Care4Rare Year 3 (April 2015- March 2016)

Report for Fondation de l'Ataxie Charlevoix-Saguenay

Care4Rare is pleased to share our progress over the third year of this four year project, which is detailed in the enclosed report.

One of our Care4Rare activities is to identify therapeutic leads for rare diseases and ARSACS is included as one of the disorders studied in this pipeline. We have utilized a number of approaches to identify drugs that affect SACS expression:

- Bioinformatics approaches using online databases developed for this purpose
- An FDA drug screen using human fibroblasts (skin derived cell line), to identify compounds that may increase the expression of the SACS mRNA
- An FDA screen using neuronal cells for diseases, such as ARSACS, that impact the central and peripheral nervous systems

A number of hits from these screens were extensively followed-up. The most promising was identification of Nilotinib upregulating ARSACs mRNA in cultures of primary mouse neurons. However, unfortunately, as is sometimes the case, the protein expression did not correspond to the mRNA expression, ruling this out as a treatment possibility (see attached report).

We have identified a cellular phenotype (appearance) in fibroblasts derived from ARSACs patients (abnormal appearing mitochondria):

- Plans are currently underway for a 3000 drug screen for ARSACs fibroblast phenotype modulators; this will look for drugs that correct this abnormality.

We thank you for your continued support.

Sincerely,

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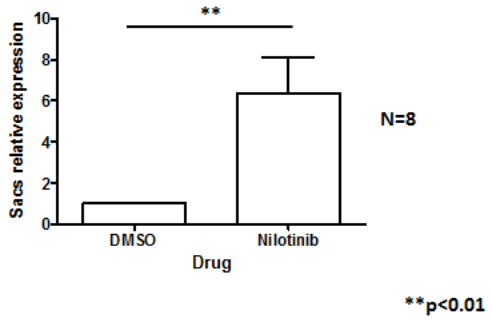
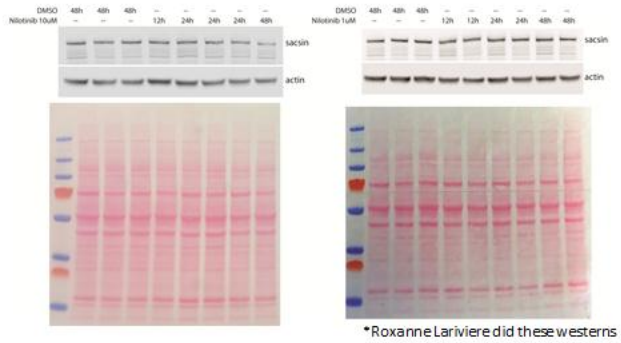
ARSACs Data Summary for Nilotinib

Nilotinib upregulates Sacs mRNA in mouse cortical neurons



We identified a dramatic upregulation of ARSACs mRNA with Nilotinib in a drug screen of primary mouse neurons. This was the only hit among 240 FDA approved drugs that showed increased mRNA (screen was performed by Jeremiah Hadwin in Alex MacKenzie's lab).

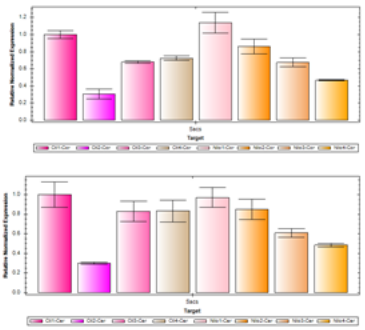
Nilotinib does not upregulate Sacsin protein in mouse cortical neurons*



*Roxanne Lariviere did these westerns

Unfortunately, the induction was not confirmed in mouse cortical cells at the protein level (Western blot analysis was conducted by Roxanne Lariviere in Bernard Brais' lab).

Nilotinib does not upregulate Sacs mRNA in C57BL6 Cortex and Cerebellum



- C57BL6 treated with 25mg/kg Nilotinib (p.o.) for 8 days.
- P=4

Unfortunately, the induction of mRNA was also not seen in brain cortex and cerebellum in mice treated with this drug (analysis was performed by Jeremiah Hadwin in Alex MacKenzie's lab).

