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14. ABSTRACT

We will determine how ASD-associated mutations in *EEF1A2* impact protein synthesis and result in deficits in neuronal development and synaptic function in human neurons. Using human induced pluripotent stems cell (hiPSC)-derived neurons as a model, the CRISPR-Cas9 system will be utilized to recapitulate ASD-associated mutations in EEF1A2 observed in patients. The hiPSCs will then be differentiated into neurons using Neurogenin-2, a master transcription factor capable of inducing differentiation into excitatory neurons in under two weeks. Using this platform, the effect of ASD-associated mutations on neuronal function will be studied. First, we will determine the impact of three ASD-associated *EEF1A2* mutations on protein synthesis in neurons, given the central role that EEF1A2 plays in protein synthesis. Moreover, we will perform ribosome profiling to determine the translatome and measure the elongation rate and translational efficiency associated with each *EEF1A2* mutation. Then we will determine the impact of each *EEF1A2* mutation on neuronal development and morphology, and synaptic function. The results of these studies will advance our understanding of the role translation elongation plays in neuronal development, and how its dysregulation leads to ASD-associated pathophysiologies.

15. SUBJECT TERMS

autism spectrum disorder (ASD), translation elongation, human induced pluripotent stem cells (hiPSC)-derived neurons, eukaryotic elongation factor 1A2 (EEF1A2), neuronal development, neuronal morphology

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Introduction

Protein synthesis is a fundamental process in all living cells and is highly regulated to accommodate the specific needs of each cell type. Dysregulated protein synthesis has been demonstrated to underlie several syndromic forms of autism such as fragile X syndrome (FXS) and tuberous sclerosis complex (TSC), both of which result from defects in genes that regulate protein synthesis. Moreover, mouse models of FXS and TSC exhibit defective synaptic function and ASD-like behaviors. Recent studies have shown that Eukaryotic Elongation Factor 1A2 (EEF1A2), the translation elongation factor responsible for GTP-dependent transport of aminoacyl-tRNAs to the elongating ribosome, is mutated in patients with autism spectrum disorder (ASD), intellectual disability and epilepsy. Elongation factor 1A has two isoforms: EEF1A1 is ubiquitously expressed and EEF1A2 is expressed only in neurons and myocytes. It is unclear why another isoform is needed in these specific cells, but it has been shown that EEF1A2 is critical for neuronal survival. The *wasted* mouse, a mouse model with a homozygous deletion of *Eef1a2*, was shown to exhibit neuron degeneration, tremors, loss of muscle bulk, and gait abnormalities after weaning. EEF1A2 has been also shown to bundle actin and microtubules independently of translation, a process known to be critical for neuronal development and migration. Taken together, these findings suggest that EEF1A2 plays a critical role in neuronal development and function.

We will determine how ASD-associated mutations in *EEF1A2* impact protein synthesis and result in deficits in neuronal development and synaptic function in human neurons. Using human induced pluripotent stems cell (hiPSC)-derived neurons as a model, the CRISPR-Cas9 system will be utilized to recapitulate ASD-associated mutations in EEF1A2 observed in patients. The hiPSCs will then be differentiated into neurons using Neurogenin-2, a master transcription factor capable of inducing differentiation into excitatory neurons in under two weeks. Using this platform, the effect of ASD-associated mutations on neuronal function will be studied. First, we will determine the impact of three ASD-associated *EEF1A2* mutations on protein synthesis in neurons, given the central role that EEF1A2 plays in protein synthesis. Moreover, we will perform ribosome profiling to determine the translatome and measure the elongation rate and translational efficiency associated with each *EEF1A2* mutation. Then we will determine the impact of each *EEF1A2* mutation on neuronal development and morphology, and synaptic function. The results of these studies will advance our understanding of the role translation elongation plays in neuronal development, and how its dysregulation leads to ASD-associated pathophysiologies.

Key Words

autism spectrum disorder (ASD), protein synthesis, translation elongation, human induced pluripotent stem cells (hiPSC)-derived neurons, eukaryotic elongation factor 1A2 (EEF1A2), neuronal development, neuronal morphology, synaptic function

Accomplishments

Herein I will describe the research accomplishments associated with each task and subtask that was outlined in the approved Statement of Work.

Major goals of project

Major task 1 in the Statement of Work was to determine generate the *EEF1A2* mutant hiPSC lines. This was to be completed in years 1 and 2. Major task 2 in the Statement of Work was to determine whether ASD-associated mutations in *EEF1A2* alters protein synthesis in i^3Neurons. These experiments were to be completed in years 1-2. Major task 3 was to determine whether ASD-associated EEF1A2 mutations alter the neuronal morphology and synapse formation of i^3Ns. These experiments were to be completed in year 3.

Accomplishments under the major goals

For major task 1, the first subtask in the Statement of Work was to transfect hiPSCs with Cas9, guide and repair template and use flow to sort GFP-positive single cell clones*.* The second subtask 2 was to expand approximately 200 clones in 96 well plates test for the correct mutation using restriction length polymorphism detection. The third subtask was to confirm mutation with Sanger sequencing and assess for any off target edits.

We have completed all three subtasks in major task 1 for the G70S mutation and the E122K mutations, and plan to complete this for the D252H subtask in the summer of 2022.

