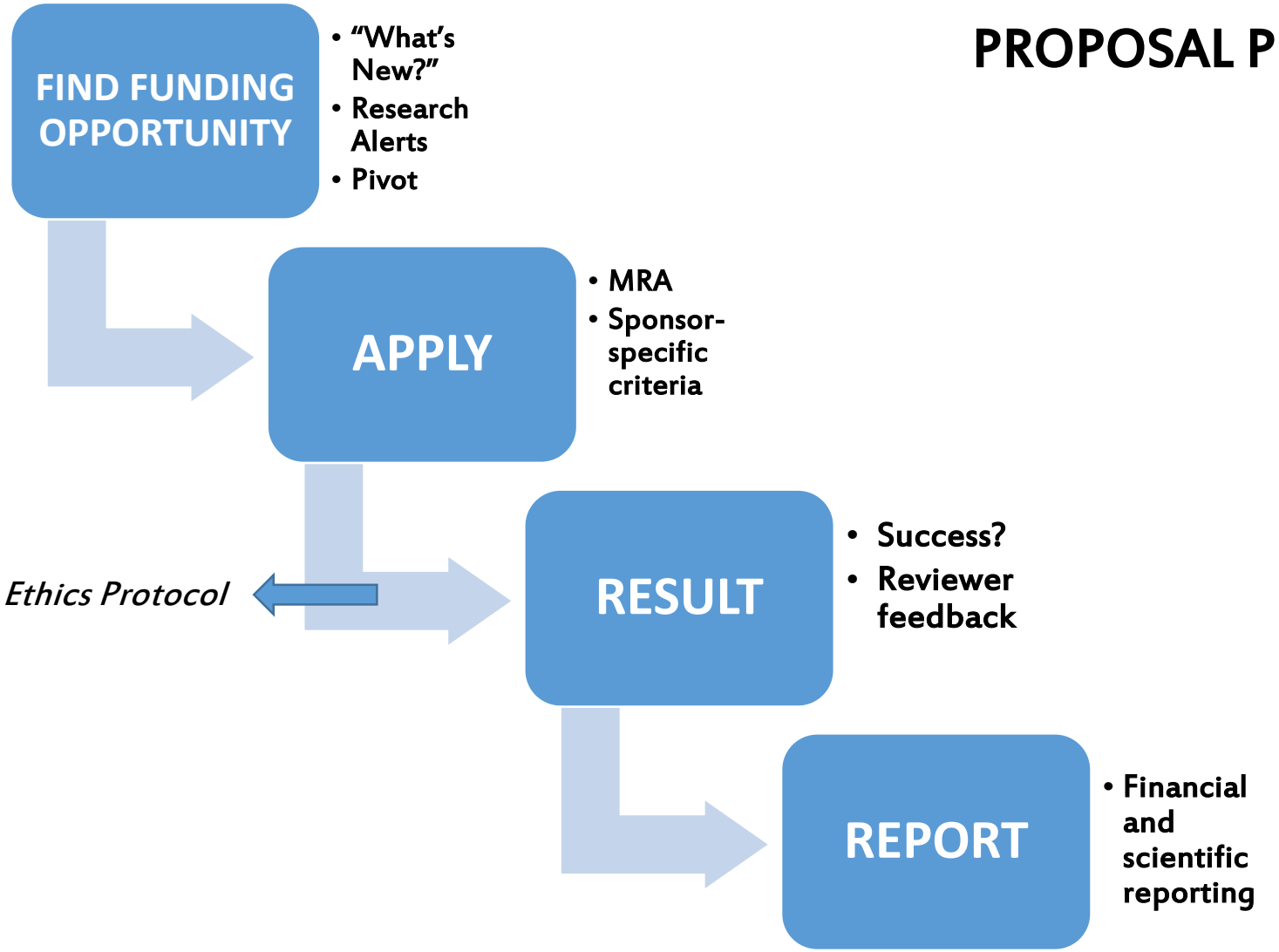
Office of the Vice Dean, Research & Innovation

Applying for & Administering Research Funding

UNIVERSITY OF TORONTO
FACULTY OF MEDICINE

PROPOSAL PROCESS



Sources for Finding Funding Opportunities

- "What's New in Research Funding" – Faculty of Medicine e-newsletter
- Research Alerts – Central OVPRI communications tool
- Pivot – 3rd party grant database (ProQuest)
- Federal and provincial websites, colleagues, and Google

