Pathophysiology of APOL1-associated kidney disease: lessons from animal models

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No disclosures
Overview

- Introduction to APOL1 associated kidney disease
- Developing mouse models to recapitulate APOL1 kidney disease
- Characterizing APOL1 transgenic mice
- Utilizing APOL1 transgenic mice to identify therapeutic avenues
- Discuss findings and future directions
APOL1 in Chronic Kidney Disease

- African-Americans represent 13% of US population, yet constitute 32% of dialysis patients
APOL1 in Chronic Kidney Disease

• African-Americans represent 13% of US population, yet constitute 32% of dialysis patients

• Increased CKD risk is attributed to two genetic variants in Apolipoprotein L1 (APOL1), G1 and G2, present in > 3 million African Americans.

• APOL1 risk alleles significantly increased the risk for FSGS, lupus nephritis, sickle cell disease, hypertensive renal disease, and HIVAN.

Odds ratio for FSGS: 10.5
Odds ratio for Hypertensive ESRD: 7.3
Odds ratio for HIV associated kidney disease: >80
Association of Trypanolytic ApoL1 Variants with Kidney Disease in African Americans

Giulio Genovese, David J. Friedman, Michael D. Ross, Laurence Leerdam, Pierrick Uzureau, Barry I. Freedman, Donald W. Bowden, Carl D. Langefeld, Taras K. Oleksyk, Andrea L. Uscinski Knob, Andrea J. Bernhardy, Pamela J. Hicks, George W. Nelson, Benoit Vanhollebeke, Cheryl A. Winkler, Jeffrey B. Kopp, Etienne Pays, and Martin R. Pollak

Science  August 13, 2010
Question:
Do APOL1 variants cause kidney disease?

Problems:
1. Only humans, gorillas and baboons have APOL1 (fish, mouse and rats do NOT have APOL1)
2. In humans APOL1 is secreted by liver; widely expressed (serum, kidney and vasculature). The role of APOL1 and the disease-causing cell type remain unclear.
3. Heterogeneity in APOL1 disease development - 20% of people with two risk alleles develop kidney disease. What is the trigger?
Establishing causality between risk alleles and kidney disease

Podocyte-specific driver

GFP

TRE- GFP-APO1

G0/G1/G2 APO1

NPHS1 rtTA

TRE- GFP-APO1

rtTA

TRE- GFP-APO1

rtTA

TRE- GFP-APO1
Podocyte specific inducible APOL1 mice recapitulates human disease

1. Functional changes

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Beckerman et al. Nature Medicine 2017
Podocyte specific inducible APOL1 mice recapitulates human disease

2. Structural changes

<table>
<thead>
<tr>
<th>NephrinrtTA/TREG0APOL1</th>
<th>NephrinrtTA/TREG1APOL1</th>
<th>NephrinrtTA/TREG2APOL1</th>
</tr>
</thead>
</table>

Beckerman et al. Nature Medicine 2017
Mice with renal tubule-specific/ liver-specific APOL1 risk allele expression appear normal

**Albumin Cre**

**Rosa flox STOP rtTA**

Liver specific expression

**TRE- GFP-APOL1**

Kidney tubule expression

Human circulating APOL1 range is 386 – 15,483 ng/ml
Heterogeneity in APOL1 associated kidney disease: Genetics + environmental stimuli

Hypothesis: Phenotype development is induced by increased APOL1 risk variant expression
The phenotype development is APOL1 dose dependent in risk allele mice
Conclusions I

The phenotype development in mice strongly correlates with human APOL1 kidney disease.

Glomerular APOL1 risk allele expression correlates with toxicity.
Podocytes are highly differentiated cells and rely heavily on autophagy for cellular survival.
APOL1-risk-allele cells show reduced autophagic flux: Cellular stress?

APOL1 high risk genotype

- ER stress
- DNA damage
- Late endosome
- Mitochondrial stress
- Stress activated signaling

Autophagic content (folds LC311/GFP)

Autophagic flux (folds LC311 SQ/S)
Increased expression of inflammasome pathway components in APOL1 associated CKD

**Inflammasome**:

Inflammasomes are a group of cytosolic multiprotein complexes, classically consisting of:
1. a sensor protein (NLRP3)
2. the adaptor protein (ASC)
3. downstream effector (caspase-1)

Inflammasome activation promotes **Pyroptosis**, a type of cell death caused by cell swelling and cell lysis.
Increased expression of inflammasome pathway components in APOL1 associated CKD

- APOL1
- NLRP3
- Pro-Caspase1
- Cleaved-Caspase1
- GAPDH

Nphs1; rtTA; APOL1 (3 wk dox)

G0 APOL1

G2 APOL1

Beckerman et al. Nature Medicine 2017
Conclusions II

The phenotype development in mice strongly correlates with human APOL1 kidney disease.

Glomerular APOL1 risk allele expression correlates with toxicity.