Executive Summary

The more than 7,000 single-gene inherited rare disorders, while individually rare, are collectively common, affecting as many as 3 percent (~3 million) Canadians, and contributing significantly to national morbidity, mortality and healthcare costs. Moreover, as many as half of these rare diseases are undiagnosed and most are currently untreatable. Care4Rare aims to improve outcomes for patients with rare diseases, building upon the infrastructure and discoveries of the highly successful FORGE Canada (Finding of Rare Disease Genes) project. As the natural successor to FORGE, Care4Rare, with collaborators in provincial health services and industry, aims to enhance the clinical care of patients and families with rare diseases by facilitating accurate molecular diagnoses, pre-symptomatic management of diseases, and more effective genetic counseling. In addition, we will validate a low-cost generalizable approach to the systematic exploration of potential therapeutics to set the stage for clinical trials. The Care4Rare project was launched on April 1, 2013.

In the first three years of Care4Rare, 668 proposed disorders were approved for study and 1932 patients and family members were recruited. 900 samples for 462 disorders have undergone exome sequencing. Mutations in known genes were identified for 108 disorders and 46 novel or potential novel genes have been discovered. The remaining disorders continue to be analyzed and data shared to enable discovery. We are part of the leadership team of Matchmaker Exchange, a group focused on establishing a coordinated network to link existing genotype/phenotype rare disease databases for gene discovery; a special issue in Human Mutation was published in October 2015 devoted to the Matchmaker Exchange including multiple articles connected to Care4Rare. The CCMG position statement for Canada on the clinical indications for genomic sequencing and best practice guidelines for the return of incidental findings, authored by Dr. Boycott, Care4Rare members and national collaborators, was published in the Journal of Medical Genetics in July 2015. Translation of exome sequencing to clinical laboratories is on-going at our major sites, enabled by our pilot study which has sequenced 45 samples thus far in clinical laboratories.

Patient cell lines have been obtained for 37 disorders for downstream evaluation of potential therapies. Seventeen promising drug-gene interactions are currently being evaluated in mouse models. We have performed and analyzed an additional drug screen of neuronal cells using a FDA-approved drug panel and RNA-sequencing; 68 positive drug-gene interactions were identified from this dataset. A cellular phenotype assay was developed for lysine degradation disorders whereby cell death can be induced in patient fibroblasts and this screen successfully revealed a potential therapeutic response to fenofibrate. We have also identified a significant accumulation of specific phospholipids in DDHD2 patient cell lines (HSP54) which may represent a screenable biomarker of disease.

The outcomes of Care4Rare's research program are being disseminated with 32 manuscripts published and 15 manuscripts in preparation.

Activity 1: Disease Gene Identification and Molecular Diagnoses

Activity 1a: **Patient selection and deep-phenotyping**.

- 668 proposals were reviewed and approved for study.
- 1,932 samples were collected from patients and family members.
- Deep-phenotyping in the PhenomeCentral database is occurring for all patients.
- We are part of the leadership team of Matchmaker Exchange, an international group focused on establishing a coordinated network to link existing databases that hold genotype and phenotype information for the purposes of gene discovery.

Activity 1b: Genomic sequencing and analysis.

- We completed exome of 900 samples for 462 disorders.
- Causative mutations in known genes were identified in 108 families.
- Forty-six novel or potential novel genes have been identified.
- We have started accepting exome data for re-analysis from patients with a clinical exome performed that were not solved. Potential novel genes identified in these datasets are being added to PhenomeCentral for matchmaking.

Activity 1c: **National data coordination**.

- Data is being deposited into the national data coordination centre.
- Consortium members and collaborators can request access to the data on a per patient basis or as a control dataset.
- Collaborators can also request a search of the FORGE/C4R database for variants in genes of interest or they can enter their phenotypic and exome variant file into PhenomeCentral.

Activity 1d: **Validation of novel disease genes**.

