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Histone modifications and FMR role in Fragile X syndrome: A review

Debarati Roy Chowdhury[1], Salmataj S A[1], Pushanjali Bhat[2]

[1]Department of Biotechnology, Manipal Institute Technology, MAHE, Manipal
[2]Department of Chemistry, Manipal Institute Technology, MAHE, Manipal.

**Corresponding author's E-mail: Debarati Roy Chowdhury*

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Abstract

Fragile X syndrome is the most common cause of genetically acquired mental disability and one of the most frequent single-gene disorders. FXS serves as an excellent model disorder for studies investigating disturbed molecular mechanisms and synapse function. FXS is an X-linked disorder that affects approximately 1 in 4000 males and females, with females experiencing milder cognitive deficits. It is caused due to expansion of trinucleotide CGG repeat above the normal range (>54 repeats) in the 5' noncoding region of the fragile x mental retardation 1 (FMR1) gene located in the q27.3 loci of the X chromosome, which results in hypermethylation of CpG islands and eventually to transcriptional silencing of the gene hence suppressing the production of the Fragile X mental retardation protein (FMRP). The lack of FMRP, involved in multiple aspects of mRNA metabolism in the brain causing intellectual disability, autism spectrum disorder, and deregulation of multiple pathways, is thought to be the direct cause of the FXS phenotypes. The purpose of this review is to summarize the basic models used to study the aspects of fragile x syndrome, the relation of FMR1 gene and FMRP with the disease, histone modifications, and various targeted molecular therapy used for cure.

Keywords: *X chromosome, FMRP, FMR1 and FXS*

1. Introduction

Fragile X Syndrome is an X linked dominant disorder caused due to mutations in the fragile x mental retardation 1(FMR1) gene and hence leading to the absence of fragile x mental retardation protein(FMRP). It was first discovered in the 1940s (; Everaers, RalfRosa, 2017). FXS is a multigenerational disorder and the most common type for causing intellectual disability after Down's Syndrome (Dean et al., 2016). Intellectual disability/mental retardation (ID) is the failure to develop sufficient cognitive and adaptive levels, causing a lack of skills necessary for day-to-day living. This is normally reflected in learning, maturation, and social adjustments. About 20% of ID cases are due to FXS (Dean et al., 2016). An affected female will have 50% affected children but an affected male will have all affected daughters and all normal sons (Dean et al., 2016). The females have a benefit over the males as they have the advantage of an unaffected X chromosome. FXS is found in 1:4000 males and 1:8000 females (Dean et al., 2016; Koukoui & Chaudhuri, 2007).

The expansion of a trinucleotide repeat of cytosine, guanine, guanine (CGG Repeat) above normal levels in the 5' untranslated region of the FMR1 gene, located at Xq27.3, is responsible for the development of the symptoms related to FXS (Hagerman et al., 2017; Jiraanont et al., 2016; Koukoui & Chaudhuri, 2007). The expansion is next to the promoter and hence interferes with the transcriptional activities. The stability of this region depends on the length of the CGG repeat. CGG expansion leads to hypermethylation of the region which leads to functional silencing of the FMR1 gene and no production of FMRP (Salcedo-Arellano et al., 2020). Other ways such as deletion and point mutation can also be the reason for the cause of the disease. Along with intellectual disabilities, there are number of characteristic features of patients with FXS, physical features include the elongated face, prominent ears, and forehead, flat feet, velvety skin; connective tissue anomalies include low muscle tone, hyperextensible joints, macro-orchidism, and behavioral abnormalities include anxiety, hyperactivity, social withdrawal, autism spectrum disorder etc,. (Dean et al., 2016; Salcedo-Arellano et al., 2020).

The diagnosis of the disease is done by FMR1 DNA testing and identification of CGG repeats. This involves PCR tests and the southern blotting of blood samples which can identify the size of both permutation and full mutation alleles (Hagerman et al., 2017). Evaluation of methylation levels in the FMR1 gene is important as it is directly linked to the building of the cognitive abnormalities. The advanced genotyping tools have reduced the labor work and time consumed and also increased the efficiency of the tests and results (Dean et al., 2016; Hagerman et al., 2017). An FMR1 DNA testing should be done in children who don't build a verbal speech by the age of 2-3 years, or any child that shows signs of intellectual disability of ASD without a known reason. Diagnosis is important as it will lead to new treatment opportunities including targeted therapy, and also the diagnosis will affect the family members of the patient. Cascade testing (that is DNA testing of other family members) is recommended so that if anyone is tested with , and expanded FMR1 region they can avoid it from spreading to their offspring (Hagerman et al., 2017). The management of FXS is usually led by pediatrician, neurologist, and psychiatrist with expertise in this field. Several therapies, like speech and language improvable, physical therapy, pharmacological therapy, and behavioral therapy can be useful for the betterment of the patient (Hagerman et al., 2017). Adequate sleep is important for optimal learning development, and functioning.

