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Review Article

New Insights into the Mechanism of Pathogenesis of Fragile X-Associated Premature Ovarian Failure

Ranjana Rana, Mona Sharma and Ashutosh Halder*

Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, India

***Corresponding Author:** Ashutosh Halder, Professor and Head, Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, Delhi, India.

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Abstract

One of the common genetic defects linked to Premature Ovarian Failure is FMR1 gene premutation (trinucleotide repeats). However, it is unclear how this trinucleotide repeats number produces ovarian failure phenotype. The aim of this review is to highlight the advances made in understanding the mechanism by which FMR1 premutation causes POF. POF due to FMR1 gene premutation may be caused by three mechanisms: RNA toxic gain-of-function, repeat-associated non-AUG translation creating cryptic protein FMRpolyG, and transcription of lncRNAs, particularly FMR4 and FMR6, which have been linked to FMR1 instability and ovarian dysfunction.

Keywords: *FMR1 Premutation; Premature Ovarian Failure; RNA Toxicity; Non-AUG Translation; Long Non-Coding RNA*

Abbreviations

POF: Premature Ovarian Failure; FMR1: Fragile X Mental Retardation 1; FRAXA: Fragile Site A on the X Chromosome; FXPOF/FXPOI: Fragile X-Associated Premature Ovarian Failure/Insufficiency; FXTAS: Fragile X-Associated Tremor/Ataxia Syndrome; RAN: Repeat Associated Non-AUG; lncRNA: Long Non-Coding Ribonucleic Acid

Introduction

In humans, the Fragile-X mental retardation 1 (FMR1) gene is found on chromosome X, and the gene mutation is classified as a trinucleotide expansion disorder. Fragile X syndrome, Fragile X associated premature ovarian failure/insufficiency, and Fragile X associated tremor/ataxia syndrome are all linked to increasing CGG repeats in the FMR1 gene promoter region. According to ACMG recommendations, there are two pathogenic FMR1 alleles: premutation alleles with 55–200 repeats and complete mutation alleles with > 200 repeats (Figure 1) [1].

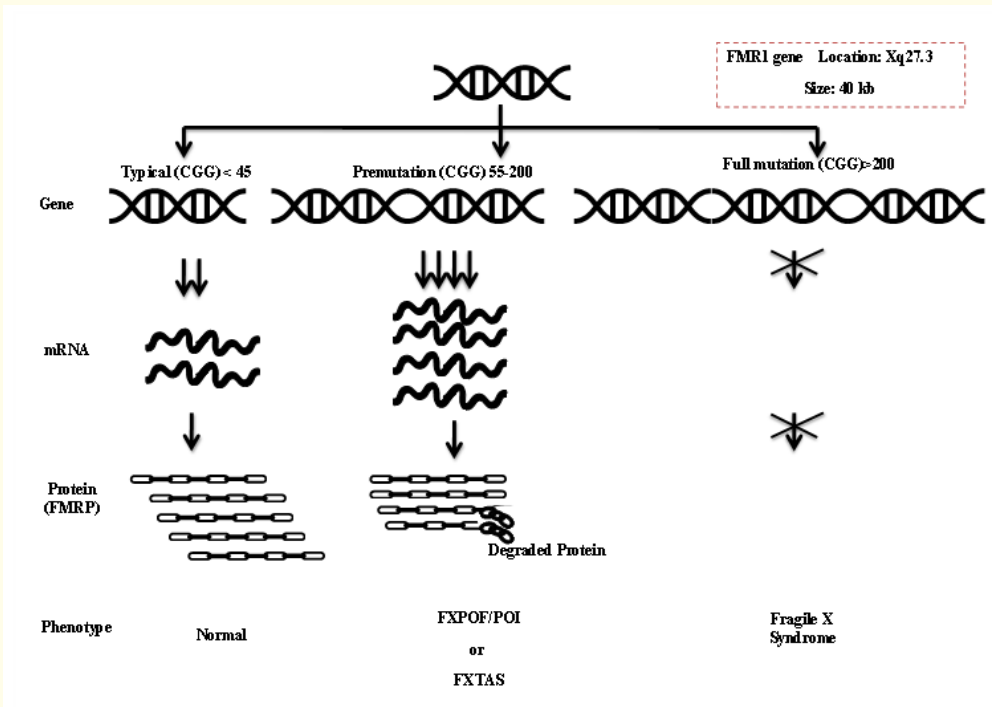

Figure 1: *Genotype-phenotype co-relation of FMR1 locus: Normal phenotype has <45 repeats with normal mRNA and FMRP levels. Premutation has 55-200 CGG repeats with elevated levels of mRNA and normal or slightly decreased FMRP. Whereas, in full mutation which is characterized by >200 repeats, the 5′ UTR is hypermethylated which leads to transcriptional silencing of FMR1 gene and hence no mRNA or protein is formed.*

