Genetics/ Genomics and HIV Nephropathy

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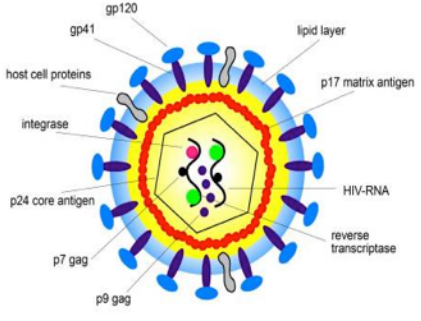
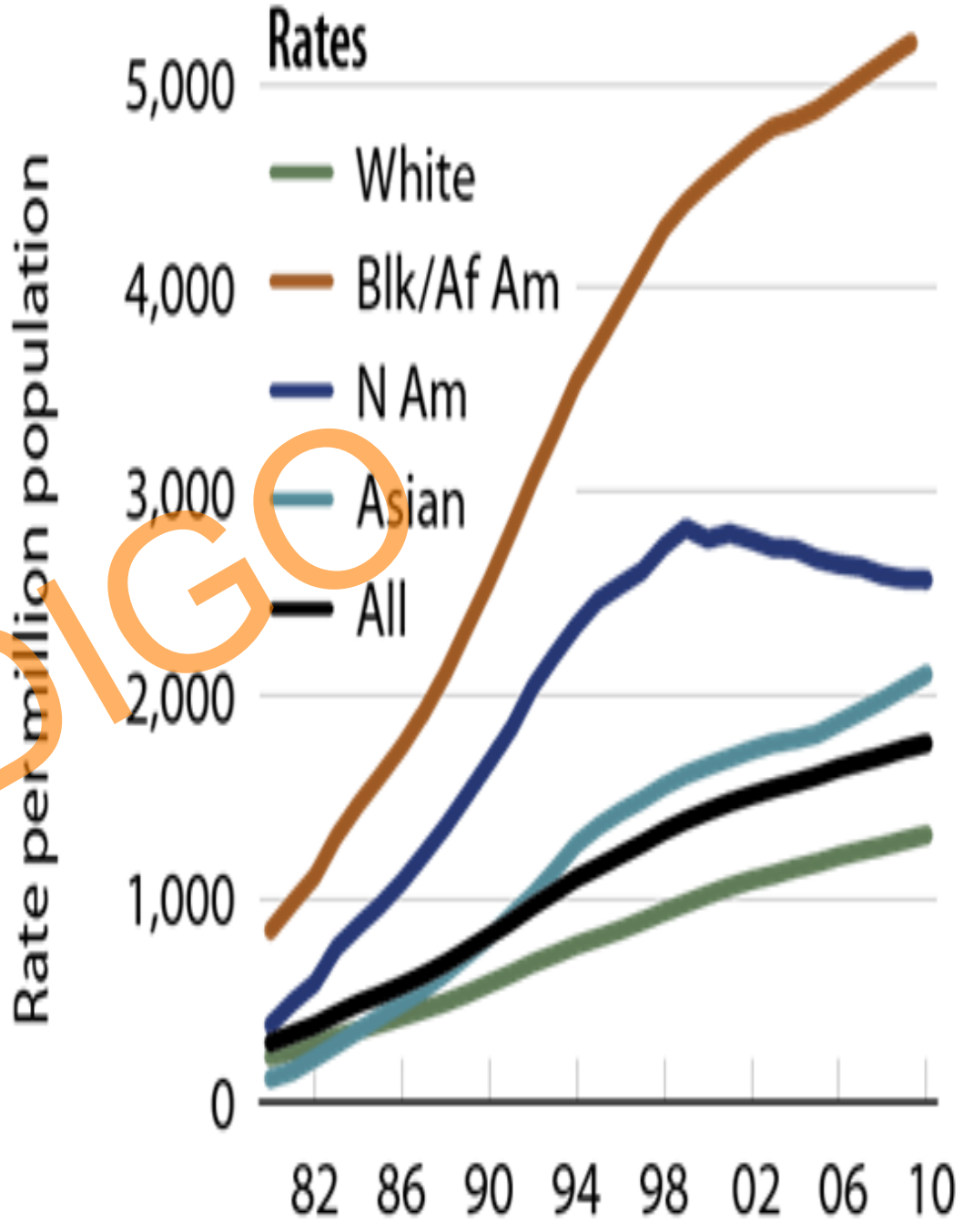


KDIGO Conference
Yaounde, Cameroon
18-20 March 2017

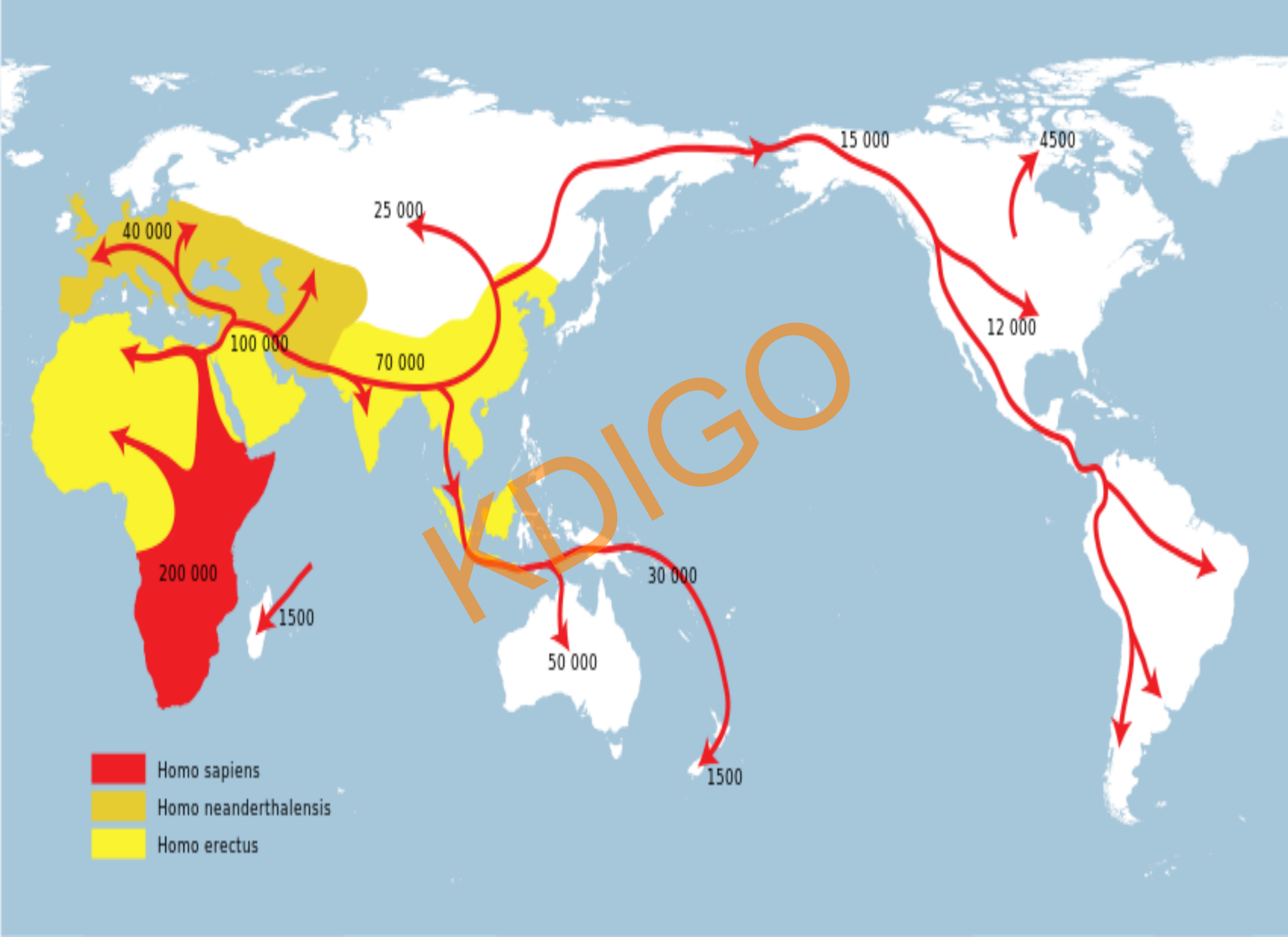
UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

