# Lafora Workshop

Sanford Consortium of Regenerative Medicine, University of California San Diego on June 12-13, 2014

The workshop was directed by Dr. Jack Dixon, organized by Dr. Carolyn Worby and Kim Rice, and was sponsored by Chelsea's Hope Lafora Research Fund. It included a welcome address by Linda Gerber, a presentation and video titled "Kristen's Story" by Kim and Jim Rice, a keynote address by Dr. Berge Minassian, eleven scientific presentations, and a round table discussion. More than 25 researchers, postdoctoral fellows, and students directly involved in Lafora research, and several Lafora parents attended the workshop.
Here are the highlights from the presentations without scientific jargon as much as possible. I stressed the significance of the research to potential clinical treatments. As such, it might downplay the significance of a lot of exquisite fundamental research that was presented.

**Dr. Berge Minassian (keynote speaker)**

It is safe to assume that everybody knows (of) Dr. Minassian so he doesn't need a long introduction. I would just say that he is a professor in the Department of Pediatrics at University of Toronto and a neurologist at The Hospital for Sick Children. His lab has discovered the genes responsible for Lafora disease, EPM2A encoding laforin and EPM2B, encoding malin, and hypothesized that they have a crucial role in glycogen metabolism. He is a strong proponent of the theory that the formation of Lafora bodies (LB) from poorly branched glycogen (polyglucosan chains) is the main cause of the Lafora disease.
His lab showed in two seminal papers that mice with either Lafora disease mutations and mutations that reduce the glycogen production indeed did not develop the disease. They followed these mice well into old age, over two years, and they remain absolutely healthy! Moreover, this path of attacking the disease has been confirmed by two other labs, lead by Dr. DePaoli-Roach and Dr. Guinovart (more on that later). This independent confirmation will be extremely important when we will have to get approval for human tests.
The crucial insight from these studies is that reducing glycogen production by 30-50% prevents the formation of LB and most importantly prevents the disease from developing. To reduce the production of glycogen three targets have been identified: the primer for the glycogen molecule, glycogenin (GYG), the protein targeting to glycogen (PTG) that activates glycogen synthase, and glycogen synthase (GS) itself, the enzyme that adds glucose molecules to glycogen.

Without getting into technical details, there are three stages in the production of an enzyme or protein:


Thus, there are three levels where one can interfere, and all 3 are active research areas in Dr. Minassian's lab.

**Gene level:**
*CRISPR* is the newest technique in gene manipulation inspired by bacterial defense against viruses. It was developed at MIT by Dr. Feng Zhang and is a hot commodity. As of now it cannot be used to repair genes, just to silence them. You can imagine *CRISPR* as a very precise pair of scissors that can be targeted to a specific gene in a cell, making it inactive. Dr. Minassian approached Dr. Zhang last fall and initiated a research project for Lafora targeted *CRISPR*. Dr. Zhang delivered recently the *CRISPRs* against GS, PTG, and GYG with more than 50% efficiency! The next step is to test them in cell cultures and in mice, by employing an effective delivery method.

Through another collaborator, Dr. Brian Kaspar from Ohio State University, Dr. M. has access to a new adeno-associated virus called AAV9 that has very good attributes for gene therapy: it can cross the blood brain barrier (BBB); can populate large percentage of the brain without generating a negative reaction; does not integrate in the genome (no risk of complications due to altered DNA); stays active for long periods of times; is large enough to host CRISPRs. Dr. Kaspar has shown good results using AAV9 in mice with Amyotrophic Lateral Sclerosis (ALS).
Dr. Minassian has experiments running using AAV9 and CRISPR in cell cultures and soon in mice. He is very excited and optimistic about these experiments.

Also at the gene level, AAV9 is employed to transport normal EPM2A and EPM2B genes in mice with LD. The virus will activate the genes inside the cell producing the missing laforin and malin protein and restoring their functionality in the glycogen metabolism. If successful this can be an effective cure for LD!

