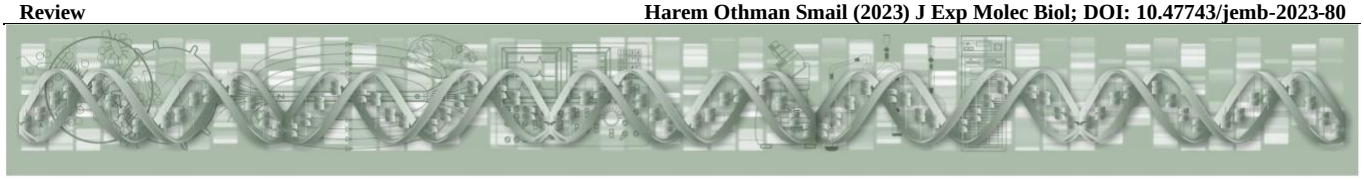

The molecular basis of the Fragile X syndrome

Harem Othman Smail

Department of Biology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region-F. R. Iraq
Email: harem.Othman@koyauniversity.org

Abstract

This analysis aimed to clarify the molecular basis of fragile X syndrome and explain the role of genetic material in the genetic disease's development and treatment. Fragile X syndrome is an X-linked mutation inheritance disorder. The mutated gene is called FMR-1. This is important for normal brain development and synaptic plasticity, which was verified and recognized in 1991, and this has become a hope for more clarification. FMR1 influences the translation of messenger RNA (mRNA), but identifying functional targets was complex and directly related to translational control and showed that dysregulated translation initiation signalling was observed for the FMR1 gene in the FMR1 knockout mouse model of FXS.

Because of the epigenetic alteration, such as hypermethylated at the DNA promoter region, and chromatin modification, such as H3K9 methylation, the FMR1 gene can be imprinted. Still, their mechanisms of aberrant epigenetic marks play a role in the etiology of many neurodevelopmental disorders, some of which we still do not fully understand and need to show more. The opportunities for epigenetic markers to map and alter epigenetic marks and the potential for therapies based on epigenetics and noncoding manipulation. For neurodevelopmental and behavioral conditions, including mental retardation, autism, anxiety, and mood disturbance, FMR1 loss of control is a model.

Most studies have focused on the new and effective approach for Fragile X syndrome, which is Gene therapy is unarguably the definitive way to treat and possibly cure genetic diseases. Many of them are under clinical trial, but more studies, such as the CRISPR/Cas9-based method, should be approved. Adeno-associated viral (AAV) vectors are highly effective for generating models. Most research is used in the mouse model of fragile X syndrome, where AAVs have been used to express fragile X mental retardation protein (FMRP), which is missing or highly reduced in the disorder. The vast expansions need southern blotting in myotonic dystrophy. Fragile X is diagnosed by a form of Southern blotting that relies on the size and the FRAXA gene's methylation status. Almost always, genetic testing is performed by PCR. The few Southern blotting uses include checking for significant destructive gene rearrangements and complete mutations of fragile X and myotonic dystrophy. Expansions suppress the expression of closely adjacent genes, causing loss of function. A named FMRI (fragile-X mental retardation syndrome) gene cDNA probe. Some unique molecular mechanisms, such as CGG expansion in Fragile-X syndrome, can make a particular sequence change in a gene far more probable than any other change.

Keywords: X-linked intellectual, FMR-1 triplet expansion region, RNA-binding protein, X mutation, DNA methylation, and Gene therapy

Introduction

Fragile X syndrome (FXS) is a genetic disorder believed to raise the likelihood of cognitive dysfunction and socio-emotional difficulties (Bartholomay *et al.,* 2019). The mental, behavioral, and physical phenotypes differ by sex, with the X-linked inheritance of the mutation affecting males more strongly (Crawford *et al.,* 2001). Owing to the full mutation, the fragile X mutation that segregates in a family is most frequently detected by a child with fragile X syndrome, with symptoms such as developmental delay or mental retardation (Sherman *et al.,* 2005). In 1969, in 3 generations of a family, Lubs first identified a separate fragile site on the X chromosome segregating with intellectual disability. In 1991, the association of the Xq27.3 fragile site with X-linked intellectual disability was confirmed (Ciaccio *et al.,* 2017).