CKD prevalence

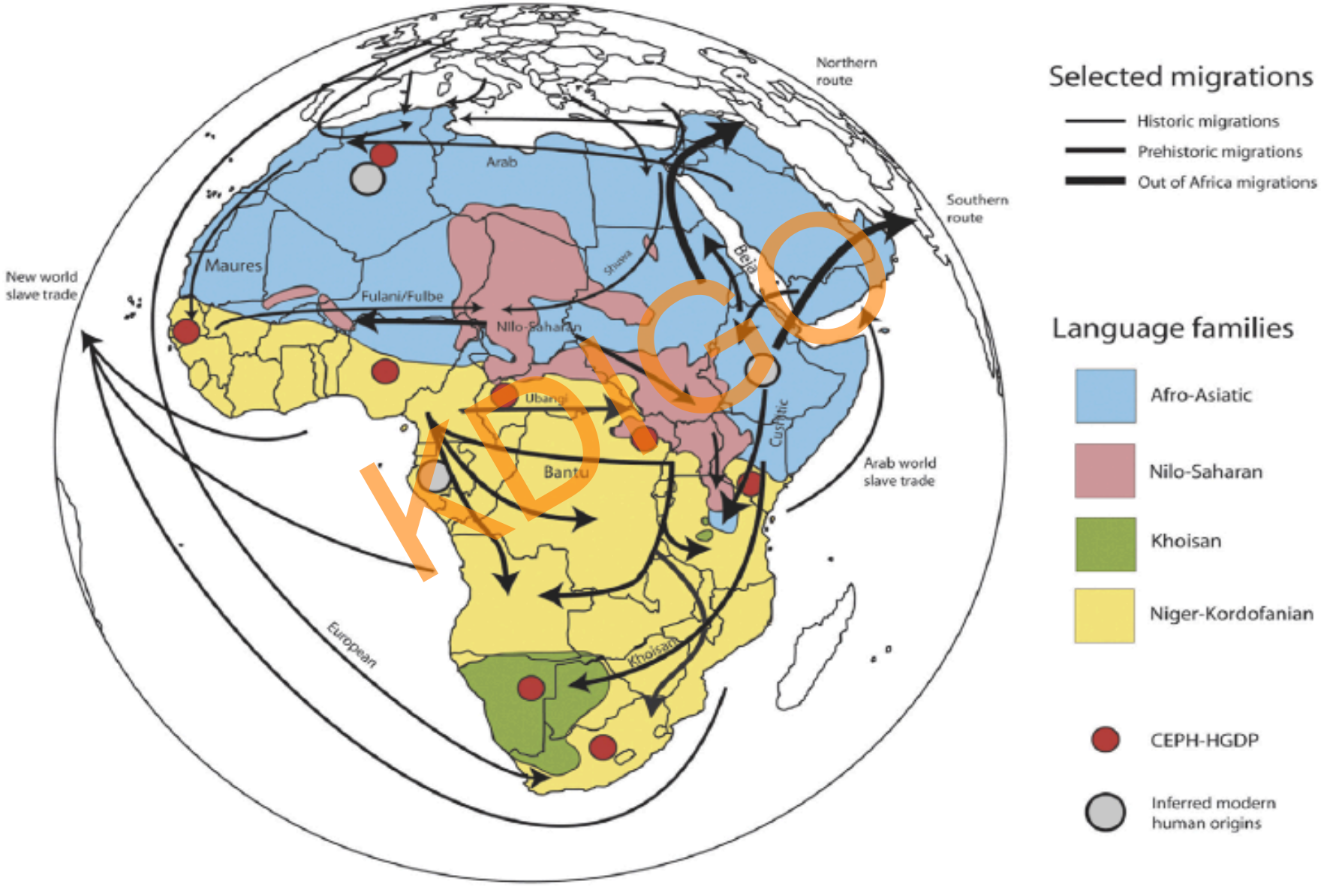
- Estimated 3,2million people on RRT, with CKD incidence growing by 6% annually (WHO)
- Cumulative lifetime risk for CKD varies by ancestry
- African descent are the most affected (4X more likely than of European origin)
- HIV CKD 18-50X increase in people of African descent



Rates adjusted for age and gender (USRDS 2012)


15 000
4500
25 000
40 000
12 000
100 000
70 000
200 000
30 000
1500
50 000
1500
Homo sapiens
Homo neanderthalensis
Homo erectus

Human diversity, migration and origins



Floyd Reed and Sarah Tishkoff

Current Opinion in Genetics & Development 2006, 16:597–605

Renal histology in HIV infection- Africa

	JHB[1]	JHB[2]	JHB[3]	DBN[4]	CT[5]	CT[6]	Nigeria[7]
N	99	84	636	37	145	192	10
HIVAN (%)	27	15	25.8	83	55	57.3	70
IC Disease (%)	21	9	9.6			8.3 21.9*	
Other GN (%) [FSGS]	41	27 [13]	27.3 [14.5]	7	15.9	12.5	
Tubulo-Int Disease (%)		21	6.3	10	13		70
Other (%)	10	15	4.9		16		

1. Gerntholtz et al. Kidney Int. 2006; 69: 1885-1891
2. Rahmanian S. MMed, Wits 2015
3. Diana et al. WCN 2015 poster
4. Han et al. Kidney Int. 2006; 69: 2243-2250
6. Swanepoel & Okpechi. Port J Nephrol & Hypert. 2011; 25: 11-15
6. Wearne et al. NDT 2012.
7. Emem et al. Nephrol Dial Transplant. 2008; 23: 741-746

