SYDNEY BRENNER INSTITUTE FOR MOLECULAR BIOSCIENCE

AWI-Gen
Africa Wits-INDEPTH Partnership for Genomic Research

APOL1 - Background, evolutionary context and in Longitudinal cohort AWI-Gen

Brandenburg, Jean-Tristan

KidneyGen Africa

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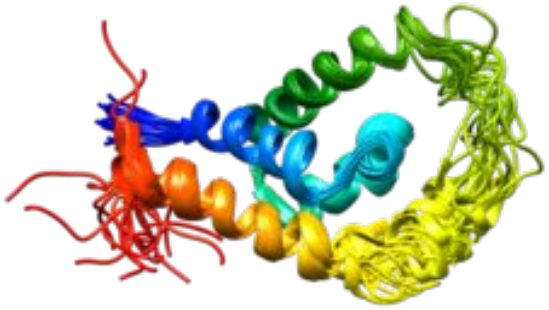
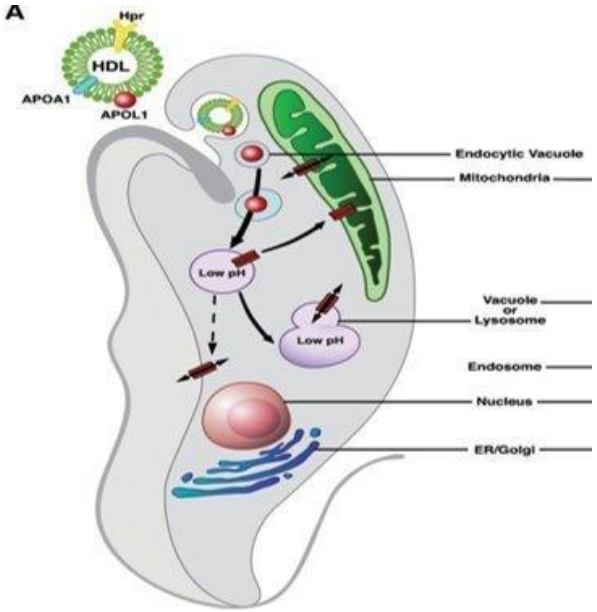
Scientific Interest

- **I use bioinformatics, statistics, and population genetics, and develop tools and methods** to explore the **genetic and multi-omics mechanisms underlying chronic kidney disease and cancer**.
- **Previously:**
  - Developed **individual-based models** to understand how **complex evolutionary processes shape genetic diversity**.
  - Demonstrated that **independent introductions and admixture events** contributed to the **adaptation of European maize and its American counterparts**.
- **More recently:**
  - Studied the **prevalence of diseases and associated risk factors**.
  - Conducted **genetic association studies**.
  - Developed **analytical tools and methods**.
  - Investigated the **genetic basis of cancer, including rare mutations**.
  - Worked across multiple phenotypes: **cancer, chronic kidney disease, hypertension, and HIV**.

APOL1 : genes function

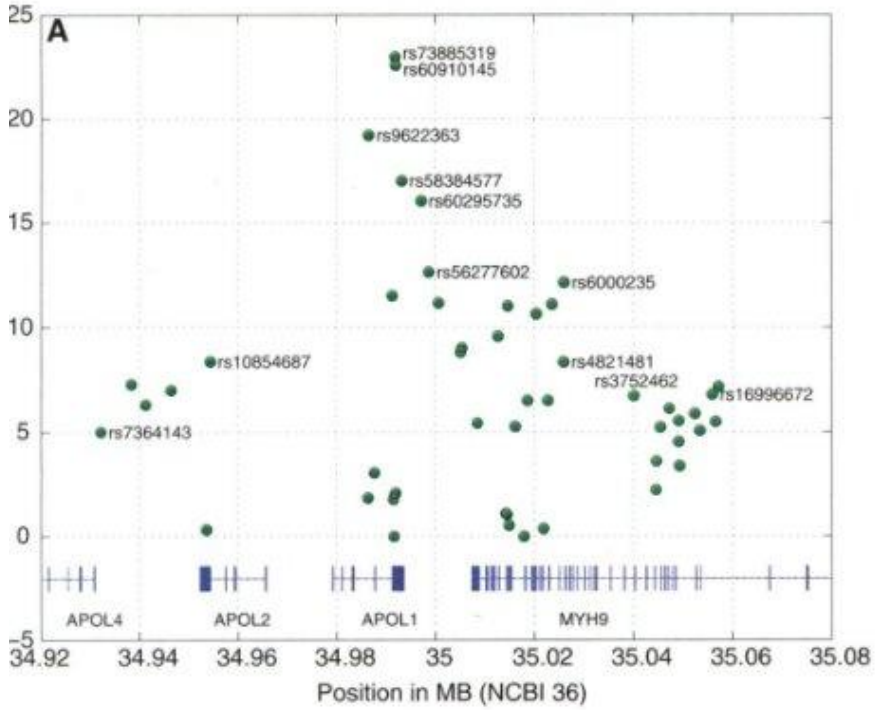


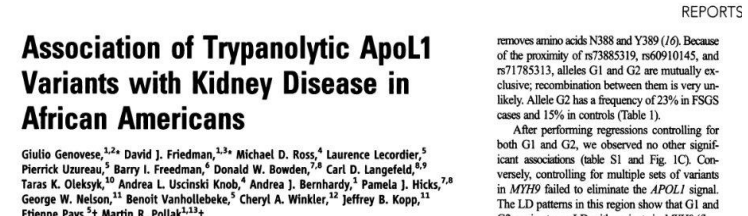
- Apolipoprotein L1 (APOL1) encodes a protein involved in innate immunity and lipid-associated pathways.
- Innate immune role : APOL1 forms pores in lysosomal membranes of Trypanosoma parasites
- Confers protection against African trypanosomiasis (sleeping sickness) where there is a high prevalence in SSA
- Expression profile :
  - Highly expressed in kidney cells (podocytes, endothelial cells)
  - Also expressed in liver and immune cells
- Physiological function Involved in membrane trafficking, autophagy, and cell survival
- May regulate ion transport and apoptosis



APOL1 – association in African American

- **Seminal discovery of APOL1 risk variants (Genovese et al., 2010)**
- In 2010, Genovese *et al.* made the seminal discovery that **two mutually exclusive exonic variants** of the gene encoding the trypanolytic factor **apolipoprotein L1 (APOL1)** on chromosome 22 are strongly associated with kidney disease in individuals of African ancestry.
- Association analyses were performed in focal segmental glomerulosclerosis (FSGS) cohorts using logistic regression for the allele in *APOL1* regions.
- The study compared **205 idiopathic biopsy-proven African-American FSGS cases** with **180 African-American controls**, using **Fisher's exact test**.
- Carrying **two APOL1 risk alleles (G1 and/or G2)** was strongly associated with increased FSGS risk.





