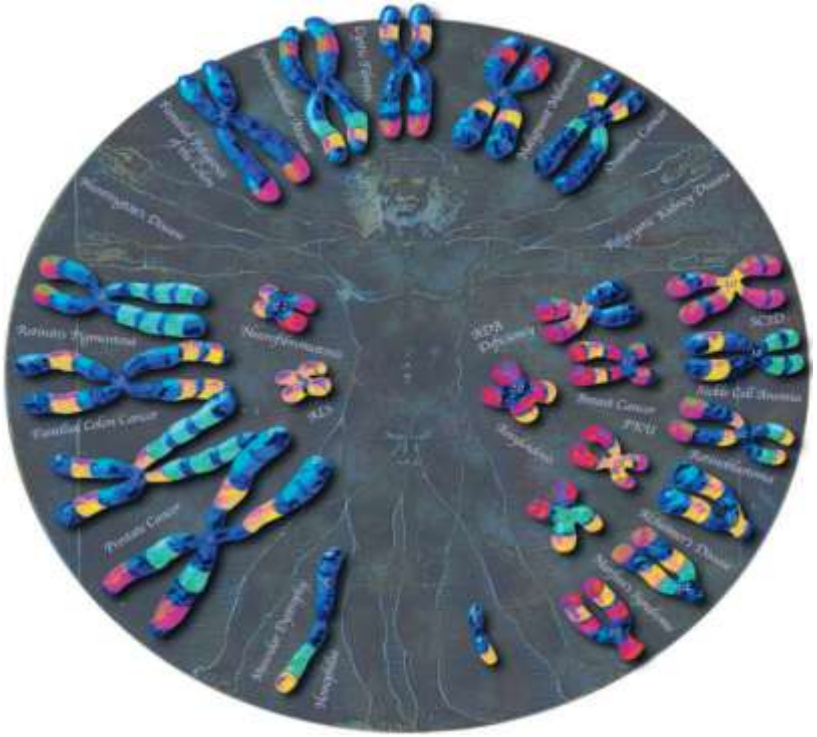
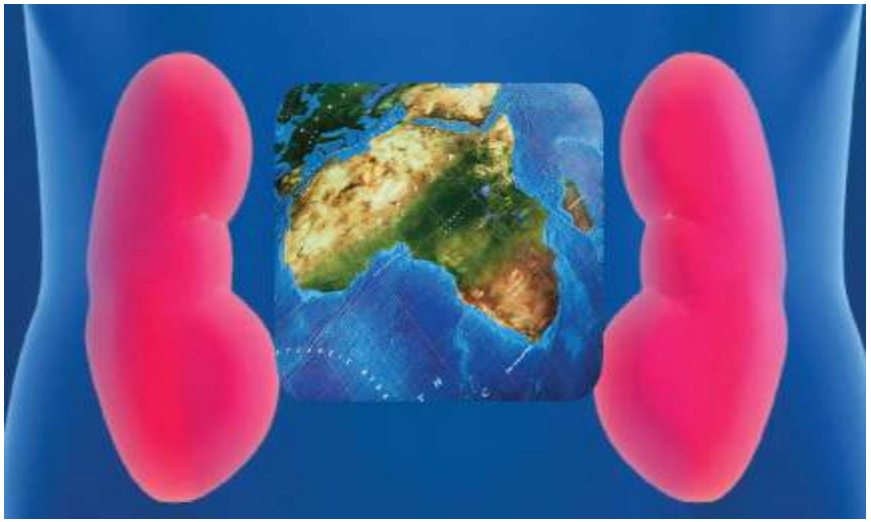
PART II

Mapping Genes in Kidney Health and Disease





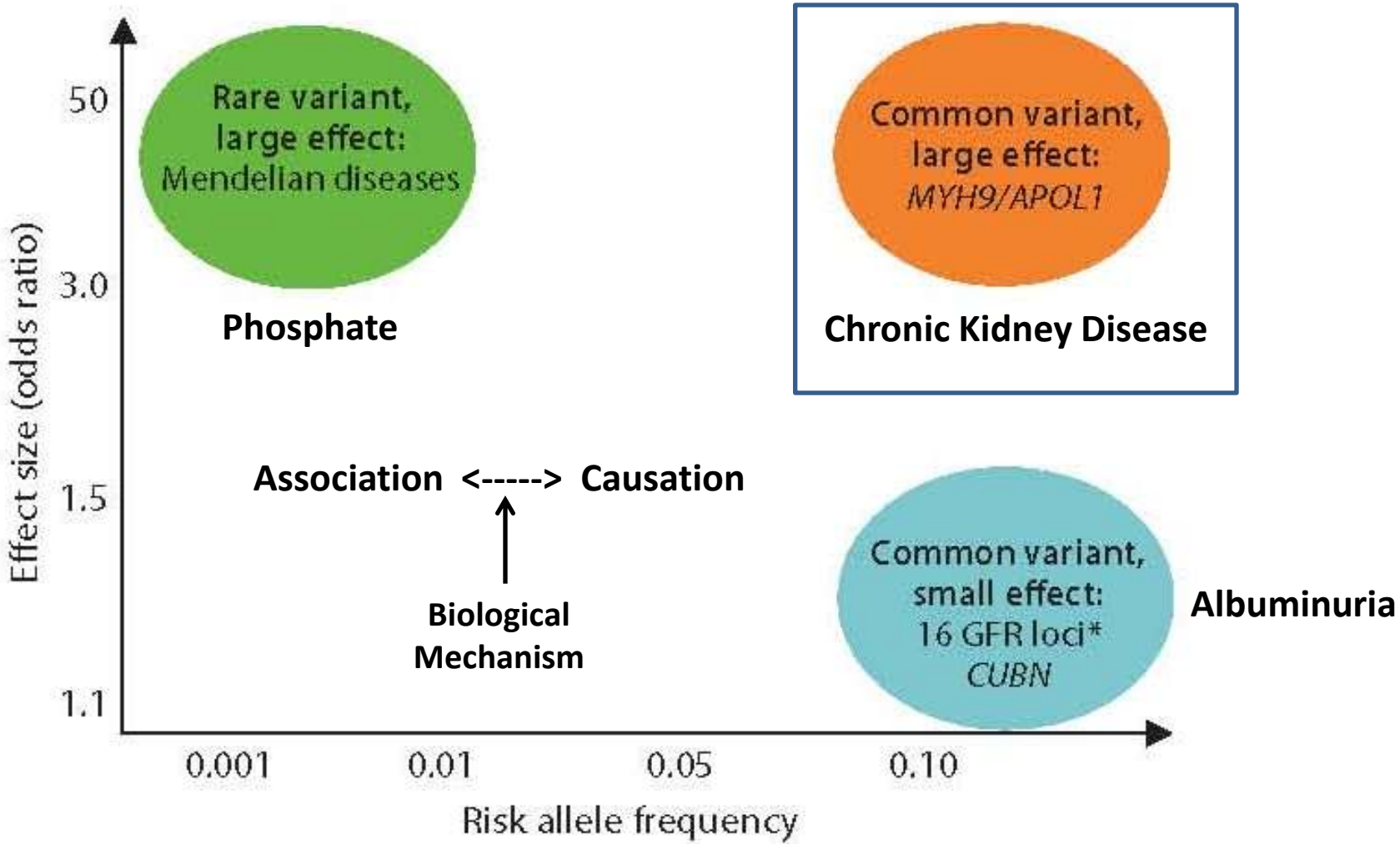
CLASSIFICATION OF INHERITED DISEASES

Monogenic – Mendelian Inheritance patterns

Complex disease and phenotypes

Polygenic – non-Mendelian Inheritance patterns

Relation of effect size and risk allele frequency of DNA sequence variants associated with kidney function and disease risk phenotypes



Adapted from Kidney and Blood Pressure Research 2011:34:225-234

APOL1 Nephropathy – A Case Study in African Population Genetics

A story of population based common disease risk gene discovery

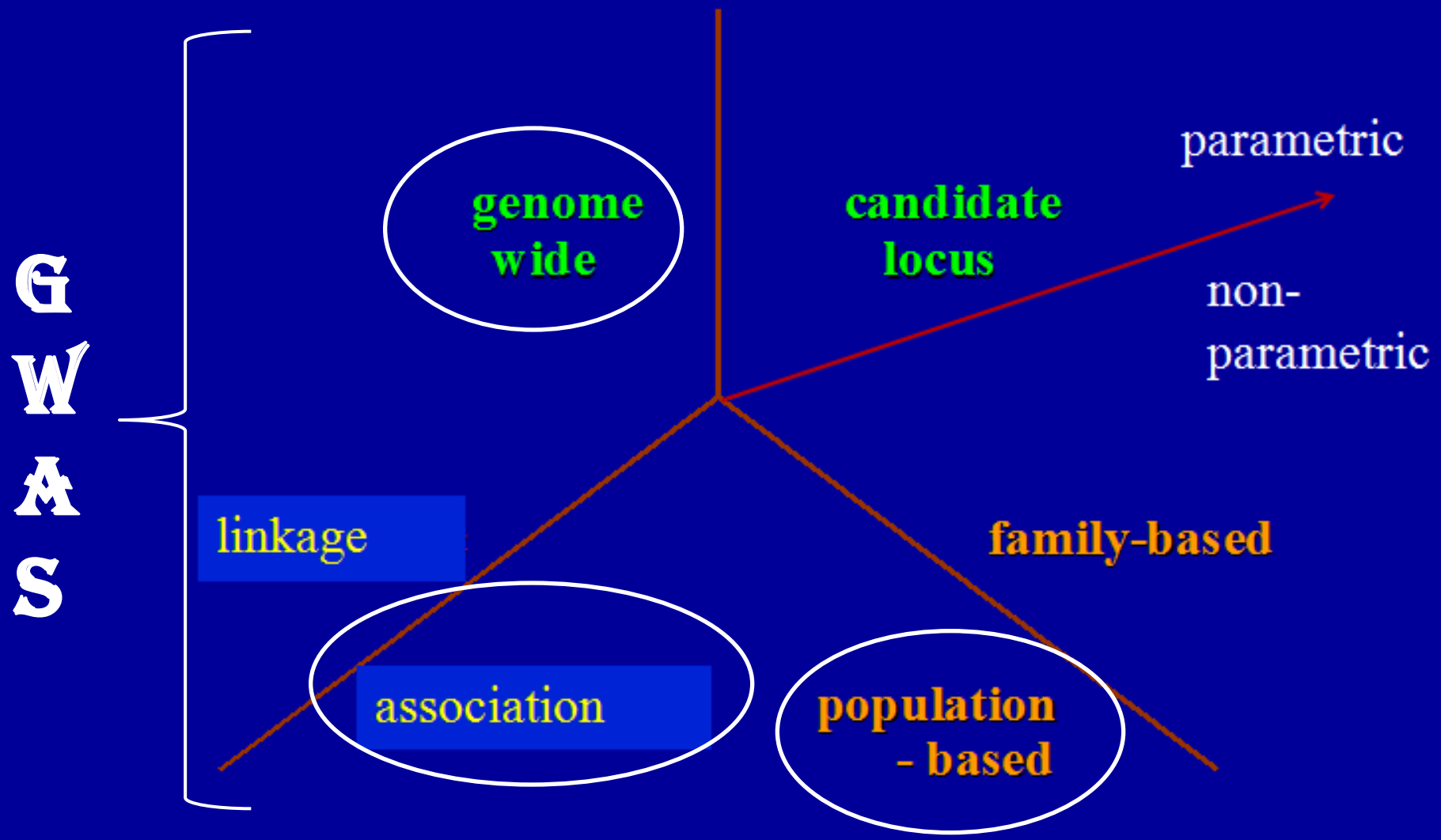
Recovering "Missing Heritablity"

