**August 2016 Funded Microgrants**

**17-46 Proof-of-principle study: Evaluation of novel drug candidates for the treatment of Epidermolysis Bullosa**

Epidermolysis bullosa (EB) is a rare inherited connective tissue disease characterized by abnormal skin fragility and blistering. This is associated with pain (comparable to severe burns), chronic open wounds, infections, scarring and an increased risk of cancer. We have identified two amino acid based compounds which have the capacity to address the 3 main problems associated with EB. We propose to test variations of these two compounds in our proprietary experimental model systems and test the most effective ones in animal models. The funds requested by this application would produce research data that may further lead to the larger project with attraction of angel investor(s) and appropriate funding necessary to develop a novel treatment for Epidermolysis Bullosa, rare overlooked but devastating disease. This collaborative project may lead in the future to discovery of compounds effective for open wound management after burns, trauma or other degenerative skin diseases.

**17-3 Improved diagnosis of microvillus inclusion disease using new microscopic techniques**

Microvillus inclusion disease is a very rare and severe genetic bowel disorder that affects infants and young children. Patients suffer from unstoppable diarrhea and do not tolerate normal food. The procedure to diagnose MVID is difficult and not fail proof. An early and correct diagnosis, however, is critical to start appropriate treatment, without which patients die during infancy. Therefore, better diagnostic tools are urgently needed. We will determine the potential of a novel technology for a fail proof diagnosis of this devastating disease.

**17-6 Raising the potential for cure: CRISPR/Cas9-mediated gene editing to correct arginase-1 deficiency**

Our research focuses on arginase-1 deficiency, a rare metabolic disorder caused by mutation of the gene encoding the arginase-1 enzyme. This disorder is commonly seen in young children showing progressive neurological and intellectual impairment, as well as persistent growth retardation. If untreated, this disorder can lead to excessive accumulation of toxic substances in the body, resulting in massive damage of the brain. Currently, there is no cure except palliative treatment to alleviate the symptoms, including life-long drug administration and a low-protein diet. The aim of our research is to develop a new treatment strategy to cure this disease. Specifically, we are using patient-derived stem cell technology in combination with a cutting edge gene-editing technique called CRISPR/Cas9 to develop a targeted gene repair approach. Cells from an arginase-1 deficient patient’s skin sample are converted to “embryonic-like” cells known as induced pluripotent stem cells, which can be genetically modified to restore the function of arginase-1 in a laboratory setting. These gene-edited cells are subsequently converted into liver cells, where arginase-1 is found in a healthy person. Our ultimate goal is to introduce the corrected liver cells back into patients to stop the progression of the disorder, or, if performed early enough, to correct the genetic disorder. This gene repair strategy using patient-derived cells will be directly applicable in the development of personalized medicine**.**

**Results -** Our research focuses on a rare disorder known as arginase-1 deficiency caused by mutation in the gene encoding the arginase-1 enzyme. This disorder is commonly seen in young children showing progressive neurological and intellectual impairment, as well as persistent growth retardation. If untreated, this disorder can lead to excessive accumulation of toxic substances in the body, resulting in massive damage of the brain. Currently, there is no cure except palliative treatment to alleviate the symptoms, including life-long drug administration and low-protein diet. Our research aimed to develop a new treatment strategy to cure this disease. Specifically, we applied patient-derived stem cell technology in combination with a cutting edge gene-editing technique called CRISPR/Cas9 for targeted gene correction. Currently, we have obtained skin samples from three arginase-1 deficient patients carrying different mutation. The cells derived from the skin were converted to “embryonic-like” cells known as induced pluripotent stem cells as a model to exploit CRISPR/Cas9-mediated gene editing. These cells were maintained indefinitely in culture and were characterized by their ability to be differentiated into any cell type, including liver cells where arginase-1 is found. Our results have shown proper targeted gene repair in patient-derived cells under laboratory settings. Ongoing work includes further validation of our findings and refining our differentiation protocol to convert the gene-edited cells into liver cells for functional studies. We anticipate that our gene repair strategy using patient-derived cells will have a direct applicability in the development of personalized medicine.

