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Fragile X syndrome- insight into what we know and prospects

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Abstract:
Fragile X syndrome is a dominantly inherited genetic disease and is a consequence of the *FMR1* gene mutation located on the X chromosome. The number of patients affected by this disease with full mutation is estimated at 1 in 4000 men and 1 in 8000 women, however, the number of carriers with premutation is far greater. Depending on the mutation of the *FMR1* gene and levels of its products FMRP a variety of symptoms can be observed including- intellectual disability, mental retardation, lowered behavioral adaptation, autism, and dysmorphic features such as a long face with a broad forehead and prominent ears. The treatment is mostly symptomatic- managing comorbidities and an emphasis on psychological therapy. The objective of this paper is to sum up the most up-to-date knowledge regarding the pathogenesis, treatment- current and clinically tested, and novelties in the Fragile X syndrome

Keywords: Fragile X syndrome, autism, neurodevelopmental disorders, FMRP, *FMR1*

1. Introduction:

Fragile X syndrome (FXS, OMIM #300624) is an X-linked dominant genetic disorder caused by the mutation of the Fragile X Mental Retardation 1 (*FMR1*) gene. [1] It is the most common cause of single-gene autism spectrum disorder (ASD) and is believed to be responsible for 2-6% of all autism cases. The disease primarily affects the nervous system resulting in mental retardation, lowered socio-behavioral adaptation, and intellectual disability (ID) [2] . The dysmorphic features present in the FXS include a long face with a broad forehead, prominent jaw and ears, and a high-arched palate. [3] The psychiatric profile of the patients includes hyperactivity, anxiety, and avoidance of socialization. [4] The general prevalence of the full mutation of FXS is estimated at around 1 in 4000 men and 1 in 8000 women. The discrepancy between men and women is a consequence of the location of the *FMR1* on the X chromosome [5] . The sex differences contribute to the phenotypic outcome; ID is observed in 85% of male and 25% of female patients.[6] Due to the genetic nature of the disease, there is no causal therapy and the patients are treated symptomatically- undergo rehabilitation and therapy to improve their independence in adult life.[7]

2. Purpose:

We aimed to gather the most up-to-date knowledge regarding Fragile X syndrome, screenings, and treatments (available or undergoing clinical studies).

3. Materials and methods:

We performed a literature review regarding Fragile X syndrome, focusing on the holistic approach to the disease using PubMed, and Google Scholar databases and publications primarily from 2017-2022. The following keywords were used: Fragile X syndrome, autism, and neurodevelopmental disorders.

4. Literature review

4.1 Pathogenesis

The FXS is a consequence of the *FMR1* mutation in the Xq27.3 chromosome 5' untranslated region. Normally there are about 30 *CGG* triplets in that gene, however, in FXS, there are increased numbers of triplets leading to DNA methylation and production impairment of the Fragile X Mental Retardation Protein (FMRP) [8] . This protein is involved in the development of synapsis and neurons. Moreover, it organizes mRNA transport and controls its products via a feedback loop [3] . It is present in the cytosol of neurons, near ribosomes. It is considered a suppressor of mRNA translation initiation and elongation of nascent proteins. The mutation effects of the *FMR1* can be observed in Fragile X-associated tremor (FXTAS) macro and microscopically as neurodegenerative defects in the white matter, astrogliosis, and excessive iron accumulation. Even though the FXS and FXTAS are of different etiology it shows the importance of *FMR1* in the proper development of the nervous tissue. [4] Some researchers suggest that FMRP controls ion channels modulating neural excitability. In addition to the roles mentioned above the FMRP seems to fine-tune the proteome depending on the needs of the cells or synapses. [9] The number of CGG triplets determines the severity of the *FMR1* mutation and If the patient has more than 200 CGG triplets in the *FMR1* gene a full onset of symptoms can be observed. [10] Generally, 6 to 44 repeats of CGG are considered normal in the *FMR1* gene. Repeats between 44 and 54 are "gray zone", 55 and 200 are recognized as a premutation, and >200 CGG triplets are considered a full mutation. Premutation alleles are unstable and there is a high risk of their expansion to full mutations in subsequent generations. This risk is dependent on the number of CGG repeats and it is estimated that in the case of alleles with >99 repeats it increases to 100% in future generations. [11] Depending on the length of altered *FMR1* and level of methylation, the activation of *FMR*1 and levels of FMRP vary therefore causing mosaicism. The heterogenicity on the molecular level is visible in the clinical phenotype of FXS- some patients with mutated *FMR*1 gene might not suffer from FXS or have mild symptoms [12] In each woman cell, one of the X chromosomes is inactive as a consequence of methylation in embryonic development. Some researchers suggest that female patients with FXS rarely experience severe forms of the disease as the X inactivation ratio in FXS-affected patients skewed from 50%:50% to 30%:70% reducing the number of mutated cells. Homozygous alleles are met in women born from consanguineous couples and are rare.[13]

