The nephrocyte actin and tubulin cytoskeleton networks model slit diaphragm structural defects pertaining to podocyte pathogenesis

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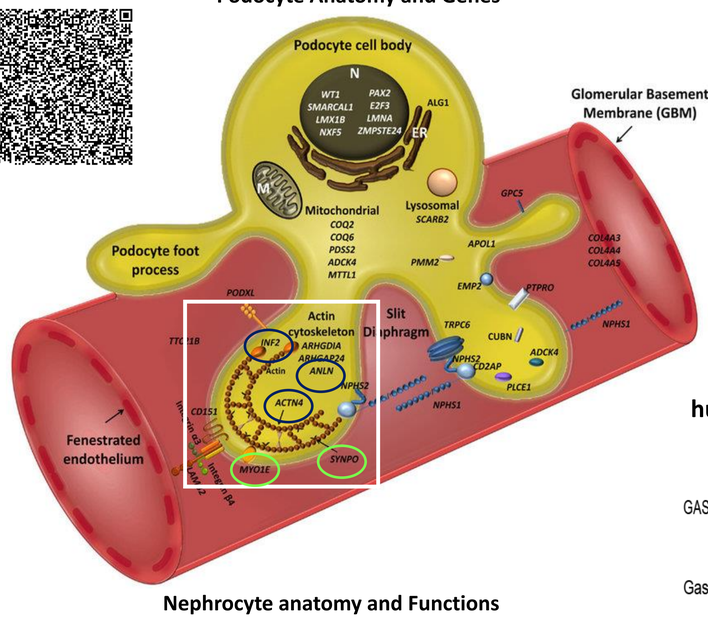
Abstract

- The nephrocyte is the structural and functional equivalent of a human podocyte in *Drosophila melanogaster,* particularly the slit diaphragm
- The nephrocyte was used to determine the relationship between nephrotic function, the actin and tubulin cytoskeletons, and their shared binding proteins
- Of 18 shared binding proteins between the actin and tubulin cytoskeletons, the knockdown of Pigs results in the most dramatic phenotype
- Immunofluorescent staining was used to see morphological differences in actin, tubulin, dual actin and tubulin binding protein, and insulin signaling genes
- This information can be used to aid further research into causes and solutions to renal diseases such as Nephrotic Syndrome and Diabetic Nephropathy

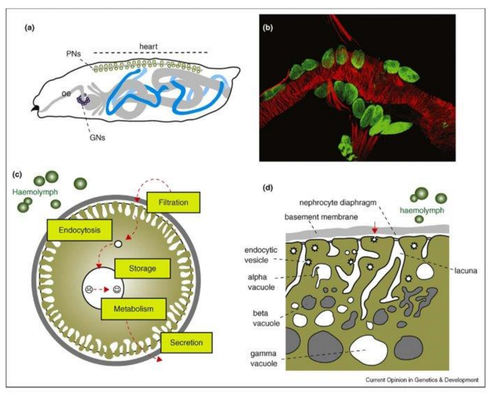
Introduction

References

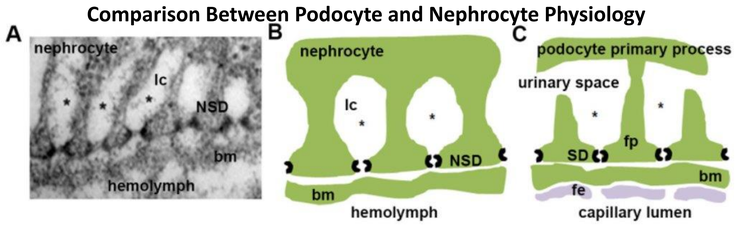
Podocyte Anatomy and Genes



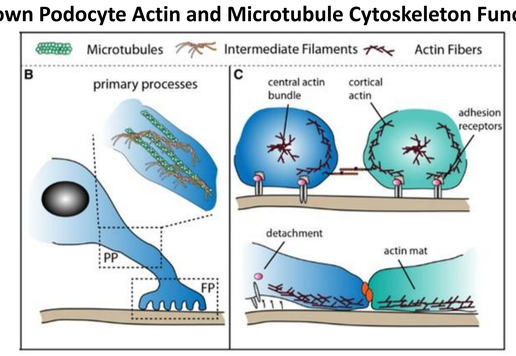
Nephrocyte anatomy and Functions



Comparison Between Podocyte and Nephrocyte Physiology



Known Podocyte Actin and Microtubule Cytoskeleton Functions



Results

Figure 1: Mutations to Tubulin genes and Insulin-signaling genes cause morphological differences in the Slit Diaphragm, the Actin cytoskeleton, and the Tubulin