Publication and Dissemination of Research Findings

Invited oral presentation “Arginase gene-editing in mouse and human induced pluripotent stem cells”, Queen's University-Ottawa Hospital Research Institute Joint Research Meeting (September 2016).

Invited oral presentation “Pre-clinical gene editing for arginase-1 deficiency”, Urea Cycle Disorders Consortium Meeting, Washington DC, USA (July 2017).

**17-17 Genetic testing and treatment in patients with rhabdoid tumour predisposition syndrome: a retrospective review**

Rhabdoid tumour predisposition syndrome (RTPS) is a rare hereditary tumour predisposition syndrome predominantly affecting children less than two years of age. Patients with hereditary cancer or tumour predisposition syndromes have a change in a gene (or genes) putting them at greater risk of developing various cancers and/or tumours. Individuals with RTPS are susceptible to developing benign and malignant tumours in various organs; most commonly manifesting as rhabdoid tumours (RT) termed atypical teratoid/rhabdoid tumours in the brain and malignant rhabdoid tumours in the kidneys. RT are very aggressive malignant cancers with a median survival of less than one year. Recently, studies have shown that correct diagnosis and intensive treatment can lead to long term survival for patients. We propose a retrospective chart review to summarize testing strategies, disease manifestations, treatment and outcome for all RTPS patients tested and/or counselled in the Cancer Genetics Program at the Hospital for Sick Children. This study would add to our knowledge of RTPS disease progression and effective treatment strategies to improve patient care and disease outcome and aid in creating a surveillance protocol for at risk individuals.

**Results -** Rhabdoid tumour predisposition syndrome (RTPS) is a rare hereditary cancer syndrome associated with the predisposition to develop aggressive and often fatal rhabdoid tumours in early childhood. As diagnostic tools and treatment strategies continue to advance, patients diagnosed with RTPS are living longer, but their lifetime risks and cancer susceptibilities remain relatively unknown. Surveillance guidelines to date focus on imaging the kidney and CNS until the age of 4. There are no comprehensive surveillance guidelines for early tumour detection in long-term survivors diagnosed with RTPS. Our research focused on a patient cohort of 60 individuals: 33 probands and 27 family members, referred to the Cancer Genetics Program at the Hospital for Sick Children for consideration of RTPS. 39% (13/33) of individuals were diagnosed with RTPS of which 38% (5/13) remained alive over the age of 4, reinforcing the need to extend current surveillance guidelines to reflect this aging patient cohort. 7.7% (1/13) presented with a second rhabdoid tumour manifestation outside the kidney and CNS. This not only illustrates the need to extend the length of surveillance beyond the age of 4, but also implement surveillance guidelines to account for RTPS manifestations outside the kidney and CNS. Therefore, in addition to current surveillance guidelines in place, we recommend an annual brain/spine MRI from age 5 onward and the addition of a whole body MRI until age 5. This research illustrates the importance of surveillance for early detection of RTPS manifestations by taking into account this aging and evolving patient cohort. This research has been drafted to publish in a scientific journal and was presented at Genetics Research Day 2017 and Rare Disease Day 2017 at the Hospital for Sick Children.

**17-2 SCN1A mutations causing Dravet Syndrome: Triggers and prevention**

Dravet syndrome is a severe form of epilepsy that affects young infants. Dravet syndrome can delay development and cause sudden death. The disease is also notable because seizures can be triggered by fever or increases in the temperature of the infants’ surroundings. The underlying causes include inherited defects in a sodium channel protein which is involved in the generation of electrical signals in the brain. Defects in channel function alter these electrical signals and can lead to seizures. Currently there is a lack of data on how temperature affects these defective proteins. Our goal is to determine how different mutant channels underlying Dravet Syndrome respond to elevated temperatures. This study will lead to improved understanding, seizure prevention and treatment.

