

- *T.b. gambiense*
  - West & Central Africa
  - Chronic disease
- *T.b. rhodesiense*:
  - East & Southern Africa
  - Acute disease
- *T.b. brucei*:
  - Not human infective
  - Across tsetse belt

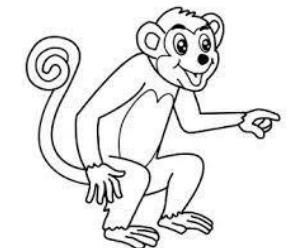
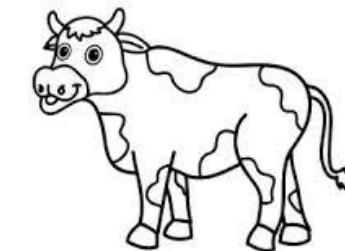
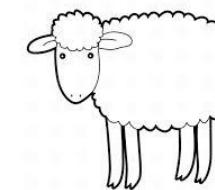
# Co-evolutionary arms race

There is a wide range of trypanosomes that infect a wide range of animals

*T. brucei*

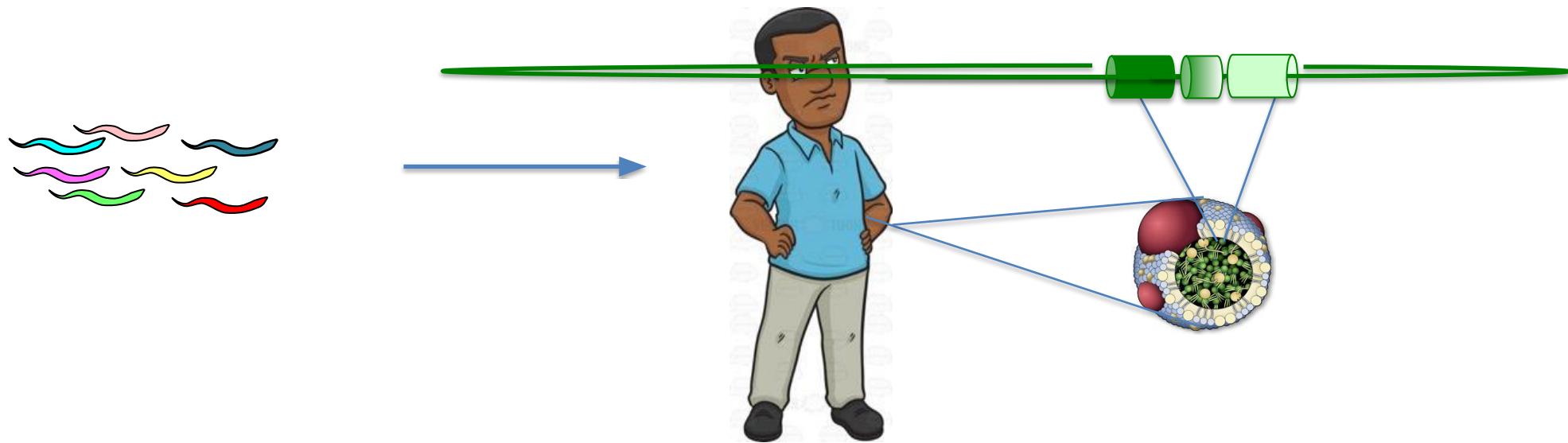
*T. congolense*

*T. vivax*



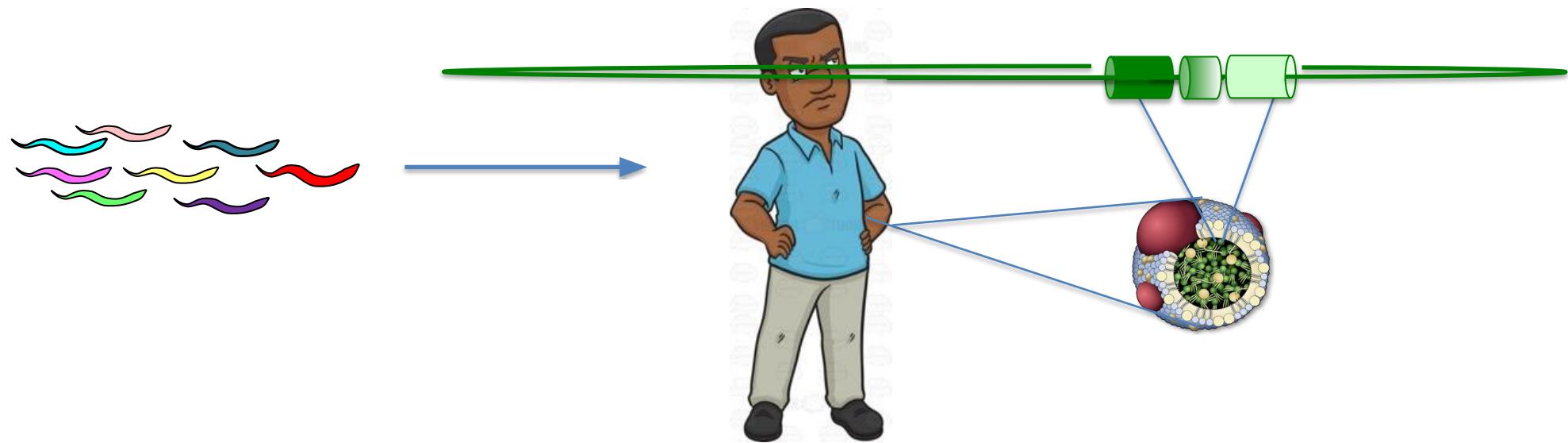
# Co-evolutionary arms race

Humans developed resistance in the form of a toxic protein, APOL1, hidden in a High Density Lipoprotein Trojan horse



# Co-evolutionary arms race

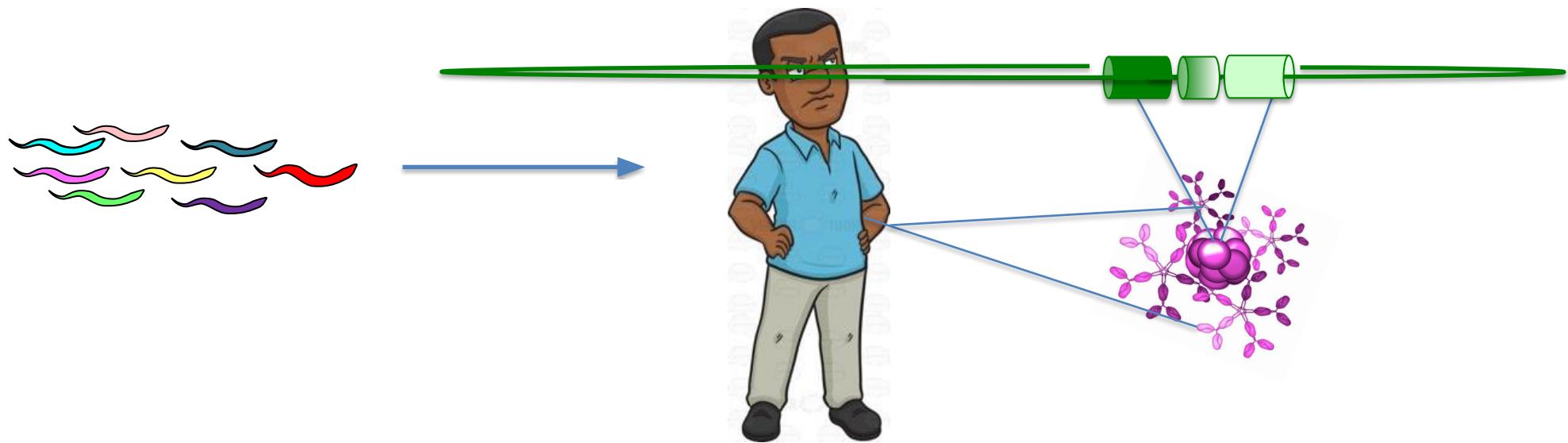
Some West African strains developed resistance by failing to internalize APOL1



*T.b. gambiense*

# Co-evolutionary arms race

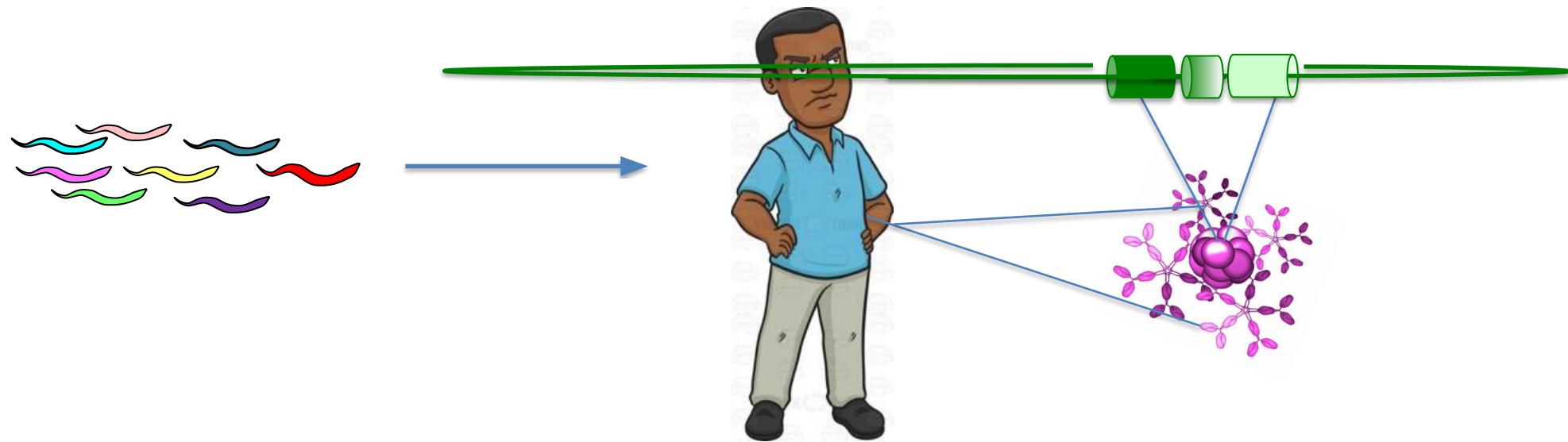
Humans find another Trojan horse to deliver the protein



*T.b. gambiense*

# Co-evolutionary arms race

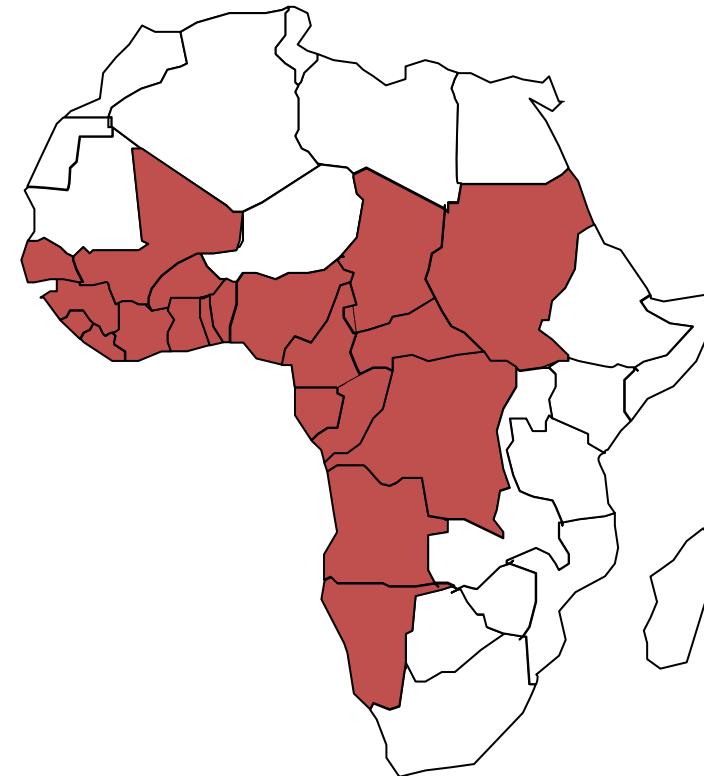
Some West African parasite strains developed resistance to APOL1 itself – at least 3 different mechanisms, TgsGP, pH increases CP activity, membrane rigidity