For many medical conditions related to FXS, including epilepsy, and chronic otitis media (O.M.), patients with FXS can be seen in clinical settings (Kidd *et al.,*2014). Hyperactivity is the most common behavioral concern in children with FXS. In approximately 80 percent of people with FXS, attention deficit hyperactivity disorder (ADHD) is diagnosed (Cowley *et al.,* 2016). Significant progress has been made in both the clinical aspects of the condition and its mechanistic basis; hence, when offering anticipatory guidance, primary care physicians must be familiar with these developments (Visootsak et al., 2005).

The animal model study has resulted in the development of possible novel pharmacological therapies that address the fragile X syndrome's underlying molecular defect rather than the resulting symptoms (Hagerman *et al.,* 2014). Although there is evidence of physical symptoms, mostly premature menopause, and mild external features of the fragile X syndrome among premutation carriers, premutation is associated with no effects (Mazzocco 2000). As highlighted by metabotropic glutamate receptor antagonists and gamma-Aminobutyric acid receptor modulators, knowledge of its pathophysiology has led to the creation of several targeted FXS therapies (Sutherland *et al.,* 1991).

Prevalence of Fragile X syndrome

Soy is ingested in different ways by many adults and children, but little knowledge is available on possible neurological side effects. Prior work suggests a correlation in mouse models of neurological disease and children with autism between the

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ingestion of soy-based diets and seizure prevalence (Westmark *et al.*, 2020). Over the past ten years, rapid advances in the genomic medicine world, including introducing whole-genome sequencing into state-funded/subsidized healthcare systems, such as the NHS (National Health Service), now suggest extending population-level genomic screening services is at least technologically feasible (Boardman 2020). The most common hereditary cause of mental retardation is Fragile X syndrome (FXS, OMIM 300624), affecting 1:3000–1:4000 males and 1:6000–1:8000 females (Hantash *et al.*, 2011).

Around one in 2,500 is the frequency of the complete mutation allele (Cordeiro *et al.*, 2011). Therefore, it is estimated that 37,000 males (1 in 3,847) and 38,400 females (1 in 3,847) bear the fragile complete X mutation within the U.S. population (Kronk *et al.*,2010). However, data on the relative prevalence, frequency, and severity of problem behaviors shown by FXS boys are minimal compared to mixed-etiology I.D. boys who also demonstrate problem behaviors (Hall *et al.*, 2016). Therefore, there will be inadequate fragile X mental retardation protein (FMRP) in the affected person. Lower repeat numbers are referred to as "premutation", ranging from 55 to 200, whereas those between 45 and 54 are referred to as "intermediate", and those between 35 and 44 are referred to as "in the high normal range" (De *et al.*,2014).

Molecular basis of Fragile X syndrome

Fragile X mental retardation gene (FMR1)-coded protein reduction causes fragile X syndrome. This genetic disorder causes several developmental issues, including learning disabilities, cognitive impairment, and behavioral disorders (Lyons *et al.*, 2015). Currently, clinical participation in males and females bearing the FMR1 premutation is a real health issue (Mila *et al.*, 2018). Many of the common fragile sites, such as those induced by APH, a DNA polymerase alpha inhibitor, are distributed with a heterogeneous composition across megabases of DNA. These regions are also enriched with high versatility and low stability for A + T-rich sequences and sequences (Yudkin *et al.*, 2014).

In 8 of four cosmids contiguous YAC DNA, a gene (FMR-1) was identified that expresses a 4.8 kb message in the human brain (Verkerk *et al.*,1991). The Fragile X Mental Retardation 1 (FMR1) gene encoding the FMR1 protein is essential (Yrigollen *et al.*, 2012). Most cases of FXS result from the expansion of a CGG·CCG repeat in the 5′ UTR of the FMR1 gene that leads to gene silencing (Kumari and Usdin 2010). Patients with FXS have more than 200 CGG trinucleotide repeats. On the other hand, although premutation carriers (55 to 200 repeats) are not affected by the classic FXS phenotype, they can have other medical, psychiatric, and neurological problems (Saldarriaga *et al.*, 2014). The lengthening of the CGG repeat, the cause of FXS, is hypothesized to occur with the addition of length-specific interruptions (e.g., AGG, CGA, or CGGG) at the distal end of the CGG array with incremental additions of smaller CGG arrays(Greenblatt *et al.*,2018).

