



# Nuances in Lupus Nephritis: An APOL1 Story

Ashira D. Blazer, MD MSCI

Assistant Professor Medicine, Division of Rheumatology

University of Maryland Baltimore

# Instructor Disclosures

In compliance with ACCME Standards for Commercial Support of CME activities...

**Ashira D Blazer**

I have these relevant financial relationships to disclose:

|    | <u>Company</u> | <u>Nature of Relationship</u> |
|----|----------------|-------------------------------|
| 1. | GSK            | Consulting Fees, Honoraria    |
| 2. | Genentech      | Consulting Fees, Honoraria    |

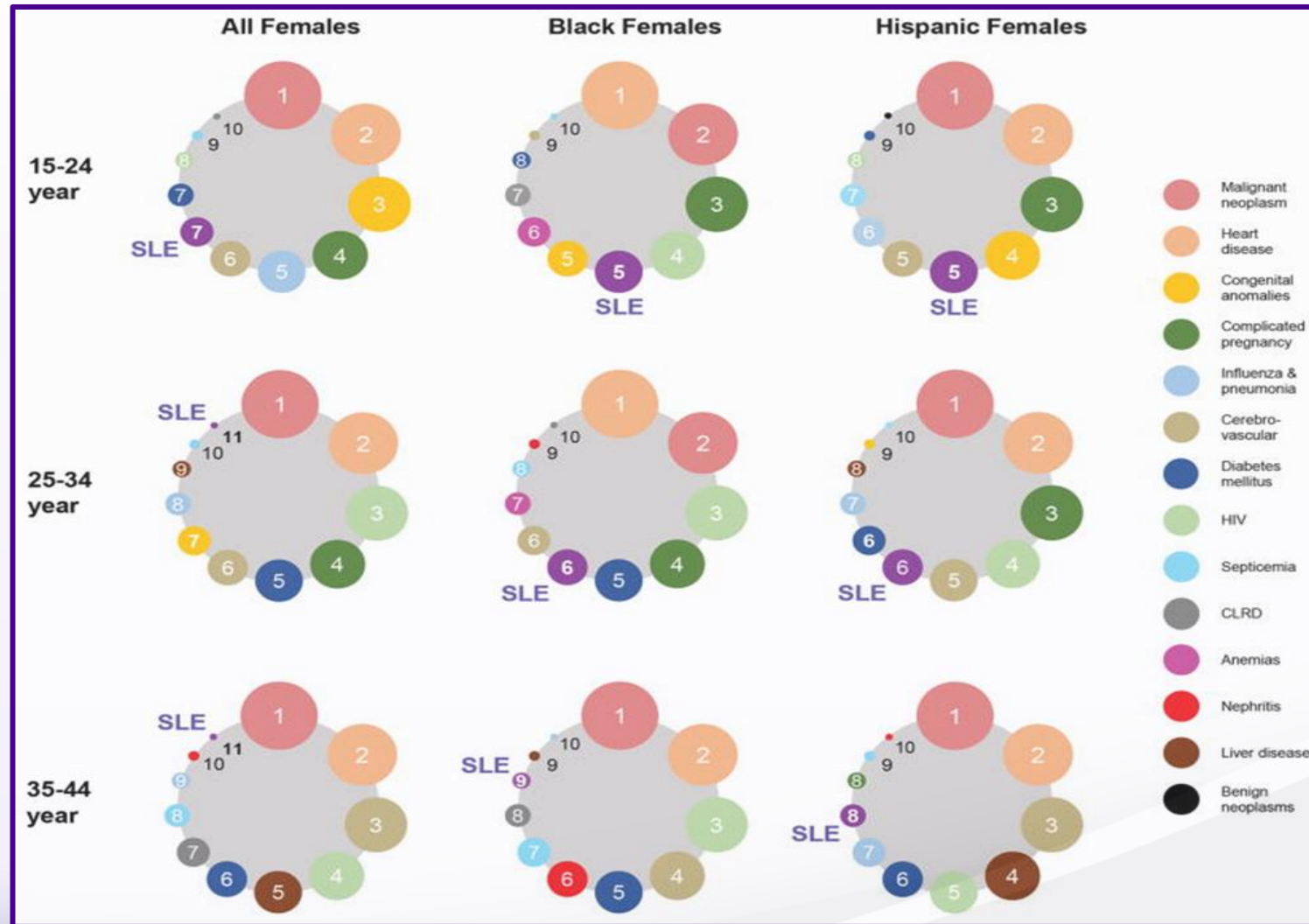
Grant Support:

K23AI163359 (NIH/NIAID)

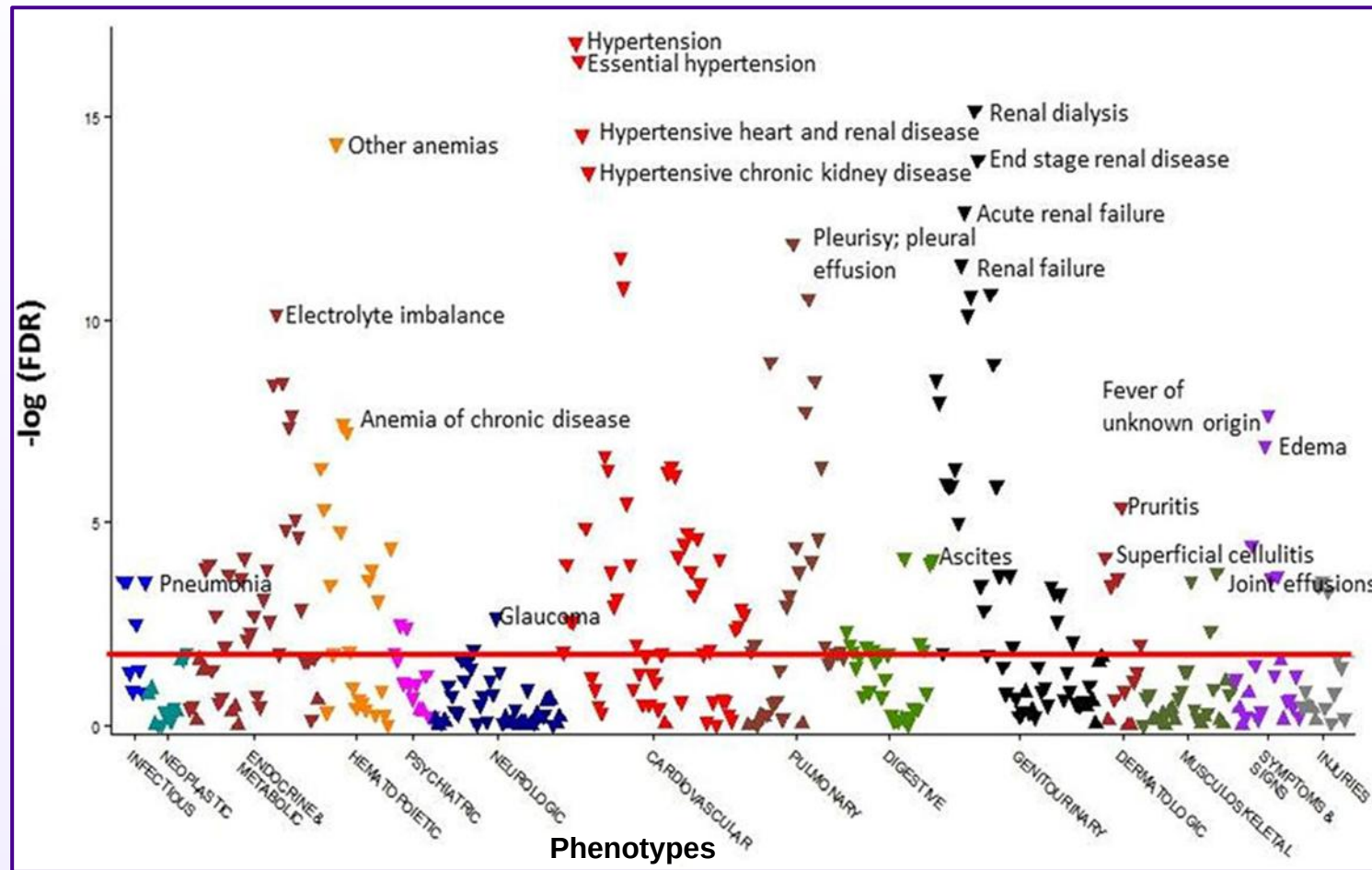
Lupus Research Alliance CDA



# SLE is a Major Cause of Morbidity and Mortality Especially in Minority Women



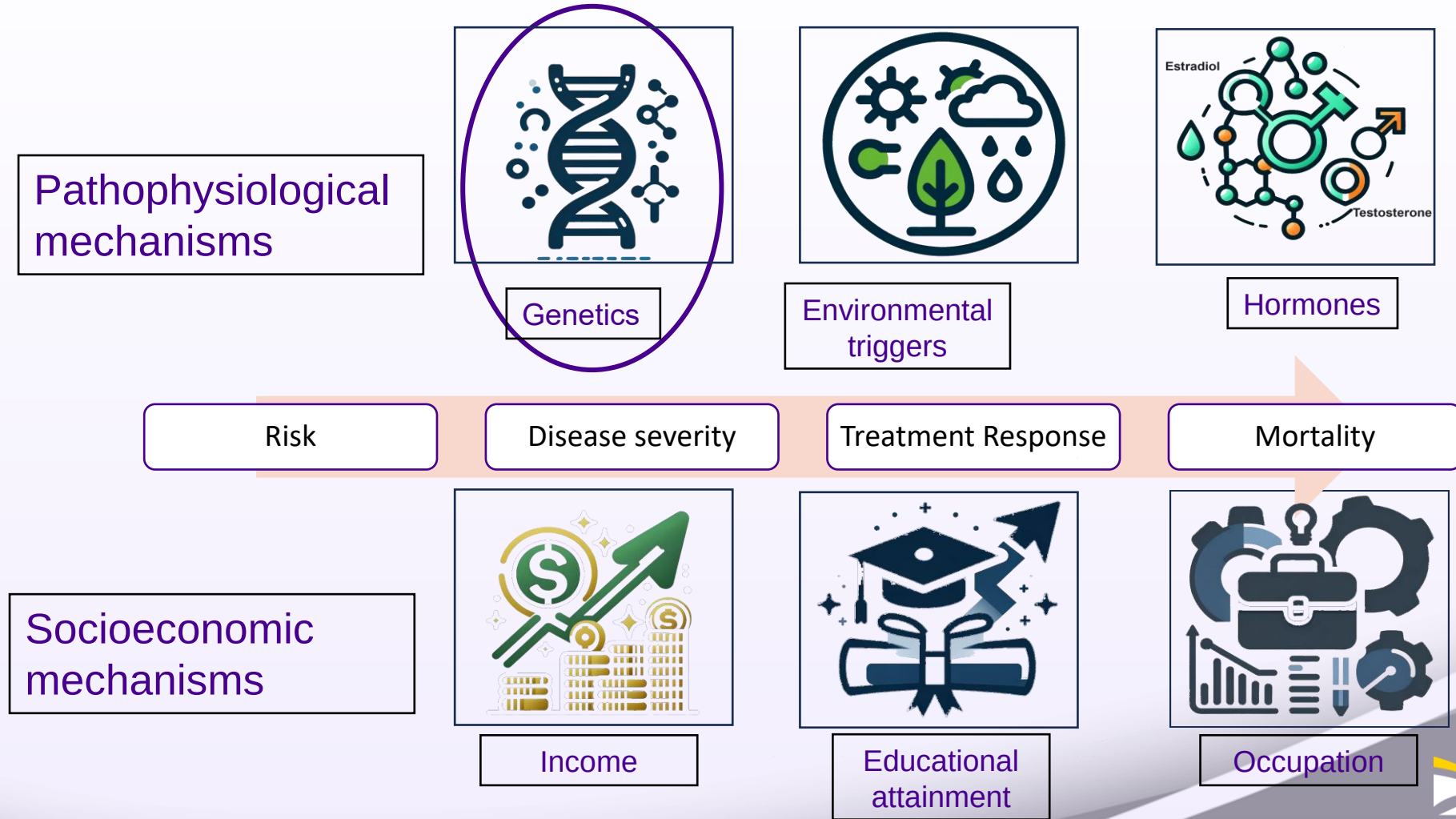
# Patients of Black African Descent Exhibit Increased Burden of Comorbidities



- ▼ More common in patients of Black African descent
- ▲ More common in White patients

|                       | Black African<br>(n=270) | White (n=715) | P Value |
|-----------------------|--------------------------|---------------|---------|
| Current age ± SD      | 44 ± 17                  | 53 ± 17       | P<0.001 |
| Age at Diagnosis ± SD | 35 ± 16                  | 43 ± 17       | P<0.001 |