*T.b. gambiense*

## *T.b. gambiense*

West and Central Africa  
>97% HAT cases  
Chronic disease  
Homogenous population (ameiotic), emerged ~10Kya

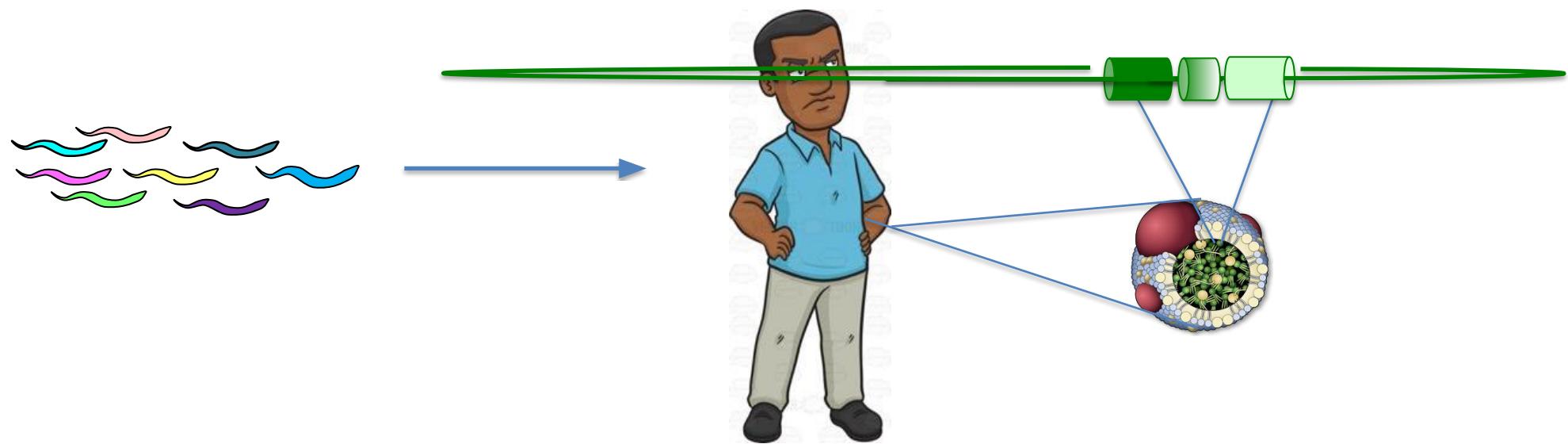


Wasting  
Skin pathology  
anaemia



# Co-evolutionary arms race

Some East African strains independently developed resistance to APOL1 by expressing SRA that binds to APOL1 neutralizing it.



*T.b. rhodesiense*

East Africa

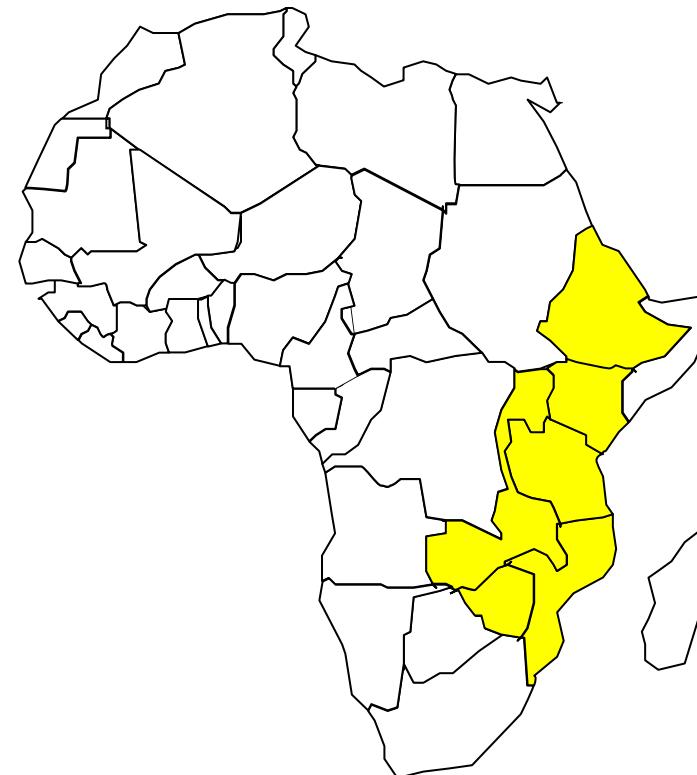
<3% HAT cases

Acute disease

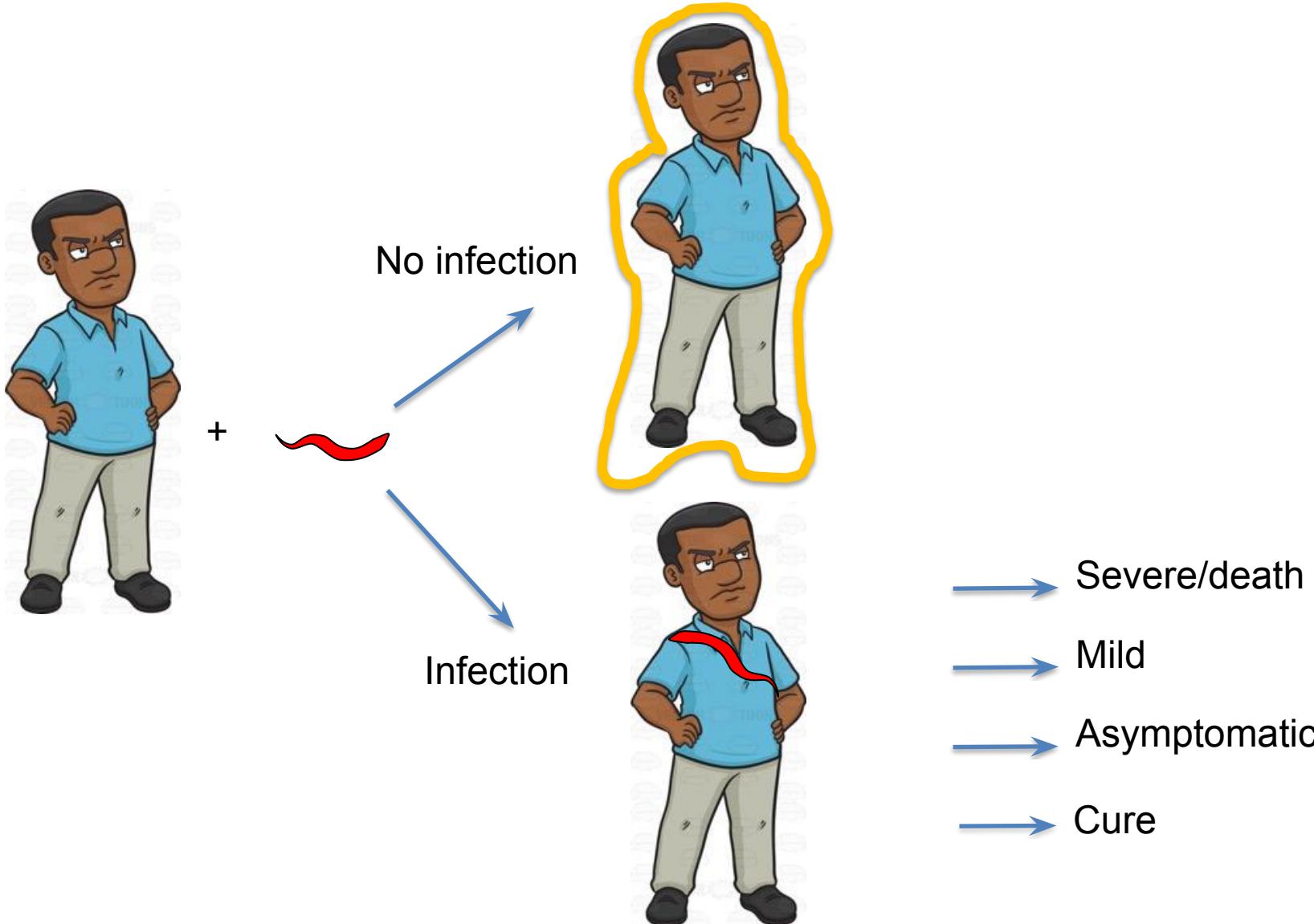
Heterogeneous population (mating with local *T.b. brucei*)

Zoonotic

Recently emerged



# Arms race is still on going....





Human genetics component that determines disease susceptibility and severity.

## TrypanoGEN+ consortium

### Trypanosomiasis and schistosomiasis



Inauguration of the TrypanoGEN Network at Makerere University

Workshop

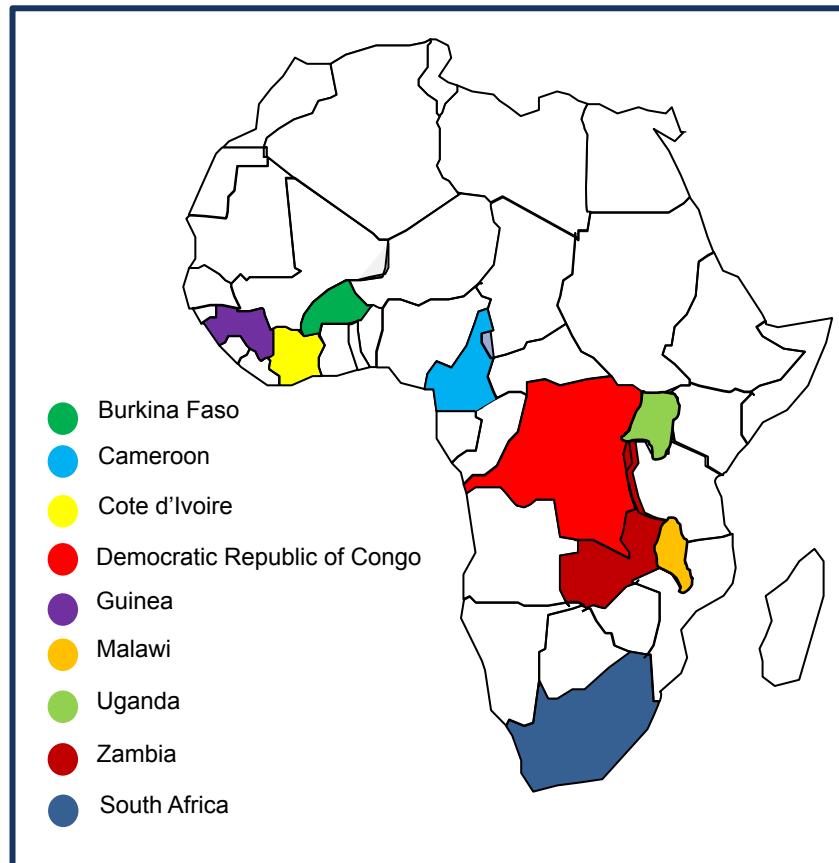
Held at Imperial Royale Hotel Kampala, Uganda 28<sup>TH</sup> 31<sup>st</sup> May, 2013





# TrypanoGEN+

We are working in the countries where trypanosomiasis and schistosomiasis are endemic.