Mutations cause the most common inherited human autism spectrum disorder in the Fragile X mental retardation 1 gene (FMR1). FMR1 affects the translation of messenger RNA (mRNA), but it has not been easy to establish realistic targets (Bardoni and Abekhoukh 2014). The FMR1 encoded protein, Fragile X mental retardation protein (FMRP), is an RNA-binding protein with a significant role in translational regulation. CYFIP1/2 (cytoplasmic FMRP interacting protein) proteins are strong candidates for intellectual disability among the FMRP interactors (Hoeffer *et al.*, 2012).

The Fragile X Mental Retardation Gene (FMR1) encoded protein (FMRP) is an RNA-binding protein linked to translational regulation. Dysregulated translation initiation signaling was recently observed in the Fmr1 knockout mouse model of FXS (Handt *et al.*, 2014). As the FMR1 coding region study is not included in normal molecular research, the prevalence of point mutations causing FXS is not well known (Maurin *et al.*, 2014). The deregulation of translation/transport/stability of these mRNAs has a cascading effect on many pathways in the absence of FMRP, resulting in the final phenotype. The proposal of an RNA (fragile X premutation rCGG repeat)-mediated gain-of-function toxicity model for fragile X syndrome has led to several lines of evidence (Li and Jin 2012). Since FMRP is associated with polyribosomes (ribosome clusters, protein-synthesizing molecular machines) and neuron-specific mRNA, it is believed to play an important role in the post-transcriptional control of neuron gene expression (Jayaseelan and . Tenenbaum 2012). FXTAS neuropathology consists of moderate brain atrophy and cerebellum degeneration, including middle cerebellar peduncle (MCP) hyperintensity, loss of Purkinje neuronal cells, deep cerebellar white matter spongiosis, Bergman gliosis, and swollen axons (Sellier *et al.*,2014). Recent studies have provided increased support for the role of FMRP in translational repression via ribosomal stalling and the microRNA pathway. The detection of signalling pathways such as PI3K and mTOR downstream of FMRP-regulating group 1 metabotropic glutamate receptors (mGluR1/5) was explicitly emphasized in neurons. New research also indicates that presynaptic dysfunction and abnormal adult neurogenesis are triggered by FMRP failure (Wang *et al.*, 2012).

Relation between Fragile X syndrome and autism

Different genetic forms of autism are hypothesized to share a similar increase in the cerebral cortex's excitation inhibition (E-I) ratio, triggering hyperexcitability and excess spiking (Langberg 2020). The most common type of heritable mental retardation and the known leading cause of autism is Fragile X syndrome (FXS) (Dölen *et al.*, 2007). FMR1 exposes fresh and unforeseen clinical presentations and molecular pathways 15 years after its discovery. FMR1 loss of control is a model for neurodevelopmental and behavioral disorders (Jacquemont *et al.*, 2007). including mental retardation, autism, anxiety, and mood disturbance. Recent research by Licznerski et al. indicates that the inner mitochondrial membrane proton leak is elevated by mutant FMRP linked to Fragile-X syndrome, leading to increased metabolism and protein synthesis changes that cause impaired synaptic maturation and autistic behaviors (Mithal and Chandel 2020).

