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Department of
Kinesiology and Physical Education

Département de
kinésiologie et d'éducation physique

Benoit J GENTIL, PhD,
Assistant Professor,
Dep of Kinesiology and Physical Education
Associate Professor,
Dep of Neurology and Neurosurgery
McGill University
475 Pine Avenue West, Room 210
Montreal, Quebec, Canada H2W 1S4
benoit.gentil@mcgill.ca

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Fondation de l'Ataxie de Charlevoix-Saguenay

Annual report
Project «Therapeutic Approaches for ARSACS»
Principal investigator responsible of the project: Benoit J Gentil
PI: Dr Benoit J Gentil and Dr Heather Durham

Personnel and collaborators involved:

- Sandra Minnotti, technician,
- Zacharie Cheng-Boivin, PhD student
- Alexandre Paré, PhD Student
- Caitlin Atkinson, MSc Student
- Azhari Sami, undergraduate student
- Lucas Eric, undergraduate student

Dr Saddikot Abbas (Montreal Neurological Institute)
Gerardo Ramos, PhD student
Dr Armstrong Garry (Montreal Neurological Institute)
Dr Herrera Federico (University of Portugal)

Collaborations: We have been contacted by Dr Graham's group (University of Saskatchewan and funded by the ARSACS Foundation) and are currently collaborating with his team by providing brains from the Sacs-/- and Sacs+/+ mice and important analysis of brain structures affected in ARSACS in order to understand the role of metals in ARSACS. The results will be presented at the ARSACS symposium in Oct 2023.

Introduction: The ARSACS foundation has been supporting our research for several years. With this support, we have been able to generate cellular models of the disease (neuronal models derived from knockout mice), and to identify cellular phenotypes characteristic of the pathology and molecular biomarkers. Developing biomarkers and understanding the role of sacsin was a crucial step in the development of therapeutic strategies. As such, we published a foundational article 'Sacsin, mutated in the ataxia ARSACS, regulates intermediate filament

assembly and dynamics' in FASEBJ identifying the effect of sacsin domain on neurofilament assembly. In fact, together with our collaborators, we demonstrated that intermediate filament bundling is a key characteristic of ARSACS. We contributed to two other foundational publications in the journal Human Molecular Genetics: 'Altered organization of the intermediate filament cytoskeleton and relocalization of proteostasis modulators in cells lacking the ataxia protein sacsin' and 'Sacs knockout mice present pathophysiological defects underlying autosomal recessive spastic ataxia of Charlevoix-Saguenay'. We developed peptides to replace sacsin loss of function aiming to derive therapeutic compounds and published in Int J Mol Sci. ***Our strategy over the last years aimed at developing a replacement peptide and gene therapy and to investigate the role of these peptides. Because sacsin as multiple chaperones domains. potential of alternative therapies already in development in our laboratory to promote protein chaperoning.***

For the last competition our specific **goals** were**:** To obtain preclinical proof-of-concept for ARSACS treatments based on: 1) a protein/gene replacement approach and 2) drug treatment using HDAC inhibitors.

Defining minimal functional sequences of sacsin: Akin to development of mini-dystrophin constructs for treatment of muscular dystrophy, the reduction of sacsin size (525kDa) for gene replacement therapy is necessary in order to package into current adenoviral vectors, which can contain a cDNA sequence to encode a 110kDa protein at best. Bearing this in mind, we designed and expressed in cultured *Sacs*$^{-/-}$ motor neurons miniconstructs, which are within the range for viral packaging. Efficacy of these miniconstruct in resolving IF bundling was confirmed in ARSACS patient fibroblasts carrying the homozygous mutation 8844delT. **This construct has been packaged into an AAV9 vector.** Transduction of spinal cord cultures successfully resolved NF bundles in motor neurons and proof of principle has been shown in mice models.

Alternative drug therapies: Since sacsin contains domains with homology to other molecular chaperones (HSP90 and DNAJ) and provides a chaperoning function for NF, we showed that other protein chaperones, like HSP70, or HSP inducers, including celastrol, are efficient in resolving NF bundles[1]. Because celastrol has a short therapeutic window, we pursued experiments with the chaperone co-inducer arimoclomol, currently in clinical trial for ALS and other disorders. Co-inducers require that at least some stress response already has been initiated by the disease process. Arimoclomol was effective only when neurons were prestressed with heat shock, suggesting that a priming stress heat shock response is not induced in ARSACS neurons. As an alternative strategy, we used histone deacetylase (HDAC) inhibitors to remodel the 'openness' of chromatin and to facilitate transcription of chaperone genes. The balance of histone acetylation and deacetylation is determined by histone acetyltransferases and HDACs, acetylation generally being permissive for transcription.

