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Characterization of Novel Genes of Importance for Renal Glomerular Function and Disease

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Thesis for Doctor of Philosophy in medical sciences
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Abstract

Glomerular kidney diseases are a major health care burden. The glomerular filtration barrier consists of three layers: the slit diaphragm that bridges the interlocking foot processes of the podocytes, the glomerular basement membrane and fenestrated endothelial cells. The filtration barrier is permselective to plasma macromolecules based on size, shape, and charge. The molecular makeup of the filtration barrier determines its permselectivity. Knowledge about the molecular mechanisms of the glomerular filtration barrier has been gained with the study of genes mutated in humans and animal models of glomerular kidney disease.

In the thesis work, we performed a proteome analysis of healthy glomeruli in mice using two-dimensional gel electrophoresis coupled to mass spectrometry. A total of 232 unique proteins were identified from 414 gel spots. This study provided a snapshot of the glomerular proteome that can serve as reference for future glomerular protein biomarker studies.

We describe the expression and physiological function of the gene Glcci1 in zebrafish. Histological analysis of Glcci1 showed expression in podocytes and mesangial cells. *In vivo* and *in vitro* studies demonstrated that Glcci1 expression is induced by glucocorticoids. Depletion of Glcci1 by morpholino knockdown resulted in the development of pericardial edema and defects in glomerular filtration. Our results suggest a role for Glcci1 in glomerular injury and proteinuria.

Knockdown experiments of the paralogs Plekhh1 and Plekhh2 in zebrafish resulted in gross morphological changes in the glomerulus, including thickening of the glomerular basement membrane and disorganization of the podocyte foot processes associated with a defective filtration barrier. These results suggest a role for Plekhh1 and Plekhh2 in regulating podocyte foot process morphology in zebrafish. We further characterized Plekhh1 and Plekhh2 in knockout mouse models. Single knockouts of Plekhh1 and Plekhh2 do not develop any apparent phenotype. Plekhh1 and Plekhh2 deficient mice were intercrossed to produce mice lacking both genes. This yielded fewer than expected number of double knockout offspring, suggesting functional redundancy. Ultrastructural analysis of surviving double knockout mice did not reveal changes in glomerular morphology suggesting that Plekhh1 and Plekhh2 are largely redundant for kidney function in mice.

These results give insight into glomerular biology and pathomechanisms of kidney disease that might provide a basis for translational research in the future.

Keywords: Kidney glomerulus, podocytes, slit diaphragm, proteinuria, zebrafish, knockout mouse.

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