My Research Applications (MRA)

- Mandatory automated system for tracking, reviewing, and approving grant applications from University of Toronto
- Internal web tool – log on with UTORID
- MRA is required **in addition to** submission to granting agency, e.g. CIHR
- Tracks: co-PI's, ethics, overhead, location of research, and more…

OVDRI – Proposal Development Team

- **Daniel Harney**, PhD, Grants & Awards Editor

- **Cindy Faber**, Research Services Officer

Funding applications are approved by:

- **Dr. Richard Hegele**, Vice Dean, Research & Innovation
  - Based on approval by Departmental Chair(s)

www.medicine.utoronto.ca/research

Jeremy Knight, Manager, Research Administration & Operations
jeremy.knight@utoronto.ca

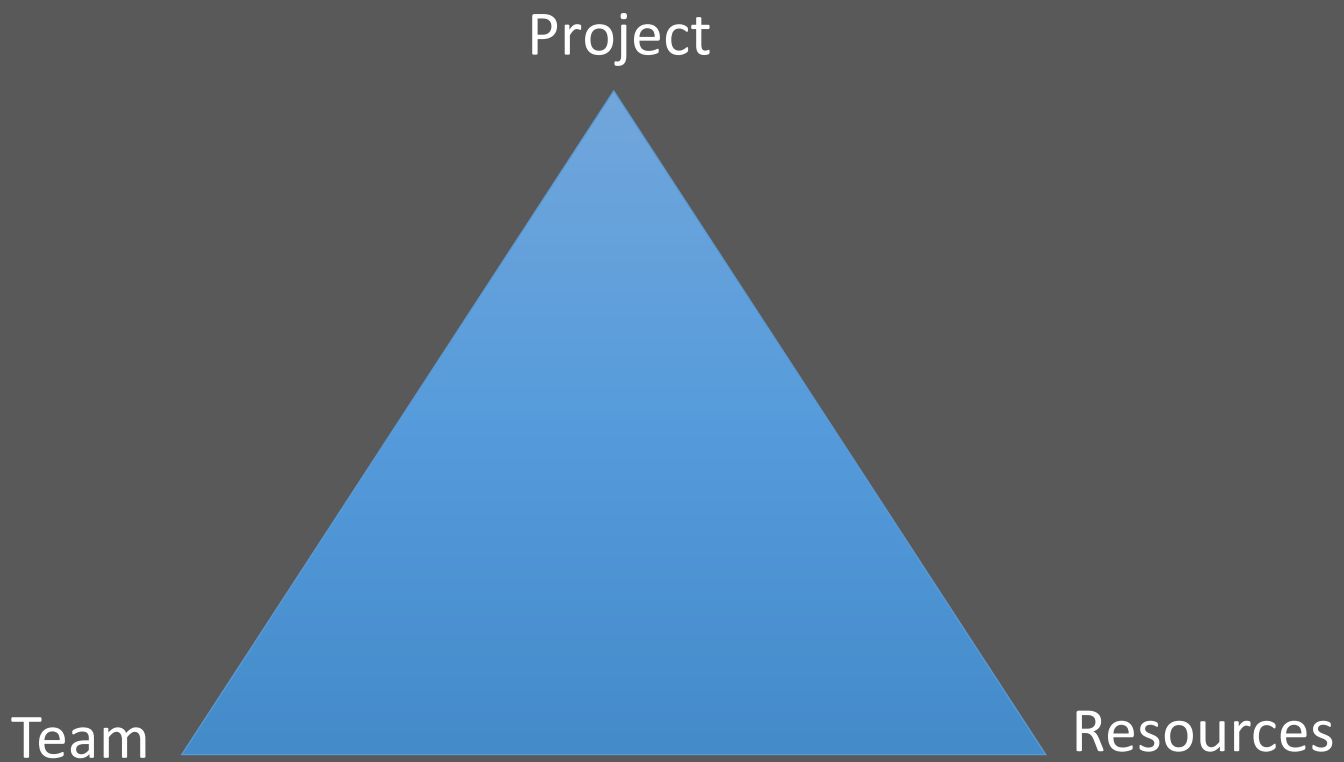
UNIVERSITY OF TORONTO
FACULTY OF MEDICINE

UNIVERSITY OF TORONTO
FACULTY OF MEDICINE

GRANT FUNDAMENTALS AND PROPOSAL DEVELOPMENT

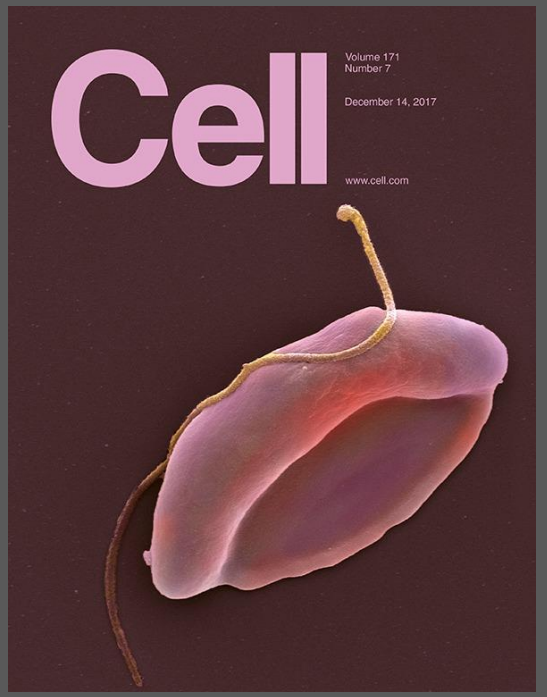
Daniel Harney, PhD
Grants and Awards Editor
Office of the Vice Dean, Research & Innovation

Pre-Writing Fundamentals



The Proposal Triangle

Scientific Paper



Grant Proposal

CIHR IRSC

Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

TENSE

Backward-facing text vs. Forward-Facing text

PURPOSE

To inform vs. To persuade

TONE

Dispassionate vs. Conveying Excitement and Urgency

DICTION

Jargon/Technical vs. Multidisciplinary/Lay

How do Reviewers Read Grant Proposals?

Top Five Reviewer Critiques

UNCLEAR

"The science is hindered by confusing sentence structure. The spelling errors are particularly frustrating to encounter."

Cardiovascular Proteomics and Molecular Therapeutics

Cardiovascular disease is the leading cause of suffering and death in Canada. A new challenge is the emerging epidemic of *heart failure* where one in five Canadians will eventually die from heart failure. The molecular basis of heart failure is complex and poorly understood. This project is aimed at providing a detailed mechanistic insight into this disease and identifying the