Genetic Variability in ZIP12, a Candidate Gene for Neurological Disorders

Introduction:

ZIP12, encoded by the gene slc39a12, is a protein that transports zinc into cells of the nervous system. A previous study demonstrated that removal of this gene from frog (*Xenopus tropicalis*) embryos leads to neural tube defects and inhibition of this gene impaired development in mouse neurons [1]. However, no previous studies have examined whether human genetic variation in ZIP12 may reduce its function. The goal of this study is to determine if these specific polymorphisms in ZIP12 that are present in the human population actually lowers its ability to transport zinc. This information may show whether individuals with these specific polymorphisms could have predispositions to neurodevelopmental disorders or degenerative brain diseases like Alzheimer's disease or dementia.

Experimental approach:

I will introduce different variants of human ZIP12 into cells and measure their ability to transport zinc in order to determine whether some variants have lower zinc transport ability. I will use the bacteria E.coli. to grow plasmid DNA with 21 specific polymorphisms of ZIP12 which changes a single amino acid in the protein. This portion of the work has already been begun. Next, I will transfer the plasmid DNA to Chinese hamster ovary cells using a process called transfection. Once the plasmid DNA has been transferred to the cells, they will start producing the normal version of human ZIP12 (control) or different polymorphic variants (experimental). In order to measure zinc in the cells, the zinc in the cells will be dyed with a fluorescent dye called Zinpyr-1. I will use a microplate reader to measure the Zinpyr-1 fluorescence of each sample and determine which variants transported the least amount of zinc compared to the control (normal version of ZIP12). Cells that fluoresce the least would have polymorphisms that were negatively affecting the transport of zinc and indicate that these polymorphisms do not function well.

Expected Results:

This experiment will identify which of the 21 polymorphisms have the lowest function through the measurement of dyed zinc. The polymorphisms with the lowest function should transport the least zinc and the polymorphisms with the highest functionality should transport the most zinc.

Timeline:

In the first month, I will produce enough of the plasmid DNA to transfect the cells, learn how to culture cells, and use the plate reader. In the next three months, I will learn how to transfect the cells and stain the cells with Zinpyr-1 to measure zinc transport. This experiment should be completed in one semester.

Literature Cited:

[1] Chowanadisai W, Graham DM, Keen CL, Rucker RB, Messerli MA. Neurulation and neurite extension require the zinc transporter ZIP12 (slc39a12). Proc Natl Acad Sci U S A. 2013 Jun 11;110(24):9903-8.