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Review Article

FMR1 Key Biomarker in Fragile X Syndrome- A Comprehensive Review

Smitha Nayak[1], Ramdas Bhat[2]*

[1]UG scholar, Department of Pharmacy Practice, Srinivas College of Pharmacy, Valachil, Mangalore.

[2]Associate Professor, Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Mangalore.

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Corresponding Author:
ramdas21@gmail.com

Abstract

Fragile X Syndrome (FXS) is a complex neurodevelopmental disorder associated with intellectual disability, behavioral challenges, and various physical manifestations. Central to understanding FXS is the Fragile X Mental Retardation 1 (FMR1) gene, pivotal in the disorder's pathogenesis. This review examines FMR1 as a key biomarker in FXS, drawing on recent research insights. The FMR1 gene, located on the X chromosome, encodes fragile X mental retardation protein (FMRP), crucial for synaptic function and brain plasticity. Mutations in FMR1, including CGG repeat expansions, cause FXS, resulting in cognitive and behavioral impairments. Advances in molecular genetics elucidate FMR1 dysfunction and its roles in FXS phenotypes. Diagnostic approaches involve DNA testing for FMR1 mutations, essential for accurate diagnosis and genetic counseling. FMR1 serves as a biomarker for disease monitoring and treatment evaluation. Emerging therapies targeting FMR1-related pathways offer promise for FXS intervention, highlighting FMR1 as a therapeutic target. Understanding FMR1's roles in FXS pathophysiology informs precision medicine approaches. Elucidating FXS's molecular basis and leveraging FMR1 as a biomarker aim to advance diagnostics, refine therapies, and improve outcomes. This review underscores FMR1's pivotal role in FXS research and clinical practice, emphasizing its potential as a key biomarker for guiding precision medicine interventions.

Introduction

Fragile X syndrome (FXS), the most common form of inherited intellectual disability and monogenic cause of autism spectrum disorders, is mainly caused by the expansion of CGG trinucleotide repeats in the 5′ untranslated region of FMR1 gene [1]. Estimates report that FXS affects approximately 1 in 2,500 to 5,000 men and 1 in 4,000 to 6,000 women. Affected men display varying degrees of symptoms ranging from mild to severe. Due to compensation by the unaffected X chromosome, only one-third of female carriers with a full mutation (FM) have ID; the majority have normal IQ, although learning difficulties and emotional problems are common [2]. In some rare cases, less than 1%, FXS occurs due to other defects that lead to loss of function of the gene, such as deletions or point mutations. The FMR1 gene normally codes for the fragile X mental retardation protein (FMRP) and therefore silencing of the gene leads to loss of expression of the protein. FMRP is an RNA binding protein that is involved in several processes including neuronal plasticity and functioning of neuronal networks. Healthy individuals have less than 45 CGG repeats. Expansion to 46–54 repeats is considered the gray zone and individuals with 55–200 repeats are considered to have the premutation (PM) [3]. Carriers of the PM can present with conditions such as fragile X-associated primary ovarian insufficiency (FXPOI) in females, neuropsychiatric conditions such as anxiety and depression, recently recognized as fragile X-associated neuropsychiatric disorders (FXAND) and fragile X-associated tremor/ataxia syndrome (FXTAS). In this review we will focus on FXTAS, a movement disorder characterized by tremor and or ataxia, cognitive involvement, neuropathy, and autonomic dysfunction in individuals with the PM [4]. Given the X-linked nature of the mutation, males also tend to have stronger clinical presentations than females4. FMRP remains the most valuable biomarker for FXS, since it can be used to identify fragile X individuals and to predict, to some extent, their cognitive functions [5].

Etiology

Fragile X syndrome (FXS) is caused by a loss-of-function mutation in the FMR1 gene, located at Xq27.3, which contains an unstable CGG repeat sequence in the upstream promoter region and encodes an RNA binding protein [6]. In the normal population, this triplet repeat has 5-55 repeats, allowing transcription and translation, but when it expands to 56-200 repeats (premutation), the gene becomes meiotically unstable, leading to increased transcription of FMR1 mRNA [6]. Although premutation carriers don't have FXS, they may experience a gain-of-function phenotype, including early menopause in women and tremor-ataxia syndrome in men. The presence of AGG interruptions within the repeat region stabilizes the trinucleotide repeat, while their absence increases size variability upon meiotic transmissions, suggesting the involvement of the brain-expressed FMR-7 gene in the phenotypic expression of FXS [7].