role of calcium and calcium-binding proteins in the development of heart failure. During the course of my existing CRC, we completed a large scale proteomic investigation of mouse and human models of heart failure as part of a Genome Canada Project entitled "Protein Expression Profiling Platform for Heart Disease Biomarker Discovery". I propose to extend our findings from this study by developing advanced protein-protein interaction (PPI) map of protein networks affected in heart failure. We will use this information to identify novel cellular targets for development of therapeutic interventions and manipulate these targets in an attempt to alter the course of disease. My specific interest and expertise in this project is the application of cellular imaging, mass spectrometry, protein–protein interaction analyses to identify key protein interactions, cardiac stem cell systems, and viral expression methods. This project will be undertaken using mammalian cell lines, human and mouse stem cells, primary cardiac muscle-derived cell cultures, and patient-derived material, to understand cardiac function and disease. Our initial focus will be the identification of protein pathways and interactions involving critical cardiac proteins; however, we will be able to extend this research platform rapidly to other key proteins found to be upregulated significantly in cardiomyopathy. The emphasis will be on discovering novel protein interactions critical for understanding the progress of cardiomyopathy. The ultimate aim of this project is to provide clinically relevant therapeutic methods that can be applied to the diagnosis and treatment of heart failure in human patients.

The Specific Aims of this research proposal can be grouped under the following headings: (1) Identify signaling mechanisms involved in PLN-based cardiac disease; (2) identify protein interactions involving other proteins involved in Ca^{2+}-regulation; and (3) use mouse and human stem cells to investigate cardiac development and disease.