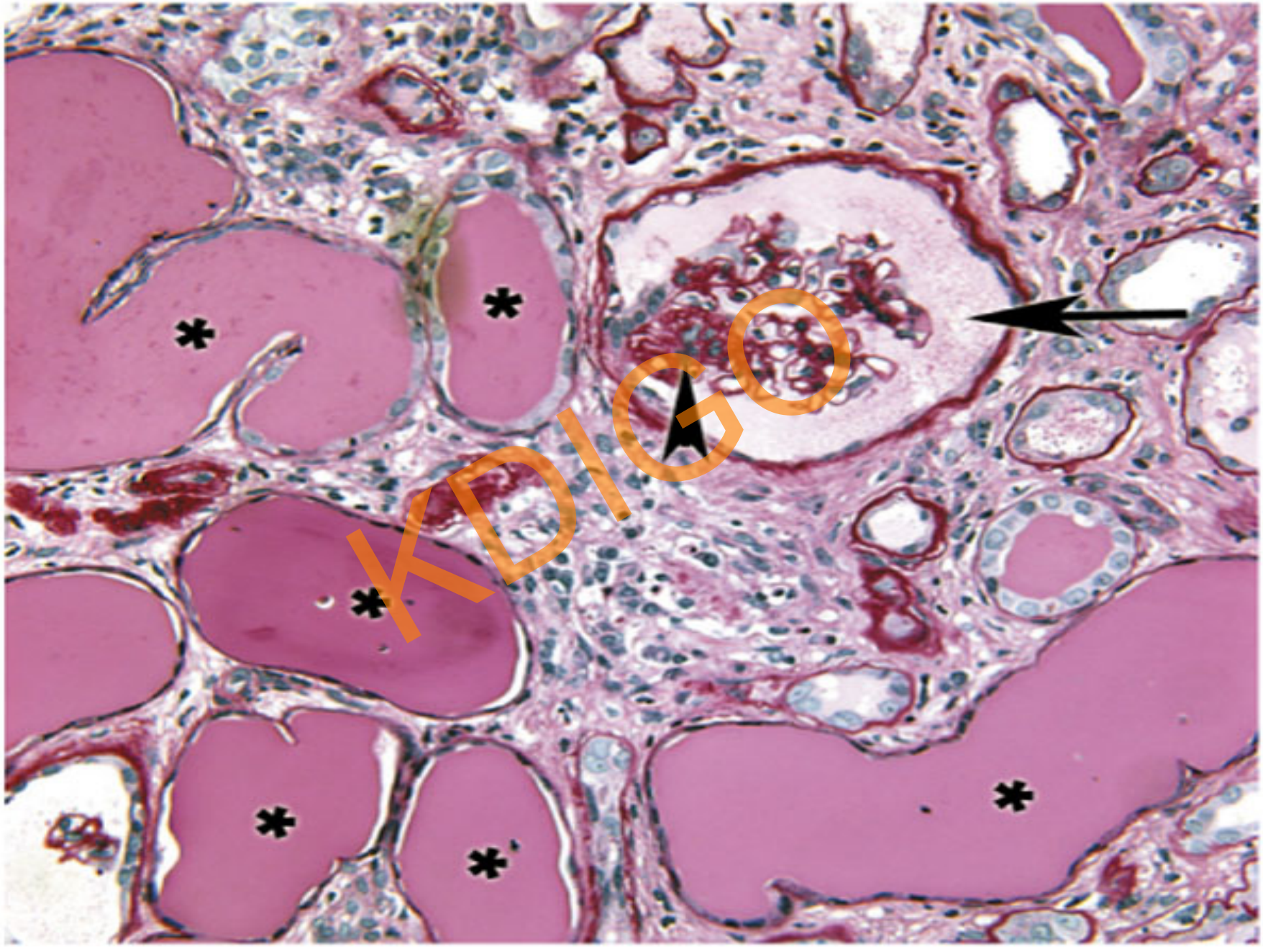
Abbreviations

JHB= Johannesburg

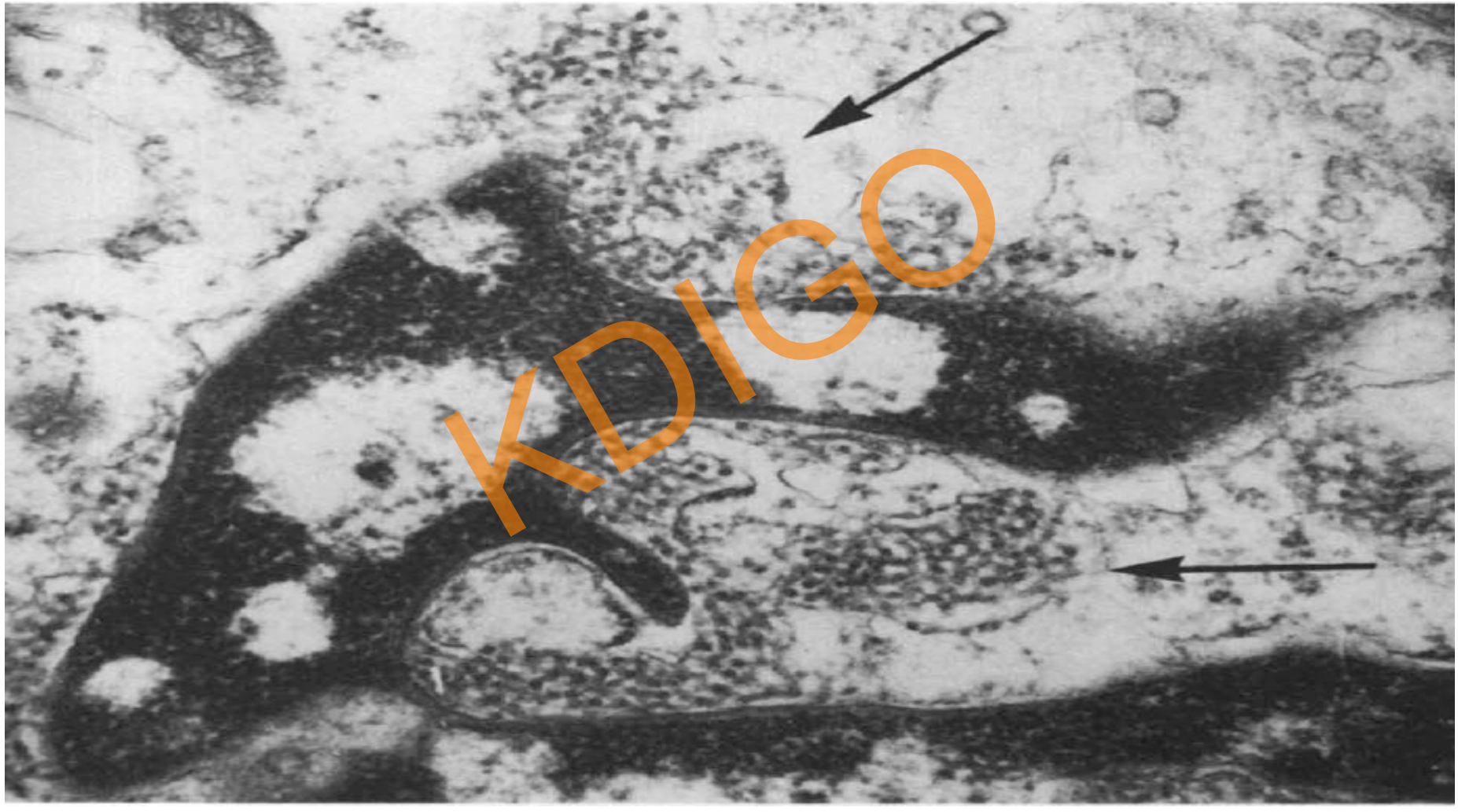
DBN= Durban

CT= Cape Town

[*=HIVAN+ICD]