Association is not Causation

Nothing in biology (or medicine) makes sense except in the light of evolution

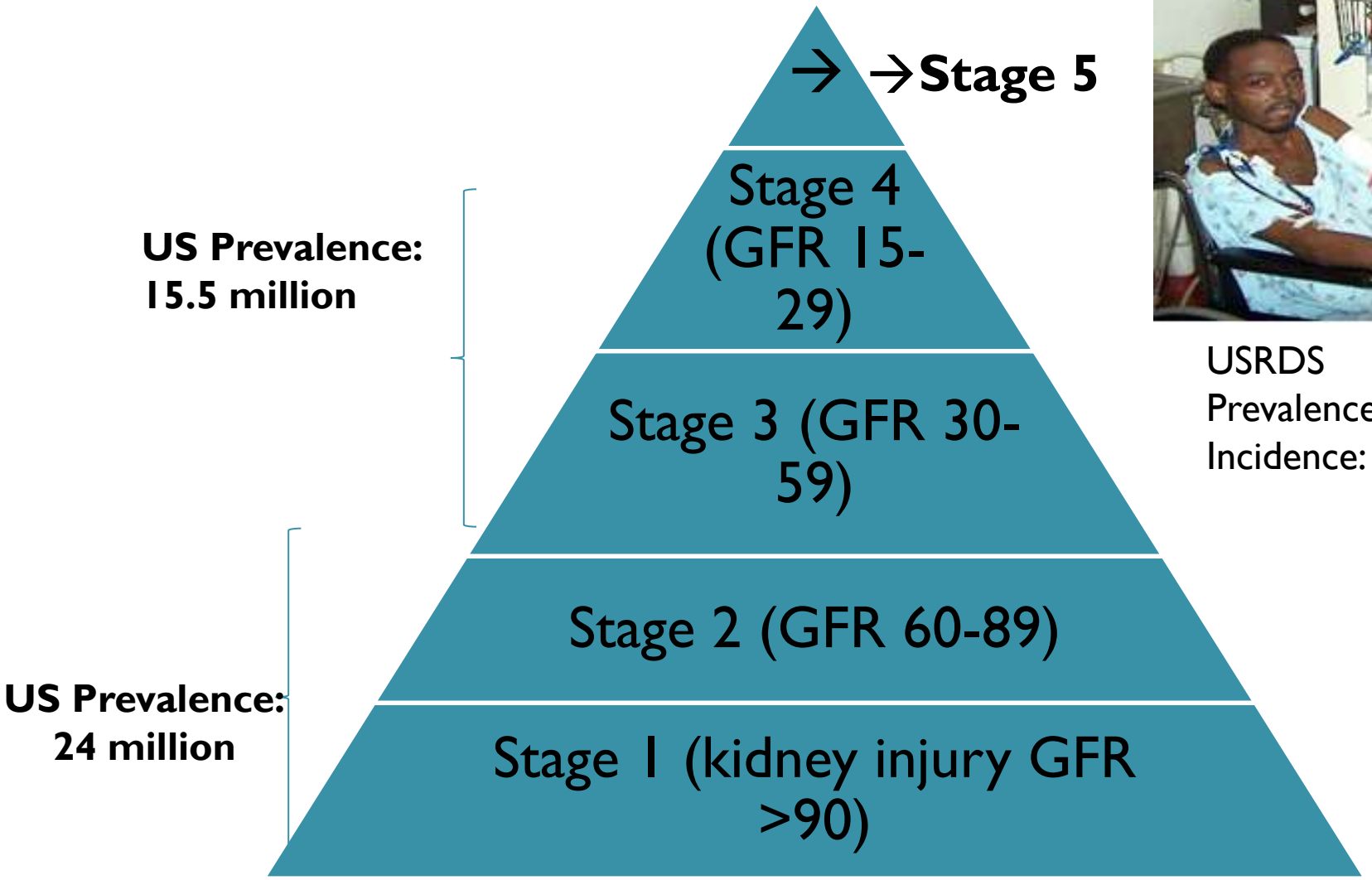
Theodosius Dobzhansky

Disease Gene Mapping



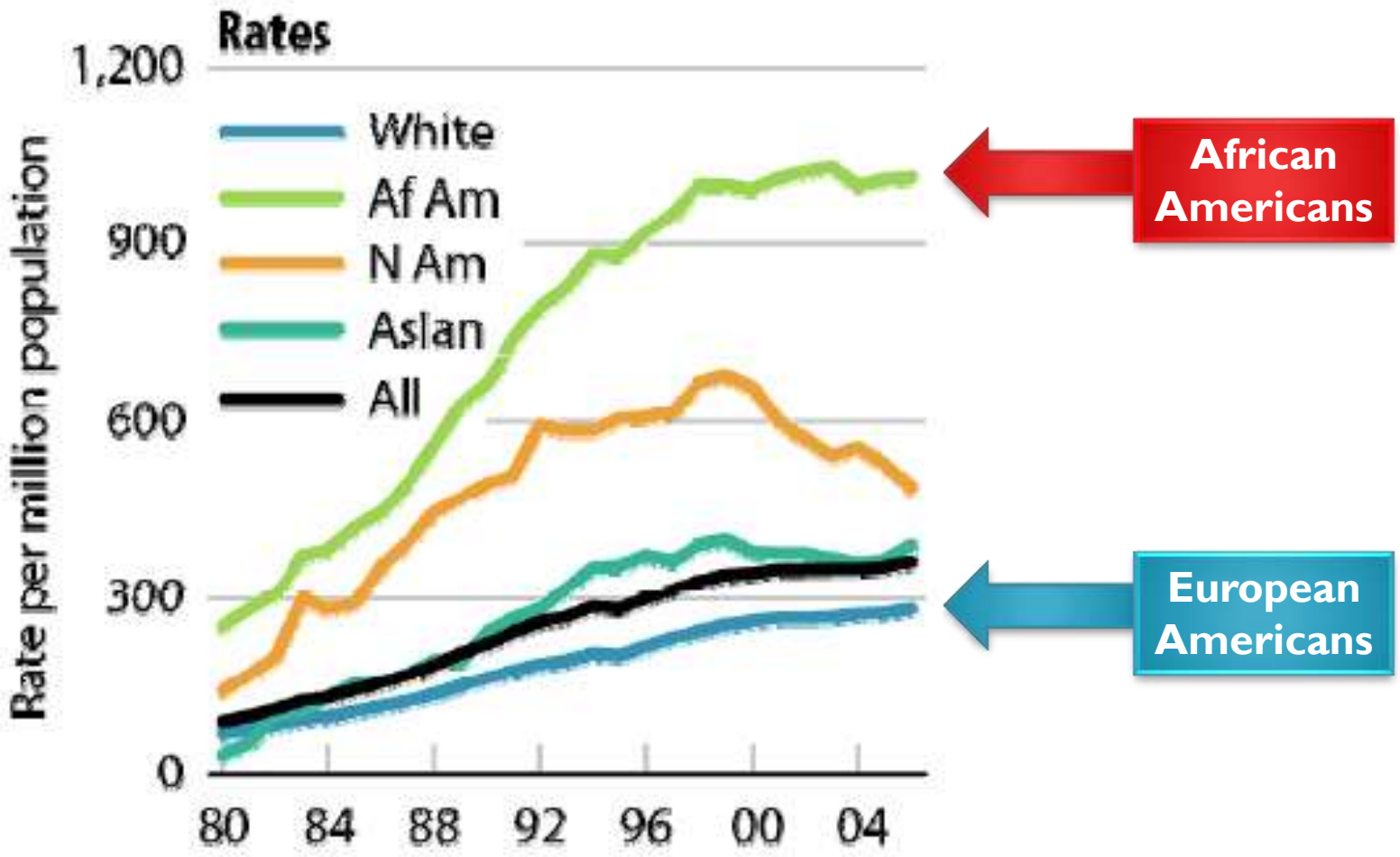
End Stage Kidney Disease

USRDS
Prevalence 584,000
Incidence: 110,000

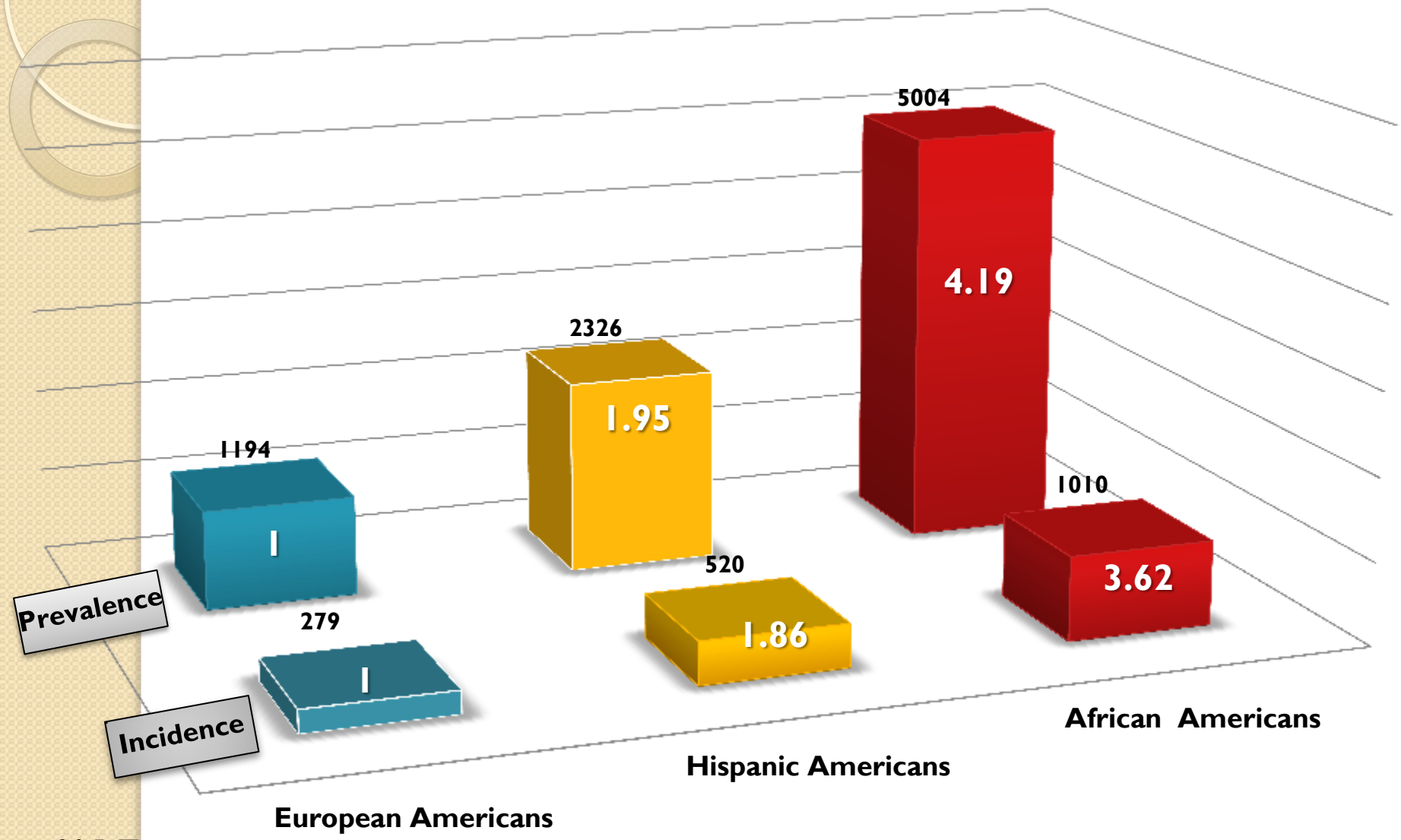


Chronic Kidney Disease

ESKD incidence rate of different US populations 1980-2006

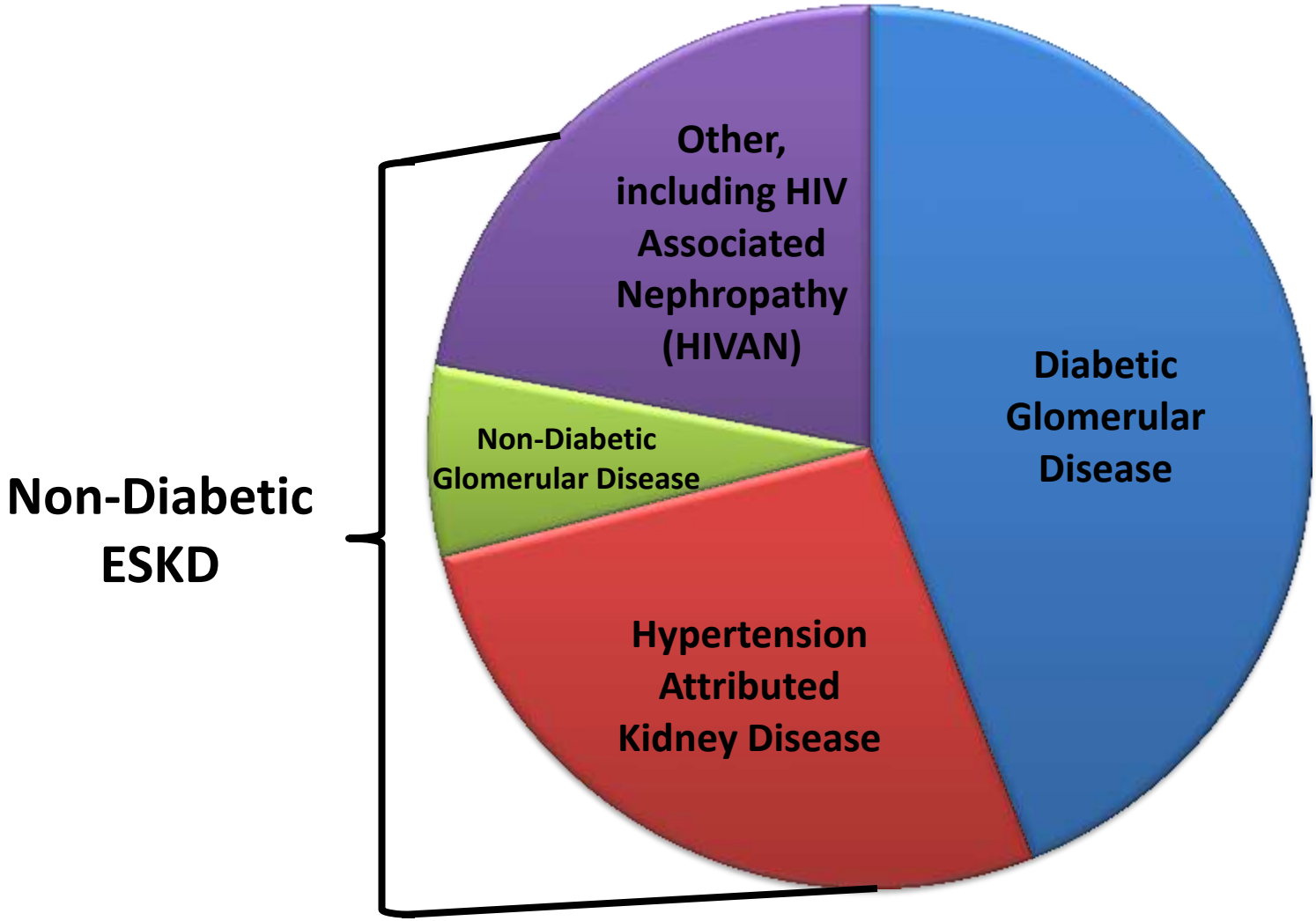


ESKD Incidence and Prevalence Rates (USRDS, 2006)



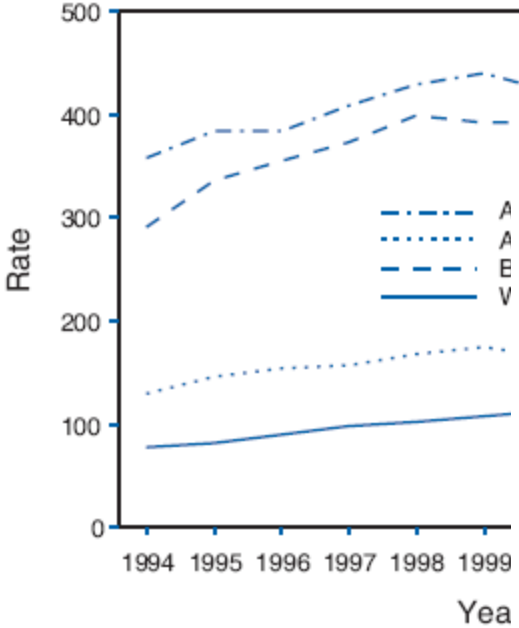
	European Americans	Hispanic Americans	African Americans
%AF	<1	31.2 (24.3)	83.4 (16.3)

The Major Etiologic Categories of ESKD



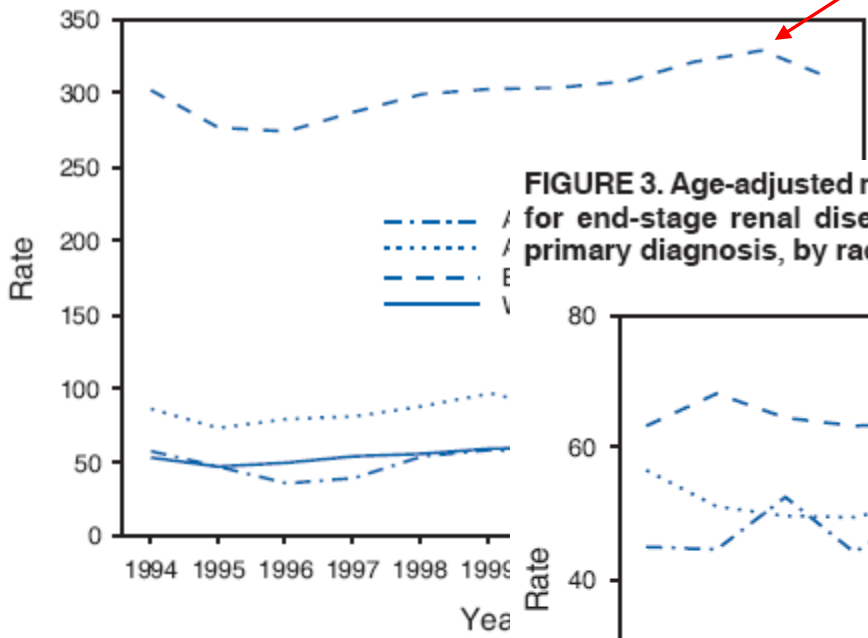
Population Disparity for all Etiologic Categories of CKD and ESKD

FIGURE 1. Age-adjusted rates* of persons who initiated therapy for end-stage renal disease with diabetes as the primary diagnosis, by race† — United States, 1994–2004



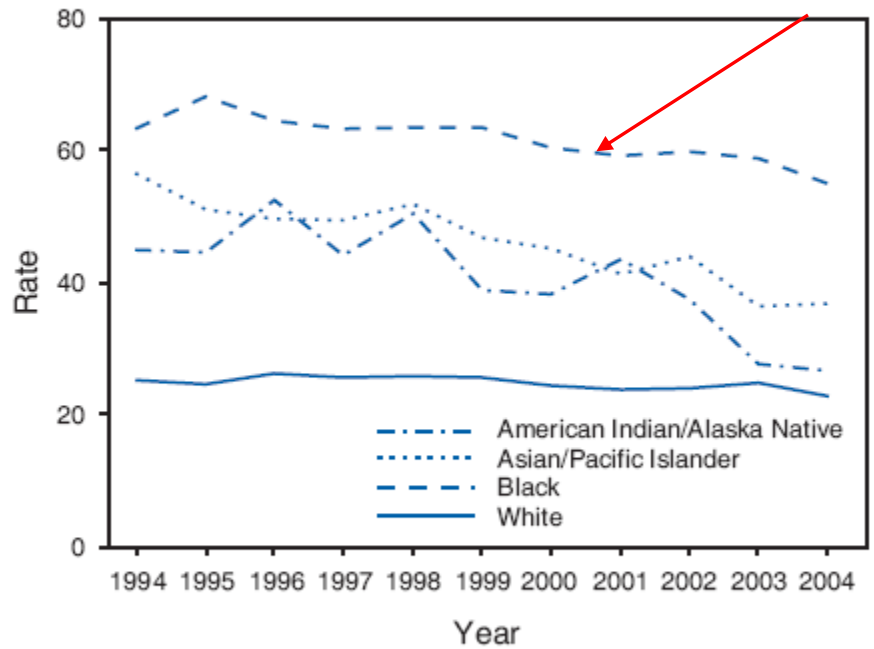
*Per 1 million population. Age adjusted to the
†Race-specific estimates include person
Hispanic origin.

FIGURE 2. Age-adjusted rates* of persons who initiated therapy for end-stage renal disease with hypertension as the primary diagnosis, by race† — United States, 1994–2004



