SCIENTIFIC COMMENTARY

Bringing fragile X-associated neuropsychiatric disorders into the phenotypic fold of premutation conditions

This scientific commentary refers to 'Modelling fragile X-associated neuropsychiatric disorders in young inducible 90CGG premutation mice' by Çalışkan *et al.* (https://doi.org/10/109/brain/awaf203).

The work by Çalışkan and co-workers[1] in this issue of *Brain* is an eye opener for researchers in the fragile X premutation field.

Carriers of the fragile X premutation—defined as an expansion of 55–200 CGG repeats in the *FMR1* gene, compared to >200 repeats in the full mutation—were originally believed to be "non-penetrant" or clinically unaffected. The mothers of children with fragile X syndrome (FXS) are typically intelligent, often brilliant, and do an amazing job managing the challenges of raising their children with intellectual disability and autism. In the 1990s, it was noted that about 20% of these carrier mothers experienced menopause before the age of 40—a condition now recognized as fragile X-associated primary ovarian insufficiency (FXPOI). Subsequently, boys with the premutation were found to have higher rates of autism and/or attention-deficit/hyperactivity disorder (ADHD) compared to controls without the premutation, even within the same families.[2]

At first these symptoms were thought to result from early silencing of the *FMR1* gene, as in FXS. However, subsequent molecular studies showed just the opposite: the gene was producing excessive levels of *FMR1* mRNA, as described in a landmark paper by Tassone and colleagues in 2000. This discovery marked the beginning of a broader understanding of premutation-related RNA toxicity. The field evolved further with the identification of five carrier males who developed tremor and ataxia in later life—symptoms now attributed to fragile X-associated tremor/ataxia syndrome (FXTAS).[3] Since then, the field has expanded rapidly with many more patients diagnosed worldwide. Characteristic patterns of white matter disease were identified—including the middle cerebellar peduncle (MCP) sign—and intranuclear inclusions were

discovered not only in neurons and astrocytes throughout the CNS, but also in peripheral neurons and organs.[4] These findings may help explain peripheral symptoms of FXTAS, including erectile dysfunction, cardiac arrhythmias, oesophageal dysmotility, and neuropathy.[4]

Pathological studies have now firmly established FXTAS as a neurodegenerative disorder, with devastating astrocyte loss throughout the brain.[5] Definitive diagnostic criteria include tremor, ataxia, white matter disease, and intranuclear inclusions. However, prior to the current study, no-one had considered that these inclusions might also occur in children with the premutation.

Up to half of all premutation carriers develop one or more neuropsychiatric conditions—such as depression, anxiety, obsessive compulsive disorder, ADHD, or insomnia—a clinical profile now referred to as fragile X-associated neuropsychiatric disorders (FXAND).[6] These symptoms may emerge in childhood but are more commonly seen in adults (Figure 1). Although initially thought to result from the stress of raising a child with FXS (and this can be truly stressful), FXAND is also seen in carriers without affected children. In addition, autoimmune problems are more prevalent among female premutation carriers and can exacerbate FXAND symptoms.[6]

In their new study, Çalışkan and colleagues[1] open our eyes to viewing the spectrum of premutation involvement as a continuum throughout life. In earlier work, they used the doxycycline (DOX)-inducible 90 CGG-repeat premutation mouse model to reveal the motor symptoms of FXTAS in aging. Now, using the same model, they provide insights into the pathophysiology and mechanisms underlying FXAND.[1]

They show that induction of the 90 CGG repeat *in utero* leads to significant hyperactivity in the mouse at 4 weeks of age—corresponding to adolescence in humans—compared to controls without induction. Notably, they also observed intranuclear inclusions in the basolateral amygdala (BLA) and ventral hippocampus by 4 weeks, although the number of inclusions was much lower than in aging premutation mice. By 7 weeks, which represents young adulthood, they saw an increase in plasticity and excitability in the BLA, an increase in gamma oscillations in the ventral hippocampus, and recurrent epileptiform activity with carbachol perfusion. Although the number of parvalbumin-positive interneurons increased during adolescence, their numbers declined by 12 weeks of age in the CA3 and CA1 regions of the ventral hippocampus. At this age, the mice also showed anxiety-like behaviours in laboratory tests, such as avoidance of brightly lit or open spaces.

To better understand the molecular mechanisms leading to these behavioural changes, the authors used mass spectrometry to conduct proteomic analysis of the ventral hippocampus. They identified both upregulation and downregulation of proteins that impact oxidative stress and cellular respiration, with patterns resembling those associated with ADHD in adolescence and anxiety and depression in adulthood. Although these abnormalities were reversible with inactivation of the DOX induction, this temporal progression suggests that changes to neural networks within the limbic system underlie the behavioural phenotypes that mirror FXAND in humans.