Despite the fact that women with the premutation have a low probability of getting Fragile X-associated tremor/ataxia syndrome, POF is a fairly common. Much of the study into the molecular processes underpinning the pathophysiology of FMR1-related illnesses has concentrated on the neurological manifestations, but the clarity of the molecular mechanisms of Fragile X associated tremor/ataxia syndrome could aid researchers in elucidating the molecular mechanisms of Fragile X associated POF/POI. So far, three potential ovarian dysfunction routes in FMR1 permutation carriers have been discovered. The first is the RNA toxic gain-of-function pathway, which results in the loss of function of over 30 RNA-binding proteins. The second is linked to non-AUG translation (RAN), which produces a cryptic polyglycine-containing protein (FMRpolyG) and the third is linked to new lncRNAs [2]. This report highlights the research advances made to understand the above-mentioned mechanisms.

Fragile X-associated premature ovarian insufficiency/failure

In the literature, the terms premature ovarian insufficiency (POI) and premature ovarian failure (POF) are frequently interchanged to describe a range of illnesses defined by hypergonadotropic amenorrhea. The clinical spectrum is divided into three stages: occult, biochemical, and overt, regardless of the terminology employed. The overt stage, often known as POF, denotes total ovarian function loss [3]. POF is a very diverse illness with many different etiologies. Genetic reasons are thought to contribute to about 25% of cases with POF [4]. Reduced gene dosage and poor DNA repair are two genetic pathways involved in the pathophysiology of POF. For example, BMP15, which plays a key role in oocyte maturation, appears to bypass X-chromosome inactivation [5] and mutations in the gene have been linked to POF, and it's a possible gene with a gene dosage impact [6]. In recent years, with the help of NGS, many genes involved in meiosis and DNA repair have been shown to affect ovarian reserve [7]. These could cause ovarian failure by reducing the pool of primordial follicles, increasing ovarian follicle atresia, or preventing follicle maturation [8,9].

Over the years, various gene mutations have also been linked to the pathogenesis of POF, out of which FMR1 gene premutation is one of the candidates. Fragile-X premutation occurs in about 16% of POF [10]. Also, an increased risk of premutation of about 14% was observed with a family history of POF which is comparatively more than spontaneous POF, where the risk is about 2% [10]. The prevalence of FMR1 gene premutation varies within different populations [11-14]. In addition, the CGG repeats length of FMR1 gene premutation has a non-linear relationship with POF penetrance [15]. When compared to the general population, women with FMR1 gene premutation have approximately five years of early menopause [16].

About 29 CGG repeats are the most common repeats observed in the general population while women with midrange repeats (70 - 90) have been found to be at the highest risk for the development of POF/POI [17,18]. The physiological mechanisms by which fragile X-associated POF develops are unknown, but it has been demonstrated using mice models that premutation alleles do not prevent the formation of the primordial follicle pool, but rather impair overall development and survival [19]. In addition, X chromosome inactivation and smoking have been investigated to explain the incomplete penetrance of POF among premutation carriers [20,21].

An irreversible consequence of POF/POI is infertility. It is caused by decreased oocyte reserve due to either increased atresia of the follicles or the presence of low ovarian reserve at birth itself. However, some women with fragile X-associated POF have an intermittent ovarian function and conceive naturally [22]. As CGG repeat instability can be inherited from the mother to the next generation, women with fragile X-associated POF may have a male child with fragile X syndrome due to CGG repeat increase, from premutation to mutation scale [23].

The RNA toxic gain-of-function mechanism in fragile X associated POF

Depending on the number of repeats, an aberrant CGG expansion in the 5′UTR region of the FMR1 gene causes a variety of hereditary diseases (Figure 1). CGG expansion in the 5′UTR region of the FMR1 gene in premutation carriers is associated with increased FMR1 transcription but reduced FMR protein synthesis [24]. As a result, RNA toxicity may play a role in the pathogenesis of fragile X-associated

POF or fragile X-associated tremor/ataxia syndrome thus, RNA toxicity may play a role in the pathogenesis of fragile X-associated POF/ fragile X-associated tremor/ataxia syndrome. Kenneson observed that the transcription (RNAs) of the FMR1 gene in permutation carriers was proportional to the size of CGG trinucleotide repeats [24]. According to this model, enlarged CGG repeat transcripts are exported from the nucleus to ribonucleoprotein complexes that do not bind to the ribosome's 40S subunit, causing translation to be disrupted and FMR 1 protein levels to be reduced. Messenger RNA production rises to compensate for low levels of FMR protein [25]. If the excess mRNA is not degraded, RNA aggregates accumulate, which trap CGG binding proteins and prevent them from completing their normal function [26-28].

