

## INTERIM PROGRESS REPORT

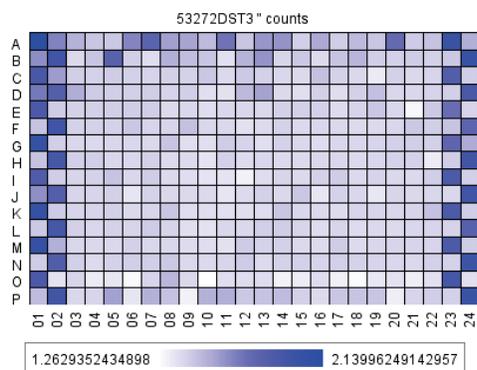
This is a summary of work performed during the 6-month period July 1 - December 31, 2013

### **Specific Aim 1: To determine the functional and biochemical consequences of *ATP1A3* mutations associated with AHC.**

We have nearly completed the biochemical assessments of three *ATP1A3* mutations (D801N, E815K, G947R). Our data demonstrate convincingly that two of these mutations (E815K, G947R) have severely impaired trafficking such that the *ATP1A3* protein does not reach the cell surface where it is needed to function. This defect in trafficking to the cell surface contributes to a loss-of-function of these two mutations. The other mutation (D801N) only has a mild or moderate trafficking impairment and therefore we conclude that other functional defects are caused by this allele.

### **Specific Aim 2: To identify drugs or drug-like small molecules that can rescue the functional and biochemical defects caused by *ATP1A3* mutations.**

We completed the optimization of a fluorescence thalium uptake assay in cells stably expressing *ATP1A3* mutants as a platform for performing drug screens. We designed the assay with results of Specific Aim 1 in mind. Specifically, we have designed the assay to allow us to test compounds for their ability to 'rescue' trafficking defects in mutant *ATP1A3* and restore activity. Mutation E815K gave the largest 'signal' in our assay optimization trials and therefore we chose this mutation for the pilot screening phase. Recently, we completed the first pilot screen of a collection of 729 drugs and drug-like molecules provided by the NIH. We observed that 37 of 729 (5%) of the compounds exhibited measurable activity (Fig. 1). Soon we will complete the remainder of the planned screening experiments when we examine the Spectrum collection of ~2,000 approved drugs and related compounds. Following these initial screens, we plan to perform a validation assay on all 'hits' in both E815K and G947R cell lines.



**Figure 1** – Pattern of fluorescence intensities across 384-wells containing the E815K cell line exposed to different compounds from the NIH library. The two edge columns are control wells. Darker purple indicates higher *ATP1A3* activity.

### **Specific Aim 3: To elucidate electrophysiological effects of *ATP1A3* mutations using iPS cell-derived neurons from AHC patients.**

Over the past year the Ess laboratory has generated dozens of iPS cell lines from fibroblasts obtained from patients AHC who have a well-characterized clinical course. We are completing the validation steps required to ensure that we have produced high quality stem cells that are capable of making all cell types as well as ensuring no additional genetic changes were introduced during the reprogramming process. We chose to focus on the three most common mutations seen in AHC as this

is expected to most rapidly lead to the identification of new therapies.

While validation studies are continuing for certain lines, we are already using cell lines that have passed validation tests and are transforming them into neuronal cells. While we have made progress

in making neurons from AHC patient-derived stem cells, we need to continue optimizing robust and reproducible methods for making specific types of neurons, for example inhibitory GABA expressing neurons. The next several months will be devoted to generating AHC neurons and enabling electrophysiological experiments described in Aim 2. We will also optimize cryopreservation methods so we can freeze and ship the AHC neurons to Chicago where the George lab will be after March 1, 2014.

## LAY SUMMARY

We have strong evidence that among the three most common ATP1A3 mutations associated with AHC, two of them (E815K, G947R) cause a severe defect in the ability of the ATP1A3 protein to reach the cell membrane where it's needed to function as an ion pump. Based on these findings, we have optimized a drug-screening assay to identify drugs and drug-like chemical compounds that are capable of 'rescuing' this defect. We are in the process of screening ~2,800 compounds including all FDA-approved drugs and other drug-like compounds that have been given safely to humans. Preliminary results suggest that up to 5% of the tested compounds may have some activity, but other steps will follow to validate these findings.

We have made considerable progress in developing a cell model of AHC using patient-derived stem cells that can be converted to nerve cells in the laboratory. The main effort now is devoted to perfecting the process of converting to nerve cells and this will be followed by studies to examine the electrical properties of the cells. We are also planning to test the effect of any compounds identified by the drug-screen on the activity of ATP1A3 in the AHC nerve cells.

Thank you for your generous support of this research. Please let us know if you have any questions or concerns about these plans or our progress.

Sincerely,



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