Optimizing Precision Treatment Targeting for Genetic Epilepsy

Final Progress Report (12 month)

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Public Statement

Hypothesis and Specific Aims: Discovering the genetic cause of an epilepsy paves the way for development of precision therapies that directly target the affected gene or protein. This gives hope for more effective, side effect-free treatments. However, the vast majority of these are still in development, and more work is needed to deliver them in the most effective way. Variants in the KCNT1 gene cause severe childhood epilepsies. At the molecular level, they cause the potassium channel it encodes to be overactive. New drugs and gene therapies have been developed to inhibit the channel and show promise in mouse models, but not all KCNT1-expressing cells contribute to disease. Identifying which cells to treat for maximum benefit is critical because treating the wrong cells can needlessly disrupt brain activity, lead to side effects, and undermine treatment goals. Using a mouse model of KCNT1 epilepsy, **we are testing whether therapies can be improved by restricting them to severely affected cells and brain areas.**

Previous work in our lab indicates that an epilepsy-causing KCNT1 variant most severely impacts cells called inhibitory interneurons. **The first aim of this study is to test whether treating *only* these cells improves outcomes by suppressing seizures and other abnormal activity without otherwise disrupting brain function.** To do this, we will use a gene therapy agent to treat either inhibitory interneurons alone, or all neurons, and measure how well each strategy controls seizures and normalizes brain activity. We also know that a few areas in a brain structure called the cortex predictably generate epileptic activity. **The second aim of this study is to determine whether restricting a drug treatment to the most severely affected area can improve outcomes in the same way**. We will treat either the whole mouse or just the most severely affected cortical area with a KCNT1 channel-blocking drug and compare outcomes to learn which is better at restoring cortical activity to the 'normal' range.

Results: This study builds on our progress in understanding basic disease biology to address a critical next step in improving therapies: targeting them to the right place. Over the past year, we made significant progress toward achieving both aims described above. The most significant, and surprising, result we obtained over the past year came from our experiments addressing Aim 2. We discovered that a specific pharmacological inhibitor of the KCNT1 channel (Prax-2904) that was previously reported to reduce seizures and interictal spikes in a mouse model of *Kcnt1*-related epilepsy (P905L) was unable to suppress epileptic activity in our mouse model (Y796H) when infused directly into the brain region where seizures originate, and at a concentration well above the free brain concentrations achieved in the other study. This is despite seeing a strong drug effect on individual brain cells in-vitro.

For the first aim we encountered delays that put us behind schedule, but still made progress. We are happy to report that we have finally received the viral shRNA construct this award allowed us to design and manufacture. We are currently conducting experiments to ensure that the construct is working as intended to reduce levels of KCNT1. Once the construct is validated we will begin testing on our mouse model as planned. This work will be funded by existing sources in the Weston lab at Virginia Tech.

Potential Impact: Our finding on the lack of efficacy of the drug raises important questions and concerns about predicting therapeutic utility based on drug studies using individual cells and specific animal models. It is critical moving forward to investigate how the drug impacts the electrical activity of nerve cells in the intact brain to understand where the discrepancy between in vitro and in vivo effects arises from. We are currently testing two additional KCNT1 inhibitors, one from a repurposing screen and the other from a pharmaceutical company. Comparing this data to our data from Prax-2904 will allow us to better assess whether inhibition of KCNT1 activity after disease onset is able to treat seizures. If these compounds also show limited efficacy, we will treat younger animals, as we suspect that epilepsy in older animals may become uncoupled from direct KCNT1 gain of function.