#ThinkRare – Biomarker analysis to bridge preclinical and clinical research in rare neurodevelopmental disorders like CDKL5 Deficiency Disorder and Fragile X Syndrome

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ULYSSES NEUROSCIENCE

Introduction

Neurodevelopmental disorders (NDDs) can be rare and ultra-rare conditions characterised by severe intellectual and physical disabilities impacting the daily life of patients, their caregivers and their immediate families. Due to the rarity of some of these disorders, they have not received sufficient attention in terms of treatment and drug discovery, despite the fact that they usually have very clear genetic and molecular underpinnings.

Our **#ThinkRare** research currently focuses on the pathogenesis and treatment of the rare genetic NDDs **CDKL5 Deficiency Disorder (CDD)** and **Fragile X Syndrome (FXS)**, both of which are due to mutations of specific genes located on the X chromosome namely *Cdkl5* and *Fmr1*, respectively. CDD is an ultra-rare and highly debilitating NDD associated with **early-onset seizures and severe global developmental delay**. FXS is also associated with **developmental delay** along with a psychological profile that includes **autism-like traits and anxiety**.

#ThinkRare prioritises the concept of **patient-centricity** in order to better bridge the translational gaps between the day-to-day experience of the individual, the clinic and the preclinical research that underlies drug discovery efforts for CDD and FXS. Patients, caregivers and associations **actively participate in the design of our preclinical and clinical research** with the aim to improve the translational aspect of our projects. Understanding the patient needs and the community's specific profile allows for **greater integration of the patients into research**.

The **#ThinkRare** team is exploring central and peripheral biomarkers including microtubule proteins (i.e., alpha-tubulin post-translational modifications (PTMs)) and inflammatory molecules (i.e., a panel of cytokines /chemokines). Alpha-tubulin PTMs drive microtubule dynamics which is required for synaptic plasticity in neurons. Such peripheral biomarkers can be easily translated between preclinical and clinical research spaces, with centrally-derived biomarkers from animal models providing an insight into key pathological processes, to help understand these disorders and to determine novel and effective therapeutic targets.

Methods:

- *Cdkl5* and *Fmr1* knock-out (KO) mice were taken from our established colonies at Ulysses Neuroscience Ltd. *Fmr1* KO mice underwent behavioural testing using the marble burying test and the 3-chamber sociability test. All mice were euthanised, and plasma and brains were sampled for molecular analysis.
- Plasma from CDD patients (n=11; ages 6-27) along with matched control volunteers (n=9; ages 27-49) was collected in the US and Italy (CDD). Plasma from FXS patients (n=11; ages 5-12) along with matched control volunteers (n=11; ages 4-12) was collected in Argentina and Chile.
- Alpha-tubulin PTMs were measured in plasma and in brain tissue using InfraRed Western Blotting (Odyssey CLx, Li-Cor).

Preclinical Results

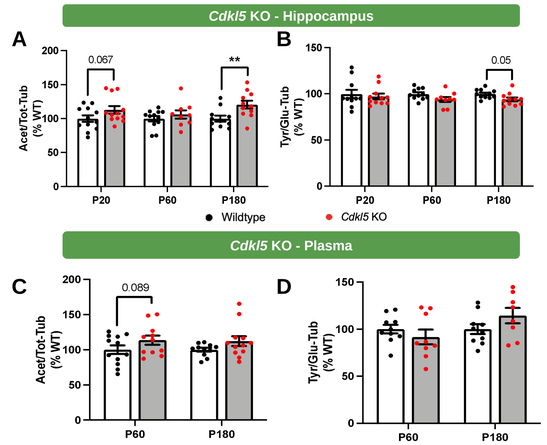

Figure 1. Alpha-tubulin PTMs as a biomarker of CDD in a mouse model. In the hippocampus of *Cdkl5* KO mice, there are significantly **(A)** higher levels of acetylated/total alpha-tubulin (Acet/Tot-Tub), and **(B)** decreased levels of tyrosinated/detyrosinated alpha-tubulin (Tyr/Gly-Tub). In plasma, **(C)** Acet/Tot-Tub trends towards an increase in *Cdkl5* KO mice. **(D)** Plasma Tyr/Glu-Tub did not show a difference between groups. ** $p < 0.01$ vs. WT control.

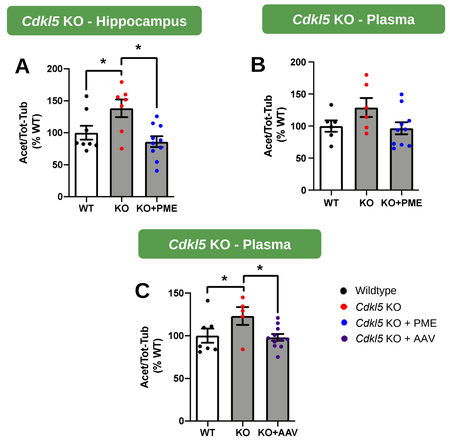

Figure 2. Therapeutic engagement confirmed using alpha-tubulin PTMs in *Cdkl5* KO mice. (A) Treatment of *Cdkl5* KO mice with pregnenolone-methyl-ether (PME) significantly rescued Acet/Tot-Tub to WT levels in the hippocampus. **(B)** In plasma, this effect for PME followed the same trend but did not reach significance. **(C)** Delivery of an AAV designed to restore Cdkl5 protein in the brain significantly rescued Acet/Tot-Tub in the periphery. * $p < 0.05$ vs *Cdkl5* KO group.

