Convincing Clinicians to Use Functionalized Genomic Medicine

Howard J. Jacob, Ph.D.
Executive Vice President for Medical Genomics
Chief Medical Genomics Officer
Faculty Investigator
@hudsonalpha
@howardJacob_phd

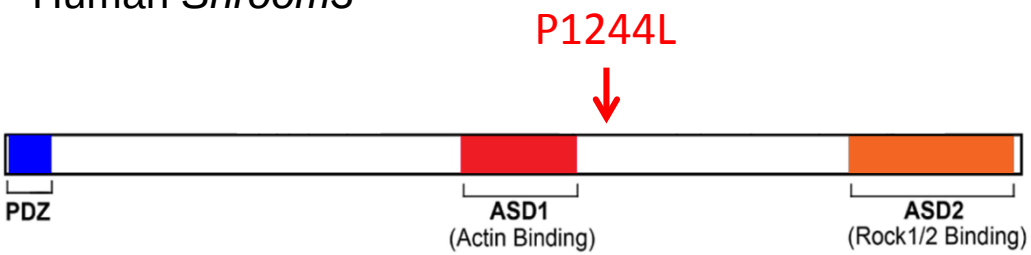
HUDSONALPHA
INSTITUTE FOR BIOTECHNOLOGY

Overview of my talk

- GWAS and Clinical Sequencing are changing how we practice and will practice medicine and research.
- Levels of Evidence
  - Traditional: QTL to Gene in animal models
  - From QTL to Gene using GWAS
  - From GWAS gene to variants
  - Testing a Variant of Uncertain Significance (VUS).
- Summary and Conclusions

Patient within the CKDgen

Human *Shroom3*



P1244L This variant is a VUS

```
Danio_JX455752                 RPIPQHFRSKSSPVENV-SQDFLARDLQ
Xenopus_shroom3                -PPALHVRSRSSPASDMKSREYMSRQEV
Gallus_shroom3_880_1062        IAYVPVHTRSRSSPTADKNHQDLLRRESS
Human_shroom3_883_1066         LAGPVHVRSRSSPATADKRQDVLLGQDS
Chimp_Shroom3_622_768          LAGPVHVRSRSSPATADKRQDVLLGQDS
Rat_shroom3_890_1074           LTVPVHVRSRSSPTSDKKGQDVLLREDS
Mouse_shroom3_881_1060         LAVPVHVRSRSSPTSDKKGQDVLLREGS
                                     * **:***.     :: :  :
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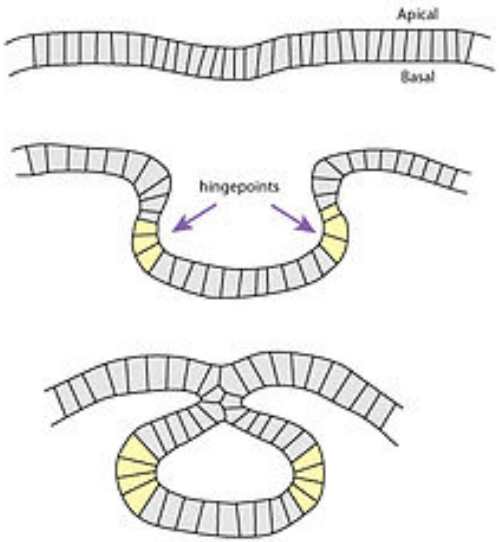
What data would you require to say this variant causes Chronic Kidney Disease in a Medical Record?