*Per 1 million population. Age adjusted to the
†Race-specific estimates include person
Hispanic origin.

FIGURE 3. Age-adjusted rates* of persons who initiated therapy for end-stage renal disease with glomerulonephritis as the primary diagnosis, by race† — United States, 1994–2004



*Per 1 million population. Age adjusted to the 2000 U.S. standard population.
†Race-specific estimates include persons of both Hispanic and non-

High Rates of Kidney Disease among HIV Positive African Americans

The most striking discrepancy is a > 10 fold greater risk for Kidney Disease (HIVAN) in HIV infected African Americans compared to HIV infected European Americans

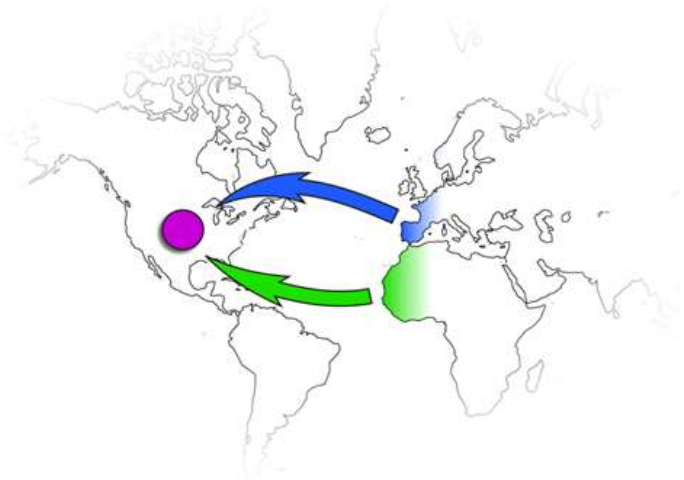
- **Population disparities not readily attributable to socio-economic or environmental factors**
- **Familial clustering of CKD and ESKD of varying etiologies in African Americans (Freedman 1999)**

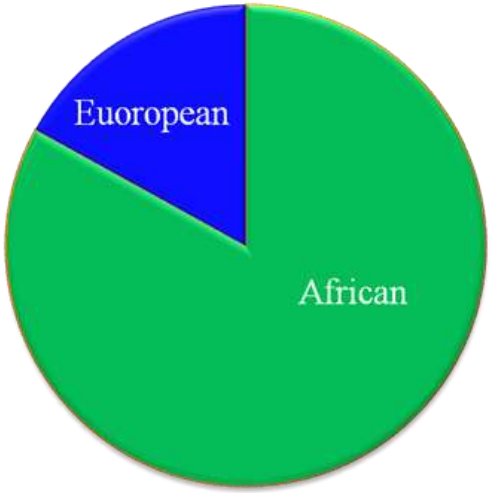
OVERARCHING GENETIC RISK VARIANT

(Freedman 1999)

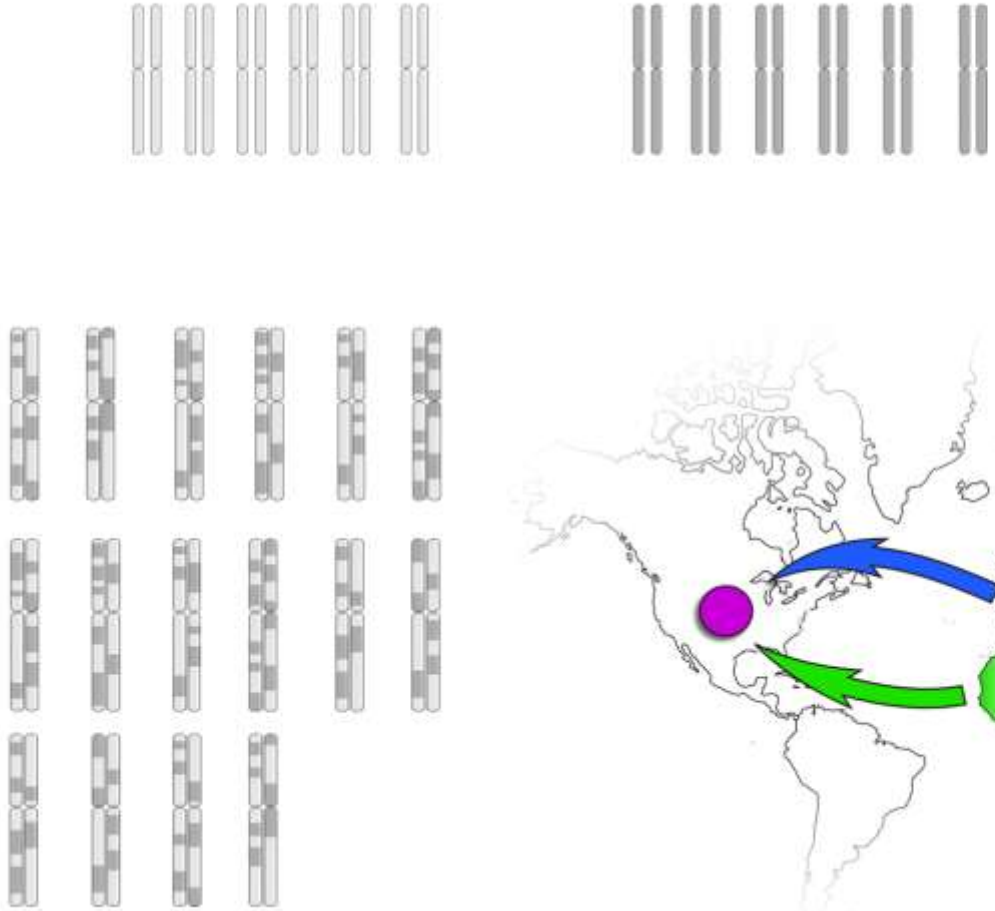
THE TRAGEDY

12 million Africans principally from three regions of western and southern Africa were forcibly translocated to the Americas in ~ 400 years of tragic slave trade (~ 1.5 M did not survive the voyage)