# TrypanoGEN+



Uninfected



infected



V



Asymptomatic



cases

V



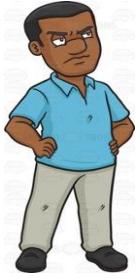


- Plasma samples from individuals in several HAT-endemic foci
- Controls, cases, seropositives
- Genotyping data is available

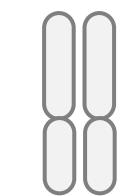
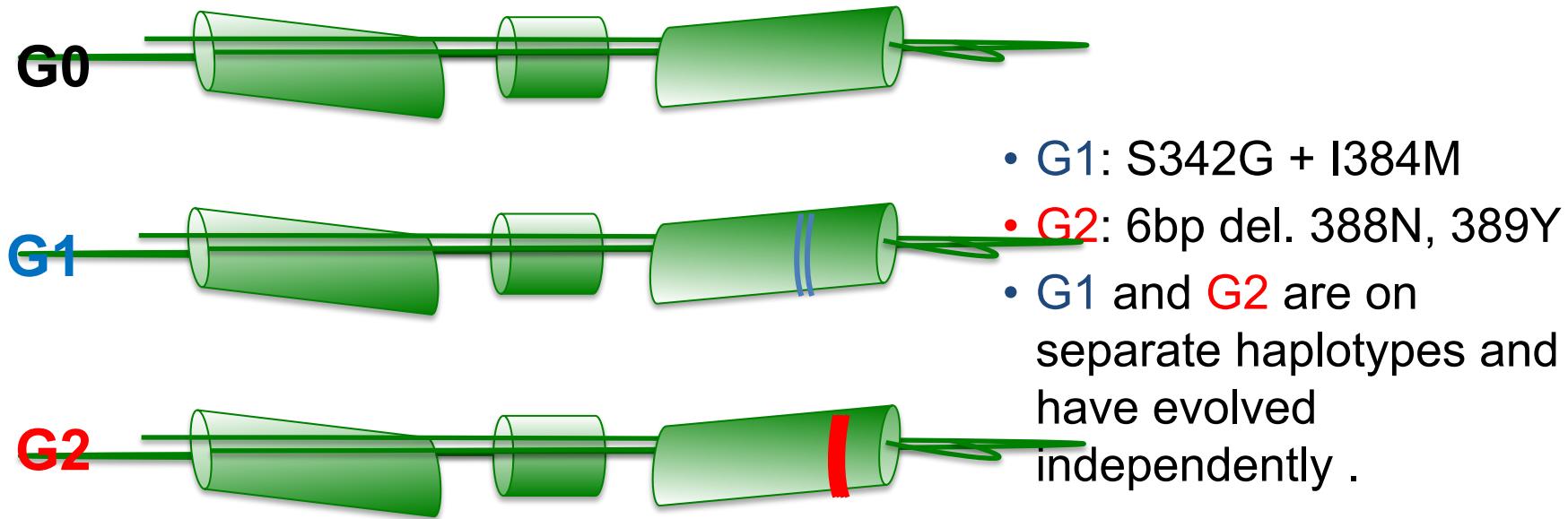
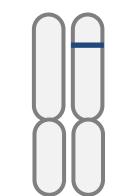
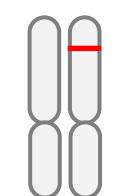
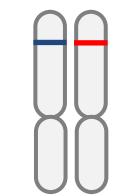
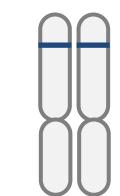
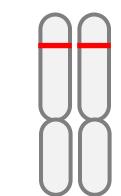
Iiboudo et al. *Plos NTDs*, 2017



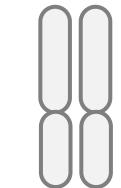
# TrypanoGEN+



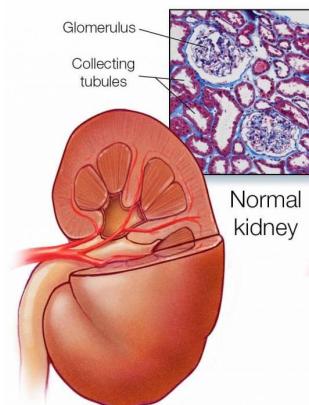
## G1 and G2 are APOL1 genetic variants

**G0G0****G0G1****G0G2****G1G2****G1G1****G2G2**

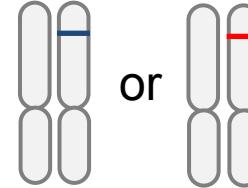
0 variant



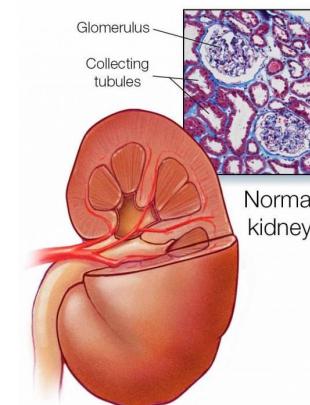
**G0G0**



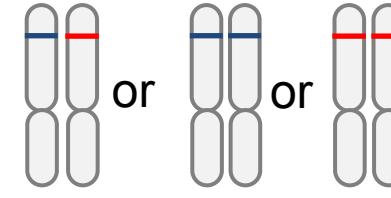
1 variant



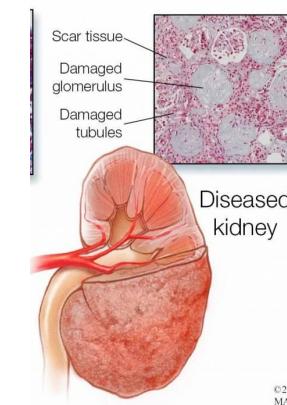
**G0G1**   **G0G2**



2 variants



**G1G2**   **G1G1**   **G2G2**



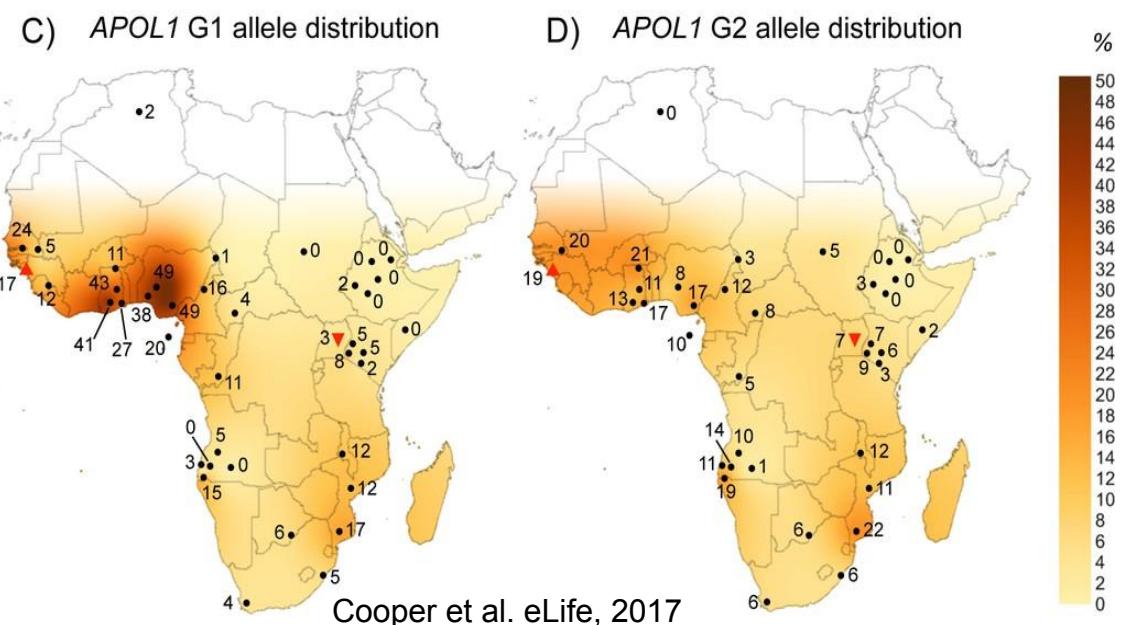
©2015  
MAYO



G1 (red), G2 (orange), G0 alleles (light blue)

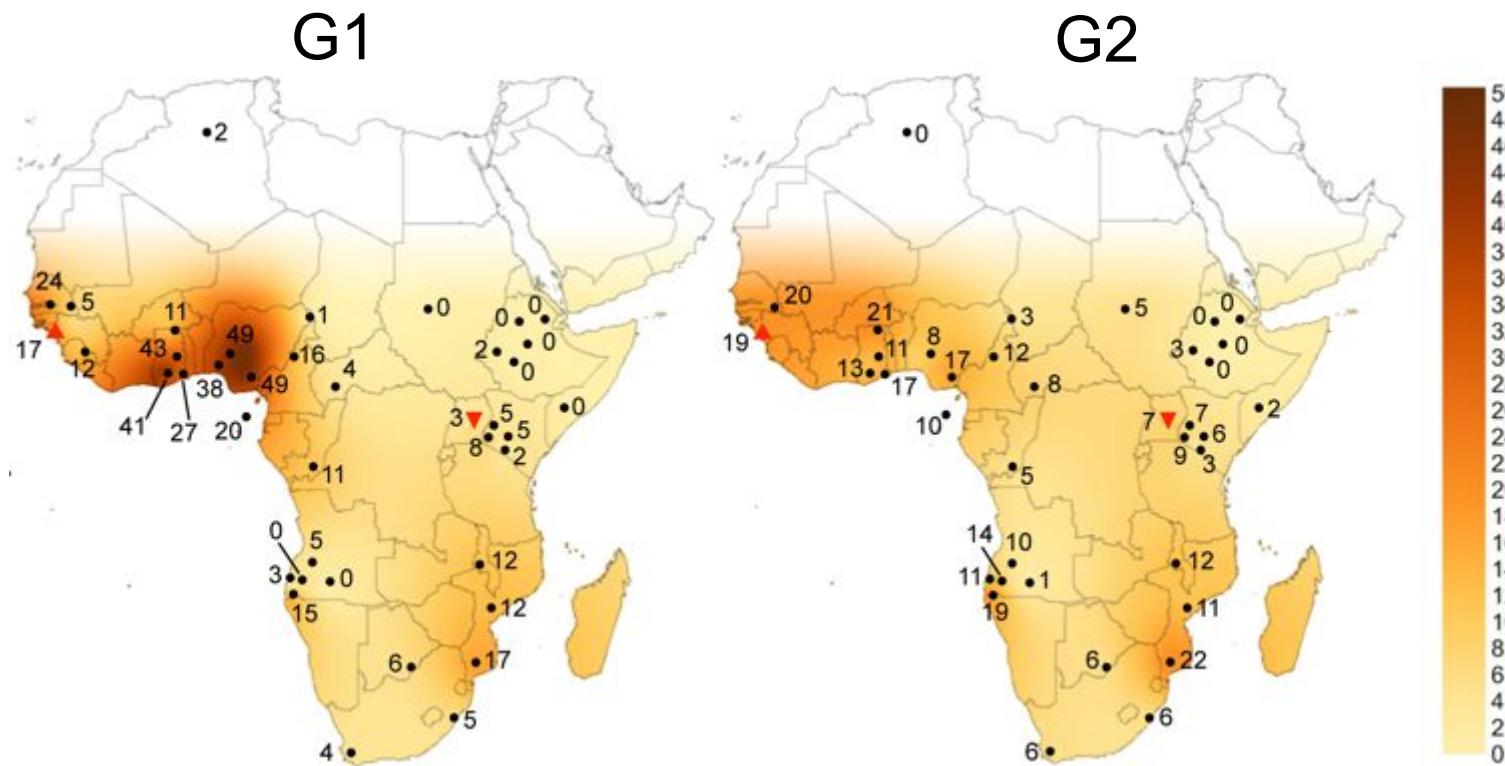
- Africans and African Americans (AA)

- G1 is highest in West Africa ~ 49%
- G2 is more homogenous (~22%) in West and East Africa



# Hypothesis

A heterozygous advantage model: APOL1 variants have been selected in the African population for resistance to infection by trypanosomes resulting in an increased risk of kidney disease.





# Association between APOL1 and *T.b. rhodesiense* Trypanosomiasis

G1 is underpowered due to few G1G1.