Models

FMR1 Knockout (KO) Mouse Model

This being one of the major pre-clinical models to study the aspects related to FXS and the importance of FMR1 in various mechanisms. The knockout mice are present in the C57BL/6 background and do not possess FMR1 protein (Ding et al., 2014). Hemizygous male and homozygous female KO mice were used to generate a mutant type. The presence of the KO allele was done using PCD and southern blotting analysis (The Dutch-Belgian Fragile X Consorthium et al., 1994). Different assays that were carried out were. behavioral tests, cognitive function analysis, audiogenic seizures- FMR1 KO models showed wild running, death, seizures and the startle reflex is decreased due to overall decrease of neuronal network (The Dutch-Belgian Fragile X Consorthium et al., 1994); open field exploration test- KO models had more movement and larger distance covered hence showing the hyperactivity; light/dark test- used to measure latency to enter by the mice and also the KO mice crossed the line between the light/dark section multiple times hence showing the more exploratory behavior (Ding et al., 2014); passive avoidance test, etc. Morris water maze is one of the widely used tests to examine the deficit in spatial learning with abnormalities in long term potentiation, along with decreased GluR1 expression in the synapses on the cortex (Kooy, 2003). A mild deficit was noticed especially in the reverse phase where the hidden platform was suddenly changed. This is linked to the hippocampal defects due to the size of the intra- and infra pyramidal mossy fiber terminal fields (IIPMF) which is noticed to be reduced in KO mouse (Kooy, 2003). Macro-orchidism is significantly observed which gradually increases over time, with heavier tested weight in KO models than the control mice due to an increased amount of interstitial tissue (The Dutch-Belgian Fragile X Consorthium et al., 1994). This model is hence suitable and valid to study the role of FMR1 and help in developing gene therapy for FXS in humans.

DNAzyme Based Model

Created larval model for FXS of zebrafish mainly focusing on the early development window (Medishetti et al., 2020). DNAzyme is an oligonucleotide that enzymatically cleaves complementary RNA sequences as a specific site (Medishetti et al., 2020). FMRP is thought to be expressed at very early stages in the embryo and the central important thing in the development and differentiation of the neural circuit. Zebrafish FMRP is a highly conserved homolog of the human FMRP. One of the advantages of selection zebrafish for the model is the easy access of the embryo at 0-6 hours post-fertilization (hpf) where early phenotypes can be used for the study (Medishetti et al., 2020). This model was created to be a high throughput, inexpensive, novel method for knockdown of FMRP and this was done so by catalytic DNA mediated degradation of fmr1 mRNA in 0 days post-fertilization(dpf) zebrafish embryos. Cloning of the fragments FMR1 ORF(400bp) and mGluR5 ORF into a pUC57 vector under a T7 promoter was done. Zebrafish were managed in circulating reverse osmosis water at 28 ± 1°C with a 12h light/dark cycle (Medishetti et al., 2020). The FMR1 ORF segment was amplified by using PCR. Zebrafish embryo was collected and injected with 10ml of the solution using microinjection, following electroporation. DNAzyme levels were measured with PCR and 2μg RNA was used to synthesize the cDNA, following western blot. Various studies like behavioral studies, open field test(OFT), drug and treatment pattern was done to study symptoms of FXS and model validation (Ng et al., 2013). The delivery of the enzyme was done to maintain minimum lethality. This was present in vivo up to 72 hours with 20% on the injected amount. The level of FMR1 and FMRP in treated embryos reduced by 60-80% up to 24hpf and subsequently got