FMR1 is an X-linked gene that is expressed in germ cells of the fetal ovary and has been suggested to cause diminished ovarian reserve [29]. It is suspected that RNA secondary structures may form in mid-range CGG repeat carriers, causing cell malfunction in the ovaries and eventually diminished ovarian reserve [30]. An increase in FMR 1 gene mRNA expression has also been observed in the ovaries of premutation mouse models and the granulosa cells of mature follicles in premutation human carriers [31].

Anti-Mullerian hormone (AMH) is a reliable biomarker for ovarian reserve and it was observed that in FMR1 premutation carriers, the AMH receptor levels were high along with high FMR1 mRNA expression, concluding that the premutation may lead to dysregulation of AMH expression levels [32]. Few proteins that bind and are sequestered by CGG have been discovered within intranuclear inclusions in the brain, and this discovery has aided in understanding the role of RNA gain-of-function toxicity in the pathogenesis of fragile X-associated tremor/ataxia syndrome. The discovery of SRC associated with mitosis of 68 kDa (SAM68) in CGG RNA aggregates is particularly significant in terms of fragile X-associated POF. SAM68 has been linked to the regulation of FSH and LH receptor mRNA splicing and it has been found to be abundant in ovarian follicles [33]. SAM68$^{-/-}$ are highly sub fertile with a reduced number of growing follicles [34]. It was observed that premutation carriers have high mRNA levels of SAM68 as compared to control [35]. These studies suggest that RNA-gain-of-function toxicity might be responsible for causing fragile X-associated POF.

The repeat associated non-AUG translation (RAN) in fragile X associated POF

The Eukaryotic central dogma includes the transcription of mRNA from DNA and subsequent translation of mRNA into protein involved in various structural and regulatory roles. But an error in translation can result in an inactive or deleterious protein that can hinder overall cellular fitness. It is thus critical for the ribosome to initiate at an appropriate codon and terminate at a specific codon. The normal translation start codon is AUG with three stop codons UAA, UGA and UAG [36]. The discovery of repeat-associated non-AUG (RAN) translation in 2011 put this theory to the test, adding still another layer of complexity to the molecular mechanisms underlying repeat disorders [37]. RAN translation has been observed in a variety of microsatellite expansion mutations. In fragile X-associated tremor/ ataxia syndrome, RAN translation is initiated within the 5′UTR that can occur in at least two reading frames, yielding either a polyglycine product, named FMRpolyG, or a polyalanine product (FMRpolyA). FMRpolyG and ubiquitin immunostained mural granulosa cells from FMR1 premutation carriers were observed that demonstrated FMRpolyG aggregates [38,39]. Therefore, this may provide some evidence that FMRpolyG is associated with the premutation-related pathology, however, more data is needed to establish whether these inclusions are pathologically related to fragile X-associated POF.

Role of lncRNAs in the pathogenesis of fragile X-associated POF

LncRNAs (long non-coding RNAs) are non-coding regulatory RNAs that are longer than 200 nucleotides. They lack many of the mRNA signature motifs yet are engaged in a variety of cellular tasks such as chromosome architectural regulation, transcriptional regulation, mRNA turnover, and translation [40]. The FMR1 gene locus also transcribes several lncRNAs, including FMR4, FMR5, FMR6, and FMR1-AS, which is intriguing. Because FMR4 and FMR6 are thought to influence FMR1 stability, splicing, subcellular localization, and translational efficiency in fragile X-associated tremor/ataxia syndrome, they have been also linked to fragile X-associated POF [41]. The number of oocytes retrieved and the expression of lncRNA-FMR6 in cumulus granulosa cells of patients with the premutation was found to have

a negative linear relationship [42]. These findings indicate that in ovary granulosa cells of females with fragile X-associated POF, the accumulation of FMR6 may result in ovarian dysfunction.

Conclusion

Researchers have been able to better grasp the genesis of fragile X-associated POF by studying fragile X-associated tremor/ataxia syndrome and other expanded-repeat illnesses. However, there is still disagreement over how RNA toxicity or cryptic FMRpolyG formation leads to disease/POF. The abnormal expression of lncRNAs has also been identified in fragile X-associated POF, but the pathology remains unknown. Furthermore, the onset and location (cellular and sub-cellular) of effect in the ovary leading to atresia and failure is still unknown besides reasons for incomplete penetrance of FMR1 premutation.

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