Heart Failure. Heart failure is a severe cardiac disease characterized by low cardiac output, circulatory congestion, both systolic and diastolic dysfunction, and pathological remodeling of the heart. However, the critical early events that impair myocyte performance are unclear. Several signaling pathways have been shown to be involved in the induction of pathological remodeling and heart failure, and many of these pathways are linked to impaired cardiac sarco(endo)plasmic reticulum (SR/ER) Ca^{2+} cycling. In cardiac muscle, Ca^{2+} release is mediated by Ca^{2+}-release channels (ryanodine receptors; RyRs) that tap the Ca^{2+} store in the lumen of the sarcoplasmic reticulum (SR) and plasma membrane Ca^{2+} channels (dihydropyridine receptors; DHPRs) that tap the high concentrations of Ca^{2+} in the extracellular space. Relaxation is achieved by lowering of the myoplasmic Ca^{2+} concentration through the combined activity of the Ca^{2+}-ATPase (SERCA), the plasma membrane Ca^{2+}-ATPase (PMCA) and Na^{+}/Ca^{2+} exchanger (NCX). The failing heart is characterized by impaired removal of cytosolic Ca^{2+}, reduced loading of the cardiac SR, and defective SR Ca^{2+} release. Therefore, the potential role of Ca^{2+} regulatory proteins has been of particular interest in finding a mechanistic solution to the process of heart failure, and is a major focus of our research program.

Previously, with Drs Litsa Kranias (U.Cincinnati) and Jon and Christine Seidman (Harvard), we identified 3 inherited PLN mutations that result in dilated cardiomyopathy (DCM), referred to as PLN-R9C, PLN-L39Stop and PLN-RDel14. The first PLN mutation resulted in a conversion of Arg9 to Cys (PLN-R9C) and it was linked to the dominant inheritance of dilated cardiomyopathy in a large American family with a probability greater than 10,000 to 1 that the mutation was causal of the disease (LOD score >4)[1]. The second PLN mutation, a T116G point mutation that resulted in conversion of leucine 39 to a premature stop codon (L39stop) was identified in two Greek families with hereditary heart failure[2]. A third human mutation, PLN R14Del, which results in the complete removal of the Arg14 codon was found in an extended Greek family with extensive cardiomyopathy[3]. Pathological evidence indicates substantial degeneration of the cardiac muscle of a deceased patient, while echocardiography shows impaired cardiac function in patients harboring the mutation. Transgenic mice overexpressing the R14Del mutant in cardiac muscle results in poor survival with dilated phenotype[3].

Role of the SR/ER in regulation of Ca2+ heart failure. A central characteristic of nearly all cardiomyopathies is significant disruption of intracellular calcium homeostasis[4], which results in considerable biochemical changes, structural remodeling, and general deterioration of the muscle. The mechanisms involved in this abnormal calcium regulation are not understood, nor are all of the downstream signaling responses activated under these pathological conditions. SERCA and PLN are two proteins found in the SR with clear roles in cardiac physiology and disease, but there are many additional cardiac proteins, both in the SR and other organelles, that are involved in regulating calcium in muscle Recently, we have initiated a systemic proteomics approach to investigate proteins and pathways in heart failure, ultimately identifying and quantifying more than 6000 ventricular proteins in five various models of cardiomyopathy. These have included: the R9C model of direct progression to dilated cardiomyopathy[1], two models of progression to cardiac hypertrophy - an aortic banding model [5,6] and a constitutively activated calcineurin (CnA) transgenic model [7], a model of viral-induced cardiomyopathy [8] and a model of myocardial ischemia/infarction [9]. In the first 3 of these models, we acquired proteomic profiles at timed intervals as the diseased hearts progressed directly to dilation or through hypertrophy and dilation to failure. The results of the R9C model have been published [10] and provided the foundation for our proposed investigations described here. Of these proteins, a significantly enriched group was related to disrupted calcium regulation, cytoskeletal remodeling, transcriptional regulation, and ER stress responses, consistent with several previous studies [11-13]. The role of these proteins in the development of cardiac disease is unclear, and elucidating the significance of these proteins is of major interest.

We have demonstrated that PLN mutations can cause human dilated cardiomyopathy [3,19]. However, the mechanisms involved are unclear. It is our goal to clarify how the PLN mutations induce Ca^{2+} overload, how that Ca^{2+} causes ER stress, how ER stress induces apoptosis and dilated cardiomyopathy, and how intervention might alter or prevent their lethal progression.