- A potential novel gene for Pelizaeus-Merzbacher-like disease (559), Developmental delay (607), and unclassified inherited bone marrow failure (672) were identified. These are being followed up by searching for additional patients by Matchmaker Exchange and functional data.
- A novel phenotype associated with FGFR1 mutations, Encephalocraniocutaneous lipomatosis (571), was published in AJHG.
- UNC80 was published as a novel gene causing developmental delay, hypotonia, and vocal cord web (593).
- Manuscripts are being prepared for the following disorders with novel genes identified and validated in multiple families: Galloway-Mowat (284), Sprintzen-Goldberg (216), Ciliopathy (317), Developmental delay, neuropathy, and scoliosis (462), Alopecia and dwarfism (299), Acrogeria (361).
- Manuscripts are being prepared for the following disorders with genes validated by functional studies: Multicystic kidney disease (393), and Extreme microcephaly (428).
- Functional studies are ongoing for candidate genes for the following disorders: SHORT-like syndrome (182) David syndrome (320b), Cowden-like syndrome (398), Metaphyseal

chondrodysplasia (592), , Microcephaly (725), Lethal microcephaly-cerebellar hypoplasia (338), Developmental delay and HSP (391), Congenital myopathy and skeletal dysplasia (418), Mitochondrial myopathy (466), Hypomyelination and movement disorder (638), Congenital Disorder of Glycosylation (420), Neurodevelopmental delay (475), and Cerebellar ataxia (483).

Activity 2: Clinical Implementation of Exome Sequencing: Proposed Changes

Activity 2a: Analysis and interpretation of exome sequencing data in the clinical setting.

- We have identified a 96.5% concordance between exome and clinical standard Sanger sequence based identification of coding variants in 20 patients (295 variants). A manuscript outlining these findings has been accepted in MGGM.
- The clinical genomic sequencing pilot is underway in the clinical laboratories (CHEO (Ontario), Ste Justine (Quebec) and ACH (Calgary)).

Activity 2b: **Estimating the value and economic consequences of exome sequencing - GE3LS activity**.

- Six focus groups were conducted across Canada in 2014 (Quebec, Ottawa, Calgary) and included 15 parents of children with rare diseases and 8 adults with rare diseases.
    - The focus groups highlighted various medical, social and personal contributors to the challenges experienced by rare disease patients in their quest for a diagnosis.
    - The data reveals that the impact of rare disease diagnosis is multifold; ranging from medical to social to personal. A manuscript on the focus group results is currently being prepared.
- Interviews were also carried out with clinicians across the country to estimate the value of exome sequencing.An article was published regarding the interview results in Clinical Genetics “The clinical utility of whole-exome sequencing in the context of rare diseases – the changing tides of medical practice.”
- A Discrete Choice Experiment (DCE) survey designed for parents of children of rare disease has been developed. It is designed to determine the personal utility of a rare disease molecular diagnosis, the price parents would be willing to pay, and how long they would be willing to wait for a clear diagnosis.
    - A recruitment letter for the online survey was mailed to 104 parents of children with rare genetic diseases in Alberta. The online survey was completed by 22 eligible individuals.
    - Based on the results from this pilot, the final changes have now been made to the survey and the modifications will be submitted to REBs at both the University of Calgary and CHEO for final approval. Recruitment for the final study will begin in May 2016.
- Our estimation of the cost of rare diseases is underway under the auspices of the Institute of Clinical Evaluative Science (ICES).
    - We will compare the cost of static and progressive genetic diseases to chronic disease and healthy controls and will include estimates of the costs before and after diagnosis.

- Six-hundred and seventy-five charts have been reviewed thus far, of these, 298 met study criteria.
- Data collection has been completed and has been transferred to ICES. The data analysis plan has been reviewed by both C4R and ICES.
- ICES has begun pulling data on these 298 individuals and has started their analysis.

Activity 2c: **Development of tools and guidelines for integration of exome sequencing into the clinic**.

- The CCMG position statement for Canada on the clinical indications for genomic sequencing and best practice guidelines for the return of incidental findings, authored by Dr. Boycott, Care4Rare members and national collaborators, was published in the Journal of Medical Genetics in May 2015.
- A generalizable clinical consent has been developed for use in the clinic and is now available for implementation; it received REB approval at CHEO in December 2015.
- We are currently working on pamphlets and patient information sheets, pictograms, and web tools.