The key message from this research is that the RNA toxicity of the premutation begins early, continues through adolescence and adulthood, and leads to dysregulation of the limbic system, which likely drives FXAND. Early intervention is therefore critical. We have seen adults in their 40s and 50s who have neurological symptoms, and others who are asymptomatic but show evidence of white matter disease in the CNS prior to developing symptoms of FXTAS.[7,8] It is likely that these white matter changes are also part of the continuum of premutation involvement that represents the beginning of neurodegeneration, and these individuals could benefit from treatment before the onset of full FXTAS. Currently, we recommend exercise to reduce inflammation and stimulate neurogenesis, antioxidants to reduce oxidative stress, and mitochondrial boosters such as CoQ10 and epicatechin.[9] Sigma-1 receptor agonists may also hold promise for future use.[9] Depression and anxiety are likely to benefit from treatment with selective serotonin-reuptake inhibitors. The findings of Caliskan and colleagues underscore the need for early diagnosis and treatment of FXAND and for the development of preventative therapies for FXTAS.

One significant caveat with studies using inducible mouse models is that the CGG-repeat-containing mRNA is generally expressed at levels that may be higher than endogenous levels in human carriers. The use of a DOX-inducible transactivator under the control of a strong promotor like PrP raises the possibility that many of the early-life features observed in the current study may be specific to overexpression. Indeed, in the *Fmr1* knock-in mouse model using the native promoter, intranuclear inclusions generally do not appear before about 30 weeks of age.[10] Thus, it will be important in future studies to compare the current results with the early phenotype of the *Fmr1* knock-in mouse.

Randi Hagerman

MIND Institute, UCDH, Sacramento, CA, USA

E-mail: rjhagerman@ucdavis.edu

Funding

R.H. is funded by NICHD HD036071 for her research in premutation carriers.

Competing interests

R.H. is carrying out a trial of Zatolmilast funded by Tetra/Shionogi Pharma and a trial of CBD funded by Zynerba/Harmony Pharma in patients with fragile X syndrome.

References

1. Çalışkan, Enrile Lacalle S, Kul E, *et al.* Modelling fragile X-associated neuropsychiatric disorders in young inducible 90CGG premutation mice. *Brain.* Published online 2 June 2025. https://doi.org/10.1093/brain/awaf203

2. Farzin F, Perry H, Hessl D, et al. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. *J Dev Behav Pediatr*. Apr 2006;27(2 Suppl):S137-44. doi:10.1097/00004703-200604002-00012

3. Hagerman RJ, Leehey M, Heinrichs W, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology*. Jul 10 2001;57(1):127-30. doi:10.1212/wnl.57.1.127

4. Hunsaker MR, Greco CM, Spath MA, et al. Widespread non-central nervous system organ pathology in fragile X premutation carriers with fragile X-associated tremor/ataxia

syndrome and CGG knock-in mice. *Acta Neuropathol*. Oct 2011;122(4):467-79. doi:10.1007/s00401-011-0860-9

5. Dufour BD, Bartley T, McBride E, et al. FXTAS Neuropathology Includes Widespread Reactive Astrogliosis and White Matter Specific Astrocyte Degeneration. *Ann Neurol*. Mar 2024;95(3):558-575. doi:10.1002/ana.26851

6. Hagerman RJ, Protic D, Rajaratnam A, Salcedo-Arellano MJ, Aydin EY, Schneider A. Fragile X-Associated Neuropsychiatric Disorders (FXAND). *Front Psychiatry*. 2018;9:564. doi:10.3389/fpsyt.2018.00564

7. Famula J, Ferrer E, Hagerman RJ, et al. Neuropsychological changes in *FMR1* premutation carriers and onset of fragile X-associated tremor/ataxia syndrome. *J Neurodev Disord*. Mar 23 2022;14(1):23. doi:10.1186/s11689-022-09436-y

8. Liani V, Torrents C, Rolleri E, et al. Premutation Females with preFXTAS. *Int J Mol Sci*. Mar 20 2025;26(6)doi:10.3390/ijms26062825

9. Sodhi DK, Hagerman R. Fragile X Premutation: Medications, Therapy and Lifestyle Advice. *Pharmgenomics Pers Med*. 2021;14:1689-1699. doi:10.2147/PGPM.S338846

10. Willemsen R, Hoogeveen-Westerveld M, Reis S, et al. The *FMR1* CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. *Hum Mol Genet*. May 1 2003;12(9):949-59. doi:10.1093/hmg/ddg114

Figure legend

Figure 1 Timeline for premutation conditions including fragile X-associated neuropsychiatric disorders (FXAND), fragile X-associated tremor/ataxia syndrome (FXTAS) and autoimmune diseases.