Association of Trypanolytic ApoL1 Variants with Kidney Disease in African Americans

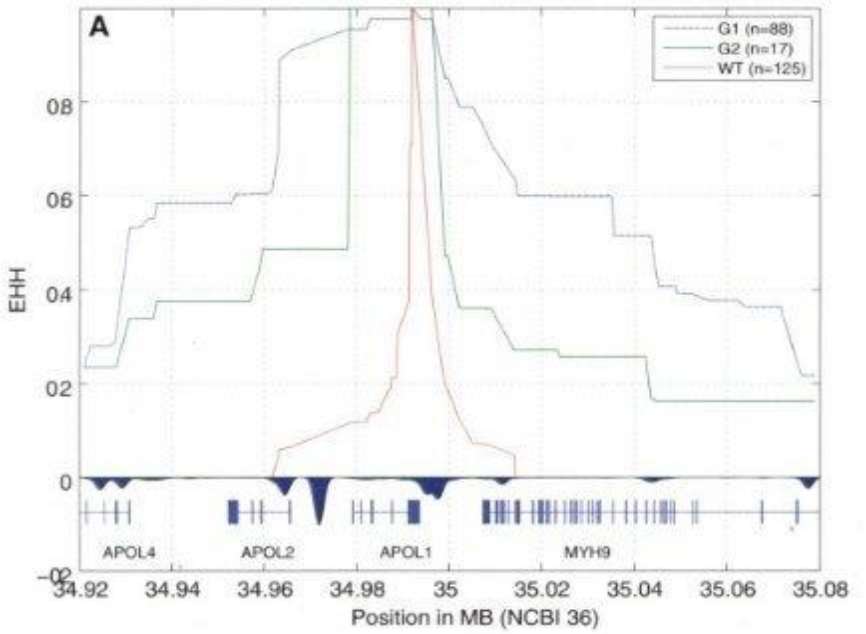
Giulio Genovese,[1,2]* David J. Friedman,[1,3]* Michael D. Ross,[4] Laurence Lecordier,[5] Pierrick Uzureau,[5] Barry I. Freedman,[6] Donald W. Bowden,[7,8] Carl D. Langefeld,[8,9] Taras K. Oleksyk,[10] Andrea L. Uscinski Knob,[4] Andrea J. Bernhardy,[1] Pamela J. Hicks,[7,8] George W. Nelson,[11] Benoit Vanhollebeke,[5] Cheryl A. Winkler,[12] Jeffrey B. Kopp,[11] Etienne Pays,[5]† Martin R. Pollak[1,13]†

removes amino acids N388 and Y389 (*16*). Because of the proximity of rs73885319, rs60910145, and rs71785313, alleles G1 and G2 are mutually exclusive; recombination between them is very unlikely. Allele G2 has a frequency of 23% in FSGS cases and 15% in controls (Table 1).

After performing regressions controlling for both G1 and G2, we observed no other significant associations (table S1 and Fig. 1C). Conversely, controlling for multiple sets of variants in *MYH9* failed to eliminate the *APOL1* signal. The LD patterns in this region show that G1 and

APOL1 – association in African American

- Natural selection analyses were conducted with the Yoruba population using Extended haplotype homozygosity :
  - evaluated for the three APOL1 alleles (G1, G2, and wild-type G0).
  - EHH was computed by combining HapMap phase 3 genotype data with the authors' genotype data for the APOL1 alleles.
- Results showed long-range haplotypes surrounding G1 and G2 compared with G0, consistent with recent positive selection, likely driven by protection against Trypanosoma infection.



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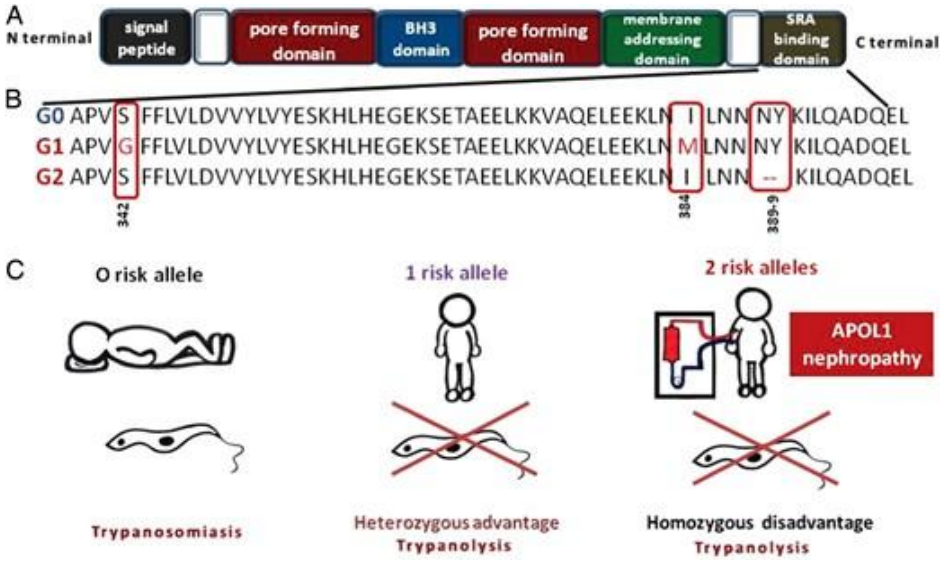
Genetic diversity is shaped by multiple evolutionary forces

- **Sources of genetic diversity**
  - **New mutations** introduce novel genetic variation and increase diversity.
- **Demographic processes**
  - **Genetic drift**: random fluctuations in allele frequencies, especially strong in small populations.
  - **Bottlenecks and migration**: population size reductions and movements can strongly reshape genetic diversity.
- Natural selection acting on traits and genes
- **Negative (purifying) selection**
  Negative selection removes deleterious mutations from a population, maintaining the stability of biological structures and functions by eliminating alleles that reduce fitness.
- **Positive (Darwinian) selection**
  Positive selection increases the frequency of advantageous genetic variants because they confer a survival or reproductive advantage.
- **Balancing selection**
  Balancing selection maintains multiple alleles within a population at higher frequencies than expected by mutation alone, preventing their loss through genetic drift.
  - **Heterozygote advantage**
  - When heterozygous individuals have higher fitness than either homozygote.
  - Example: The HBB rs334 variant (sickle cell trait), where heterozygotes are protected against malaria while avoiding severe sickle cell disease.
  - **Antagonistic (context-dependent) selection**
  - When an allele increases fitness in one context or environment but decreases fitness in another The APOL1 G1 and G2 risk variants are maintained in African populations because they provide protection against African sleeping sickness (Trypanosoma brucei). While carrying one risk allele confers a survival advantage, inheriting two risk alleles substantially increases the risk of chronic kidney disease (CKD), including focal segmental glomerulosclerosis and HIV-associated nephropathy.

APOL1 G1 / G2

- **Two risk variants (alleles)** of the *Apolipoprotein L1 (APOL1)* gene
  - **G1 haplotype**
  - **G2 haplotype**
- **G1 and G2 haplotypes emerged in West Africa**, defined by **three specific genetic variants**
- **High frequency in individuals of sub-Saharan African ancestry**, including African residents and populations of African descent worldwide
- **Evolutionary Trade-Off**
- **APOL1 G0 (ancestral allele)**
  - Reduced ability to lyse *Trypanosoma brucei gambiense* and *T. b. rhodesiense*
- **APOL1 risk variants**
  - **G1**: Confers resistance to *T. b. gambiense*
  - **G2**: Confers resistance to *T. b. rhodesiense*
- **Negative consequence**
  - **G1/G2 variants are associated with an increased risk of chronic kidney disease (CKD)**
  - **Carrier of 2 risk variants have an in increase risk of CKD**