**Results -**Dravet Syndrome (DS) is an inherited form of severe myoclonic epilepsy. DS seizures are most commonly triggered by increased body temperature. Mutations in the genes that encode voltage-gated sodium channels (VGSC) are causally associated with DS. The traditional perspective has been that nonsense mutations in VGSC genes cause a loss of VGSC function (LOF), and that this LOF underlies Dravet Syndrome. Our research on 15 novel DS missense mutations identified from patients in the United Kingdom, Ireland, and Australia, reveal a new perspective for DS. Whereas some of these novel mutations also result in LOF, others cause either a gain of function (GOF) or a mixture of GOF and LOF. Unlike most previous studies, our experiments have been conducted at physiological temperatures. We are in a unique position to relate this plethora of biophysical data with the clinical data collected by our colleagues. Our results are significant in that, whereas one set of pharmaceutical interventions may be appropriate for LOF, a completely different set of drugs may be appropriate for GOF. Our results, coupled with the clinical observations, will direct development of future therapies for DS patients, particularly those that have been intractable, and help relieve the enormous burden of this devastating rare disease. Funding from the Rare Disease Foundation for our work on DS was used to conduct preliminary experiments and leveraged to obtain additional funding from Dravet Canada. We hope to further build on this success and eventually secure federal funding for our research.

**17-25 Explaining disease variability in CADASIL through genotype analysis**

CADASIL is a hereditary disease which leads to stroke and dementia. Some CADASIL patients get their first stroke before the age of 30, whereas others remain without clear symptoms well into their seventies. The causes of this variability are not well understood. At this moment, therefore, we cannot predict disease course for an individual CADASIL patient. Our aim is to better understand the causes of CADASIL disease variability, in order to allow for better counselling of CADASIL patients and their family members. Specifically, we will investigate how and to what extent the location of the genetic defect influences disease severity.

**17-26 Testing the acceptability of a digital empowerment toolkit co-created with patients with congenital hypogonadotropic hypogonadism (CHH)**

Patients with rare diseases are dispersed and face many health challenges. It is often hard for them to find information about managing their condition that is clear and easy to understand. We have brought together doctors, nurses, specialists in genetics and researchers to focus on a particular rare disease (CHH). Patients live with their disease every day, and because of this, they are experts too. We have invited patients to be partners in our work to improve care for CHH. Together we have made patient education materials and this project will test if patients find these materials acceptable. After, we will provide materials in 16 languages on our website as part of a digital online empowerment toolkit so that patients all over the world can learn about CHH, find expert care and connect with other patients to improve their health and wellbeing.

**Results -**Patients with rare diseases are dispersed and face many health challenges. It is often hard for them to find information that is clear and easy to understand. We recognize that patients are experts in their own disease because they live with it every day. We invited patients to be partners to improve care for congenital hypogonadotropic hypogonadism (CHH). We brought together doctors, nurses, genetic specialists, researchers and patients to co-create educational materials. These materials help patients and families better understand CHH and learn how they can take care of their health. We used the internet to reach patients with CHH and evaluate the co-created patient education materials.Working with patients, we made a document that was easy to read, easy to understand and highly accepted. We translated it in 20 languages and shared the materials in three ways: 1) we informed doctors at healthcare meetings and conferences, 2) we distributed materials to patient organizations using social media, and 3) we made all the information freely available on the web as part of an online ‘empowerment toolkit’. The toolkit (available at [www.gnrhnetwork.eu](https://www.gnrhnetwork.eu/)) helps patients learn about the condition, find expert care, participate in research and connect with other patients for support to improve their health and well being. This project showed the importance of working with rare disease patients as partners. Importantly, this project had a global reach and helps patients with CHH around the world.

**Project Outputs -**Developing and evaluating rare disease educational materials co-created by expert clinicians and patients: the paradigm of congenital hypogonadotropic hypogonadism. COST Action BM1105, Badiu C, Bonomi M, Borshchevsky I, Cools M, Craen M, Ghervan C, Hauschild M, Hershkovitz E, Hrabovszky E, Juul A, Kim SH, Kumanov P, Lecumberri B, Lemos MC, Neocleous V, Niedziela M, Djurdjevic SP, Persani L, Phan-Hug F, Pignatelli D, Pitteloud N, Popovic V, Quinton R, Skordis N, Smith N, Stefanija MA, Xu C, Young J, Dwyer AA.Orphanet J Rare Dis. 2017 Mar 20;12(1):57. doi: 10.1186/s13023-017-0608-2. PMID: 28320476. [Article Here](https://ojrd.biomedcentral.com/articles/10.1186/s13023-017-0608-2)