**RNA level:**
Dr. Minassian mentioned his collaboration with ISIS Pharmaceuticals, which specializes in anti-sense drugs that bind to the mRNA produced by that, effectively turning that gene "off". He was excited to report that they delivered Anti-Sense Oligonucleotides (ASOs) that bring GS, PTG, and GYG down by 50%, the target he requested. ISIS has tested these in cell cultures and in normal mice. Dr. M. was not allowed to show the actual results due to a non-disclosure agreement (NDA), but he has already injected ASOs in the brains of Lafora mice. The drawback of ASOs is that they cannot cross the BBB. Thus, they need to be injected in the brain ventricles or by a spinal tap at 2-3 months interval.
A note on ISIS Pharmaceuticals: currently has one approved drug (KYNAMRO for hypercholesterolemia), and various compounds in clinical trials for a variety of diseases such as Crohn's disease, psoriasis, asthma, and cancer. They are collaborating with Biogen ($100 millions project) to develop antisense drugs for neurological disorders, which will advance the brain delivery methods for ASOs.

**Protein Level:**
From Dr. Minassian updates we all know that his lab and collaborators are involved in a massive automatic screening of small molecules that can cross the BBB (more than 190,000) to find GS inhibitors. In addition, Dr. M. has entered in an agreement with an unnamed pharmaceutical company to share 5,000 chemical compounds that, in their tests, were showing GS reduction. Unofficially, because he is restricted by NDA to show the actual

results, he shared with us that 40 of those compounds indeed reduced the GS in the test assays! While he called this "fantastic progress" he was quick to caution that it does not mean automatically that those compounds are safe to be use in patients. Indeed he does not even know what those compounds are since they are identified just by a code. The company has the option to pursue the treatment with Dr. M. or they might just disclose to him what their chemical composition is. Right now Dr. M. is already embarked in the next step, determining the dosage required to produce 50% suppression of GS in cell cultures. After that of course they will be tested on mice for safety and effectiveness. Dr. Minassian is optimistic about this treatment avenue as the first line of attack on the disease. In his opinion this would allow the fastest translation to human tests.

**Dissolving Lafora Bodies:**
Most of the researchers now agree that poorly branched glycogen accumulates into insoluble polyglucosan chains that form the LB that in turn produce neuronal degeneration. The research projects enumerated above all aim to reduce the production of glycogen in the brain and stop LB formation, halting the disease. Yet, to effectively reverse the disease, the Lafora bodies have to be removed. It was hypothesized at the Workshop that the cells might have cleaning mechanisms that are simply overwhelmed in LD. If the glycogen production is reduced they might slowly clean up the LB. Nevertheless, Dr. Minassian's lab works on research to dissolve LB using amylase. The challenge is to deliver functional amylase to the brain. Two vehicles are now under investigation. AAV9 loaded with amylase was already injected in mice and the results are pending. The second involves CRM197 a mutated, non-toxic diphtheria toxin (DT) that is supplemented with amylase. The diphtheria toxin crosses the BBB and infects the neurons. It is hoped that this mutated version will safely transport amylase into the brain.

**Dr. Anna DePaoli-Roach**
Professor Emeritus, Department of Biochemistry and Molecular Biology, Indiana University School of Medicine.

Her lab works on understanding the mechanisms of Lafora disease. The lab also produced the first mice lacking PTG, paving the way for Dr. Minassian studies on the effect of glycogen production on LB formation.
Her work independently confirmed that reducing glycogen presence in neurons rescues Lafora disease, and answer affirmatively that Lafora bodies are causative of the Lafora disease. An experiment treating Lafora mice rapamycin+verapamil (increasing autophagy, the cell cleaning mechanism), showed no effect on the progression of the disease.
Her lab works independently of Dr. Minassian to identify GS inhibitors by screening small molecules from the ChemBridge libraries (193,000 compounds). She announced finding 29 potentially active molecular compounds, as well as 5 active compounds from the list of 1,200 FDA approved drugs. More tests are required before on cell cultures and mice to confirm the results.

**Dr. Joan Guinovart**
Group Leader at the Institute for Research in Biomedicine, and professor in the Biochemistry and Molecular Biology Dept., University of Barcelona, Spain.

Working with cultured neurons, genetically modified mice and flies, his lab found that neurons contain low amounts of glycogen and the mechanism to metabolize it. He showed that mice without glycogen were less able to learn, and that glycogen has positive effects in neuronal survival in stress induced by hypoxia, and in situations that require intense brain activity.
His work with Lafora mice showed that reducing glycogen synthase by 50% prevents the disease, independently confirming Dr. Minassian's results by a different method. Moreover, mice without Lafora mutations but with increased PTG expression (increased glycogen production) showed formation of accumulations similar to LB.
Their work also revealed that glycogen accumulation is the cause for the neurodegeneration, as opposed to the hypothesis that blames autophagy impairment.