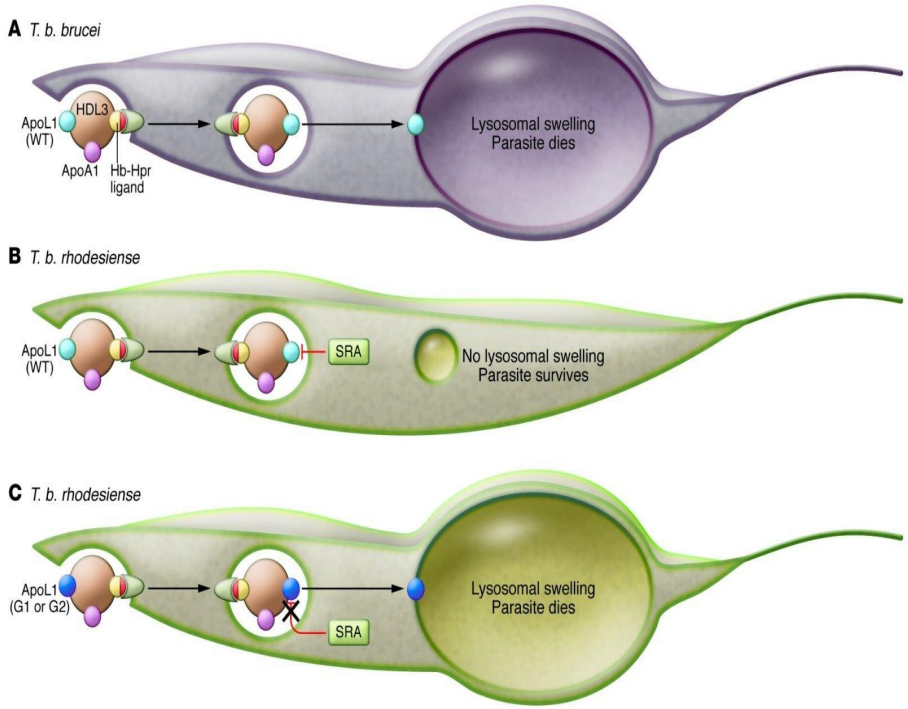
APOL1 G1 / G2 : Allele definition

Haplotype	rs73885319 (p.S342G)	rs60910145 (p.I384M)	rs71785313 (p.N388_Y389del)	Description
G0 (Reference)	A (REF)	T (REF)	AATAATT (REF)	Reference haplotype — no risk variants; low-risk.
G1	G (ALT)	G (ALT)	AATAATT (REF)	Contains two missense mutations (S342G and I384M) on the same haplotype; high-risk.
G2	A (REF)	T (REF)	A (6 bp deletion)	In-frame deletion removing N388 and Y389; high-risk.

- rs73885319, rs60910145 and rs71785313 position define G0, G1 and G2

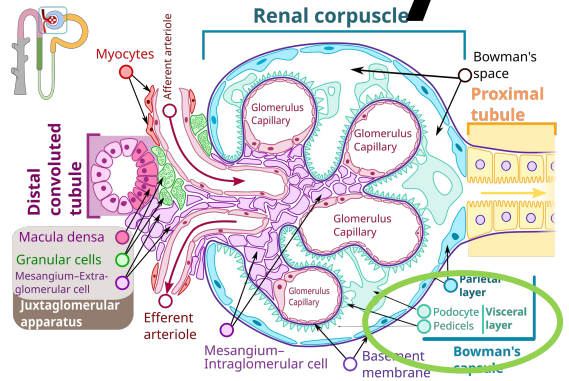
APOL1 and Trypanosoma lyse

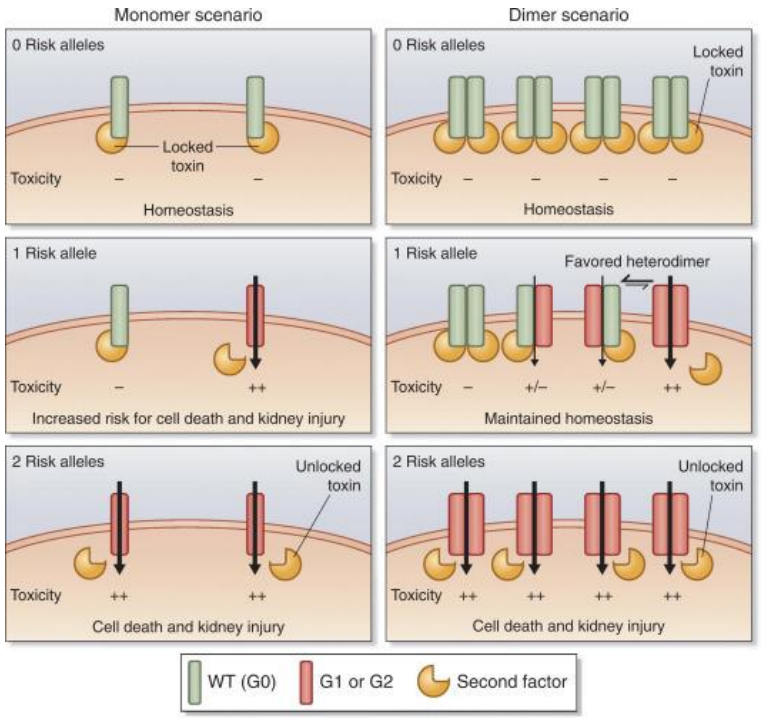
- APOL1 is a **human innate immune protein** that kills Trypanosoma parasites
- After uptake, APOL1 **forms pores in the lysosomal membrane**, causing parasite lysis
- Some trypanosomes as T. gambiense and T. rhodesiense and express SRA, which neutralizes APOL1
- **G1 (missense) and G2 (6-bp deletion)** variants prevent SRA binding



APOL1: Molecular Function and Kidney Toxicity

- **APOL1 (Apolipoprotein L1)** functions as a **pH-dependent ion channel**
- It regulates the flux of cations, including **potassium (K^+), sodium (Na^+), and calcium (Ca^{2+})**
- **APOL1 risk variants** show an increased tendency to form ion channels in **human kidney podocytes**, leading to:
  - Increased cation permeability
  - **Potassium depletion**
  - **Calcium influx**
  - Cellular stress and **cytotoxicity**
- These cellular effects contribute to **podocyte injury** and the development of **chronic kidney disease (CKD)**
- The **APOL1 dimerization model** is currently the **most supported hypothesis** to explain APOL1-associated CKD
  - Dimer formation enhances channel activity and cellular toxicity

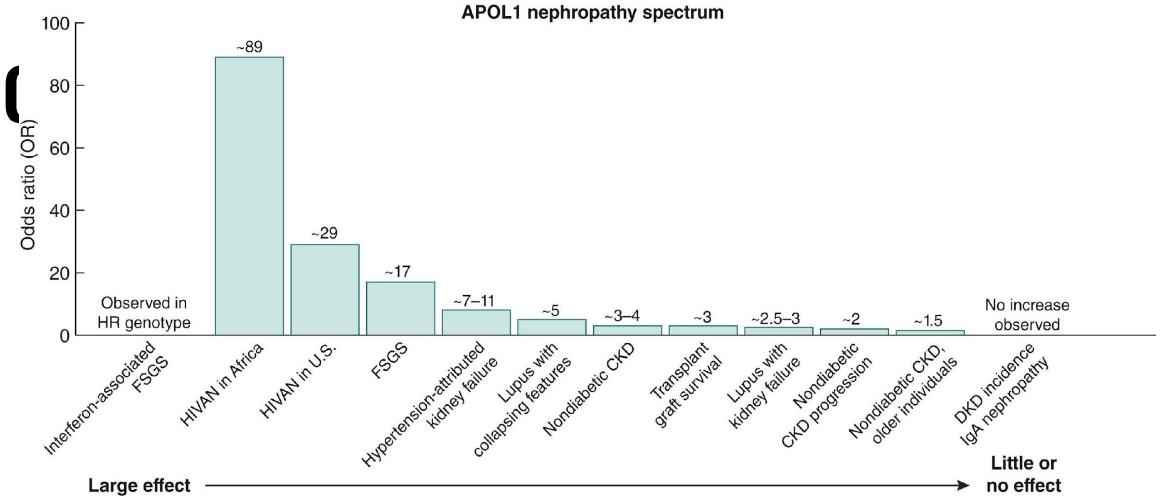




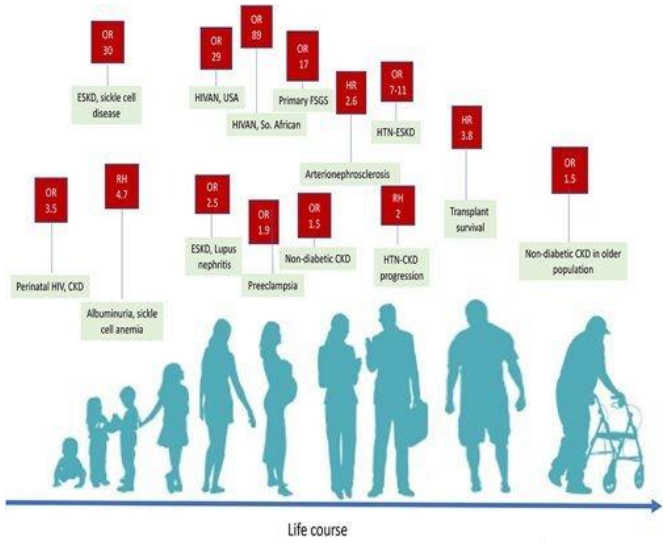
APOL1 toxin, innate immunity, and kidney injury Sophie Limou et

APOL1 and Kidney toxi(

- Most studies have focused on African American populations.
- More recently news study had been done using sub Saharan population.
- Odds ratios (ORs) and hazard ratios (HRs) show high variability across studies.
- The observed impact depends on sample size and population genetic and environmental background :
  - HIV-associated nephropathy (HIVAN) is a severe, rapidly progressive kidney disease caused by HIV infection in individuals with two risk variants (G1 or G2) of the APOL1 gene,in SSA and AA showed between OR of 39 and 89
  - Longitudinal studies report lower ORs compared with HIVAN and hypertension.