ER stress is the result of an imbalance between the load of unfolded proteins entering the ER and the capacity of the cell to handle the load [14-17]. The cellular responses to ER stress, involving changes in the synthesis or the activation of hundreds of gene products, are to lower protein synthesis and transport into the ER and to increase the capacity of the ER to handle the load. However, if homeostasis cannot be re-established, apoptosis, or programmed cell death pathways are triggered to protect the cell from mis-folded proteins. Thus, *surmountable* ER stress is balanced against *insurmountable* (lethal) ER stress. These signaling pathways are referred to as the unfolded protein response or UPR. An imbalance between the load of unfolded proteins entering the ER and the capacity of the cell to handle the load initiates 3 responses; 1[a] a reduction in the protein load by lowering protein synthesis and transport into the ER; 2

Cardiovascular disease is the leading cause of suffering and death in Canada. A new challenge is the emerging epidemic of *heart failure* where one in five Canadians will eventually die from heart failure. The molecular basis of heart failure is complex and poorly understood. This project is aimed at providing a detailed mechanistic insight into this disease and identifying the role of calcium and calcium-binding proteins in the development of heart failure. During the course of my existing CRC, we completed a large scale proteomic investigation of mouse and human models of heart failure as part of a Genome Canada Project entitled "Protein Expression Profiling Platform for Heart Disease Biomarker Discovery". I propose to extend our findings from this study by developing advanced protein-protein interaction (PPI) map of protein networks affected in heart failure. We will use this information to identify novel cellular targets for development of therapeutic interventions and manipulate these targets in an attempt to alter the course of disease. My specific interest and expertise in this project is the application of cellular imaging, mass spectrometry, protein–protein interaction analyses to identify key protein interactions, cardiac stem cell systems, and viral expression methods. This project will be undertaken using mammalian cell lines, human and mouse stem cells, primary cardiac muscle-derived cell cultures, and patient-derived material, to understand cardiac function and disease. Our initial focus will be the identification of protein pathways and interactions involving critical cardiac proteins; however, we will be able to extend this research platform rapidly to other key proteins found to be upregulated significantly in cardiomyopathy. The emphasis will be on discovering novel protein interactions critical for understanding the progress of cardiomyopathy. The ultimate aim of this project is to provide clinically relevant therapeutic methods that can be applied to the diagnosis and treatment of heart failure in human patients.

which results in the complete removal of the Arg14 codon was found in an extended Greek family with extensive cardiomyopathy[3]. Pathological evidence indicates substantial degeneration of the cardiac muscle of a deceased patient, while echocardiography shows impaired cardiac function in patients harboring the mutation.

Project Summary

Cardiovascular disease is the leading cause of suffering and death in Canada. A new challenge is the emerging epidemic of *heart failure* where one in five Canadians will eventually die from heart failure. The molecular basis of heart failure is complex and poorly understood. This project is aimed at providing a detailed mechanistic insight into this disease and identifying the role of calcium and calcium-binding proteins in the development of heart failure. During the course of my existing CRC, we completed a large scale proteomic investigation of mouse and human models of heart failure as part of a Genome Canada Project entitled "Protein Expression Profiling Platform for Heart Disease Biomarker Discovery". I propose to extend our findings from this study by developing advanced protein-protein interaction (PPI) map of protein networks affected in heart failure. We will use this information to identify novel cellular targets for development of therapeutic interventions and manipulate these targets in an attempt to alter the course of disease. My specific interest and expertise in this project is the application of cellular imaging, mass spectrometry, protein–protein interaction analyses to identify key protein interactions, cardiac stem cell systems, and viral expression methods. This project will be undertaken using mammalian cell lines, human and mouse stem cells, primary cardiac muscle-derived cell cultures, and patient-derived material, to understand cardiac function and disease. Our initial focus will be the identification of protein pathways and interactions involving critical cardiac proteins; however, we will be able to extend this research platform rapidly to other key proteins found to be upregulated significantly in cardiomyopathy. The emphasis will be on discovering novel protein interactions critical for understanding the progress of cardiomyopathy. **The ultimate aim of this project is to provide clinically relevant therapeutic methods that can be applied to the diagnosis and treatment of heart failure in human patients.**

1. Aims

The Specific Aims of this research proposal can be grouped under the following headings: (1) Identify signaling mechanisms involved in PLN-based cardiac disease; (2) identify protein interactions involving other proteins involved in Ca^{2+}-regulation; and (3) use mouse and human stem cells to investigate cardiac development and disease.