Activity 2d: **Educational program for trainees in Genetics**

- This program is being delivered as 4 modules, each an hour in length, and includes a combination of pre-recorded PowerPoint sessions and live sessions using the remote presentation system, Telegraph.
- Module 1: Technical Aspects, Module 2: Ethical Issues, Module 3: Clinical Reporting, Module 4: Clinical Cases.
- The course is directed towards the training of CCMG fellows, RCPSC Medical Genetics trainees, as well as those established Medical Geneticists expressing an interest in this learning opportunity.
- Teaching to various other audiences have also occurred including medical students and attendees at the annual CCMG meetings (2015, 2014).

Activity 3: Re-positioning of Clinic Ready Compounds to Treat Rare Diseases

Activity 3a: Identification of disorders and patient resources for therapeutic investigation

- Eighty target genes have been prioritized based upon the genetic pathology and clinical tractability (see Table 4; Activity 3 Summary). Additional target genes are added as opportunity arises; more may follow based on outcomes from Activities 1 and 2 and the neuronal screen described below.
- Commercial repositories such as Coriell or our extensive clinical network are utilized to recruit the patients to obtain or establish the relevant cell lines to validate drug-gene responses. We currently have 68 cell lines for 26 rare diseases which have been collected on an as-needed basis.

Activity 3b: Identification of clinically promising drug-gene interaction

- >1,900 potential gene-drug interactions in fibroblasts were tested by qPCR.

- After validation of drug-gene interactions was performed utilizing different (therapeutic) concentrations, multiple cell lines, and/or different time points; 24 demonstrated at least a modest response (at the transcriptional level) to therapeutic doses.
- Mouse cortical neuron cultures represent a much more sensitive and physiological relevant cell type to test drug responses (majority of diseases of interest impact CNS; a greater proportion of the transcriptome is expressed when compared with fibroblasts as well as a greater transcriptional response to drug). We performed a drug screen of primary mouse neuronal cells using 230 blood-brain penetrant FDA-approved drugs and the RNA-seq data has been analyzed.
- For our disease genes of interest, 68 positive drug-gene interactions were identified from this dataset.
- Validation by qPCR in multiple biological replicates has verified at least 9 of these interactions to-date.
- In addition, based on RNA-seq data and validated by qPCR, Formoterol appears to down-regulate Pmp22 which may have therapeutic implications for Charcot-Marie-Tooth Disease which is associated with over-expression of PMP22 (primarily due to gene duplication).

Activity 3c: Proof-of-mechanism of clinically promising drug-gene interactions

- Successful cellular phenotyping was used for identification of PPAR agonists as a potential therapeutic for lysine degradation disorders pyridoxine-dependent epilepsy (PDE) and Glutaric Acidemia type I.
- We will soon be receiving an extensive "chemogenomic" library of drugs (2883 compounds) from Pfizer which will enable us to screen cell phenotype responses to perturbation of almost all known cellular pathways. This library will be used in conjunction with our FDA panel to identify therapeutic targets for a number of diseases of interest with a scorable cellular phenotype.
- Significant accumulation of specific phospholipids in DDHD2 patient cell lines (HSP54) may represent a screenable biomarker of disease (identified in collaboration with Dr. Steffany Bennett). The relationship between this finding and pathogenesis of disease is ongoing. To aid in these assessments, we have generated iPS-derived neurons from patient fibroblasts.
- For ARSACS we have discovered that patient cell lines demonstrate abnormal levels of ROS, which may indicate an underlying pathogenic trait of this disease. Screening for modulation of this phenotype will be performed using our libraries.
- In addition to the original 11 Phase II shelved drugs provided by Pfizer, an additional 37 will soon be received for use in our screens.
- Based on extensive in vitro data we have now shifted our emphasis to testing drug-gene interactions in vivo. 17 different drugs have now been tested in vivo so far and over 200 drug-gene interactions tested.
- Dexamethasone treatments for FBN2 and ITPR1 induction were unsuccessful.
- Isotretinoin appears to result in activation of the SMAD3 pathway as determined by induction of known downstream Smad3 transcriptional targets in heart/aorta tissues.