Structure of fmr1 gene

The FMR1 gene, spanning 40 kb with 17 exons, encodes a 3.9 kb mRNA and is inactivated by the methylated CGG expansion in fragile X patients [8]. The CGG repeat, located in the first exon's 5' untranslated region, is part of a CpG island crucial for FMR1 expression. Alternative splicing affects exons 12, 14, 15, and 17 [8]. The resulting protein, FMRP, contains RNA-binding domains, nuclear localization and export signals, and regulates translation of RNAs involved in synaptic plasticity [9]. Premutation alleles are unmethylated, producing normal FMRP levels, but premutation females are more likely to experience attention problems, anxiety, depression, and developmental delay [11]. Reduced FMRP levels, despite elevated mRNA, suggest a feedback response to impaired translation [13]. FMRP associates with ribosomes via large RNP particles containing other proteins, and its absence is pivotal to the fragile X syndrome, as evidenced by FMR1 deletions and point mutations in affected patients [15].

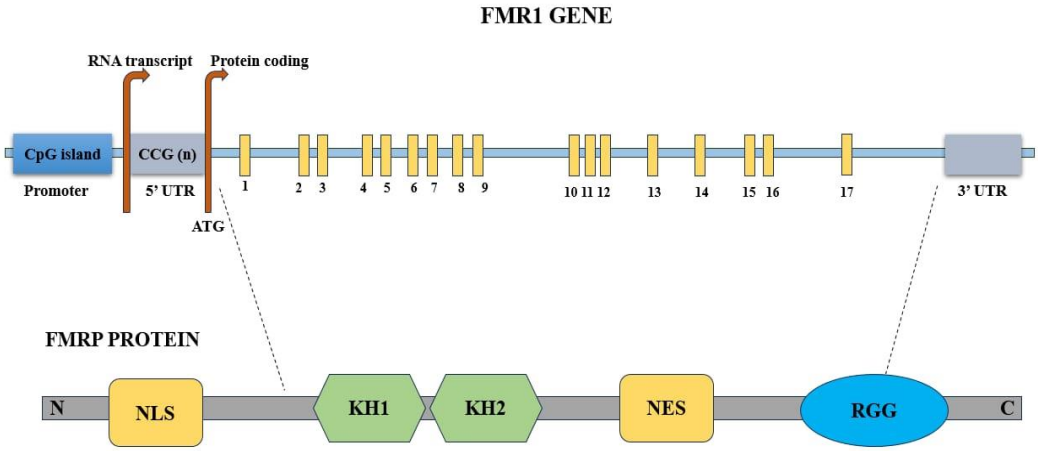

Fig 1: Structure of FMR1 gene

Clinical presentation of fragile-x syndrome

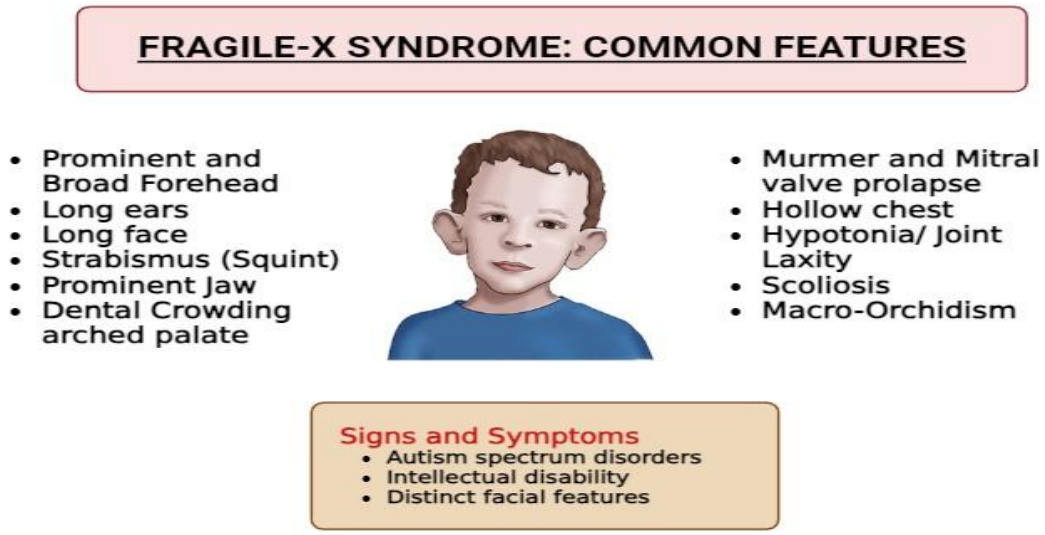

Fig 2: Clinical presentation of Fragile-X Syndrome

Fragile X syndrome (FXS) is characterized by distinct physical features, including a long face, large ears, loose connective tissue, and macroorchidism, which become apparent after puberty [16]. Movement disorders, such as hand flapping and stereotypies, and cognitive deficits leading to moderate-to-severe mental retardation (average IQ of 40) are common [17] [18]. Hypotonia, developmental delay, and attention-deficit hyperactivity disorder (ADHD) are also frequently reported [19].

Affected individuals display a wide range of anxiety symptoms that fit multiple different types of anxiety disorders of the Diagnostic and Statistical

Manual IV (DSM IV) [22]. Females who are affected by fragile X syndrome (FXS) can have significant physical, neuropsychological, and emotional involvement [20]. Fragile X premutation carriers experienced cessation of menses prior to age 40 years more commonly than did noncarriers or control women when all women older than age 21 years were considered [21]. Individuals with FXS often have co-occurring conditions like autism spectrum disorder (ASD), which affects approximately 50-60% of individuals with FXS [23]. Sex differences influence the severity of the FXS phenotype, with intellectual and developmental disability affecting 85% of males and 25% of females [24]. Joint hypermobility and prominent ears are also common features [23].