Heart Failure. Heart failure is a severe cardiac disease characterized by low cardiac output, pulmonary congestion, both systolic and diastolic dysfunction, and pathological remodeling of the heart. However, the critical early events that impair myocyte performance are unclear. Several signaling pathways have been shown to be involved in the induction of pathological remodeling and heart failure, and many of these pathways are linked to impaired cardiac sarco(endo)plasmic reticulum (SR/ER) Ca^{2+} cycling. In cardiac muscle, Ca^{2+} release is mediated by Ca^{2+}-release channels (ryanodine receptors; RyRs) that tap the Ca^{2+} store in the lumen of the sarcoplasmic reticulum (SR) and plasma-membrane Ca^{2+} channels (dihydropyridine receptors; DHPRs) that tap the high concentrations of Ca^{2+} in the extracellular space. Relaxation is achieved by lowering of the myoplasmic Ca^{2+} concentration through the combined activity of the Ca^{2+}-ATPase (SERCA), the plasma membrane Ca^{2+}-ATPase (PMCA) and Na^{+}/Ca^{2+} exchanger (NCX). The failing heart is characterized by impaired removal of cytosolic Ca^{2+}, reduced loading of the cardiac SR, and defective SR Ca^{2+} release. Therefore, the potential role of Ca^{2+} regulatory proteins has been of particular interest in finding a mechanistic solution to the process of heart failure, and is a major focus of our research program.

2. Research Plan

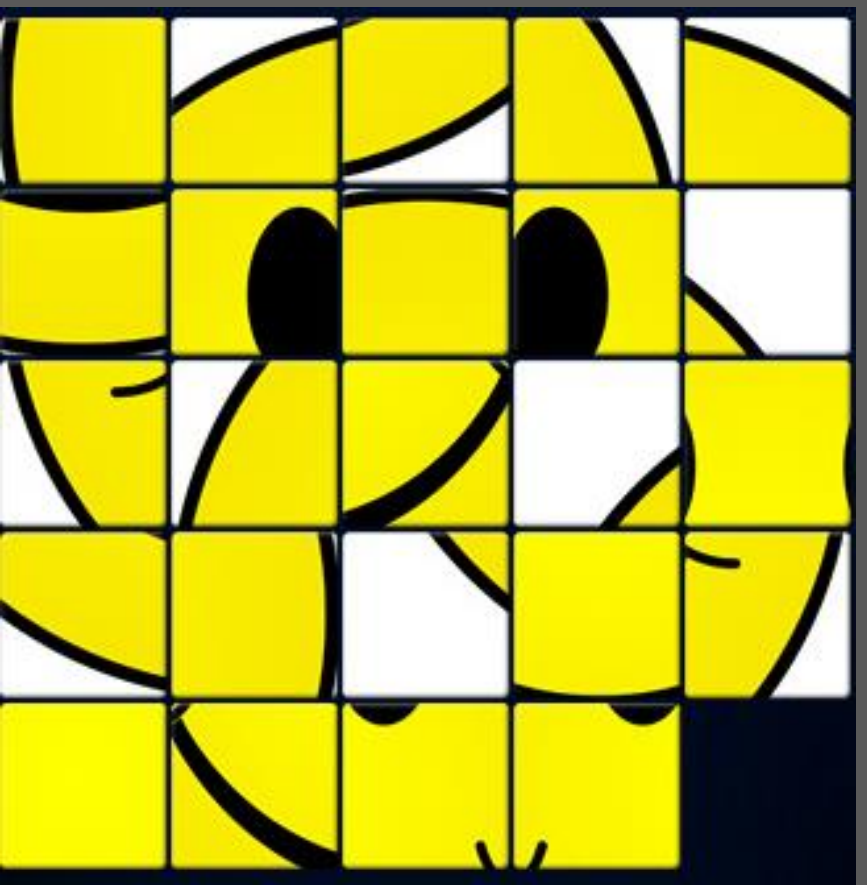
Previously, we identified 3 inherited PLN mutations that result in dilated cardiomyopathy (DCM), referred to as PLN-R9C, PLN-L39Stop and PLN-RDel14. The first PLN mutation resulted in a conversion of Arg9 to Cys (PLN-R9C) and it was linked to the dominant inheritance of dilated cardiomyopathy in a large American family with a probability greater than 10,000 to 1 that the mutation was causal of the disease (LOD score >4)[1]. The second PLN mutation, a T116G point mutation that resulted in conversion of leucine 39 to a premature stop codon (L39stop) was identified in two Greek families with hereditary heart failure[2]. A third human mutation, PLN R14Del, which results in the complete removal of the Arg14 codon was found in an extended Greek family with extensive cardiomyopathy[3]. Pathological evidence indicates substantial degeneration of the cardiac muscle of a deceased patient, while echocardiography shows impaired cardiac function in patients harboring the mutation. **Transgenic mice overexpressing the R14Del mutant in cardiac muscle results in poor survival with dilated phenotype[3].**

