**Kidney function below par: The Key Role of the PAR-1 Receptor in the Podocyte in circulating factor Nephrotic Syndrome.**

Great progress has been made over the last few years in the molecular mechanisms of genetic nephrotic syndrome. However, the physiopathology of idiopathic nephrotic syndrome has been difficult to elucidate. The role of a hitherto unknown circulating factor or factors in idiopathic nephrotic syndrome is well established. Whilst genetic forms of nephrotic syndrome respond well to kidney transplant, patients with the idiopathic form fare less well: with post-transplant disease recurrence leading to graft loss in up to 60% of cases. There is a clear need for a more targeted approach in this form of the disease. Currently, research is focussed on identifying the circulating factor(s). Indeed, there have been several candidates published in the literature over the years.

Work previously published by our group suggest a role for serine proteases. Human podocytes in culture respond to post-transplant relapse plasma by phosphorylating VASP, a known actin cytoskeleton regulator. This response is not seen when the podocytes are treated with remission plasma from the same patient. The response could also be blocked by co-incubating the cells with protease inhibitors. Crucially, the response was blocked by knock down of the Protease Activated Receptor 1 (PAR-1)

To definitively establish a direct role for PAR-1 in proteinuria, a transgenic mouse was developed that expresses a podocyte specific constitutively active form of PAR-1. We hypothesise that this replicates over exposure to a circulating protease and hence causes idiopathic nephrotic syndrome.

Usually, the PAR-1 receptor is proteolytically activated by serine proteases (thrombin, trypsin etc), and rapidly recycled. When the PAR-1 receptor is made phospho-null intracellularly, the mutant form of the protein not recycled. It remains active and hence continues to stimulate downstream signalling.

Transgenic mice were generated expressing this mutant PAR-1. The transgene was inserted at the Rosa 26 locus. These mice were crossed with Pod-Cre mice to establish a mouse line in which the PAR1 is switched on throughout development and Pod-rtTA Tet-O-Cre miceto establish a mouse line in which the PAR1 can be switched on inducibly.. Kidneys were harvested and processed for histological staining and EM as indicated.

Wild-type human podocytes treated with a PAR-1 agonist *in vitro* showed pro-migratory signalling events and, indeed, a more motile phenotype than untreated podocytes.

The developmental PAR-1active mice were born normal, and died consistently between the ages of 39 and 45 days. They demonstrate proteinuria from the age of 14 days. The level of proteinuria increases considerably over time. By day 40 they have significantly higher blood urea and creatinine (mean 70mMol/L, 60μMol/L respectively), suggesting the mice die of renal failure. EM analysis showed a significantly thickened GBM and foot process effacement. PAS and Masson’s Trichrome staining over a time-course ranging from 8 to 40 days demonstrated an initial hypercellularity within the Bowman’s capsule at 8 days that appear to reduce by 14 days. By 30 days there is clear evidence of fibrosis and by 40 days there is both fibrosis and sclerosis. The inducible PAR-1 Active mice show a similar phenotype although less severe.

This work clearly demonstrates the role of PAR-1 in the pathogenesis of idiopathic nephrotic syndrome. The similarities between the phenotype in the transgenic mice and human FSGS support this PAR-1 overactive mouse as the first model of a circulating factor nephrotic syndrome.