- For PDE (and GAII), in vivo treatment with Fenofibrate reduces the accumulation of toxic metabolites (biomarkers) that have been hypothesized to contribute to the clinical manifestations of this disease. We observe a response in liver and serum but, likely due to poor blood-brain barrier penetration, we are yet to demonstrate a direct effect on brain tissues. Different dosing regimens and alternative PPAR agonists are currently being studied.
- Fenofibrate has also been shown to induce many gene targets of interest in the liver but unfortunately not in the disease relevant tissues. These include Hprt, Ddhd2, Grn, and Cpt2. In the case of Hprt and Ddhd2, both demonstrate modulation at RNA and protein level.
- Other drugs that have had positive drug-gene interactions that are currently being tested in mice include Rosiglitazone, Pindolol, Tranylcypromine, Gemfibrozil, Mebendazole, Biperiden, Cyproheptadine, Deferasirox, Pirinixic acid, Levothyroxine, Formoterol, Quercetin and Luteolin.
- Scn1a Dravet syndrome mouse colony (developed in collaboration with the Toronto Centre for Phenogenomics) will be established at our facility in the near-future for detailed analysis and testing of potential therapeutics.

Activity 4: Facilitating National Efforts in Rare Diseases

Activity 4a: **Orphanet Canada**

- Care4Rare is overseeing data entry into Orphanet Canada (http://www.orpha.net/national/CA-EN/index/homepage/), our affiliate of the international Orphanet database for rare diseases (http://www.orpha.net/consor/cgi-bin/index.php).
- Data entry of all molecular diagnostic labs and most cytogenetic and biochemical diagnostic labs has been completed and are continually being updated.
- Orphanet Canada data Quality Assessment is being conducted in parallel with these initiatives.
- Efforts to increase the profile of Orphanet Canada included obtaining baseline information for Canadian utilization of Orphanet website thru Google analytics, contacting the executive co-chair of the Consumer Health Information Providers Interest Group to introduce Orphanet Canada, and presenting in various clinical venues such as the CAGC conference (2015).

Activity 4b: **Regulatory framework for novel therapies for rare disorders**.

- With an overarching goal of facilitating a regulatory approval process for orphan disease products, research and comparative analyses of orphan drug policies, relevant regulations, and the proposed Canadian orphan drug regulatory framework was undertaken.
- Review and analysis of recent amendments to the Food and Drugs Act, comparative review of orphan drug policies (focus on U.S., Europe, and Australia), comparison with

Canada's draft orphan drug regulatory framework, and survey of incentives for orphan drug development have all been completed and will be the subject of a final report.

- Survey of incentives for orphan drug development was completed.
- A Masters student completed a literate review on incentives for orphan drug development.
- An article regarding pharmacogenomics and orphan drug legislation was published in the Journal of Law and Biosciences "Orphan Drug Incentives in the Pharmacogenomics Context: Policy Responses in the U.S. and Canada".
- A manuscript regarding special access programs was completed and submitted for publication.
- Research on registries for rare disease and patient engagement in drug approval processes continues.

Activity 4c: **Canadian Strategy for Rare Diseases**.

- To facilitate the development of a formal Canadian strategy on rare disease, we have participated in a number of national meetings; meetings were held as part of the annual Canadian Organization for Rare Diseases meeting in March 2015 and March 2016
- Recommendations were made regarding the scope of the national plan from various stakeholders.To facilitate the development of a formal Canadian strategy on rare disease, we have participated in a number of national meetings; the sixth meeting was held as part of the annual Canadian Organization for Rare Diseases meeting on March 5, 2015.
- Based on these recommendations, we have as actively participated in the writing of the national strategy which is now on line (http://www.raredisorders.ca/documents/CORD_RD-Strategy_3MAR15_WITHCOVER.pdf). The Framework outlines five goals that collectively aim to optimize outcomes of rare diseases in Canada. Canada's Rare Disease Strategy was launched on May 26, 2015.