European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 07, Issue 07, 2020

Fragile X Syndrome: Epigenetics marks in the Therapy

Shreya shreshtha[1], Raghu N[2], Gopenath TS[3], Parthiban R[4], Prathibha Rajashekara S[5], Kanthesh M Basalingappa[6]

[1,2,6]Division of Molecular Biology, School of Life Sciences, JSS Academy of Higher Education and Research, SS Nagara, Mysuru-570015.
[3]Department of Biotechnology & Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, SS Nagara, Mysuru-570015.
[4] Department of Clinical Laboratory Services & Translational Research, Malabar Cancer Centre, Thalassery. Kerala
[5]Department of Botany, JSS College, Ooty Road, Mysuru-570025.
Email ID: kantheshmb@jssuni.edu.in

Abstract: Fragile X syndrome (FXS) occurs due to the absence of expression of the fragile X intellectual retardation protein and it is an inherited syndrome. FXS is the monogenic form which involves a single gene, or we can say that one of the intellectual disabilities and generally called the most important reason for autism. The main cause of FXS is the deficiency of an RNA-binding protein known as Fragile X Mental Retardation Protein (FMRP). This RNA-binding protein is encrypted by the gene called Fragile X Mental Retardation Gene 1 (FMR1) gene. Multiple roles have been assigned to this protein including shipping of the RNA to translational management of mRNAs. FMRP is the protein that helps the dispatch and translation of lots of mind mRNAs by modulating an activity-based etiquette. Due to the lack of this RNA-binding protein, highbrow disability occurs which is a result of disorganization of a couple of neuronal pathways. In the present scenario, FXS efficient treatment is not available. Here in this paper, we are discussing the FMR1 gene recovery as a probable advance toward the diagnosis of FXS and pressurize on the small molecules which suppresses essential pathways for silencing of a gene which leads to the success in treating FMR1 gene allied disabilities. In conclusion, the therapeutic techniques which have visible foremost success in FXS using genetic and epigenetic manner. There is first-rate progress in basic, preclinical, and translational clinical studies that has explained a lot of molecular, cellular, and system-degree defects in FRAXA which has led to the discovery of some encouraging therapeutic techniques and analysis of this syndrome.

Keywords: Fragile X Syndrome, FMRP, highbrow disability, recovery, mRNAs, epigenetic.

1. INTRODUCTION:

The fragile X condition (OMIM +309550) is the most widely recognized acquired type of Intellectual disability. It has been achieved by an increase in a polymorphic repeating of CGG triplet, in the publicist of the FMR1 quality, including more than 200 repeats. It is a genetic disorder, occurs due to the gene which is mutated on X-chromosome. It has scientific names like Martin Bell syndrome, FRAXA syndrome, Marker X syndrome [1]. Fragile X syndrome is an inherited X-linked dominant pattern. The affected male has an elongated face, big and inflated ears, and macroorchidism besides learning disabilities as well as cognitive impairment. It also includes autism spectrum disorder affecting social and communication interactions along with medical problems epilepsy and frequent otitis media in children [2]. Moreover, there is complicacy in learning, as well as issues which include lack of attention, anxiousness, and autism, are concurrent attributes.[3]

Fragile X syndrome (FXS) is the foremost reason for inherited mental retardation which affects nearly 1 in 5000 males and 1 in 4000 to 8000 females [4]. Due to the mutations in the fragile X mental retardation 1 (FMR1) gene, active fragile X mental retardation protein is deficient (FMRP) which leads to FXS [5].

2. FMR1 GENE:

On the elongated arm of the X chromosome, the FMR1 gene is situated (fig 1). The DNA location is present at the beginning of this FMR1 gene which varies from person to person. Most of the cases of FXS occur due to the CGG trinucleotide repeating units which is a result of mutation of DNA fragment. In this FMR1 gene, CGG triplet repeat is dilated within the 5' UTR of the gene. All the alleles consisting of these repeating units are termed as full mutation (FM) alleles. Due to the alliance of these alleles with epigenetic changes, transcriptional gene silencing occurs [5,6,7].