G2 is associated with protection.



# Association between APOL1 and *T.b. gambiense* Trypanosomiasis

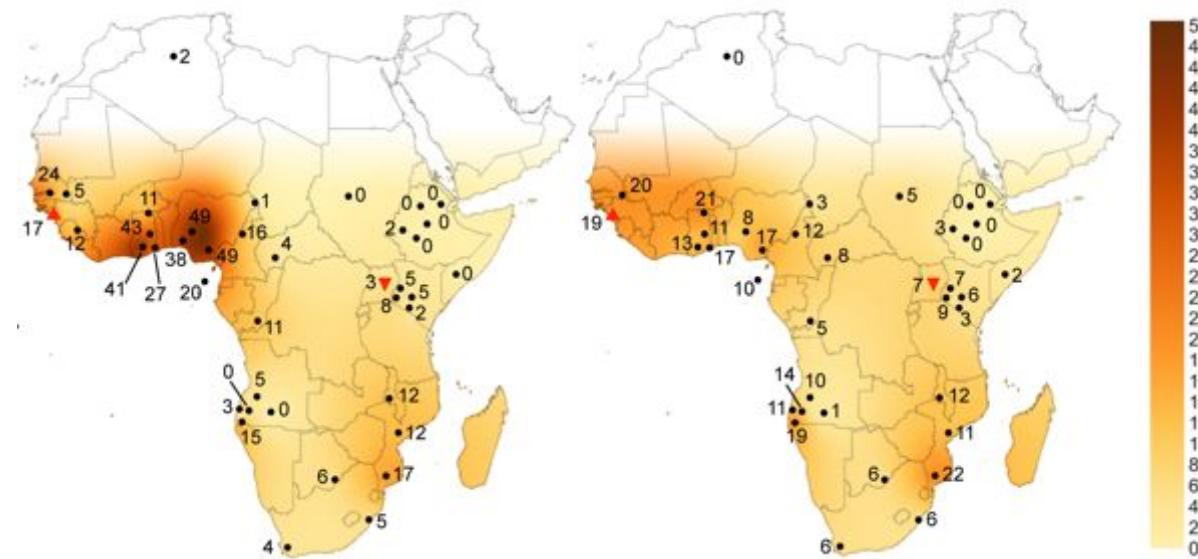
G1 no association with infection.

G2 no association with infection.



# Association between APOL1 and Trypanosomiasis is complex

	G1	G2
<i>T.b. rhodesiense</i>	No association	↓ Risk infection OR=5
<i>T.b. gambiense</i>	↓ Risk severe disease OR=3	↑ Risk severe disease OR=0.35



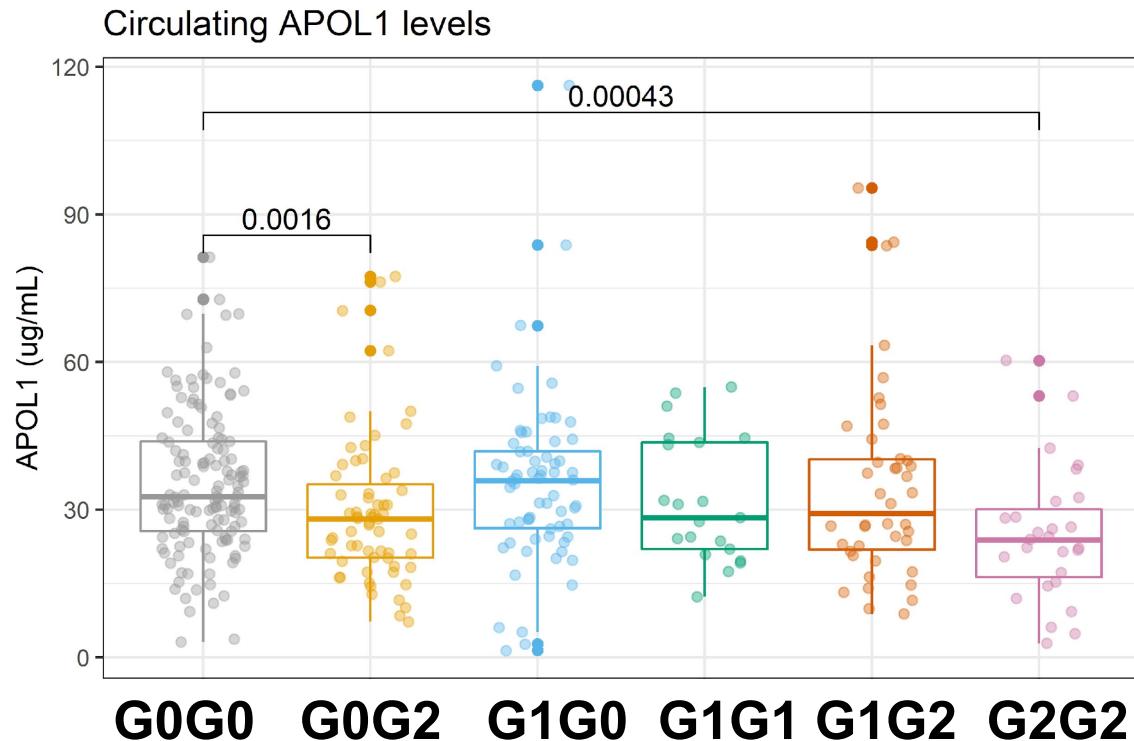
2 polymorphisms just 4 amino acids apart give opposing phenotypes for 2 different diseases.  
G1 could have selected by *T.b. gambiense*. G2 unlikely to have been selected by trypanosomes.

# How does APOL1 affect trypanosomes?

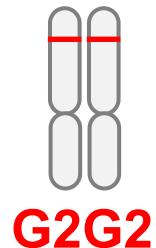
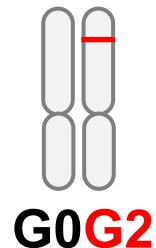
- Do the different variants affect APOL1 concentration?
- Do the different variants kill the different trypanosome species?

- Selected 350 individuals from TrypanoGEN biobank representing the 6 different APOL1 genotypes.
- Measured the amount of circulating APOL1 using allele specific mass spec.

# Does APOL1 concentration differ in people with APOL1 variants?



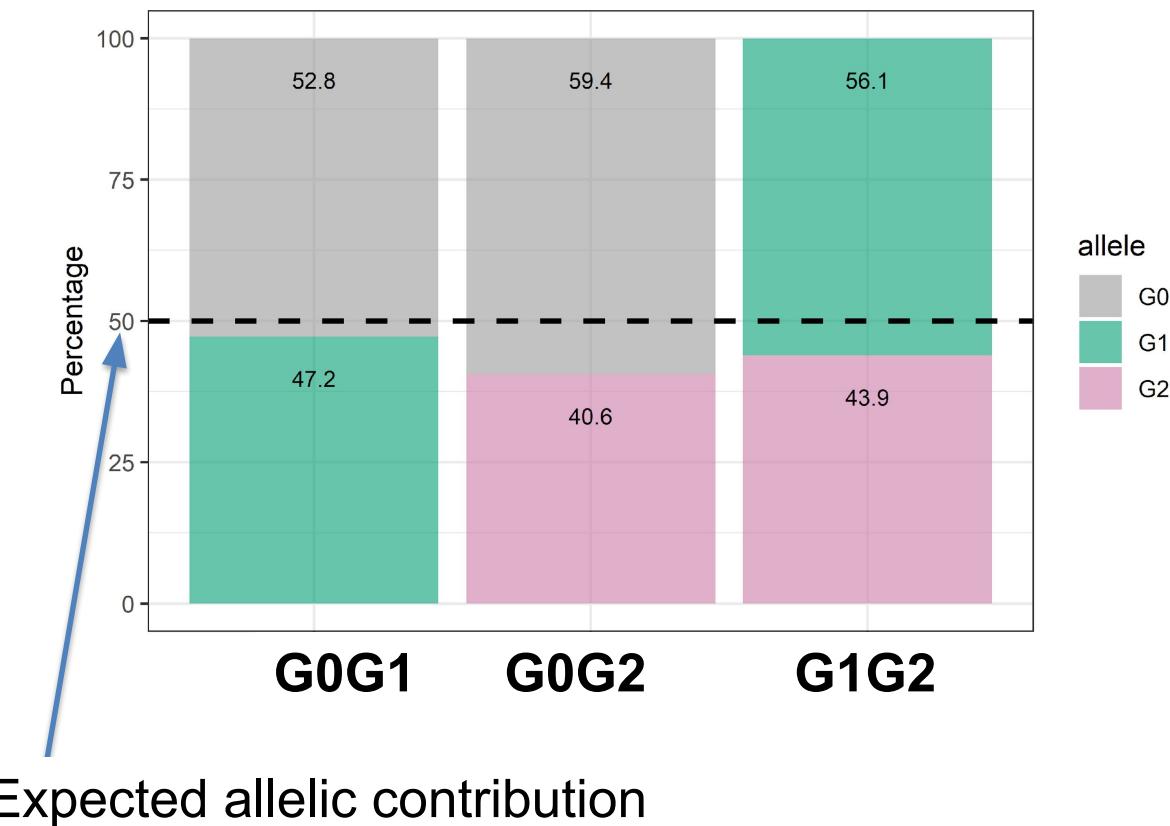
	Mean $\pm$ SEM [APOL1] ug/mL
G0/G0	35.5 $\pm$ 1.3
G0/G2	29.6 $\pm$ 1.9 <sup>a</sup>
G1/G0	35.6 $\pm$ 2.2
G1/G1	31.9 $\pm$ 2.8
G1/G2	34.7 $\pm$ 3.1
G2/G2	24.8 $\pm$ 2.6 <sup>a</sup>



Plasma APOL1 is lower in individuals carrying G2

## G2 allele contributes less to plasma in heterozygotes

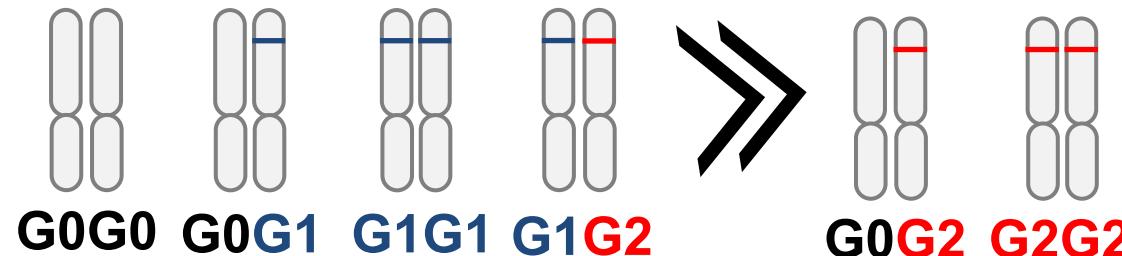
Percentage allele contribution to APOL1 levels in heterozygotes



	Mean [allele] ug/mL
G0	17.9
G1	17.6
<b>G2</b>	<b>13.4*</b>

## Summary

- The level of APOL1 is lower in plasma from G0G2 and G2G2 individuals compared to those with other genotypes
- G2 variant contributing significantly less than either G1 or G0 to circulation