Boys with Fragile X Syndrome (FXS) are at high risk of experiencing signs of attention deficit/hyperactivity and symptoms of the autism spectrum, but their experiences have not been observed over time (Doherty *et al.*, 2020). Despite this convergence,

due to inconsistency in diagnosis approaches in the literature, our understanding of autism spectrum disorder symptoms and the seriousness of fragile X syndrome is minimal (Haebig *et al.,* 2020).
A higher degree of depressive symptoms than the other groups of fathers was reported by the fathers of sons or daughters with ASDs. There was a lower degree of pessimism recorded by fathers of sons or daughters with D.S. than by the other fathers. No community variations were found in the paternal coping style. Group variations in paternal depressive symptoms and pessimism were partly linked to differences in paternal age, behavioral issues of the infant, risk of additional disabled children, and maternal depressive symptoms (Hartley *et al.,* 2012). Concerning lower-order (motoric) limited, repetitive behaviours and social approaches, the behavioural phenotype of FXS + Aut and iAut is most similar but varies in more nuanced types of restricted, repetitive behaviours and some behaviours of social response. Such results demonstrate the general phenotypic variability of autism and its unusual presence in an etiologically distinct condition (Wolff *et al.,* 2012). Matrix metalloproteinase 9 (MMP-9) is one of the proteins elevated in FXS, and in the Fmr1 knockout mouse model of FXS, minocycline decreases excess MMP-9 activity. Via randomized therapy trials, both minocycline and mGluR5 antagonists are currently being tested in patients with FXS. In around 10% of males and 2-3% of females, premutation (55-200 CGG repeats) may also lead to the mechanism of autism (Wang *et al.,* 2010). There are many advantages to researching the occurrence and stabilization of autism in infants with FXS, such as clarifying the fundamental causes of autism development in FXS and strengthening similarities and distinctions between co-morbid FXS with autism and I.A. Infant studies in both I.A. and FXS were explored as well as findings and consequences for practice and future research (McCary and Roberts 2013).

Molecular Diagnosis of Fragile X syndrome

Prenatal Diagnosis was based on cytogenetic detection of the fragile X chromosome acquired through cordocentesis in cultured amniotic fluid, chorionic villus cells, or fetal blood. The prevalence of misdiagnosis is around 5% due to unusual false positive and false negative diagnoses more commonly (Tassone 2015). Although with some limitations, southern blot and PCR analysis were replaced by cytogenetic analysis, which was the method of Diagnosis in the early 1990s (Sofocleous *et al.,* 2009).
Molecular and immunocytochemical methods are used in diagnostic approaches. For most laboratories, Southern blot, which makes it possible to detect mutations and assess methylation status in a single test, remains the technique of choice (Gold *et al.,* 2000). The combined use of PCR to amplify normal- and premutation-length alleles and Southern analysis to detect fully expanded alleles and evaluate methylation is required for unequivocal molecular characterization of the FMR-1 triplet expansion region. A simplified molecular diagnostic test based on fluorescent methylation-specific PCR may be an effective alternative or complement to Southern blot analysis for diagnosing Fragile X syndrome (Zhou *et al.,* 2006). In three families, the pfxa3 probe confirmed the cytogenetic Diagnosis, rediagnosing the other three as non-fragile X. A further two families had the clear expression of a separate fragile site susceptible to folate, FRAXE, similar to FRAXA, but not associated with fragile X syndrome and not detectable by the pfxa3 probe. Subsequent referrals were obtained from additional family members or members of new families for whom related markers did not predetermine carrier status. In these 222 additional cases, a direct diagnosis of pfxa3 for the 135 females was confirmed by dose review with the control probe (Mulley *et al.,* 1992). Changes in the repeat length of FMR1, such as full mutation and premutation, could then be observed. Therefore, the proposed standardization has proven successful in diagnosing FXS, encouraging families to obtain adequate genetic counseling (Gigonzac *et al.,* 2016). It is best practice to use a tool that detects the full range of expansions when examining relatives (including prenatal Diagnosis) in a family with any known fragile X condition due to expansion. The study must state that rare cases of point mutation or deletion can not be identified while testing the FMR1 gene in population screening or rare cases of CGG expansion mosaicism (MoMN), if the tool used, can not detect the full range of expansions (Biancalana *et al.,*2015).