**Lawrence Goldstein, Ph.D.**
Department of Cellular and Molecular Medicine, University of California San Diego.

Presented work on a neurodegenerative disease that has similarities with Lafora. His work in producing neurons carrying the mutations of the disease from epithelial cells by making stem cells has good potential in creating Lafora affected neurons for cell culture studies.

**Pascual Sanz, Ph.D.**
Biomedicine Institute of Valencia, Spain

His group seems to support the theory that LBs are not the actual cause of the disease. Using fibroblasts from LD patients and samples from LD mouse brains, they found mitochondrial alterations, signs of oxidative stress and a deficiency in antioxidant enzymes. He is working on finding molecular compounds to reduce the oxidative stress. Personal note: NAC (N-Acetyl-Cysteine) has antioxidant effect.
In the discussion that followed his presentation most scientists agreed that oxidative stress is present, but it is caused by glycogen accumulation.

**J. Machado-Salas, M.D., Ph.D.**
West Los Angeles Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, CA.

He studied the Lafora bodies in mouse brains. He identified LBs of type I, that appear first, are irregular in shape and in large numbers, as well as LBs of type II, that appear later, are spherical in appearance with complex architecture, and their number is much less. His hypothesis is that the LB type II are in fact the results of the neurons processing the LB type I. Also, he sowed large differences in the number of LBs in different areas of the brain.

**José M Serratosa, M.D., Ph.D.**
Research Center for Rare Diseases (CIBERER), Madrid, Spain

He studied the sensitivity of Lafora mice to the administration of convulsant drugs of different doses. Not surprisingly the Lafora mice were more sensitive (had seizures faster and at lower doses) that healthy mice. Since this is a standard test by which the anti-epileptic treatments are

evaluated, his work paved the way to show the effectiveness of LD. It will be very useful in persuading the NIH and FDA to agree on human tests.

**Matthew S. Gentry, Ph.D.**
Department of Molecular and Cellular Biochemistry, Center for Structural Biology, College of Medicine, University of Kentucky

His research is at the fundamental scientific level. He is trying to understand the exact molecular structure of laforin and the role of laforin in glycogen metabolism. He presented exquisite detailed models of laforin and showed the possible impact of several of the Lafora mutations in its functionality. His work is very important in understanding Lafora disease and could lead to different treatment avenues. In his own words: ”Our hope is that we can [] identify some small molecules that will be beneficial for some mutations. I see this type of work as the second wave of translational work. My hope is that the first wave is successful and we can devise a treatment that slows disease progress while we continue to work on this second wave.“

**Oliver Kötting, Ph.D.**
Institute of Agricultural Sciences, ETH Zurich, Switzerland

He studied the plant starch metabolism that is the equivalent of glycogen metabolism in animals. He recently switched to studying Lafora bodies. In his work he purified LBs and identified what proteins and enzymes are present in LBs. The goal is to identify all proteins involved in LBs formation. Most importantly he developed a method to purify LBs and this is very valuable service for other researchers. I know that Dr. Minassian plans to use purified LBs from Oliver for his amylase research.

**Felix Nitschke, Ph.D.**
University of Potsdam, Potsdam, Germany

He is another convert from plant metabolism research, working now with Dr. Minassian in Toronto. He studied in detail the functionality of laforin on glycogen and provided insight from comparisons with plant starch metabolism. His work has a significant contribution to understanding exactly how LD develops.

**Peter J. Roach, Ph.D.**
Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis IN

He discovered that LBs are formed from hyper-phosphorylated glycogen, and introduced the hypothesis that laforin is involved in glycogen metabolism. His current work is on the actual mechanism by which the GS is building up the glycogen molecule and the effect of lack of laforin on this process. Very important fundamental research, that could open additional treatment avenues.

**Antonio V. Delgado-Escueta, M.D.**

West Los Angeles Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, CA

He did not present any scientific work; his presentation was about funding a potential opportunity for epilepsy through NIH, to create a “Center without walls”.

**Round table discussion:**
Dr. Dixon led the round table discussion and started by asking for opinions regarding the usefulness of the Symposium. All scientists agreed that it was very beneficial for them and voted to organize it every two years. The next one in 2016 is going to take place again in San Diego. The funding opportunity proposed by Dr. Escueta was discussed, but no consensus was reached. Dr. Gentry was charged with reporting the Workshop proceedings to the NIH Rare Disease program director.