# Does serum from different APOL1 genotypes lysis trypanosomes?

*T.b. gambiense*      West African human infective

*T.b. brucei*      West and East Africa- not human infective

*T.b. rhodesiense*      East African – human infective

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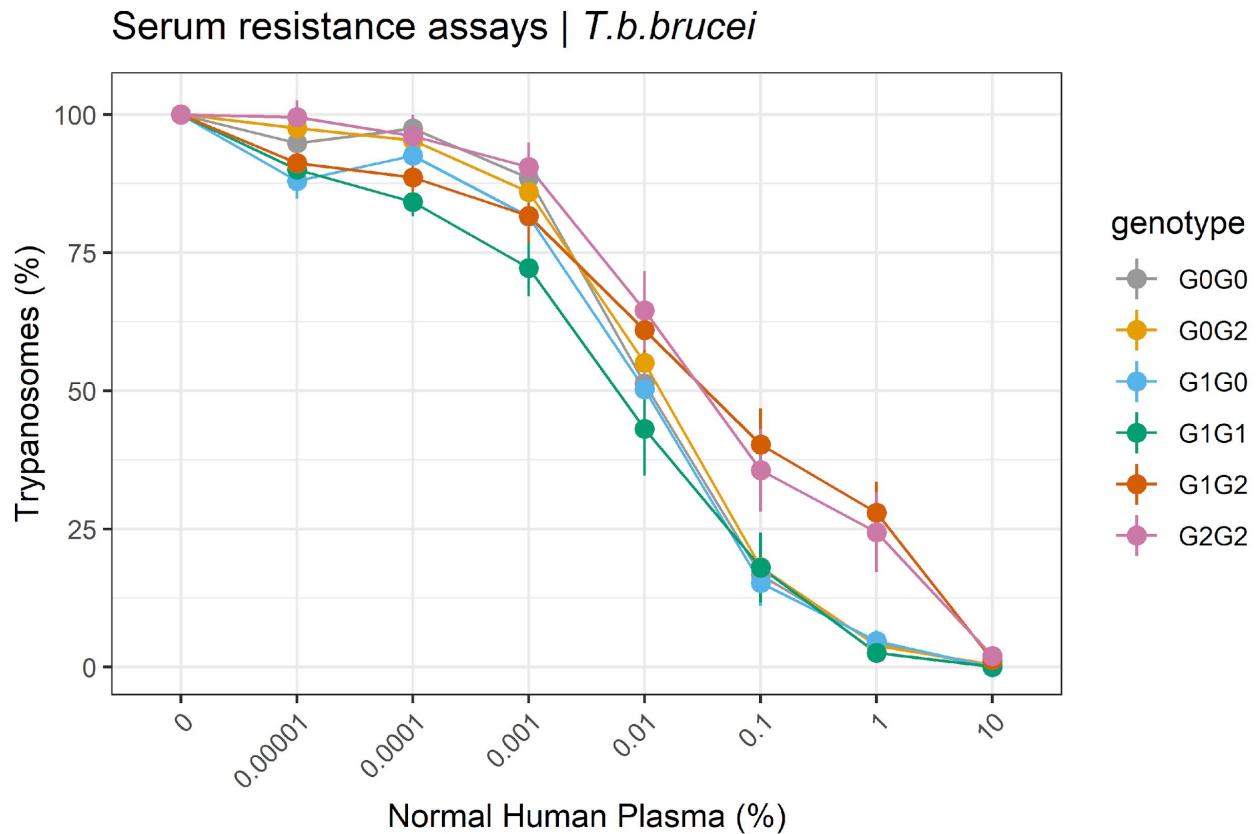
- *T.b. gambiense* is refractory to lysis by plasma regardless of APOL1 genotype.
- The protective association of G1 against *T.b. gambiense* HAT is not a result of parasite lysis.
- Indirect mechanism requiring further investigation.

# Does serum from different APOL1 genotypes lysis trypanosomes?

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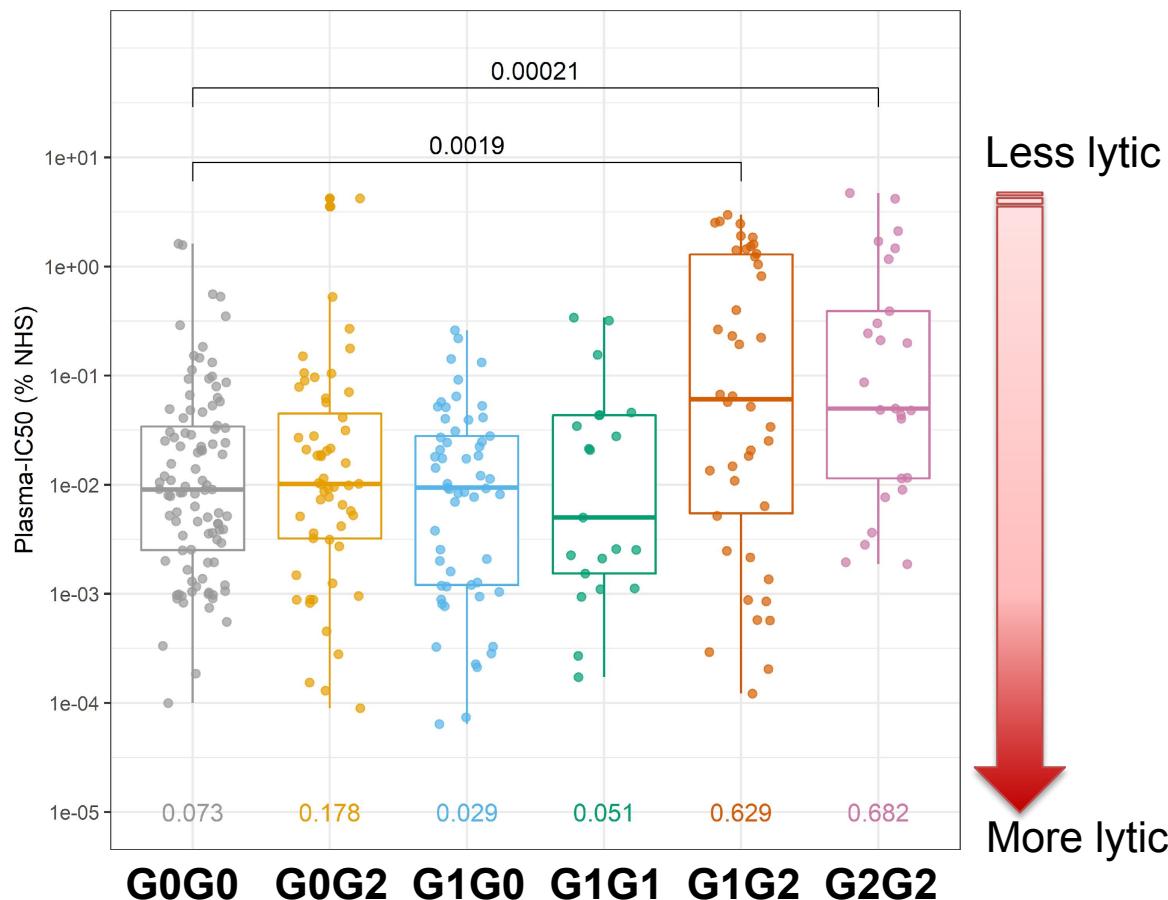


Plasma regardless of APOL1 genotype can lyse *T.b. brucei* at 10% (v/v).

Mean serum APOL1 concentration is 100x more than is required to lyse *T.b. brucei*. It may be feasible to developing APOL1-targetting kidney disease treatments that balance therapeutic benefit while maintained trypanosome resistance.

# G2-containing sera are less lytic to *T.b. brucei*

Lytic potency against *T.b. brucei* | Plasma\_IC50 (% plasma)

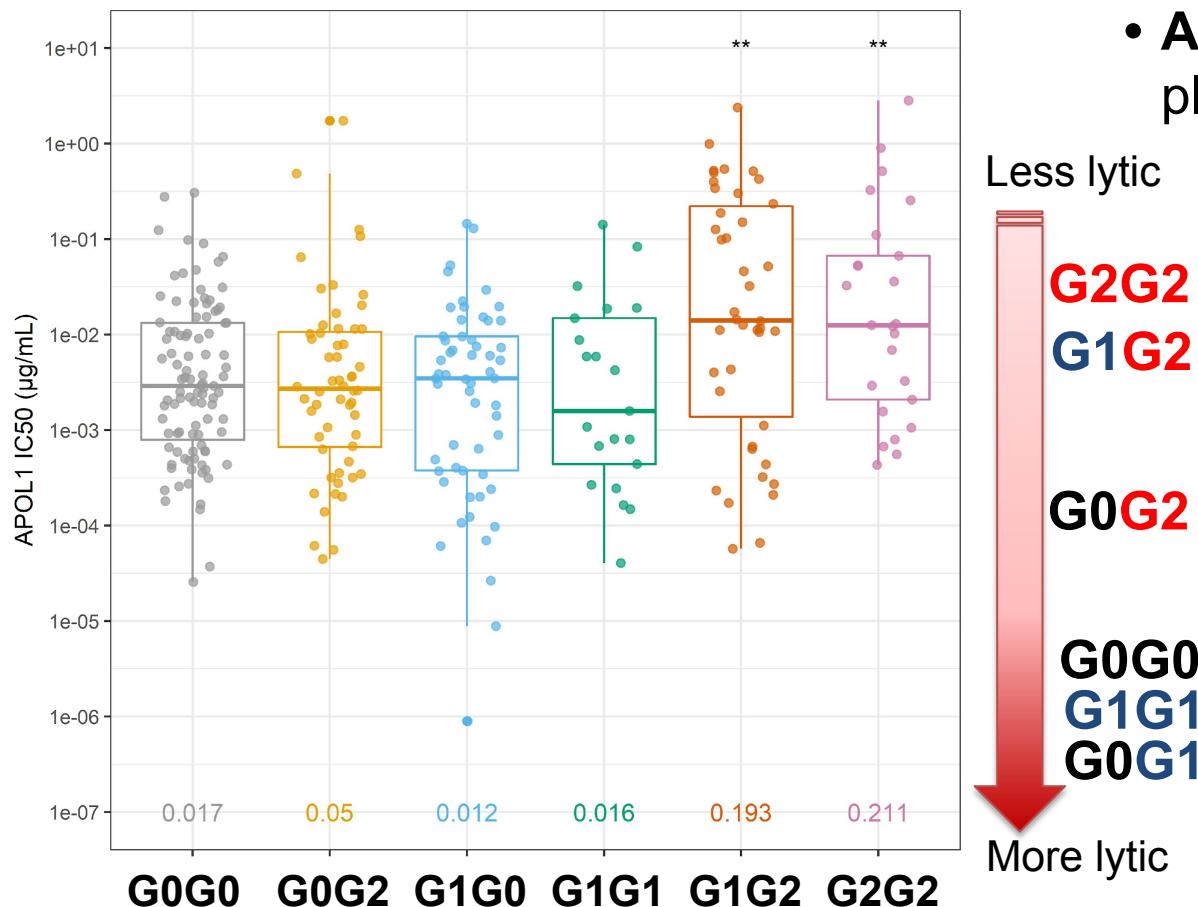


Less lytic  
More lytic

- Compared to G0/G0, plasma from individuals with **G1/G2** and **G2/G2** are significantly less lytic to *T.b. brucei*

# G2-containing sera are less lytic to *T.b. brucei*

Lytic potency against *T.b. brucei* | APOL1\_IC50 (ug/mL)



- APOL1\_IC50:  
plasma\_IC50 × [APOL1]