Subsequent admixture with Europeans to current genome wide African American population average of ~83% African

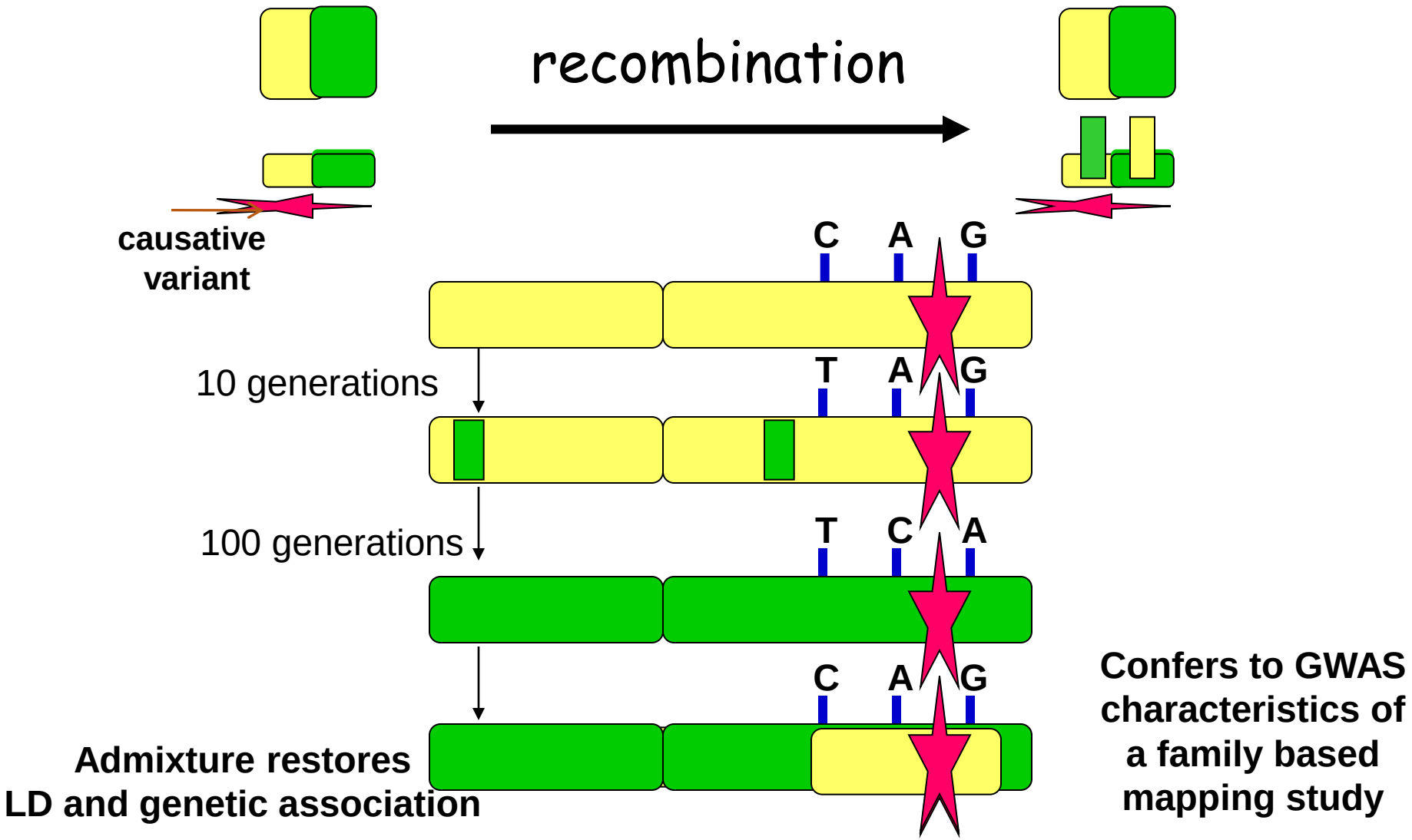


Admixture Generates Blocks of Linkage Disequilibrium (LD) greatly facilitating population-based gene mapping

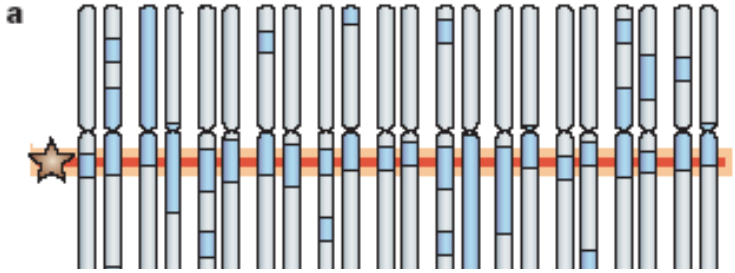
Identify "ancestry" of chromosomal regions using DNA markers whose allele frequencies differ markedly between parent populations

(Ancesty Informative Markers - AIMS)

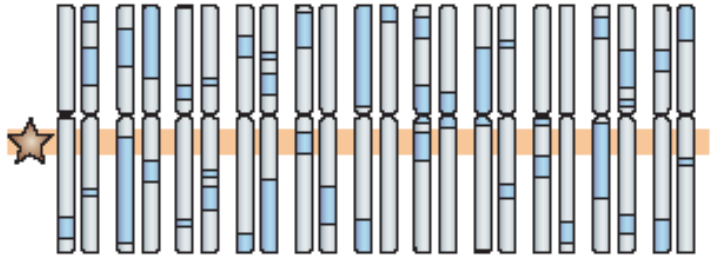
Recombination Breaks Down LD



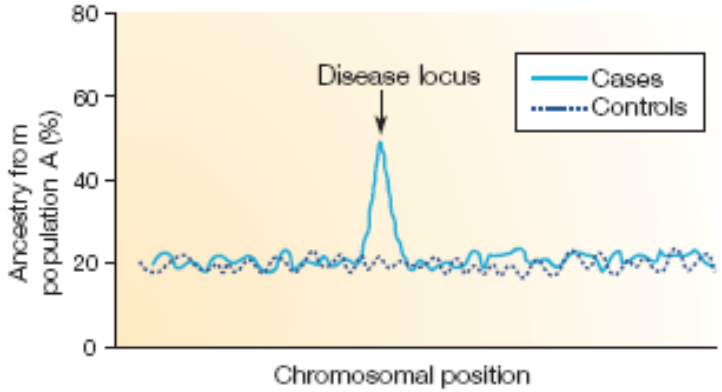
Mapping by Admixture Linkage Disequilibrium (MALD)



Cases: admixed population with differential disease risk



Controls: subjects or other chromosomal regions



Adapted from Smith and O'Brien 2005

In the case of a parent population specific common variant which confers common disease risk– the genomic regions containing that variant should be significantly enriched in markers which "paint" the ancestry of the region in "cases" compared to "controls" (or rest of genome)

Admixture Mapping

Feasibility depends on:

- **Admixture LD**
- **Population disparity in disease frequency**
- **Common risk variant(s) that have risen to high frequency in the at risk population**

  (Common Variant – Common Disease)

Disease Examples (partial list)

Higher relative risk in African-Americans		
Lupus nephritis with systemic lupus erythematosus	3.13	(1.21–8.09)
Myeloma	3.14	(2.00–4.93)
Dementia	3.21	(2.18–4.73)
Prostate cancer	2.73	(2.13–3.52)
Hypertensive heart disease	2.80	(2.03–3.86)
Pregnancy-related death	2.65	(1.73–4.07)
Hypertension	2.61	(2.09–3.27)
Focal segmental glomerulosclerosis	2.49	(1.05–5.95)
Intracranial haemorrhage	2.10	(1.44–3.06)
Non-insulin dependent diabetes	1.99	(1.60–2.48)
End-stage renal disease	1.87	(1.47–2.39)
Stroke	1.57 1.30–5.00[‖]	(1.27–1.94) (1.00–1.61)
Hypertensive retinopathy	1.48	(1.08–2.03)
Lung cancer	1.48	(1.30–1.67)
HIV progression	1.41	(1.06–1.86)

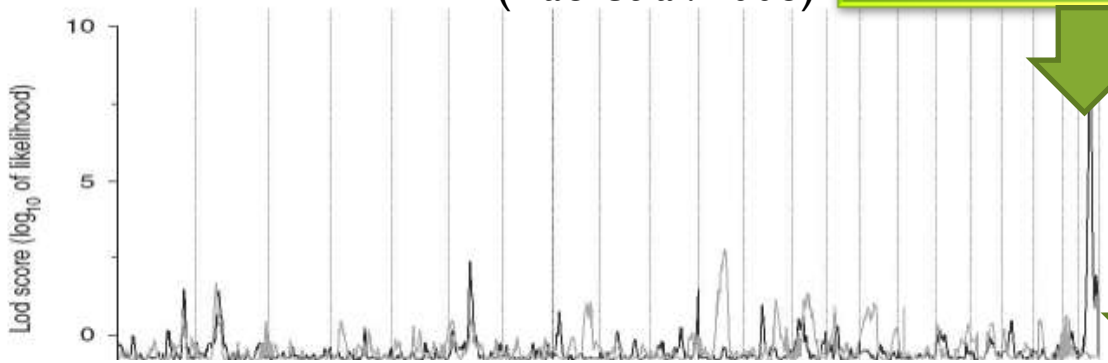
Smith and O'Brien 2005

End Stage Kidney Disease in African Americans: Admixture Scan

Chromosome22 q12-13

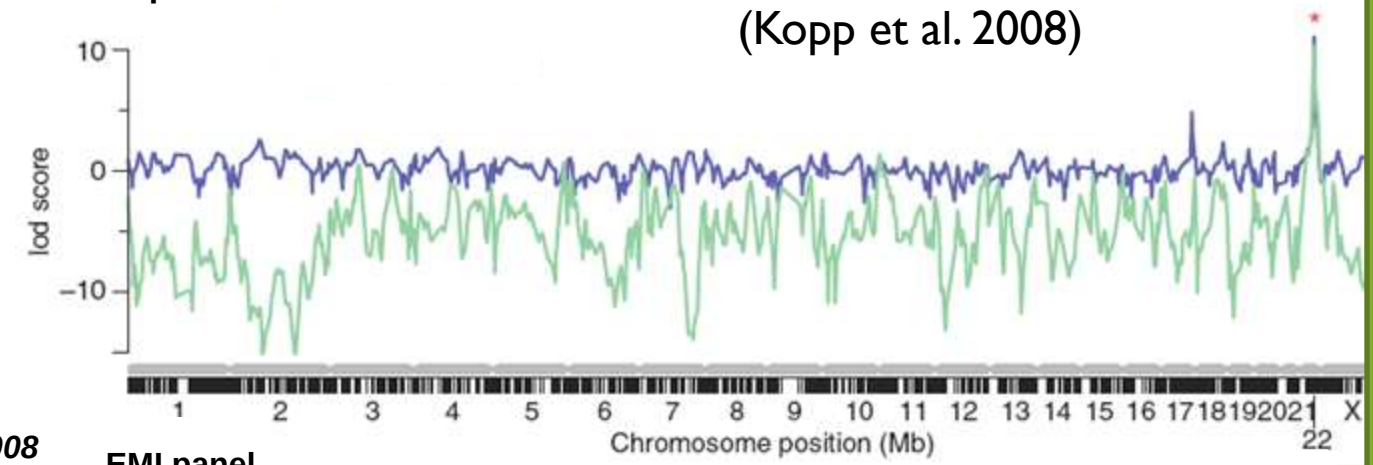
Smith panel

(Kao et al. 2008)