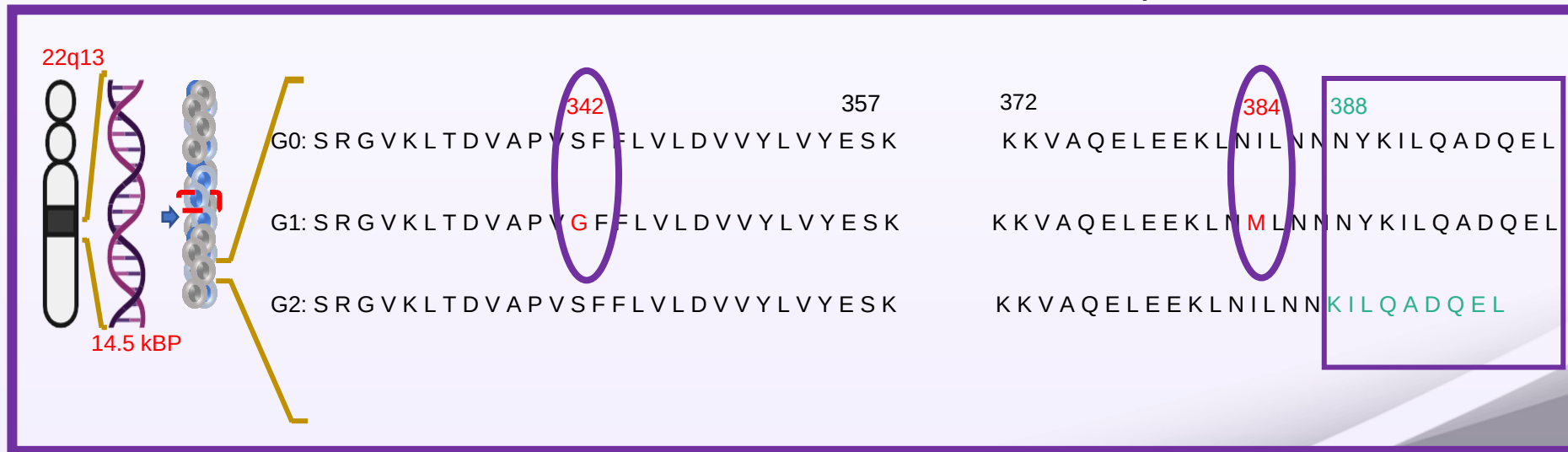
# The Etiological Framework in SLE Health Disparities Reveals Multiple Causes



(Falasinnu T 2017)

# Two Coding Variants in the Apolipoprotein L1 (APOL1) Gene Associate with Renal and Vascular Adverse Outcomes in African Ancestry Populations

- APOL1 polymorphisms:
  - Reference allele: G0
  - First risk variant: G1 / Second risk variant: G2
- Variants confer amino acid substitutions/deletions near the protein C terminus



- Disease Associations:
  - Renal: FSGS, HIV associated Nephropathy, SLE Nephritis
  - Vascular: Microvascular stroke, Peripheral vascular disease, Cardiovascular death

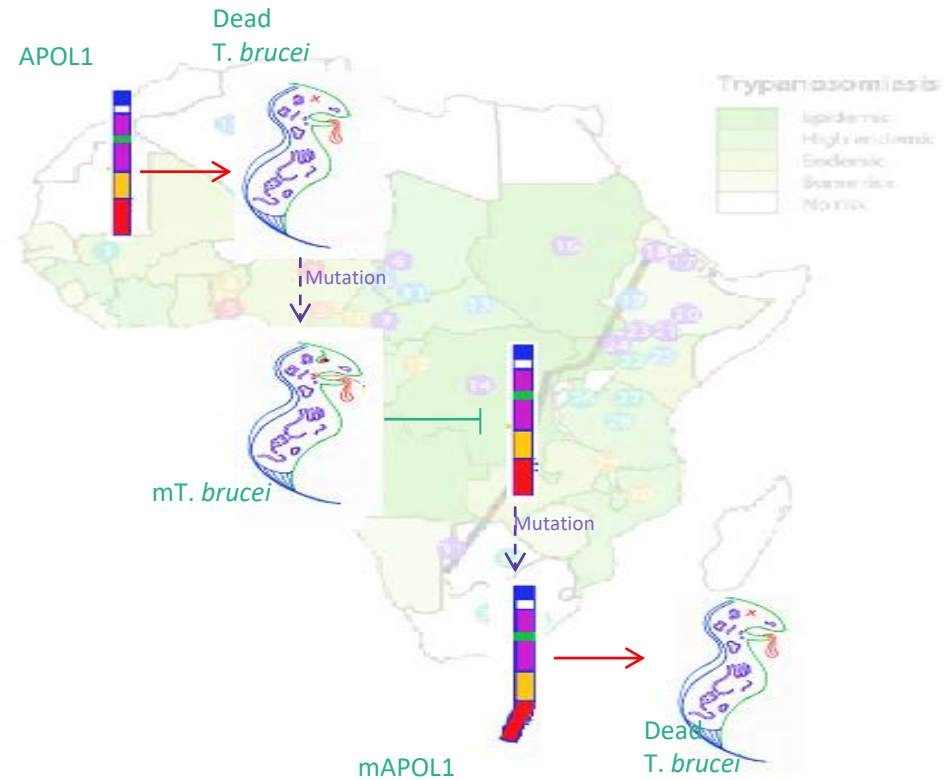
# Extracellular Variant Functions Confer Evolutionary Fitness: Resistance to African Trypanosomiasis

APOL1

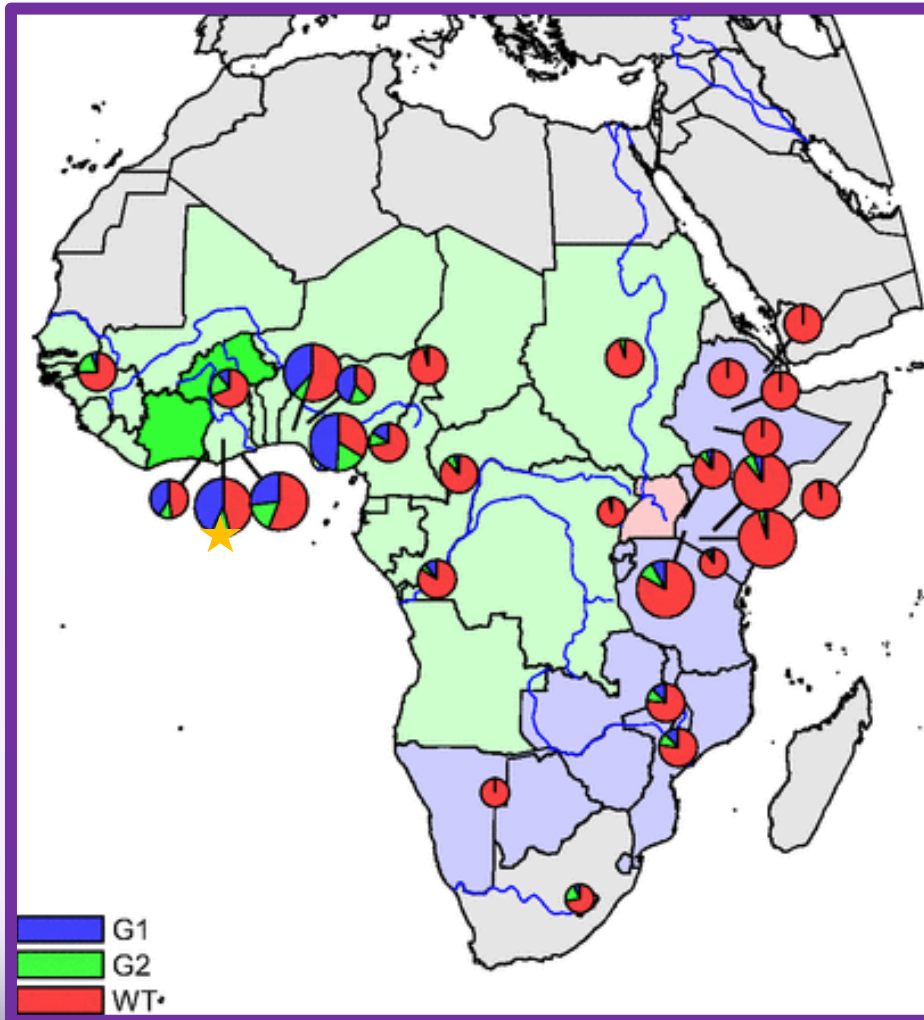


Trypanolytic Factor

## Evolutionary Pressures



# Apolipoprotein L1 (APO L1) Gene Mutations: High Allelic Frequencies in those of Recent West African Heritage

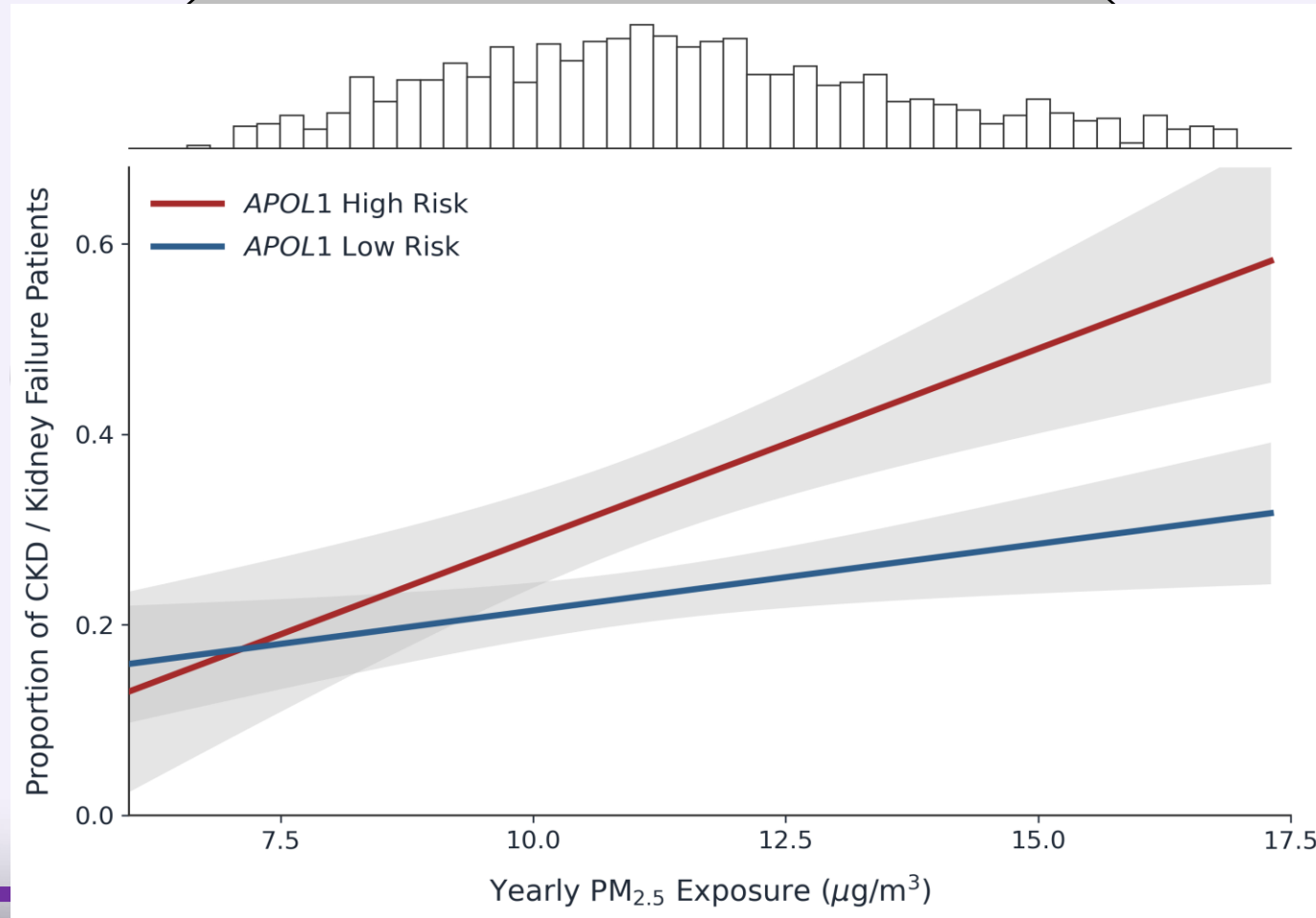


- These mutations offer resistance to African trypanosomiasis
- Evolutionary advantage comes with the risk of progressive renal and cardiovascular disease
- Allelic frequencies are high
  - Ghana
    - Asante: G1: 40.9% G2: 12.9%
    - Bulsa: G1: 11.4% G2: 21.4%
  - African Americans
    - New York: G1: 20.9% G2: 13.4%
    - Southern USA: G1: 19.7% G2: 13.4%

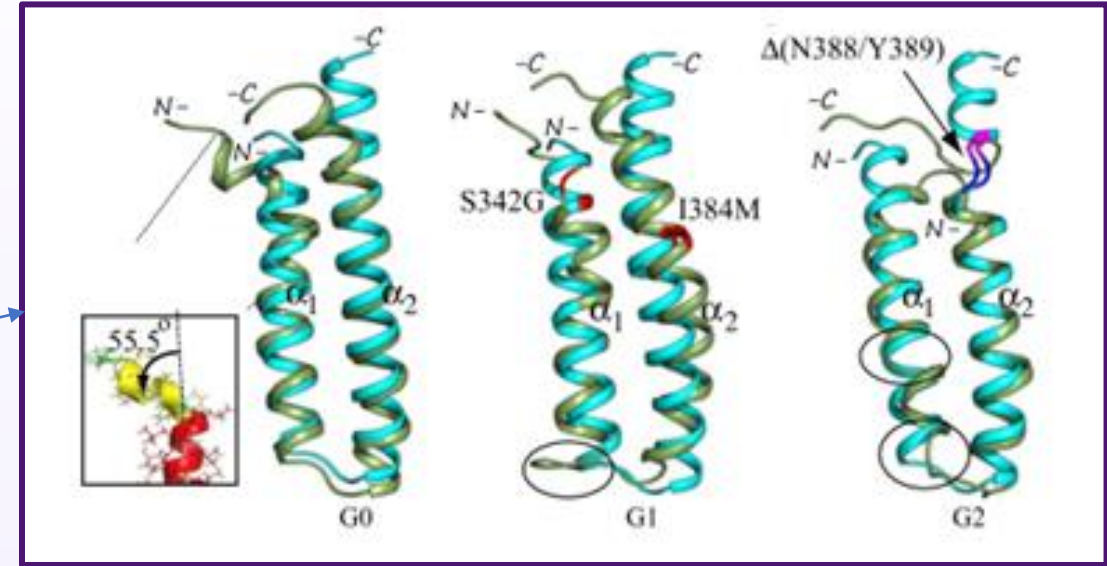
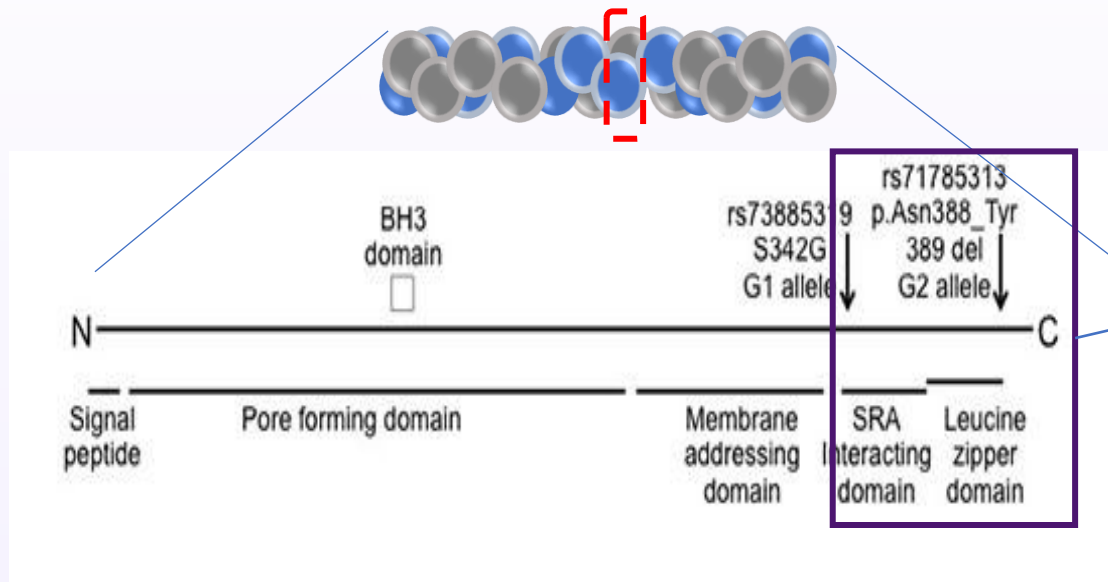
# APOL1 High Risk Genotype CKD Associations are Context Dependent