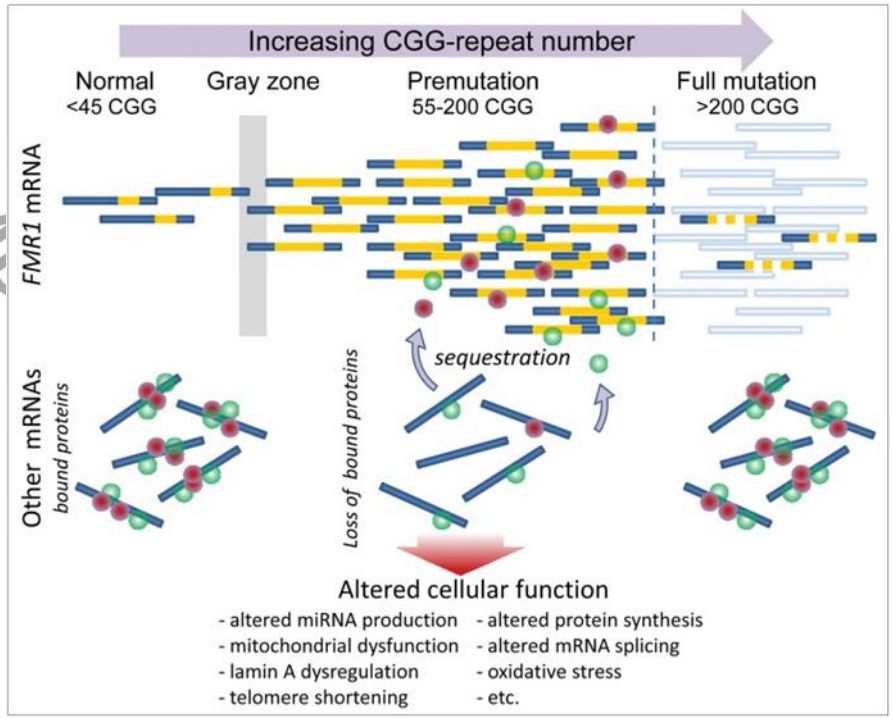

Figure 1: With increasing CGG-repeat length (gold segments) in the premutation range, FMR1 mRNA levels rise and undergo a transition to dramatically decreased levels in the full mutation range due to the FMR1 promoter region hypermethylation. In some instances, methylation mosaicism in the full mutation spectrum results in the continued development of low-to-moderate mRNA levels. RNA toxicity in the premutation range is thought to occur by direct sequestration of one or more RNA binding proteins that would normally be connected by direct sequestration of one or more RNA binding proteins with other mRNAs binding to the expanded CGG-repeat portion within the FMR1 mRNA. In turn, sequestration contributes to the loss of those proteins' normal function(s), which may include, among other functions, splice modulation, and control of the development of miRNA. It is suspected that RNA processing dysregulation contributes to different types of downstream cellular dysregulation(Hagerman *et al.*,2012).

Epigenetics of Fragile X syndrome

In complex processes, such as genomic imprinting and transcriptional regimen regulation, epigenetic modifications play a role. Moreover, these modifications are important for optimal brain growth and behavioral performance. Indeed, aberrant epigenetic marks play a part in the etiology of many neurodevelopmental disorders, some of which we still do not completely understand (Doherty and Roth 2020).

In the 5 'region of the fragile X mental retardation1 (FMR1) gene, the primary molecular defect in this disorder is the expansion of a CGG repeat, leading to de novo methylation of the promoter and inactivation of this otherwise normal gene, but little is understood about how these epigenetic changes occur during development (Hecht *et al.*,2017). Most FXS patients have an expansion over 200 repeats of (CGG)n sequence ("full mutation" (F.M.)) located in the 5′UTR of the FMR1 gene, resulting in local DNA methylation (methylated "full mutation" (MFM)) and epigenetic silencing. The absence of the FMRP protein is responsible for the clinical phenotype of FXS(Tabolacci *et al.*,2020).

Gene silencing and the degradation by DNA methylation and chromatin remodeling of gene products (Smail 2016, Smail 2019, Shitik *et al.* 2020, Smail et al. 2022, Smail 2023). The restrictive H3K9 methylation mark is enriched in FXS patient cells by the FMR1 gene and is, therefore, a possible drug target. However, its relation to the silencing process is uncertain (Kumari *et al.*,2020). To control cell fate decisions, neural development includes orchestrating complex modifications in gene expression. This control is highly affected by epigenetics, not specifically clarified by genomic knowledge alone (Salinas *et al.*, 2020).

