Syrens: A Neurolinguistic study to assess cognitive abilities and develop composite biomarkers in Fragile X Premutation Carriers

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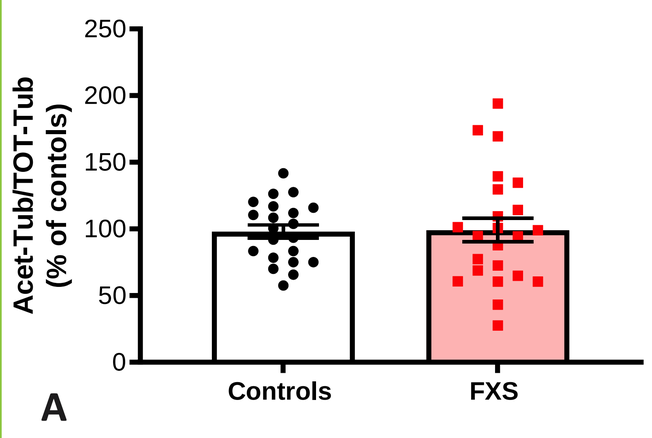
Background

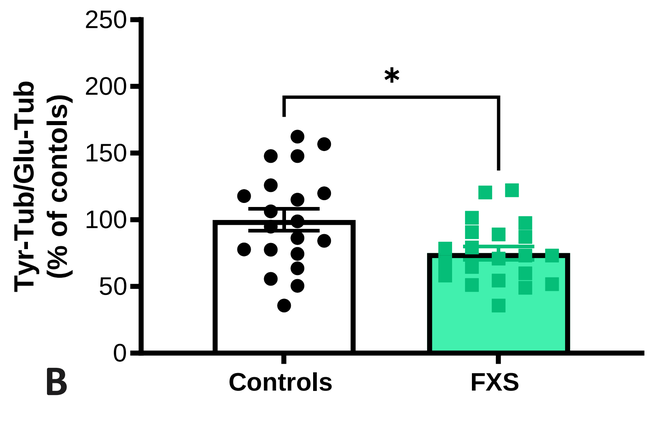
- Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability, and it is caused by mutations of the *Fmr1* gene that produces a protein fundamental for brain development and plasticity (FMRP). FXS is linked to the full mutation of the gene (more than >200 CGG nucleotide repeat expansion), while the premutation (55-200 CGG repeat expansion) can lead to various conditions including a Parkinson-like neurodegenerative disorder named Fragile X Tremor Ataxia Syndrome (FXTAS).
- More studies targeting both FXS children and premutation carriers are needed to account for the quality of life of all people affected by the gene mutations, which can only be achieved by including both parents and children in the studies.
- We aim at developing a composite biomarker of disease exploring different measures as biomarkers of disease progression are needed to anticipate diagnosis and accelerate treatment discovery.
- Microtubules are a fundamental structure of the cytoskeleton involved in many cellular processes, including the facilitation of processes underlying synaptic plasticity. FMRP is associated to Microtubule Dynamics (MT). α-Tubulin Post Translational Modifications (PTMs) are a marker of MT related to synaptic plasticity.
- Measuring levels of α-Tubulin PTMs in plasma samples is potentially reflective of the global state of microtubule health in the body. Acetylated/Total α-Tubulin is a marker of less dynamic microtubule. Tyrosinated/Detyrosinated α-Tubulin is a marker of more dynamic microtubule.

Study 1. Dysregulation of microtubule dynamics (MT) as a potential biomarker in Fragile X Syndrome (FXS).

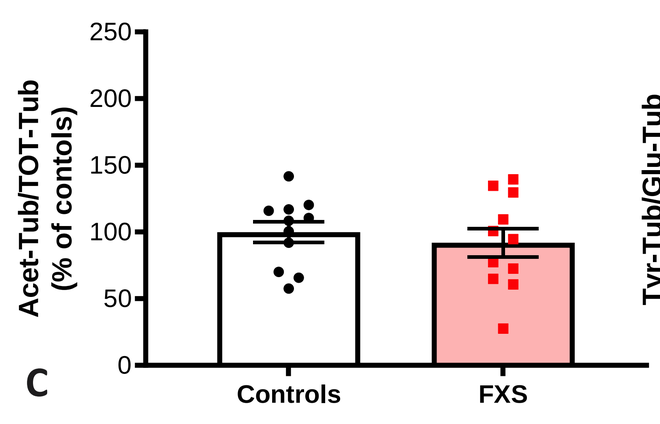
In a preliminary study, we received Fragile X plasma samples of children and adults with matched controls from FRAXA Research foundation, which we analysed for alpha-tubulin PTMs.
The results showed a dysregulation of alpha-tubulin PTMS in adult patients compared to controls.