Macroenvironment

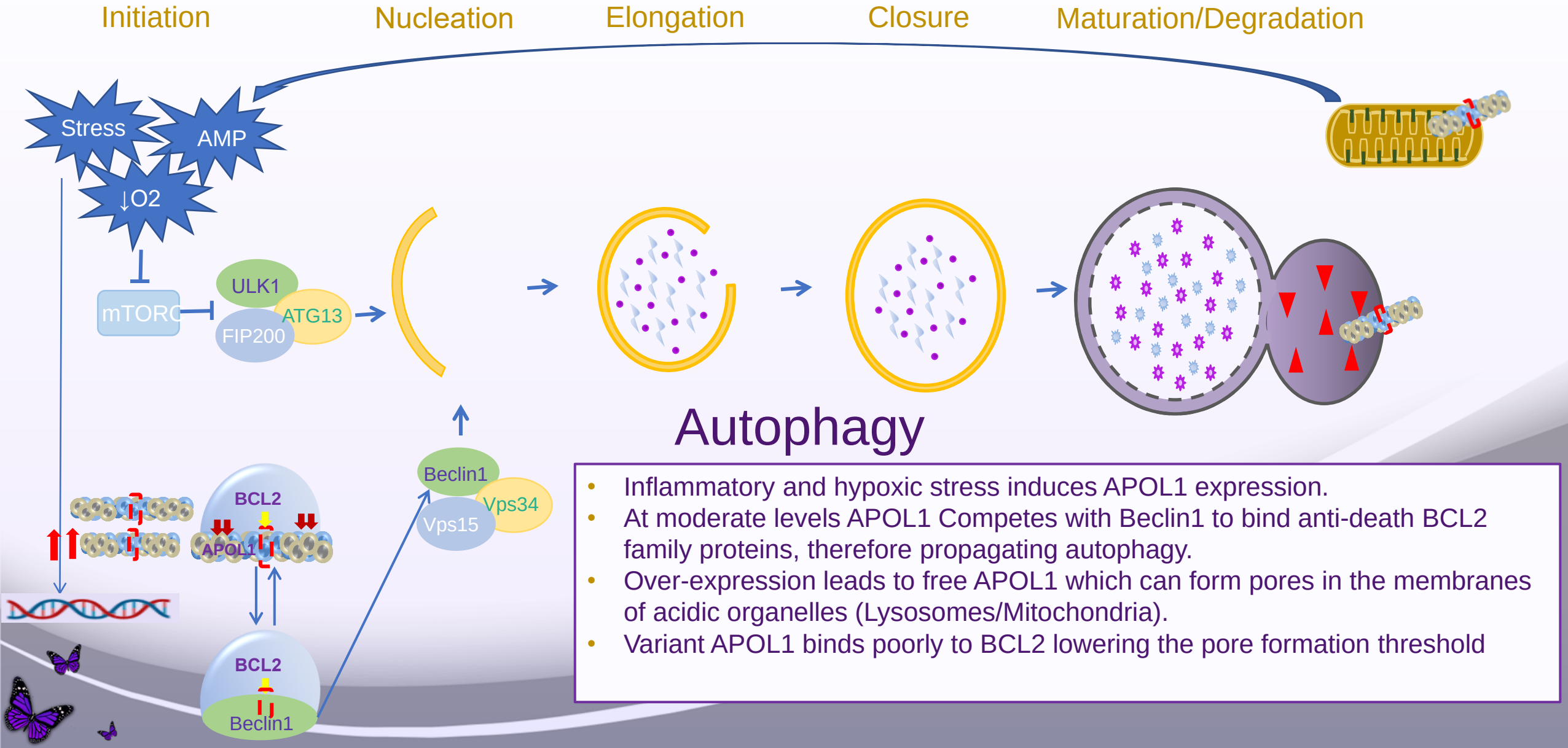


# Intracellular Apolipoprotein L1: A Five Domain BH3 Only Pro-Autophagic Protein



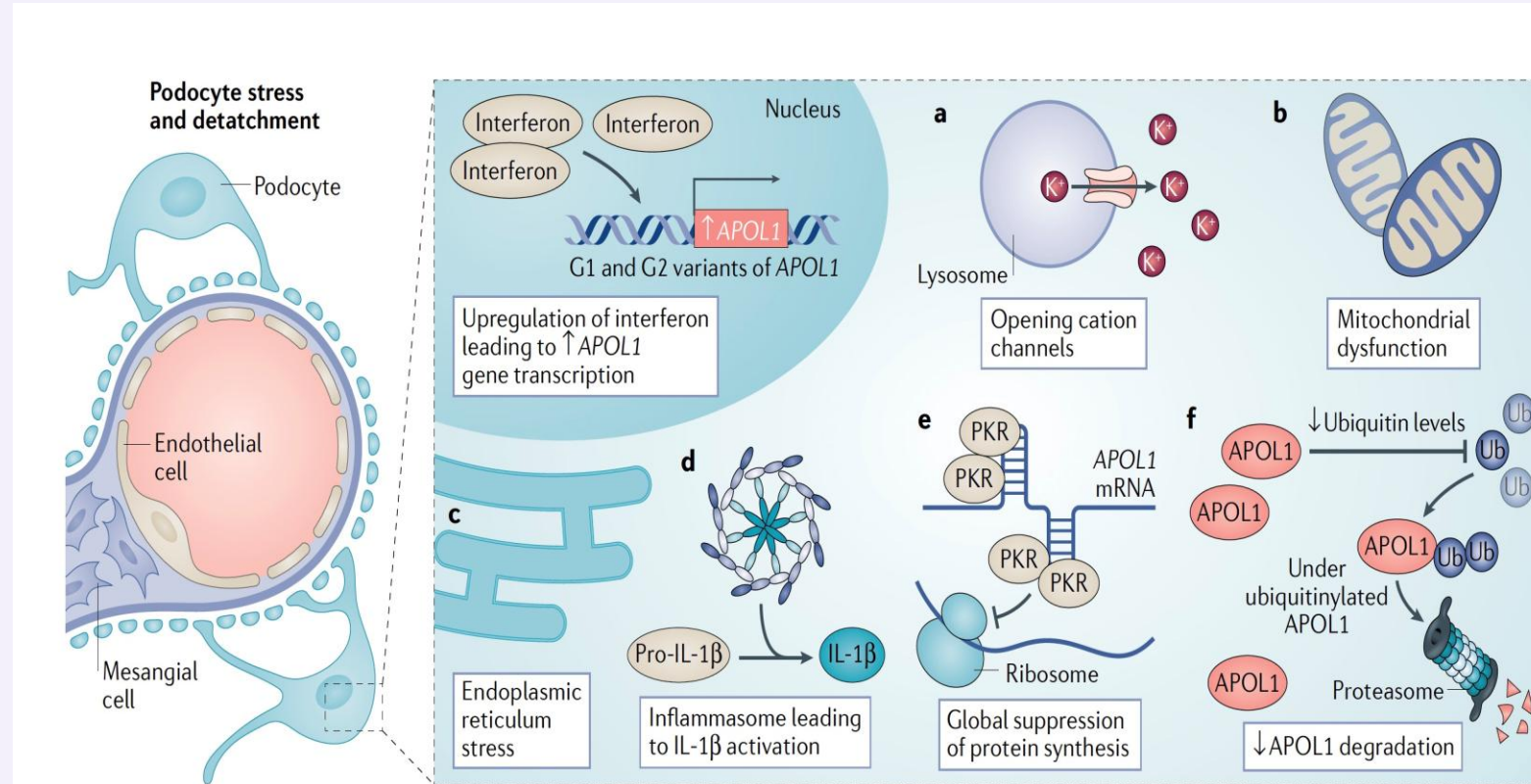
- BH3 Domain: autophagy initiation
- Pore Forming Domain
  - Extracellular: Trypanolytic
  - Intracellular: Transmembrane Pore formation
- Membrane Addressing Domain: pH Sensitive, traverses phospholipid bilayers
- SRA Associated: Coiled-coil alpha helix important for off target binding
  - In serum: trypanosome binds to neutralize
  - Intracellular: anti-death BCL2 family proteins

# Toxicity Model: APOL1 Variants May Upset the Autophagy/Pore Formation Balance



# The APOL1 High Risk Genotype is Thought to Cause a Podocytopathy Through Pore Formation in Lysosomes and Mitochondria

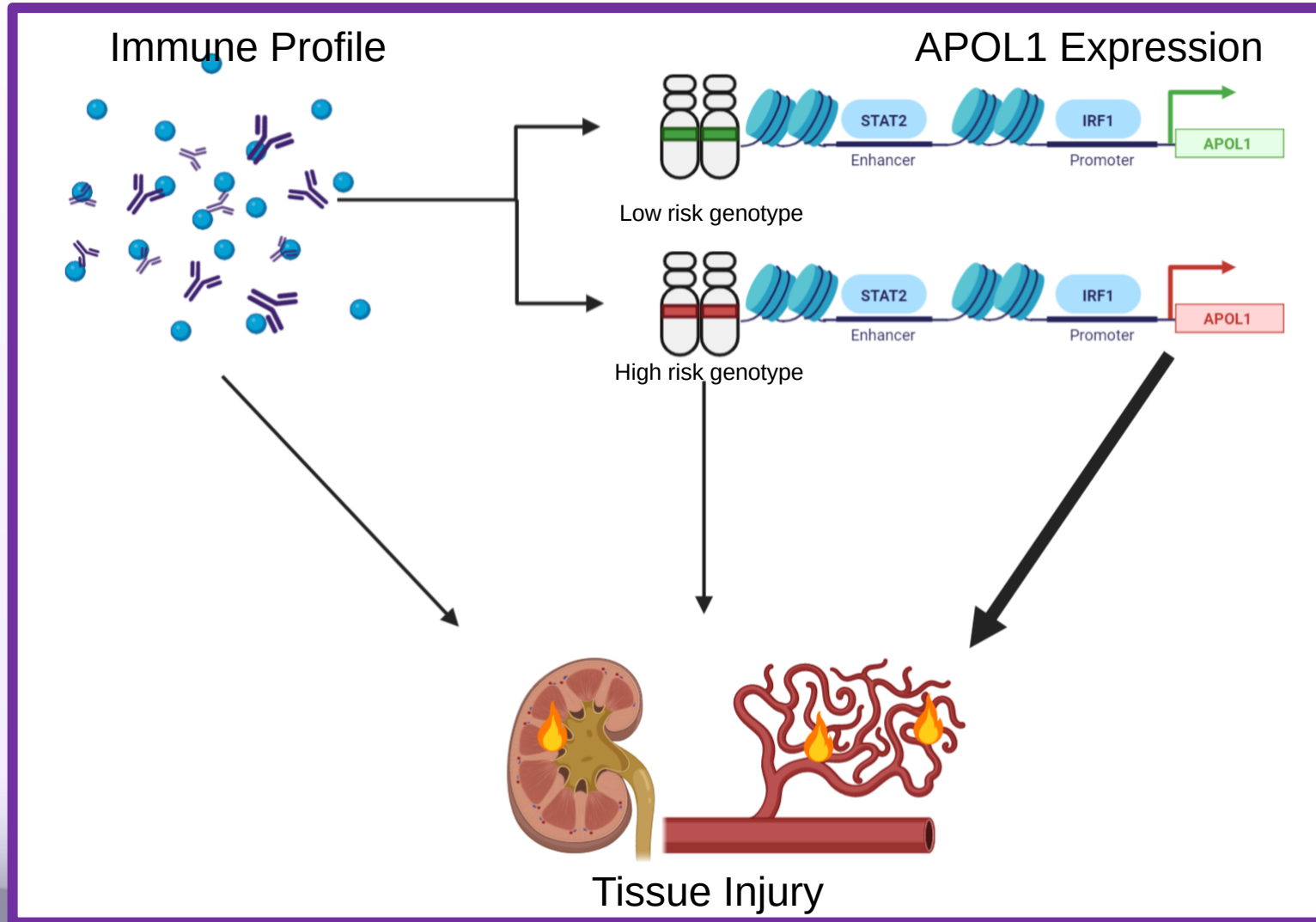
- a. Pore formation and cation flux in the lysosome
- b. Pore forming domain causes mitochondrial dysfunction
- c. Endoplasmic reticulum stress
- d. Activation of the NLRP3 inflammasome
- e. Suppression of protein synthesis through binding protein kinase R
- f. Reduction of ubiquitin levels causing retention of intracellular proteins