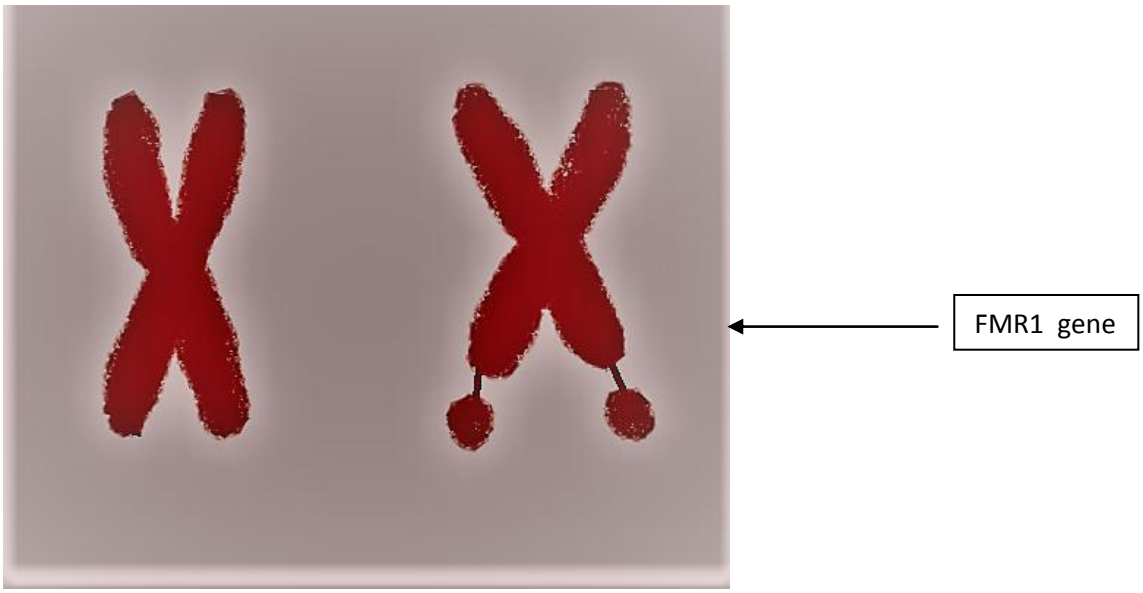

FIGURE 1: FRAGILE X GENE.

3. CAUSES:

FXS occurs due to an expansion of CGG trinucleotides which is known as full mutation and on the promoter region FMR1 gene, repeats are located on the Xq27.3 loci. The CGG triplet repeat of the FMR1 gene has a variation in the DNA sequence throughout the population. The time this triplet repeat outreaches 200 in number and methylation of the promoter takes place which results in the translation of its encoded protein called FMRP and leads to expression deficit of the gene. FMRP being an RNA-binding protein is involved in various distinctive steps of mRNA metabolism which includes shipping of RNA as well as the translational regulator. It additionally helps in neural development by controlling much synaptic protein synthesis and deficiency of these proteins results in several brain-related disabilities [8]. Premutation carriers are those which has 55–200 CGG triplet repeats inside theFMR1gene [9]. Due to the premutation in females, they are more likely to suffer from fragile X-associated primary ovarian insufficiency (FXPOI) Which means infertility and onset of menopause earlier than the age 40 [10,11]. The risk of premutation to complete mutation allele transition relies upon the maternal FMR1 alleles [12]. The repeating length of CGG triplet with 45-54 number are known as intermediate or grey zone alleles of FMR1 gene. Still, it is not clarified that intermediate alleles are associated with an elevated risk of ailment or not [9].

Epigenetics and the therapy of FXS:

In the FXS treatment, the evidence of epigenetic marks that inert FMR1 are reversible has led to the process which can be achieved therapeutically. Moreover, the treatment is targeted for the elimination of DNA methylation or histone rectifications which is conserving the FMR1 locus in a transcriptionally interstate. The drugs namely 5-azacytidine and 5-aza deoxycytidine are responsible for taking off DNA methylation. Also, these two drugs have been subjected to the treatment of myeloid leukaemia, and they also identify the therapeutic fate by the inadequately silenced suppressor gene. These drugs were applied to the cell lines of FXS patients and at the locus of the gene, FMR1 eliminate DNA methylation is removed and to repair incomplete FMRP production histone rectifications were moved to the transcriptionally active configuration [5,7,13].