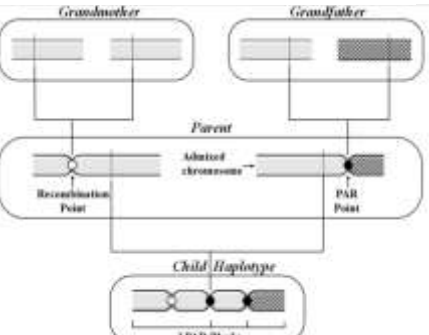
Smith panel

(Kopp et al. 2008)

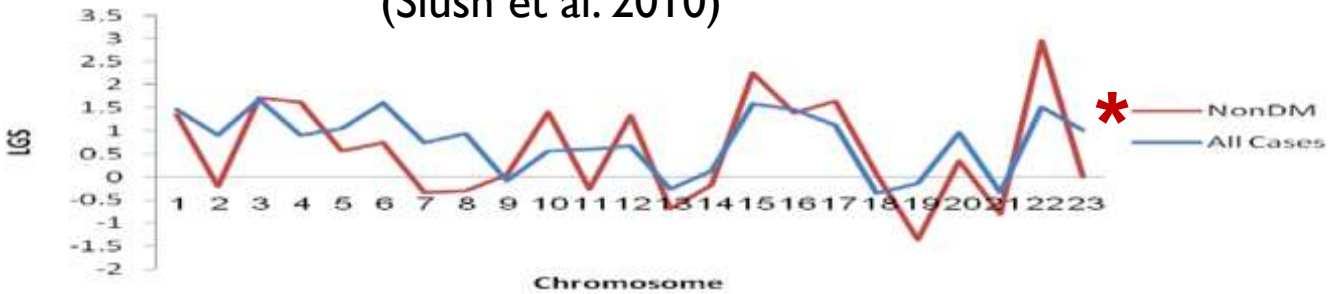


Bercovici 2008
Genome Research

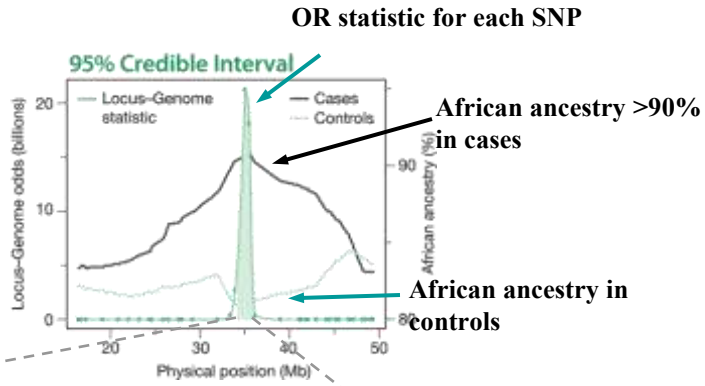
EMI panel

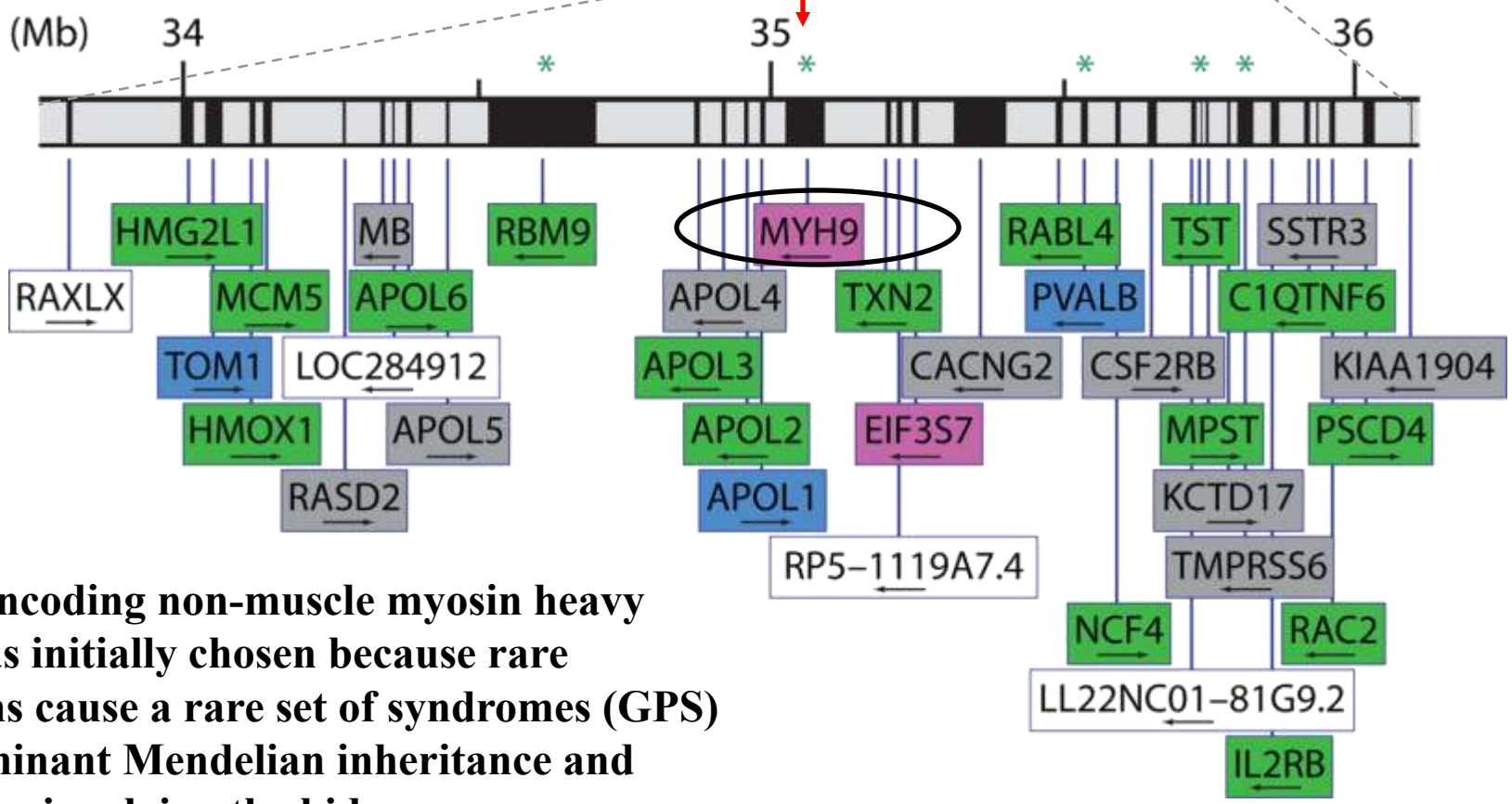


(Slush et al. 2010)



Admixture peak: >30 genes were found in the a 2mMb 95% interval

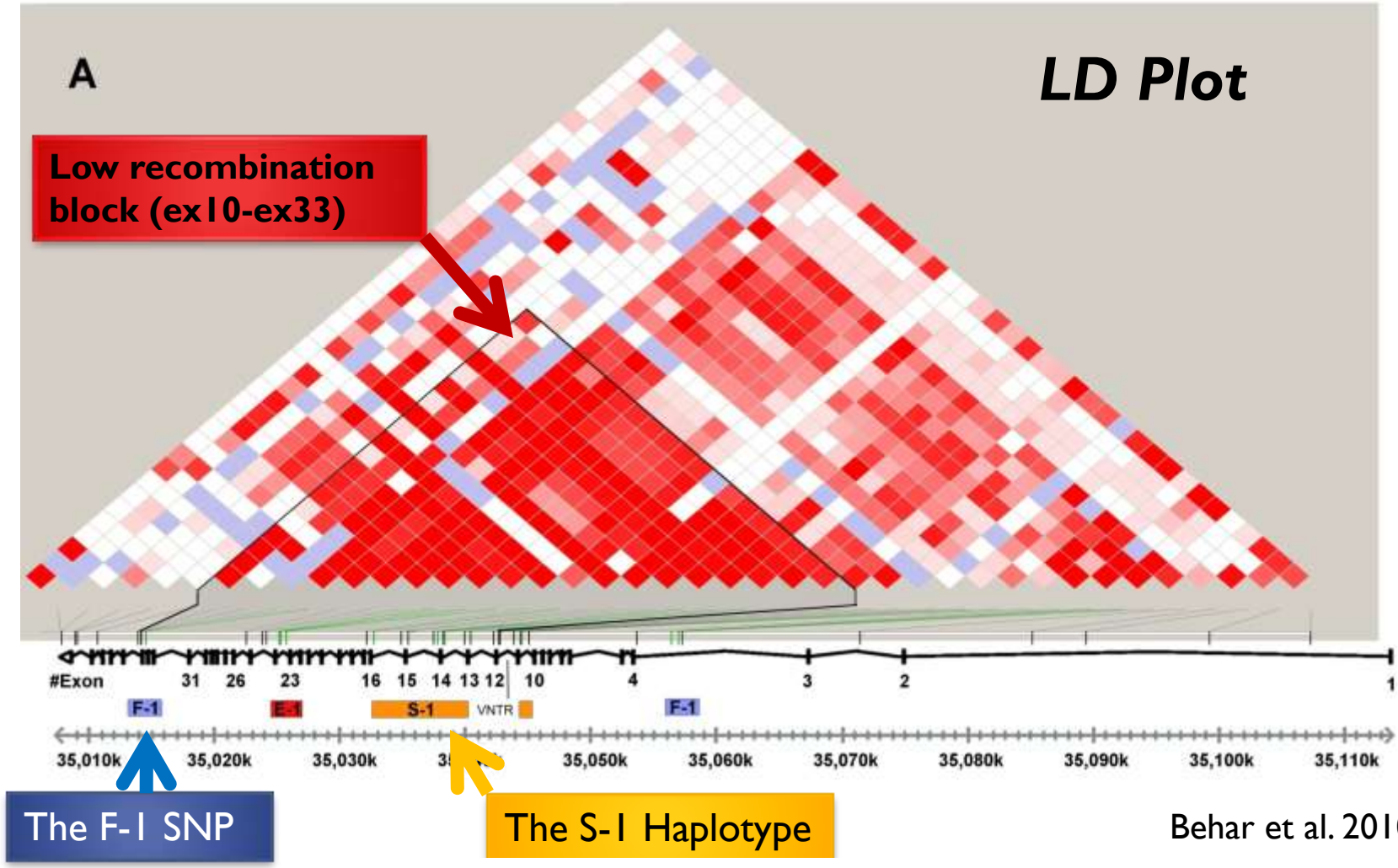




MYH9 encoding non-muscle myosin heavy chain was initially chosen because rare mutations cause a rare set of syndromes (GPS) with dominant Mendelian inheritance and sometimes involving the kidney

Adapted from Kopp et al 2008 and NIDDK 2010, Kao et al 2008

Multiple Groups Conducted Fine Mapping Using a "Case-Control" Candidate Locus Association Study Design to Identify Disease Risk Markers for Non-Diabetic End Stage Kidney Disease