A roadmap for developing an online virtual empowerment toolkit for patients with congenital hypogonadotropic hypogonadism. A Dwyer. 2017. Radiz 5th Rare Diseases Summer School. Zurich, Switzerland – June 2017

**17-33 Imaging resilience in a rare brain disease**

Leukoencephalopathies are rare diseases of brain white matter which impact a startling range of neurologic functions and impair overall quality of life. Remarkably, there are even fewer cases where a leukoencephalopathy is highly suspected, but the patient is clinically normal. We seek to study an intriguing case of a “clinically silent,” suspected leukoencephalopathy using advanced, quantitative diffusion MRI to further characterize the patient’s white matter pathologies and to identify structural neural mechanisms accounting for her remarkable resilience. We believe we will provide our patient with some insight into her unique diagnostic conundrum. We also expect that our findings may inform providers that patient-specific resilience or reserve may significantly alter prognoses even in the face of severely abnormal brain imaging, and this can not only be implied, but quantified using advanced diffusion MRI.

***The above research grant was generously supported by Global Genes (www.globalgenes.org)*.**

**17-39 Developing a next generation therapeutic approach for Glioblastoma Multiforme using patient-derived brain tumor initiating cells**

Glioblastoma Multiforme (GBM) is a rare genetic disorder and the most aggressive malignancy among all gliomas. Cutting edge technology has allowed scientists to isolate brain tumor initiating cells (BTICs) from GBM patients. BTICs are a population of cells that demonstrate stem cell like properties and differentiate into the advanced GBM tumors. Typically, BTICs survive surgical, drug and radiation therapeutic interventions and are the underlying cause for the GBM relapse. Current front-line GBM therapeutics are designed to damage the DNA of aggressively proliferating cancer cells. These cancer therapeutics induce the genetically hard-wired process of programed cell death (apoptosis), which plays a critical role in removing tumor cells from the human brain. However, BTICs and fully transformed GBM tumor cells circumvent apoptotic cell death by overproducing inhibitor of apoptosis proteins (IAPs) and hence do not respond to the front-line GBM therapeutics. This emphasizes an urgent need for developing novel therapeutic approaches to combat GBM. In this study, we propose to evaluate IAP-targeted experimental therapeutics using BTICs derived from GBM patients. To this end, we will use chemical compounds that specifically degrade IAPs and thereby mediate apoptosis in BTICs. This therapeutic approach has the potential to effectively kill GBM tumor cells and also to prevent reoccurrence of more aggressive GBM.

**Results -** Taking a combinatorial therapeutic approach, we treated patient-derived BTICs (a panel of 4 lines obtained from the Hotchkiss Brain Institute) as well as several established GBM lines with Smac-mimetic compounds (SMCs) and other pharmacological inhibitors, that specifically target translation of IAPs, to activate apoptosis. We found that the U343 (established GBM line) was resistant to LCL-161 (monomeric SMC) + Tumor Necrosis Factor α (TNF- α) treatment. However, this cell line was sensitive to Birinapant (bimeric-SMC) + TNF-related apoptosis-inducing ligand (TRAIL) treatment. We also performed Western blot analysis from the treated cells and measured the levels of IAPs. These anti-apoptotic proteins were downregulated in the SMC + TNF- α/TRAIL-treated cells. Moreover, we found that established GBM cell lines and BITICs were sensitive to the mammalian target of rapamycin (mTOR) inhibitors (PP242 and AZD2014). These pharmacological inhibitors inhibit the translation of IAPs and thereby induce apoptosis. We further performed Western blot analysis to confirm if the levels of IAPs are decreased in mTOR inhibitors treated cells. Indeed, the levels of all IAPs were decreased with the treatment of mTOR inhibitors. Furthermore, we have established a procedure to image BTICs in our lab and performed live cell imaging for caspase activation, which confirms the apoptotic cell death. We have also optimized a protocol for measuring apoptosis using fluorescence activated cell sorting (FACS). In addition to the IAPs-targeted therapeutics, we also evaluated the effect of Ribavirin on the survival of BTICs. Ribavirin is an FDA-approved drug for the treatment of Hepatitis C virus infection. Ribavirin targets mammalian mRNA translation machinery. In our low-throughput drug screen, Ribavirin with or without SMCs showed significant growth inhibition/cell death of BTICs lines. ***These are significant findings which will lead to the preclinical mouse models (orthotopic xenograft) studies for 1) Birinapant + TRAIL, 2) Ribavirin + Birinapant, and 3) Birinapant + AZD2014 treatments.***