Nomination of *SHROOM3* by GWAS

- One of the most reproducible risk loci
- Renal function of *SHROOM3* is not known
- 11 GWAS have reported *SHROOM3* variants as being associated with markers of chronic kidney disease
  - Glomerular filtration rate
  - Albuminuria
- Association observed in virtually all populations tested, including European and East Asian

Shroom3

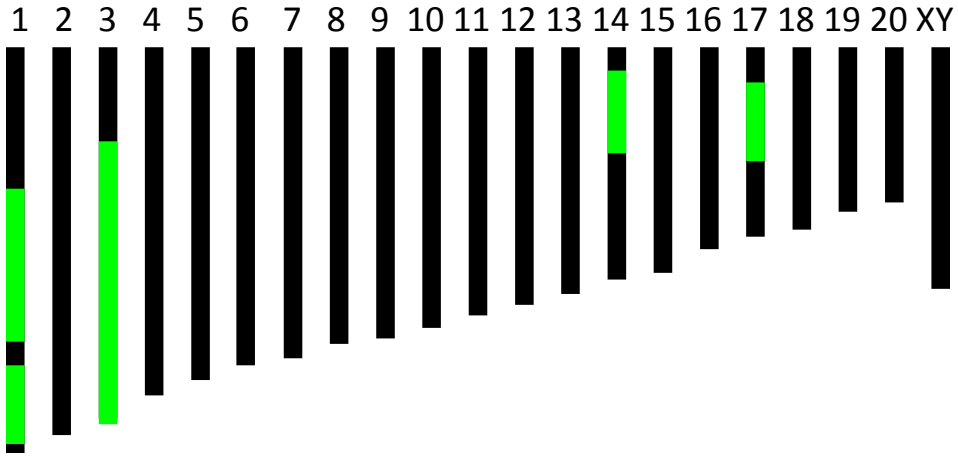
- *Shroom3 e*ncodes a cytoskeletal protein that plays a critical role in epithelial cell morphogenesis
- First identified as an important factor for neural tube closure
- Homozygous *Shroom3* null mice are embryonic lethal due to neurulation defect



RAT DATA

QTL to Variant

Renal failure 1-5 (Rf-1-5)

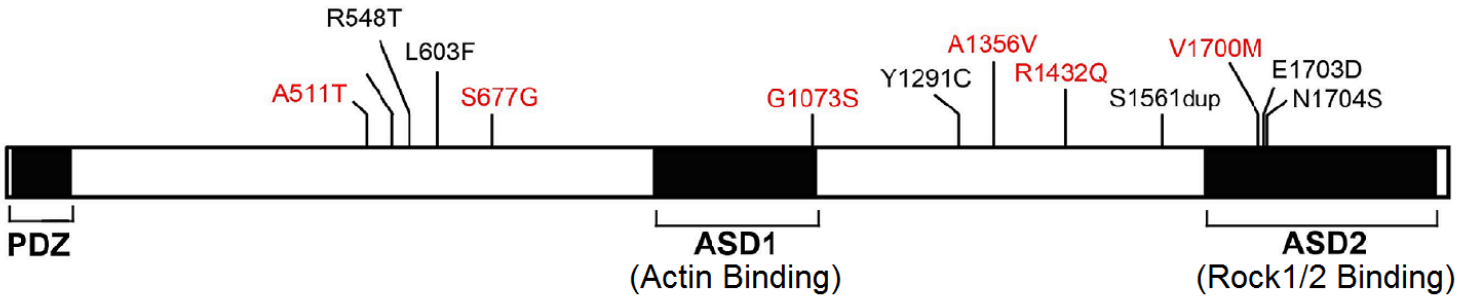


QTLs≈	Chromosome	QTL Size	Phenotypes	Human QTL
Rf-1	1q55	30 Mb	FSGS, UPV/UAV, Palb	ESRD in AA* CCr #
Rf-2	1q32	35 Mb	UPV/UAV	Familial FSGS&
Rf-3	3q1-q2	D3mit4	FSGS, UPV/UAV, Palb	
Rf-4	14p1-q1	14 Mb	FSGS, UPV/UAV, Palb	Cr, CCr, GFR% Diab. Neph. !