KDIGO

Glomerular endothelial cell containing tubulo-reticular inclusions



D'Agatti et al, Kidney Int. 1989; 35: 1358-1370

MYH9 and CKD

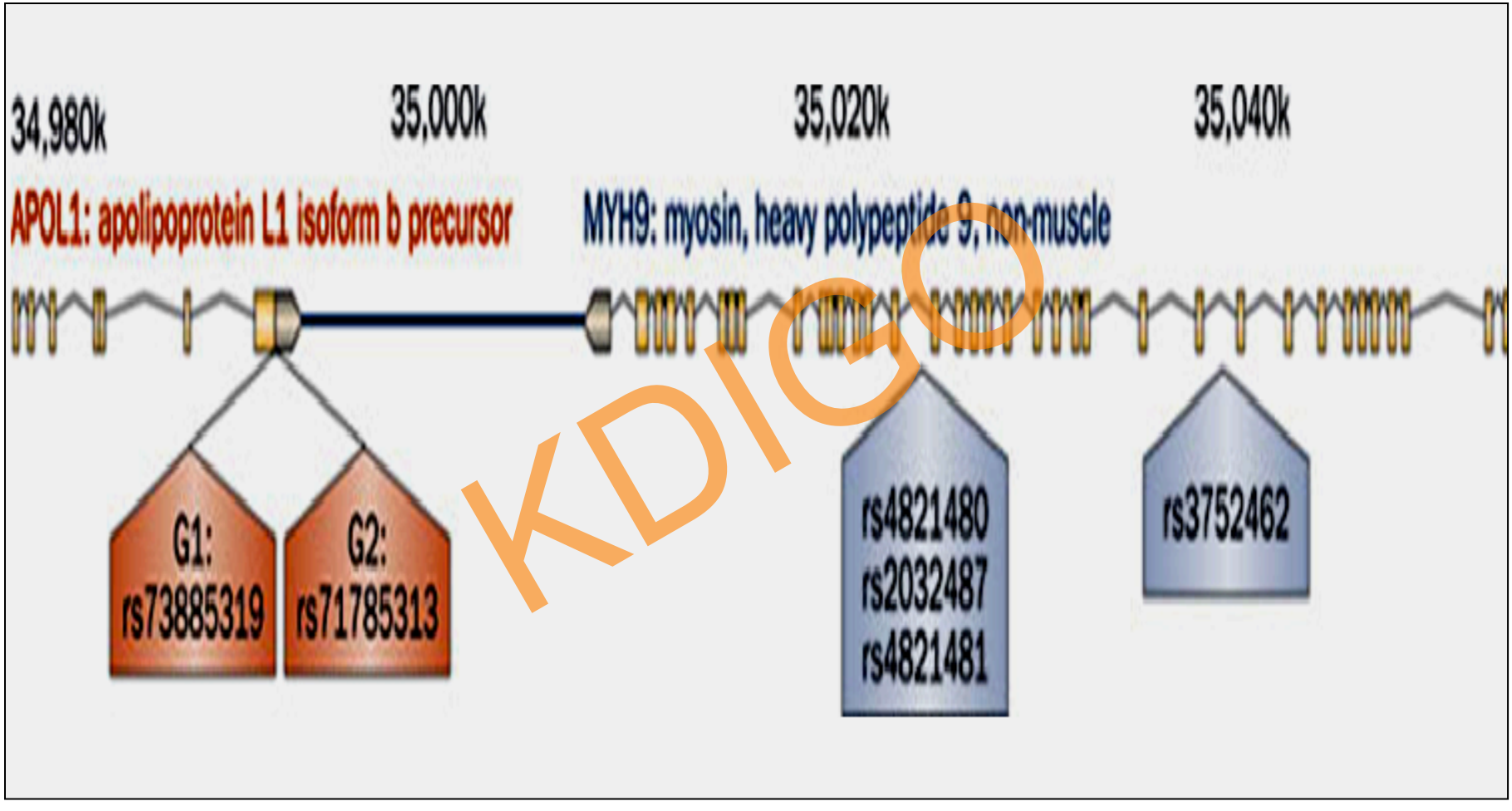
- *MYH9* encodes myosin heavy chain 9, a component of non-muscle myosin IIA, is localized to chromosome 22q12 .
- Myosin IIA is expressed in many tissues and cells, including podocytes
- The *MYH9* risk haplotype contributes to risk for idiopathic FSGS (OR 4) and HIV-associated CGN (OR 6)
- The risk variant accounts for all or nearly all of the increased risk for FSGS (80%) and HIV-associated CGN (100%) that characterize African Americans.
- However, detailed sequencing and genotyping did not identify specific functional mutations in *MYH9*

Kopp JB *et al.* (2008) *Nat Genet* 40: 1175–1184

APOL1 gene

- Further studies *(Tzur et al. 2010 and Genovese et al. 2011)* showed novel missense mutations with predicted functional African ancestry effects in the *APOL1* gene
- *APOL1* is found adjacent to *MYH9* (~ 20.0kb 3' downstream of *MYH9*)
- Codes for Apolipoprotein L1 protein
  - Soluble factor human serum with HDL particles
  - Role in trypanosomal lysis
  - Autophagic cell death
  - Lipid metabolism

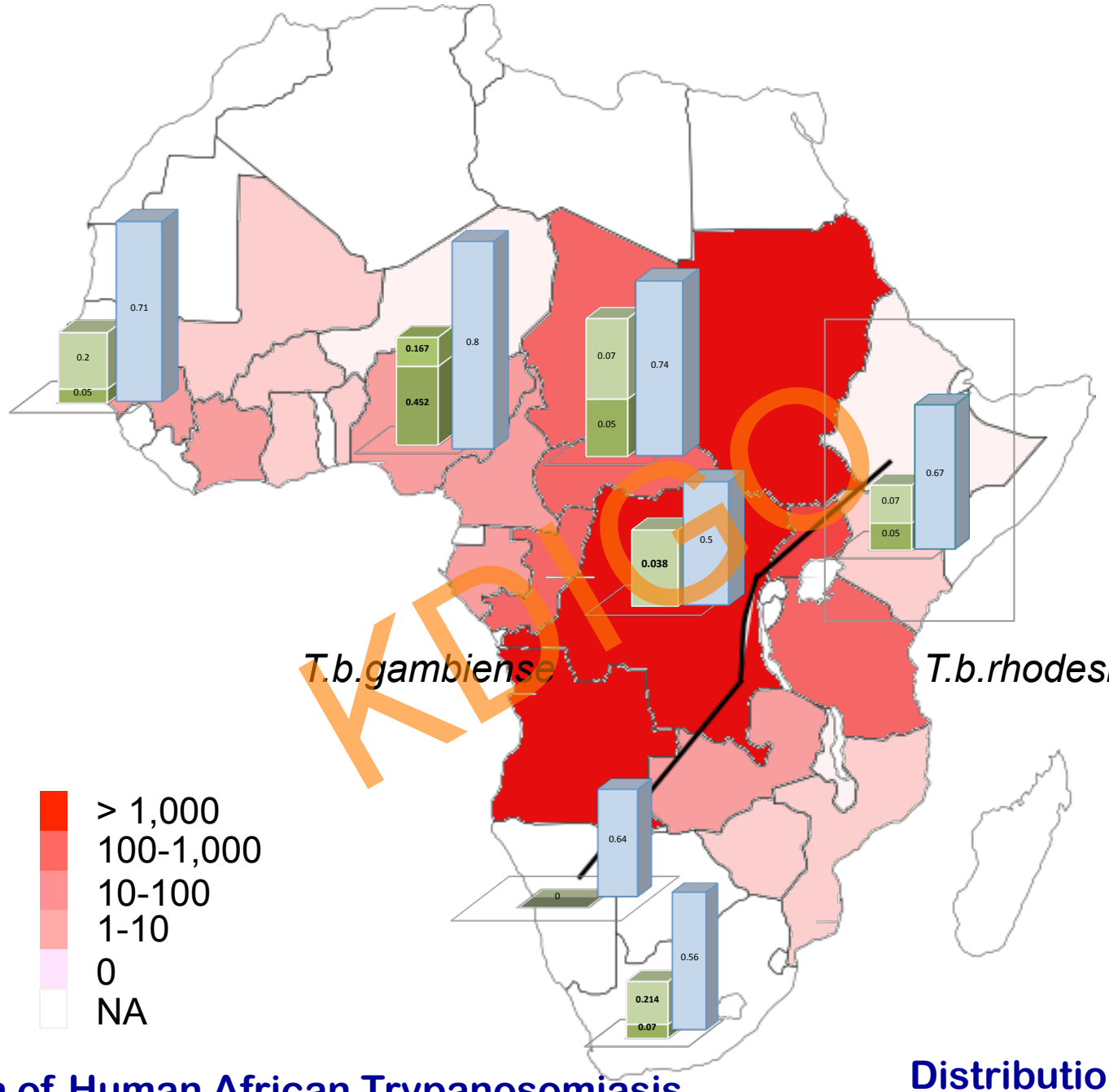
APOL1 and *MYH9* gene regions on Chromosome 22