Pathogenesis

FXS is the most common form of inherited intellectual disability (ID) and monogenic cause of autism spectrum disorder (ASD). The prevalence rate of FXS is estimated at 1 in 5000 males and 1 in 8000 females [23]. It is caused by the full mutation (FM) of the FMR1 gene, which is characterized by the excessive expansion of CGG trinucleotide repeats (≥ 200) in the 5' untranslated region (UTR) of the gene. These expanded CGG triplet repeats are hypermethylated with consequent transcriptional gene silencing, halting gene expression, thereby resulting in a reduction or absence of FMRP. Although this is considered the main cause of FXS, many of the numerous molecular mechanisms involving FMRP and some physiological consequences presenting as FXS are yet to be discovered [24]. It is well known that mild to moderately low FMRP levels are associated with less severe symptoms, such as moderate emotional dysregulation and learning difficulties, often with a normal IQ, as is seen in some girls with FXS. Deficient levels of FMRP or lack of its synthesis are associated with more severe forms of ID, as is common in males with FXS. The number of CGG trinucleotide repeats expands with each subsequent generation, growing from premutation in women (PM; 55–200 repeats) to a FM in their offspring [25]. For PM alleles with more than 99 CGG repeats, the risk of transition from PM to FM approaches 100%. Individuals with PM have a normal IQ, while it is observed that female PM carriers have a high probability of having a child with FXS. It has also been shown that neurons with PM undergo earlier cell death in culture, with heightened toxin sensitivity. For example, these neurons are more vulnerable to toxins in the environment, such as alcohol and pesticides, and they die more readily in cell culture. "Gray zone" or intermediate alleles of the FMR1 gene (45–54 CGG repeats) could be considered precursors for PM alleles. The tendency of trinucleotide repeats to expand with each generation is why this genetic change is referred to as a 'dynamic mutation [25,26].

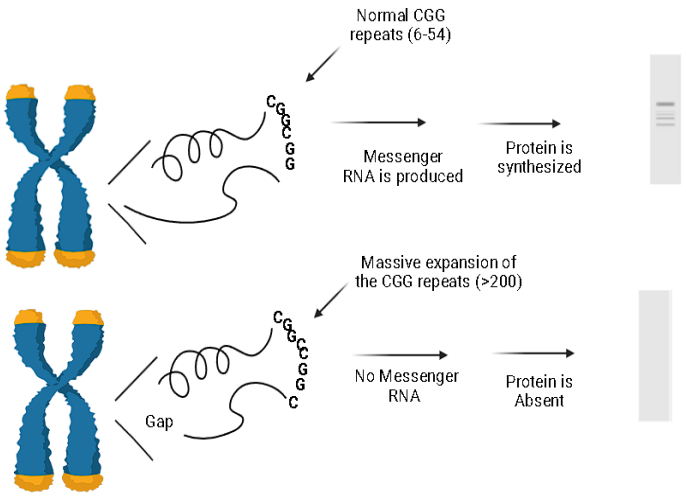

Fig:3 Pathogenesis of Fragile X Syndrome.