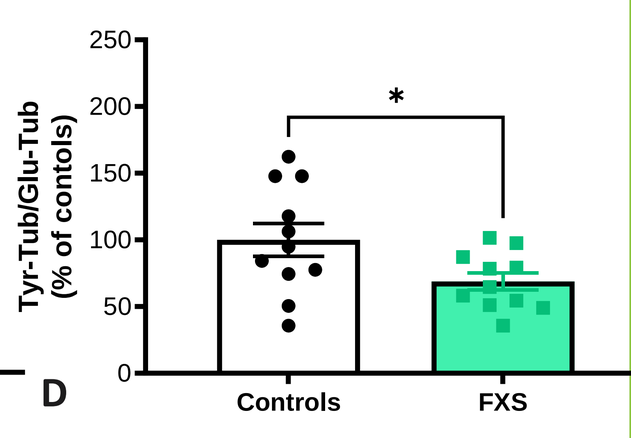
α-Tub Post Translational modifications (PTMs) in children and adults



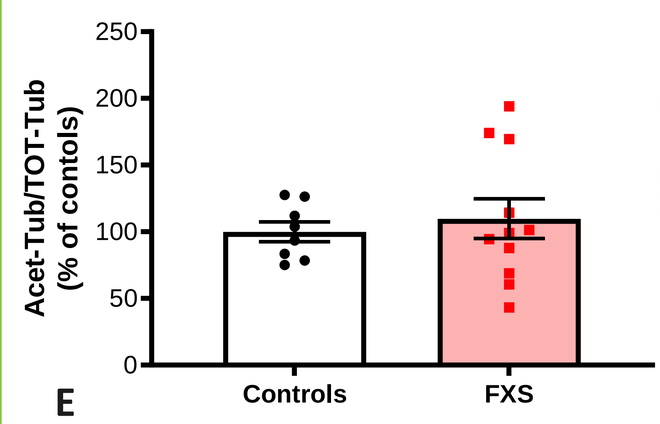


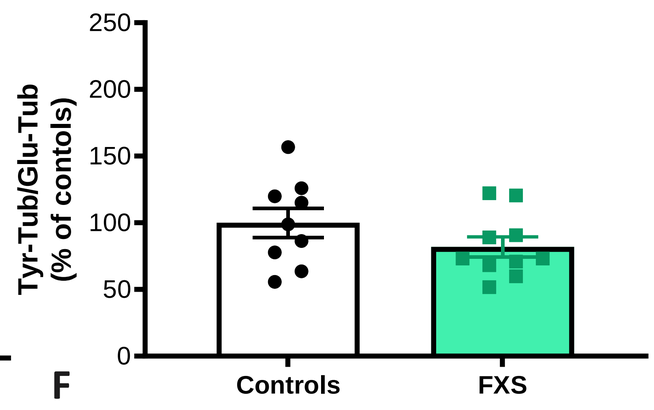
α-Tub Post Translational modifications (PTMs) in adults