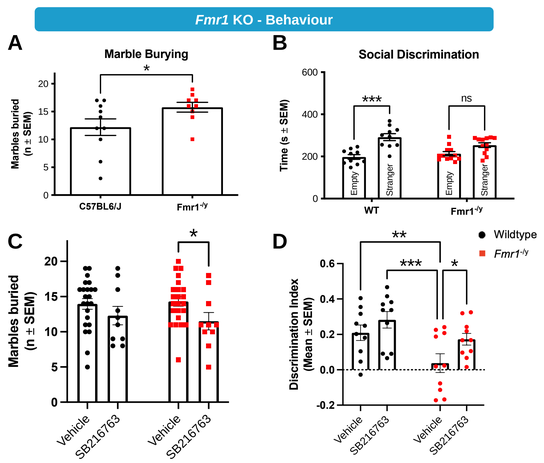

Figure 3. Behavioural alterations in *Fmr1* KO mice alleviated by pharmacological treatment. *Fmr1* KO mice show **(A)** increased marble burying and **(B)** reduced preference for a stranger in the 3-chamber sociability test. Chronic treatment with the GSK-3β inhibitor SB216763 **(C)** significantly reduced marble burying and **(D)** significantly increased levels of social interaction.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. WT control.

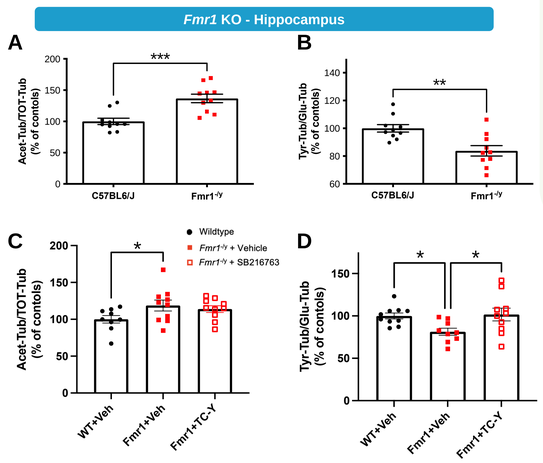

Figure 4. Alpha-tubulin as a biomarker of drug engagement in *Fmr1* KO mice. *Fmr1* KO mice show significant **(A)** increases in Acet/Tot-Tub and **(B)** decreases in Tyr/Glu-Tub in hippocampus. Treatment with a protein kinase inhibitor (TC-Y) had **(C)** no effect on Acet/Tot-Tub but **(D)** rescued Tyr/Glu-Tub. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. WT control.

Clinical Results

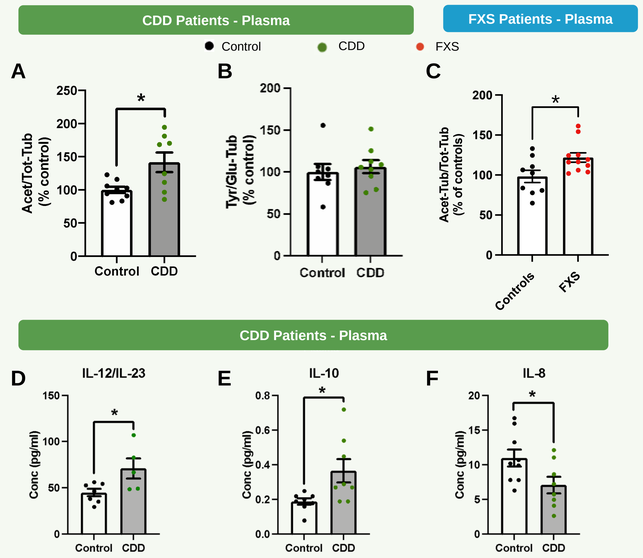

Figure 5. In patients with a NDD, alpha-tubulin PTMs are altered in a similar manner to mouse models. Plasma from patients with CDD and FXS were compared to matched controls on measures of alpha-tubulin PTMs and, for CDD only, inflammatory markers. **(A)** Acet/Tot-Tub is significantly higher in CDD patients compared to matched control levels. **(B)** There was no difference observed in Tyr/Glu-Tub. **(C)** FXS patients show a similar increase in Acet/Tot-Tub as seen in patients with CDD. CDD patients showed a consistent shift in cytokines with significant increases in **(D)** IL-12/IL-23 and **(E)** IL-10, and **(F)** a significant decrease in IL-8. * $p < 0.05$ vs matched controls.

- These results suggest that **microtubule dynamics can be used as a translational biomarker** in NDDs such as CDD and FXS.
- CDD and FXS are associated with reductions in synaptic plasticity, supporting the validity of using alpha-tubulin PTMs as indicators of synaptic integrity.
- In mouse models of CDD and FXS, these **disruptions in microtubule dynamics respond to pharmacological and genetic interventions.**
- Finally, the translational value of alpha-tubulin PTMs is clear given that they can be **detected not just in the brain but also in plasma**. This allows **direct comparison of molecular and functional changes** in rodent models with patients.