Less lytic

**G2G2**  
**G1G2**

**G0G2**

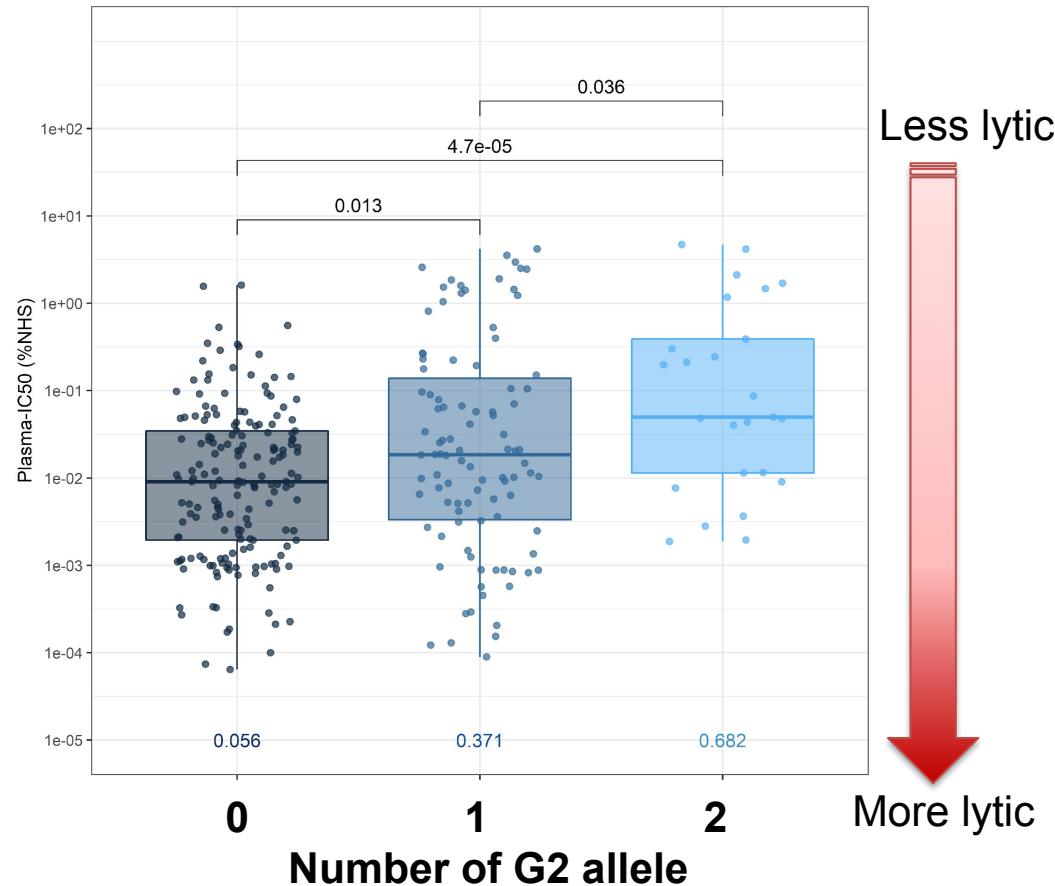
**G0G0**  
**G1G1**  
**G0G1**

More lytic

Taking [APOL1]  
into account

- APOL1 genotype accounts for 14% of the variation in lysis of *T.b. brucei*.

G2 carriage on Lytic potency against *T.b. brucei* | plasma\_IC50 (%)



- There is less G2 in a plasma.
- G2 is intrinsically less lytic to *T.b. brucei*.

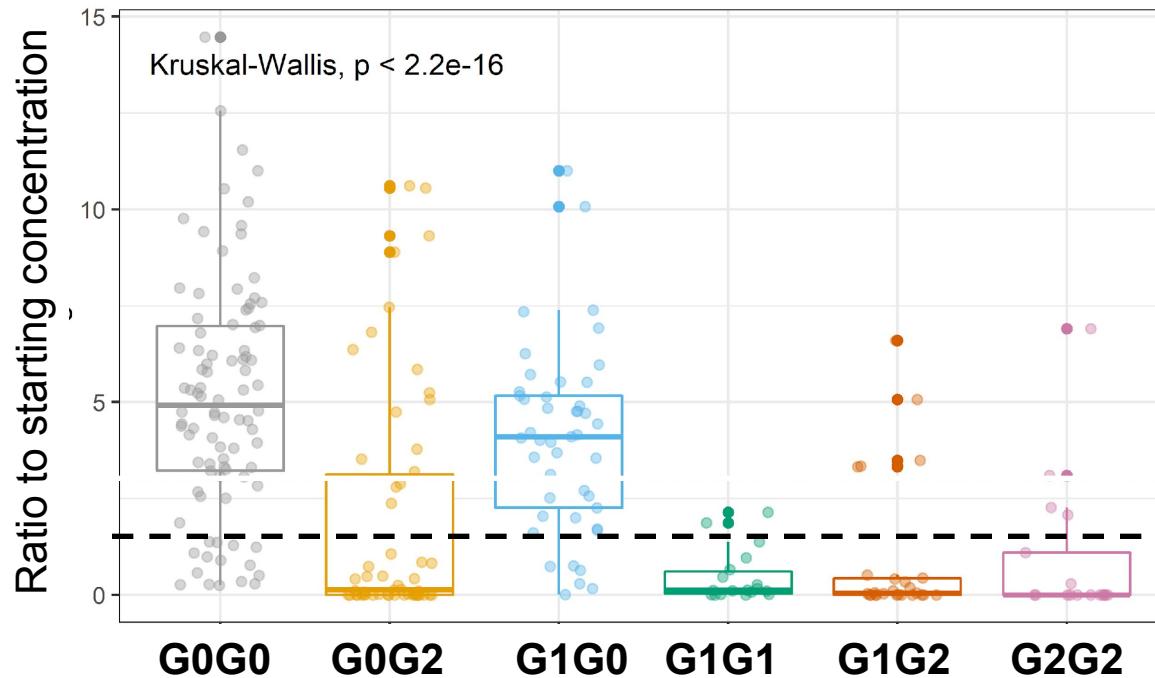
# Does serum from different APOL1 genotypes lysis trypanosomes?

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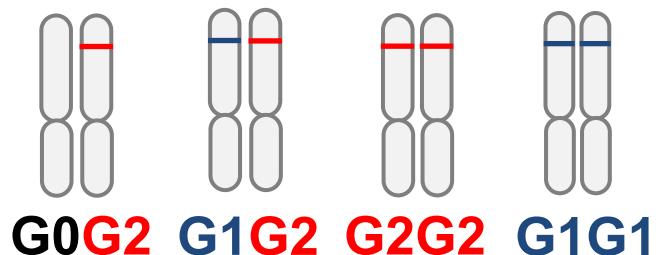
Plasma resistance assays at 10% plasma | *T.b. rhodesiense*



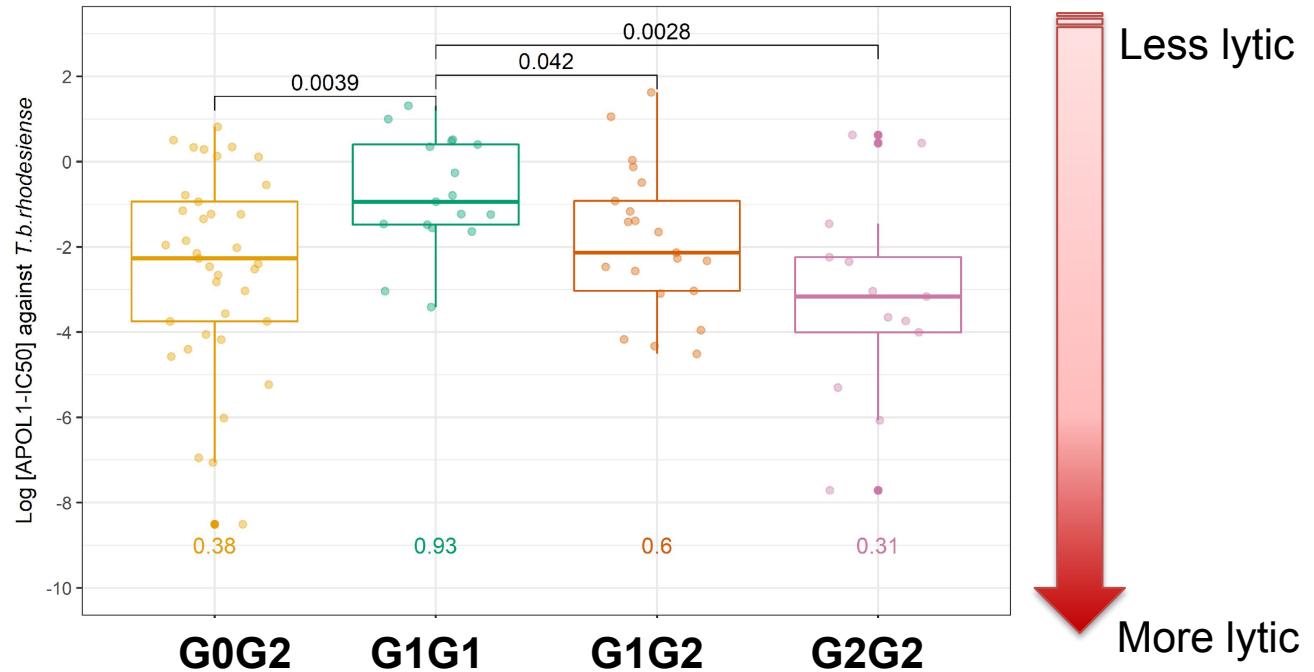
Consistent with the association study that G2 is protective.

Starting concentration

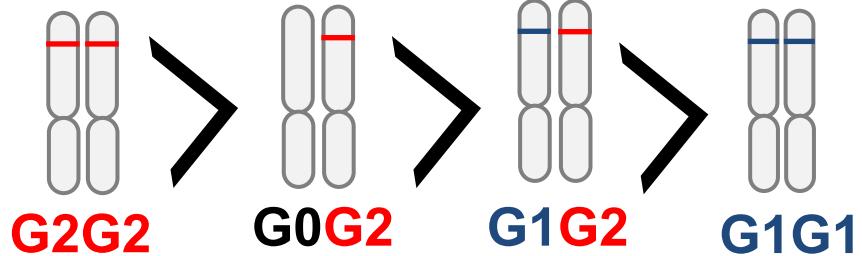
Plasma with at least one copy of G2 or two copies of G1 lysed *T.b. rhodesiense*



Lytic potency against *T.b.rhodesiense* | APOL1\_IC50 (ug/mL)



- (mean) Lytic potency:

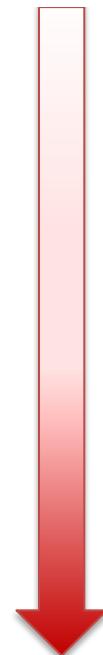


G2 is less toxic to *T.b. brucei* and there is less of it in serum.

*T.b. brucei*

**G2G2**  
**G1G2**  
**G0G2**  
**G0G0**  
**G1G1**  
**G0G1**

No lysis



*T.b. rhodesiense*

**G0G0**    **G0G1**  
  
**G1G1**  
**G1G2**  
**G0G2**  
**G2G2**

G2 is highly toxic to *T.b. rhodesiense* even if there is less of it in serum.

	Mean [APOL1]	[IC50] <i>T.b. rhodesiense</i>	[IC50] <i>T.b. brucei</i>	<i>Tbr/Tbb</i>
<b>G0/G0</b>	35.5	-	0.017	-
<b>G0/G1</b>	35.6	-	0.012	-
<b>G0/G2</b>	29.6	0.38	0.050	8x
<b>G1/G1</b>	31.9	0.93	0.016	58x
<b>G1/G2</b>	34.7	0.60	0.193	3x
<b>G2/G2</b>	24.8	0.31	0.211	1.5x

- Depending on the APOL1 genotype, between 1.5- and 60-fold more of [APOL1] is required to lyse *T.b. rhodesiense*.