 **Use of fund and trainees involved in this project:** This microgrant fund was used toward purchasing reagents required for the project. A postdoctoral fellow (Dr. Joseph Ross) and a graduate student (Divya Sharma) were trained to undertake this project.

**Dissemination:** This microgrant support was acknowledged in 1 poster and 3 oral presentations. This data will be used in a manuscript that we are planning send for publication by December 2017.

**17-47 Validating the genetic cause of a familial case of non-syndromic strabismus using the mouse model**

Strabismus refers to crossing of the eyes and can affect up to 5% people in the general population. It can lead to loss of depth perception and double vision. In some cases, a lazy eye can develop, which may cause blindness. Strabismus can also have negative psychological effects, impacting an individual’s performance both at school and at work. In recent years, more genetic discoveries were found through the sequencing technology. Many genes are thought to be involved in strabismus but few have been located and there is no test to identify children early on before they encounter problems. We have identified a family with 7 generations affected by strabismus in what is called a dominant pattern of heredity, scanned their entire DNA, and narrowed the cause down to 3 possible genetic changes. This is a very rare family and the identified genetic changes have never been reported. We are applying for funds to determine which of the 3 genetic changes are causing strabismus in the family. We will do this by studying microscopic structures of the eye and brain as well as vision-associated behaviour in a mouse that is missing the most likely gene. Pinning down the responsible gene in this family will provide a gene test to diagnose family members early on, before problems occur. Moreover, it will lead to an improved understanding of strabismus genetics and possibly identify a gene responsible for other strabismus cases.

**Results -** These changes were modeled in zebrafish and no effects were seen so the mouse studies described above were not undertaken. Thus, this study did not go ahead and all funds were returned to the RDF and BCCHF microgrant program.

**17-48 The disease mechanism of a novel G-protein-linked developmental disorder**

Intellectual disability has a large number of genetic causes, many of which still remain unknown. In five children with intellectual disability, we have identified mutations in a gene that performs multiple critical roles in cells and is particularly important for the regulation of cell movement. Preliminary evidence strongly suggests that this is a new disorder that has been not described previously. However, further studies are needed to prove causality. We propose to study the effect of the five genetic changes on the function of this gene and on cells. This will help to –

1. Establish the causal link between this gene and intellectual disability
2. Establishing causality will help in providing accurate diagnosis and counselling to the families.
3. Provide understanding into the mechanism of this novel disease.
4. Understanding the mechanism will help in opening opportunities to develop novel treatment. Of note, a number of compounds that can alter the function of this gene are already known.
5. The data generated will provide a strong foundation for future studies.

**17-49 Sanfilippo B syndrome: DNA repair of mutation E153K using CRISPR-Cas9 techniques**

Sanfilippo syndrome is a genetic metabolic disease hallmarked by the accumulation of undegraded heparan sulfates in the brain causing neural degeneration and a significantly shortened lifespan. Treatment by injection of the missing enzyme is ineffective as the administered enzyme fails to reach the brain because of the presence of an anatomic structure known as the blood-brain barrier. We propose to correct the gene defect in patient-derived induced pluripotent stem cells using the newly emerged technique CRISPR-Cas9, followed by converting the stem cells to neuronal and other cell types for potential therapeutic treatment.

