1.1 Fragile X-associated tremor/ataxia syndrome (FXTAS)

FXTAS is a progressive neurodegenerative disorder characterized by problems with movement and cognition [1]. FXTAS is a late-onset disorder, usually occurring after age 50, and its signs and symptoms gets worsen with age [2]. In addition to neuropathy, thyroid dysfunctions, hypertension, immune dysfunction and cardiac arrhythmias are also observed in some FXTAS patient [3]. The prevalence of FXTAS is estimated to range between 1 in 110 to 250 in females and 1 in 260 to 800 in males [4], [5]. However, due to incomplete penetrance it is estimated that 1 in ~3000 male and 1 in ~5000 female are at risk of developing FXTAS, with disease symptoms getting more pronounced with age [6]. This condition affects males more frequently and severely than females. Affected individuals have areas of damage in the part of the brain including cerebellum, cerebral and cerebellar white matter, frontal cortex, and hippocampus which can be analyzed with magnetic resonance imaging (MRI).

At the histopathological level, FXTAS is characterized by neuronal cell loss and presence of large ubiquitin-positive intranuclear inclusions in both neurons and astrocytes [7], [8]. Rare ubiquitin positive inclusions are also observed in non-CNS organs like kidney and thyroid [9], At the genetic level, FXTAS is caused by the presence of 55 to 200 CGG repeats located within the 5’ untranslated region(UTR) of the Fragile X mental retardation1(*FMR1)* gene (OMIM *309550), located on the q-arm of the X chromosome [10],[11]. The *FMR1* gene encodes FMRP which is RNA binding protein involved in regulation of transport and local translation of mRNAs in brain and is essential for synaptic plasticity and neuronal development [12], [13]. An expansion of CGG trinucleotide repeats within the 5’-UTR of *FMR1* causes different neuropathological conditions based on the number of CGG repeats. FXTAS, Fragile X-associated primary ovarian insufficiency (FXPOI) and Fragile X-associated Neuropsychiatric Disorders (FXAND) are caused by expansions of 55 to 200 CGG repeats (premutation, PM), while Fragile X syndrome (FXS) is caused by expansions greater than 200 CGG repeats (full mutation, FM) [14], [15].

1.2 Expansion of CGG repeats at 5’UTR of *FMR1* gene

FXTAS belongs to the Fragile X family of disorders which are mainly caused by presence of expanded CGG repeats at the 5′ untranslated region(UTR) of the Fragile X mental retardation

1(FMR1) gene. The name Fragile X refers to a fragile site found on the long arm of the X chromosome [16]. There are 25-45 CGG repeats observed under normal condition, while exceeding repeats from 45-55 considered as gray zone as these alleles are more likely to be unstable upon transmission to next generation [17], [18]. FXTAS is also referred as premutation condition(PM) where number of CGG repeats falls in the range of 55-200. While under full mutation condition, number of CGG repeats increases to more than 200 which leads to fragile X syndrome [19]. Expanded CGG repeats tends to form secondary RNA structures which decreases the efficiency of FMRP translation causing decreased levels of FMRP in premutation carriers [20]. Whereas in FXS, the (CGG)n exceeds more than 200 (full mutation, FM) and the upstream promoter region of the *FMR1* gene gets hypermethylated [16],[21],[22]. This causes transcription silencing and the gene product fragile X mental retardation protein, FMRP, is not produced. Hence, deficiency of FMRP in neurons ultimately leads to mental retardation and intellectual deficits in FXS patients [23]. Although, different hypothesis have been proposed, mechanisms of CGG repeat instability and associated pathogenesis are still not clear.