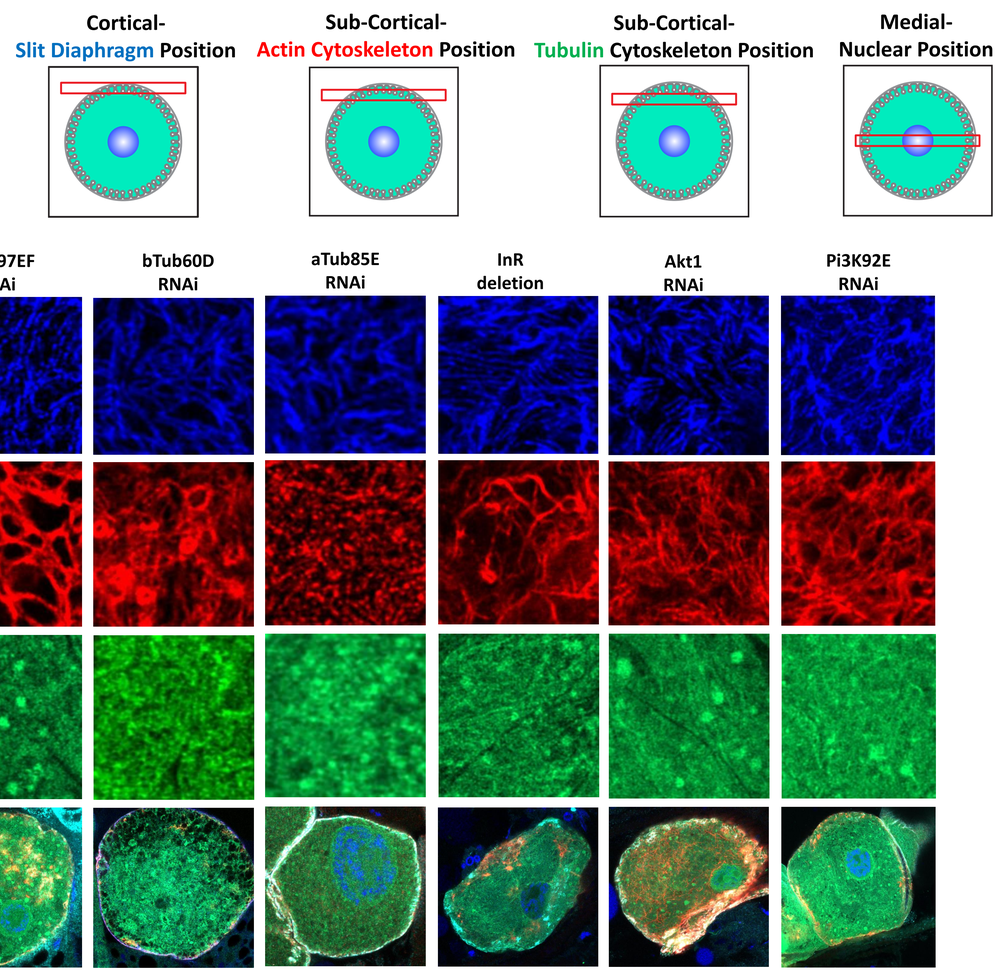
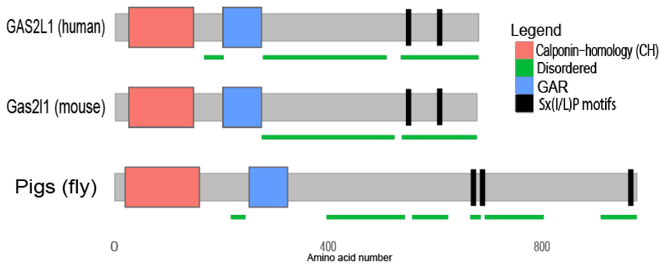


Figure 2: Pickled Eggs (Pigs) is an Actin-binding and Tubulin-binding protein, has conserved domains in humans and mice, and highly expressed in both embryonic and adult nephrocytes



Fly Gene	Human homologue	Mouse homologue	Max DIOPT score	Actin-binding domain	Microtubule lattice-binding domain	Microtubule plus-end binding motif
Pickled eggs	GAS2L1	Gas2l1	11	Calponin-homology (CH)	GAS2	Sx(I/L)P