**Results -** I am pleased to submit this progress report to the Rare Disease Foundation summarizing the progress made in the above project during the past 12 months as follows:

* Establishment and optimization of the CRISPR-Cas9 techniques -  Using the *HPRT* gene as a control to establish the experimental conditions for CRISPR-Cas9 transfection and gene editing, we have successfully transfected both the Sanfilippo B cultured skin fibroblasts and iPSCs (induced pluripotent stem cells) reprogrammed from the Sanfilippo B fibroblasts. We noted that upon transfection, the gRNA complimentary to *HPRT* successfully targeted and induced double stranded DNA breaks that resulted in insertions/deletions (indel) in the HPRT gene. This was accomplished by using a gRNA that targets *HPRT* with the CRISPR-Cas9 riboprotein complex during lipofectamine transfection and subsequent genomic cleavage detection analysis using T7 endonuclease. The results showed that the CRISPR-Cas9 cleavage efficiency was at 22%, a value within the norm using this protocol.
* Design of gRNA for CRISPR-Cas9 gene editing and correction of mutation E153K in the N-acetyl-glucosaminidase (*Naglu*)gene  -Using the [CHOPCHOPv2](http://chopchop.cbu.uib.no/updates.php) *in silico* program, we have designed 4 gRNAs in either the sense or antisense orientation that are within 25 bp of *Naglu* mutation E153K with minimal (less than 2 sites) or no off-targeting genome cleavage. We then performed CRISPR-Cas9 lipofectamine transfection using each of the above gRNAs in separate experiment, followed by subsequent genomic cleavage detection analysis by T7 endonuclease to determine *Naglu* targeting cleavage and indel formation efficiency. We noted that the CG contents in the gRNA is crucial in determining the targeting and cleavage of *Naglu,* and indel formation decreased substantially when the CG contents in the gRNA exceeded 65%. We also noted that optimal cleavage efficiency at about 30% was achieved when the gRNA CG contents was 50-60%. We have submitted an abstract summarizing this work for presentation at the 2018 World Symposium on Lysosomal Storage Diseases. This is the first documentation on the impact of CG contents in the gRNA in CRISPR-Cas9 gene editing.
* Design of oligonucleotide donor correction template for repair of mutation E153K - We have designed an asymmetrical single strand oligonucleotide donor correction template that has been documented to improve the homology-directed repair efficiency by six-fold (Richardson et al, *Nature Biotechnology* 4:339, 2016). We then proceeded to transfect cultured Sanfilippo B fibroblasts using CRISPR-Cas9 and the gRNA with the highest *Naglu* cleavage efficiency (please refer to Section 2 above), for mutation E153K correction. Preliminary results showed that the transfection was successful with an overall 28% *Naglu* DNA cleavage and indel formation efficiency. Upon expansion of cell culture, we will screen for cells in which the mutation was repaired by using Naglu enzyme activity assay and immunoblot analysis. If successful, we will proceed to correct mutation E153K in Sanfilippo B iPSCs for potential cell replacement therapy.

**17-56 Delineating a new syndrome caused by mutations in exon30/31 of the *CREBBP* gene**

We recently reported on 11 patients with intellectual disability with a change in *CREBBP*, the gene that, if altered, usually causes a syndrome called Rubinstein-Taybi syndrome (RSTS). The patients that were described by us, however, did not, or only in a very limited manner, resemble RSTS. Some patients did resemble one another quite strikingly, and all had an alteration in a specific region of the *CREBBP* gene. We now aim to gather data of more patients to characterize this new syndrome in a more detailed way. This will be important in order to provide patients and their families with optimal information and clinical care.

**17-57 Genetics of severe autosomal recessive early-infantile onset epileptic encephalopathy**

Early infantile epileptic encephalopathies (EIEEs) are a group of extremely rare and severe brain disorders of early age with poor prognosis and high rate of morbidity and mortality. Many of EIEEs have now been recognized to be due to a genetic cause. Making a genetic diagnosis in (EIEE) is important for several reasons: first, it provides families and healthcare professionals insight into disease management and risks for further complications. It also helps discussion about recurrence risk for future pregnancies and family planning and influences medication decisions. Finally, it allows insight into how and why the disease manifests and provides avenues for drug development. However, currently for the majority of families with EIEE, and in particular autosomal recessive types, no genetic causes have been identified since studying these forms of disease in general is more challenging in outbred westernised populations. In this study we propose to employ a powerful and efficient approach, homozygosity mapping, to hunt for elusive causes of the disease in four consanguineous families with multiple affected individuals which would significantly increase the chance of finding the mutations in these families. We believe any new finding from this study would lead to diagnoses of many other patients with undiagnosed EIEEs worldwide.