restored over a course of 2-3 days (Medishetti et al., 2020). Similar to human FXS phenotypic abnormalities, changes like tail deformities, bent notochord, and craniofacial abnormalities were observed in the DNAzyme treated embryos (Medishetti et al., 2020). Shaoling behavior was tested and shoaling preference was used to test the social behavior deficiency of the knockout model (Wu et al., 2017). OFT test was used to measure the anxiety levels and it was noticed that the affected population preferred to stay away from the open central area and stick close to the peripheral walls of the petri dish indicating increased anxiety and their total speed and distance swam was noted (Medishetti et al., 2020; Ng et al., 2013). For the passive avoidance test a red bar stimulus was given on the lower half of the petri dish, it was noted that reaction to stimulus in the control group preferred being in the upper half during the stimulus as compared to FXS larvae which did not react to the stimulus hence portraying lack of cognition and reaction to an aversive stimulus (Medishetti et al., 2020). The FXS group also showed increased circling relative correlation to the repetitive behavior observed in FXS and ASD. The protection of embryo from FMRP reduction in the early stages can be a good measure in reducing the impact of the disease (Medishetti et al., 2020). These results indicated that changes and treatment done in the early stages can help in the rescue of the abnormalities caused. This model also projected the DNAzyme based knockout of the fmr1 gene, thus creating a model for FXS.

Fmr1 and Its Role

Fragile X mental retardation 1 is a human gene that codes for fragile X mental retardation protein (FMRP). Changes and expansion in the FMR1 gene in the primary cause of the disease since the altered functioning of the gene results in no production of the FMRP and thus causing all the defects. FMR1 is also responsible for the premature ovarian failure and fragile X- associated tremor (Oostra & Willemsen, 2009). The gene consists of 17 exons spanning 38 kb of DNA (Dean et al., 2016). Expansion of the trinucleotide repeat CGG (polymorphic) in the 5’ untranslated region of FMR1 gene located in q27.3 chromosome loci of the X chromosome suppressed the translational activities and hypermethylation. FMR1 gene is classified into 4 allelic forms, normal allele (5–44 repeats), intermediate allele (45–54 repeats), pre-mutation (PM) allele (55–200 repeats), and full mutation (FM) allele (>200 repeats) (Dean et al., 2016; Pietrobono et al., 2005). FXS patients contain >200 CGG repeats which is the full mutated allele that is usually hypermethylated and this methylation extends up to the promoter region. The pre-mutated alleles can transform into fully mutated alleles on maternal transfer during meiosis in several generations (Oostra & Willemsen, 2009).Many mechanisms have been proposed over the year for the reason behind the expansion of the repeat, one of them being the slippage of the replication fork which happens dude to loop formation by the unpaired bases which in turn leads to expansion or contraction in the next round of replication (Oostra & Willemsen, 2009). However, slippage alone is not the reason for the expansion.

Unmethylated FMR1 genes are transcriptionally active but methylation leads to the silencing of the FMR1 gene and the promoter region (Koukoui & Chaudhuri, 2007). The change in the environment of the FMR1 gene from transcriptionally active chromatin to inactive heterochromatin due to the loss of 4 sites: a palindrome, two GC boxes, and a cAMP response element present in the promoter region of the gene (Garber et al., 2006). DNA methylation happens during early embryonic stages and leads to gene silencing by inhibition of transcription binding factor or through inducing chromatin changes at the site of methylation (Coffee et al., 2002). Methylation of the CpG islands is also one of the important factors for the expression of the fragile X site. Somatic mosaicism is often observed in the CGG repeats (Flannery et al., 1995). Some protein has also been known to bind to the CGG repeats and hence they either create or destroy protein binding sites (Flannery et al., 1995).

Internal structure analysis of the FMR1 gene has shown that it is an array of CGG repeats with regularl space AGC trinucleotides giving a definite structure (Mila et al., 2018). Most commonly in a population, they have one or two interruptions of AGG in contrast to the permutation alleles which have no or few AGG interruption which in turn possess a higher risk of unstable transcription (Pietrobono et al., 2005). These may be the precursors following further spillage which leads to the fragile X chromosome. It was noticed that the fragile X site occurs at the location of delayed replication which resulted due to disturbance in condensation of the chromosome in the G2 phase of the cell cycle (Hansen et al., 1993). Replication of the CGG repeats is very difficult because of the higher melting temperature of the methylated region which could be the manifestation of the fragile site. But it was noticed that the delayed replication time was due to stalling from both the 3’ and 5’ end sides of the repeats (Hansen et al., 1993). The CGG expansion has the capability of inactivating the replicating origin thus switching the timing to a later time.