Rf-5	17p1-q1	D17mit12	UPV/AUV	

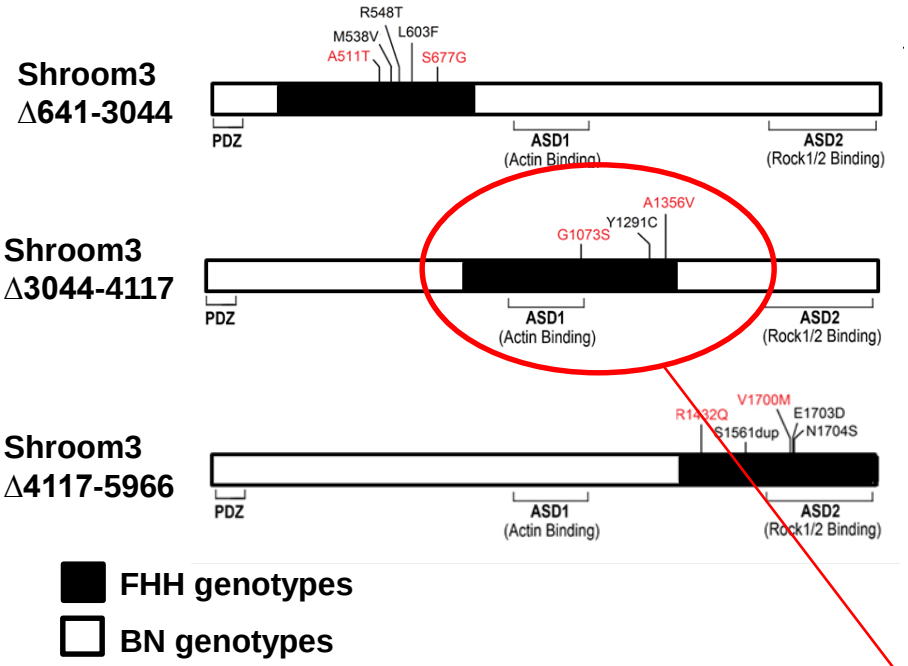
The FHH *Shroom3* allele harbors coding variants, compared to Brown-Norway (BN) control

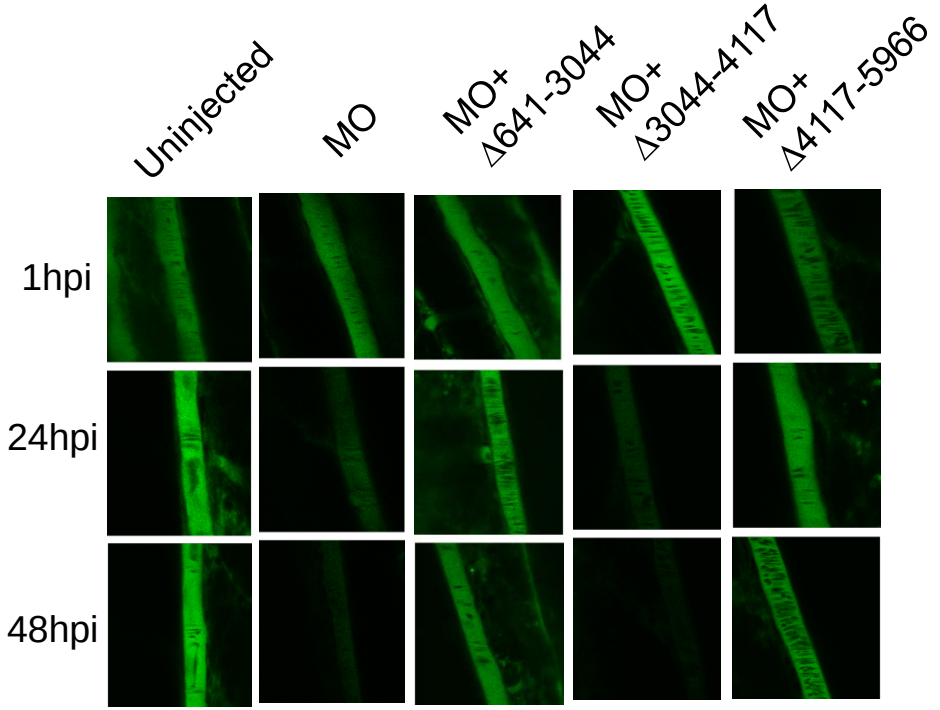
Fawn-Hooded Hypertensive (FHH)

- Glomerular hypertension
- Proteinuria
- Focal segmental glomerular sclerosis
- Podocyte effacement



Schematic of *Shroom3* protein





G1073S, Y1291C, and A1356V are potential candidate variants

Now would you put in the Medical Record?