	<i>T.b. brucei</i>	<i>T.b. rhodesiense</i>	<i>T.b. gambiense</i>
<b>G0/G0</b>	✓	✗	✗
<b>G0/G1</b>	✓	✗	✗
<b>G0/G2</b>	✓	✓	✗
<b>G1/G1</b>	✓	✓	✗
<b>G1/G2</b>	✓	✓	✗
<b>G2/G2</b>	✓	✓	✗

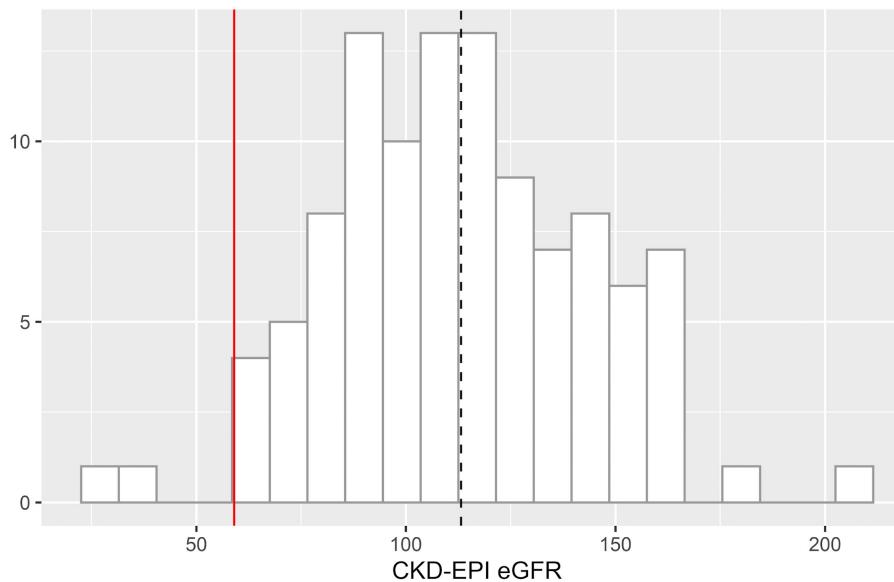
✓ lytic

✗ non-lytic

## Summary

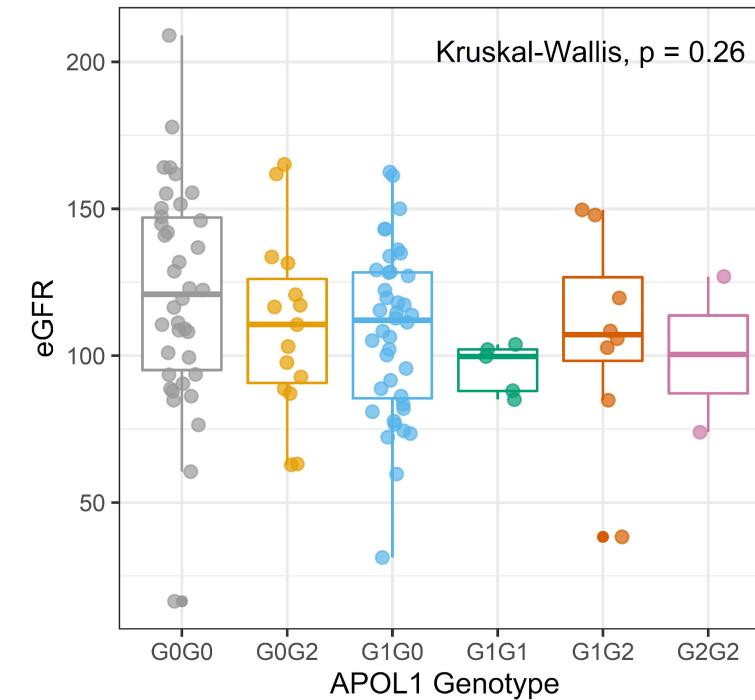
- APOL1 genotype rather than plasma levels accounts for the variation in lysis of both *T.b. brucei* and *T.b. rhodesiense*
- Increasing G2 variant has contrasting effect on the lytic potency of plasma against *T.b. brucei* and *T.b. rhodesiense*.
- Evolutionary adaptation towards G2's broader activity but lower efficiency.
- Plasma APOL1 levels are approximately 100-fold and 10-fold more than is required to effectively lyse *T.b. brucei* and *T.b. rhodesiense*, respectively.
- Therapeutic window is smaller for *T.b. rhodesiense*. Potential to render resistant (G2G2, G0G2, G1G2, G1G1) individuals susceptible to *T.b. rhodesiense* infections if reduce APOL1 10-fold.

Distribution of eGFR in an African cohort

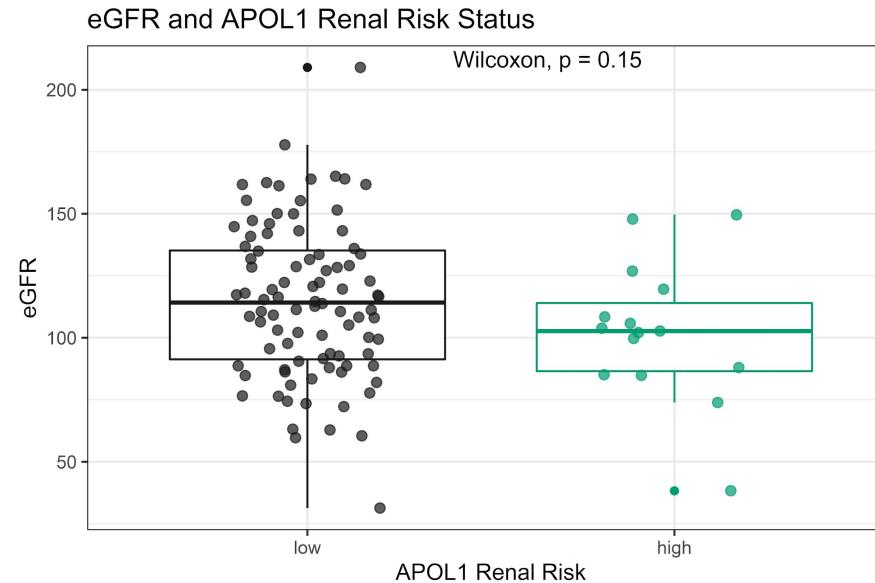


- Red line: cut off for CKD stage 3
- Dotted line: mean eGFR in the cohort

eGFR across APOL1 genotypes



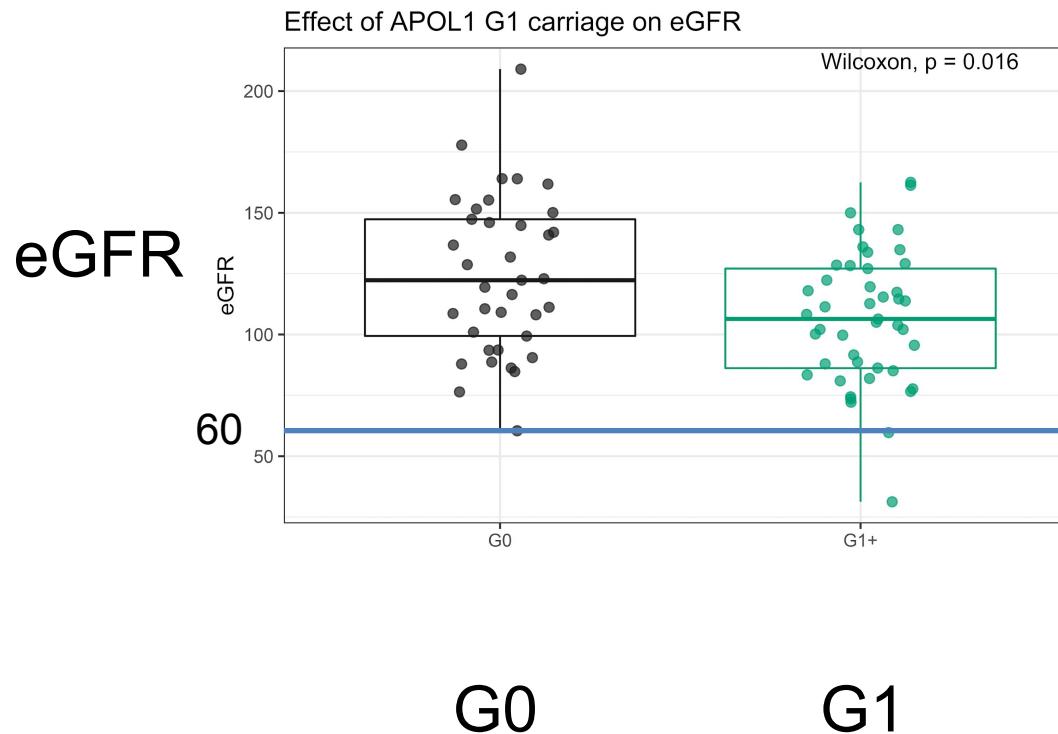
- No difference in eGFR across APOL1 genotypes