Diagnosis

DNA analysis is the preferred method for testing Fragile X Syndrome (FXS), crucial for isolated cognitive impairment cases and known carriers. Prenatal testing via amniocentesis is recommended after 15 weeks' gestation, with caution advised for CVS due to potential methylation status issues [23,26].

Molecular diagnosis has evolved since the FMR1 gene's discovery in 1991, shifting from cytogenetic analysis to more sensitive PCR-based approaches. These methods measure CGG repeat size and assess FMR1 gene methylation status, enhancing diagnostic accuracy [26,27].

Risk assessment for FXS involves evaluating FMR1 gene mutations through genetic evaluation, family history analysis, and diagnostic testing. PCR-based techniques are preferred for their sensitivity. Genetic counseling is crucial in providing information about inheritance, transmission risk, and management options [27,28].

Treatment

Pharmacological Therapy:

FXS treatment includes various medications targeting specific symptoms. Sertraline (SSRI) is used for anxiety, starting early in life, based on serotonin deficits in ASD brains [22,28]. Metformin, typically for diabetes, shows potential for FXS through AMPK-dependent and independent pathways [23,25]. Acamprosate, used for alcohol abstinence, may affect glutamate and GABA neurotransmission [27,28]. Lovastatin inhibits RAS-MAPK-ERK1/2 activation, potentially preventing epileptogenesis [24,28]. Minocycline inhibits MMP-9 activity, improving synaptic connections [28]. Intranasal oxytocin may improve social communication skills [29].

Non-Pharmacological Therapy:

Non-pharmacological approaches are crucial for FXS management, adapting strategies from ID and autism interventions. These include psychological evaluations, positive behavioral support, functional behavioral analyses, relaxation training, and desensitization techniques. Speech and language therapy, occupational therapy, social and welfare support, special education, and vocational guidance are also employed. While these form the core of FXS management, medications like SSRIs for anxiety and methylphenidate for attention deficits may complement non-pharmacological interventions, though their efficacy requires further research [24,30].

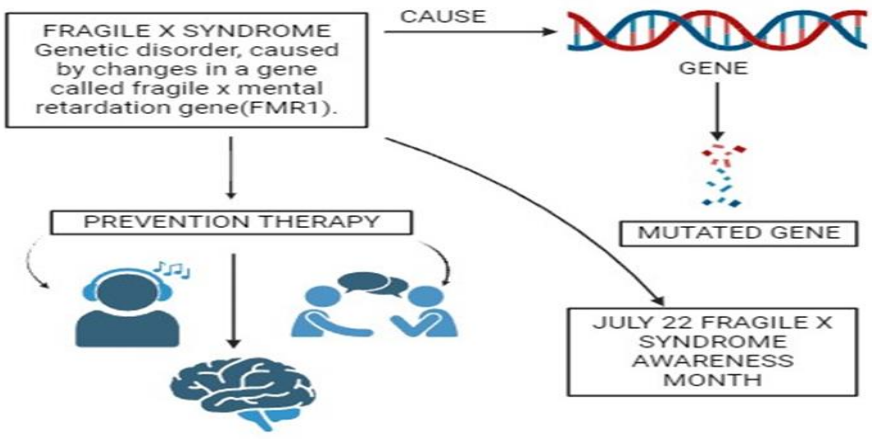

Fig 5: Non-pharmacological therapy of Fragile X Syndrome

Current advancements in targeting fmr1 in fragile-x syndrome

Fragile X Syndrome (FXS) research is rapidly evolving, offering hope through innovative approaches targeting the absence of FMRP caused by FMR1 gene mutation [1]. Gene therapy, including CRISPR-Cas9, aims to increase FMRP production and correct mutations [30]. Researchers are exploring mGluR5 Positive Allosteric Modulators to enhance remaining FMRP function [31], investigating gut-brain connections for microbiome-based therapies [32], and unraveling complex signaling pathways to develop targeted drugs [33]. Artificial intelligence integration enables personalized treatment plans [34], while non-invasive techniques like TMS and tDCS are being explored to modulate brain activity [35]. Combining these approaches offers a comprehensive strategy for treating FXS, potentially providing synergistic effects [30-35].