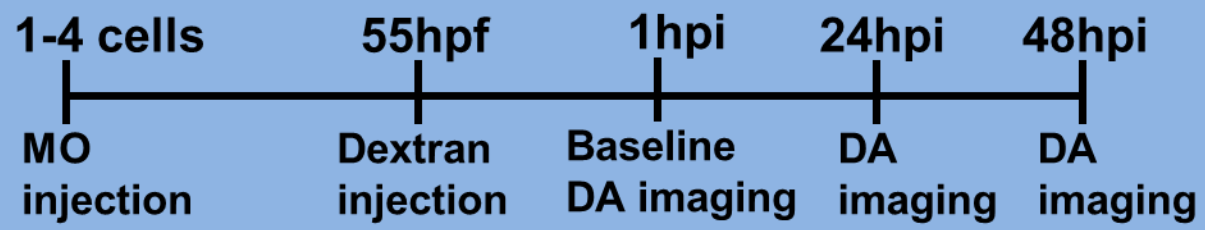
1. GWAS nominated *Shroom3*.
2. QTL data in the rat.
3. The same mutation was in the ACI and FHH. Shows how “normal” can carry alleles causing disease.
4. Gene Editing used to test, find and validate the casual mutation

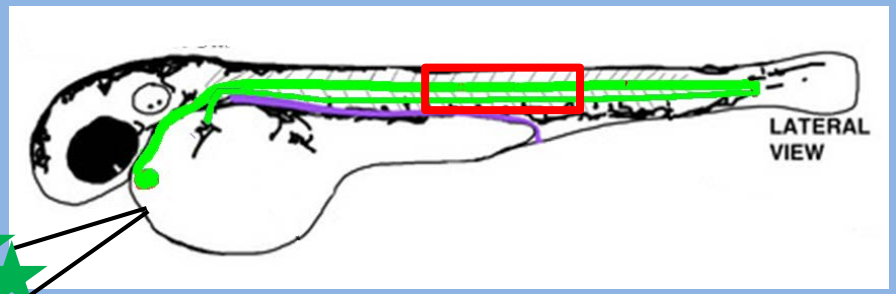
ZEBRAFISH DATA

Variant to likely function

Dissecting *Shroom3* function using zebrafish pronephros

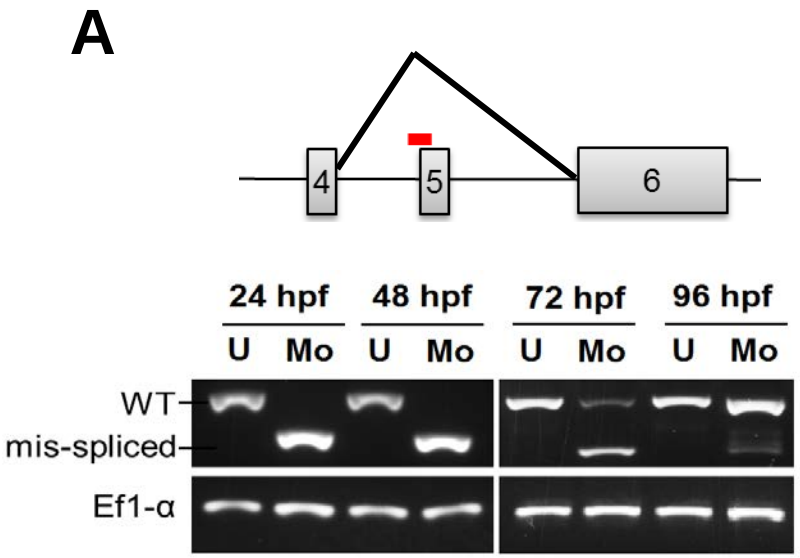
Study design



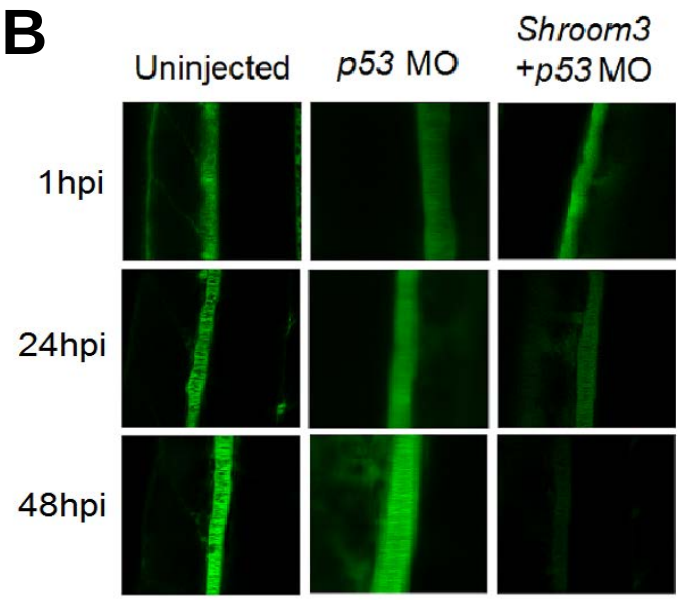


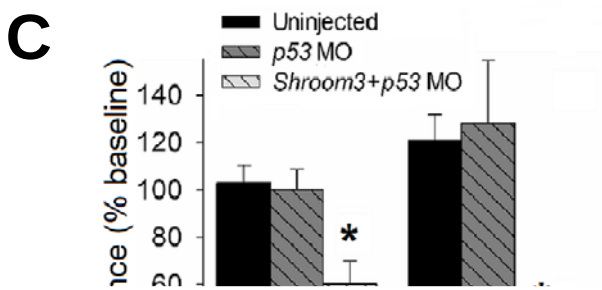
70-kDa FITC dextran

