Convincing Clinicians to Use Functionalized Genomic Medicine

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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

What data would you require to say this variant causes Chronic Kidney Disease in a Medical Record?

This variant is a VUS

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danio_JX455752</td>
<td>RPIPQHFRSKSSEPVENV−SQDFLARDLQ</td>
</tr>
<tr>
<td>Xenopus_shroom3</td>
<td>PPALHVRSSPASDMKSRMSRQEV</td>
</tr>
<tr>
<td>Gallus_shroom3_880_1062</td>
<td>IAYVPVHTSSRPATADKNHQDLLLLRESS</td>
</tr>
<tr>
<td>Human_shroom3_883_1066</td>
<td>LAGPVHVRSSPATADKRQDVLLGQDS</td>
</tr>
<tr>
<td>Chimp_Shroom3_622_768</td>
<td>LAGPVHVRSSPATADKRQDVLLGQDS</td>
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<tr>
<td>Rat_shroom3_890_1074</td>
<td>LTVPVHVRSSPSSDKKGQDVLLRED</td>
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<tr>
<td>Mouse_shroom3_881_1060</td>
<td>LAVPVHVRSSPSSDKKGQDVLLREGS</td>
</tr>
</tbody>
</table>
Nomination of SHROOM3 by GWAS

• One of the most reproducible risk loci
• Renal function of \textit{SHROOM3} is not known
• 11 GWAS have reported \textit{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* encodes 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)

<table>
<thead>
<tr>
<th>QTLs</th>
<th>Chromosome</th>
<th>QTL Size</th>
<th>Phenotypes</th>
<th>Human QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf-1</td>
<td>1q55</td>
<td>30 Mb</td>
<td>FSGS, UPV/UA, Palb</td>
<td>ESRD in AA* CCr #</td>
</tr>
<tr>
<td>Rf-2</td>
<td>1q32</td>
<td>35 Mb</td>
<td>UPV/UAV</td>
<td>Familial FSGS&amp;</td>
</tr>
<tr>
<td>Rf-3</td>
<td>3q1-q2</td>
<td>D3mit4</td>
<td>FSGS, UPV/UA, Palb</td>
<td>Cr, CCr, GFR% Diab. Neph. 1</td>
</tr>
<tr>
<td>Rf-4</td>
<td>14p1-q1</td>
<td>14 Mb</td>
<td>FSGS, UPV/UA, Palb</td>
<td></td>
</tr>
<tr>
<td>Rf-5</td>
<td>17p1-q1</td>
<td>D17mit12</td>
<td>UPV/AUV</td>
<td></td>
</tr>
</tbody>
</table>
The FHH *Shroom3* allele harbors coding variants, compared to Brown-Norway (BN) control

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

Fawn-Hooded Hypertensive (FHH)

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**

- **1-4 cells**
  - MO injection

- **55hpf**
  - Dextran injection

- **1hpi**
  - Baseline DA imaging

- **24hpi**
  - DA imaging

- **48hpi**
  - DA imaging

70-kDa FITC dextran
Knockdown of *Shroom3* by morpholino caused increased glomerular permeability

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

(B) Quantification of dextran fluorescence.

* *p* < 0.05 vs uninjected and *p53* MO.
Glomerular filtration barrier prevents leakage of high molecular weight proteins.

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

**Hypothesis**

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

Images are downloaded from [http://ecofts.uklibk.ac.at](http://ecofts.uklibk.ac.at)
Podocyte-specific *Shroom3* knockdown caused increased glomerular permeability and podocyte effacement.

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

(C) Quantification of podocyte injury.  
**p<0.01 ***p<0.001 vs podocin:GAL4 control.
Now would you put in the Medical Record?

1. GWAS nominated *Shroom3*.
2. QTL data in the rat
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*
Now would you put in the Medical Record?

1. GWAS nominated *Shroom3*.
2. QTL data in the rat
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.
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

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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<sup>−/−</sup>) 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<sup>+/−</sup>) 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.

Received June 5, 2015. Accepted January 14, 2016
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?
Acknowledgements

**Jacob Lab at MCW**
Howard Jacob, PhD
Jozef Lazar, MD, PhD
Melinda Dwinell, PhD
Caitlin O’Meara, PhD
Mike Flister, PhD
Jeremy Prokop, PhD
Carol Moreno, MD, PhD
**Nan Cher (Flo) Yeo**
Matthew Hoffman
Angela Lemke
Allison Sarkis
Bryce Schuler
Becky Schilling
Akiko Takizawa, PhD
Sharon Tsaih, PhD
Michael Tschannen
Jaime Wendt
Sasha Prisco
Allison Zappa

**Link lab**
Brian Link, PhD
Kerry Veth, PhD
Michael Cliff

**Drummond lab**
Iain Drummond, PhD
Ritu Tomar, PhD

**Freedman Lab**
Barry Freedman, PhD
Donald Bowden, PhD
Jason Bonomo

**Lombard lab**
ACKNOWLEDGMENTS

• Chris O’Donnell
• Dan Levy
• Caroline Fox

• PhysGen Knockout Team
  ○ Allen Cowley
  ○ Melinda Dwinell
  ○ Dave Mattson
  ○ Julian Lombard
  ○ Carol Moreno Quinn
  ○ Jozef Lazar

• Hartmut Weiler
  ○ Shawn Kalloway
  ○ Jamie Foeckler

• Abraham Provoost

• Norbert Hubner, the MDC

• EuTRANS