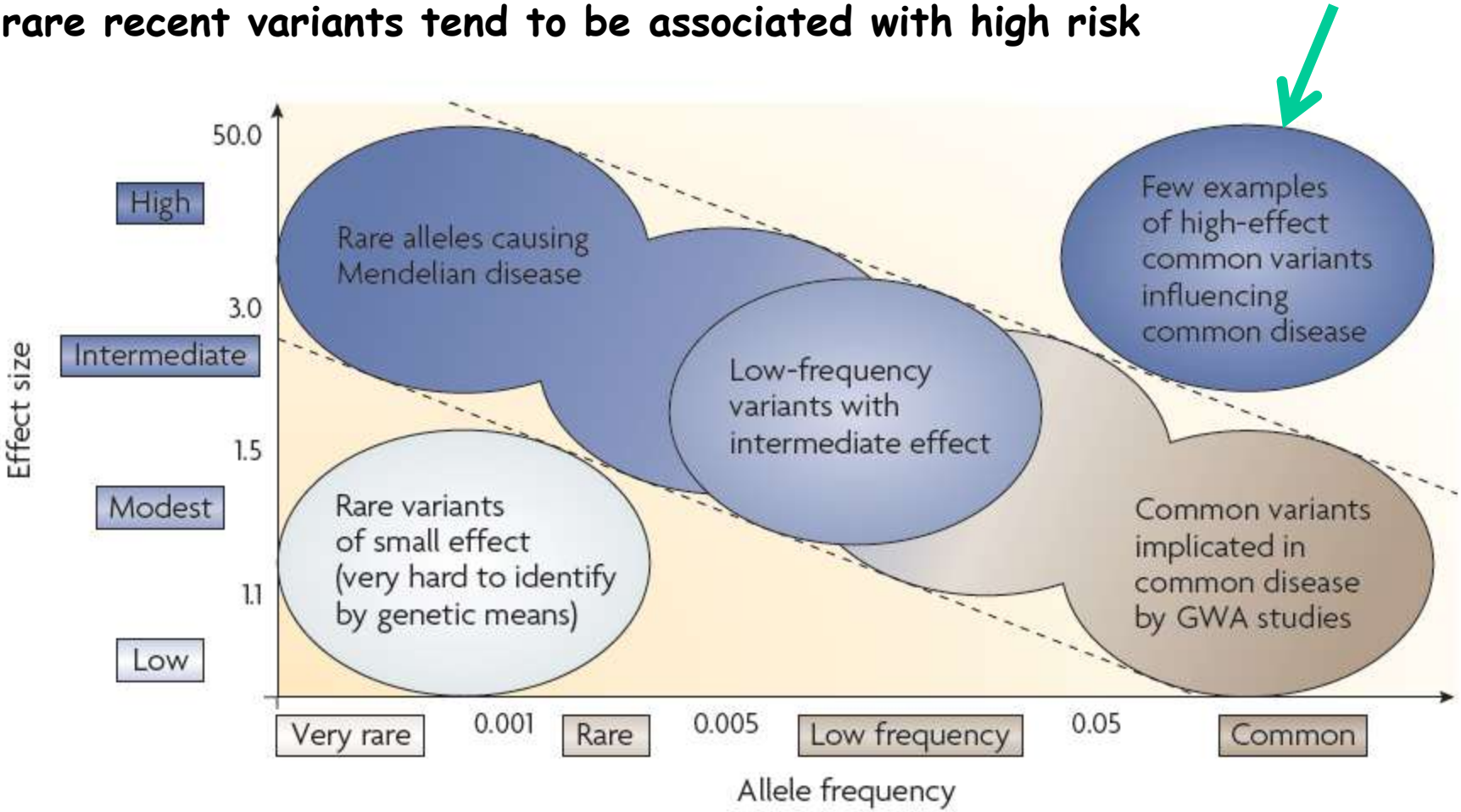
Behar et al. 2010

Odds Ratios, p values, and ROC parameters among the strongest ever seen for a common variant associated with a common disease

Disease Associated Variants

"Missing Heritability"

- common ancient variants tend to be associated with low risk
- rare recent variants tend to be associated with high risk



Antoonorakis et al.et al. NRG 2010

***Common variant with very high OR* (> 10)**

PAR and Explained Fraction exceed those for smoking and lung cancer

Find the "causative" pathogenic mutation in MYH9 which confers disease risk

- **Molecular and cellular approaches using identified SNPs**
- **Resequencing within MYH9 in healthy and affected**

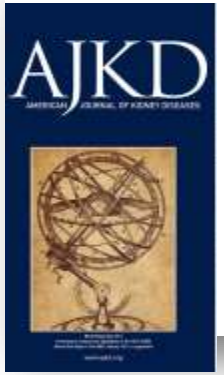
Shay Tzur (doctorate student)

Thinking Out of the Box

- **Clinical Observation**
- **1000 Genomes Data Mining**
- **Evolutionary Biology**

Ethiopian Jews (Beta Israel)
Low Risk for Kidney Failure – specifically HIVAN

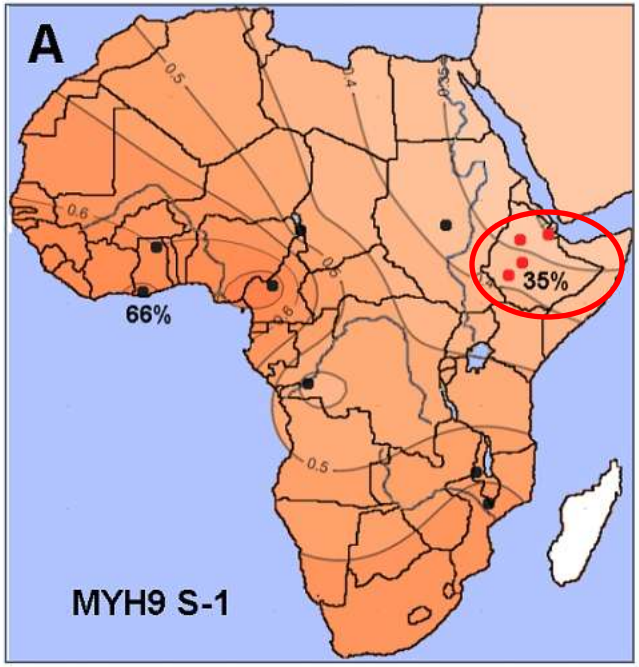
2006



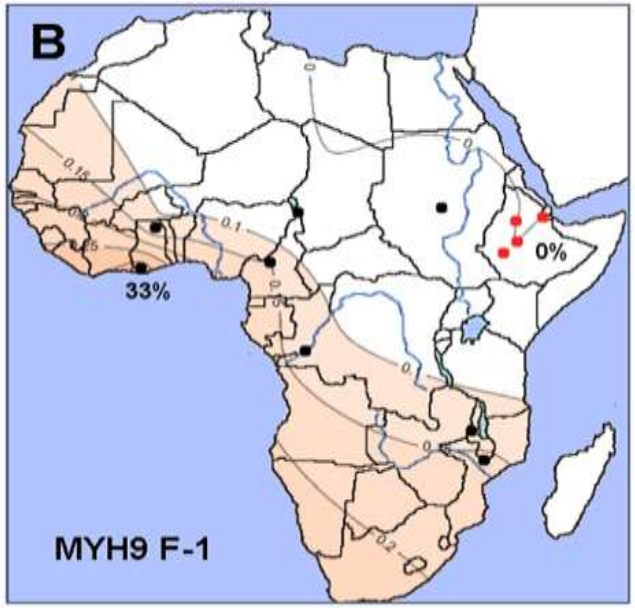
Absence of HIV-Associated Nephropathy in Ethiopians

Doron M. Behar, MD, Liran I. Shlush, MD, Carcom Maor, MPA, Margalit Lorber, MD, and Karl Skorecki, MD

We now know also this to be valid for Ethiopian non-Jews
(collaboration with Yonas Hailiesellasie and Dawit Wolday – U. of Addis Ababa)

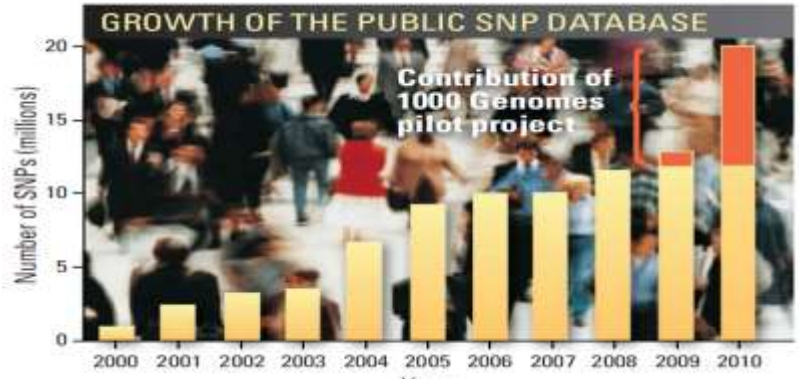


MYH9 S1 "best" Tag SNP *present* at allele frequency of *0.35* in Ethiopia



MYH9 F1 SNP 3' centromeric in the gene *absent* in Ethiopia

Resequence the Region



- Much more strongly associated with diease phenotypes that any variants in MYH9
- Explain the associations observed with MYH9
- Encodes apolipoprotein L1
- Circulates in certain HDL particles

MYH9

1000 Genomes showed no candidate functional variants

1000 Genomes showed functional variants which were very highly associated with disease

APOL1 (15kbp)