Over time researchers noticed abnormalities in functioning cells with loss of the *FMR1* gene. They have become anchor points for further investigation of the mutation and its consequences in living organisms. The mGluR theory states that the lack of FMRP hyperactivates the mGluR5- mediated pathway. The research was done on animals with FMR1 Knock-out (KO) models using mGluR5 antagonists resulted in the perseverance of neurons, synapses, and behavioral improvements. Unfortunately, during the clinical trial, no beneficial effects of the treatment were noticed.[14]

For some time, researchers have put under scrutiny kinase proteins and their role in FXS. In numerous *FMR1* KO animal models abnormalities regarding; protein kinase B, calcium/calmodulin-dependent protein kinase type II subunit alpha, diacylglycerol kinase kappa, focal adhesion kinase, glycogen synthase kinase 3, p21 activated kinases, p90 ribosomal protein S6 kinase was found to be of decreased or increased activity. [15] Although no research was done on living humans, these models and data collected from them could direct future directions of research.

4.2 Symptoms

The clinical phenotype is various and depends on several factors such as sex, activeness of the *FMR1* gene, and its products. At birth, most neonates do not exhibit physical traits of the disease and are diagnosed later in life when most common physical dysmorphic features as well as mental retardation and traits of ASD are most visible. [16] The most typical dysmorphic features have been collected in Table 1. The neurobehavioral phenotype includes impaired visual-motor functions that may underline structural and functional abnormalities of visual pathways. This inability to correctly manipulate objects explains poor outcomes of neuropsychological tasks measuring IQ. [17] Boys with mild or severe FXS typically have IQ scores <50, while women are usually less affected. What is interesting, the IQ score of adolescents and adults with FXS is statistically lower than

younger children with FXS. It is being questioned whether the difference derives from the usage of different tests at different ages as opposed to deterioration and loss of skill. Even though patients with FXS have more expressive language than patients with ASD, they still face many communicative problems. Amongst them are linguistic receptive skills, pragmatics, and functional skills. Other mental disorders suggested by researchers include lowered levels of attention, common anxiety diagnosis, ASD, and other behaviors including aggression, self-harm, and hypersensitivity to sensory stimuli.[18]

Clinical symptoms and traits		Occurrence
Physical	Narrow face	83% (more visible in adults)
	Prominent ears	~75%
	High arched palate	94%
	Macrocephaly	51-81%
	Prominent jaw	80% of adults
	Hypermobility of joints	In 50-70% of observed children
	Obesity	50-61%
	Soft skin	~50%
Common comorbidities	Strabismus	8-40%
	Otitis media	47-97% (mostly in younger children)
	Bicuspid valve abnormalities	3-12%
	Seizures	16-20%

Table 1. Most common features of FXS noticeable upon physical examination and history taking. [2,3,19]

4.3 Treatment and screening

As of now, there is no specific cure for FXS due to the genetic nature of the disease and its complexity. Thus the treatment is mostly symptomatic with an emphasis on behavioral interventions. Lifestyle modifications such as avoidance of alcohol and environmental toxins is should be introduced to patients with full mutation as well as with premutation. For depression and anxiety pharmacological therapy with selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) as well as cognitive behavioral therapy is used. Other methods of managing the symptoms include avoiding oxidation stress because of its neurodegenerative effect and maintaining an active lifestyle. [20] Obesity commonly observed in patients with FXS has been successfully treated using metformin.[21]