Progress in Current Year:

We **published** a manuscript to Int J Mol Sci. describing the efficiency of a cell-penetrating peptide derived from sacsin, reporting *the role of sacsin DNAJ domain in NF assembly*. In addition to a role of DNAJ in NFL disassembly *in vitro*, we demonstrated the direct role of

DNAJ in preventing the *in vitro* assembly of NFL and in breaking down NF bundles into segments amenable to autophagy. We gave access to the preprint of this manuscript through the BioRiV platform and my ResearchGate profile to speed up the knowledge dissemination process. The J domain of sacsin disrupts intermediate filament assembly | bioRxiv.
Using GST-DNAJ in a pull down assay, we identified several proteins involved in Golgi-ER trafficking, which shed light on the function of sacsin in organelle trafficking and autophagosome formation and the virus escape of the degradation system. These preliminary data are being used to leverage the ARSACS funding and were granted a funding from Neurosphere, the McGill platform dedicated to innovation and partnership in neuroscience research. This was the work of Alexandre Paré and was published in his MSc thesis Thesis | Development of an active peptide derived from the Sacsin J domain and identifying new functions of Sacsin in ARSACS models | ID: cc08hn06d | eScholarship@McGill. We also prepared a manuscript who will be accessible with an open access at BioRiV.

*We moved forward in the development of **peptide** and **gene therapy strategies***. We have now produced all the cell-penetrating peptides and testing of the efficiency in resolving IF bundles in fibroblasts and motor neurons in culture. Similarly, we tested the our construct packaged in a clinical grade gene therapy vectors. We have tested the efficiency of our viral vectors in mice models of ARSACS and observed a delay of onset of some symptoms while other were not progressing.

We are writing a manuscript showing the therapeutic potential of HDAC inhibition in ARSACS. Treatment with the pan HDAC inhibitor SAHA (Vorinostat) was sufficient to resolve vimentin IF bundles in ARSACS patient's fibroblasts and NF bundles in cultured *Sacs*$^{-/-}$ motor neurons. The more selective HDAC6 inhibitor, Tubastatin A, efficiently resolved NF bundles in 6 week-old cultured *Sacs*$^{-/-}$ motor neurons, but the class I HDAC inhibitor RGFP109 did not. Interestingly acetylation of tubulin and histone3 was reduced in ARSACS patient's fibroblasts, suggesting altered acetylation/deacetylation balance in ARSACS.

Summary: We have compared the efficiency of a cell-permeant peptide and AAV gene vector for gene therapy in cells. We obtained the proof of principle of the use of gene therapy in mice. In parallel, we finished to investigate the role of HDAC6 inhibition as a therapeutic approach.

Training of talents: The grant contributed to the professional development of Alexandre Paré as a MSc student in the Integrated Program in Neuroscience of McGill University who is leading the project. We have been recruiting to move forward our work (full list of personnel provided above). In addition, the ARSACS project was used to initiate several undergraduate students enrolled in Kinesiology honors program to research.

Knowledge dissemination:
Knowledge dissemination:

As mentioned, we have submitted one comprehensive manuscript for publication and we are working on two new manuscripts. We will continue our strategy to post preprints on the BioXriv, an **open science server** in addition to being published in peer reviewed journals. To our knowledge, we have been pioneer in an open communication of our data amongst the reseracher funded by the ARSACS foundation.

Presentations: Dr Gentil presented his work at Genethon and at the RareDIG Disease Day.

Gentil B.J. (2023) RareDIG Rare Disease Day, Montreal, Canada. A researcher perspective on rare diseases. Audience: scientists, students, physicians and patients

Funding leverage strategy:
The ARSACS grant was also used to leverage funding in order to increase the research potential of our group. As such, we used the dollar-match strategy and preliminary data of the ARSACS project to obtain funding for our students or apply to funding opportunities. We secure the first level grant of Neurosphere.

Grants:
Benoit Gentil. Ignite Neurophere McGill University. 2023. Development of a gene therapy for ARSACS and intermediate filament disorders. $50,000

Awards:
Sami Azhari McGill University (Tassone research award $5,800, 2023)

We also have been in contact with biopharmaceutical companies.

Awareness strategy: Our group has an active program on ARSACS and his committed to develop therapeutic strategy and to identify function of sacsin. We welcome undergraduate students during their honor project, which contributes to raise awareness on ARSACS amongst our promising student. Several of these students have been enrolled in medicine, physiotherapy or in pharmacy, which will bring more awareness toward ARSACS and future allies in disciplines in contact with ARSACS patients. In addition, in the context of his course EDKP449 (Exercise Physiopathology), Dr Gentil gives a lecture on ARSACS in order to train future kinesiologists.

Benoit GENTIL