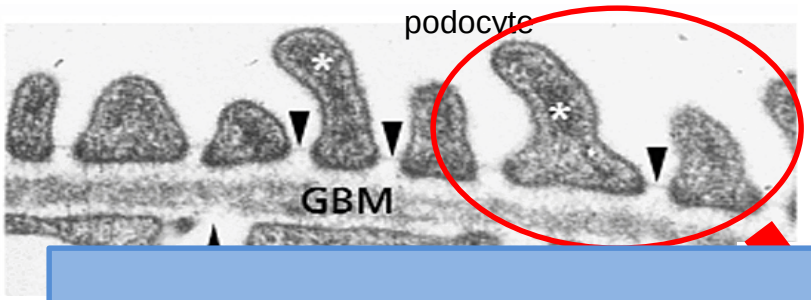
Knockdown of *Shroom3* by morpholino caused increased glomerular permeability



(A) MO blocks proper splicing of *Shroom3* transcript in zebrafish.



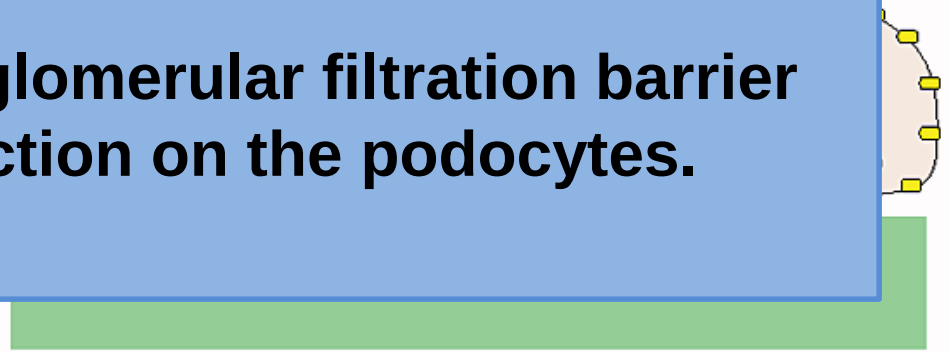




Glo
lea
pro

Hypothesis

***Shroom3* regulates glomerular filtration barrier function via its action on the podocytes.**

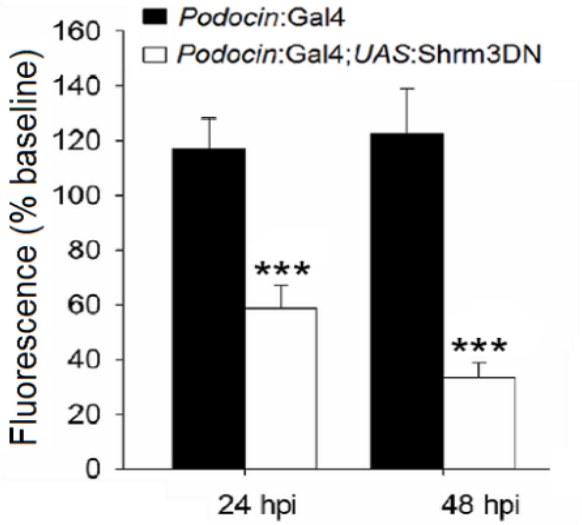


- Actin cytoskeletal signaling regulates the podocyte integrity
- Disruption of podocyte cytoskeletal network leads to glomerular injury and proteinuria