Epigenetics research applications are evaluated, including opportunities to map and modify epigenetic marks (Smail et al.2022).the possibilities for epigenetic and noncoding RNA manipulation-based therapies (Henshall 2020). Silencing the FMR1 promoter through an epigenetic process involving CGG repeat DNA methylation and the regulatory regions surrounding it. The reduction in FMR1 transcription is related to the loss of the FMR1 protein needed for normal brain growth (Kraan *et al.*, 2019). Given the lack of sufficient cellular and animal models that can completely recapitulate the molecular features characteristic of disease pathogenesis in humans, the timing and mechanisms of FMR1 epigenetic gene silencing and repeat instability are far from being understood (Abu and Eiges 2019). More than ~200 CGG repeats result in transcriptional silencing, and the FMR1 encoded protein, FMRP, is absent in the 5' untranslated portion of the FMR1 gene. FMRP is an activity-dependent RNA-binding protein that regulates the transport and translation of various mRNAs in the brain(Kumari *et al.*,2019). Methylation mosaicism individuals produce more FMRP than individuals with complete mutation alleles completely methylated. Besides, CGG repeat numbers in the premutation range and FMRP expression are inversely related; hence in

addition to complete mutation alleles, individuals with size mosaicism who bear premutation alleles will also likely produce more FMRP than non-mosaic individuals. Elevated FXS FMRP levels are associated with fewer clinical symptoms and positively correlate with I.Q. (Rajaratnam *et al.*,2017).

Gene therapy of Fragile X syndrome

Unquestionably, gene therapy is the definitive way to treat genetic disorders and potentially cure them. In theory, a simple idea in theory, even when directed to easily accessible somatic cell systems, has proven difficult to realize in practice. Gene therapy for diseases in which the target organ is the central nervous system (CNS) poses much greater challenges, and multiple vectors and approaches to brain delivery are under study (Rattazzi *et al.*,2014). Since no existing clinical therapies are directly aimed at the underlying neuronal defect due to the absence of FMRP, new effective therapeutic methods may be opened up (d'Hulst and Kooy 2009). The research community agreed to rethink the methods and procedures used, introducing improvements in both the preclinical and the clinical arenas in the face of disparities in expertise and the lack of therapies for FXS patients. This specific problem discusses some of the changes being made to find appropriate therapies for FXS in the field (Kumari and Gazy 2019).

In identifying therapeutic strategies and repurposing medicines for neurological disorders, data show a good value of transcriptome-based computation and suggest trifluoperazine as a possible treatment for FXS(Ding *et al.*,2020). Despite comprehensive studies using animal models, understanding how FMRP controls human brain development and function remains a major challenge. Human pluripotent stem cells (hPSCs) provide powerful platforms to research human disease processes and test possible therapies. Genome editing, especially the CRISPR/Cas9-based method, is highly effective (Zhao and Bhattacharyya 2020).

In the mouse model of fragile X syndrome, numerous studies have examined the efficacy of adeno-associated viral (AAV) vectors. AAVs have been used to express a fragile X mental retardation protein (FMRP) absent or highly reduced in the condition. These studies have shown several efficiencies in various experiments, from absolute correction to partial rescue to no effect (Hampson *et al.*, 2019). Neurodevelopmental conditions such as Rett Syndrome, Fragile X, and autism still face major hurdles to overcome before the effects of viable human gene therapy can be considered(Gray *et al.*,2013). offers the first evidence of the theory that gene therapy in the FXS mouse model can correct particular behavioral anomalies (Gholizadeh *et al.*,2014). Guide RNA-mediated CRISPR-Cas nucleases are a powerful mammalian genome engineering technology. In cell-based models, CRISPR-Cas9-dependent editing of mutated genes causing Huntington's disease and fragile X syndrome has recently been accomplished, heralding the first step towards transforming this technology into viable neurological disease therapeutics (McMahon and Cleveland 2017). Several recent studies suggest minocycline as another possible route of FXS clinical treatment in addition to the promise of mGluR5 pathway therapies (Tabolacci and Chiurazzi 2013). The potential application of selective treatment of genetic disorders through epigenetic methods Clinical studies are underway to transfer outcomes to humans in animal models of FXS, posing complicated problems with the design of trials and outcome measures to test cognitive improvement that may be correlated with therapy(O'Donnell and 2002). Recent trials of novel FXS therapies have highlighted several challenges, including subpopulations with possibly differential therapeutic responses, the lack of specific outcome measures capturing the full range of improvements, A lack of biomarkers that can control whether a particular mechanism is sensitive to a new drug and whether the response is associated with clinical improvement in patients with FXS (Jacquemont *et al.*, 2014). In reversing cellular and behavioral phenotypes and restoring proper brain connectivity in mouse and fly models, the use of metabotropic glutamate receptor (mGluR) blockers and gamma amino-butyric acid (GABA) agonists is successful (Hagerman 2012).