- **S342G and I384M** ← G1 missense risk haplotype

LD 279/280 Chromosomes

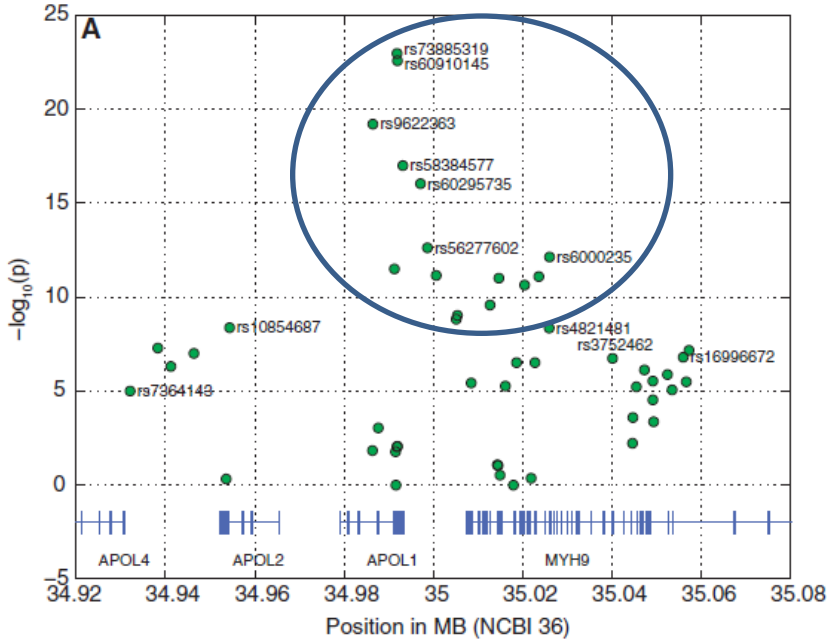
del.N388/Y389 ← G2 nonsense deletion

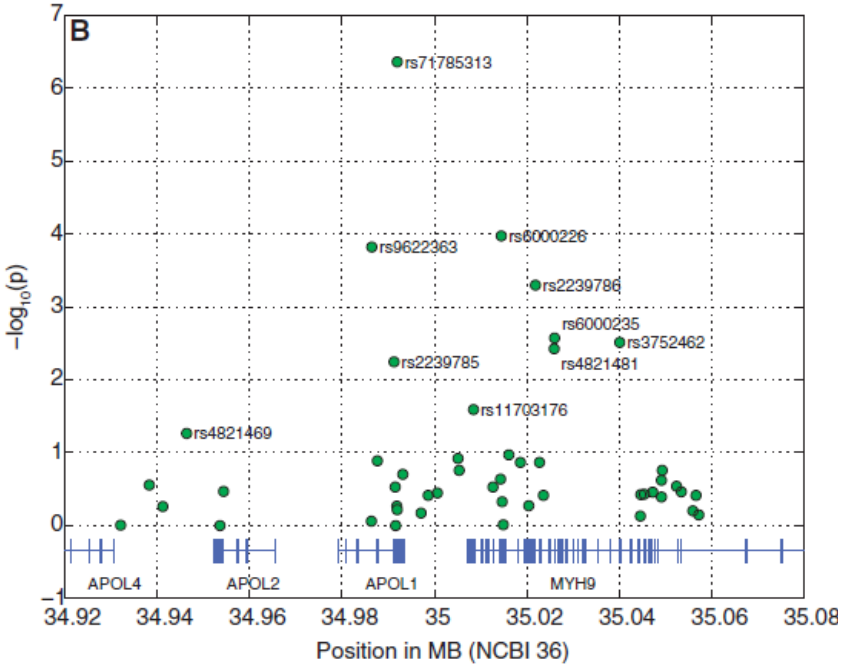
Tzur et al 2010

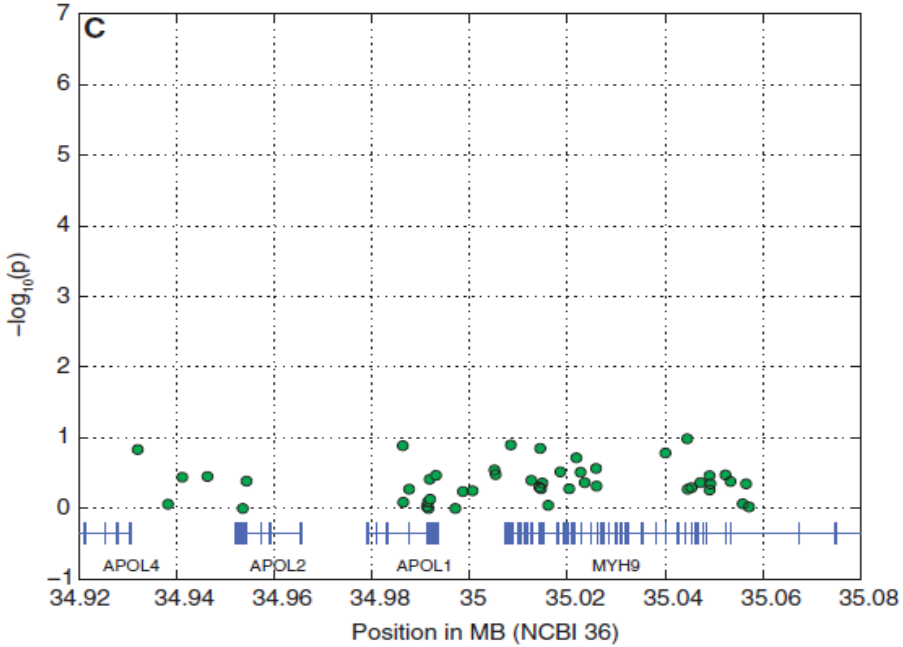
Genovese et al. July 15, 2010

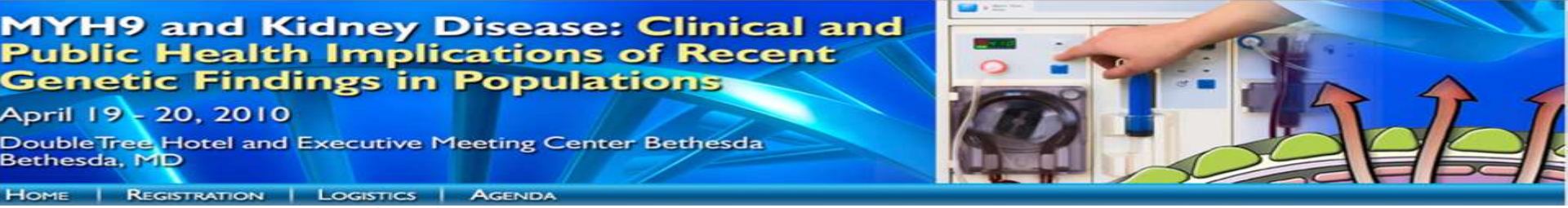
Genovese et al. 2011

The MYH9 Signals are attenuated to near insignificance when association is conditioned upon APOL1 G1 + G2









Thinking Out of the Box

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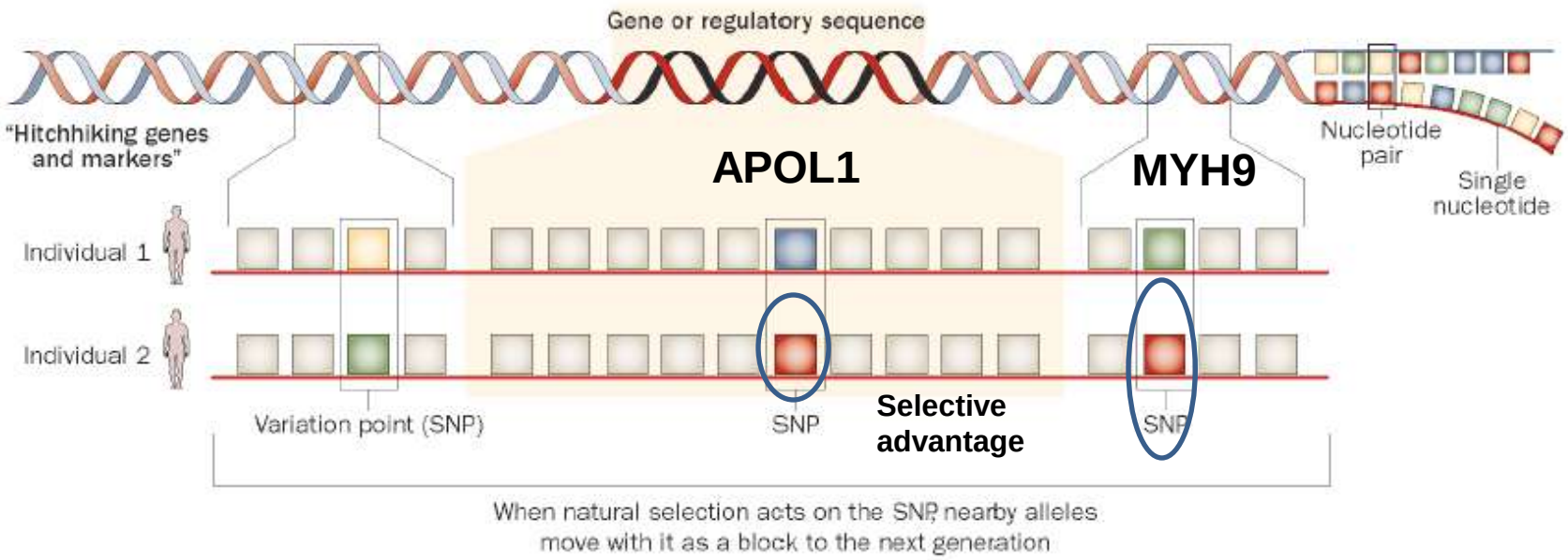
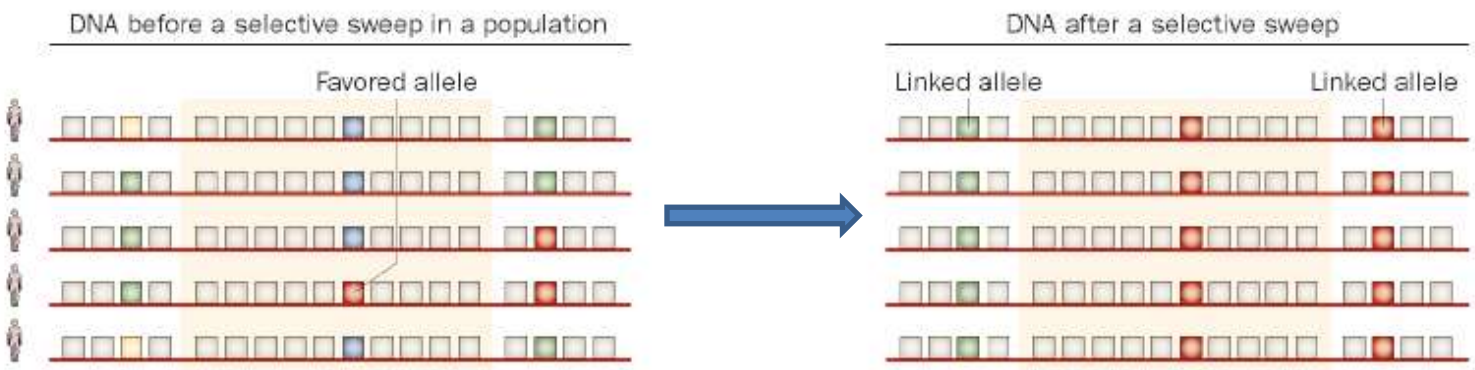
- **1000 Genomes Data Mining**
- **Evolutionary Biology**

"Nothing in Biology Makes Sense Except in the Light of Evolution" Theodosius Dobzhansky

Positive Selection and Hitchiking

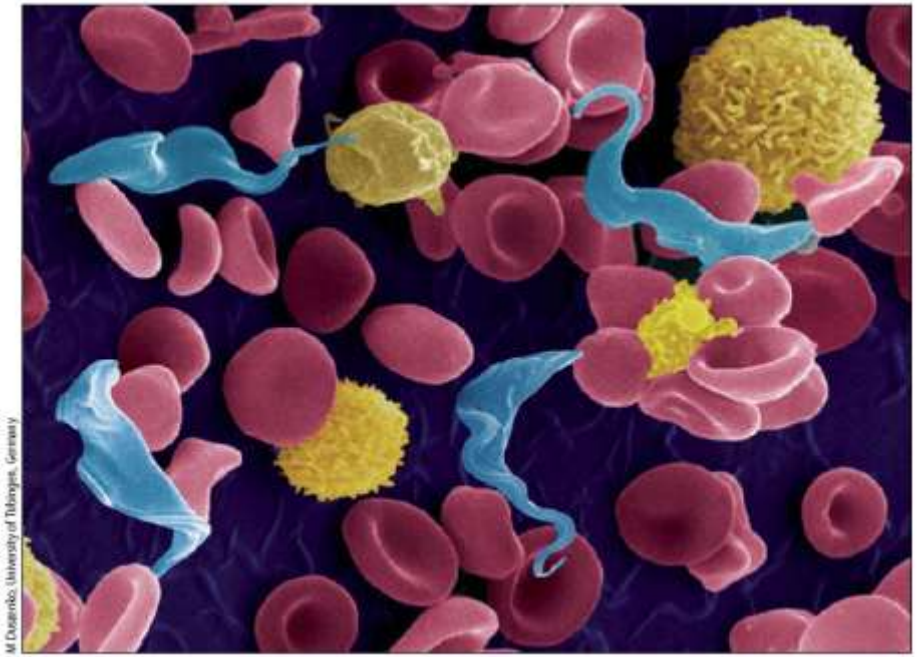




Adapted: Jonathan K Pritchard, Owen Gildersleeve
Scientific American 2010

Brun 2010

Human African Trypanosomiasis
African Sleeping Sickness

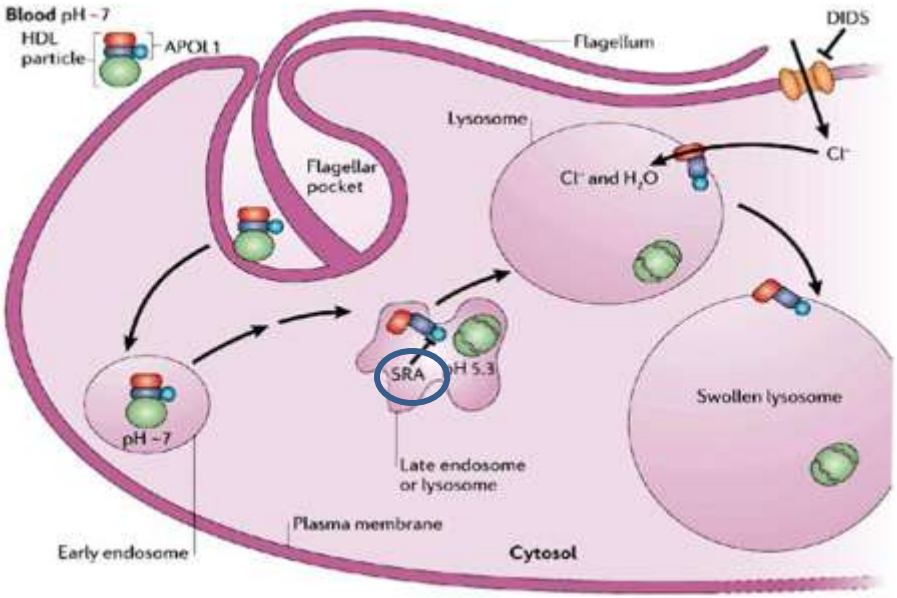


M Dzierzko, University of Tübingen, Germany

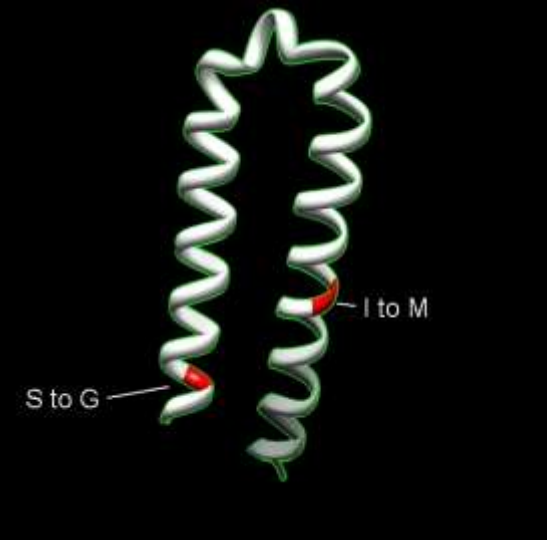
Figure 4: Trypanosomes among blood cells

Pays and colleagues and Raper and colleagues had shown that most human APOL1 efficiently kills many (but not all) Trypanosoma species

SRA – present in T. Brucei Rhodesiense binds, sequesters and confers resistance to APOL1

