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

CLAIRE MARTIN, on Wed. December 13, 2017 at 9:30 a.m. in SSC 2315

(Advisor: Dr. Nina Jones)

Thesis Title: **Characterization of signaling pathways regulating nephrin endocytosis in kidney podocytes: novel roles for Nck and ShcA adaptor proteins**

Examination Committee:

Dr. A. Nassuth, Dept. of Molecular and Cellular Biology (Chair)
Dr. N. Jones, Dept. of Molecular and Cellular Biology
Dr. R. Moorehead, Dept. of Biomedical Science
Dr. S. Ryan, Dept. of Molecular and Cellular Biology
Dr. Jean-Francois Côté, Institut de recherches Clinique de Montréal (IRCM)

Abstract: The podocyte is a unique epithelial cell type that contributes to the kidney's blood filtration barrier. Filtration selectivity is largely determined by podocyte slit diaphragms, intercellular junctions found laterally between neighbouring cells. These create a discerning molecular sieve that blocks the loss of essential blood components into the urine, a common and detrimental complication of kidney disease. Nephrin makes up the core of the slit diaphragm and it must withstand significant insult as a function of its barrier role. Although regulation of nephrin expression on the cell surface is essential for its function, little is known about the mechanisms responsible for its maintenance. Using comprehensive cell and animal modeling, we sought to investigate the role of disrupted nephrin turnover in kidney dysfunction and to determine the regulatory mechanisms that govern this pathway. We demonstrate that nephrin tyrosine phosphorylation is commonly disrupted in the injured podocyte, and that this results in loss of filtration selectivity, which is associated with disrupted interactions between nephrin and the cytoskeletal adaptor Nck. We characterize a requirement for multivalent nephrin/Nck interactions during nephrin's endocytosis in cells, which is attributed to Nck's ability to recruit actin and dynamin. Targeted disruption of nephrin/Nck interactions also inhibits nephrin's turnover in mice and this contributes to kidney disease during aging. Intriguingly, aberrant nephrin phosphorylation and endocytosis is observed in several models of acute injury, and this is associated with heightened levels of the ShcA phosphotyrosine adaptor, a protein not previously described within the podocyte. We determine that ShcA overexpression induces a feed-forward mechanism to stimulate nephrin's internalization, leading to barrier breakdown. Notably, ShcA expression is also upregulated in several forms of human kidney disease, supporting the relevance of this mechanism in humans. Collectively, this work has verified that deregulation of nephrin turnover results in filtration barrier demise, and identifies Nck and ShcA as two novel mediators of nephrin endocytosis. Integration of these pathways into the larger framework of nephrin trafficking, a rapidly growing area of interest, remains an important objective for future work.

Curriculum Vitae: Claire obtained her B.Sc. in Biomedical Science at the University of Guelph in 2011, and began her MSc in the laboratory of Dr. Nina Jones in September of the same year. In the fall of 2012, Claire transferred to the PhD program.

Awards: Dr. Donald Robert Phillips Molecular and Cellular Biology Scholarship (2017); NSERC CGS D (2014-2015/ 2016-2017; disruption due to maternity leave); American Society of Nephrology Kidney STARS Scholarship (2015); CIHR Travel Scholarship (2015); NSERC MSFSS (2015); OGS (2013-2014); NSERC CGS M (2011-2012)

Publications: Martin CE, New LA, McNeilly R, Keyvani Chahi A, Mitro A, Lu P, Blasutig IM, Jones N. Multivalent interactions between nephrin and Nck modulate nephrin's endocytosis. *Under review at J Am Soc Nephrol.*

Martin CE, Petersen KA, Aoudjit L, Tilaq M, Hardy R, Quaggin SE, Takano T, Jones N (2017) ShcA adaptor protein promotes nephrin endocytosis and is upregulated in proteinuric nephropathies. *J Am Soc Nephrol.* Epub ahead of print 2017 Oct 10.

> Article highlighted in Nature Reviews Nephrology: Carney EF (2017) Podocytes: ShcA regulates nephrin turnover. *Nat Rev Nephrol*. Epub ahead of print 2017 Oct 30.

New LA, Martin CE*, Scott RP*, Platt MJ, Keyvani Chahi A, Stringer CD, Lu P, Samborska B, Eremina V, Takano T, Simpson JA, Quaggin SE, Jones N (2016) Nephrin Tyrosine Phosphorylation Is Required to Stabilize and Restore Podocyte Foot Process Architecture. *J Am Soc Nephrol.* 27(8):2422-35. *(*denotes equal contributions).*

> Article highlighted in Nature Reviews Nephrology. Carney EF (2016) Podocyte biology: Phosphorylation preserves podocytes. *Nat Rev Nephrol*. 12(4):197.

Keyvani Chahi A, Martin CE, Jones N (2016). Nephrin Suppresses Hippo Signaling through the Adaptor Proteins Nck and WTIP. *J Biol Chem.* 291(24):12799-808.

Clouthier DL, Harris CN, Harris RA, Martin CE, Puri MC, Jones N (2015) Requisite role for Nck adaptors in cardiovascular development, endothelial-to-mesenchymal transition, and directed cell migration. *Mol Cell Biol*. 35(9):1573-87.

> Article highlighted as a spotlight article, selected by the editors.

Martin CE*, New LA* and Jones N (2014) Advances in slit diaphragm signaling. *Curr Opin Nephrol Hypertens.* (4):420-30. *(* denotes equal contributions).*