**Low:**  
G0G0,  
G0G1,  
G0G2

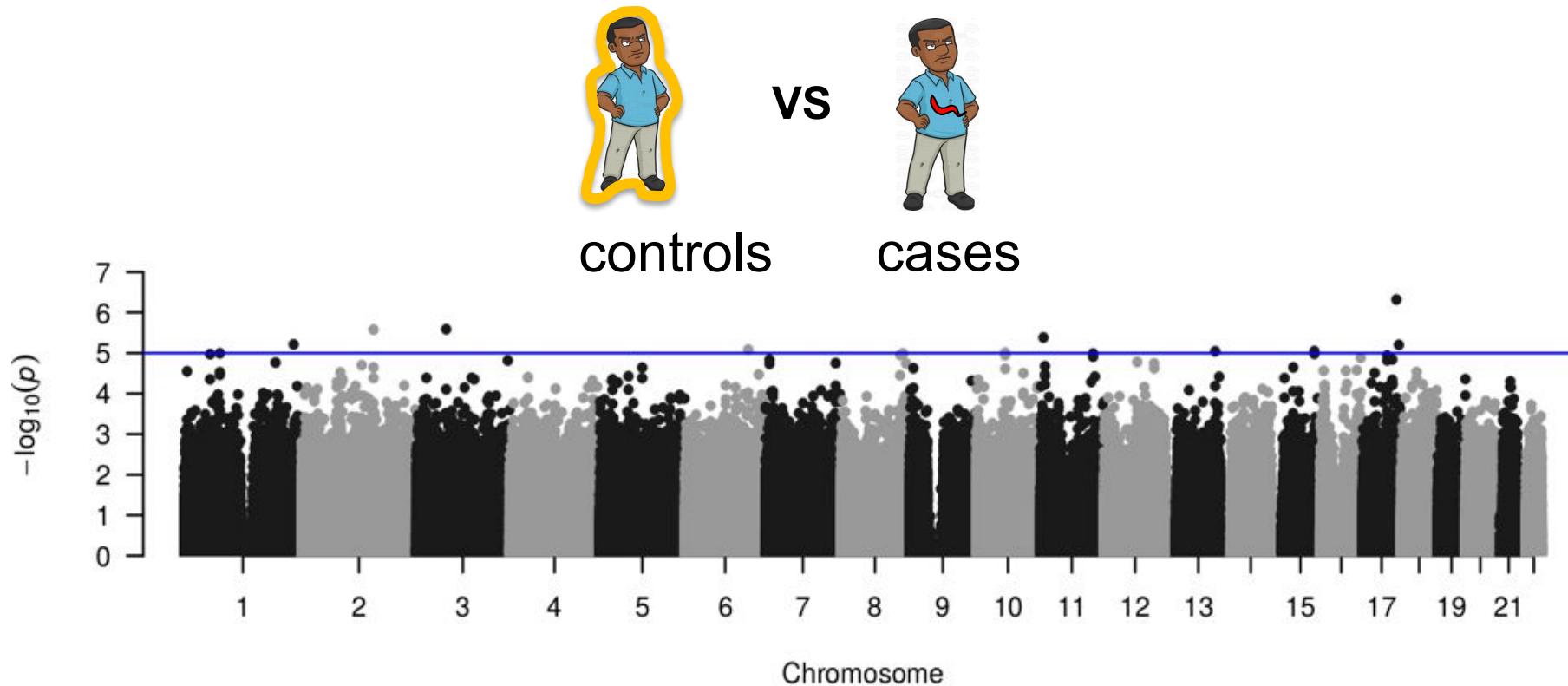
**High:**  
G1G1,  
G2G2,  
G1G2

- Carriage of G1 (G0G1, G1G1, G1G2) associates with significantly lower eGFR



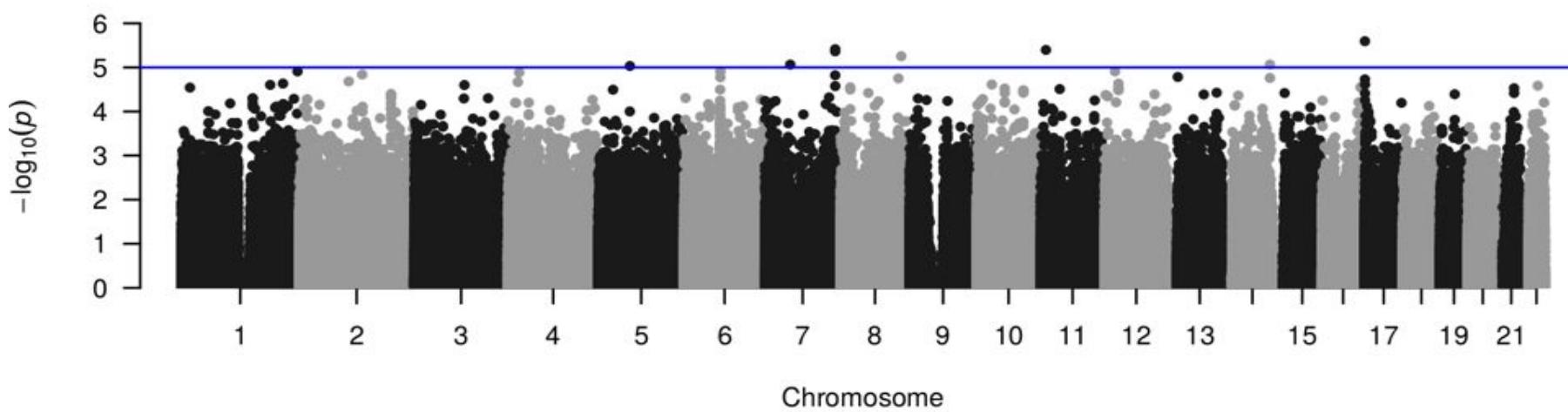
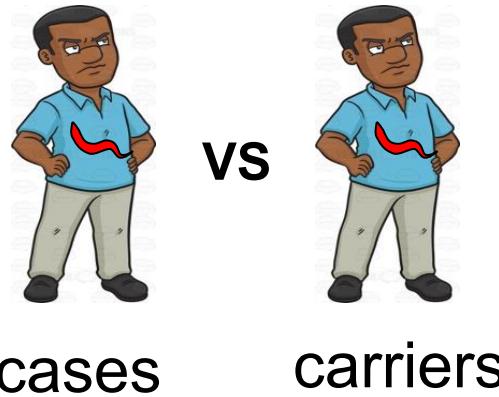


# Other genes associated with resistance to trypanosomiasis





# Other genes associated with disease severity



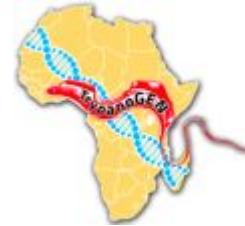


# GWAS top hits

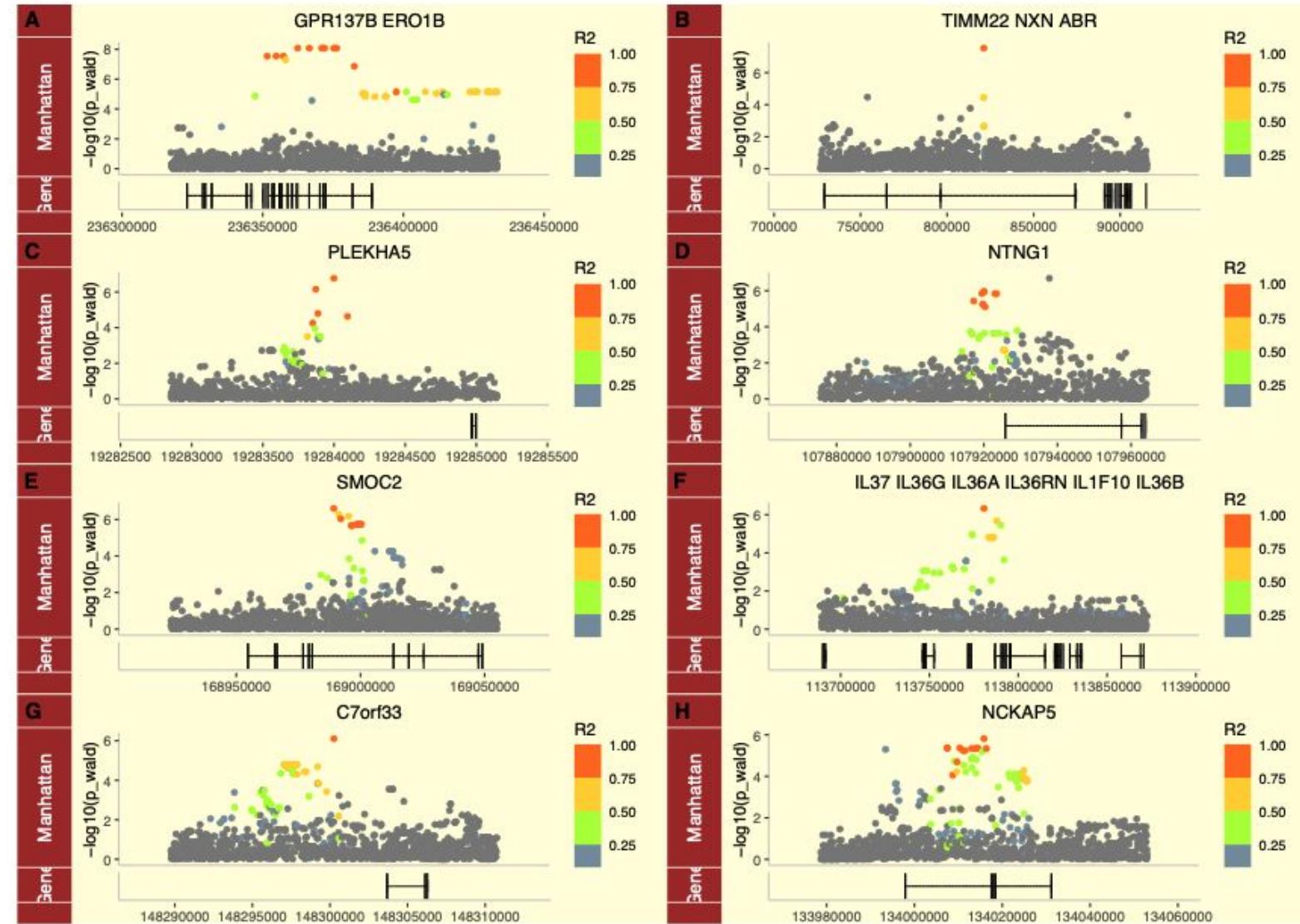
Fine map loci from discovery cohort.

Impute and test in validation cohort.

APOL1 is on the long list but does reach genome-wide significance.

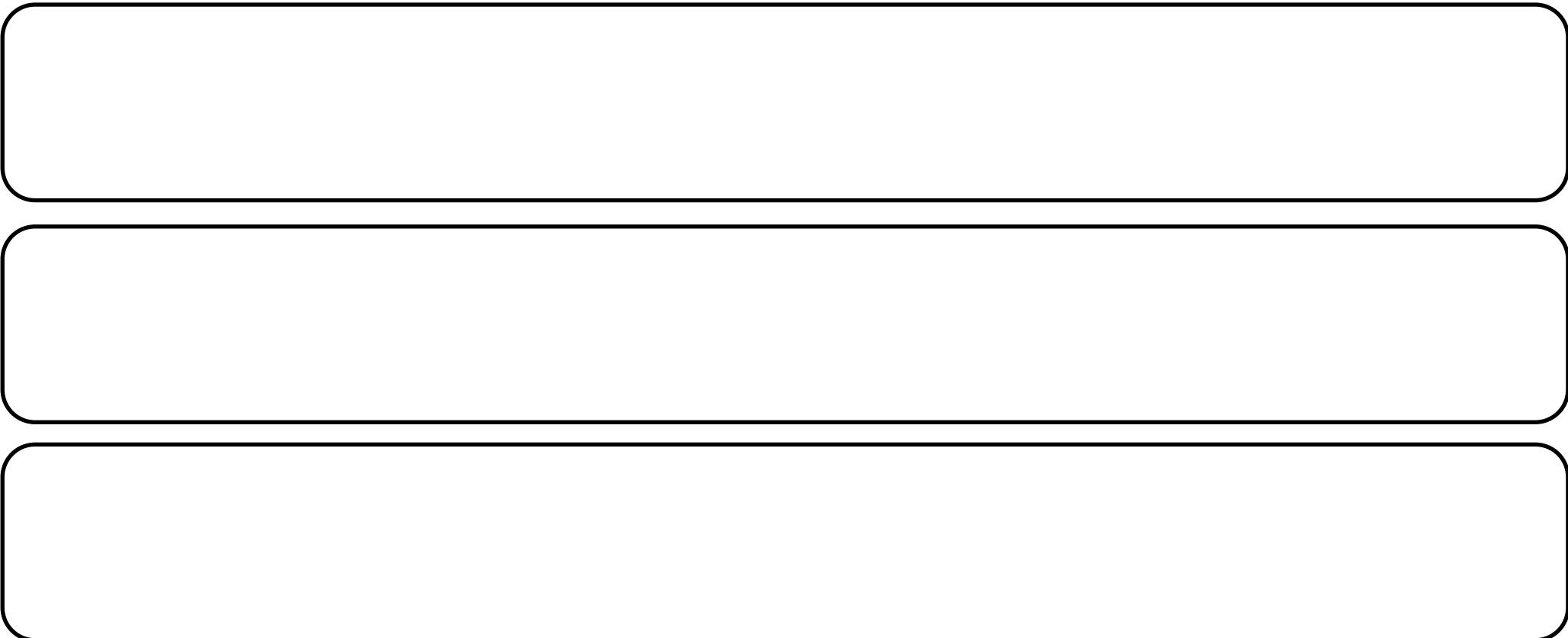


# GWAS top hits: beyond APOL1

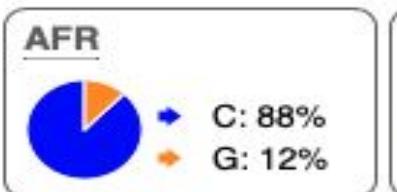




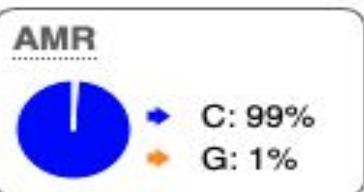
# Top hit: SMOC2



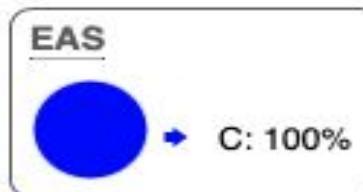
African



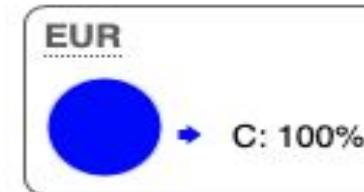
American



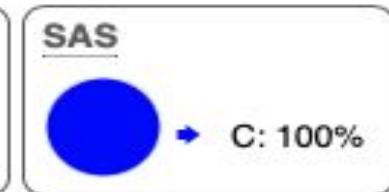
East Asian



European



South Asian





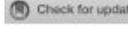
## Top hit: SMOC2

Second gene linking trypanosomiasis to kidney disease.



# SMOC2 interacts with *APOL1*

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## SMOC2 gene interacts with *APOL1* in the development of end-stage kidney disease: A genome-wide association study

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# Future directions

Interaction with APOL1.



# Thanks: TrypanoGEN



PhD students and postdocs



Patients and controls

Enock Matovu

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