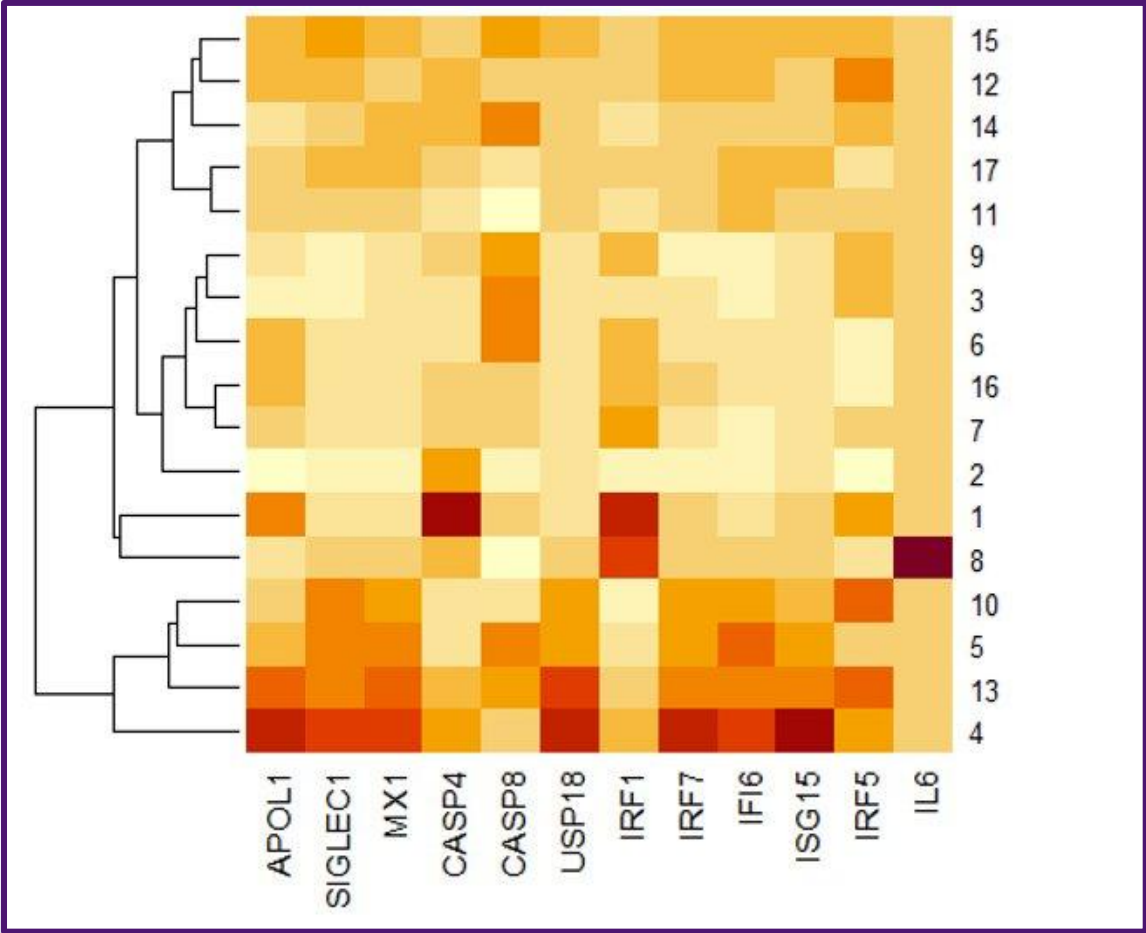
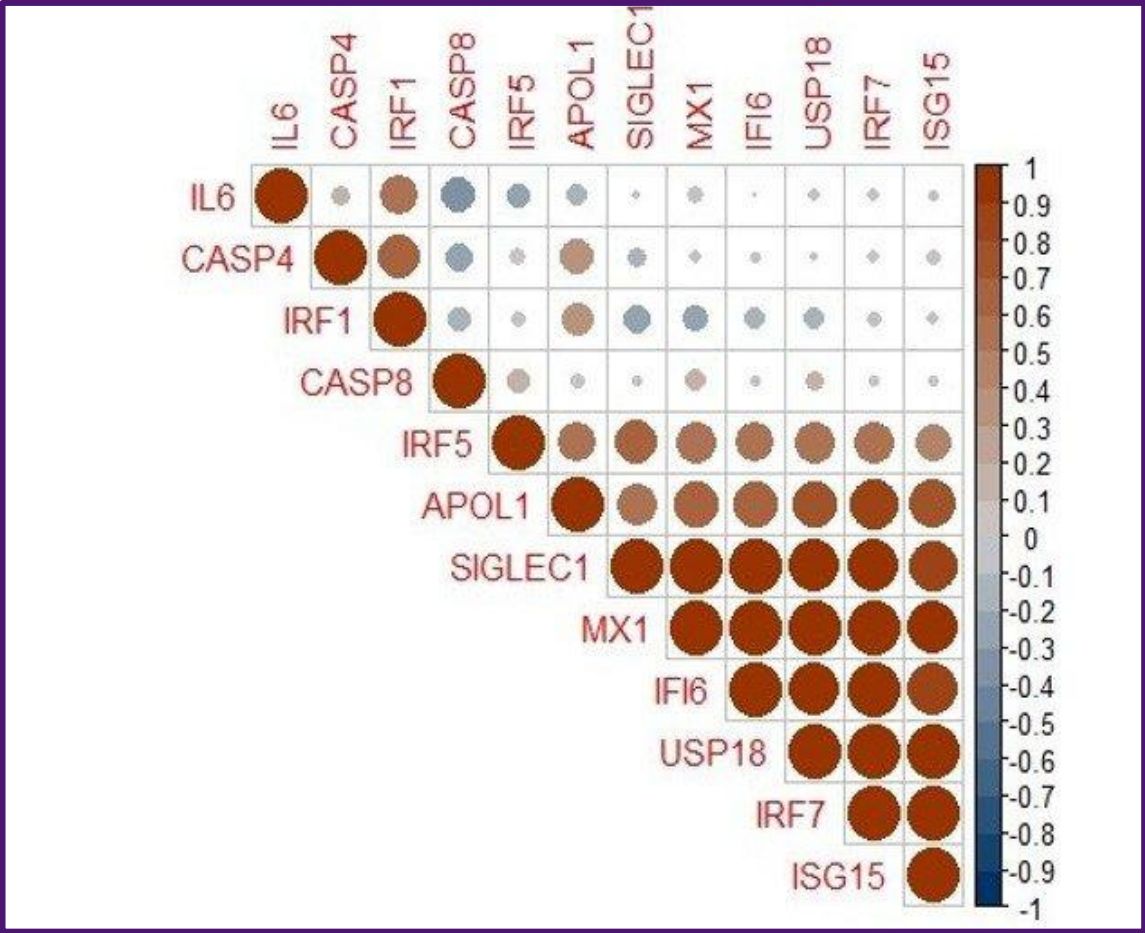
# Does the Inflammatory Milieu of SLE Act as a Second Hit In the Relationship Between APOL1 HRG and Kidney Injury?



# Can We Leverage SLE Monocytes to Understand the Tissue Microenvironment in APOL1 Variant-Associated Cytotoxicity?



# SLE PBMCs: APOL1 Expression Co-clusters with Interferon Stimulated Gene Expression

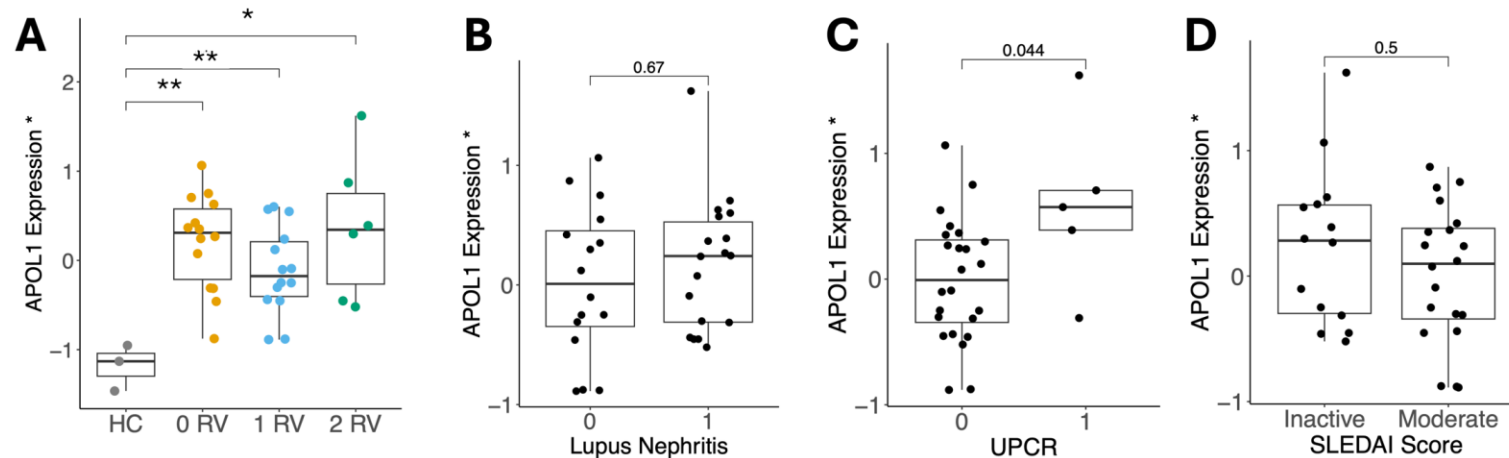


# Monocyte APOL1 expression is elevated in SLE and active proteinuria



## Key findings

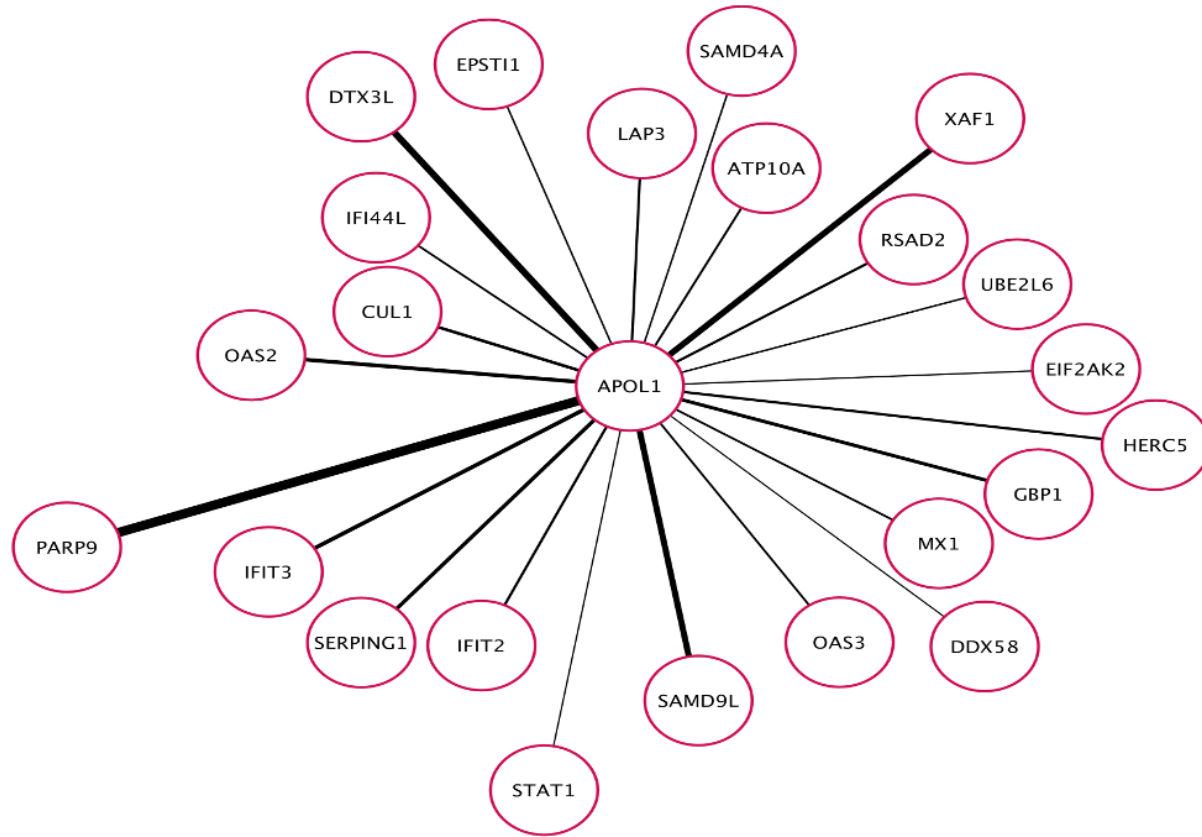
- SLE across genotype**  
0, 1, and 2 RV groups each showed higher expression than healthy controls
- Active proteinuria**  
Higher APOL1 expression with active proteinuria  $p=0.044$
- Other clinical groups**  
No difference by nephritis status or SLEDAI category  $p=0.67$  and  $p=0.5$