APOL1 kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference Akinlolu O. Ojo et al.



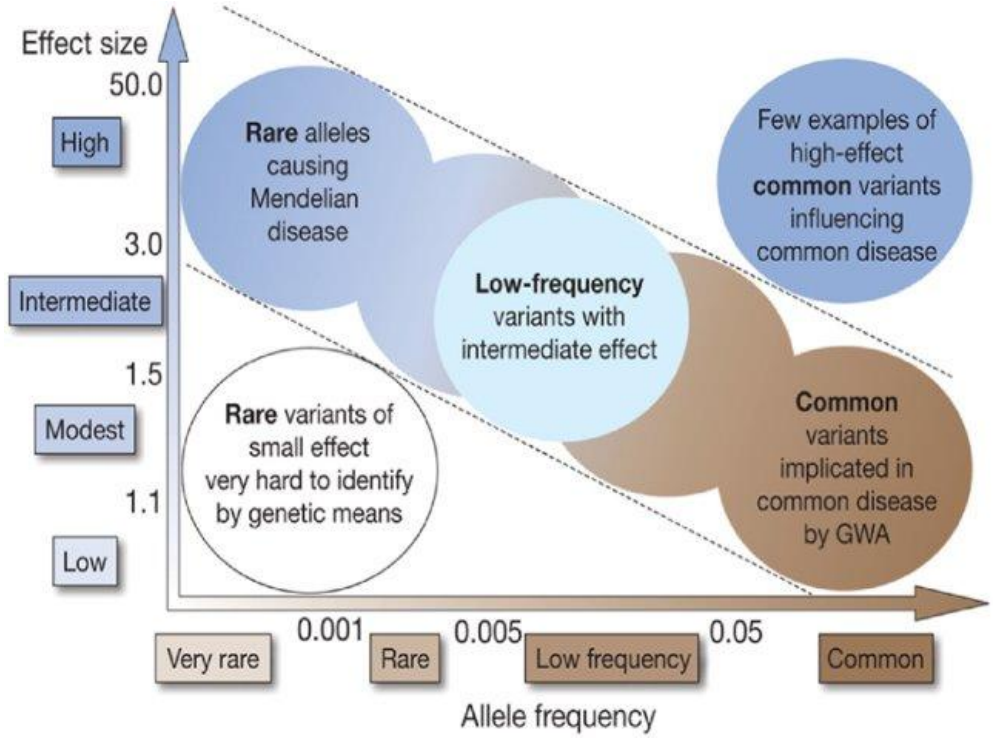
INVITED REVIEW ARTICLE

Kidney disease and APOL1

Aminu Abba Yusuf[1,†], Melanie A Govender[2,†], Jean-Tristan Brandenburg[2,†] and Cheryl A Winkler[3,†*,†]

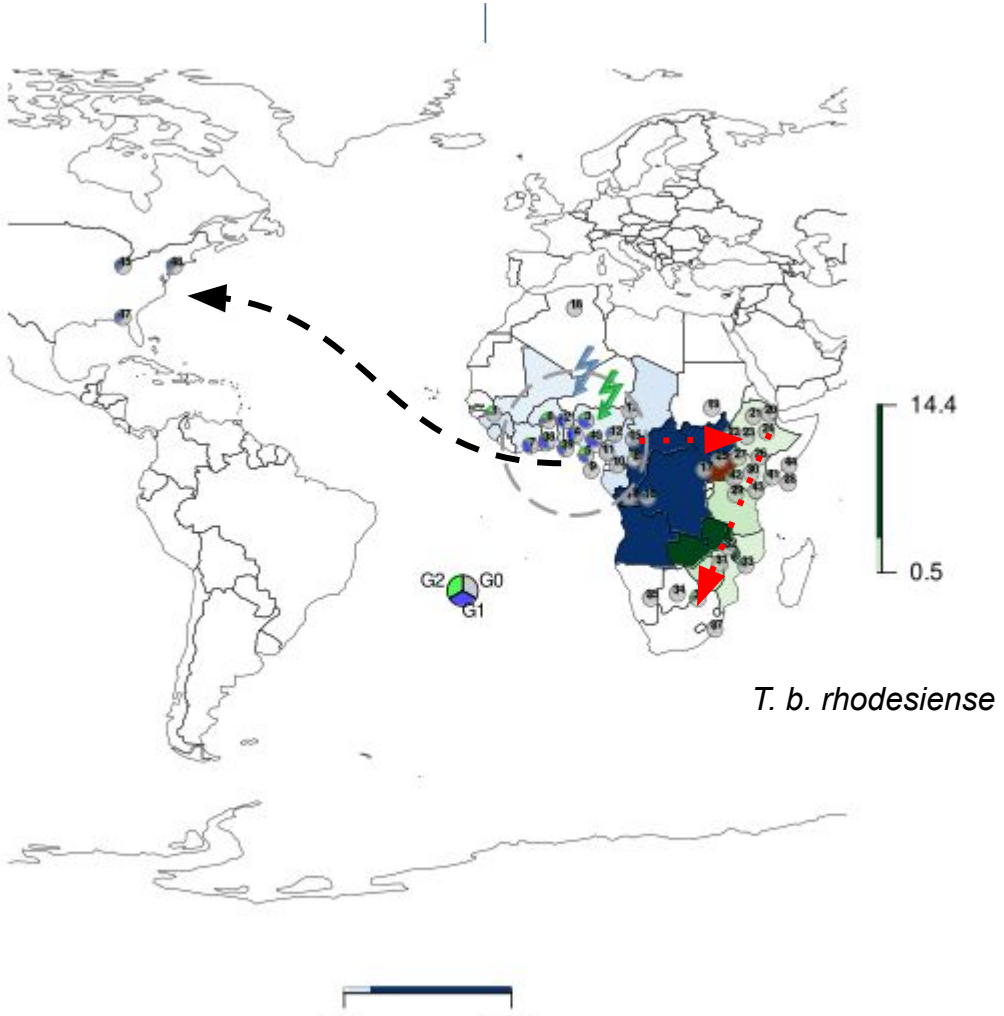
Genetics and variability of diseases and phenotype

- Disease associations are often conceptualized in two dimensions : allele frequency and effect size.
- Highly penetrant alleles for Mendelian disorders are extremely rare with large effect sizes : Example BRCA 1 in breast cancer
- While most GWAS findings are associations of common SNPs with small effect sizes.
- APOL1 had a high penetrant genes but need a secondary hits (i.e. background phenotype)



Frequency – Geographic Distribution & Selection

- APOL1 risk alleles are present in **populations of African ancestry** and in individuals with **African admixture**
- **G1 and G2 variants arose in West Africa, Bantu ethnicicy between 5,000 and 10,000 year ago**
- **Positive selection** for G1/occurred in **West and parts of East Africa**, driven by T. b. gambiense and G2 by T. b. rhodesiense exposure
- **Allele spread within Africa** occurred via **population movements, including the Bantu expansion**
- **African American populations** carry G1/G2 alleles due to the **transatlantic slave trade**
- **Frequency in G1, G2 is far to be uniform inside Sub Saharan population or non resident SSA.**