Drawz, P. E. & Sedor, J. R. (2011)

APOL1 gene variants associated with CKD

- G1 risk haplotype: S342G (**rs73885319**), and I384M (**rs60910145**)
- G2 risk haplotype: N388_Y389del (**rs71785313**)-\TTATAA
- These variants are protective against *Trypanosoma brucei rhodesiense* and *T. b. gambiense*
- Mainly attributed to **natural selection as a result of Trypanosomiasis** (sleeping sickness) endemic in Africa hence provided an evolutionary advantage



Distribution of Human African Trypanosomiasis
(*T.b gambiense* and *T.b rhodesiense)*

Distribution of MYH9 and APOL1 risk haplotypes

Kasembeli et al. World J Nephrol 2015 May 6; 4(2): 295-306

APOL1 and CKD

- APOL1 gene variants G1 and G2 are in strong linkage disequilibrium with MYH9
- risk of FSGS (HIV and non-HIV associated) and hypertensive-end stage kidney disease in people of African descent
- may be more important in the genesis of these renal diseases
- APOL1 is involved in autophagy (a major protective factor against podocyte aging and glomerular injury)

Kronenberg et al, 2011; Genovese et al, 2010

APOL1 risk alleles and risk for kidney disease among African Americans

	Results			
	N	OR or RR	% with 2 RA	References
Case-control studies				
HIV-associated nephropathy	54	29 (13-68.5)	72	Kopp et al[2]
Primary FSGS	217	17 (11-26)	72	Kopp et al[2]
Lupus collapsing glomerulopathy	26	5.4 (0.4-12.1)	50	Larsen et al[22]
Lupus with end-stage kidney disease	855	2.7 (1.8-4.2)	25	Freedman et al[23]
Sickle cell disease nephropathy	520	3.4 relative risk for proteinuria	45	Ashley-Koch et al[24]
Hypertension-attributed nephropathy	675	2.6 (1.8-3.6); 4.6 (3.1-6.8) in progressors	23	Lipkowitz et al[25]
Case-only biopsy series from HIV-positive patients				
HIV-associated nephropathy	60	–	62	Atta et al[26]
HIV + FSGS	35	–	63	Fine et al[27]
HIV immune complex kidney disease	31	–	3	Fine et al[27]

Reviewed in Limou et al. Adv in Chr Kidney Dis. 21: 426-433, 2014

APOL1 in CKD

- **Hypertension**
  - OR 7 [Genovese et al. Science 2010]
  - 2.57 in AASK Study (incr to 6.29 in advanced CKD)[Lipkowitz et al. Kidney Int 2012]
- **FSGS** : OR 17 [Kopp et al. JASN 2011]
- **HIVAN**:
  - OR 29 [Kopp et al. JASN 2011]
  - OR 89 in our SA Study [Kasembeli et al. JASN 2015]
- **Children with perinatal HIV infection:**
  - 3X incr odds of CKD with high risk genotype
  - median age of 8.8 vs 14.3 years in those with 0 or 1 risk allele

[Purswani et al. JAIDS 2016]

APOL1 in HIVAN, CKD and Controls in South Africa

78.9% of the HIVAN subjects were homozygous (G1/G1 or G2/G2) or compound heterozygotes (G1/G2), compared to 3.7% of the HIV (+) controls *($P=1.2x10^{-14}$)* and 1.9% of the population controls *($P=8{\cdot}9x10^{-16}$)*

***APOL1* genotype distribution among HIVAN, HIV (+) CKD, and HIV (-) CKD cases and Controls**

GENOTYPE	HIV (+) Cases and Controls			HIV (-) Cases and Controls	
	HIVAN N (%)	CKD N (%)	Controls N (%)	CKD N (%)	Controls N (%)
0 risk allele	**2 (5.3)**	22 (56.4)	**34 (63.0)**	26(66.7)	**36 (66.7)**
1 risk allele	6 (15.8)	12 (30.8)	18 (33.3)	12(30.8)	17 (31.5)
G0/G1	5 (13.2)	4 (10.3)	4 (7.4)	6 (15.4)	7 (13.0)
G0/G2	1 (2.6)	8 (20.5)	14 (25.9)	6 (15.4)	10 (18.5)
2 risk alleles	**30 (78.9)**	5 (12.8)	**2 (3.7)**	1 (2.6)	**1 (1.9)**
G1/G1	8 (21.0)	1 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)
G1/G2	19 (50.0)	2 (5.1)	0 (0.0)	1 (2.6)	0 (0.0)
G2/G2	3 (7.9)	2 (5.1)	2 (3.7)	0 (0.0)	1 (1.9)
Total	**38**	**39**	**54**	**39**	**54**

*The single HIV (+) CKD subject carrying the $G1^{+M}$ (A-G-I) haplotype is excluded from the table.

Association between *APOL1* risk alleles and various glomerular diseases

Glomerular Disease	HIV-Positive Patients and Controls					HIV-Negative Patients and Controls				
	No. of *APOL1* Risk Alleles			OR (95% CI)	*P* Value	No. of *APOL1* Risk Alleles			OR (95% CI)	*P* Value
	0	1	2	2 versus 1 or 0 risk alleles		0	1	2	2 versus 1 or 0 risk alleles	
Controls (*n*=108)	34 (63)	18 (33)	2 (4)	—	—	36 (67)	17 (32)	1 (2)	—	—
HIVAN (*n*=38)	2 (5)	6 (16)	30 (79)	89 (17.7 to 912)	1.2×10^{-14}	—	—	—	—	—
Other CKD (*n*=78)	22 (57)	11 (28)	6 (15)	3.8 (0.6 to 42)	0.13	25 (64)	13 (33)	1(3)	1.4 (0.02 to 11)	>0.99
FSGS (*n*=22)	9 (69)	3 (23)	1 (8)	2.1 (0.03 to 44)	0.48	5 (56)	3 (33)	1 (11)	6 0.3 (0.08 to 527)	0.26
HIVICK (*n*=12)	4 (33)	5 (42)	3 (25)	5.6 (0.4 to 86)	0.13	—	—	—	—	—
Other GN (*n*=27)[a]	7 (70)	3 (30)	0 (0)	0.0 (0 to 30)	>0.99	10 (59)	7 (41)	0 (0)	0 (0 to 124)	>0.99
Other kidney diseases (*n*=17)[b]	2 (50)	0 (0)	2 (50)	21 (0.2 to 2029)	0.11	10 (77)	3 (23)	0 (0)	0 (0 to 210)	>0.99

Kasembeli et al. J Am Soc Nephrol. 26: 2882-2890, 2015

APOL1 predicts histopathology in HIV-related kidney disease?

