Fragile X syndrome:
Physiopathological aspects and therapeutic perspectives

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I. Introduction

The Fragile-X-Syndrome (FXS) is a genetic syndrome, which causes inherited mental retardation. The disease was identified in 1991. It is the second leading syndrome causing mental retardation, after the Down syndrome. It concerns 1/4000 male and 1/8000 female. Besides mental retardation, it causes learning disabilities and almost half of the children, suffering from this disease, are also diagnosed autistic. FXS patients also present physical dysmorphia. The mutation is a monogenic dynamic mutation. In the 5' UTR part of FMR1 gene, the repeats of the CGG sequence is normally quantitatively stable. An increase of this number can lead to 2 state of the mutation: the premutation and the full mutation.

The inheritance of the FXS is X-linked, but doesn't follow its usual pattern. Indeed, the anomalous pattern that FXS follows is called the Sherman Paradox; the frequency of the disease increases with each new generation and is sex-dependent. Women suffering from full mutation produce oocytes carrying the fully mutated X chromosome and, thus, are able to transmit the full mutation. In this case their children have a 50% risk of being affected. In case of premutated female, the length of repeats frequently enhanced during the meiosis due to the mutation's instability, thus those females may transmit the full mutation to their children.

However, in male, the length of the repeats does not enhance during the meiosis, thus premutated male are able to pass only a premutation to their daughters. Moreover it appears that the mutation in FMR1 gene has a mosaic distribution; individuals carrying a premutation or a full mutation have variability in the CGG sequence's length. Studies showed that germinal cells are only carrying the premutation. Hence, male with a full mutation passes only a premutated gene to their daughters.

In this thesis, we briefly review the genetic defect, the diagnosis and the therapeutic aspects of the Fragile X syndrome.

II. Genetic defect

a. The FMR1 gene

The mutated gene responsible for Fragile X syndrome is the FMR1 (for Fragile X Mental Retardation 1) also called FRAXA. It is located on the long arm of chromosome X on 27.3 locus (Xp27.3) coding for the Fragile X mental retardation protein (FMRP). The gene is made of 39,177 bases (base pairs 147,911,950 to 147,951,126) divided in 17 exons spanning 38 kb. Within the 4.4 kb of FMR1 transcript, a CGG trinucleotide repeat is located at the 5′-untranslated region (5′UTR). Among normal individuals, this CGG repeat is highly polymorphic in length and content, and ranges from 5 to 40 CGG repeats. We find 3 types of abnormal alleles:

- full mutation (FM) with more than 200 repeats
- premutation (PM) with 55 to 200 repeats
- gray zone mutation with 45 to 54 repeats

The FXS caused by a FM is characterized by the absence of FMR1 mRNA levels and thus an absence of FMRP. The high number of repeats causes the hypo-acetylation of the histones and the hyper-methylation of the CpG Island in the gene's promoter region causing transcriptional silencing. It has also been shown that FMR1 mRNA's trinucleotide repeats binds to the CGG-rich DNA fragment, hence preventing the transcription of the gene\(^1\).

Meanwhile, individuals with a PM are characterized by an elevation of FMR1 mRNA levels up to 2-10 times more than individuals with a normal range of CGG repeat. Studies have shown that the premutated gene induces enriched acetylated histones on the gene's promoter initiating frequent transcription\(^2\).

It has been shown that the repetition of CGGs is not the only mutation causing FXS. Occasionally FXS results from point mutations or deletions within or around the FMR1 locus\(^3\).

Figure 1 - FMR1 gene in Fragile X syndrome - National Fragile X Foundation website
b. FMRP

FMRP is composed of 632 amino acids and its mass is 71174 Da. It is an ubiquitous protein but mostly found at high levels in the brain and testis. It regulates the expression of other protein located in synapses. Immunochemistry was used to locate the protein, which seems to be functional mostly in the neurons and spermatogonia’s cytoplasm. The protein is expressed at early stage of the fetus development cholinergic neurons of the nucleus basalis magnocellularis and in pyramidal neurons of the hippocampus, its lacking probably contribute to the pathogenesis of the mental retardation observed in FXS. Its expression in spermatogonia suggests that FMR1 is necessary for germ cell proliferation.