With expanding knowledge about the *FMR1* gene and FMRP using KO models many therapeutic strategies have been proposed. One direction of research is based on the reduced production of GABA in the FXS. A potent GABA-B selective antagonist- Arbaclofen was the subject of a phase 3 randomized, placebo-controlled study in 297 individuals- both adults and children. This drug showed no potential benefits in adults and adolescents, however, in children, Arbaclofen showed statistically significant improvement at the highest dosage group. General assumptions of the trial failed, but Arbaclofen proved to be beneficial to some younger patients with FXS.[22]

Other strategies for searching for an effective drug for FXS include matrix metalloproteinases pathways, serotonin pathways, oxytocin, and endocannabinoid pathways. [14] The latter-mentioned mechanism revolves around the usage of cannabidiol (CBD). The endocannabinoids bind to the G-protein-coupled receptors, cannabinoids 1 and 2 (CB1 and CB2). The usage of CBD bypasses the affected of FXS endogenous cannabinoid loss. The literature states cases and a small sample of groups that benefited from CBD treatment with improvement in language skills decreased levels of anxiety with little to no adverse effects. [19] A transdermal cannabinoid- ZYN002 in phase 1/2 was used to treat behavioral aspects of FXS. The twelve patients who completed the 12-week treatment period experienced a meaningful reduction of hyperactive behavior, social avoidance, and general anxiety and slightly lowered depressed mood and compulsive behavior. [23]

Another explored pathway of FXS pathology is the Insulin-Like Growth Factor 1 (IGF1) pathway. KO animal models in *Drosophila* flies and mice embryos underline the role of *FMR1* in-memory processing and synapse maturation. [14] [24] An analog of IGF1- the amino-terminal tripeptide called Trofinetide has recently been the subject of clinical trials due to promising results on animal models. A double-blind, placebo-controlled, randomized clinical study using the drug mentioned above was performed on a group of patients aged from 12 to 45 years old with full mutation FXS. At 70 mg/day of Trofinetide was generally well tolerated and meaningful, positive changes were noticed by caregivers and clinicians. [25]

Lately, novel therapeutic strategies have been proposed to address the core issue of the FXS- loss of FMRP. The adeno-associated viral vectors have been under scrutiny for their possible usefulness in gene therapy of the FXS. This approach to restoring the *FMR1* gene function using viral vectors had been tested on animal models with promising results. The research conducted on *FMR1* KO models in mice with viral vector genome therapy alleviated long-term depressive symptoms and was proved to correct expression levels of proteins regulated by

FMRP. The introduced genes via the viral therapy were detected 7 months after injection proving their stability in cells. [26] These viral vectors were used only on animal models and no data relating to humans has been published however this research brings hope in finding a causal treatment for FXS.
There is no prevalent screening program for newborns. Most diagnoses are set when behavioral symptoms appear. Nonetheless, some organizations such as Early Check provide expanded screening panels for conditions thought to benefit from earlier identification. Access to presymptomatic individuals with permutations and their families could help manage better family planning, earlier rehabilitation, and treatment, and could provide researchers with data necessary for creating future therapeutic approaches.[27,28]

5. Conclusions:

FXS is a genetic disorder with high prevalence in society. Clinicians and psychologists will likely at some point in their career treat a patient with an *FMR1* gene mutation. As described previously patients with this mutation can exhibit a variety of symptoms due to numerous possible mechanisms of mutation and loss of functions of the FMRP. A correct diagnosis can be difficult sometimes as there are no pathognomic signs of the disease. The genetic diversity of the disease can pose a diagnostic hardship as some patients with FXS don't have a typical *CGG* expansion. For those patients, more thorough testing including high-resolution melting of the FMR1 coding sequence, array-based sequencing, massively parallel sequencing, and sequencing of target custom capture libraries should be performed. [29] The families of the patients with FXS should be genetically counseled as well as the disease is inherited dominantly.
As mentioned above, no causal treatment has been discovered, however latest advances in understanding the mechanism of the disease may bring results in the future. As of now many therapies including genomic therapy and novel drugs are being tested- unfortunately with mediocre results and apply only to some patients.
General awareness among medical field workers about the FXS should be raised as early and correct diagnosis enables proper management- pharmacological therapy, psychological therapy, and behavioral interventions. The families of the patients with FXS should be genetically counseled as well as the disease is inherited dominantly.

Disclosure

The authors declare no conflict of interest.

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