APOL1 risk alleles

2: FSGS(76%); HPT neph(10%); DN(10%)

1: ICGN(47%); FSGS(23%); DN(9%); other(23%)

0: ICGN(40%); FSGS(12%); DN(28%); HPT neph(8%)

Fine et al. J Am Soc Nephrol 23: 343-350, 2012

Absence of HIVAN in Ethiopians

Ethiopians

- High frequency of E and S cluster *MYH9* risk variants
- Zero allele frequency for G1 and G2 *APOL1*
- No HIVAN

Behar et al. Am J Kid Dis 47: 88-94, 2006;
Rosset et al, Nat Rev Nephrol 7: 313-326, 2011

APOL1 Risk Variants and Other Characteristics Associated with Persistent Proteinuria in Unadjusted and Adjusted Logistic Regression

Variables	Unadjusted (n=1285)			Adjusted†					
				Without AIDS History* (n=902)			With AIDS History* (n=304)		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
2 vs. 0/1 *APOL1* risk allele	4.18	2.26, 7.74	<0.001	5.92	2.57, 13.67	<0.001	1.11	0.21, 6.02	0.90
Age, per 10 year older	1.59	1.21, 2.09	0.001	1.11	0.70, 1.75	0.65	1.23	0.64, 2.35	0.54
History of injection drug use	0.86	0.26, 2.83	0.81	1.16	0.24, 5.65	0.85	‡	---	---
Hypertension	2.48	1.55, 3.95	<0.001	2.17	1.04, 4.51	0.04	1.93	0.79, 4.74	0.15
Hepatitis C virus co-infection	1.75	1.09, 2.80	0.02	1.18	0.56, 2.48	0.65	1.08	0.44, 2.64	0.86
History of clinical AIDS	1.77	1.10, 2.84	0.02	---	---	---	---	---	---
HAART since last visit	0.82	0.48, 1.41	0.48	1.26	0.54, 2.94	0.58	1.20	0.37, 3.84	0.76
CD4+ cell count, per ln100 cells/mm^3 higher	0.70	0.57, 0.85	<0.001	0.69	0.47, 1.00	0.05	0.87	0.61, 1.23	0.43
HIV-1 RNA level, per ln1000 copies/ mL higher	1.14	1.05, 1.24	0.003	1.06	0.91, 1.23	0.47	1.23	1.01, 1.51	0.04
eGFR, per 10 mL/min/ 1.73 m^2 lower	1.20	1.10, 1.32	<0.001	1.16	1.02, 1.32	0.03	1.14	0.94, 1.38	0.17

*P-interaction=0.06

†Adjusted for PCs 1–3 and all listed covariates

‡History of injection drug use among women with a history of clinical AIDS perfectly correlated with the presence of persistent proteinuria

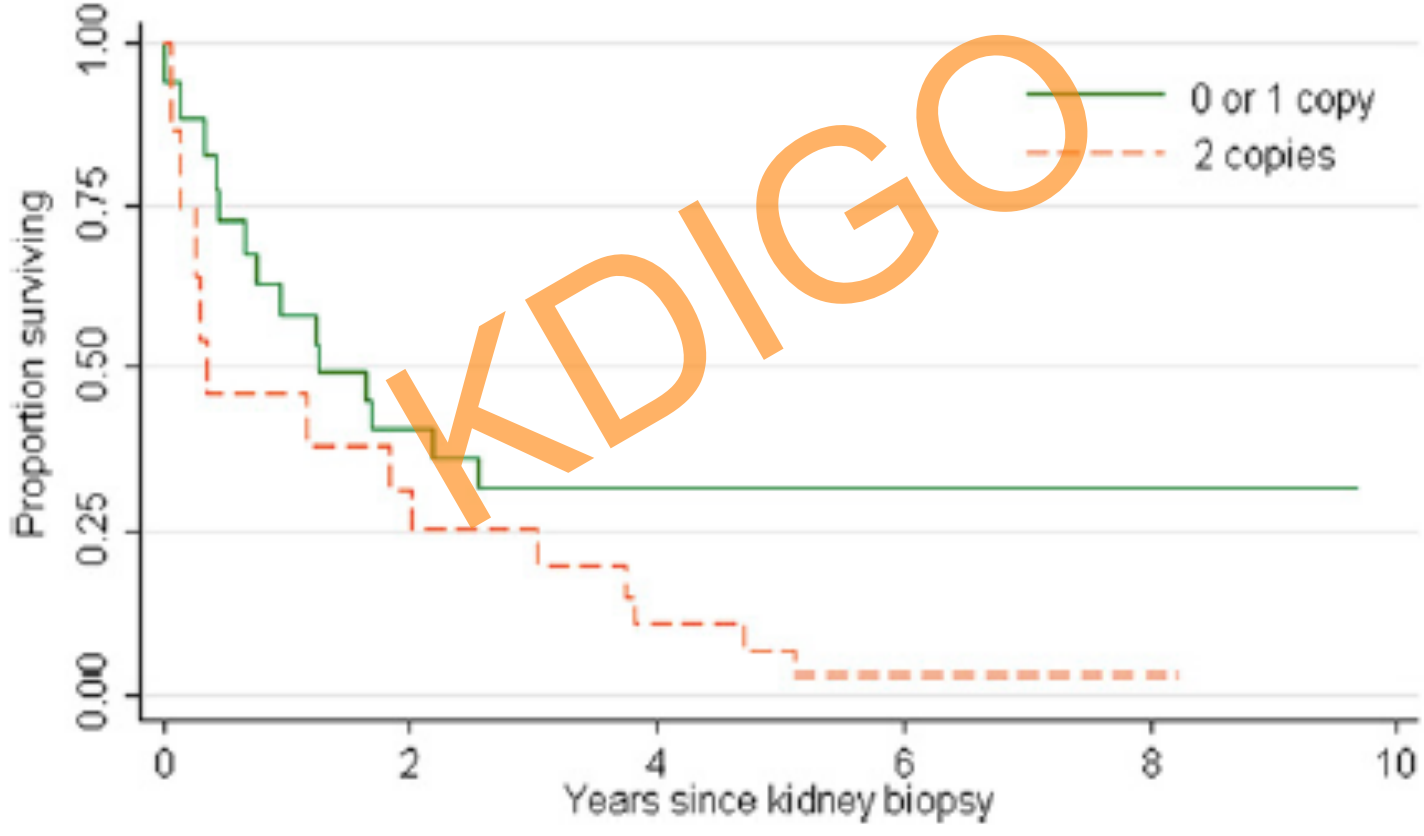
Abbreviations: PC, principal components; HAART, highly active antiretroviral therapy; eGFR, estimated glomerular filtration rate

Estrella et al. Kidney Int 84: 834-840, 2013

ESRD risk associated with *APOL1*

- 2 *APOL1* risk alleles
  - More rapid progression to ESRD
  - 2x greater risk of ESRD
- Greater risk of ESRD with
  - Lower baseline kidney function
  - Proteinuria
    - Fine et al. J Am Soc Nephrol 23: 343-350, 2012