FMR1 gene silencing:

When research carried out with the FXS patient cells then provided most of the knowledge concerning the benefaction of several epigenetic alterations in the gene silencing of the FMR1 gene. FMR1 gene has transcriptional functions which are controlled by the epigenetic marks which encompass DNA methylation and modification of the N terminal rears of the histone proteins related to the promoter. Generally, it has been shown that transcription activities are hypomethylated which are beneficial for acetylated histones. The FMR1 gene silencing of FXS patient cells is interrelated with the efficacious heterochromatin tags which consist of H3 di-methylated histone at lysine 9 (H3K9me2) and H3 tri-methylated histone at lysine 27 (H3K27me3), as well as integral heterochromatin tags which includes H4 tri-methylated histone at lysine 20 (H4K20me3) and H3 tri-methylated histone lysine 9 (H3K9me3) [14]. At the FMR1 locus H3K9me2 and H3K27me3 are assigned predominantly,

which is an integral heterochromatin tag range close to CGG triplet repeats and inherent expanded repeats imply the signal for their deposition [15,16,17]. However, this similar process of repeat mediates heterochromatin arrangements is not well-known in FXS, however, recent observations include a method of FMR1 mRNA. It has been explained that the outside of the protein-coding sequence the CGG expansion takes place so, the FMR1 gene reactivation should be essential in the progress of treatment in FXS. Therefore, in the perception of the epigenetic silencing method which is very important to determine if it is plausible, then to bring out the most favourable reactivation strategy. By using the in-vitro technique in FXS patients the expression of the FMR1 gene can be restored by way of inhibiting DNA with the aid of treating it with 5-aza deoxycytidine (AZA) [14]. Anyhow, AZA treatment does no longer clear away the FMR1 alleles reactivation of repressive histone methylation labels. This recommends that the accumulation of histone methylation labels occur are before DNA methylation or their accumulation develops DNA methylation self-sufficiently. Thus, the silenced allele is beneficial for histone alteration features of each functioning and integral heterochromatin [14-17].

The evidence in FXS induced pluripotent stem cells and treatment with 5-azacytidine leads to partial DNA demethylation, histone modification, and FMR1 gene partial transcriptional activation [18]. Still, there are numerous difficulties with the access as most of these induced epigenetic changes are brief, and once the drug is removed the methylation comes to a normal state within 3-4 weeks as well these agents are cytotoxic, mutagenic, and they can be blended only with dividing cells as they do not have cell specificity. There is another way is to target histone alterations with the help of histone deacetylation inhibitors such as 4-phenylbutyrate, sodium butyrate, and tri-Cho statin A, and these induce histone acetylation anyhow, there is no result in reactivation of FMR1 by using histone deacetylase inhibitors alone [19]. Also, there are different drugs available namely L-acetyl carnitine and valproic acid, considered an important treatment option in FXS, and these drugs are generally regarded as histone Deacetylates. In the study of the fruit fly model of FXTAS, it has been explained that the small molecules which are histone deacetylase inhibitors interfere with histone proteins acetylation and offer the chance of FMR1 chromatin repression and helps in restoring FMR1 transcription to the normal range [20].

4. ROLE OF CRISPER IN GENE EDITING:

Conclusively more targeted advances are needed which allows minimum cytotoxicity in selective regulation of FMR1. The evolution of the CRISPR/Cas9 gene-editing tool has been considered important in the treatment of FXS and here there are a couple of ways that are explained. First, in pluripotent stem cells of FXS patients, this CRISPR/Cas9 gene-editing tool has been used to dock the expanded CGG triplet repeats, which results in the demethylation of the FMR1 promoter and reactivation of FMRP production. Lately, the Cas9 tool, with no alterations in the sequence of DNA has been allowed to target editing of DNA methylation. By this strategy, some research has been proven that each of the FMR1 CGG expansion is demethylated and, that the FREE1 region in FXS-induced pluripotent stem cells supports sensible FMR1 chromatin derepression, reactivation of FMRP expression, and to protect the aberration in FXS neurons which are maybe electrophysiological. [21,22]

Primal treatment and FXS screening in infants:

A newborn infant FXS screening is considered as an implicit approach for parents to prevent the second offspring from the syndrome. Yet there is a question in achieving this as these methods are quite expensive as utilizing CGG triplet determining and has an implicit drawback of identifying premutation expansions. Due to these expansions, intellectual disability does not get affected but meanwhile, these are linked with a threat of late-onset disorders [23]. There are ways which use epigenetics in avoiding these drawbacks. In newborn male infants screening for anomalous methylation can be done by using a real-time polymerase chain reaction test which can determine the potentiality of this approach [24]. In research, it has been suggested that the frequency of full mutation is 1 in 5161 in the US male common population which set a comparable frequency using CGG determining. Female with a methylation test for full mutation was identified as 82%, but it was not able to find out the difference between clinically affected females and non-penetrant full mutation carriers.