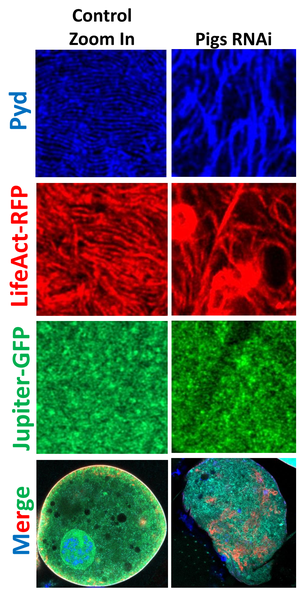


Table 1: Dual Actin-Binding and Tubulin-Binding Genes Previously Related to Kidney Diseases

Human Symbol	H2F Fly Symbol	F2H Max DIOPT score	Actin Binding	Tubulin Binding	Nephrotic Syndrome	Kidney Phenotype	DisGeNET Phenotype
HDAC6	HDAC6	14	Yes	Yes	No	Yes	Polycystic kidney diseases
INO80	Ino80	14	Yes	Yes	No	Yes	Chronic kidney diseases
LRRK2	Lrrk	8	Yes	Yes	No	Yes	Renal cell carcinoma; Conventional (clear cell) renal cell carcinoma; Chromophobe renal cell carcinoma; Sarcomatoid renal cell carcinoma; Collecting duct carcinoma of the kidney; Papillary renal cell carcinoma
NCALD	Nca	13	Yes	Yes	No	Yes	Diabetic nephropathy

Table 2: Actin Cytoskeleton and Tubulin Cytoskeleton Binding Proteins Previously Related to Nephrotic Syndrome

Human Symbol	H2F Fly Symbol	F2H Max DIOPT score	Actin Binding	Tubulin Binding	Nephrotic Syndrome	Kidney Phenotype	DisGeNET Phenotype
ACTN4	Actn	Actn	13	Yes	No	Yes	**Diabetic nephropathy**; Focal glomerulosclerosis; Proteinuria; **Nephrotic syndrome, focal and segmental glomerular lesions**; **Nephrotic syndrome, steroid-resistant, autosomal recessive**; **Nephrotic syndrome with focal and segmental hyalinosis**; **Nephrotic syndrome with focal and segmental sclerosis; Nephrotic syndrome with focal glomerulonephritis**; Focal segmental glomerulosclerosis 1
ANLN	scra	scra	10	Yes	No	Yes	Focal glomerulosclerosis**; Nephrotic syndrome, steroid-resistant, autosomal recessive**; Focal segmental glomerulosclerosis 8
AVIL	Gel	Gel	13	Yes	No	Yes	**Nephrotic Syndrome 1; Steroid-resistant Nephrotic Syndrome**
CFL1	CG6873: :tsr	CG6873::tsr	11	Yes	No	Yes	**Diabetic nephropathy; Nephrotic syndrome**; Hypertensive nephropathy
DAAM2	DAAM	DAAM	15	Yes	No	Yes	**Nephrotic Syndrome 4**
GSN	Gel	Gel	13	Yes	No	Yes	**Nephrotic syndrome; Chronic kidney disease stage 5**
INF2		form3	7	Yes	No	Yes	Focal glomerulosclerosis; **Nephrotic syndrome**; Proteinuria; Kidney failure; Hyalinosis, segmental glomerular; Renal glomerular disease; **Nephrotic syndrome, steroid-resistant, autosomal recessive**; Focal segmental glomerulosclerosis 5; Focal segmental glomerulosclerosis, not otherwise specified
MYO1E		Myo31DF	14	Yes	No	Yes	Focal glomerulosclerosis; **Nephrotic syndrome; Nephrotic syndrome, steroid-resistant, autosomal recessive**; Focal segmental glomerulosclerosis 6
SYNPO		CG1674	2	Yes	No	Yes	Focal glomerulosclerosis; Focal segmental glomerulosclerosis, not otherwise specified; **Nephrotic Syndrome**

Currently Known

- 79 Actin and Tubulin Binding proteins already related to kidney-related diseases – ClinVar and DisGeNET databases
- 31 are Tubulin-binding only
- 43 are Actin-binding only
- 4 are dual binding proteins
- 9 are related directly to Nephrotic Syndrome, but all are actin-binding

Conclusions

- The knockdown of Tubulin genes effect the SD, actin, and tubulin structures
- The knockdown of important insulin signaling pathway genes results in the structural disruption of the SD, the actin cytoskeleton, and the tubulin cytoskeleton
- There are known binding protein mutations that result in both Nephrotic Syndrome and Diabetic Nephropathy, including those that bind to both actin and tubulin cytoskeletons
- Drosophila melanogaster is a viable model organism to study podocyte defects pertaining to the Actin and Tubulin Cytoskeletons

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