Practical exercise : APOL1 – Exercise

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Understanding for *APOL1* Renal Risk 'Genotypes' are derived

Day 2: KidneyGenAfrica: 1st Training Workshop

Purpose:

a. To use raw genotype data to derive APOL1 risk haplotypes and genotypes (G0, G1 and G2; G0/G0, G1/G0, G2/G0, G1/G2, G1/G1 and G2/G2)
b. To understand allele and genotype frequencies
c. To understand genetic association and causality

Background information:

It is made up of haplotypes that involve 3 different genetic variants. Two single nucleotide variants and one indel.
Each variant is defined by a 'rs' number, the nucleotides involved (A/G/C/T) and the amino acid changes (S-serine; G-glycine; I-isoleucine ; M-methionine; N-asparagine; and Y-tyrosine).

Three loci that are each bi-allelic are involved

Locus	Alleles	Genotypes
rs73885319	A or G	A/A or A/G or G/G
rs60910145	T or G	T/T or T/G or G/G
rs71785313	ins or del	ins/ins or ins/del or del/del

Haplotypes (defined by the sequence of alleles at the three loci on a single chromosome):

Haplotype	rs73885319 A/G S342G	rs60910145 T/G I384M	rs71785313 TTATAA(ins)/ del N388Y389/del
G0	A	T	ins
G1	G	G	ins
G1	G	T	ins
G1	A	G	ins
G2	A	T	del

High Risk Genotypes – as usually referred to in the literature:
G0/G0, G1/G0, G2/G0, G1/G2, G1/G1 and G2/G2

Data as generated in the laboratory (either in a single genotype experiment or an array). The H3Africa array included rs73885319 and rs60910145, but not rs71785313). Therefore if using array data, rs71785313 has to be inferred (imputed) using a statistical probabilistic approach.

Part A: Converting genotype data into APOL1 high risk 'genotypes'

Individual	rs73885319 A/G	rs60910145 T/G	rs71785313 TTATAA/del	
ID 1	A/A	T/T	ins/ins	
ID 2	A/G	T/T	ins/ins	
ID 3	A/A	A/T	ins/del	
ID 4	A/A	T/T	del/del	
ID 5	A/A	T/G	ins/ins	
ID 6	A/G	T/G	Ins/ins	
ID 7	A/G	T/T	Ins/del	
ID 8	A/G	T/T	Ins/ins	

Haplotypes (2 filled in as examples – complete the rest of the table)

Individual	Haplotype 1	Haplotype 2	Diplotype	APOL1 risk 'genotype'
ID 1	A-T-ins	A-T-ins	A-T-ins/ A-T-ins	G0/G0
ID 2	A-T-ins	G-T-ins	A-T-ins/ G-T-ins	G0/G1
ID 3				
ID 4				
ID 5				
ID 6				
ID 7				
ID 8				

Questions:

1. Could you resolve all the ‘genotypes’ – actually haplotypes?
2. What are the allele frequencies for each of the three loci individually?
3. What are the *APOL1* G0, G1 and G2 frequencies and the diplotype frequencies?

Part B: Effects of APOL1 ‘genotypes’ on phenotype
Examine the following tables and figures and answer questions

Study 1: Brandenburg JT, Govender MA, Winkler CA, Boua PR, Agongo G, Fabian J, Ramsay M. Apolipoprotein L1 High-Risk Genotypes and Albuminuria in Sub-Saharan African Populations. Clin J Am Soc Nephrol. 2022 Jun;17(6):798-808. doi: 10.2215/CJN.14321121. Epub 2022 May 16. PMID: 35577564; PMCID: PMC9269651.

