The Fragile X Messenger Ribonucleoprotein 1 Regulates the Morphology and Maturation of Human and Rat Oligodendrocytes

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Presenter: Wei-Hsiang Hsu **Date/Time**: 2025/09/18, 16:20-17:10
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Background: Fragile X Syndrome is the most common cause after Down syndrome for intellectual disability, affecting 1 in 4000 males and 1 in 5-8000 females globally. These patients lack FMRP, an RNA-binding protein that targets hundreds of transcripts within the brain to regulate their translation. As a result, CNS neuroglia are delayed to mature or to support neuron activity in the postnatal stage of development. In the pursuit of new drugs that aim to restore FMRP, more research is needed on the human model wherein the *Fmr1*-KO mouse model was insufficient, for FXS in humans is caused by (1) abnormal 5'-UTR CGG expansion-induced promoter methylation and silencing and (2) alternative splicing that creates non-functional isoforms of FMRP, but hardly (<1%) by deletions, point mutations or other non-trinucleotide repeat mutations.

Objective: With the advent of stem cell technology, study the human oligodendrocytes derived from clinical biobank samples, parallel to traditional gene-knockout models.

Results: With HITS-CLIP data that sequenced FMRP co-precipitated targets, they identified the target transcriptome in the oligodendrocyte lineage cells as enriched in synaptic assembly and cytoskeletal-microtubule organization functions. They analyzed *Pak1*, *Tppp* and 5 other targets by qRT-PCR on *Fmr1*-KO postnatal rat oligodendrocyte cultures and found *Tppp* with reduced relative expression, which manifested in their less mature oligodendrocytic morphology – less and shorter branches from the cell body. They found a decrease in MBP^+ cells, although not in $O4^+$ cells. As for the OPCs, they did not find a difference in counts of either homeostatic or proliferating cells. Human oligodendrocyte cultures were made using OPC derived from glial spheres expanded from human ESCs (control vs *FMR1*-KO) and iPSCs, which were lung fibroblasts from an affected case (aborted fetus) in Coriell Institute 1985, whose cord blood lymphocytes had been tested 9/50 in FUdR fragile-X screening. An isogenic iPSC control was generated by repairing its *FMR1* gene. In either pair, the KO or affected OL were less mature in their morphology, and *PAK1* transcript levels were reduced in $O4^+$ cells. In vivo, they traced individual myelinating oligodendrocytes and found *Fmr1*-KO rats had lower counts of myelin sheaths per cell, although their internodal length was normal. To answer whether this delayed growth was caused by OL itself or resulted from neuron-glia interactions, they grafted *FMR1*-KO human OPC into the neocortex of immunodeficient shiverer mice and still observed less myelin sheaths by area, even in a neighborhood of FMRP-complete neurons. Similarly by grafting *Fmr1*-KO rat OPC on an organotypic slice of shiverer mice, they counted less myelin sheath formation, concluding that FMRP-deficient effects in postnatal OL are cell-autonomous in nature.