Dravet Canada has awarded a total of $247,500 towards research into the care, cure, treatment and understanding of Dravet spectrum disorders.

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In partnership with RDMM (we each contribute $12,500 towards the project)

2017 - David Dyment

"Towards personalized medicine in Dravet syndrome"

The overall goal of this project will be the creation of a catalog of drug(s) that act to reduce seizure frequency and mortality in a mouse model of Dravet syndrome. These FDA-approved drugs can then be taken forward for pre-clinical study. To meet this overall goal, several deliverables of each Aim will need to be achieved.

Aim 1: We plan to have an inventory of 12 stable transfectants that express the altered $Na_v1.1$ channels (4 are underway). These mutations will be selected from individuals diagnosed with Dravet syndrome in Canada. These transfectants will be used to screen the 220 FDA-approved drugs and we plan to have a short list of drugs that "normalize" the electrophysiological properties of these aberrant cells. It is difficult to predict the final number of drugs that will have a beneficial effect, although we will plan to include anti-epileptic medications and it might be reasonable to predict 10-15 drugs (5-7%). These drugs will then be used to test neuronal networks derived from the induced neurons of the same Dravet patients. We will also be testing these same 10-15 drugs on the cortical networks derived from the mouse model. We plan to deliver a final catalog of 1-5 re-purposed drugs that reduce excitability and can be taken forward to Aim 3.

Aim 2: The goal at the end of Aim 2 will be to have a new and fully characterized mouse model of Dravet syndrome. This will be a model based on a missense mutation derived from a patient versus knock-out or conditional knock-out models. To achieve this we will measure seizure frequency, duration, severity and EEG in the mouse (n=12 mice). We will compare neurobehavioral testing results between 12 $Scn1a^{H939R/+}$ carrier mice and 12 WT littermates. These standardized tests will be SHIRPA, the rotarod, fear conditioning, open field and novel object recognition test. Pathology of the cortex and hippocampi will also be performed and this will include whole cell electrophysiology experiments as per descriptions of previous mouse models.

Aim 3. Lastly, we will assess seizure frequency, duration and severity in mice that have been exposed to the candidate therapies identified in Aim 1, and those mice not exposed to treatment. We will also perform neurobehavioral testing between $Scn1a^{H939R/+}$ mice as in Aim 2. The deliverable at the completion of Aim 3 will be to have a safe drug that reduces excitability in heterologous cells, dissociated neuronal networks, and finally an in vivo mouse model that can be taken forward to pre-clinical study.

2017 - Deborah Kurrasch

The main deliverable from this work will be a greater understanding of whether Dravet models share some overlapping pathophysiology that can be potentially targeted by a small molecule or if screening in a particular genotype will uncover genotype-specific drugs. As stated by Henrik Klitgaard, VP & Head Neuroscience Discovery at UCB Pharma, there is a place for blockbusters and minibusters, we just need to know which is which. As more labs seek to use zebrafish as a model to uncover antiseizure drugs, it becomes imperative to have a better understanding as to whether our genetic models are uncovering drugs specific for a particular genotype in the patient, or will act more broadly. We will publish our

findings and share our reagents (zebrafish lines, drugs, etc) with other researchers to facilitate research into this question.

2016 – Drs. Pierre Drapeau and Éric Samarut (Research Center of the University of the Montreal Hospital Centre)
Thanks to the equipment bought with the catalyst grant (automated recording chamber for zebrafish), we were able to detect and quantify an epileptic phenotype in the gabra1 mutant fish. This allowed us to thoroughly characterized this phenotype.
Moreover, it will allow us to identify new potential therapeutical anti-epileptic drugs in a high-throughput fashion (96 fish at a time).
The chd2 model has been paused because of a maternity leave. As soon as the master student will get back, we will characterize their phenotype as we did for gabra1 mutants.
We are now trying to unravel the molecular mechanisms of gabra1 epileptogenesis. In the next 6 months, we expect to have key pathways identified. We also plan to test potential therapeutic compounds in this model.
Also, we plan to characterize the chd2 phenotype using our automated recording chamber.
We have presented our findings so far in a poster at Epilepsy Day, Montreal Neurological Institute; and oral presentation at Neurosymposium 2017, Montreal; ad will be giving an oral presentation at the European Zebrafish Meeting 2017, Budapest

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2017 and 2016

$5,000 each year as "continuing funding" to **David Hampson** to secure $600,000 grant through CIHR (Canadian Institutes for Health Research) to further his study into "Gene Therapy in a Mouse Model of Dravet Syndrome".

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2016 Dravet Canada Research Award

$35,000 towards **Dr. Danielle Andrade** and her team studying "Long-term outcomes in Dravet Syndrome".
The first objective of our work is to investigate the progression of cognitive and behavioral changes along the course of Dravet syndrome (DS), especially after adolescence. The specific hypothesis behind the proposed research is that there is neurocognitive and behavioral decline as patients age. So far most research assessed the cognitive status in pediatric Dravet patients. Therefore, there is scant information about how subjects diagnosed with DS evolve into adulthood, from the neuropsychological standpoint.
Our second objective is to evaluate how adults with Dravet syndrome respond to medications traditionally used to treat children with DS. So far most studies evaluating the treatments for Dravet syndrome have been done in children. However, in our experience, adults with DS do not react in the same manner to the medications typically used for children with DS. Furthermore, the adults with DS have a different seizure severity and have new motor problems. So far there are no studies exploring how adults with DS should be treated and the treating neurologist will typically follow the treatment suggested for children. This may or may not be appropriate and it is therefore important to understand how adults with DS respond to medications.