**17-59 Genetic investigation of hereditary unexplained pediatric-onset neuromuscular disorders in five consanguineous families**

Two common questions ask by parents of children with any rare genetic diseases including neuromuscular disorders (NMDs) is: “Why did this happen to our child?” and “Will this happen again in future pregnancies?”  Although precise information through molecular testing is available for the vast majority of patients with NMDs, for some forms there is still no answer at the present time. Many neuromuscular conditions have a genetic cause, hence knowledge about the cause of disease is important for accurate diagnosis and management, as some NMDs respond best to early intervention. We propose to use state-of-the-art genomic technologies (genome wide SNP arrays and DNA sequencing data) to find the elusive NMD-causing gene in 5 undiagnosed consanguineous families. It’s well established that identifying mutations in rare autosomal recessive hereditary disorders is much easier in patients from consanguine families with multiple affected individuals. The findings from our research would lead to defining new NMD genes which would directly benefit the families under study as well as other patients waiting for a diagnosis worldwide.

**17-61 Rapid drug discovery in genetic models of CHARGE syndrome**

CHARGE Syndrome (CS) is an autosomal-dominant genetic disorder characterized by a complex array of birth defects, for which there are no cure. We have recently completed a high-throughput drug screen of 3850 clinical approved molecules in a *C. elegans* model of CS and have identified several small molecules that can suppress the dysfunctional phenotype caused by mutation in the CS gene, *CHD7*. We now aim to validate the efficacy of the candidate compounds in a zebrafish model of the CS. Lead compounds will subsequently be tested through other funds for preclinical tests in mammalian CS models and ultimately in clinical tests. Our findings may assist in accelerating the development of drugs for the treatment of CS.

**17-63 Drug correction of molecular defects in KATP channel mutations that cause hyperinsulinsim**

Potassium ion channels, which are essential for a healthy pancreas, are formed from two different proteins: (1) the Kir6.2 protein that forms the channel pore and (2) the SUR1 protein that controls pore opening and closing. Changes in the *SUR1* gene that result in defective channels cause hyperinsulinism (HI), a rare (1/50,000 live births) and severe disease that results in excessive insulin in the blood, and is treated by partial or total removal of the pancreas leading to severe health consequences. If left untreated HI causes brain damage. We will determine, at a molecular level, how changes in SUR1 (called mutations) cause the potassium channel to stop working, which will allow us to design specialized drugs to treat HI caused by specific mutations. Mutations can change the 3-D arrangement of atoms in SUR1 and how SUR1 physically associates with other parts of the channel. These changes reduce how well SUR1 senses the cellular concentration of ATP (the energy currency of cells) and/or disrupt transport of the channel from where it is made inside the cell to the cell membrane where it functions. We will focus on the regions of SUR1 called the nucleotide binding domains (NBDs). The NBDs use the energy of ATP to control pore opening and closing, and contain HI-causing mutations. Using a technique similar to medical MRI, we will be provide a 3-D picture describing (1) which parts of the NBDs bind candidate drugs and (2) how mutations alter the NBD architecture. The ability of drugs to correct defects in the SUR1 mutants will be addressed through studies that examine the transport of newly-made channels to the cell surface and with experiments that measure opening and closing of the channel pore. The molecular level knowledge provided by our studies will address how SUR1 mutations cause hyperinsulinism, but most importantly how well potential drugs work, promoting the design of new drugs to treat the disease.

**Results -** Potassium ion channels, which are essential for a healthy pancreas, are formed from two different proteins: (1) the Kir6.2 protein that forms the channel pore and (2) the SUR1 protein that controls pore opening and closing. Changes in the SUR1 gene that result in defective channels cause hyperinsulinism (HI), a rare (1/50,000 live births) and severe disease that results in excessive insulin in the blood, and is treated by partial or total removal of the pancreas leading to severe health consequences. If left untreated HI causes brain damage.