Lately, a thriving diagnosis of full mutation in the newborn by examining blood spots from males and females by FREE2 methylation testing was achieved. It also demonstrated the reasonable price and ability to predict prognosis [22-24]. Ongoing studies are going on to approve the viability of this attempt for screening for each male and female newborn infants from FXS. However, it is fascinating as it is related to primal diagnosis and screening of newborn infants, research have recommended that motifs in methylation of FMR1 gene in the family are not determinate however, it can be altered over the times. It has been observed that in females, there are chances of having confirmation for favourable selection concerning their age which favours cells which has normal FMRI allele on the active X chromosome [25,26]. While it conflicts in adult males were targeted FMR1 methylation assay indicates that it may be raised moderately with age [27]. Moreover, all these are essential techniques in grasping of FMR1 activity by epigenetic alterations, as wells as in determining the adequacy of FMR1 methylation abundance, mostly in FXS females, it is regarded as a constituent of diagnostic testing. In the upcoming research, methylation alteration and its association with various tissues should establish, including Central Nervous System and these modifications are whether capable for prognosis should not be determined.

5. CONCLUSION:

In FXS, the mutation is hereditary, and the search for its therapy and treatment is still ongoing and this is not only limited to FXS, however, but this may also be generalized for all neurodevelopmental disorders. The growth of FMR1 CGG triplet repeat to a full mutation brings out the overflow of epigenetic co-occurrences including FMR1 promoter methylation and alterations of related histones in FXS. Due to the lack of FMRP or maybe due to the inactivation of other non-coding RNAs which are normally transcribed from FMR1 locus, mediates the downstream consequences elsewhere within the genome. Enhanced perception in the development gives a viable explanation for the inter-individual interpretation seen in the kids having FXS. The evolution of epigenetic-based therapies has unlocked many ways that are going to be helpful in the search for FXS treatment. These exams are highly sensitive and are essential for the screening of newborn infants which may presume a long duration of

prognosis. Still, large-scale research is needed to completely demonstrate the tests scientifically. There has been a lot of encouraging results which are provided by epigenetic therapies for fragile X including cell model system however, for now, it is not geared up for in vivo use. For these genetic diseases, many modifying therapies and medicines are still developing and maybe soon we will achieve the goal.

Author Contributions: Shreya S (SS) and Kanthesh BM (KBM) conceptualized the study. Shreya S (SS), Raghu N (RN), and T. S. Gopenath (TSG) drafted the Manuscript. Parthiban R (PR), Prathibha Rajashekara S (PRS), and Kanthesh BM (KBM) helped with the Manuscript and Discussion.

Acknowledgments: The authors would like to acknowledge the Management of JSS Academy of Higher Education & Research, Mysuru, Karnataka, for supporting the basic research ideas and also for the resources provided.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

6. REFERENCE:

[1] Martin JP, Bell J. A pedigree of mental defect showing sex-linkage. Journal of neurology and psychiatry. 1943 Jul;6(3-4):154.
[2] Harris SW, Hessl D, Goodlin-Jones B, Ferranti J, Bachman S, Barbato I, Tassone F, Hagerman PJ, Herman K, Hagerman RJ. Autism profiles of males with fragile X syndrome. American Journal on Mental Retardation. 2008 Nov;113(6):427-38.
[3] Kidd SA, Lachiewicz A, Barbouth D, Blitz RK, Delahunty C, McBrien D, Visootsak J, Berry-Kravis E. Fragile X syndrome: a review of associated medical problems. Pediatrics. 2014 Nov 1;134(5):995-1005.
[4] Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB, Moine H, Kooy RF, Tassone F, Gantois I, Sonenberg N, Mandel JL, Hagerman PJ. Fragile X syndrome. Nature reviews Disease primers. 2017 Sep 29;3(1):1-9.
[5] Pieretti M, Zhang F, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. Absence of expression of the FMR-1 gene in fragile X syndrome. Cell. 1991 Aug 23;66(4):817-22.
[6] Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang F, Eussen BE. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell. 1991 May 31;65(5):905-14.
[7] Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST. DNA methylation represses FMR-1 transcription in fragile X syndrome. Human molecular genetics. 1992 Sep 1;1(6):397-400.
[8] Banerjee A, Ifrim MF, Valdez AN, Raj N, Bassell GJ. Aberrant RNA translation in fragile X syndrome: From FMRP mechanisms to emerging therapeutic strategies. Brain Research. 2018 Aug 15;1693:24-36.