Role of the SR/ER in regulation of Ca2+ heart failure. A central characteristic of nearly all cardiomyopathies is significant disruption of intracellular calcium homeostasis[4], which results in considerable biochemical changes, structural remodeling, and general deterioration of the muscle. The mechanisms involved in this abnormal calcium regulation are not understood, nor are all of the downstream signaling responses activated under these pathological conditions. SERCA and PLN are two proteins found in the SR with clear roles in cardiac physiology and disease, but there are many additional cardiac proteins, both in the SR and other organelles, that are involved in regulating calcium in muscle Recently, we have initiated a systemic proteomics approach to investigate proteins and pathways in heart failure, ultimately identifying and quantifying more than 6000 ventricular proteins in five various models of cardiomyopathy. These have included: the R9C model of direct progression to dilated cardiomyopathy [1]; two models of progression to cardiac hypertrophy - an aortic banding model [5,6] and a constitutively activated calcineurin (CnA) transgenic model [7], a model of viral-induced cardiomyopathy [8] and a model of myocardial ischemia infarction [9]. In the first 3 of these models, we acquired proteomic profiles at timed intervals as the diseased hearts progressed directly to dilation or through hypertrophy and dilation to failure. The results of the R9C model have been published [10] and provided the foundation for our proposed investigations described here. Of these proteins, a significantly enriched group was related to disrupted calcium regulation, cytoskeletal remodeling, transcriptional regulation, and ER stress responses, consistent with several previous studies [11-13]. The role of these proteins in the development of cardiac disease is unclear, and elucidating the significance of these proteins is of major interest.

We have demonstrated that PLN mutations can cause human dilated cardiomyopathy [3,19]. However, the mechanisms involved are unclear. It is our goal to clarify how the PLN mutations induce Ca^{2+} overload, how that Ca^{2+} causes ER stress, how ER stress induces apoptosis and dilated cardiomyopathy, and how intervention might alter or prevent their lethal progression. ER stress is the result of an imbalance between the load of unfolded proteins entering the ER and the capacity of the cell to handle the load [14-17]. The cellular responses to ER stress, involving

INCOHESIVE

“The arguments provided in the translation section don’t align with the activities and scope of the proposed projects and requested infrastructure enhancements.”



INSIGNIFICANT

“There was no mention of what will happen if the proposal is not funded.”

Rick Hansen Institute
Institut Rick Hansen

THE REALITY OF SPINAL CORD INJURY

Spinal cord injuries (SCI) have a devastating impact on the health and well-being of individuals. Many would categorize SCI as one of the greatest survivable catastrophes experienced by a human being. Health care services for people who sustain a SCI are highly specialized and complex. Regardless of cause or age at injury, SCI has far reaching consequences for individuals and their families.

SOME FACTS ON SCI IN CANADA

People living with SCI in Canada

86,000

121,000 projected by 2030

Number of new cases of SCI each year

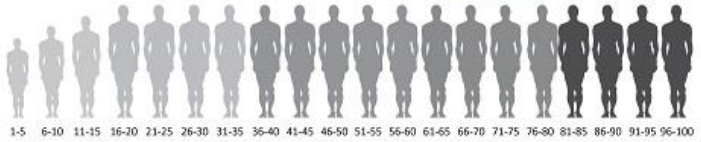
4,300

5,800 a year by 2030

Traumatic SCI occurs most commonly in males between the ages of

20 - 29

years old



...as the population ages more injuries will occur from falls.

COST OF TRAUMATIC SCI

Financial care requirements over a lifetime for each individual can vary from

$1.5 Million
PARAPLEGIA

$3.0 Million
QUADRIPLEGIA

The estimated economic cost of traumatic SCI for newly injured Canadians is

$2.7 Billion
per year

health care, equipment and modifications, long-term care. Costs are even greater when including those with chronic injuries.