APOL1 risk alleles -> defective autophagy -> inflammasome activation.
Hypothesis: APOL1-induced cytotoxicity is mediated by inflammasome signaling

- APOL1 high risk genotype
- ER stress
- DNA damage
- Stress activated signaling
- Mitochondrial stress
- NLRP3-Caspase-1 inflammasome
- Active Caspase-1
- Mature IL1-β
- Inflammatory pyroptosis
  - Cell swelling
  - Cell lysis
Role of NLRP3 in G2 APOL1-induced kidney injury

1) Albuminuria
2) Histology
3) Kidney injury markers

Dox diet at 4.5 weeks for 3 weeks
NLRP3 knock out leads to improvement in renal function

Albumin Creatinine Ratio (mg/mg)

Baseline Week1 Week2 Week3
0 10 20 30
40 50 75 100
125 150
*** * **
G2; Nephrin rtTA; Nlrp3 (+/+)
G2; Nephrin rtTA; Nlrp3 (-/-)

Relative APOL1 transcript level

0.0 0.5 1.0 1.5
G2; Nlrp3 (+/+)
G2; Nlrp3 (-/-)

Blood Urea Nitrogen

Serum Urea Nitrogen (mg/dL)

0 50 100 150 200
Wildtype
G2; Nephrin rtTA; Nlrp3 (+/+)
G2; Nephrin rtTA; Nlrp3 (-/-)
## *

Serum Creatinine

Serum Creatinine (mg/dL)

0 1 2
Wildtype
G2; Nephrin rtTA; Nlrp3 (+/+)
G2; Nephrin rtTA; Nlrp3 (-/-)
# *

ACR correlated with APOL1 expression
NLRP3 knock out leads to improvement in renal fibrosis

![Wildtype PAS](image1)

![G2 APOL1; Nlrp3 WT PAS](image2)

![G2 APOL1; Nlrp3 KO PAS](image3)

![Wildtype Sirius Red](image4)

![G2 APOL1; Nlrp3 WT Sirius Red](image5)

![G2 APOL1; Nlrp3 KO Sirius Red](image6)

**% Sirius red positive area**

![Wildtype](image7)

![G2; Nephrin rtTA; Nlrp3 (+/+)](image8)

![G2; Nephrin rtTA; Nlrp3 (-/-)](image9)

**Relative transcript level**

![Wildtype](image10)

![G2; Nephrin rtTA; Nlrp3 (+/+)](image11)

![G2; Nephrin rtTA; Nlrp3 (-/-)](image12)
Role of Caspase-1 in G2 APOL1-induced kidney injury

1) Albuminuria
2) Histology
3) Kidney injury markers
Caspase-1 knock out leads to improved renal histology

Graph showing the comparison of Albumin Creatinine Ratio (mg/mg) between G2; Nephrin rtTA; Casp-1 (+/+) and G2; Nephrin rtTA; Casp-1 (-/-) mice over the weeks of baseline, week 1, week 2, and week 3. The graph indicates a significant improvement in ACR in Caspase-1 knockout mice.

Images showing histological sections of renal tissue for Wildtype, G2 APOL1; Casp-1 WT, and G2 APOL1; Casp-1 KO mice stained with PAS and Sirius Red. The images illustrate the differences in renal histology between the wildtype and knockout groups.
Interplay between Caspase1 and NLRP3 implies pyroptosis pathway in APOL1 cytotoxicity

<table>
<thead>
<tr>
<th></th>
<th>Nlrp3 (+/+)</th>
<th>Nlrp3 (-/-)</th>
<th>Casp-1 (-/-)</th>
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</thead>
<tbody>
<tr>
<td>NLRP3</td>
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<td>Caspase1</td>
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<tr>
<td>GAPDH</td>
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</tbody>
</table>

**Relative transcript level in kidney**

- **Ctrl**
- **Nlrp3 WT**
- **Nlrp3 KO**

* indicates a significant difference.
APOL1-induced cytotoxicity is mediated by inflammasome signaling.

- **APOL1 high risk genotype**
- ER stress
- DNA damage
- Stress activated signaling
- Mitochondrial stress

Resulting in:
- NLRP3-Caspase-1 inflammasome
- Active Caspase-1
- Mature IL1-β
- Inflammatory pyroptosis
- Cell swelling
- Cell lysis

Additional notes:
- Autophagosome
- Late endosome
The phenotype development in mice strongly correlates with human APOL1 kidney disease.

Glomerular APOL1 risk allele expression correlates with toxicity.

APOL1 risk alleles cause defective endolysosomal trafficking blocking autophagy.

Expression of APOL1 risk allele in mice podocytes induces expression of inflammasome components.

Knocking out NLRP3 and Caspase1 significantly attenuates G2APOL1-induced kidney disease in mice.

**Future direction:** To determine if pharmacological inhibition of Caspase-1 and NLRP3 could reduce APOL-1 mediated kidney disease in mice.
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