UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

MICHAEL JONES

On Monday, August 14, 2023 at 9:30 a.m. (online)

Thesis Title: **Divergence of disease: a characterization of actin residue R312 and its relation to hypertrophic and dilated cardiomyopathy onset**

Examination Committee:

Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. John Dawson, Dept. of Molecular and Cellular Biology
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Tami Martino, Dept. of Biomedical Sciences
Dr. Peter Hwang, Faculty of Medicine and Dentistry, Medicine Dept., University of Alberta (External Examiner)

Advisory Committee:
Dr. John Dawson (Advisor)
Dr. Nina Jones
Dr. John Dutcher

Abstract: Abstract: Cardiovascular disease (CVD) is one of the leading causes of death globally, and many of these have been found to be genetic rather than the result of a poor lifestyle. One such heritable CVD is known as cardiomyopathy (CM), which can be further differentiated into hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). HCM results in a thickening of the left ventricular wall, while DCM results in a thinning of the left ventricular wall. Mutations which have been shown to contribute to CM are found in the sarcomere proteins, which make up the functional unit of our muscle. The predominant theory for CM onset is that mutations within these sarcomere proteins result in changes in the force and sliding velocity of the sarcomere, causing the heart muscle to undergo increased/decreased power output. The consistent upregulation/downregulation of the power output of the cardiac tissue is thought to result in changes in ventricular myocardium size. Within the mutations on actin that have been linked to CM, those on residue R312 are of interest, because of the fact that R312 substitutions have been linked to both HCM and DCM. Here, we classified two mutations of cardiac actin on this residue: R312C and R312H, linked to HCM and DCM respectively. We also aimed to produce a method for testing novel CM therapeutics on the various actin mutations linked to CM. We found that R312C and R312H produced similar biochemical effects, with increases in basal thin filament velocity under low calcium and rightward pCa (-log[Ca2+]) curve shifts. We also discovered the R312C and R312H variants demonstrated a reduction in tropomyosin elongation rates, which is a main regulatory protein in the sarcomere. Taken together, it is clear that the R312-ACTC variants disrupt the regulatory components of the sarcomere. Due to the similar biochemical features, with varying levels of severity, we hypothesize that the disease differentiation is likely due to R312H-ACTC progressing from HCM to DCM, rather than disease differentiation from onset. We also demonstrated changes to the pCa curves of WTRec-ACTC (Wildtype recombinant actin) with the addition of trifluoperazine (TFP), a calcium sensitizer, as

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well as changes to heart rate and survivability of zebrafish (*Danio rerio*). This therapeutic trial resulting from TFP addition allows us to follow a similar framework for future CM therapeutics soon to be available to patients suffering from CM. Hopefully, this research will contribute to changes in the way those suffering with CM are treated and improve their quality of life.

Curriculum Vitae: Michael completed his Bachelor of Science (Honours) in Biochemistry at the University of Guelph in April 2019. He then began his PhD in Molecular and Cellular Biology at the University of Guelph in May 2019 under the supervision of Dr. John Dawson.

Awards: Graduate Tuition Scholarship (2019-2023)

Publications: **Jones, M. R.**, Tran, C., Singh, J., and Dawson, J. F. (2022) A gradient of force generation at rest differentiates cardiomyopathy outcomes with variants of actin located at the same residue. *Journal of Molecular and Cellular Cardiology Plus*. 10.1016/J.JMCCPL.2022.100023

Prill, K., **Jones, M. R**., Steffensen, K., Teng, G. Z., and Dawson, J. F. (2023) Increasing the calcium sensitivity of muscle using trifluoperazine-induced manipulations in silico, in vitro and in vivo systems. *Arch Biochem Biophys*. 10.1016/J.ABB.2023.109521

Jones, M. R., Steffensen, K, Pace, A., Misini, E., Dawson, J. F. (2023) Analysis of Regulation Impairments Resulting from Mutations on Residue R312. In preparation to be submitted for peer review.