CANADIANS WITH TRAUMATIC SCI

COMPARED TO THE GENERAL POPULATION

Are re-hospitalized

2.6x
more often

Require contact with a physician

2.7x
more often

Require home care services

30x
more hours

Have a far shorter life expectancy

15 - 30
fewer years

Sources:
Dryden et al. 2004, "Utilization of health services following spinal cord injury: a six year follow-up study"

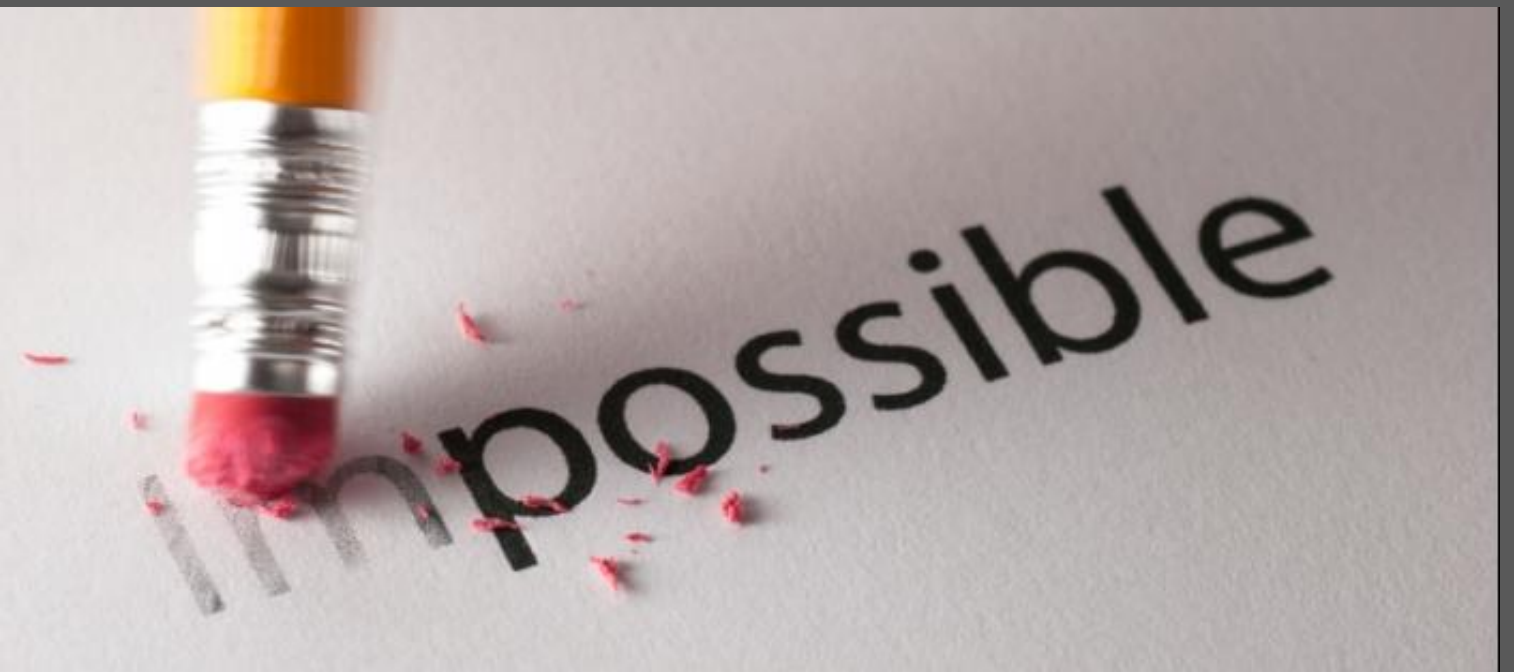
Krueger et al. 2013, "The economic burden of traumatic spinal cord injury in Canada"

Noonan et al. 2012, "Incidence and prevalence of spinal cord injury in Canada: a national perspective"

Urban Futures Institute, 2010, "The Incidence and Prevalence of Spinal Cord Injury in Canada"

INFEASIBLE

"It's not clear to me that the PI has the expertise or the time to complete the 3 ambitious proposed projects in the 5-years of the grant."



4

LACKING IMPACT

“The proposed activities don’t go beyond what has been done and is still fruitlessly being done in other programs.”

5

For additional Grant and Award resources, visit our website:
http://medicine.utoronto.ca/research

Daniel Harney, PhD
Grants and Awards Editor
Faculty of Medicine, University of Toronto
dan.harney@utoronto.ca