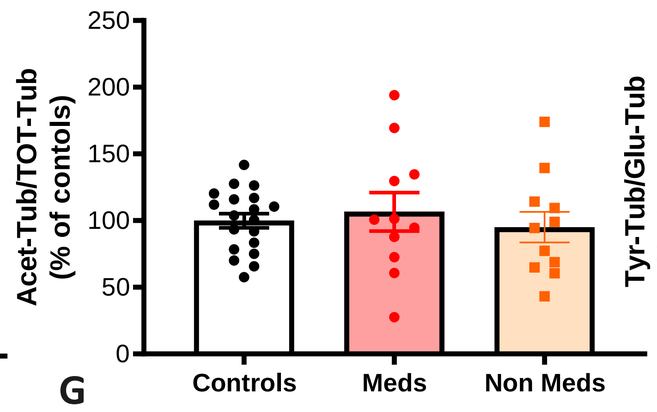


α-Tub Post Translational modifications (PTMs) in children





α-Tub Post Translational modifications (PTMs) in patients on medications



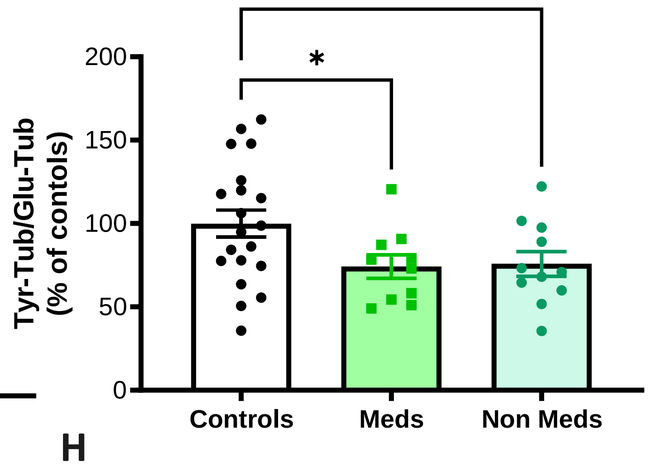


Figure1. Dysregulation of alpha-tubulin PTMS in plasma samples of FXS patients compared to controls

(A) Acetylated/Tot-α-Tub is not different between FXS Adults and Children compared to Controls. **(B)** Tyrosinated/Detyrosinated α-Tub is significantly decreased in *FXS* compared to *Controls* potentially indicating less dynamic MT. (**C)** Acetylated/Tot-α-Tub is non significantly decreased in FXS adults compared to Controls. **(D)** Tyrosinated/Detyrosinated α-Tub is significantly decreased in *FXS* compared to *Controls* potentially indicating less dynamic MT. (**E)** Acetylated/Tot-α-Tub is non significantly increased in *FXS children* compared to *Controls*. (**F)** Tyrosinated/Detyrosinated α-Tub is non significantly decreased in *FXS* compared to *Controls* potentially indicating less dynamic MT. (**G)** Acetylated/Tot-α-Tub is non significantly increased in *Patients on Medications* compared to *Controls*, indicating a potential effect of medications on Acetylated α-tubulin. (**H)** Tyrosinated/Detyrosinated α-Tub is significantly decreased both in *Patients on Medications* and non compared to *Controls*, indicating that those medications might not have an effect on Tyrosinated α-tubulin.
Data expressed as mean ± SEM. Analysed using unpaired t-tests and Mann-Whitney tests .N=8-11 per group. Outliers excluded based on +/- 2 StDev of group mean.*p<0.05 vs. healthy controls.

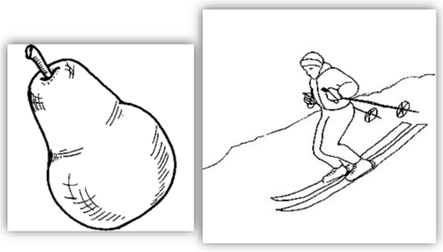
Study 2. Can early signs of cognitive decline be detected in Fragile X Premutation Carriers (FXPCs) through analysis of language abilities and plasma protein alterations?

A further study, currently targeting Fragile X Premutation Carriers with the objective of including their FXS children in the near future, focuses on the development of a composite biomarker, exploring different aspects including analysis of language abilities and plasma protein alterations.

Methods

We are recruiting 25 FXPCs and 25 healthy controls, with the objective of including their FXS children in the near future, with the aim of combining analysis of language abilities to other measures to develop a non-invasive composite biomarkers:

(A)



(B)

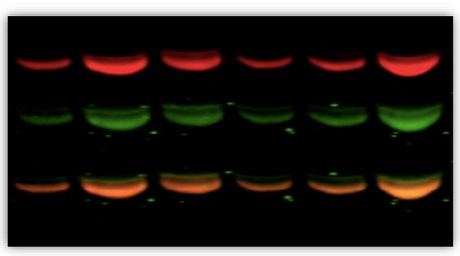
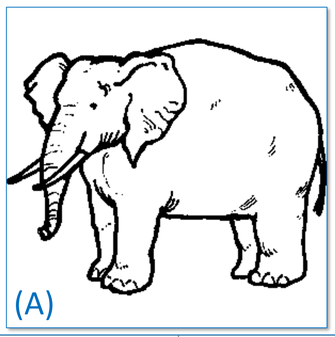