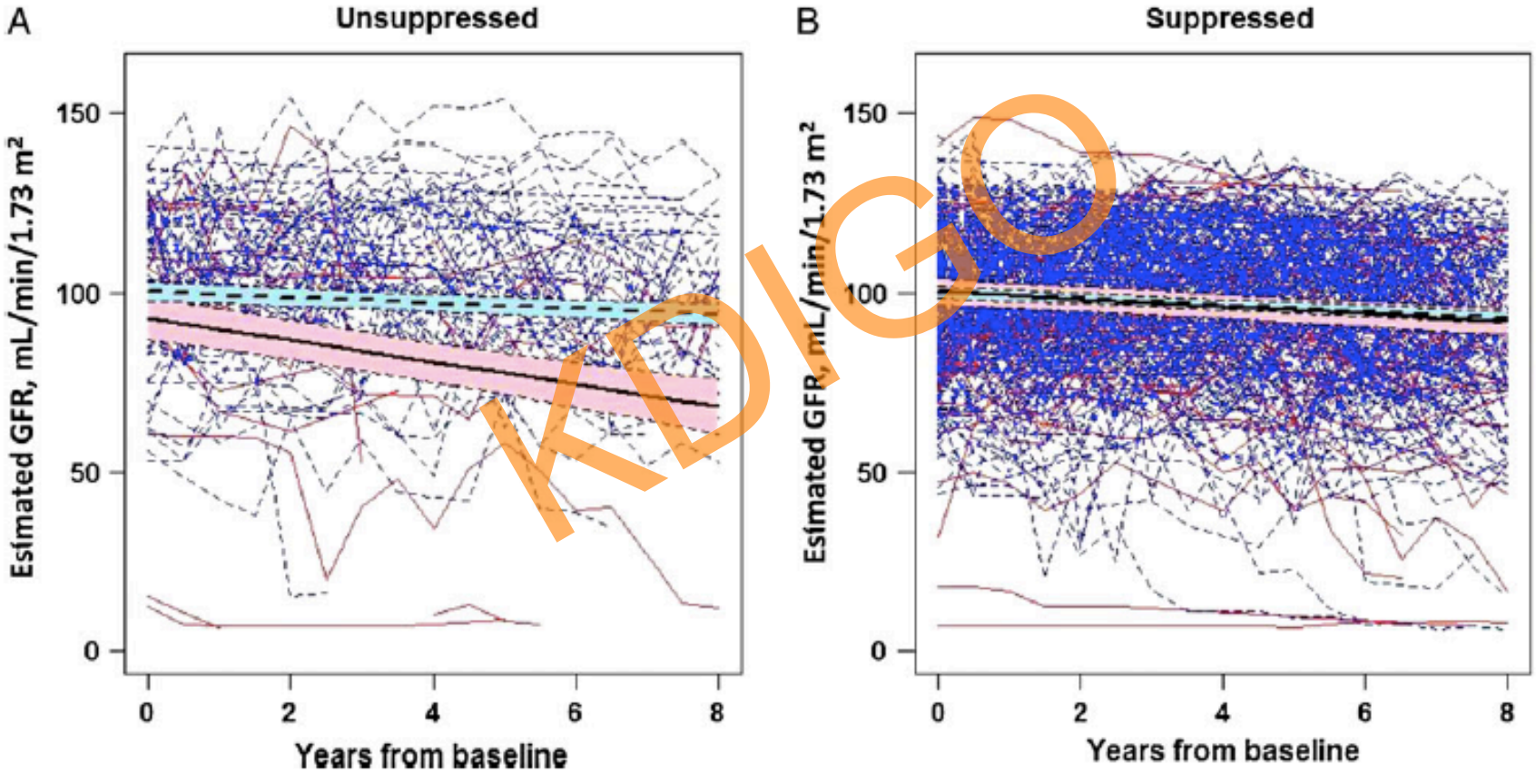
Age-adjusted renal survival by number of *APOL1* risk alleles



Response to ART

- **Response to ART**
  - Rapid progression of HIVAN to ESRD if no/under-Rx [Kalayjian, 2010; Fine, 2012]
  - 3x incr risk of ESRD in non-HIVAN in spite of effective ART/RAAS [Fine, 2012]
- **APOL1 status, viral suppression and kidney function**
  - 2.5x decline in eGFR with high risk APOL1 genotype if poor viral suppression in the Multicenter AIDS Cohort Study [Estrella, CID 2015]

Decline in renal function related to viral suppression and APOL1 risk genotype



Solid line signifies the APOL1 high-risk group while the dashed line signifies the low-risk group.

Role of *MYH9* and *APOL1* in FSGS?

- *MYH9* SNPs associated with CKD in European Americans lacking *APOL1* risk alleles
  - O'Seaghdha et al. Hum Mol Genet. 20: 2450-2456, 2011
- *MYH9* ablation in podocytes predisposed mice to adriamycin nephropathy (drug-induced model of FSGS)
  - Johnstone et al. Mol Cell Biol. 31: 2162-2170, 2011
- *MYH9* downregulated in glomeruli of patients with HIVAN
  - Hays et al. AIDS. 26: 797-803, 2012
- Both genes may independently contribute to kidney disease susceptibility, with *APOL1* more susceptible to FSGS and HIVAN

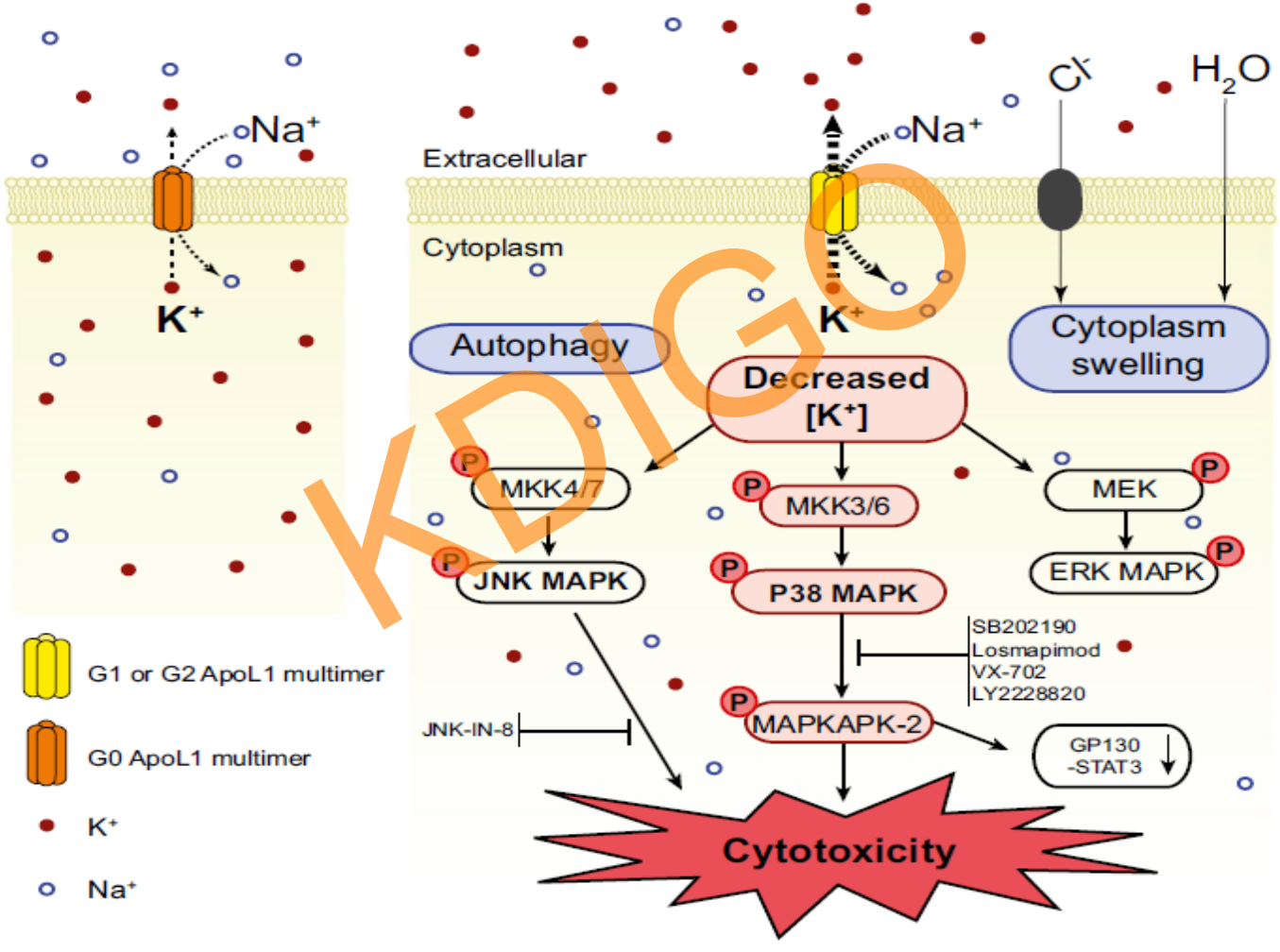
Mechanisms for kidney disease risk with APOL1

