

Moser Center for Leukodystrophies

LBSL Research Update for Patients and Families

September 2019

Overview

Since our last official update in October 2018, the LBSL research effort at Kennedy Krieger Institute's Moser Center for Leukodystrophies has grown in both size and scope. Now in its third year, our translational LBSL research program includes 17 researchers at Kennedy Krieger and The Johns Hopkins University, and encompasses both clinical and basic research in LBSL (leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation).

Our goal is to bring together highly complementary expertise from basic molecular methods, cell (and stem cell) culture, animal research, clinical outcome measure research, and machine learning methodologies through to clinical trial design.

We have been fortunate to receive support from the LBSL community, the National Institutes of Health, and other internal sources to establish, to the best of our knowledge, the most comprehensive LBSL research effort in the world.

Development of Animal Models of LBSL

As you may remember, our team spent the first two years developing an LBSL mouse model using transgenic technology. We discovered that in most scenarios, "knocking out" or eliminating the DARS2 gene in mice was not compatible with life.

After many attempts, we created a viable test model by genetically engineering mice that lack the DARS2 gene in mature nerve cells in their brains. Known as the CAMKII mice, these animals do not present like human patients with LBSL, as there is no clear motor impairment and no signs of damage to the spinal cord. Their most notable symptom is their slowly progressive hyperaggressive behavior. Along with their increased activity, they experience significant neuroinflammation and loss of neurons, and severe brain shrinkage over time, and thus serve as a model of DARS2 deficiency.

We also generated genetically modified mice that lack the DARS2 gene in oligodendrocytes, the cells that produce myelin. However, this is technically more difficult to do, and we cannot completely eliminate the gene in all myelin-producing cells. With these limitations in mind, and as far as we can tell, there is no consistently clear deficit to the brain's white matter or myelin sheath in these mice. This goes along with a standing hypothesis proposed by Professor Marjo van der Knaap, who discovered LBSL. She hypothesized that based on the pattern of the MRI findings in patients, the DARS2 gene mutation may affect nerve cells more than myelin-producing cells. We are still exploring if this is indeed the case by isolating myelin-producing cells in the dish and studying them further.

In terms of drug testing, our team has utilized the CAMKII mice to test two drug therapies:

- The first was a nanotherapy drug called Dendrimer-NAC. These tests occurred between 2017 and 2018 with no success. Although the drug is a powerful antioxidant, it does not appear to generate any meaningful changes in the mice's brains or behavior.

- The second drug is an ISR inhibitor, which inhibits the so-called integrated stress response (ISR). This is a molecular response in cells when they are exposed to stress. We have found that this pathway is overactive in LBSL mice and it is not clear whether this is a protective or harmful response. We have therefore begun testing the response of the ISR inhibitor drug in CAMKII mice. These tests began in December 2018, and studies are ongoing. So far, we have not seen any noticeable behavioral changes in the mice. However, we won't know conclusively if this drug has any benefits until early-to-mid-2020, when we are able to complete all the needed analyses.

Additionally, by performing a type of advanced genetic sequencing called RNA-seq, we have found hundreds of other molecular changes in the CAMKII mice. Most of these changes appear to be in the inflammatory processes in the brain and may be potential targets for drugs. However, we need to investigate further before drawing any conclusions. We also need to see if we can detect similar changes in the LBSL mini-brains (see below).

Over the next year, we hope to conduct additional RNA-seq to answer two key questions:

1. Is oxidative stress an important factor in LBSL?
2. Is inflammation a key player, as it appears in the initial RNA-seq data?

Stem Cell Work to Model LBSL in the Dish

While animal models are needed to test therapeutics, they do not carry the exact genetic changes found in humans. Therefore, replicating a patient's own brain cells in the lab allows us to better understand the exact biochemical mechanisms involved in LBSL. Thanks to a collaboration with Cedars-Sinai Medical Center in Los Angeles, we are now capable of making stem cells from a patient's own blood. Using a protocol developed by Dr. Mingyao Ying, an investigator at Kennedy Krieger, we can engineer these human, LBSL-specific stem cells into neuronal cells. Now that we have these engineered neurons from patients in a dish, we can monitor their development, and using a machine to study their electrical activity, we can see how LBSL neurons "fire," how they recover from stress, and how fast or slowly these cells grow processes in the dish. So far, we have studied neurons that were originally derived from our patient Ellie, and we have found that Ellie's cells appear to grow processes more slowly and fire less frequently, compared to the cells of a healthy age-matched person.