[9] Grigsby J. The fragile X mental retardation 1 gene (FMR1): historical perspective, phenotypes, mechanism, pathology, and epidemiology. The Clinical Neuropsychologist. 2016 Aug 17;30(6):815-33.
[10] Rajaratnam A, Shergill J, Salcedo-Arellano M, Saldarriaga W, Duan X, Hagerman R. Fragile X syndrome and fragile X-associated disorders. F1000Research. 2017;6.
[11] Sherman SL. Premature ovarian failure in the fragile X syndrome. American journal of medical genetics. 2000 Sep;97(3):189-94.
[12] Nolin SL, Brown WT, Glicksman A, Houck Jr GE, Gargano AD, Sullivan A, Biancalana V, Bröndum-Nielsen K, Hjalgrim H, Holinski-Feder E, Kooy F. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. The American Journal of Human Genetics. 2003 Feb 1;72(2):454-64.
[13] Kumari D, Gazy I, Usdin K. Pharmacological reactivation of the silenced FMR1 gene as a targeted therapeutic approach for fragile X syndrome. Brain sciences. 2019 Feb;9(2):39.
[14] Kumari, D,Usdin, K. The distribution of repressive histone modifications on silenced FMR1 alleles providesclues to the mechanism of gene silencing in fragile X syndrome. *Hum. Mol. Genet*.2010;19: 4634–4642.
[15] Hecht M, Tabib A, Kahan T, Orlanski S, Gropp M, Tabach Y, Yanuka O, Benvenisty N, Keshet I, Cedar H. Epigenetic mechanism of FMR1 inactivation in Fragile X syndrome. International Journal of Developmental Biology. 2017 Jun 2;61(3-4-5):285-92.
[16] Kumari, D.; Usdin, K. Polycomb group complexes are recruited to reactivated FMR1 alleles in Fragile Xsyndrome in response to FMR1 transcription. *Hum. Mol. Genet*.2014;23; 6575–6583.
[17] Colak, D.; Zaninovic, N.; Cohen, M.S.; Rosenwaks, Z.; Yang, W.Y.; Gerhardt, J.; Disney, M.D.; Jaffrey, S.R. Promoter-bound trinucleotide repeat mRNA drives epigenetic silencing in fragile X syndrome. *Science.* 2014;343:1002–1005.
[18] Tabolacci E, Pomponi MG, Pietrobono R, Chiurazzi P, Neri G. A unique case of reversion to normal size of a maternal premutation FMR1 allele in a normal boy. European journal of human genetics. 2008 Feb;16(2):209-14.
[19] Chiurazzi P, Pomponi MG, Willemsen R, Oostra BA, Neri G. In vitro reactivation of the FMR1 gene involved in fragile X syndrome. Human molecular genetics. 1998 Jan 1;7(1):109-13.
[20] Todd PK, Oh SY, Krans A, Pandey UB, Di Prospero NA. Histone Deacetylases Suppress CGG Repeat–Induced Neurodegeneration Via.
[21] Park CY, Halevy T, Lee DR, Sung JJ, Lee JS, Yanuka O, Benvenisty N, Kim DW. Reversion of FMR1 methylation and silencing by editing the triplet repeats in fragile X iPSC-derived neurons. Cell reports. 2015 Oct 13;13(2):234-41.
[22] Xie N, Gong H, Suhl JA, Chopra P, Wang T, Warren ST. Reactivation of FMR1 by CRISPR/Cas9-mediated deletion of the expanded CGG-repeat of the fragile X chromosome. PloS one. 2016 Oct 21;11(10):e0165499.
[23] Godler DE, Amor DJ, Slater HR. Methylation analysis in newborn screening for fragile X syndrome. Jama Neurology. 2014 Jun 1;71(6):800-.

[24] Coffee B, Keith K, Albizua I, Malone T, Mowrey J, Sherman SL, Warren ST. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. The American Journal of Human Genetics. 2009 Oct 9;85(4):503-14.

[25] Inaba Y, Schwartz CE, Bui QM, Li X, Skinner C, Field M, Wotton T, Hagerman RJ, Francis D, Amor DJ, Hopper JL. Early detection of fragile X syndrome: applications of a novel approach for improved quantitative methylation analysis in venous blood and newborn blood spots. Clinical chemistry. 2014 Jul 1;60(7):963-73.

[26] Godler DE, Inaba Y, Schwartz CE, Bui QM, Shi EZ, Li X, Herlihy AS, Skinner C, Hagerman RJ, Francis D, Amor DJ. Detection of skewed X-chromosome inactivation in Fragile X syndrome and X chromosome aneuploidy using quantitative melt analysis. Expert reviews in molecular medicine. 2015;17.

[27] Taylor AK, Safanda JF, Fall MZ, Quince C, Lang KA, Hull CE, Carpenter I, Staley LW, Hagerman RJ. Molecular predictors of cognitive involvement in female carriers of fragile X syndrome. Jama. 1994 Feb 16;271(7):507-14.