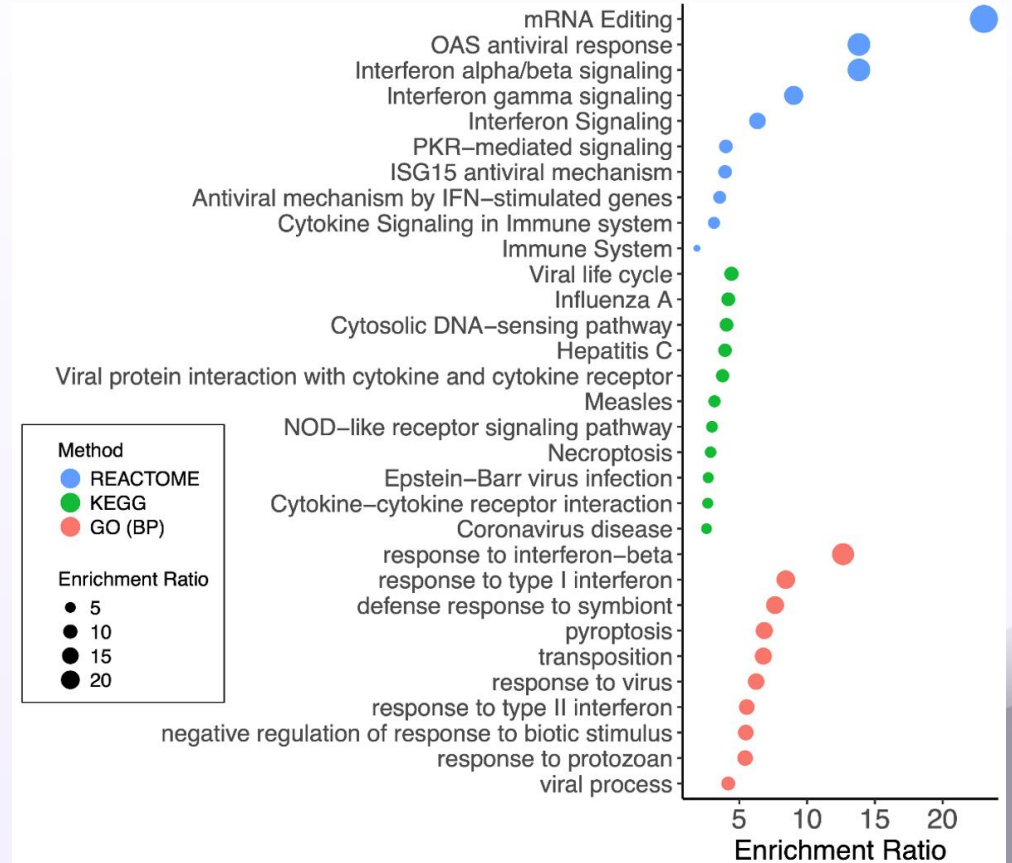
Monocyte APOL1 expression was elevated in SLE and was higher with active proteinuria.

# Gene Ontology Shows that Monocyte APOL1 Expression is Positively Linked to Interferon Signaling

A



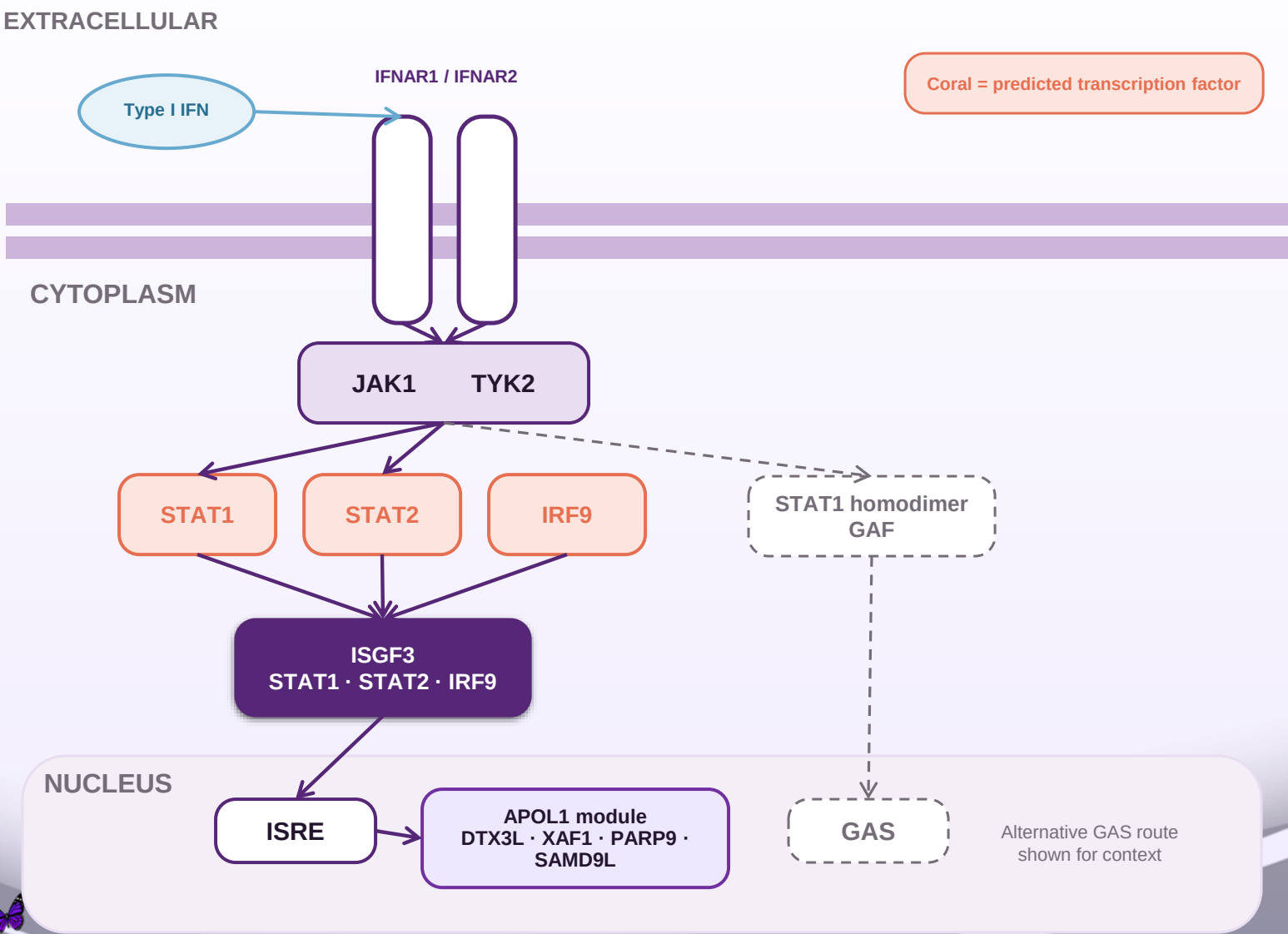
B



(A) Network visualization of the pink module. Genes directly connected to APOL1 are highlighted. The line thickness indicates genes ranked by degree centrality, defined as the number of direct network connections.

(B) Over-representation analysis of pink module genes using Gene Ontology Biological Process (GO-BP), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathway databases.

# APOL1 Expression Module Indicates ISGF3-Dependent Type I Interferon Activation



## APOL1-ASSOCIATED SIGNALS AND BIOLOGIC ROLES

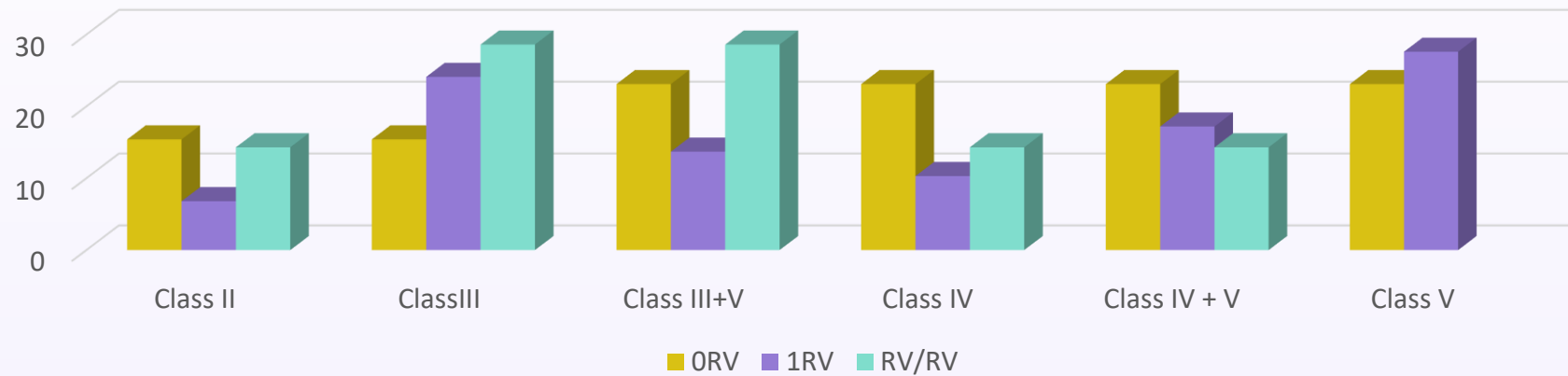
| CO-EXPRESSED TRANSCRIPTS        |                                                                                             |
|---------------------------------|---------------------------------------------------------------------------------------------|
| Gene                            | Concise role                                                                                |
| DTX3L                           | E3 ubiquitin ligase; partners with <a href="#">PARP9</a> in IFN-induced antiviral responses |
| XAF1                            | IFN-inducible proapoptotic protein that antagonizes XIAP                                    |
| PARP9                           | IFN-inducible ADP-ribosylation regulator; forms a complex with <a href="#">DTX3L</a>        |
| SAMD9L                          | IFN-inducible antiviral restriction and growth-regulatory protein                           |
| PREDICTED TRANSCRIPTION FACTORS |                                                                                             |
| Factor                          | Concise role                                                                                |
| STAT1                           | IFN signal transducer; functions in both ISGF3 and STAT1 homodimers                         |
| STAT2                           | Type I IFN signal transducer and core ISGF3 subunit                                         |
| IRF9                            | DNA-binding ISGF3 subunit that recognizes ISRE elements                                     |
| IRF7                            | Key regulator of type I interferon production and amplification                             |
| ETV7                            | IFN-inducible transcriptional repressor that limits a subset of ISGs                        |
| SPIC*                           | Heme-responsive macrophage transcription factor involved in iron handling                   |

Pathway granularity may explain different APOL1 expression among individuals with similar composite interferon signatures.

APOL1 = apolipoprotein L1    ISGF3 = interferon-stimulated gene factor 3    GAF = gamma-activated factor

# African American Cohort: Variant Homozygotes Progressed to ESRD Despite More Reassuring Biopsies

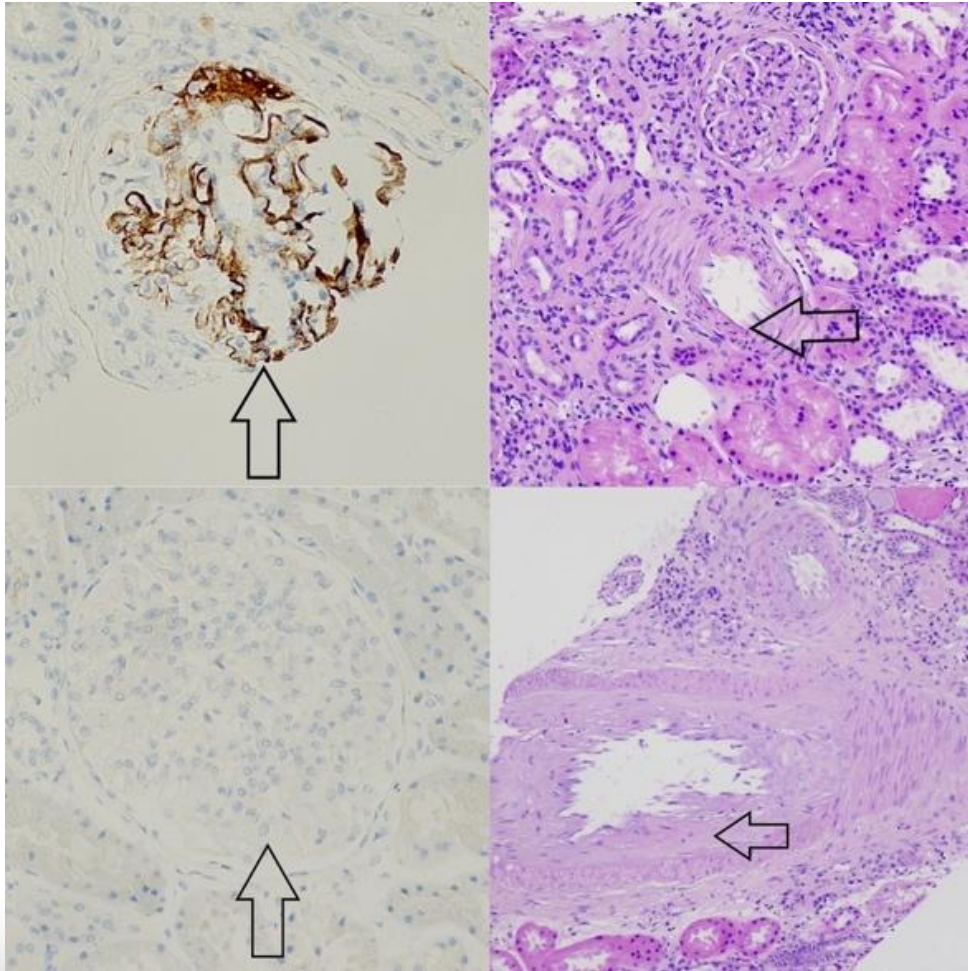
Distribution of Biopsy Class by APOL1 Genotype



|         | Activity Index | Chronicity Index | % Sclerotic Glomeruli |
|---------|----------------|------------------|-----------------------|
| ORV     | 5/24           | 3/12             | 29%                   |
| 1RV     | 6/24           | 2/12             | 14%                   |
| 2RV     | 1/24           | 1/12             | 8%                    |
| P value | 0.04           | 0.01             | 0.06                  |

|         | Creatinine | uPCR | % ESRD            |
|---------|------------|------|-------------------|
| ORV     | 1.2        | 2.5  | 18%               |
| 1RV     | 1.1        | 2.8  | 15%               |
| 2RV     | 2.2        | 4.1  | 43%               |
| P value | 0.01       | 0.04 | OR: 5.7<br>P=0.05 |

# The same histology, a different mechanism



C4d stain, 20x objective (left column). Haematoxylin and eosin, 10x objective (right column).