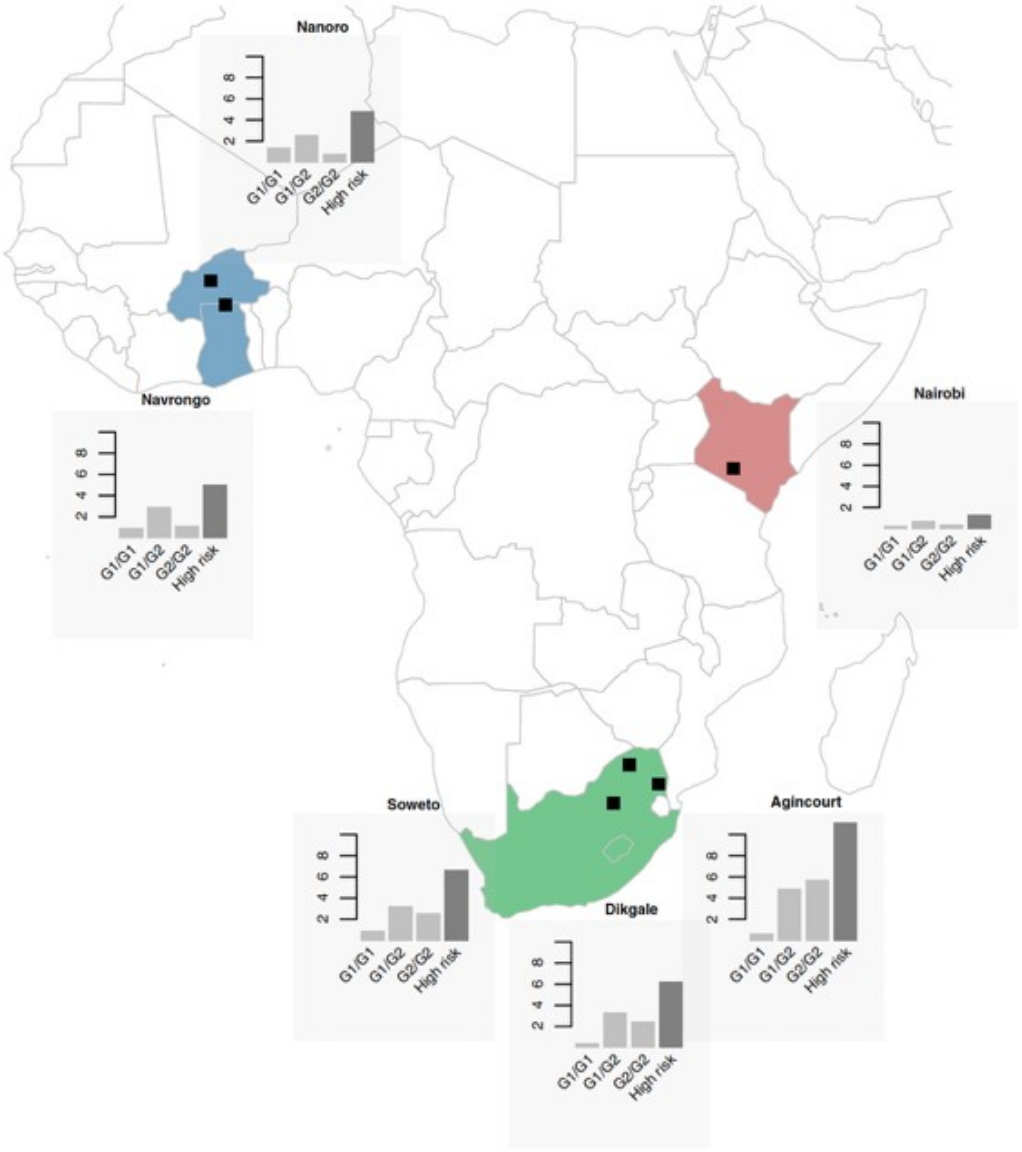


Figure 1. | **Combined "high-risk" genotype frequencies are highest in West Africa or South Africa and lowest in East Africa, with distinct differences with regard to the G1/G1, G1/G2 and G2/G2 genotypes across regions.** *APOL1* genotype (G1/G1, G1/G2, and G2/G2) frequencies (percentages) and combined "high-risk" genotypes (G1/G1, G1/G2, and G2/G2) for each sub-Saharan African region: Nanoro and Navrongo in West Africa; Nairobi in East Africa; and Soweto, Dikgale, and Agincourt study sites in South Africa.

