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*

CASEY WILLIAMSON

on Tuesday, April 23rd, 2024 at 1:00p.m. (SSC 3317)

Thesis Title: THE IMPACTS OF IMPAIRED NEPHRIN-NCK SIGNALING ON DIABETIC OUTCOMES: A CHARACTERIZATION FROM THE PANCREAS TO THE KIDNEY

Examination Committee:

Dr. Jennifer Geddes-McAlister, Molecular and Cellular Biology (Exam Chair)
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Jim Petrik, Dept. of Biomedical Sciences, OVC
Dr. Andrew Bendall, Dept. of Molecular and Cellular Biology
Dr. Darren Yuen, Dept. of Physiology and Pharmacology, University of Toronto (External Examiner)

Advisory Committee:
Dr. Nina Jones (Adv)
Dr. Jim Petrik
Dr. Jeremy Simpson

Abstract: The nephrin-Nck adaptor signaling axis is a powerful modulator of diverse cell functions expressed in pancreatic β cells and kidney podocytes, two vulnerable cell types in diabetes and diabetic nephropathy (DN). In β cells, a limited number of studies have implicated nephrin in insulin secretion. In podocytes, nephrin-Nck signaling is required to maintain actin-rich foot processes (FPs) and the slit diaphragm (SD), a filtration barrier that prevents large plasma protein excretion. To potentially elicit these functions, spatial clustering of nephrin can lead to its phosphorylation at three tyrosines within YDxV motifs which recruit Nck. The Nck adaptor paralogs, Nck1 and Nck2, share many functions downstream of these nephrin motifs, although their paralog-specific functions remain largely uninvestigated.

Importantly, the expression pattern in these cell types suggest that nephrin-Nck signaling might influence diabetic outcomes. Thus, it is first hypothesized that nephrin YDxV motifs regulate insulin secretion and glucose tolerance *in vivo*. Next, as DN is associated with mechanical strain and podocyte detachment, it is hypothesized that nephrin YDxV motifs regulate contractility and adhesion to mitigate DN injury. Lastly, evidence has implicated Nck2 in podocyte injury responses, and it is hypothesized this paralog has distinct functions to mitigate DN injury.

Using nephrin-Y3F mice, which have three phenylalanine residues substituted at YDxV tyrosines, loss of these residues did not impair glucose tolerance and led to increased insulin secretion with age. To determine mechanosignaling roles, we showed that nephrin clustering in podocytes effectively induced

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actomyosin contractility and adhesion activation in a pYDxV-dependent manner. Demonstrating this role *in vivo*, diabetic nephrin-Y3F mice had exacerbated proteinuria, glomerular hypertrophy, and evidence of increased podocyte detachment. In elucidating Nck paralog-specific roles, a transcript microarray was performed in Nck1KO or Nck2KO cultured podocytes, resulting in many overlapping transcriptomic changes that suggest their role in epithelial-mesenchymal plasticity. In contrast, diabetic Nck2KO mice, but not Nck1KOs, had pronounced proteinuria and abnormal FP ultrastructural features.

To summarize, impaired nephrin pYDxV signaling enhances insulin secretion with age, and this signaling axis, likely with the involvement of Nck2, can elicit adaptive mechanosignaling to mitigate DN injury.

Curriculum Vitae: Casey completed her Bachelor of Science (Honours) with a major in Biomedical Biology and a minor in Biochemistry at Laurentian University in Winter 2015. In Summer 2017, she completed her Master of Science in Biology at Laurentian University in the lab of Dr. TC Tai. In Fall 2017, she began her PhD research in Molecular and Cellular Biology at the University of Guelph under the supervision of Dr. Nina Jones.

Awards: Natural Sciences and Engineering Research Council of Canada (NSERC) Postgraduate Scholarship – Doctoral (2018); Department of Molecular and Cellular Biology - Roche Molecular Biochemicals Award of Excellence (2018); College of Biological Science – Graduate Entrance Excellence Scholarship (2017); NSERC Canada Graduate Scholarship – Master's Program (2016); Ontario Graduate Scholarship (2015); NSERC Undergraduate Student Research Awards (2013 and 2014)

Publications:
A Mahesaniya*, CR Williamson*, A Keyvani Chahi, CE Martin, AE Mitro, P Lu, LA New, KL Watson, RA Moorehead, and N Jones (2022). Sex differences in glomerular protein expression and effects of soy-based diet on podocyte signaling. *Can J Kidney Health Dis*. 9:20543581221121636.

CE Martin, NJ Phippen, A Keyvani Chahi, M Tilak, SL Banerjee, P Lu, LA New, CR Williamson, MJ Platt, JA Simpson, M Krendel, N Bisson, AC Gingras, and N Jones (2022). Complementary Nck1/2 signaling in podocytes controls α-actinin-4-mediated actin organization, adhesion, and basement membrane composition. *J Am Soc Nephrol*. 33:1546-1567.

J Lamothe, S Khurana, S Tharmalingam, C Williamson, CJ Byrne, N Khaper, A Kumar, and TC Tai (2021). Oxidative stress mediates the fetal programming of hypertension by glucocorticoids. *Antioxidants*. 10:531.

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CR Williamson, S Khurana, P Nguyen, CJ Byrne, and TC Tai (2017). Comparative analysis of renin-angiotensin system (RAS)-related gene expression between hypertensive and normotensive rats. *Med Sci Monit Basic Res.* 23:20-24.

S Khurana, CJ Byrne, S Mercier, J Lamothe, CR Williamson, J Grandbois, and TC Tai (2015). Role of Adrenal Hormones in the Fetal Programming of Hypertension. In G Santulli (Ed.). *Adrenal Glands: From Pathophysiology to Clinical Evidence.* Hauppage, New York: Nova Science Publishers.

*Authors contributed equally