## Biopsies done for LN May Have Less Immune Complex deposition

Top left: a glomerulus with positive C4d staining in lupus nephritis.  
Bottom left: a glomerulus from an APOL1 high-risk genotype patient with minimal C4d staining, indicating low immune complexes.

### Lupus nephritis

Immune complex mediated.

### APOL1 high-risk genotype

A kidney-intrinsic podocytopathy.

## No Current Biomarker Distinguishes These Two Entities

The ISN/RPS classification grades immune complex deposition and fibrosis. It has no category for a concurrent genetic mechanism..

# We Evaluated Time of Biopsy Serologies and Histopathologic Features in 84 Biopsies from 77 SLE Participants

| Characteristic              | 0 RV (n=30) | 1 RV (n=36) | 2 RV (n=18)      | p           |
|-----------------------------|-------------|-------------|------------------|-------------|
| Female % (n)                | 90 (27)     | 83 (30)     | 94 (17)          | 0.46        |
| Age at biopsy, mean (SD)    | 35.2 (11.0) | 29.6 (10.2) | 30.9 (13.2)      | 0.12        |
| Hypertension % (n)          | 65 (17)     | 69 (24)     | 81 (13)          | 0.53        |
| High anti-dsDNA % (n)       | 80 (20)     | 79 (26)     | <b>47 (7)</b>    | <b>0.04</b> |
| Activity index, mean (SD)   | 6.1 (5.2)   | 7.9 (6.0)   | <b>2.2 (3.1)</b> | <b>0.03</b> |
| Class 0/II/III % (n)        | 7 (2)       | 11 (4)      | <b>39 (7)</b>    | <b>0.01</b> |
| Chronicity index, mean (SD) | 3.2 (2.4)   | 2.6 (2.2)   | 1.8 (3.3)        | 0.43        |
| FPE >75% % (n)              | 72 (18)     | 57 (17)     | 62 (8)           | 0.50        |
| Proteinuria g/g, mean (SD)  | 3.0 (2.5)   | 3.5 (2.9)   | 3.8 (3.3)        | 0.61        |
| eGFR, mean (SD)             | 83.7 (41.4) | 85.0 (42.9) | 78.2 (50.8)      | 0.86        |
| ESKD % (n)                  | 20 (6)      | 19 (7)      | 22 (4)           | 0.97        |

## HRG Participants Exhibited Less disease activity

HSG Participants had:

- Less dsDNA-positive cases
- Lower activity index
- four times the rate of Class 0/II/III disease
- Similar Rates of ESKD at Time of Biopsy

84 biopsies from 77 participants. RV, APOL1 risk variants. Full characteristics in Table 1.

# Podocytopathy on Biopsy Occurred Only in APOL1 High-Risk Genotype



| Genotype          | Biopsies with a retrievable report | Podocytopathy diagnosed | %          |
|-------------------|------------------------------------|-------------------------|------------|
| 0 RV              | 18                                 | 0                       | 0%         |
| 1 RV              | 11                                 | 0                       | 0%         |
| <b>2 RV (HRG)</b> | <b>11</b>                          | <b>3</b>                | <b>27%</b> |

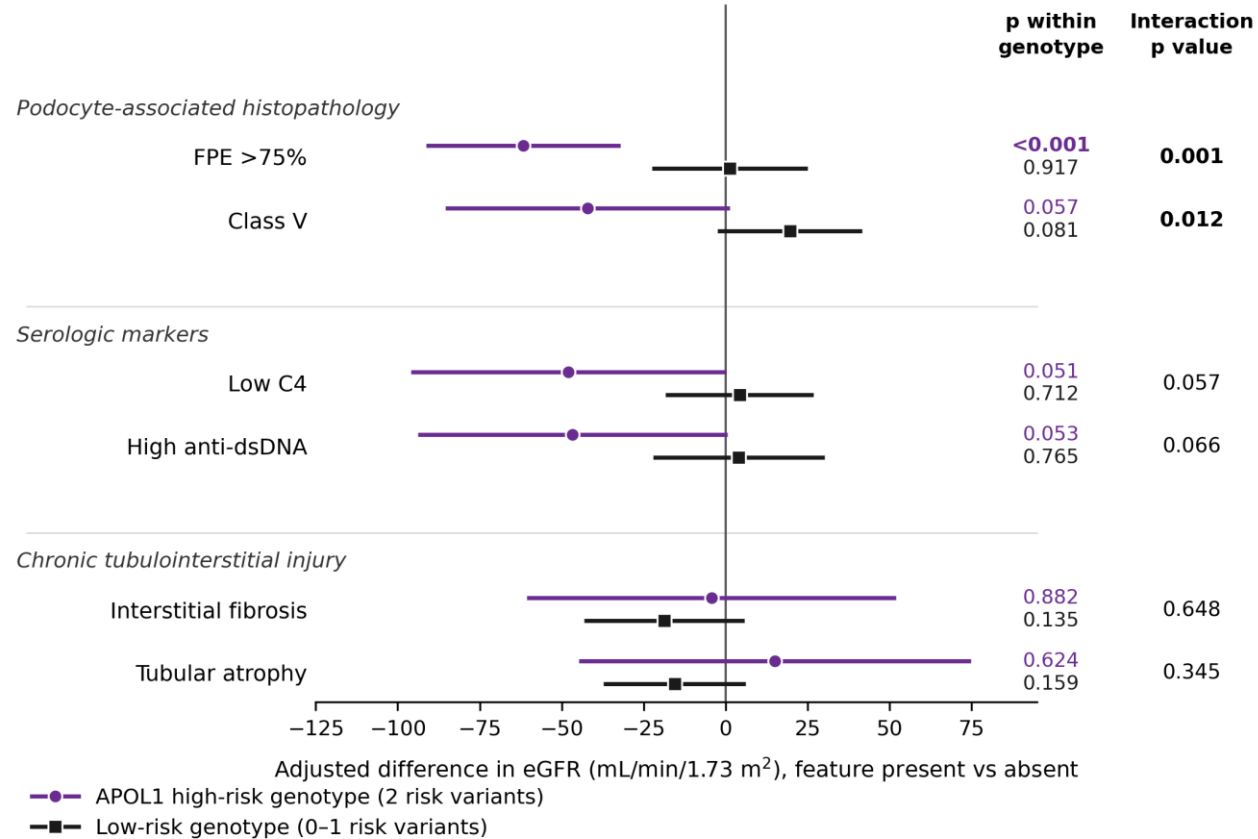
Fisher exact p = 0.017

**H1084 (G2/G2)** “Minimal change nephrotic syndrome in the setting of SLE” — noted as a form of lupus podocytopathy. >80% effacement, no immune deposits.

**H1092 (G1/G2)** “Glomerular podocytopathy suggestive of minimal change glomerular disease.” Proliferative and membranous features absent.

**5772 (G1/G2)** “Diffuse foot process effacement” — >80% effaced, noted as possible lupus podocytopathy. Class I disease, activity index 0/24.

# Increased Foot Process Effacement and Class V LN Show Genotype-Specific Associations with eGFR



## Key findings



### FPE >75%

Lower eGFR in HRG, but not LRG  
interaction p=0.001



### Class V

The association also differed by genotype  
interaction p=0.012



### Chronic injury

Fibrosis and tubular atrophy did not differ by genotype

## Mean eGFR within histopathology strata

| Feature               | HRG present | HRG absent | LRG present | LRG absent |
|-----------------------|-------------|------------|-------------|------------|
| FPE >75%              | 67.0 (8)    | 123.0 (5)  | 90.9 (33)   | 91.7 (20)  |
| Class V               | 45.5 (7)    | 99.0 (11)  | 91.0 (34)   | 79.3 (27)  |
| Interstitial fibrosis | 69.4 (8)    | 97.1 (6)   | 74.9 (30)   | 106.5 (20) |
| Tubular atrophy       | 65.0 (7)    | 90.1 (6)   | 75.6 (34)   | 101.5 (21) |

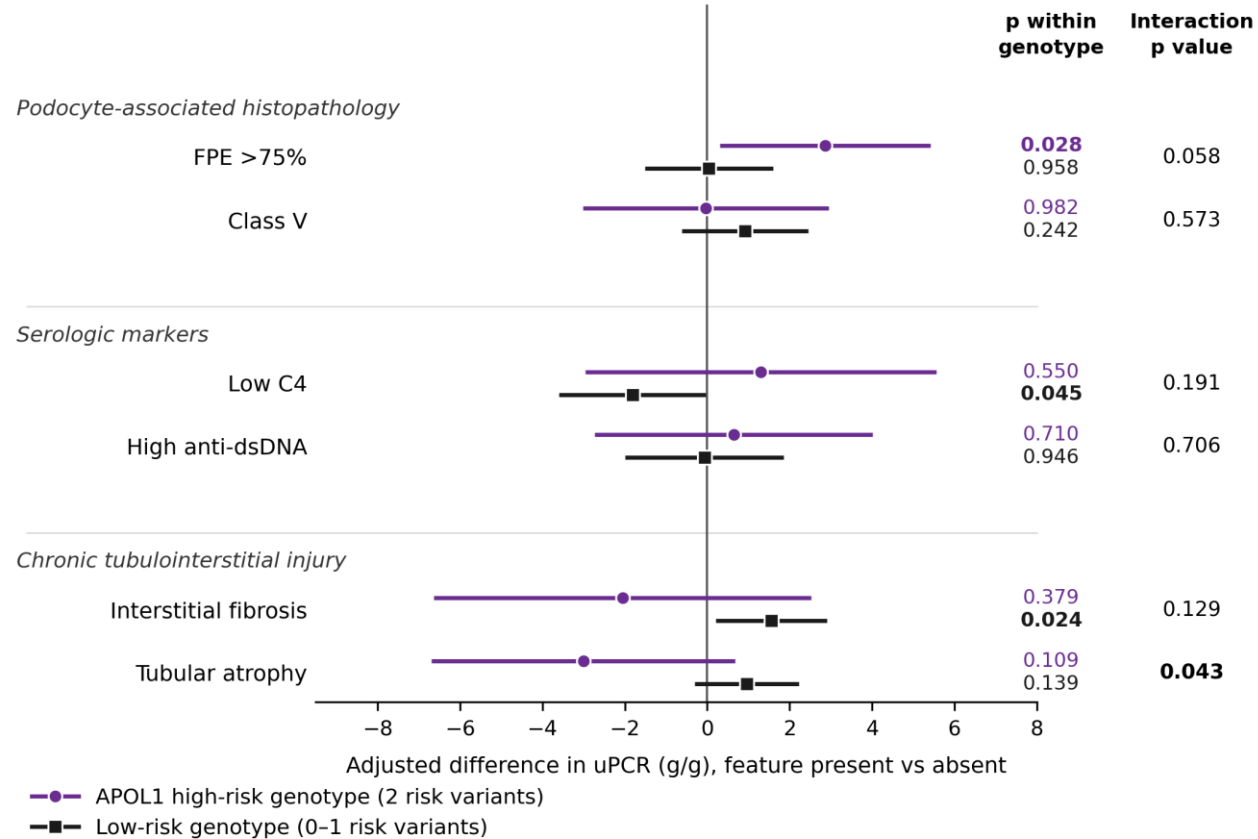
Values are mean (n) and are descriptive. The figure shows adjusted marginal effects and interaction p values.

APOL1 genotype modified the association of podocyte injury with eGFR.