Table 4. Association between *APOL1* genotype and biomarkers of kidney disease stratified by region

Genotype[a]	All	East Africa	South Africa	West Africa
Low eGFR[b]				
G0/G1	1.03 (0.73 to 1.47)	1.00 (0.29 to 2.63)	1.06 (0.61 to 1.76)	1.01 (0.58 to 1.69)
G0/G2	0.76 (0.55 to 1.05)	0.77 (0.26 to 1.88)	0.82 (0.53 to 1.25)	0.74 (0.37 to 1.34)
G1/G1	0.52 (0.07 to 3.83)	NA[c]	0 (0 to 35,130.28)	0.93 (0.05 to 4.47)
G1/G2	0.81 (0.39 to 1.69)	4.0 (0.2 to 26.4)	1.13 (0.46 to 2.38)	NA[c]
G2/G2	0.89 (0.41 to 1.94)	15.09 (0.69 to 125.23)	0.99 (0.38 to 2.18)	NA[c]
Albuminuria[d]				
G0/G1	0.97 (0.77 to 1.22)	0.79 (0.38 to 1.48)	0.82 (0.58 to 1.14)	1.3 (0.9 to 1.8)
G0/G2	1.09 (0.9 to 1.31)	1.21 (0.73 to 1.95)	1.06 (0.84 to 1.35)	1.1 (0.7 to 1.6)
G1/G1	3.87 (2.16 to 6.93)[e]	NA[c]	3.67 (1.38 to 8.82)[e]	4.93 (2.15 to 10.26)[e]
G1/G2	1.24 (0.83 to 1.87)	3.24 (0.16 to 23.79)	1.20 (0.72 to 1.92)	1.28 (0.52 to 2.66)
G2/G2	1.65 (1.09 to 2.51)[e]	4.63 (0.62 to 23.40)	1.62 (1.01 to 2.51)[e]	1.09 (0.17 to 3.71)
Composite end point[f]				
G0/G1	0.97 (0.79 to 1.19)	0.84 (0.44 to 1.50)	0.83 (0.61 to 1.13)	1.22 (0.89 to 1.65)
G0/G2	0.99 (0.83 to 1.17)	1.12 (0.69 to 1.77)	0.98 (0.78 to 1.22)	0.95 (0.67 to 1.34)
G1/G1	2.73 (1.51 to 4.96)[e]	NA[c]	2.47 (0.87 to 6.18)	3.45 (1.52 to 7.13)[e]
G1/G2	1.06 (0.72 to 1.57)	2.56 (0.12 to 19.26)	1.11 (0.69 to 1.73)	0.89 (0.37 to 1.83)
G2/G2	1.40 (0.93 to 2.11)	4.32 (0.59 to 21.73)	1.41 (0.89 to 2.17)	0.74 (0.12 to 2.52)

The associations between *APOL1* genotype and biomarkers of kidney disease are presented as odds ratios and 95% confidence intervals. For biomarkers of kidney disease, repeat measures for those with low eGFR or albuminuria were not performed, thus preventing confirmation of CKD; therefore, we used "biomarkers of kidney disease" to define these measures on a single screening. Linear and logistic mixed models were adjusted for site as a random variable and age, sex, body mass index, diabetes mellitus status, hypertension status, and HIV status as fixed variables. NA, not applicable.

[a]*APOL1* genotypes G0/G1, G0/G2, G1/G1, G1/G2, and G2/G2 were compared with G0/G0 as the reference.

[b]Low eGFR is eGFR <60 ml/min per 1.73 m^2 calculated using the Chronic Kidney Disease Epidemiology Collaboration (creatinine) equation 2009 without adjusting for the African American coefficient.