Coded by the FMR1 gene, FMRP is a RNA-binding protein interacting with 4% of human fetal brain mRNA. Ashley et al. suggested that an abnormal interaction between FMRP and a subset of RNA might result in the pleiotropic phenotype encountered in FSX.

FMRP contains typical RNA-binding motifs, which are highly conserved (RGG box and KH domains). Immunofluorescent studies showed its translational role between the nucleus and the cytoplasm. Brown and his team identified some of the genes’ mRNA which translation is altered by mutFMRP explaining some of FSX phenotype. Among those we can cite PCL1 and PPP2CA, responsible for abnormalities of the nucleus membrane or of the cell growth and division, which, occurring during the embryonic phase, may be the consequence of the clinical symptoms observed in fragile X patients.

Pre-mutated female patient present often a Fragile X-associated Premature Ovarian Failure/Insufficiency (POF1 or POI), defined as an early menopause (before the age of 40), which has never been found in FM patients.

Men, harboring Fragile X at PM range, have been shown to present progressive intention tremor, parkinsonism, cognitive decline, generalized atrophy on MRI, and impotence, associated with a level of FMRP mRNA 2-4 times higher than normal. This phenotype known as Fragile X-associated tremor/ataxia syndrome (FXTAS) affects men, and less often women, presenting the FMR1 premutation.

Overexpression of the mRNA from the premutation expanded alleles is not associated with increased levels of FMRP protein. This is consistent with the hypothesis of a translational defect form the nucleus to the cytoplasm. The association of FMR1 mRNA with the polysome is decreases as the number of CGG repeats increases. This is why, in the case of PM, even a high level of mRNA leads to normal or lower FMRP levels and clinical symptoms. Furthermore, it would appear that the accumulation of this mRNA creates intranuclear inclusions in brain and nerve cells probably interacting with the normal activity of some protein or causing the death cell. Thus, the carriers of fragile X premutation harbor mild clinical disorder that can be compared to fully mutated individuals; like learning disabilities, ADHD, intellectual disabilities, while presenting symptoms that FM patients don’t have (FXPOI and FXTAS).
c. **Genotype/phenotype correlation**

Several researchers teams showed that there is a significant inverse correlation between the IQ level and the number of cells presenting the fragile X. Rousseau et al. performed a multicentric study of this genotype-phenotype correlation on more than 300 affected families. Mental status of fragile X permuted individuals did not differ from those with a normal genotype but they could show that abnormal methylation and increased size of FMR1 CGG expansion were highly correlated with cytogenetic, facial dimorphisms, macroorchidism and mental retardation. Female fetuses with a fragile X allele do not randomly inactivate on of the X chromosome but it would seem like there is a selection process explaining the difference between the phenotype of a female and a male harboring the same number of CGG repeats in the fragile X positive chromosome. A 2014 study by Murray and his team showed an Odd Ratio of 5.4 (95% CI = 1.7-17.4; p = 0.004) for the prevalence of fragile X permutation in a 254 group of women presenting POF.

### III. Diagnosis

Nowadays, the diagnostic of the fragile X syndrome (full mutation) is based on clinical data and the identification of a loss of function in the FMR1 gene in a patient suffering from mental retardation. Two particular situations need to know this diagnostic:

- When a child has mental retardation with learning difficulties, language-acquiring deficiency, behavior disorders.
- At the beginning of the pregnancy, the obstetrician have to ask the women about family history to look for FXS or premutation of FMR1. If there are any doubts on the mother’s genotype, the molecular diagnosis has to be done.

a. **Clinical diagnosis**

The clinical diagnosis is based on the clinical symptoms we can find in the patient and/or the family history. Usually the family seeks the GP’s advice when they eventually think their child has symptoms of delayed development. This includes mentally; delays in speech, in language skills, social difficulties and hypersensitivities to certain stimulations, and physically; problems with the learning of motor skills. Mutated individuals can present anxiety, impulsiveness, seizure, depression, sleeping difficulty. Apart from the behavior side of the patient, we can also find facial and physical symptoms such as large forehead with a prominent jaw, a unusually lengthened face, joint hypermobility and pes planus (flat feet). The diagnosis is averagely made at the 36th month for boys and 42nd month for girls. Furthermore, the families usually have a history of learning disabilities. Sometimes the diagnosis is late or never made, especially for females and mild-symptomatic patients.

b. **Cytogenetic diagnosis**

Due to the fragility of the end of X's long arm, the telomere seems to be “hanging by a thread” from the rest of the chromosome and we could detect in the karyotype of patient's lymphocytes cell in a very specific cellular culture after simple blood sample. This technique is no longer used because of its high cost and low sensitivity.