Despite past negative trials, the search for effective targeted therapies continues, with 71 FXS studies listed on ClinicalTrials.gov as of October 2018 [31]. Promising agents like OV101/gaboxadol, ZYN002/cannabidiol, BPN14770, and Bryostatin-1 are being developed, though trials are complicated by subgroups of responders. Biomarkers such as EEG findings or iPSC-derived neuronal cell culture responses may aid in patient stratification for future trials [36]. Key priorities include overcoming population heterogeneity, developing improved outcome measures, and identifying treatment response biomarkers. Gene therapy approaches, including FMR1 reactivation, show promise [37]. Ongoing research also aims to identify alternative downstream targets of FMR1. This comprehensive approach holds promise for advancing FXS treatment and improving outcomes for affected individuals [31, 36, 37].

Table 1: Clinical Trials of Drugs for Fragile X Syndrome

Drug	Type	Target	Clinical Trials	Trail Results	Approval Status	References
Zatolmilast	PDE inhibitor	Modulating cAMP	Phase 2	Safe, well-tolerated; Some improvement in cognition.	Phase 3 ongoing development	[38]
Arbaclofen	GABA-B agonist	Influencing GABA signaling and FMRP protein	Phase 3	Didn't improve social avoidance; Not FDA-approved.	Not FDA-approved for FXS	[39]

Challenges in targeting fmr1 in fragile-x syndrome

Efficient delivery of therapeutic genes poses a significant challenge in gene therapy for Fragile X Syndrome (FXS), given the FMR1 gene's location on the X chromosome, which complicates access to affected cells [40]. Additionally, Epigenetic Silencing resulting from the CGG repeat expansion adds complexity to restoring normal gene function [41]. Identifying Effective Compounds for pharmacological reactivation that can reverse FMR1 gene silencing without off-target effects remains a major hurdle, exacerbated by the lack of reliable biomarkers for assessing treatment efficacy in FXS. Moreover, the

heterogeneous nature of FXS, with varying degrees of FMRP expression and therapeutic responses, complicates the development of effective treatments across various therapeutic modalities [42].

Challenges persist in Patient Selection for clinical trials, necessitating strategies to navigate FXS's heterogeneity. The absence of reliable biomarkers further complicates the evaluation of treatment efficacy, requiring innovative approaches to quantify disease modification over time and across symptom domains. Understanding FXS is impeded by its phenotypic heterogeneity, epigenetic modifications of the FMR1 gene, and the gene's high conservation, highlighting the need for collaborative efforts to address these challenges and advance FXS research and therapeutic development [43].

Future direction

The future of targeting the FMR1 gene in Fragile X Syndrome (FXS) is characterized by diverse and innovative approaches. Gene therapy emerges as a key strategy, focusing on gene augmentation to deliver healthy FMR1 copies and boost FMRP production, and gene editing using CRISPR-Cas9 to correct the CGG repeat expansion. These methods have shown promise in preclinical studies and patient-derived cells, respectively. Pharmacological reactivation of the silenced FMR1 gene is another avenue, with researchers targeting specific pathways and proteins involved in gene silencing. High-throughput screens have identified promising compounds, and targeted transcriptional activation has achieved selective FMR1 reactivation in human embryonic stem cells.

Researchers are also decoding the complex signaling pathways associated with FMRP to develop targeted drugs. Artificial intelligence integration enhances the potential for personalized treatment plans by analyzing vast amounts of data to predict treatment responses and identify new drug targets. The combination of multiple therapeutic approaches, including gene therapy, pharmacological reactivation, and other innovative strategies, offers a comprehensive and synergistic approach to treating FXS. This multifaceted strategy promises to revolutionize the treatment landscape, potentially leading to more effective and personalized interventions that could significantly improve outcomes for individuals with Fragile X Syndrome.

Conclusion

Ongoing research in Fragile X Syndrome (FXS) treatment strives to overcome challenges in gene therapy delivery and finding effective pharmacological compounds. Clinical trials face difficulties in patient selection and outcome measurement due to FXS's diverse presentation. Understanding FXS's varied symptoms and epigenetic changes is essential for developing targeted treatments. Despite obstacles, progress in molecular diagnosis and risk assessment holds promise for enhancing FXS management and improving patient outcomes.

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