1.3 Prevalence

According to the population based screening studies approximately 1 in 150-300 females and 1 in 300-800 males in the United States may have a fragile X premutation [4], [24], [25], [26]. Corollary, a report from 40,000 women in Israel revealed a prevalence of 1 in 154 which follows the almost same trend of prevalence as in USA [27]. Similarly, Colombian population study had also estimated the prevalence of premutation in 14.1 per 1000 males and 35.9 per 1000 females [28], while Japan being the country having the lowest reported prevalence of the PM allele [29]. Similarly study from Korea among the 8,641 women, 8 women found to be premutation carriers (1:1,090, 0.09%) [30]. However, it is now known that different ethnic groups show a different pattern of prevalence, for example it is less common in Asian populations [31] while it is more prevalent in Mediterranean countries [32], [33]. Estimates of prevalence may vary in Asian population where screening for premutation had been done on targeted population having one or more premutation associated phenotypes. specifically in India, where only few studies have reported the prevalence of premutation carriers considering cohort with progressive, late-onset tremor/ataxia [34]. Therefore, it becomes difficult to predict estimate of overall population.

1.4 Molecular mechanisms underlying FXTAS pathology

Studies in the last decade have identified three main mechanisms of how CGG repeats expression can be toxic for neurons: RNA mediated titration of specific RNA binding proteins, Repeat Associated non-ATG (RAN) translation of the CGG premutation into toxic proteins and altered DNA damage repair response [35]–[40]. Historically, RNA gain of function was the first model proposed to explain pathogenesis of FXTAS [36],[40],[41]. According to this, expanded CGG repeats at 5'UTR of *FMR1* mRNA may bind and titrate specific RNA binding proteins, resulting in loss of their normal functions. *FMR1* mRNA containing expanded CGG repeats binds several RNA binding proteins such as hnRNP A2/B1, DROSHA/DGCR8, Purα etc.[37],[42],[43]. Several emerging evidences indicate that translation initiation to near-cognate start codons located before the CGG premutation leads to expression of a small polyglycine-rich protein named FMRpolyG. FMRpolyG is prone to aggregation and forms large nuclear ubiquitin-positive inclusions in both cells and animals. Importantly, FMRpolyG expression appears mandatory to mediate the toxicity of the CGG premutation in cells, Drosophila and mouse models of FXTAS [45],[46]. However, how FMRpolyG causes neuronal cell dysfunctions and cell death is currently unclear. Further, *FMR1* RNA containing expanded CGG repeats can be pathogenic by creating R-loops, which are RNA: DNA hybrids formed between the nascent transcribed CGG-rich *FMR1* mRNA and the DNA[47]–[49]. Excessive R-loop formation may lead to DNA breaks, resulting in accumulation of gH2AX, a histone variant associated with DNA damage repair and decreased level of ataxia telangiectasia mutated (ATM) which is involved in DNA damage repair [35],[49].

1.5 Altered miRNA biogenesis in FXTAS

miRNAs are small noncoding RNAs which add a new layer of complexity to the control of gene expression. They play crucial role in posttranscriptional gene regulation and are known to regulate translation of the genes involved neuronal functions including both differentiation and survival of neurons [50]–[54]. Briefing miRNA biogenesis, miRNAs are transcribed by the RNA polymerase II into primary miRNA (pri-miRNAs) transcripts, which are further processed by the type III RNase DROSHA and double stranded RNA binding protein DGCR8 into precursor miRNAs (pre-miRNAs) [55]–[58]. Pre-miRNAs are then exported into the cytoplasm, where they are further processed by the DICER enzyme into mature miRNAs. In earlier study,

FMR1 premutation CGG repeats have been reported to lead to deregulation of miRNAs in the brain of the FXTAS fly model [59]. Lately, study from Berguerand NC and group have demonstrated possible cause for decreased miRNA biogenesis in FXTAS. The study showed partial sequestration of DROSHA-DGCR8 within CGG RNA foci, reduces the processing and expression of various miRNAs in neuronal cells expressing expanded CGG repeats [38].