![Figure 4](http://pageofmystery.com/ALPHAS/genetics/fragilex.html) - Phan Cl. et al. PCR analysis as a rapid screening test for diagnosis of FXS. 2006. Medecine & Health

![Figure 5](http://pageofmystery.com/ALPHAS/genetics/fragilex.html) - Karyotype of a Fragile X syndrome - http://pageofmystery.com/ALPHAS/genetics/fragilex.html

c. **Biochemical diagnosis**

There is no biochemical diagnosis available for the diagnosis of fragile X syndrome. The diagnosis is based on DNA studies.
d. Molecular diagnosis

The current standard diagnostic test involves molecular genetic techniques that detect the \textit{FMR1} gene. The exact number of CGG triplet repeats can be determined. Southern blot and polymerase chain reaction (PCR) are the 2 methods of genetic analysis that are currently available. From a simple Blood sample the DNA can be analyse. The blood test usually ranges from $300-600, and results are available in 2-4 weeks.

i. Southern Blot

The Southern-Blot is better in recognizing the full mutation and determine if the gene is deactivated (methylation).

![Southern Blot Diagram]

**Figure 6 - Fragile X Analysis by Southern Blot** - https://fragilex.org/fragile-x-associated-disorders/testing/

ii. Polymerase Chain Reaction

The polymerase chain reaction analysis can determine the number of “CGG repeats” that are present in the Fragile X gene. It is able to identify the size of the permutation, intermediate and the normal sequence. It will be the only test used in the near future, because it is less expensive and quicker.

![PCR Chromatograms]

**Figure 7 - RT-PCR chromatograms of four unrelated FRX male patients and of two controls. No peaks corresponding to FMR-1 can be observed for the four FRAS patients, while in panels 1 and 2 we can see the pic of FMR1.**

e. **Prenatal diagnostic:**

Prenatal diagnostic means testing the fetus before birth in order to diagnostic a severe disease. In this case there is a difficult decision to decide if the pregnancy should better be interrupt. Two different samples are used in the PND. The chorionic callus sampling is made after 10-13 weeks of gestation; it is a small sample of the developing placenta. The amniocentesis is made between the 15th and 20th week of gestation; it is a sample of amniotic fluid. This fluid has fetal cells that can be grown and studied for various genetic conditions.

Prenatal Fragile X DNA testing is reliable and accurate. It is also important to take a blood sample of the mother to be sure that the fetal sample is not contaminated. The lab use the method of SB and PCR like for adult testing.

### IV. Therapeutic

Unfortunately there is no curative medication for the Fragile X syndrome. Currently the only treatment are symptomatic against the ADHD symptom, the insomnia, the anxiety.. We will present the actual drugs used in the symptomatic treatment of the syndrome. Then we notice the paramedical care. Finally the actual research on the topic will be discussed.

First we will focus on the take-care of full mutated patients and then on the pre-mutated patient.

**a. Full mutated patients:**

i. **Symptomatic treatment:**

The symptomatic treatments are off-labels of several drug classes:

- **Sertraline:** This antidepressant drug is a selective serotonin reuptake inhibitor, it allow the synapse to increase its signaling capacity, because of an increase of serotonin level in the synapse. It is against anxiety, depression...

- **Minocycline:** It is an antibiotic, which induce a decrease of MMP9 expression. The expression of the enzyme is increase in the X fragile Syndrome. And it interfere with the development of synapses. This drug have been showed to be efficient against the patient anxiety, it also improve its behavior and its general mood. [Modulation of the GABAergic pathway for the treatment of fragile X syndrome – Lozano R at al.] But this drug has important side effects. It can induced graying of their permanent teeth, sometimes a lupus-like syndrome, ...