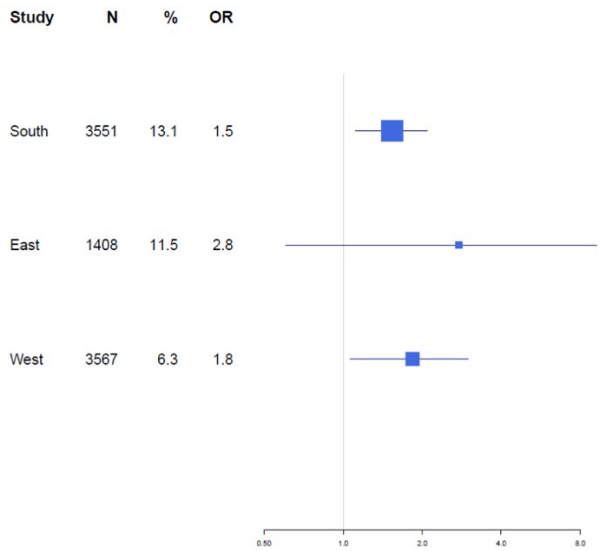
[c]Sample size was too small to perform the statistical test.

[d]Albuminuria is random spot urine albumin-creatinine ratio >3.0 mg/g.

[e]Corresponds at main section and in genotype at the total of individual with zero risk allele, one risk allele, and two risk alleles.

[f]The composite end point is low eGFR and/or albuminuria.

Supplemental Figure 1: Forest plot: association between high-risk *APOL1* genotypes (OR (95%CI)) and albuminuria by region

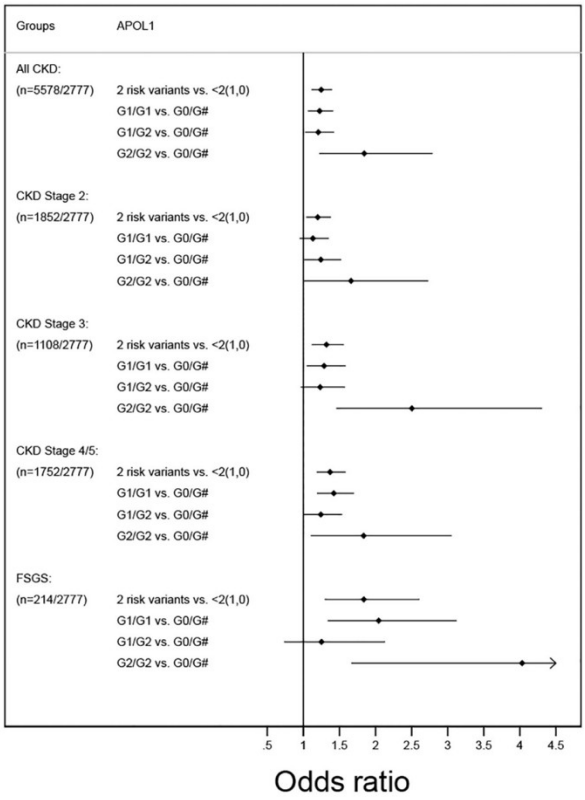


Regions: **East**: East Africa (Kenya); **West**: West Africa (Burkina Faso and Ghana); **South**: South Africa.
OR (odds ratio) with 95% CI (confidence interval).

Study 2: Gbadegesin RA, et al. H3Africa Kidney Disease Research Network. *APOL1* Bi- and Monoallelic Variants and Chronic Kidney Disease in West Africans. N Engl J Med. 2025 Jan 16;392(3):228-238. doi: 10.1056/NEJMoa2404211. Epub 2024 Oct 26. PMID: 39465900; PMCID: PMC11735277.

***APOL1* risk variants**	**OR (95% CI)**	
	Unadjusted	**Adjusted†**
All CKD cases (N=5578) vs. Controls (N=2777)		
APOL1 Risk Alleles: 2 vs. 1 and 0	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)

Associations of *APOL1* Risk Alleles and CKD among 8355 West Africans in the H3Africa Kidney Disease research network (covariates: Age, sex, BMI, MAP, HIV status, diabetes, clinical site, tobacco use and language group)



Association of ***APOL1*** high risk genotypes with CKD among 8355 West Africans in the H3Africa Kidney Disease research network

Questions:

1. What conclusions can you draw about genotype frequencies in different African populations?
2. Are the associations with the genotypes/haplotype or with the alleles?
3. Is the *APOL1* risk genotype causal of chronic kidney disease? Motivate you response
4. Do G1 and G2 contribute equally to the kidney function phenotype?