Using a technique similar to medical MRI, we have determined how changes in SUR1 (called mutations) cause the potassium channel to stop working, at a molecular level. This work, which was published in *Biochemistry* in 2017, showed that different mutations result in different defects in the channel. Some mutations cause changes in the 3-D arrangement of atoms in SUR1, and also reduce how well SUR1 senses the cellular concentration of ATP (the energy currency of cells). Other mutations cause an overall disruption in the way the atoms are arranged in 3-D space. These data shed light on the underlying molecular basis of mutations that cause HI. Further, the variability in defects caused by different changes in SUR1 underscores the need for these studies in characterization of HI disease-causing mutations. Our data that address the molecular defects of disease mutations is critical for designing and testing drugs to treat HI.

**17-66 Development of an online tool to exploit liver involvement in Congenital Disorders of Glycosylation (CDG): Liver CDG electronic Questionnaire (LCDGeQ)**

Congenital Disorders of Glycosylation (CDG) are one of the fastest growing groups among the more than 8000 rare diseases currently known. Most of the genetic defects underlying CDG result in severe disease, mental retardation and physical handicap. CDG can be associated with a broad variety of symptoms. Importantly, CDG have major liver involvement. However, little research has been performed to better understand how the liver is affected in CDG patients. This will be an unprecedented study dedicated to an area with high unmet needs among this group of rare metabolic disorders. This study will be done under the scope of the only official CDG patient led international network named CDG & Allies - Professionals and Patient Associations International Network (CDG & Allies – PPAIN).

**17-68 Severe cerebral palsy possibly caused by loss of superoxide dismutase activity**

Cerebral palsy is a broad term describing impaired motor function present from birth. Causes include loss of oxygen to the developing brain, either before or during birth, damage from infections, or genetic mutations. We evaluated two siblings affected with cerebral palsy so severe that even swallowing was difficult and suspected a genetic cause given the recurrence within the same family. Genetic testing showed a mutation in the gene SOD1 (superoxide dismutase) affecting the copies inherited from both the mother and the father. Mutations in SOD1 are already a known cause of neurological disease (Lou Gehrig’s disease, also known as amyotrophic lateral sclerosis (ALS)), but only when a certain type of mutation is inherited from a single parent, who often develops the disease as well. In this case the parents are healthy, but the children have both copies mutated. The location of the variant suggests it would be quite damaging to the function of this enzyme. Many therapies are in development to try to remove the excess free radicals generated when this enzyme is not fully functioning. One of the two siblings has already deceased and we will first prove this is indeed the cause of their disease before embarking on anti-oxidant therapy.

**17-69 Impact of mutations in a potassium channel, *KCNQ5*, a probable new cause of intellectual disability**

Intellectual disability affects about 2% of people and is most often caused by harmful changes to genes. More than 850 different genes have been found to cause intellectual disability and many more remain to be discovered. A small group of genes that cause ID encode for potassium channels, which prepare neurons (brain cell) for discharging signals to neighbouring neurons. Without complete and coordinated opening and closing of potassium channels, neurons cannot form the new networks necessary for learning. Seizures are also a frequent complication, resulting from uncoordinated firing of neurons. A pair of *KCNQ* genes (*KCNQ2 and KCNQ3*) is known to cause intellectual disability and seizures when mutated. Study of the impact of several mutations in these genes suggests that the changes cause the channel to fire too easily, and for other mutations there may not be enough firing. Certain medications, such as the recently FDA approved drug retigabine, target KCNQ channels and help keep them open to reduce over-firing. It is very important to understand the exact impact of specific mutations on KCNQ channels in order to choose medications targeted for specific effect. We have discovered a new KCNQ channel mutated in intellectual disability with or without seizures, and seek to characterize each of the four new mutations discovered to see if there is too much channel opening, or not enough.

**17-76 A novel mitochondrial therapy to treat Duchenne muscular dystrophy**

Limited therapies exist for Duchenne muscular dystrophy (DMD, ~ 1 in 3500 males) – a muscle wasting disease causing immobility, an inability to breathe, heart failure and death by teens to 20's. DMD damages essential energy-producing structures inside muscle cells known as 'mitochondria' which then trigger self-destruct mechanisms that likely contribute to muscle wasting in DMD. Using mice, this project will test a novel compound that stops this self-destruct mechanism in order to prevent muscle wasting/weakness in DMD.