1. Abstract

Millions of Americans suffer from Autism Spectrum Disorder (ASD) or have affected family members producing tremendous human and economic impacts. The origins of ASD are complex with both environmental and genetic factors playing key roles. Recent advances in genome sequencing technologies have led to the discovery of dozens of mutations in NMDA receptor (NMDAR) subunits in patients with ASD. How alterations in these genes lead to the varied manifestations of ASD is a difficult problem to address, as is the challenge of developing effective therapeutics. Both will require multifaceted approaches. NMDARs are glutamate-gated ion channels that mediate excitatory neurotransmission in the brain. Many higher order neural processes including synaptogenesis and the synaptic plasticity underlining learning and memory depend on NMDAR-mediated transmission. Missense and nonsense mutations in NMDARs are not only associated with ASD but are found in individuals with a variety of neurodevelopmental disorders including epilepsy, intellectual disability, and schizophrenia.

Currently, we study NMDAR disease variants in mammalian cell culture (Wollmuth) and NMDAR functions in developmental neurogenesis using zebrafish (Sirotkin). The goal of this proposal is to develop zebrafish ASD models to tightly synergize our efforts and study the impacts of human ASD disease variants on neurogenesis and other developmental processes using zebrafish. This would address key critiques of recent collaborative grant submissions. We chose zebrafish for these studies because of the relative ease in analyzing very early neural development (which is a key time window for ASD and other neurodevelopmental disorders) and the unparalleled capabilities to preform high-throughput behaviorally based drug screens. To take our studies to the next level, we need to precisely manipulate NMDAR genes in zebrafish to generate fish models that harbor ASD-associated mutations. The recently described prime editing technique is the most promising approach to enable us to generate targeted single nucleotide mutations that would parallel the NMDAR disease variants we are studying *in vitro*. To date, precise mutations have been generated with prime editing in zebrafish by a single group (Petri et al 2021). To use this approach on a broader scale, additional optimizations are needed. Furthermore, demonstration of our ability to make these modifications is critical to our upcoming grant submissions on ASD-associated NMDAR variants. Lastly, because prime editing can be used to make a variety of precise genomic modifications advancement of these techniques would enhance the research of other SBU investigators and provide support for their future grant submissions.