Technologies that can safely edit genes in the brains of adult animals may revolutionize the treatment of neurological diseases and the understanding of brain function. Here, they demonstrate that intracranial injection of CRISPR-Gold, a CRISPR-Cas9 ribonucleoprotein nonviral delivery vehicle, can edit genes in multiple mouse models in the brains of adult mice. CRISPR-Gold can provide ribonucleoproteins for both Cas9 and Cpf1 and can alter all major cell types in the brain, including neurons, astrocytes and microglia, with undetectable levels of toxicity at the doses used (Lee *et al.*,2018). Multiple pharmacological and genetic manipulations that target receptors, scaffolding proteins, kinases, and translational control proteins can rescue neuronal morphology, synaptic function, and behavioural phenotypes in FXS model mice, presumably by reducing excessive neuronal translation to normal levels (Richter *et al.*, 2015). The results inspired the introduction of clinical trials in patients. The targeted pathways converge in part with those of related neurodevelopmental disorders, raising hopes that the treatments developed for this specific disorder might be more broadly applicable (Braat and Kooy 2014). Such strategies have led to the development of drugs that are now in clinical trials most promisingly. The research shows how progress in understanding disorders such as FXS has led to a new age in which molecular therapy for neurodevelopmental disorders has become possible (Wijetunge *et al.*,2013). In addition, S6K1 deletion prevented immature dendritic spine morphology and multiple behavioral phenotypes, including social interaction deficits, impaired novel object recognition, and behavioral inflexibility. The results support the model that the primary causal factor in FXS is dysregulated protein synthesis and that normal translation restoration will stabilize peripheral and neurological function in FXSS (Bhattacharya *et al.*, 2012).

The prevention and care of FXS would result from concerted campaigns, between government medical professionals and the public, in multiple arenas. A specialized clinic and research center is urgently necessary to provide the latest information to Chinese medical practitioners. More publicity and education are needed to provide awareness and resources to individuals with FXS and their families (Niu *et al.*, 2017). Current research indicates that this chromosomal mutation is correlated with a host

of other concerns related to education, including learning difficulties, attention deficit disorders, speech and language deficiencies, autism traits, and behavioural disorders. A summary of the distinctive inheritance pattern demonstrates why milder manifestations of the syndrome are now being recognized and educational strategies are being applied(Santos 1992). The proportion of young people with FXS who comply with fundamental recommendations in preventive care guidelines varies depending on health status and demographic factors. For certain classes, this proportion may be increased, particularly in cases of influenza vaccination and physical activity(Gilbertson *et al.*,2019). SXF molecular prenatal diagnosis is simple and 100 percent accurate, although it is complicated from a scientific point of view and involves using different molecular techniques. The low rate of mutations found guarantees offspring from the therapeutic point of view, while molecular studies do not predict mental status' in either girl with full mutation or children with permutation(Tejada 2001).

Conclusions

From this review, I conducted the following conclusions:
The development of genetic diseases and traits linked to FMR1 gene mutations is known as fragile X syndrome. A scientist is currently focusing on a cutting-edge method for diagnosing and treating Fragile X syndrome. The FMR1 genes are silenced and inactivated by two important epigenetic mechanisms. DNA methylation and remodelling of the chromatin. Future medical care is possible thanks to gene therapy.

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