Figure5. Representation of the two measures.
(A) Language abilities (psycholinguistic tasks): The psycholinguistics tasks we designed aim at investigating if word retrieving difficulties can be a potential sign of cognitive decline [3].
(B) Plasma protein alterations: The aim is to explore if alterations in plasma-based proteins related to brain development and function can indicate abnormal process which contribute to disease progression linked to a mutation on the *FMR1* gene [4]. We will compare these results with results of FXS patients in study 1.

Language abilities (psycholinguistics task)



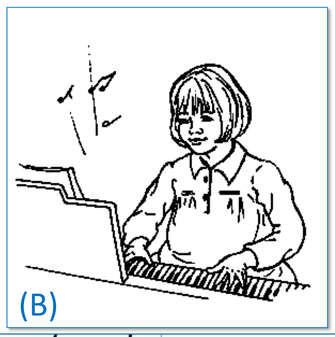


Figure 6. Picture naming task
(A) objects, (B) actions.
Espressive language task with a voice response
(A) «elephant», **(B)** «play» [5].

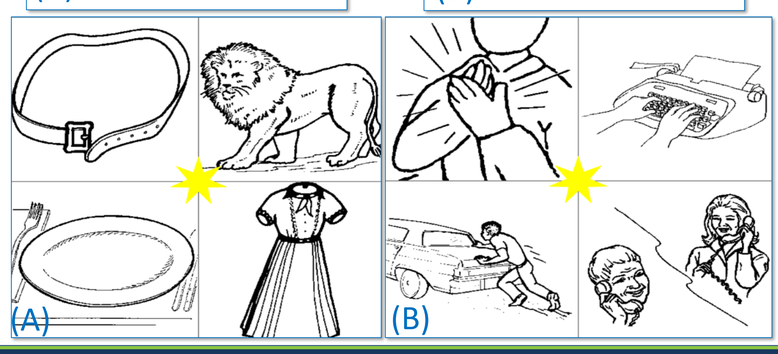


Figure 7. Visual Word Paradigm
(A) objects
(B) actions.
Receptive language task.
Auditory stimulus
(A)«lion», **(B)** «push».
Response
(A)«click on image of lion»,
(B)«click on image push»

The results are expected to provide the foundations for future studies aimed at early intervention for Fragile X Premutation Carriers.
Additionally, we believe it can provide potential future applications for the full mutation to research new treatments and interventions for Fragile X Syndrome.
Scan the QR code for more information.

References

[1] Monday, H. R., Kharod, S. C., Yoon, Y. J., Singer, R. H., & Castillo, P. E. (2022). Presynaptic FMRP and local protein synthesis support structural and functional plasticity of glutamatergic axon terminals. *Neuron*, *110*(16), 2588–2606.e6.
[2] Macoir, J., A. Lafay, and C. Hudon, *Reduced Lexical Access to Verbs in Individuals With Subjective Cognitive Decline.* Am J Alzheimers Dis Other Demen, 2019. **34**(1): p. 5-15.
[3] Bredin-Oja, S.L., et al., *Word retrieval difficulty in adult females with the FMR1 premutation: Changes over time and across contexts.* Brain Cogn, 2021. **148**: p. 105694.
[4] Barbiero, I., M. Bianchi, and C. Kilstrup-Nielsen, *Therapeutic potential of pregnenolone and pregnenolone methyl ether on depressive and CDKL5 deficiency disorders: Focus on microtubule targeting.* J Neuroendocrinol, 2022. **34**(2): p. e13033.
[5] Szekely, A., D'Amico, S., Devescovi, A., Federmeier, K., Herron, D., Iyer, G., Jacobsen, T., Arévalo, A. L., Vargha, A., & Bates, E. (2005). Timed action and object naming. *Cortex; a journal devoted to the study of the nervous system and behavior*, *41*(1), 7–25