1.6 Subcellular localization of miRNAs

The process of miRNA-induced silencing complex (RISC) mediated messenger RNA degradation occurs in the cytoplasm; however, multiple studies have reported presence of miRNAs along with RISC components in other subcellular compartments (reviewed in [60]). The organelle specific localization of miRNAs is critical to finetune the site specific expression levels of mRNAs. Considering this, an early study showed association of AGO2 with the endoplasmic reticulum (ER) [60], and subsequent studies have also confirmed the association of miRNAs with actively translating mRNAs on rough ER [61]–[63]. Cytoplasmic miRNAs were also present in processing bodies (P-bodies) and are involved in inhibition of translational and mRNA decay together with components of RISC enriched in P-bodies [64]. Interestingly, nuclear encoded miRNAs are also localize to mitochondrial outer membrane and inside the mitochondria known as mito-miRs [65],[66]. mito-miRs use the inter membrane proteins, polynucleotide phosphorylase and the polyA polymerase suggesting that nuclear-encoded miRNAs are transported via same route taken by nuclear encoded mitochondrial proteins and accumulate in the mitochondria [67].

1.7 Mito-miRs can regulate mitochondrial functions

The hypothesis of translocation of miRNAs to various subcellular compartments is emerging. Interestingly, the complimentary studies have supported some of these presumptions [65], [68]–[70]. The experimental evidences form the last decade have confirmed the presence of miRNAs in/on mitochondria from various sources including rat liver (15miRNAs,[71]), mouse liver (20 miRNAs,[72]), myotubes (20miRNAs,[73]), 143B (3 miRNAs,[74]), human muscles (46 mature miRNAs and 2 pre-miRNAs). The majority of them target nuclear encoded mitochondrial genes whereas some regulates mitochondrial genome encoded transcripts such as hsa-miR-133a targets ND1 and has-miR-130 targets COX3 [71] while miR-1 is known to regulate whole

mitochondrial transcriptome during myogenesis [75]. However, there are only few reports which have addressed their functional relevance systematically. In FXTAS condition, where secondary RNA structures (hairpin, duplexes) sequester several RNA binding proteins including proteins involved in miRNA biogenesis (e.g., DROSHA/DGCR8), which may lead to decreased pool of miRNAs and also affect their translocation to mitochondria. Thus, the role of mito-miRNAs in modulation of mitochondrial functions should be considered and needs to be investigated further to understand FXTAS pathogenicity.

1.8 Mitochondrial dysfunction in premutation

Mitochondria play a central role in the regulation of various cellular processes, including, cell survival, apoptosis, inflammation, autophagy, and calcium trafficking. They are also important for the maintenance of cellular homeostasis and energy metabolism. Therefore, mitochondrial dysfunction leads to numerous pathological conditions [76]. Mitochondrial dysfunctions are also one of the major hallmarks of neurodegenerative diseases including FXTAS. Various studies had shown abnormal mitochondrial functions in cell and mouse models of FXTAS as well as in fibroblasts and brain tissue of premutation carriers [77]–[80]. However, the cellular and molecular mechanisms triggering mitochondrial dysfunctions in FXTAS are not well understood.

miRNAs show their RNA interference activity by binding with its target mRNAs and forming RNA induced silencing complex (RISC). Considering organelle specific localization of miRNAs, mito-miRs have gained huge interest in the field of mitochondrial biology. The mitochondrial outer membrane may serve as platform for miRNAs and translational machinery where they fine tune the levels of important proteins involved in mitochondrial maintenance. On the other hand, miRNAs that are residing in mitochondrial matrix may play crucial role in regulation of mitochondrial transcript processing and ultimately mito-transcript levels.

Hence, we hypothesized that the altered miRNA biogenesis which has been already reported under premutation condition may also affect trafficking of miRNA at mitochondria. Further, miRNAs which translocate to mitochondria (mito-miRs) may regulate both nuclear DNA encoded mitochondrial targeted mRNA and mitochondrial DNA (mtDNA) encoded transcripts

and ultimately control mitochondrial function and cell death in various pathophysiological conditions. Therefore, we systematically characterized the association miRNAs with mitochondria and decipher their impact on mitochondrial function and cell death in human cells expressing expanded CGG repeats which mimics in vitro FXTAS condition.