T. b. rhodesiense

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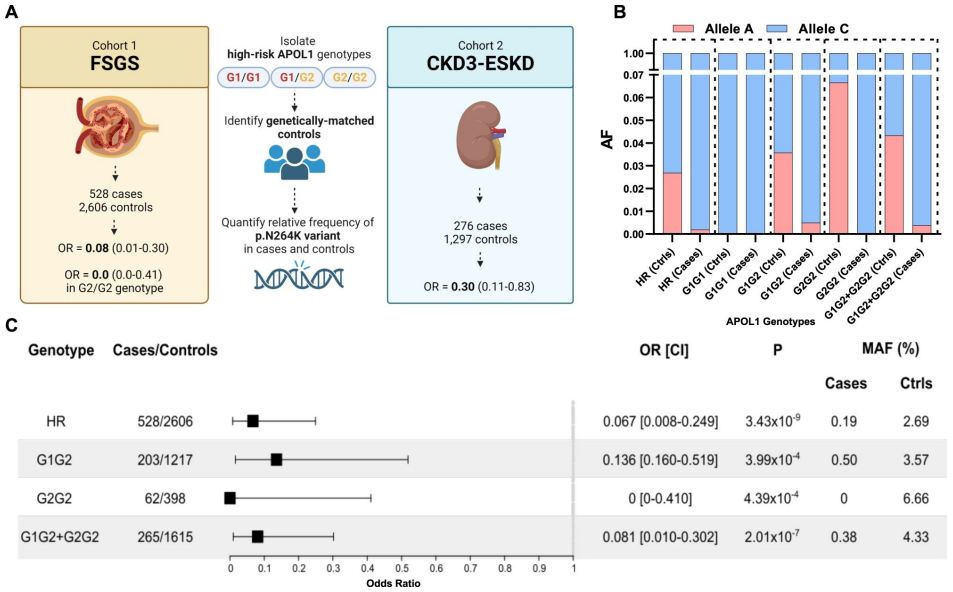
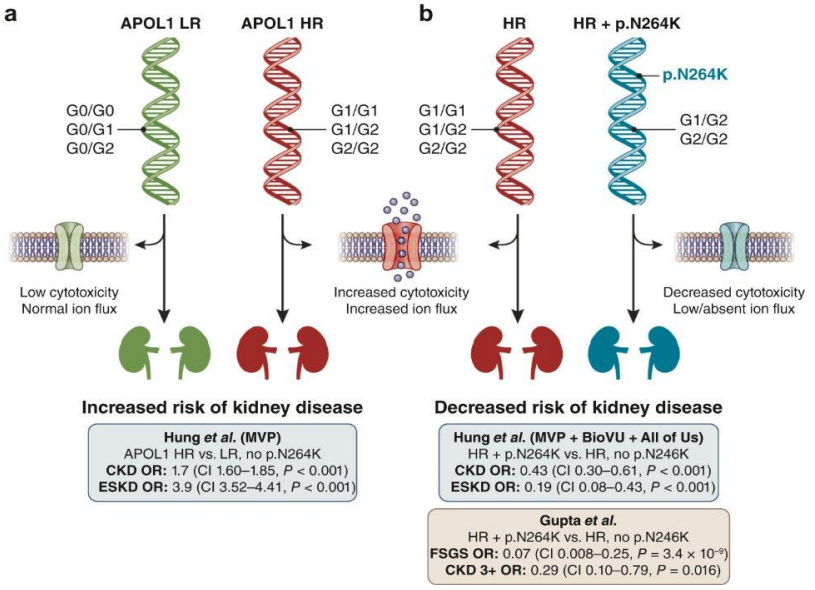
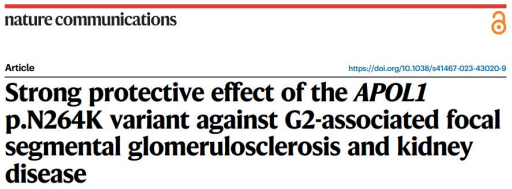
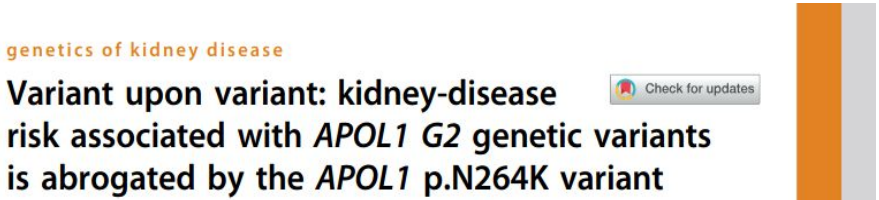
APOL1 G1, G2 and N264K

- Recent discovery :
  - *APOL1* p.N264K missense variant, when co-inherited with the G2 *APOL1* risk allele
  - reduces the penetrance of the G1/G2 and G2/G2 high-risk genotypes by rendering these genotypes low-risk.
  - Allele *APOL1* p.N264K missense variant was in higher prevalence in G2/G[1/2] was control compared to case
  - Decreased cytotxicity by absence of ion flux





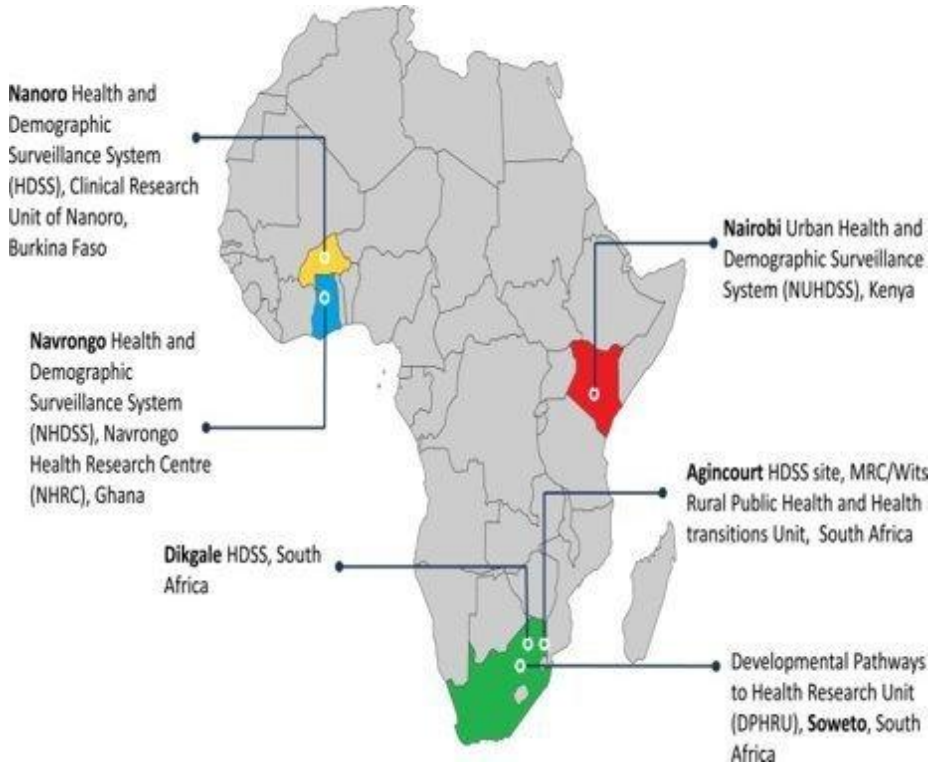




Chronic Kidney Disease and the Role of APOL1 in Longitudinal Studies in Sub-Saharan Africa: The AWI-Gen Study