# Chronic Tubular Injury Associated with Higher uPCR in LRG While >75% FPE Was Important in HRG Participants

FPE was higher in HRG, while chronic injury showed opposite genotype patterns



## Key findings



### FPE >75%

Higher uPCR in HRG (p=0.028)  
interaction p=0.058



### Interstitial fibrosis

Associations moved in opposite directions  
interaction p=0.024



### Tubular atrophy

A similar genotype difference was present  
interaction p=0.043

## Mean uPCR within histopathology strata

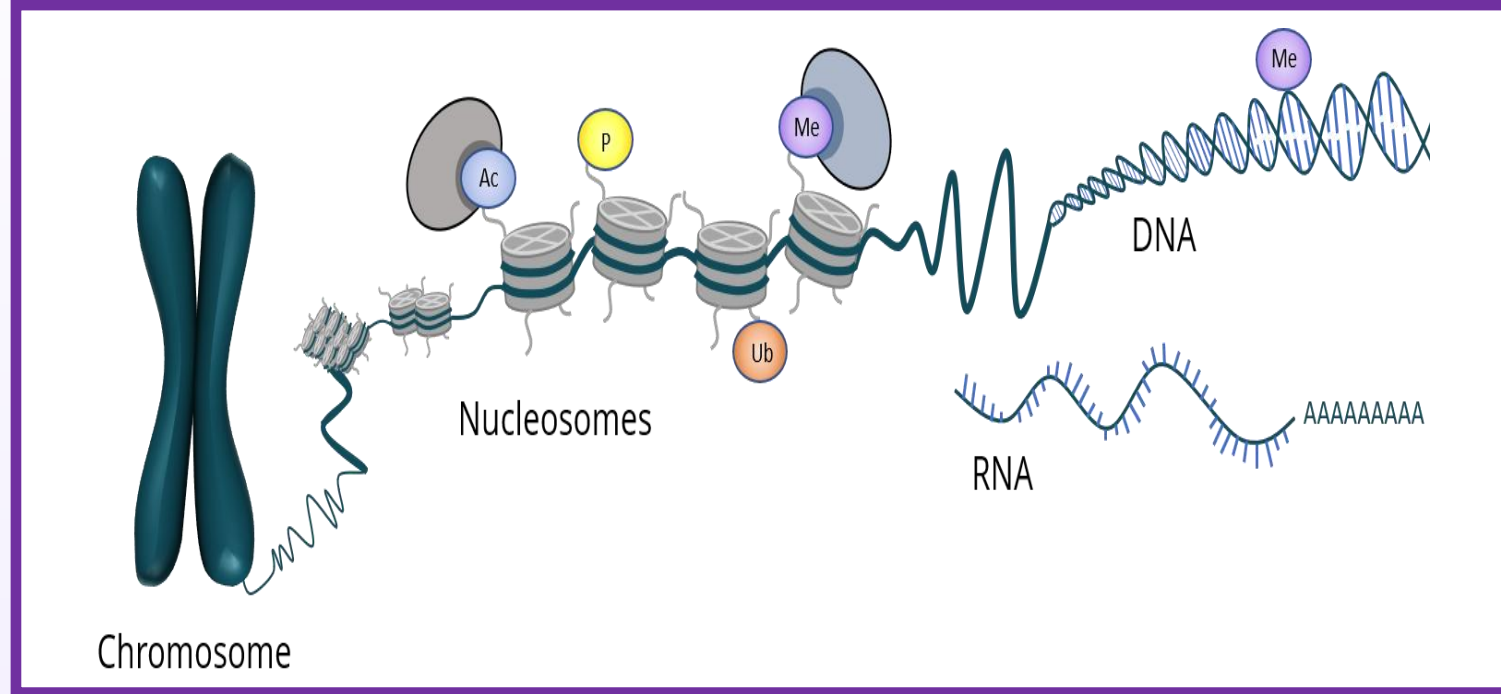
| Feature               | HRG present | HRG absent | LRG present | LRG absent |
|-----------------------|-------------|------------|-------------|------------|
| FPE >75%              | 5.4 (8)     | 1.6 (5)    | 3.1 (32)    | 2.9 (20)   |
| Class V               | 3.5 (5)     | 3.9 (11)   | 3.6 (33)    | 2.8 (27)   |
| Interstitial fibrosis | 2.5 (8)     | 4.9 (5)    | 3.7 (29)    | 2.2 (20)   |
| Tubular atrophy       | 2.6 (7)     | 5.3 (5)    | 3.7 (33)    | 2.5 (21)   |

Values are mean (n) and are descriptive. The figure shows adjusted marginal effects and interaction p values.

FPE tracked with higher proteinuria in HRG. Chronic injury associations differed by genotype.

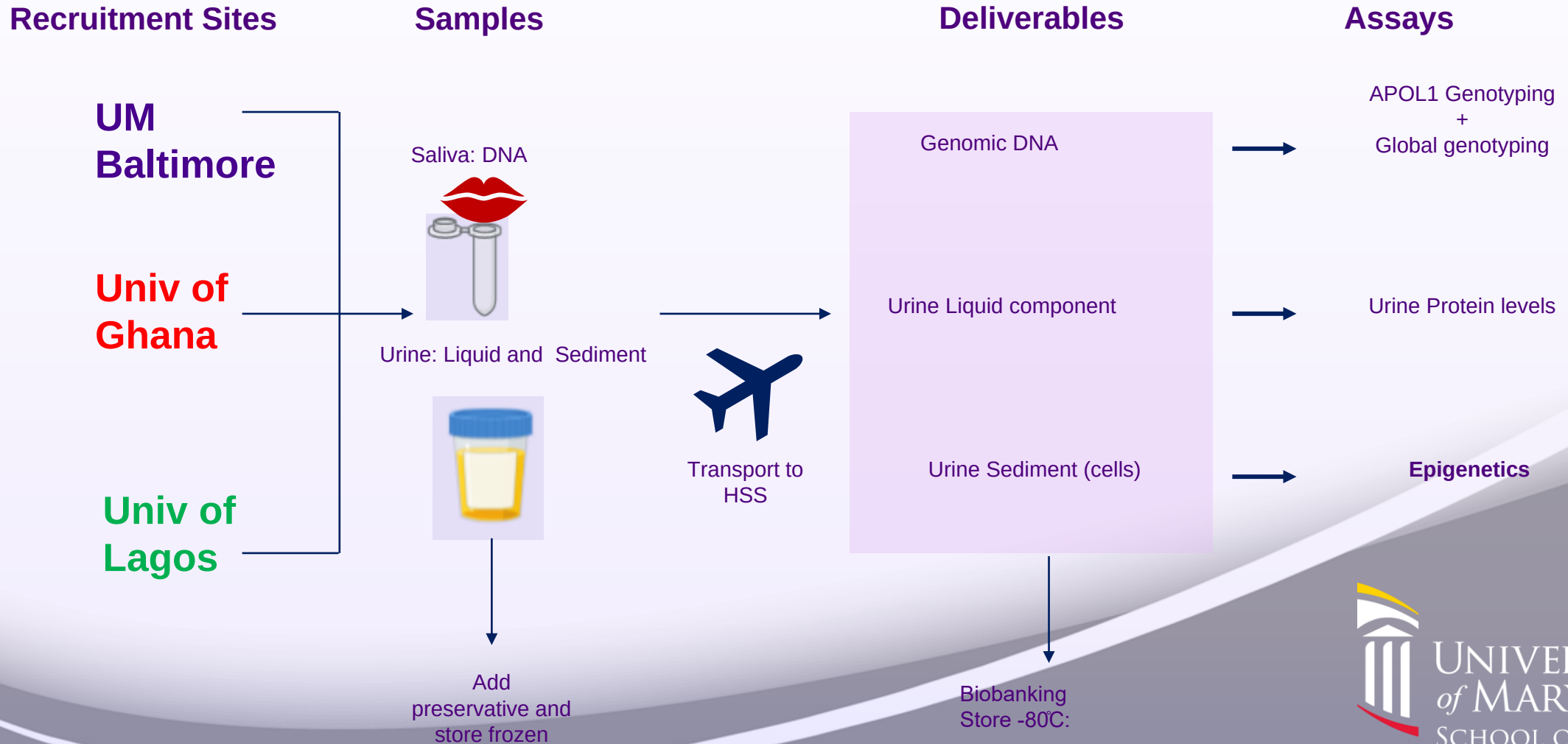
# Epigenetic Modifications Can Be Markers of Gene Expression and Cell Type

- **Epigenetics:** The ways our genetic information is packaged
- Influences which genes are active and which are inactive
- Is different in different cell types
  - Urine contains many cell types
  - These cell types can be distinguished by epigenetics
- Can change as inflammation becomes more active



- **Research Question: Can DNA present in the urine tell us whether the kidney is inflamed due to lupus or APOL1 kidney injury?**

# Pilot Study: Leveraging Ongoing Biobanking Efforts to Understand Urine Cell Epigenetics by APOL1 Genotype



# Pilot Cohort: 45 Participants of African Ancestry

| Variable                 | LN: Yes<br>(n=29) | LN: No<br>(n=12) | Healthy Control<br>(n=2) | Total<br>(N=45) | p-value |
|--------------------------|-------------------|------------------|--------------------------|-----------------|---------|
| Age At Diagnosis         | 30.4 ± 14.9       | 34.8 ± 10.6      | —                        | 31.2 ± 13.7     | 0.32    |
| Female %                 | 27 (93.1%)        | 12 (100.0%)      | 2 (100.0%)               | 43 (95.6%)      | 1.00    |
| Number of APOL1 variants |                   |                  |                          |                 | 0.41    |
| 0                        | 10 (35.7%)        | 4 (36.4%)        | 2 (100.0%)               | 18 (41.9%)      |         |
| 1                        | 10 (35.7%)        | 6 (54.5%)        | 0 (0.0%)                 | 16 (37.2%)      |         |
| 2                        | 8 (28.6%)         | 1 (9.1%)         | 0 (0.0%)                 | 9 (20.9%)       |         |
| ANA                      | 27 (100.0%)       | 11 (100.0%)      | —                        | 40 (100.0%)     | 1.00    |
| Neurologic               | 6 (20.7%)         | 2 (18.2%)        | —                        | 8 (19.0%)       | 1.00    |
| Arthritis                | 26 (89.7%)        | 9 (81.8%)        | —                        | 36 (87.8%)      | 0.60    |
| Hematologic              | 19 (65.5%)        | 5 (45.5%)        | —                        | 25 (59.5%)      | 0.43    |
| Serositis                | 15 (51.7%)        | 1 (9.1%)         | —                        | 17 (40.5%)      | 0.03    |
| Mucocutaneous            | 21 (72.4%)        | 7 (63.6%)        | —                        | 29 (69.0%)      | 0.70    |
| Discoid                  | 7 (33.3%)         | 2 (18.2%)        | —                        | 9 (26.5%)       | 0.44    |
| Malar Rash               | 8 (38.1%)         | 5 (45.5%)        | —                        | 14 (41.2%)      | 0.98    |
| Hypocomplementemia       | 14 (51.9%)        | 6 (54.5%)        | —                        | 20 (50.0%)      | 1.00    |
| Anti-Ro                  | 8 (32.0%)         | 4 (36.4%)        | —                        | 12 (32.4%)      | 1.00    |
| Anti-La                  | 1 (4.0%)          | 1 (9.1%)         | —                        | 2 (5.4%)        | 0.52    |
| Anti-RNP                 | 9 (47.4%)         | 5 (45.5%)        | —                        | 14 (45.2%)      | 1.00    |
| Smith or dsDNA           | 14 (51.9%)        | 6 (54.5%)        | —                        | 21 (52.5%)      | 1.00    |

Table 1. Clinical and serologic features by LN status. Sites: HSS, UMB, University of Ghana, University of Lagos. APOL1 genotyped by PCR.

**45**

participants enrolled across 4 sites (US & West Africa)

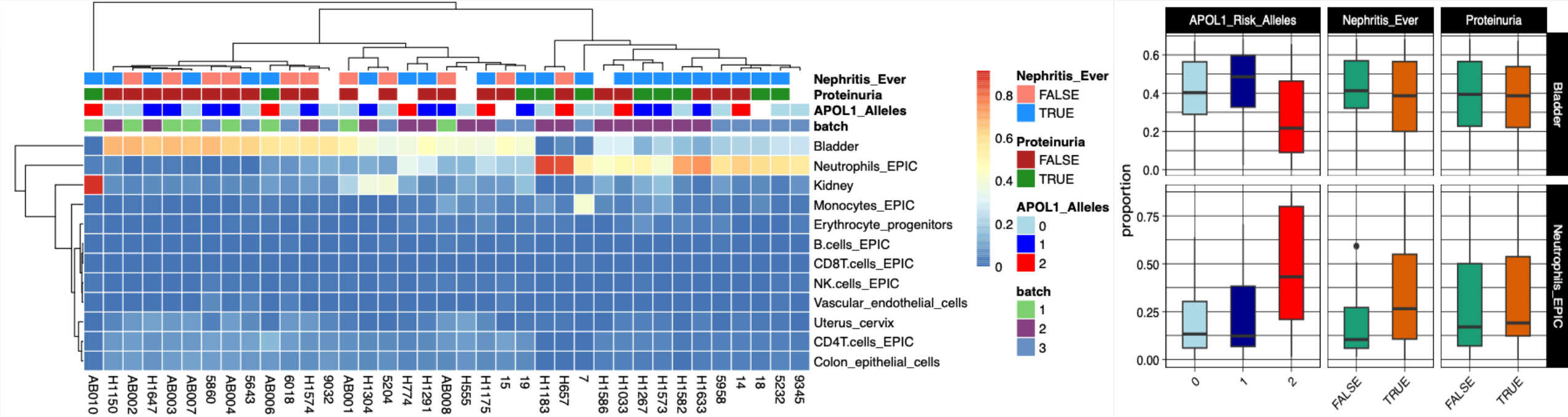
**29**

with lupus nephritis; 16 with active proteinuria

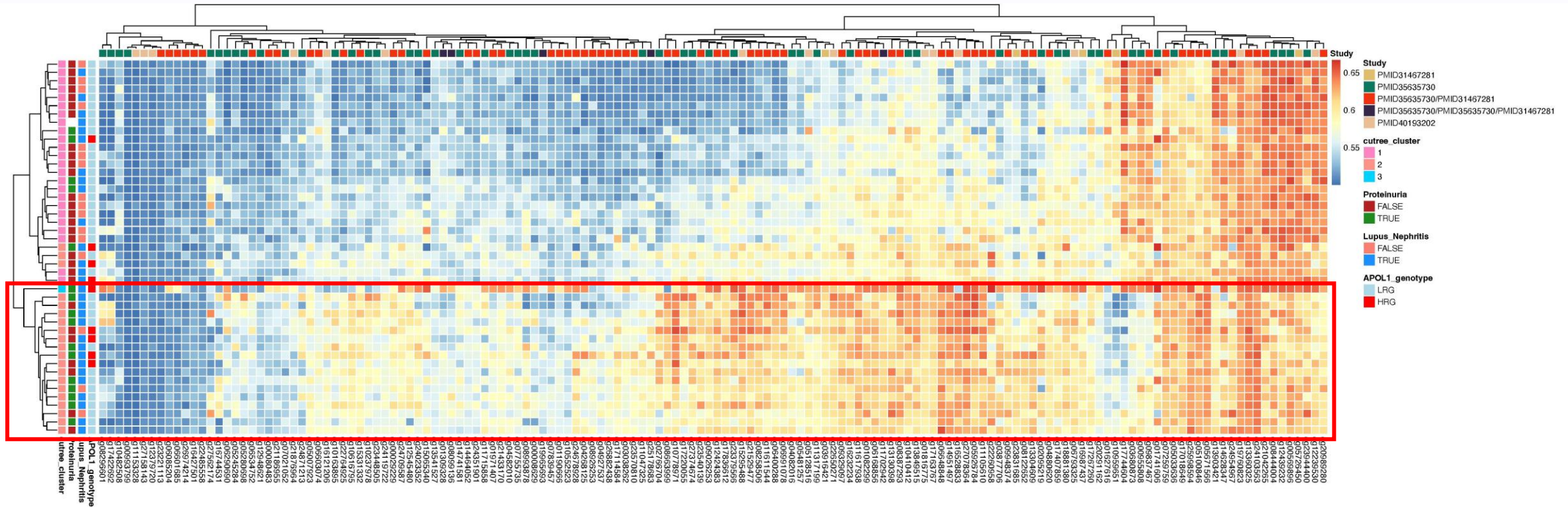
**9**

carrying the APOL1 high-risk genotype (HRG)