Also, we have been working with a collaborator at Case Western Reserve University in Cleveland, Ohio, to grow our patient blood-derived stem cells into 3D cultures. Over a period of more than 150 days, these 3D cultures develop different cell types and brain regions, aka "mini brains" or organoids. Since our last update, we have successfully developed one set of LBSL mini-brains and one set of "healthy" control mini-brains. Our initial observations point to notable differences in the size and shape of the mini-brains. As these mini-brains are quite small and take several months to grow, future work with them will be driven by our findings on the neurons. Then, we can use our findings to guide experiments involving mini-brains derived from the cells of additional patients.

While we are intrigued and excited by these observations, a one cell-to-one cell comparison does not provide enough data to draw firm conclusions. With the help of major donors, we have now started making stem cells and neurons from other patients with LBSL and from control subjects, and will be studying them in the dish over the next 12 to 18 months to see if we make the same observations. If all LBSL neurons behave abnormally, then we can expose the cells in the dish to many different drugs and determine if any drug or drug combination results in neuronal firing that grows more quickly and is more rapid.

Understanding the Consequences of Gene Mutation in LBSL

With help from Dr. Stephen Fried, a very talented young professor of biophysics at The Johns Hopkins University, we also hope to determine conclusively if the DARS2 gene mutation results in the gene being less active, or whether it gains a different function. If the DARS2 gene mutation results in the gene being less active, we can treat the animals or cells with gene therapy—basically, infecting them with a virus that has a correct copy of the gene. However, if the patient mutations result in the gene working differently, then adding an additional copy of the gene will not help. Instead, we would have to silence or shut down the mutated gene. This can be achieved by a strategy called antisense oligonucleotide (ASO) therapy.

Both gene therapy using viruses and ASO therapy are already approved for use in treating another rare disease (spinal muscular atrophy) and are being tested for use in the treatment of a series of other rare diseases. Therefore, we think there is hope that once we finish these studies, we may be able to find a path toward clinical trials for LBSL. These gene function studies will need to be done first in cells and mice. Later on, larger safety studies in bigger animals will be needed. Typically, this is a process that takes up to two decades, but we hope we can get there within the next 10 years.

Clinical (Human) Research

Because LBSL is so rare, little is known about how the disease presents and progresses over time. In April 2018, our team launched a natural history study, gathering information from patients through the review of their medical records, including past neuroimaging studies, and through repeated virtual visits using wearable technology at the participants' homes, which occurs every six months over four years. To date, we have collected baseline information on 17 patients in North America, and we are in talks with a well-known European leukodystrophy expert to replicate and expand the study overseas.

Information from this study will help us better understand the relationship between the type of DARS2 mutation a patient has (as many of you know, there are variants carried by one parent and not the other) and the clinical signs and symptoms that they experience. This information will also help us identify the most common and most important symptoms that impact quality of life for patients with LBSL. In turn, that information will be used to develop clinical outcome measures required by the U.S. Food and Drug Administration for new treatments.

We have also developed new and highly advanced MRI techniques that allow us to see very small differences in the brain and spinal cord from one patient to another. By collecting data from LBSL patient scans and comparing it with other data points in the LBSL natural history study, we hope to find correlations between variants in the mutations, their impact on the body, and their connection to a patient's clinical signs and symptoms.

Summary

With our test models built and our human studies underway, the infrastructure to start answering pressing questions about LBSL is now in place. Once we have more data, hopefully by mid-2020, we plan to work with A Cure for Ellie, a foundation started by Ellie's parents, to initiate informal conversations with the FDA about the outcome measures we have developed, and determine which measures represent the best targets for potential drug therapies.

Our next LBSL conference is set for May 28–30, 2020, in Baltimore, Maryland. Our goal at the conference is to provide even more details and new updates on each of our efforts to cure LBSL. Registration information and details are forthcoming.