AWI-Gen: Africa Wits-INDEPTH Partnership for Genomic Research

- Goal: AWI-Gen aims to understand how genes, environment, and lifestyle influence cardiovascular, and metabolic health across Africa, helping uncover insights to improve the health ofAfrican populations.
  - Project Lead: Michele Ramsay
- Study Sites (6 sites · 3 regions · 4 countries):
  - West Africa: Burkina Faso (Nanoro), Ghana (Navrongo)
  - East Africa: Kenya (Nairobi)
  - South Africa: Agincourt, Soweto, Dikgale
  - Includes both rural and urban communities
- Sample Size: ~ 12,000 participants collected between 2018
- Data Collected:
  - Genetics: H3A-GWAS array
  - Phenotypes
  - Microbiome data

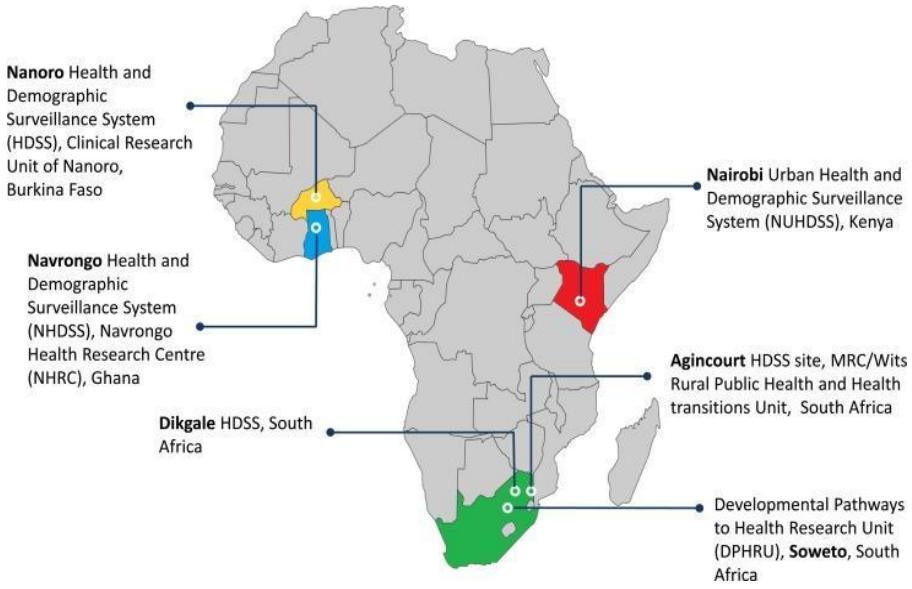


Materials : AWI-GEN studies

- Kidney markers :
  - 🧪 eGFR (serum-creatinine based)
  - 🧪 uACR (urine albumin-to-creatinine ratio)
  - 🔍 CKD definition: Low eGFR and/or Albuminuria

- Health related
  - Diabetes
  - Hypertension
  - HIV status
  - Current smoker
  - Current alcohol consumption
  - History of cardiovascular disease
  - ….

Study Sites : 6 sites, 4 countries, 3 African regions (rural + urban)



- Sociodemographic Variables
  - 👤 Age
  - 👤 Sex
  - 🏠 Socioeconomic status
  - 🎓 Education level

Kidney prevalence


FULL TEXT ARTICLE

Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study

Jaya A George PhD, Jean-Tristan Brandenburg PhD, June Fabian MD, Nigel J Crowther Prof, Godfred Agongo MPhil, Marianne Alberts Prof, Stuart Ali PhD, Gershim Asiki PhD, Palwende R Boua MSc, F Xavier Gómez-Olivé PhD, Felistas Mashinya PhD, Lisa Micklesfield PhD, Shukri F Mohamed MPH, Freedom Mukomana MSc, Shane A Norris Prof, Abraham R Oduro MBChB, Cassandra Soo MSc, Hermann Sorgho PhD, Alisha Wade DPhil, Saraladevi Naicker Prof and Michèle Ramsay Prof

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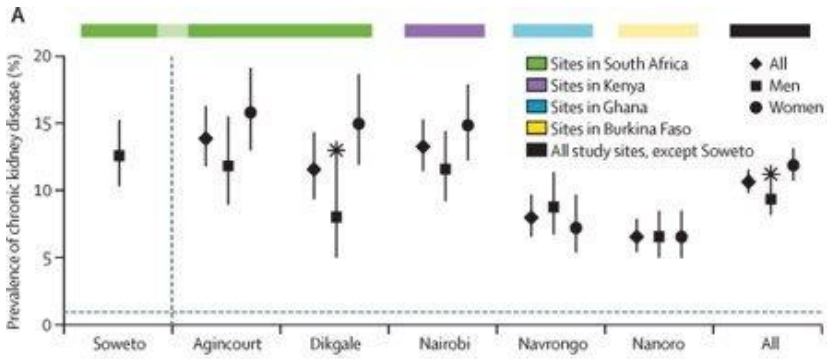
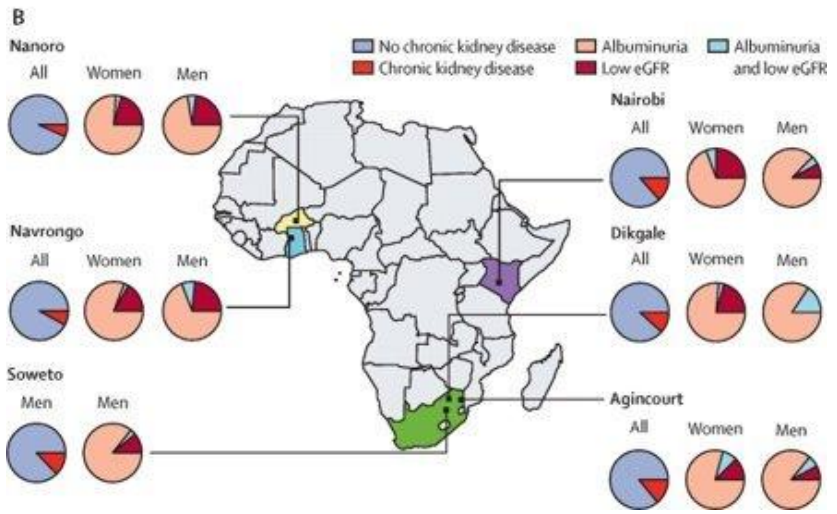
Lancet Global Health, The