$20,000 towards **Dr. Peter Ruben** and his team studying "Triggers for Dravet-Associated Seizures".
The mechanisms causing temperature sensitivity in Dravet syndrome are not well understood. Our overall goal is to understand the role that environmental triggers, such as temperature, play in seizure generation. Our present aims are to elucidate whether altered sensitivity to febrile temperatures is intrinsic to all Dravet variant NaV1.1 channels, and – if so – how this may be expected to alter the firing patterns of individual neurons expressing these mutants. The specific aim of the present proposal to Dravet.ca is to extend our past and present findings to an in vivo model of Dravet febrile seizure generation.

$10,000 towards **Dr. Abby Collier** and her team studying "Interactions between SCN1a and UGT variants affecting lamotrigine efficacy and toxicity in children with Dravet's Syndrome".
It is not well understood why lamotrigine worsens seizures in DS and whether this might be due to drug toxicity by metabolism or by channel interactions. LTG is cleared from the body almost exclusively by glucuronidation metabolism. Glucuronidation is an elimination reaction in the liver performed by the UDP-glucuronosyl transferase enzymes (UGT), and specifically for LTG: by the subtypes UGT1A4 and UGT2B7. The UGTs only develop after birth in the neonatal and pediatric liver and total liver elimination of some drugs by this pathway does not mature until late teen years. The development of pediatric LTG metabolism, and relative contribution of UGT pathways are largely unknown. We hypothesize that we can define the development of glucuronidation towards LTG in our pediatric liver archive and that we can compare UGT polymorphisms and SCN1A variants for co-associations. Together, defective SCN1A action and lower or absent UGT metabolism may make children more susceptible to LTG toxicity. This project can define the developmental drug handling of LTG and interactions between disease etiology (SCN1A) and drug handling (UGT) that may be resolved by altered drug dosing. The advantage is that children are not exposed to potentially sub-therapeutic or toxic doses of drugs but human-specific data can be gained. The outcome from this research will be to provide evidence-based dosing guidelines (dosages, dose intervals) for expanded practice with LTG to better serve the Dravet community.

$50,000 towards **Dr. Dave Dyment** and his team at the Children's Hospital of Eastern Ontario (CHEO) studying "A comprehensive approach to drug repurposing to reduce seizures in Dravet syndrome; a pilot project"
We plan to expand the potential therapeutic options for those living with Dravet syndrome. To do so, we will optimize a pipeline to re-purpose drugs that includes in vitro and in vivo models. We have generated a mouse model of Dravet syndrome in collaboration with The Centre for Phenogenomics (Toronto, Canada). The mouse model incorporates a substitution based on the genotype of an individual with Dravet syndrome living in Canada. In addition to the use of this novel mouse model system, we will also be creating induced neurons from the fibroblasts of this patient and other individuals with Dravet syndrome. The induced neurons can be cultured to form a dissociated network of neurons that may better reflect the complexity seen in the central nervous system. These innovative models will then be used to screen a library of 250 CNS-specific drugs in a novel assay system known as multichannel electrode arrays (MEAs). Any candidate drugs identified by the assay can then also be tested, in vivo, in the mice, to assess for seizure reduction. The work we propose for this grant is therefore a part of our translational research program to provide tailored treatment options to those with Dravet syndrome.

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2015 Dravet Canada Research Award

$20,000 towards **Dr. Elizabeth Donner** and her team from the Hospital for Sick Children with their project entitled"A Case-control Study of SUDEP in Dravet Spectrum Disorders Une étude cas-témoin de la SUDEP chez les personnes atteintes de troubles du spectre de Dravet"
Objective 1: Create a network of collaborators to identify DSD SUDEP cases and DSD living controls. Collaborations will be established with DSD advocacy groups and clinical experts working in DSD. Working with the North American SUDEP Registry, we will assemble collaborators from Canada, the US, Europe, Asia and Australia to optimize case ascertainment of SUDEP in Dravet. Several collaborations are already established. Each participating centre will obtain institutional research ethics board approval.
Objective 2: Determine the frequency of recognized and novel risk factors for SUDEP in DSD. A case-control cohort will be assembled.

$20,000 towards **Dr. David Hampson** and his team from the University of Toronto with their project entitled "A Pilot Study of Gene Therapy in a Mouse Model of Dravet Syndrome"
Use highly customized viral vectors to rescue abnormal CNS biochemistry and behavior in a mouse model of Dravet Syndrome. We hypothesize that viral vector-mediated gene therapy represents a viable approach to treating CNS genetic disorders such as Dravet. A huge advantage of gene therapy is that a single administration of the vector translates into long-term transgene expression (vector-mediated protein synthesis) in the CNS, thus potentially inducing comprehensive and lasting correction of the disorder. We will examine the application of viral vector therapy for correcting abnormal inhibitory neurotransmission in Dravet mice where the NaV1.1 sodium channel is mutated. The seizures, and possibly other symptoms of Dravet Syndrome, are thought to be largely induced by impaired neurotransmission in GABA-containing inhibitory neurons due to the defective NaV1.1 channels in these cells. Therefore, a specific objective is to boost the effectiveness of these neurons by selectively expressing wild-type NaV1.1 channels in GABA neurons. Success in selectively targeting vector expression to inhibitory neurons could have additional positive spin-offs by demonstrating proof-of-principle, and thereby potentially extending applicability of this strategy to other neurological/psychiatric disorders seeking to modify GABAergic neurotransmission in the brain.

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2014 Dravet.ca Research Award

$35,000 towards **Dr. J. C. Martin del Campo** and his team from Toronto Western with their project entitled
"High CBD Low THC Cannabis Sativa Extract from genetically selected Cannabis sativa as Add-on Treatment for Drug Resistant Epilepsies"
Our funding was used to prepare the way for the group of studies being coordinated under the EpLink team.