Images are downloaded from http://ecofts.uklibk.ac.at

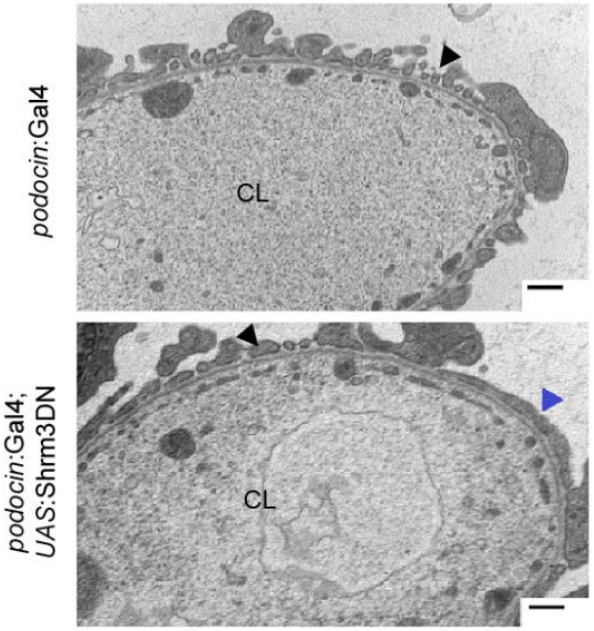
Podocyte-specific *Shroom3* knockdown caused increased glomerular permeability and podocyte effacement

A

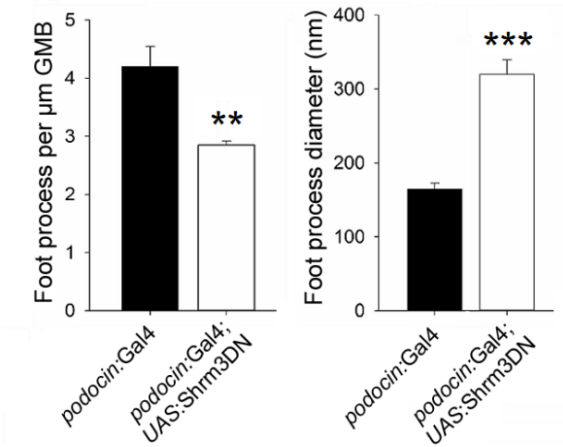


(A) Quantification of dextran fluorescence.
***p<0.001 vs *podocin*:GAL4 control .

B



C



(C) Quantification of podocyte injury.
p<0.01*p<0.001 vs *podocin*:GAL4 control.

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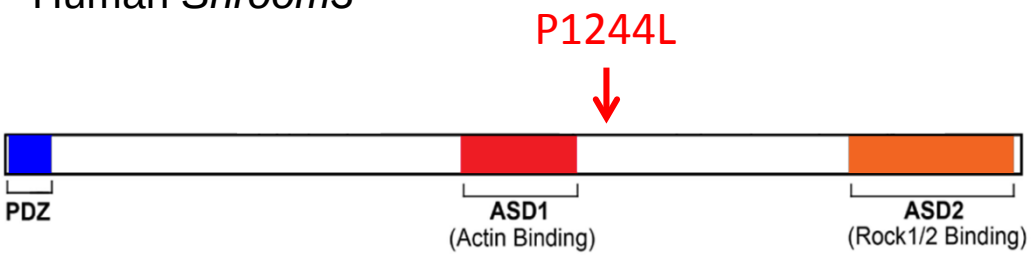
3. *Shroom3*—causes morphological changes to glomerular filtration barrier.