As seen above, expansion and methylation of CGG repeat have been the major cause of FXS, it has also been found out that point mutation, deletion of the entire FMR1 gene, and presence of no CGG repeat can also cause the disease. A rare case of germinal mosaicism (phenotypically and genotypically normal parents possessing affected children) was noticed where an entire deletion of 300kB of DNA segment that was removing the entire FMR1 gene led to FXS. It has been noticed that either small deletion (<10 kB) which results in CGG instability or large deletion (>13Mb) can occur (Jiraanont et al., 2016). Detection of no primer amplification and no hybridization by doing molecular analysis concludes the deletion of the FMR1 gene (Jiraanont et al., 2016). The large deletions occur due to crossing over of non-homologous chromosomes rather than around the homologous loci. There are some hotspots present in the CGG repeat for deletion which act as breakpoints near the 5' region which emphasize the instability of this region. The silencing of the gene also consists of four epigenetic changes: DNA methylation, histone modification, chromatin remodeling, and RNA interference (Pietrobono et al., 2005; Tabolacci & Chiurazzi, 2013). Transcription locus of FMR1 contains several non-coding RNAs (ncRNAs) including long ncRNAs which can transcribe both sense and antisense strands and the ncRNAs/lncRNAs may act as transcription regulators as well (Pietrobono et al., 2005). Hence the cognitive impairment in FXS is caused due to the changes in the FMR1 gene which in turn leads to no FMRP in the neurons leading to various ailments.

Fragile X Mental Retardation Protein (Fmrp)

Fragile X mental retardation protein is an RNA binding protein (RBP) which is involved in various steps like mRNA metabolism (D'Annessa et al., 2019), stability, localization, and translation which is critical to neuronal development, dendritic spine architecture, and synaptic plasticity (Ferron et al., 2020) thus regulating a group of proteins important for synaptic functions (Alpatov et al., 2014). FMRP acts as a repressor of translation especially at the synapses (Pietrobono et al., 2005). FMRP is present in abundance in ovaries, testes, and brain of fetal and adult tissue (D'Annessa et al., 2019) which can recognize and bind up to 4% of mRNAs present in them (De Rubeis & Bagni, 2010). FMRP and its analogs FXR1P and FXR2P which can partially compensate for the loss of FMRP, make up the small family of RNA-binding proteins with more than 60% of amino acid identity (Jin & Warren, 2003; Zalfa et al., 2003). It is known that FMRP is largely a cytoplasmic protein with only about 4% of it being in the nucleus but has a potential role (Alpatov et al., 2014). FMRP is also associated with actively translation polyribosomes in an RNA-dependent mannver via ribonucleoprotein (mRNP) particles. FMRP does not change the mRNA hence proving that FMRP is a translation regulator (Jin & Warren, 2003).

Through its multi-domain structure, it interacts with microRNAs, proteins, and non-coding regions as well (D'Annessa et al., 2019). This protein has three RNA binding domains: one RGG box rich in arginine and glycine residues two ribonucleoprotein K homology domain (KH domains- KH1, KH2) (Athar & Joseph, 2020; Chen et al., 2014; Eliez et al., 2000). The KH domains and RGG box are structurally organized among 3 terminus that is the N- terminus, C- terminus, and the central domain (D'Annessa et al., 2019). The N-terminus is responsible for most of the protein–protein interaction and RNA binding motif. It was found out that N- terminus consists of a preceding KH domain knowns as KH0 differing from KH1 and KH2. KH domains are single nucleotide-binding motifs found in tandem modules of 2-16 repeats long. The central domain consists of KH1and KH2 domain and any point mutation in KH2 can lead to severe phenotypic changes related to FXS. C- terminus contains the RGG box which is responsible for the interactions with the RNAs.

The RGG box binds with guanine (G) quadruplex forming RNA motif stabilized by K^+ ions and interaction is modulated by methylation of the arginine's in the box, hence the affinity decreases upon methylation (D'Annessa et al., 2019). G quartet are hydrogen-bonded structures formed from four guanosine residues in a square planner array stabilized by K^+ ions and disrupted by Li^+ (Jin & Warren, 2003). FMRP bind to RNAs that possess G-quadruplex structure and repress their translation (Chen et al., 2014). It has also been found that FMRP when interacted with microRNAs and RNA-inducing splicing complex (RISC) regulates the translation of mRNAs (Banerjee et al., 2018). FMRP contributes in helping the miRNA-RISC complex to recognize the RNA and repress the translation. Post translation modification of FMRP is also needed for the miRNA-RISC complex to be able to find the target RNA. It has been found out that the protein can shuttle between nucleus and cytoplasm with the help pf certain factors like Nuclear localization signal (NLS) and nuclear export signal (NES) (D'Annessa et al., 2019). it is transported into the nucleus of the neurons via its NLS where it assembles into a mRNP complex interacting with specific RNA and protein. Subsequently, FMRP-mRNP complex is transported out via NES (Feng et al., 1997; Jin & Warren, 2003). In the cytoplasm, the FMRP-mRNP can associate with ribosome or get transported to dendrites to regulate local protein synthesis.