# Patients with High risk APOL1 genotypes Have Increased Proportions of Neutrophil cfDNA in Their Urine



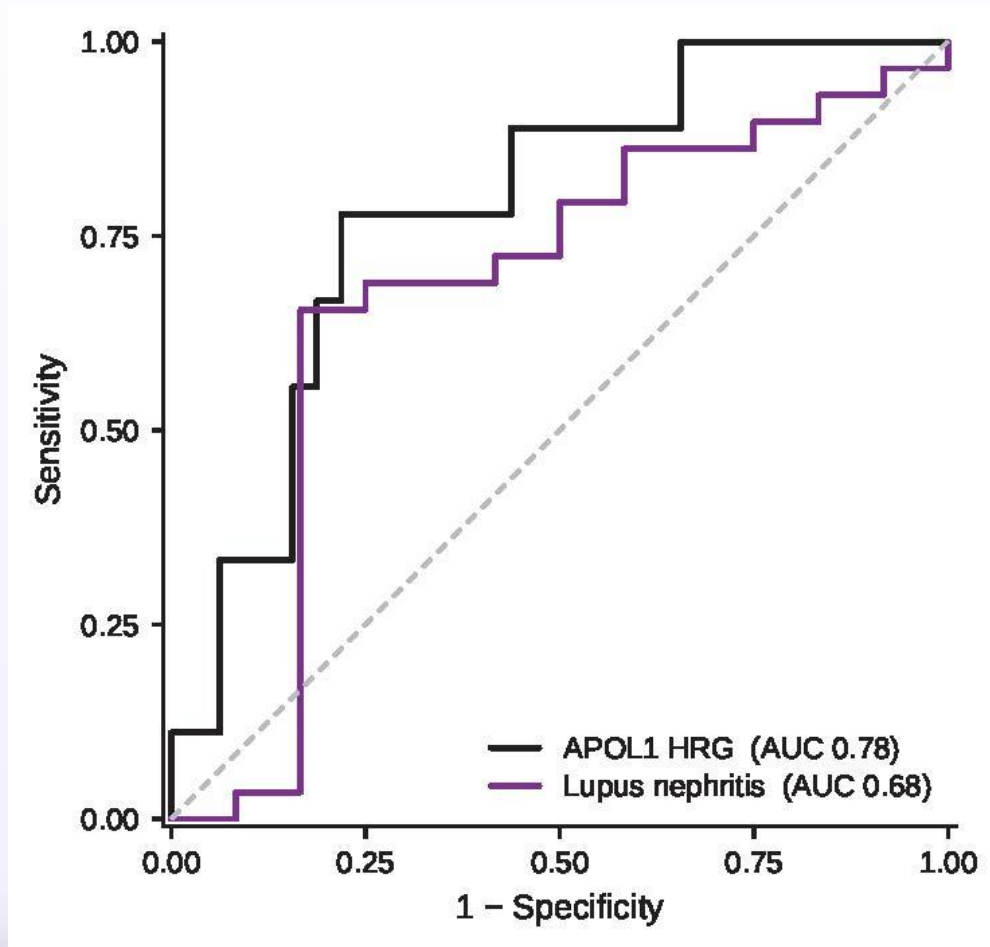
# Distinct Epigenetic Marks Distinguish Patients with Lupus Nephritis from Non-Nephritis Patients



**Updated cohort (148 differentially methylated probes).** Methylation values for previously published probes were adjusted for submission batch, neutrophil proportion, and bladder epithelial cell proportion.

**Unsupervised clustering separates three groups:** lupus nephritis (LN) patients, APOL1 high-risk genotype (HRG) patients with proteinuria, and HRG patients without proteinuria. Cluster differs by APOL1 genotype ( $p=0.03$ ) and proteinuria ( $p=0.02$ ).

# Distinct Signatures Predict LN and APOL1 HRG



PCA-based logistic regression; AUC 0.79 (HRG), 0.68 (LN).

## PC1 — lupus nephritis axis

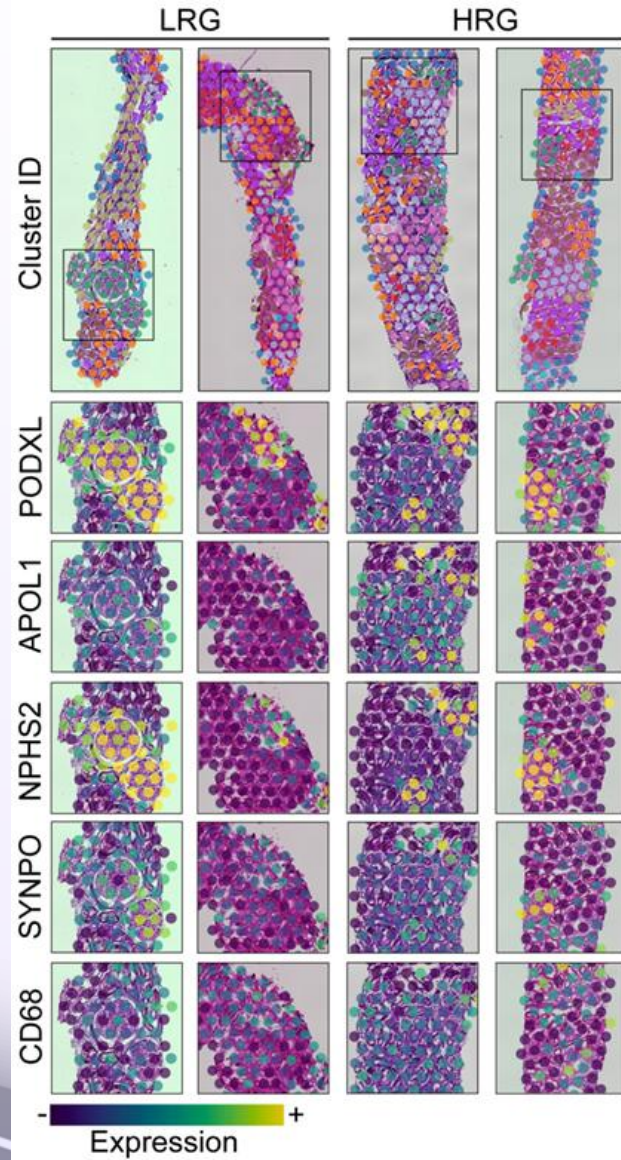
- PC1 associated with prior LN ( $\beta = 0.40 \pm 0.15$ ,  $p = 0.007$ ).
- Each unit rise in PC1  $\approx$  50% higher odds of LN.
- PC1 also associated with carrying the HRG ( $\beta = 0.64 \pm 0.28$ ,  $p = 0.02$ ).

## PC2 — APOL1 axis

- PC2 associated with HRG ( $\beta = -1.79 \pm 0.83$ ,  $p = 0.03$ ) but not LN ( $p = 0.69$ ).
- Different probe sets predict LN vs HRG — separable epigenetic signatures.

**Proof of concept that urine cfDNA methylation reflects both immune-mediated and genetically driven kidney injury**

# Future Direction: Anchoring the Epigenetic Signature In Spatial Transcriptomics from Paired Kidney Biopsies



Visium spatial profiling of matched FFPE biopsies, low-risk versus high-risk genotype

Each glomerulus will be a unit of analysis. Transcripts at or near candidate methylation loci will be tested for compartment-specific differential expression.

**PODXL** Podocalyxin — podocyte surface marker

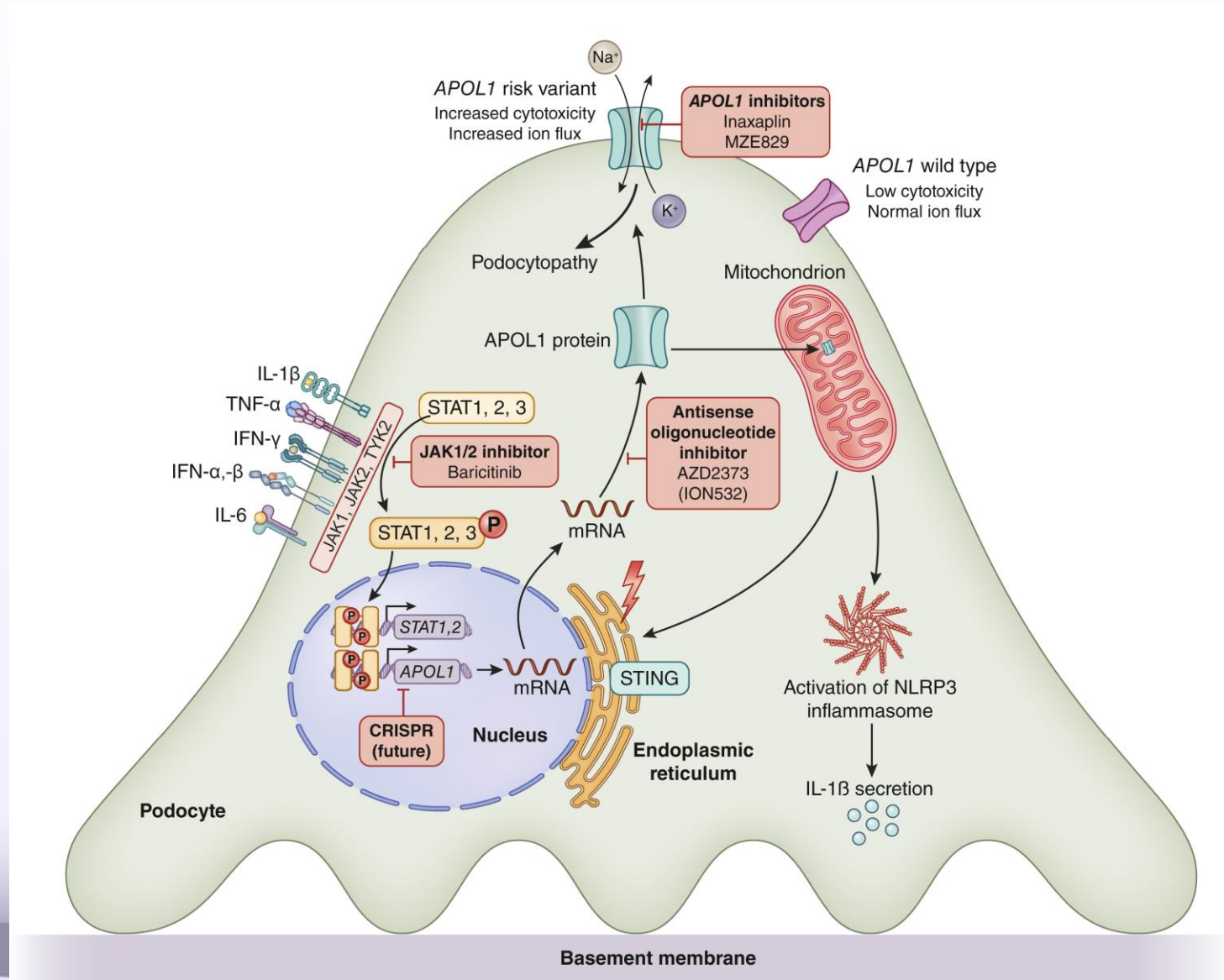
**APOL1** Expressed in the glomerulus; the gene of interest

**NPHS2** Podocin — slit diaphragm, essential for filtration

**SYNPO** Synaptopodin — podocyte actin cytoskeleton

**CD68** Macrophage marker — infiltrating immune cells

# The Current Therapeutic Landscape for Both SLE and APOL1 Nephropathy Includes JAK Inhibitors



# Translational implications

- APOE1 variant alleles are common in recent African ancestry populations and may prime carriers for tissue injury
- APOE1 Nephropathy Appears to exist along side LN
- Understanding how the inflammatory microenvironment contributes to APOE1 associated tissue injury can open new treatment options
- Wider APOE1 genetic allele testing may aid in risk stratification



# ACKNOWLEDGMENTS

## Mentor Team

Tim Niewold, MD  
Jasmin Divers, PhD  
Wonder Drake, MD PhD  
James Kaper, PhD

## Blazer Lab

Vishnuprabu Pandian  
Prajakta Shinde  
Hope Ononiwu

## NYU School of Medicine

Jill Buyon, MD  
Olugbenga Ogedegbe, MD

## Collaborators

Cristina Lanata, MD  
William Liao, PhD

## The University of Ghana

Ida Dzifa Dey, MD  
Margret Reynolds  
Festus Ankrah

## Lagos University Teaching Hospital

Olusola Ayanlowo, MD  
Uyi-Ima-Edomwonyin, MD  
Hakeem Olaosebikan, MD

## NIAID K23 Career Development Award



**Rheumatology Research Foundation**

Advancing Treatment | Finding Cures



**LUPUS  
RESEARCH  
ALLIANCE**

**Patients!!**



UNIVERSITY  
of MARYLAND  
SCHOOL OF MEDICINE