For major task 2, the first subtask was to perform SUnSET assays in i^3Neurons that contain each of three mutant *EEF1A2* mutants. The second subtask was to perform FUNCAT assays in i^3Neurons that contain each of three mutant *EEF1A2* mutants. The third subtask was to perform ribosome profiling and RNA-seq experiments from i^3Neurons that contain each of three mutant *EEF1A2* mutants. The fourth subtask was to analyze the raw RNA sequencing results for gene ontology using DAVID and subjected to Ingenuity pathway analysis.

We have already begun performing SUnSET and FUNCAT assays for the G70S and E122K mutations, and our preliminary data indicate that these ASD-associated *EEF1A2* mutations reduced de novo protein synthesis. We have begun the ribosome profiling and RNAseq experiments for these two mutations and should have the sequencing results by the summer of 2022. We also plan to conduct and complete the SUnSET and FUNCAT assays, and the ribosome profiling and RNA-seq experiments for the D252H mutant in year 2.

For major task 3 (to completed in years 2 and 3), the first subtask is to perform Scholl analysis and measure soma size, as well spine density and number of dendritic branches, of i^3Neurons with the three *EEF1A2* mutations using confocal microscopy. The second subtask is to perform live cell imaging and conduct neuronal outgrowth assays of i^3Neurons with the three *EEF1A2* mutations. The third subtask is to perform whole-cell electrophysiology experiments and calcium imaging studies with i^3Neurons containing the three EEF1A2 mutations to measure intrinsic properties, synaptic function, and calcium transients.

We plan to begin the Scholl analysis, live imaging and electrophysiology experiments for the G70S and E122K mutants in year 2, and the D252H mutants in year 3.

Summary of accomplishments

- Successfully generated of G70S and E122K mutants (both heterozygous and homozygous). Generation of D252H mutants should be completed in this summer.

- Preliminary assays indicate that de novo protein synthesis is reduced with heterozygous G70S and E122K *EEF1A2* mutations.

Opportunities for training and professional development

Nothing to report.

Dissemination of results to communities of interest

Nothing to report.

Plan on what to do during next reporting period to accomplish the goals
We will continue to conduct the experiments as outlined in the statement of work. We have made good progress toward accomplishing our goals in the first year of work and foresee no problems as we continue on in years 2 and 3.

Impact

The most common ASD-associated mutations in *EEF1A2* are G70S, E122K, and D252H. Notably, these mutations occur in or near coding regions for different functional domains of EEF1A2: G70S is in the GTPase domain, E122K is near the tRNA-binding domain, and D252H is near the actin-binding domain. The location of the D252H mutation is particularly interesting because it may affect one of the non-canonical functions of EEF1A2. EEF1A2 has a been shown to regulate cellular filopodia via tubulin and actin bundling, processes critical for neuronal migration and synapse formation. Thus, we hypothesize that the *EEF1A2* mutations may not only affect translation but also neuronal cytoskeletal regulation. However, there have been no studies exploring the consequences of *EEF1A2* mutations in neurons. Therefore, we decided to model this form of ASD in neurons derived from human induced pluripotent stem cells (hiPSCs). hiPSCs are a powerful tool to model ASD and screen therapeutics that can be readily used to study neurodevelopmental disorders in other model systems. Paired with the advent of CRISPR-Cas9 technology, the potential to model any genetic disorder is endless. We have generated multiple hiPSC cell lines expressing an inducible neurogenin-2 (NGN-2) system, where hiPSCs can be differentiated into functional neurons in under 2 weeks. These neurons are largely cortical glutamatergic neurons, form mature synapses and can incorporate into existing neural networks when transplanted into a mouse brain. We already have introduced two of the three ASD-associated EEF1A2 mutations into hiPSC-NGN2 lines via CRISPR-Cas9 and have begun to study their impact protein synthesis, neuronal morphology, and synaptic function. These studies will be the first to comprehensively study the impact of three different *EEF1A2* mutations in human neurons.

Changes/Problems

We foresee no changes in approach in years 2 and 3. We do not anticipate either problems or delays. There will be no changes that impact expenditures.

Products

There have been no publications from the Klann lab directly based on this work thus far.

The preliminary data generated in the first year of this work will be presented at the Gordon Research Conference entitled "Fragile X and Autism-related Disorders: Novel Technologies to Advance Discovery of Disease Mechanisms and Therapeutics for Fragile X and Autism", Lucca (Barga), Italy

Participants & Other Collaborating Organizations

Name: Eric Klann
Project role: Principal Investigator
Person months worked: 1.2 cal mos
Contribution to project: Design and supervise experiments, interpret data.

Name: Muhaned Mohamed
Project role: Graduate Student
Person months worked: 12 cal mos (Mr. Mohamed's salary is paid for by a fellowship).
Contribution to project: Generate ASD-associated *EEF1A2* mutants, design and perform de novo protein synthesis experiments, analyze data.

Name: Vaishnavi Shankar
Project Role: Postdoctoral Fellow
Person months worked: 5 cal mos
Contribution to project: Design and perform ribosome profiling and RNA-seq experiments, analyze data.

Name: Ela Golhan
Project Role: Research Technician
Person months worked: 4 cal mos
Contribution to project: Order supplies and chemicals, maintain inventory, analyze data.

Name: Jessica Alapin
Project Role: Postdoctoral Fellow
Person months worked: 4 cal mos
Contribution to Project: Design and perform neuronal morphology experiments, analyze data