Histone Modifications

The shutdown of FMR1 gene activity is not only because methylation in CGG region but also because of modifications made in the histone of the chromatin. It has been seen that DNA methylation leads to chromatin changes through histone deacetylases (HDACs) by a methyl CpG binding (MBD) protein like MeCP2. The H3K9 methylation is recognized by HP1 protein which recruits DNA methyltransferase which then binds to the MeCP2 (Pietrobono et al., 2005). The MeCP2 binds to the histone deacetylases and suppress the transcription activity (Coffee et al.,1999). This acetylation of the histone results in a stronger association between the histone amino termini and DNA resulting in condensed chromatin structure which excludes the transcription factor (Coffee et al., 2002). Full mutation of FMR1 that is present in FXS patients is essentially the loss of acetylation of H3 and H4 histones, reduction of lysine 4 in H3 (H3K4), and increased methylation of lysine 9 in H3 (H3K9), acetylation linked to histone H3 is linked to the transcriptional activity (Garber et al., 2006). Methylation on H3K4 also inhibits the binding of nucleosome and deacetylase repressor complex (Pietrobono et al., 2005). It has been revealed that the amount of H4 acetylation is inversely proportional to the CGG repeat length and more spread out towards the 3' end of the gene and remains insufficient to open the condensed chromatin (Coffee et al., 2002). The active promoter region contains high levels of histone acetylation and H3K4 methylation, while the silenced promoter has high-level H3K9 methylation (Gheldof et al., 2006). In fragile X cells, H3Ac, H4Ac, and H3K4Me2 at the FMR1 promoter were all reduced, whereas H3K9Me2 was increased at the transcription start site of FMR1 marking that histone modification can happen in a highly localized manner (Coffee et al., 2002). These histone modifications directly affect the chromatin conformations. Histone acetylation reduces the ability of the chromatin to form compact fibers. The cascading events that cause the FMR1 inactivation are histone deacetylation, H3K9 methylation followed by DNA methylation and H3K4 demethylation, lysine 27 on H3 is trimethylated (H3K27me3) and lysine 20 on H4 (H4K20) increases its methylation status near the CGG expansion (Pietrobono et al., 2005) but undermethylated full mutation carriers somehow cannot complete the silencing process and hence carry an active FMR1 gene (Tabolacci & Chiurazzi, 2013). The re-activation of FMR1 could be done sing azadC accompanied by increasing the amount of H3 and H4 acetylation at the 5' end. In contrast to hypoacetylated full mutation alleles, pre-mutation alleles have 1.5-2 times of normal level of acetylated H3 and H4 which results in a more open chromatin form leading to hairpin formation, stalling of 40s ribosome unit leading to translation deficit in PM alleles (Pietrobono et al., 2005).

Treatment

As we know, FMR1 silencing due to methylation and epigenetic changes is the cause for the disease. This may be cured by re-activation of the FMR1 gene which might successively end in gaining back the transcriptional activity and formation of the FMRP needed for synapses. Pharmacological treatment with 5-aza-2-deoxycytidine (5-azadC) or 5-azacytidine (5-azaC) is one of the most common ways (Shitik et al., 2020) and has been found out to be a way of for reactivating the gene. 5-azadC/5-azaC analogs of deoxycytidine are usually incorporated into the cell line during replication which irreversible block DNA methyltransferases (Tabolacci & Chiurazzi, 2013). DNA binding proteins CTCF is considered one of the possible regulators for controlling FMR1 transcription was absent in FM alleles, treatment with 5-azadC was not able to restore the CTCF transcription (Pietrobono et al., 2005). The FXS lymphoblastoid cells were treated with 5-azacD for 7 consecutive protein and hence a reduction of the protein led to reduction of FMR1 and FMR1 antisense RNA1 days. 5-azacD treatment was combined with histone deacetylase and great changes in FMR1 reactivation were noticed.