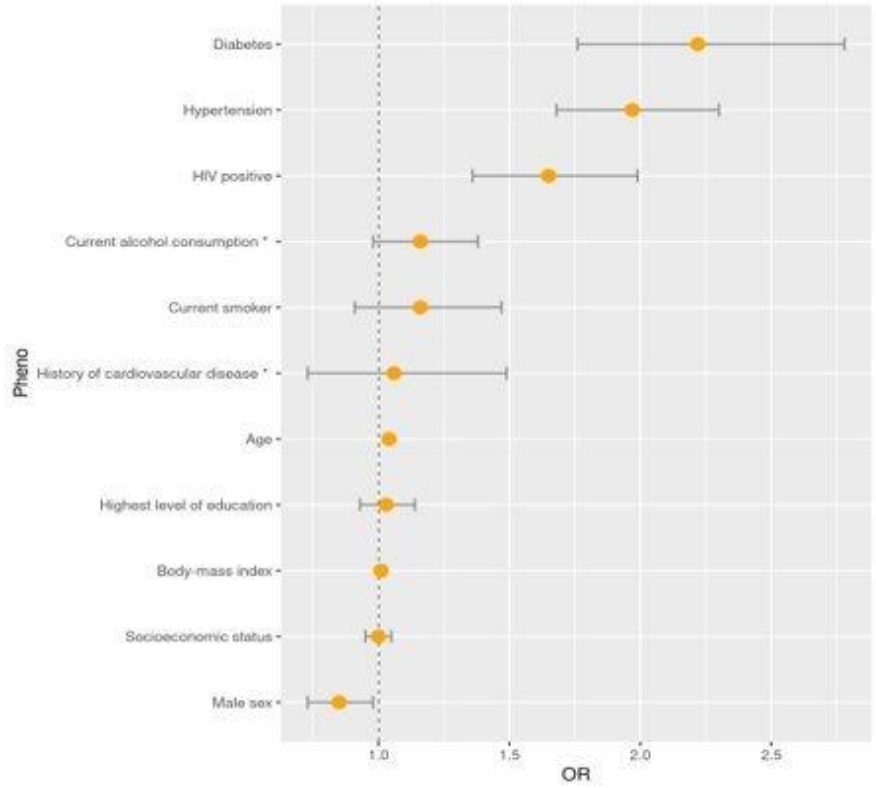
Volume 7, Issue 12

- Chronic Kidney Disease (CKD)
  - Low eGFR: eGFR < 60 mL/min/1.73 m²
  - Marker of advanced kidney disease
  - Albuminuria: uACR ≥ 3 mg/mmol
  - CKD definition: Presence of low eGFR and/or albuminuria
- Prevalence of CKD
  - Overall prevalence: 10.3%
  - Geographical variation:
    - Highest prevalence in South Africa, with 14.0% in Agincourt
    - East Africa (Nairobi): 13.4%
    - West Africa: 6.0%
- Sex differences: CKD prevalence was higher in women
- Main contributor: CKD prevalence was mainly driven by albuminuria rather than low eGFR :
  - Presence of Low eGFR was very low
  - Equation of GFR estimation had biais in SSA context.





Kidney risk factor



- Risk factors:
- Diabetes is the strongest risk factor, followed by hypertension, HIV status, age, and sex.
- Diabetes, age, and hypertension are common risk factors across all study sites,
- Current alcohol consumption, current smoking, and HIV positivity show variable associations with risk across sites.

Methods

Insertion to defined G2 are not present on array

Focus on APOL1 region

Panel with presence of del / ins for G2

Frequencies estimation and imputation effectiveness

Genotyping using h3aArray

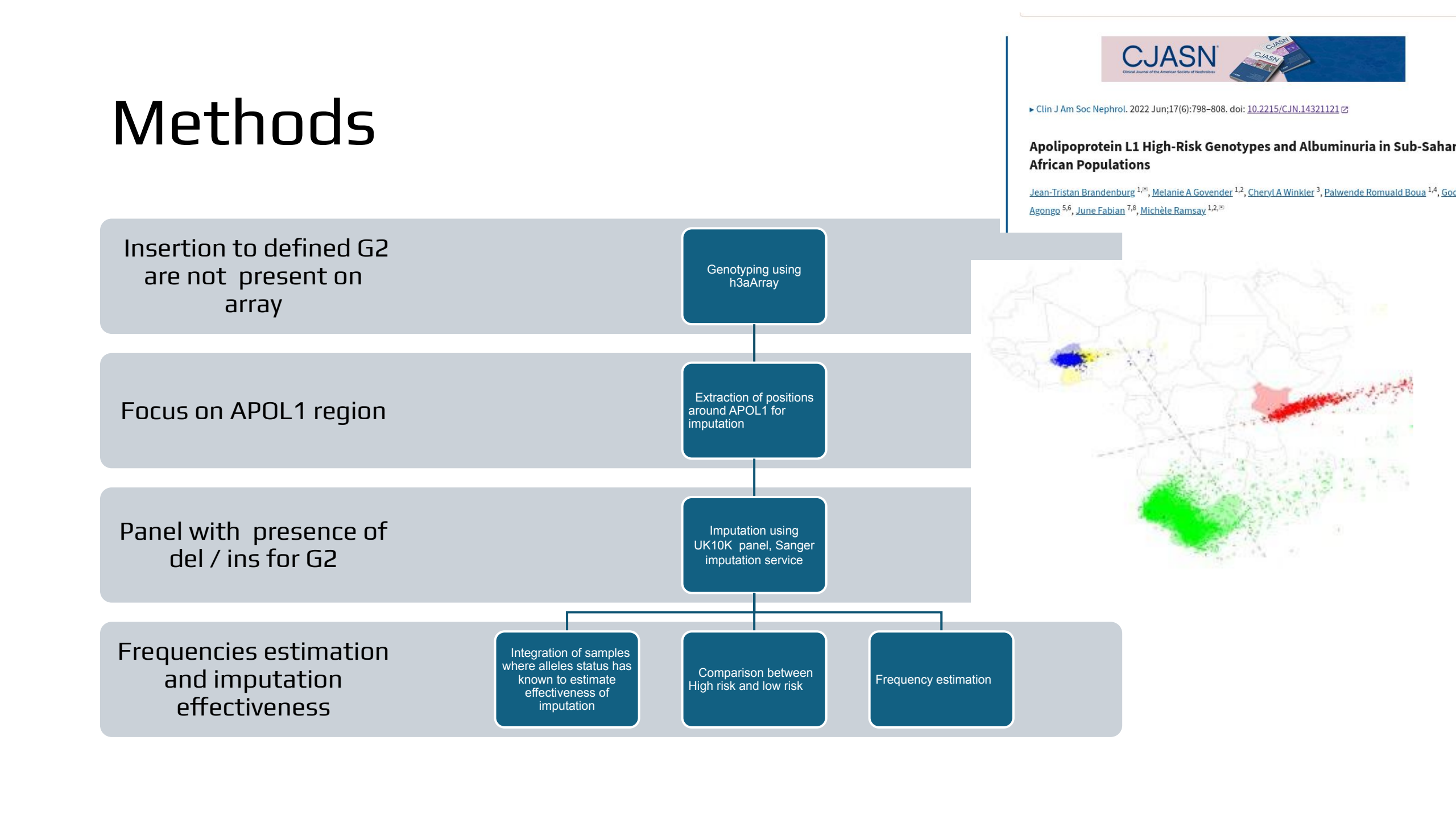
Extraction of positions around APOL1 for imputation

Imputation using UK10K panel, Sanger imputation service

Integration of samples where alleles status has known to estimate effectiveness of imputation

Comparison between High risk and low risk

Frequency estimation



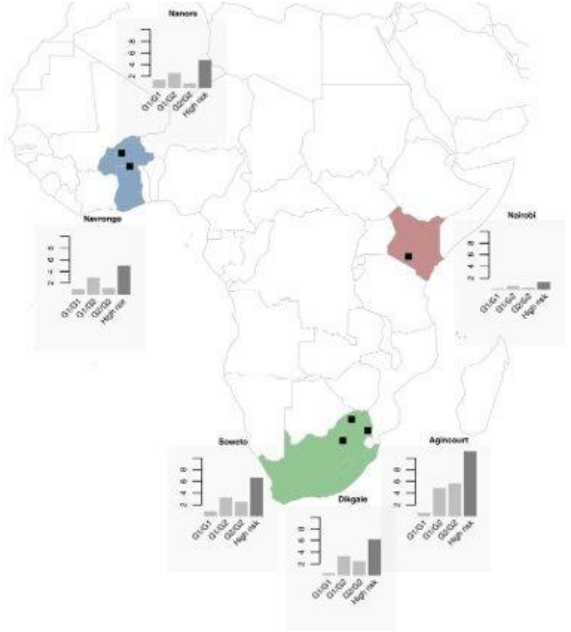
▸ Clin J Am Soc Nephrol. 2022 Jun;17(6):798–808. doi: 10.2215/CJN.14321121

Apolipoprotein L1 High-Risk Genotypes and Albuminuria in Sub-Sahar African Populations

Jean-Tristan Brandenburg [1,✉], Melanie A Govender [1,2], Cheryl A Winkler [3], Palwende Romuald Boua [1,4], Goc Agongo [5,6], June Fabian [7,8], Michèle Ramsay [1,2,✉]