- Two risk alleles required for disease phenotype
- However, biopsy-proven HIVAN
  - >1/3 had zero or one risk allele
  - APOL1 risk genotype predicted combined non-HIVAN FSGS and HIVAN (11X higher odds); HIVAN alone (3X higher odds)
  - 19x risk of progression to ESRD in those with DM
    - Atta et al, Kidney Int 2012
- 'Second hits'
  - Infections: HIV; viral hepatitis co-infection; other
    - produce IFN
    - Therapeutic IFN reported to cause collapsing FSGS
  - Gene-gene interactions
  - Illicit drug use
  - Other CKD risk factors

Mechanisms for kidney disease risk with APOL1

- *APOL1* high risk variants may cause glomerulosclerosis
- Mutated particles bind less tightly to circulating HDL3, undergo glomerular filtration and proximal tubular reabsorption and thereby cause kidney disease
- Endogenous ApoL1 in renal epithelium may cause apoptosis or autophagic cell death

APOL1-mediated cytotoxicity



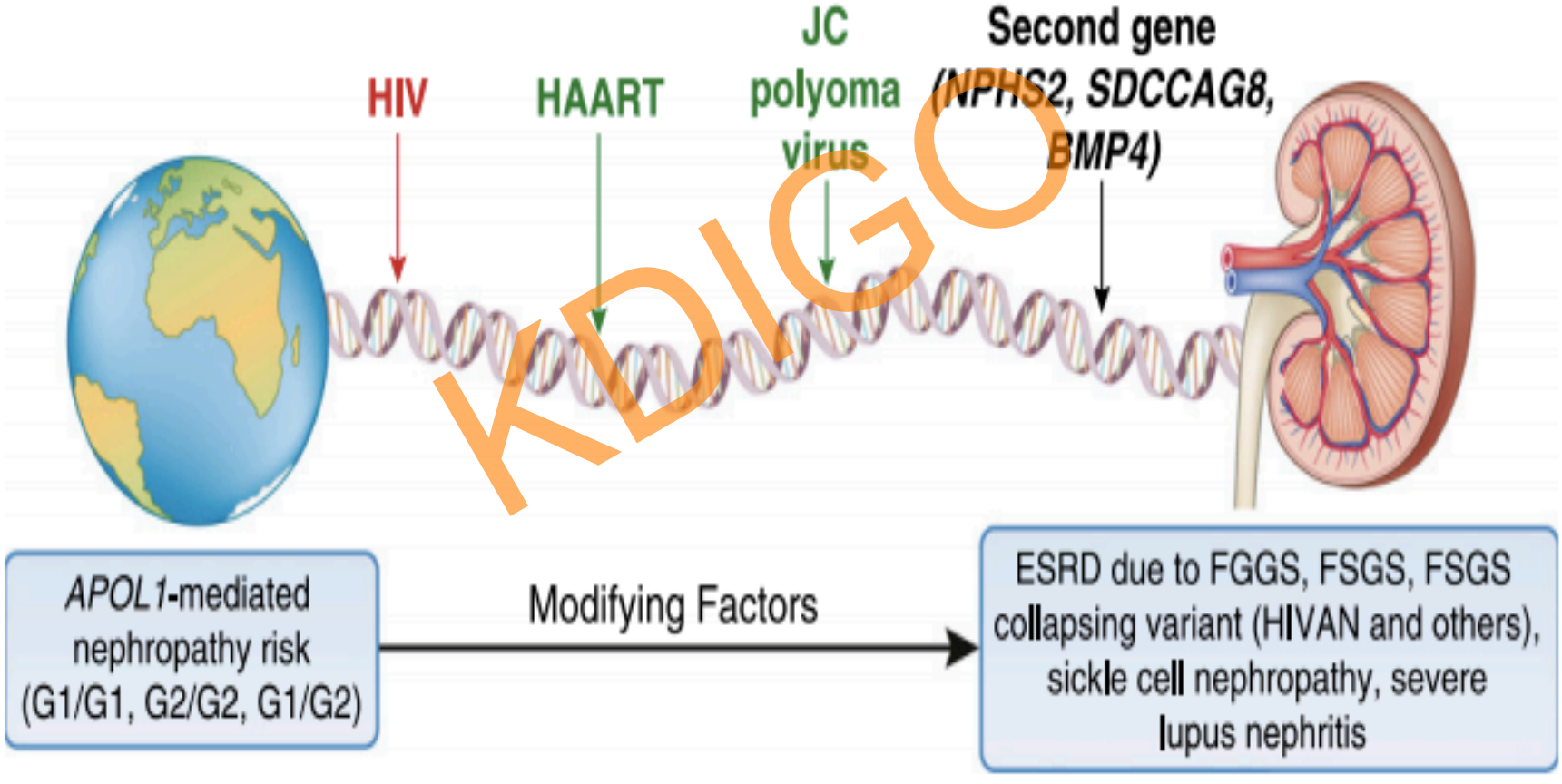
Olabisi et al. PNAS. 2016

APOL1 mediated cytotoxicity- Summary

- G1 and G2 APOL1 induce increased loss of intracellular potassium early in APOL1-induced cytotoxicity
- This loss of intracellular potassium activates SAPK pathways
- These act as mediators of cell death

These signaling activities are dependent on the dose of G1 and G2 expression

Pathways leading from genetic susceptibility to clinical kidney disease



Freedman and Skorecki. Clin J Am Soc Nephrol. 9: 2006-2013, 2014

Conclusion

- Genetic variation in APOL1 developed as an evolutionary adaptation against endemic human African trypanosomiasis resulted in resistance to the infection
- However, it has conferred increased susceptibility to chronic kidney disease in conjunction with ‘second hits’, such as HIV infection, other infections, gene-gene and gene-environmental interactions