5-azadC induces histone acetylation and increased methylation oh H3K4, reducing methylation of H3K9 which restore euchromatin configuration in the FMR1 promoter in treated FXS cells translation a methylated FM into an unmethylated full mutation (UFM) (Tabolacci & Chiurazzi, 2013). The treatment also results in partial restoration of FMRP production. The treatment in partial and induced, after the withdrawal of 5-azadC it was seen that promoter region resumed its methylated condition (Pietrobono et al., 2005). De-methylation effect in restricted to the the promoter region of FMR1. The efficiency of the reactivation is directly proportional to the amount of demethylation done by this treatment (Chiurazzi et al., 1998). Limitations related to use of 5-azadC is related to its toxicity in term of long usage and an apparent need of cell division for it to be active (Pietrobono et al., 2005). Valproic acid (VPA) which acts as a histone deacetylases inhibitor but not a DNA demethylator also been used for treatment but has shown very little modest effect as a transcriptional reactivator of FMR1 (Syndrome, 2019). The mGluR theory was considered one of the reasons for the causes of phenotypic changes related to FXS. The activation of metabotropic glutamate receptors (especially

mGlu5) stimulates the production of long term depression (LTD) stabilizing proteins and FMRP which halts the synthesis of LTD (Luo et al., 2016). This activation leads to mRNA translation at the synapses, AMPA receptors internalize and loss of FMRP leads to several protein synthesis-dependent consequences of activating mGluRs are exaggerated and loss of AMPA receptors (Dölen et al., 2007). Loss of these receptors leads to damaged spine morphology and LTD. Since the discovery of mGluR theory lots of antagonists have been found for the cure of this and in turn cure the FXS disease. The antagonists that are used for this are MPEP being the most promising and AFQ056 which has a non-competitive inhibitory mode of action (Levenga et al., 2011). Since MPEP was found to be non-specific for mGluR receptors, analogs like AFQ056 were developed for the usage (Doering et al., 2013). It was seen that the mGluR antagonists were able to rescue the abnormal dendritic spine length by reducing the spine length and changes in the width and density of the same (Levenga et al., 2011) along with a positive effect in behavioral phenotype (Pop et al., 2014). This tells us the AFQ056 could be a good antagonist is in the cure of many FXS phenotypes.

Another method for treatment is via administration of adeno-associated virus (AAV) vectors for gene delivery (Syndrome, 2019). For CNS treatment of the disease, AAV9 has been the most widely used, and studied vector (Arsenault et al., 2016). The FMR1-AAV injected contains the gene encoding the major CNS isoform of FMRP (Zeier et al., 2009). Though it is capable of affecting other areas, when injected in CNS is preferentially only affects the neurons (Morgenstern et al., 2011). The goal of this treatment is to mimic as close as possible to brain-wide expression of FMRP. After the administration of the recombinant AAV using microinjection, therapeutic protein expression increases gradually, and peaks at about 3-4 weeks post injection (Hampson et al., 2019). AAV shows efficient transduction, maintains robust transgene expression, and has high degree of safety as they do no integrate in the host genome (Zeier et al., 2009). It was noticed that after infection with AAV-FMRP, behavioral and social anxiety levels had decreased along with normal amount production of MeCP2 which was capable of correcting the expression of proteins linked to FMRP (Hampson et al., 2019). Few limitations associated with this method are large scale production, restricted room for target DNA insertion, presence of anti-AAV antibodies in human population and insertional genotoxicity that leads to oncogenesis (particularly in the liver) (Alves & Cartier, 2017; Hampson et al., 2019).

2. Conclusion

Fragile X syndrome is an X linked genetic disorder which causes phenotypic changes and mental disability. It is a monogenic syndrome with every patient having the same mutations in their gene. Various animal models have been studied to know about the symptoms, causes and targeted treatment for the disease. FMR1 gene and its protein FMRP have been identified as the main reason for the disease to occur. Methylation, expansion and epigenetic changes in the gene cause the defects which silencing the gene resulting in no production of the protein FMRP which is needed for synaptic plasticity. Various clinical treatments like FMR1 reactivation, adeno-associated virus gene therapy, pharmacological treatments are being formulated and checked for the betterment of the patients and for a stable cure of the disease but correction of effect of genetic defect is still a tremendous challenge. Various clinical therapies have been found out to be useful for the development of IQ, speech etc.

Conflict of interest

None

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