4. The same mutation was in the ACI and FHH. Shows how "normal" can carry alleles causing disease,
5. Gene Editing used to test, find and validate the casual mutation
6. With Zebrafish showed the rat mutations cause podocyte effacement—the dominant hypothesis for how CKD starts.

Need to Test the Patient's Variant

P1244L in *SHROOM3* contributes to glomerular dysfunction

Human *Shroom3*



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5. Gene Editing used to test, find and validate the casual mutation
6. With Zebrafish showed the rat mutations cause podocyte effacement—the dominant hypothesis for how CKD starts.
7. The VUS was tested in Zebrafish using gene editing and showed the same podocyte effacement and proteinuria

At the American Society of Nephrology in Nov. 2015

From an Audience of ~500 Physicians and Scientists how many agreed to put in the medical record?

Developmental Origins for Kidney Disease Due to *Shroom3* Deficiency

Hadiseh Khalili,* Alexandra Sull,† Sanjay Sarin,* Felix J. Boivin,* Rami Halabi,† Bruno Svajger,* Aihua Li,* Valerie Wenche Cui,* Thomas Drysdale,†‡ and Darren Bridgewater*§

*Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada; Departments of †Physiology and Pharmacology and ‡Pediatrics, University of Western Ontario, London, Ontario, Canada; and §Hamilton Center for Kidney Research, St. Josephs Healthcare, Hamilton, Ontario, Canada

ABSTRACT

CKD is a significant health concern with an underlying genetic component. Multiple genome–wide association studies (GWASs) strongly associated CKD with the shroom family member 3 (*SHROOM3*) gene, which encodes an actin-associated protein important in epithelial morphogenesis. However, the role of *SHROOM3* in kidney development and function is virtually unknown. Studies in zebrafish and rat showed that alterations in *Shroom3* can result in glomerular dysfunction. Furthermore, human *SHROOM3* variants can induce impaired kidney function in animal models. Here, we examined the temporal and spatial expression of Shroom3 in the mammalian kidney. We detected Shroom3 expression in the condensing mesenchyme, Bowman's capsule, and developing and mature podocytes in mice. *Shroom3* null (*Shroom3*$^{Gt/Gt}$) mice showed marked glomerular abnormalities, including cystic and collapsing/degenerating glomeruli, and marked disruptions in podocyte arrangement and morphology. These podocyte-specific abnormalities are associated with altered Rho–kinase/myosin II signaling and loss of apically distributed actin. Additionally, *Shroom3* heterozygous (*Shroom3*$^{Gt/+}$) mice showed developmental irregularities that manifested as adult-onset glomerulosclerosis and proteinuria. Taken together, our results establish the significance of Shroom3 in mammalian kidney development and progression of kidney disease. Specifically, Shroom3 maintains normal podocyte architecture in mice *via* modulation of the actomyosin network, which is essential for podocyte function. Furthermore, our findings strongly support the GWASs that suggest a role for SHROOM3 in human kidney disease.

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magnesium levels and serum creatinine levels is shroom family member 3 (*SHROOM3*).[5–7]

Shroom3 is an actin-associated protein that regulates epithelial cell shape and tissue morphogenesis. Shroom3 regulates these developmental processes by binding F actin and regulating its subcellular organization.[8,9] Shroom3 interacts and recruits Rho-kinase (Rock), resulting in the phosphorylation and activation of nonmuscle myosin II (MyoII). Activation of this Rock/MyoII signaling pathway causes localized contraction of actomyosin networks at the apical surface of the cell, resulting in changes in cell morphology.[10] During development, Shroom3 is essential for neural tube closure, gut, and lens morphogenesis[9,11,12]

Conclusions

- Sequence first ask questions later will drive much of basic research.
- Basic science at the speed of the clinic is critical.
- Need to establish new criteria for “proving” a gene and variant cause disease and therefore can be put into the medical record? Risk/Benefit considerations required?

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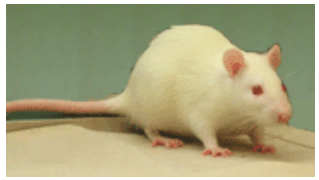


CKD Gen
CONSORTIUM