Hyperactive GABAergic mutations and Rett syndrome

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Scientific Abstract:
Rett syndrome is a debilitating neurodevelopmental disorder affecting around 1:10,000 live births. While mutations in the gene encoding for on *Methyl-CpG-binding protein 2* (*MECP2*) account for the majority of Rett cases, recently, several non-*MECP2* gene mutations have been identified in Rett syndrome individuals with normal *MECP2*. Among these are two mutations in a gene encoding for an inhibitory G-protein coupled receptor (GPCR) in the brain, the $GABA_BR$ (*GABBR2*). $GABA_BRs$ are ubiquitous in the brain and when activated by the principal inhibitory neurotransmitter GABA, they control GABA and glutamate release from presynaptic terminals, and inhibit postsynaptic signaling by hyperpolarizing cells and suppressing Ca^{2+} channels. The mutations (A567T and A707T) are located in the transmembrane domains and they may affect the structural integrity of this GPCR and G- protein activation by $GABA_BRs$. This proposal will establish the significance of these mutations for the Rett phenotype by addressing the defects that result in $GABA_BR$ and neuronal signaling *in vivo* and *in vitro*.

The molecular properties of mutant $GABA_BRs$ will be characterized *in vitro* using molecular biology, flow cytometry, live-cell confocal and Ca^{2+} imaging along with electrophysiology. Preliminary results suggest that the mutations increase sensitivity of $GABA_BRs$ to GABA at low concentrations. This has important consequences because GABABRs are mainly localized to extrasynaptic areas where ambient GABA concentrations are low. As a result, the mutant receptors are likely to be overactive in these regions and thus suppress normal synaptic transmission. At least one of the mutations (A707T) express more efficiently on the cell surface, a property which could further exacerbate the over-activity of mutant receptors. The consequences of the mutations will be directly measured by recording inhibitory and excitatory postsynaptic currents in neurons expressing the mutant receptors. Assessing neuronal dendritic morphology and spine plasticity will also explore impact on excitatory neurotransmission. Finally, basal presynaptic release and dendritic Ca^{2+} transients will be measured by expressing a genetically-encoded Ca^{2+} sensor. The efficacy of $GABA_BR$ antagonists to reverse any deficits observed in many of the experiments outlined will be tested *in vitro*.

Having established the pharmacological profiles of the mutants *in vitro*, we will create a conditional knock-in mouse model for the A567T mutation to explore its impact *in vivo*. Behavioral phenotypes of motor activity will be studied using open-field tests and limb clasping will be used to assess characteristic stereotypes similar to mid-line hand-wringing. Synaptic dysfunction resulting from the mutations will be characterized using whole cell recordings from hippocampal and cerebellar slices- areas that are key for learning, cognition and motor co-ordination. Finally, $GABA_BR$ antagonists will be assessed for their efficacy in alleviating anomalous behavioral traits in the mouse model as a novel therapeutic agent for treatment of Rett syndrome.

Our study will therefore address previously unexplored links between GABAergic neurotransmission and the Rett phenotype, involving the principal brain inhibitory GPCR. In generating a novel mouse model, we aim to find alternative therapeutic strategies for individuals with Rett syndrome who harbor these, and other, $GABA_BR$ mutations as well as Rett syndrome in general.