- **Stimulants** are the most commonly used drugs (53%), to control the ADHD symptom. If the patients do not response to the treatment they will also treated with alpha-agonist (guanfacine), which can be very useful.

- **Melatonin** is often given temporally to patients with sleep disturbance( particularly for the children).

<table>
<thead>
<tr>
<th>Symptom category</th>
<th>Prevalence</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>46-86%</td>
<td>Stimulants α2-agonists</td>
</tr>
<tr>
<td>Anxiety</td>
<td>50-71%</td>
<td>SSRIs (selective serotonin reuptake inhibitor) Other antidepressants Benzodiazepines</td>
</tr>
<tr>
<td>Aggression and self-injurious behavior</td>
<td>17-58%</td>
<td>SSRIs Antipsychotics Anticonvulsants</td>
</tr>
<tr>
<td>Irritability, mood disorders, mania, compulsive behavior, autims</td>
<td>15-52%</td>
<td>SSRIs Antipsychotics Lithium Anticonvulsants</td>
</tr>
<tr>
<td>Sleep disturbances</td>
<td>27-73%</td>
<td>Melatonin α2-agonists Atypical antidepressants</td>
</tr>
<tr>
<td>Seizures</td>
<td>10-20%</td>
<td>Anticonvulsants</td>
</tr>
</tbody>
</table>

ii. **Paramedical care:**

The fragile X syndrome leads to a severe intellectual handicap and to autonomy problem.

To improve the life conditions of the patients in this situation a pluridisciplinary team is needed, the importance of the paramedical take care is not to forget. The patients, who are suffering from X fragile Syndrome have cognitive impairments such as language difficulties, mathematic, short-term memory, executive function and visuospatial disabilities. The treatment is also based on a multidisciplinary approach. Therefore, previous drugs have to be associated with speech therapy, individualized educational plans, sensory integration occupational therapy, and behavior interventions.
iii. **Future therapeutic paths:**

The understanding of the neurobiology of FXS has led to the development of targeted treatments to better fight the symptoms.

**mGLU5 inhibitors:** Mice model (Fmr1 KO) reflects many of the biochemical, anatomical, neurophysiological and behavioral feature of the human disorder. Researchers, after bimolecular studies, have shown that there is a strong link between the mGLU5 receptor and the disease. That lead to the suggestion that mGLU5 inhibitor could be a therapeutic target. Some animal studies show that the effect of this inhibitor was useful against a broad range of phenotype, including the increase synaptic spine density, aberrant synaptic plasticity and learning memory deficits and epilepsy. This study has permit to understand that FXS doesn't induce an irreversible disruption of the brain development. Recent clinical trials have been tried. But unfortunately, they showed no therapeutic benefit in FXS patients for reasons as yet unclear.

**GABA agonist:** A other therapeutic idea to fight the symptoms of the disease is the GABA signaling path. It appears that the level of GABA is decrease in the fragile X mice model (decrease of GABA subunit receptors, decreased synthesis of GABA, increased catabolism of GABA, decreased GABAergic input in many regions of the brain). The consequence of this decrease seems to induce oversensitivity to sensory stimuli, epilepsy, social fear, anxiety and autistic behavior. So several GABA agonist have been test on these mice with promising results. Some of them have been use on human but until now just very small sample of patient. (Arbaclofen and ganaxolone). Now further studies are needed to determine the safety and efficacy of GABAergic treatment.

**b. Pre-mutated patients:**

Traditionally a carrier has been defined as an individual who has no clinical symptoms but as seen before in the case of FMR1 premutation it is not the case. Therefore fragile X premutation carriers often demonstrate incomplete penetrance. Nowadays, like for the full mutation, symptoms of the premutation are currently treated based on symptoms, rather than underlying disease mechanisms. As for the full mutation the psychosocial support from both professionals and family members is equally important than drugs.

A major challenge in searching for targeted treatment of the premutation has been the development of a premutation animal model. Researchers are searching for useful biomarkers to predict the penetrance of the disease.

The central treatment question is whether some anticipatory treatments or environmental modifications could prevent or reduce the severity of symptoms.
V. References