Imputation accuracy and frequencies

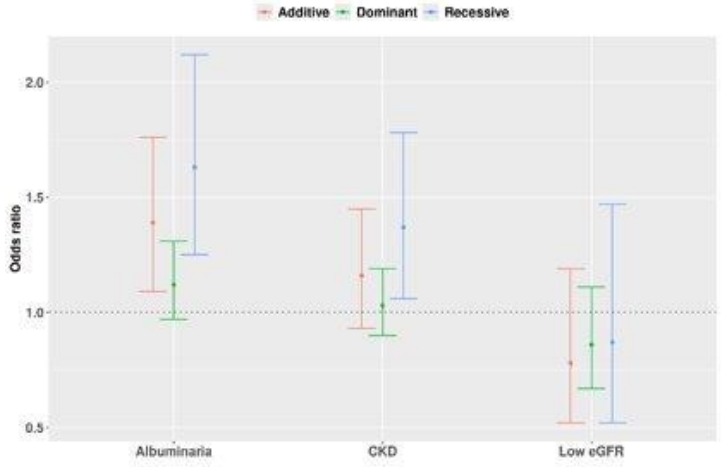
- Imputation accuracy : Overall accuracy exceeded 99%.
- APOL1 allele frequencies in AWI-GEN:
- Distribution of high-risk alleles (G1/G2): Southern Africa > West Africa > East Africa
- West Africa:
  - G1 frequency is higher than G2
  - G1 confers resistance to *Trypanosoma brucei gambiense*, which is predominantly present in West Africa
- Southern and Eastern Africa:
  - G2 frequency is higher than G1
  - G2 confers resistance to *Trypanosoma brucei rhodesiense*
- Eastern Africa:
  - Very low APOL1 risk allele frequencies
  - Samples are partially Bantu

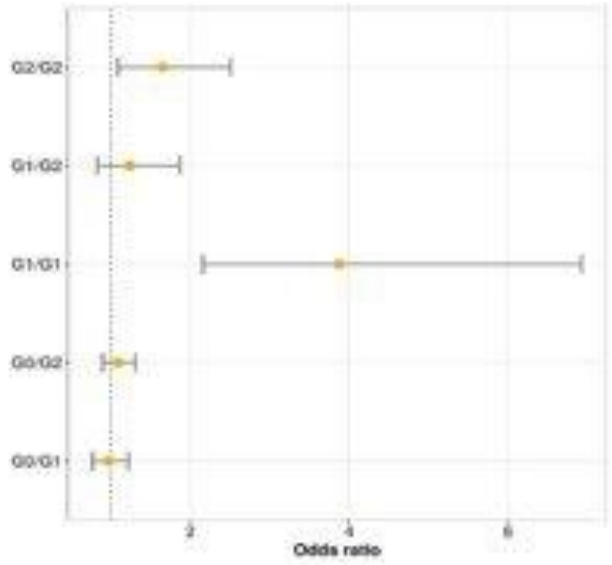


Region	G0 (%)	G1 (%)	G2 (%)	**2 risk alleles (%)**
Southern Africa	**71.5**	**9.3**	**19.2**	**8.3**
Eastern Africa	**87.8**	**5.0**	**7.3**	**1.3**
Western Africa	**77.0**	**12.2**	**10.8**	**5.0**

APOL1 and CKD

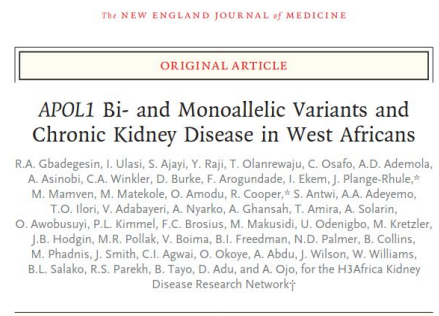
- **We analyzed the association between APOL1 diplotypes and CKD**, according to specific **CKD markers**:
  - **Low eGFR**
  - **Albuminuria**
  - **CKD defined as low eGFR and/or albuminuria**
- **Genetic models tested:**
  - **Recessive model:** presence of **two high-risk (HR) alleles** (G1 and/or G2)
  - **Dominant model:** presence of **at least one HR allele**
  - **Additive model**
- **Key findings:**
  - **APOL1 high-risk genotypes were more strongly associated with albuminuria** than with CKD overall, and **least strongly with low eGFR**.
  - **Recessive genetic models (two HR alleles)** showed **stronger and more significant associations** than additive or dominant models.
- **Genotype-specific effects:**
  - **G1/G1 individuals showed the largest increase in risk** for both albuminuria and CKD.
  - **Weaker associations were observed for G1/G2 and G2/G2 genotypes**.
- **GWAS context:** Associations **did not reach genome-wide significance**
  (p-values ≈ 10^{-3}, above the GWAS threshold of 5×10^{-8}).





APOL1 : Africa Kidney Disease Research Network - 2024

- **5,578 CKD cases** (eGFR ≤ 90 ml/min/1.73 m² and uACR > 3), including **866 with glomerular diseases**, and **2,777 controls** from **Ghana and Nigeria**.
- **APOL1 kidney risk variants (G1 and G2)** were genotyped using **custom TaqMan assays**.
- **Kidney biopsies** were performed when **eGFR reached 15 ml/min/1.73 m²**.
- **Analysis of the impact of high-risk APOL1 alleles** in the study samples on CKD.



APOL1 West africa : Africa Kidney Disease Research Network

- Overall allele frequency in the sample:
  - Compared with AWI-GEN, G1/G2 frequencies are higher.
  - Two risk alleles (high-risk genotype): 25.7% in controls vs 30.9–33.2% in CKD cases.
- Association with CKD:
  - Odds ratio (OR) ≈ 1.25 for CKD compared with controls.
  - Genotype-specific effect: G2/G2 shows the strongest association with CKD.

Population	G0 (%)	G1 (%)	G2 (%)
Overall (N=8,355)	**48.8**	**37.5**	**13.6**
Ghana (N=3,066)	**51.4**	**36.9**	**11.7**
Nigeria (N=5,289)	**46.9**	**37.9**	**15.2**
With CKD (N=5,578)	**46.9**	**38.8**	**14.3**
Without CKD (N=2,777)	**52.4**	**35.7**	**11.9**

Table 2. Association of *APOL1* Risk Alleles and Genotypes with CKD.*

APOL1 Genotypes	Odds Ratio (95% CI)	
	Unadjusted	Adjusted†
2 *APOL1* risk alleles vs. <2	1.34 (1.21–1.49)	1.25 (1.11–1.40)
G0/G1 vs. G0/G0	1.16 (1.03–1.31)	1.19 (1.04–1.35)
G0/G2 vs. G0/G0	1.18 (1.01–1.38)	1.19 (1.00–1.41)
G0/G1 and G0/G2 vs. G0/G0	1.17 (1.05–1.30)	1.18 (1.04–1.33)
G1/G1 vs. G0/G0	1.46 (1.26–1.69)	1.37 (1.16–1.61)
G1/G2 vs. G0/G0	1.40 (1.18–1.65)	1.34 (1.12–1.61)
G2/G2 vs. G0/G0	2.25 (1.52–3.34)	2.05 (1.35–3.13)

* Values are odds ratios for CKD among West African participants with the indi-

Some point of conclusion

- **The paradigm that carriers of two APOL1 risk alleles (G1/G2) have an increased risk of CKD, or that APOL1 plays a major role in CKD, is more complex than initially thought:**
- **The effect of G1/G2 increases in the presence of background phenotypes** (e.g. HIV infection, hypertension), whereas in **longitudinal studies such as AWI-Gen, the effect size is lower than that of diabetes**.
- **The p.N264K variant**, linked to the **G2 haplotype**, has been shown to **reduce or reverse the pathogenicity of G1/G2**, but this variant was **not assessed in AWI-Gen or H3AKDRN**.
- **The frequency of individuals carrying two APOL1 risk alleles varies widely across populations**, suggesting heterogeneous health impacts:
  - ~13% in African Americans
  - ~5% in West African AWI-Gen cohorts
  - ~25% in West Africa (H3AKDRN)
  - ~1.3% in East African AWI-Gen cohorts
  - ~8.3% in South African AWI-Gen cohorts
- **The relative effects of G1 versus G2 remain debated** (G1 > G2 in some studies), with **inconsistent conclusions across studies**, likely reflecting differences in population background, study design, and environmental modifiers.