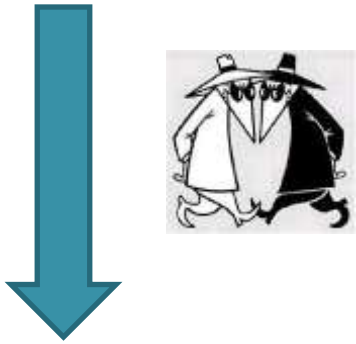


Pays et al. 2006



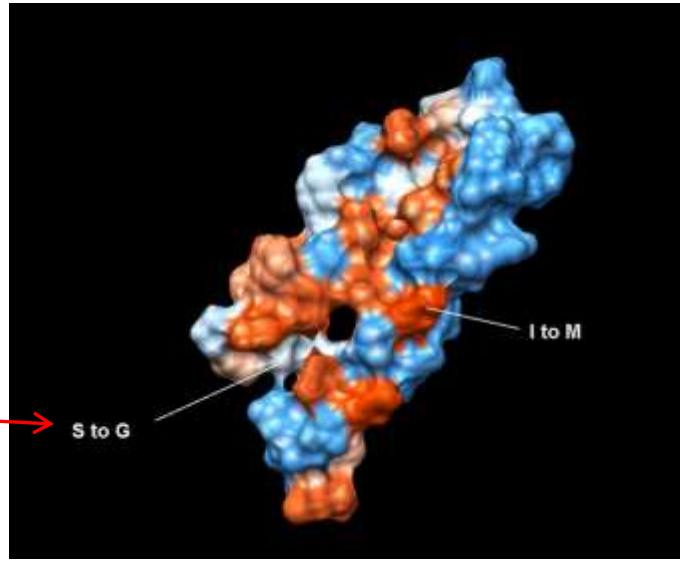
bent alpha helix

ApoL1 G1 and G2 Variants modify protein structure so as to confer protection from Trypanosoma B. Rhodesiense Sleeping Sickness in Subsaharan Africa

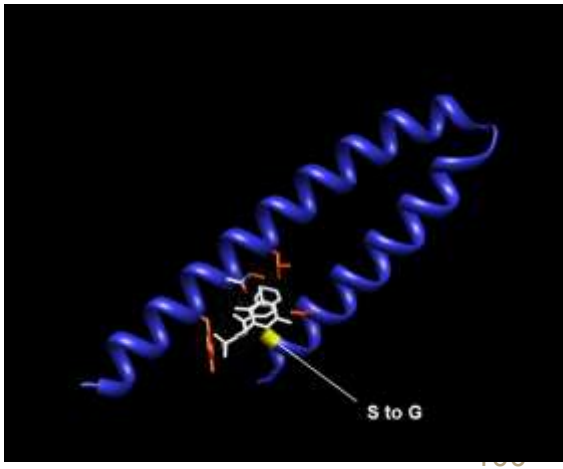


Mutation predicted to exert a disruptive effect on SRA binding domain

internal site



hydrophobic core stabilized the bend



Protein Structure Models for Apoliproteins L:

I-TASSER and CHIMERA

Tm>0.5 for all predictions

Tzur et al. 2010

Genovese et al. 2010

APOL1 Variant ***Trypanosmal Killing***

ETat 1.2R

WT

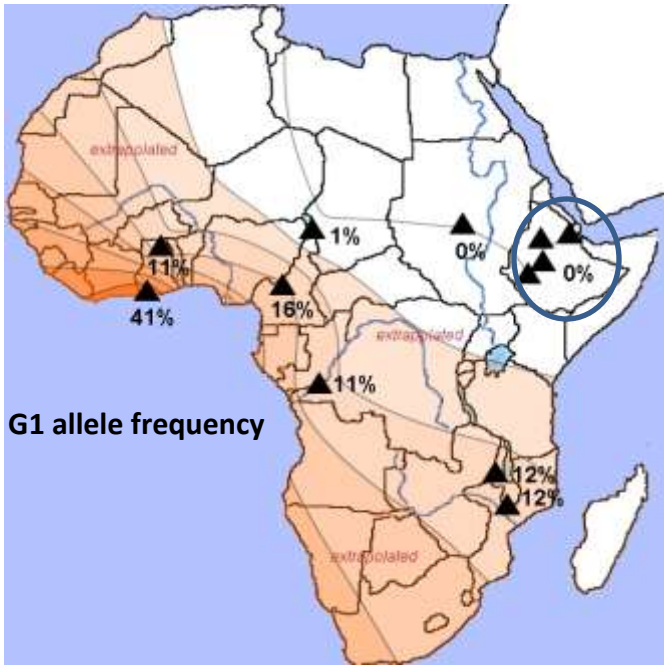
G1

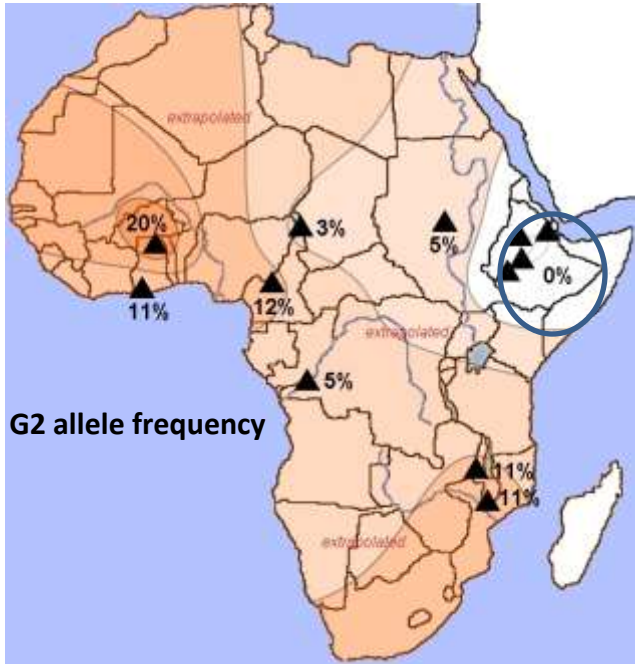
G2

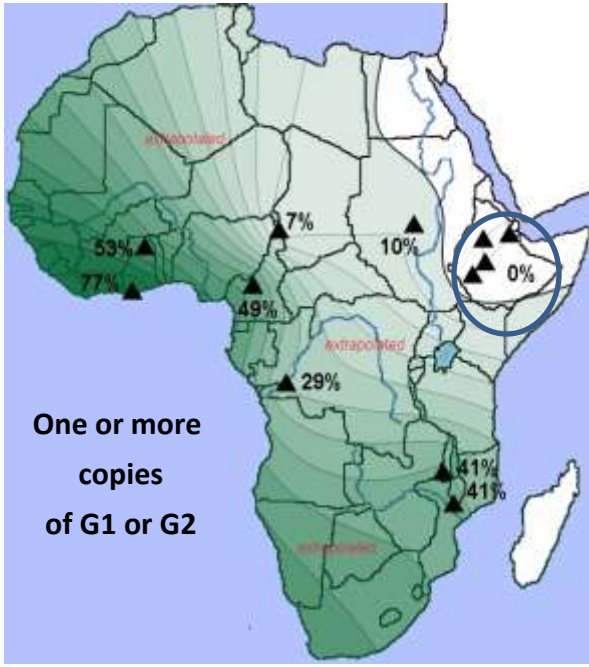
Trypanosoma Bruei Rhodesiense

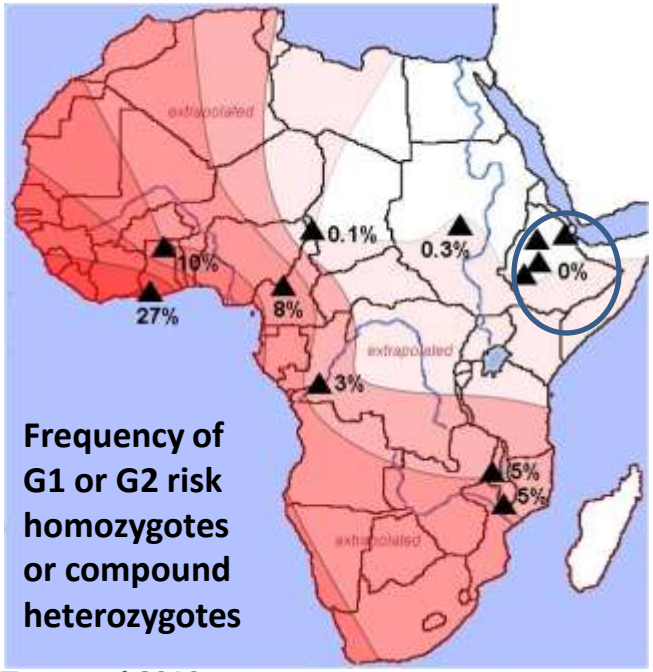
B

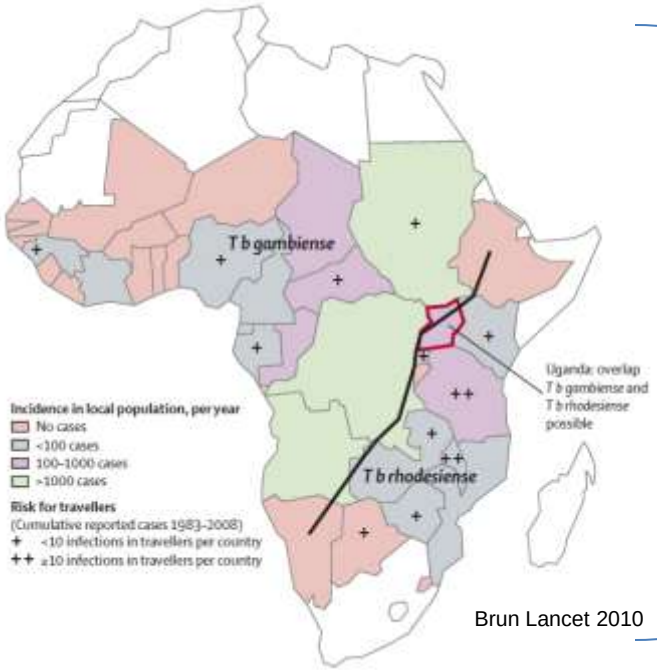
	total	unbound	% unbound
NHS			1
SS/II (WT)			6
SG/IM (G1)			3
GG/MM (G1)			8
SG/IM/id (G1 + G2)			39
id (G2)			35
dd (G2)			82











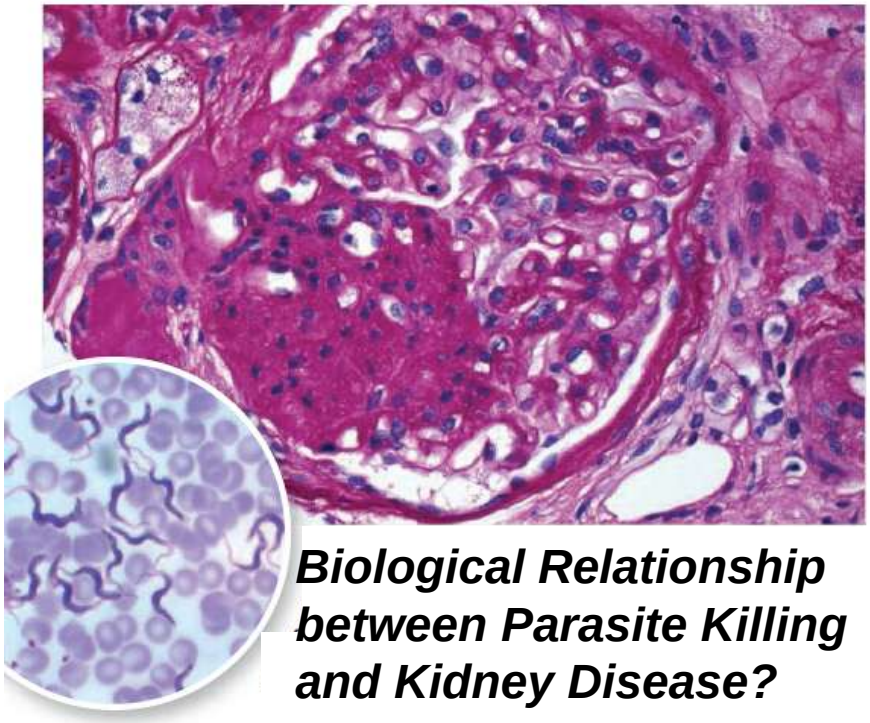
Brun Lancet 2010

G1 and G2 single copies afford full protection against T b rhodesiense

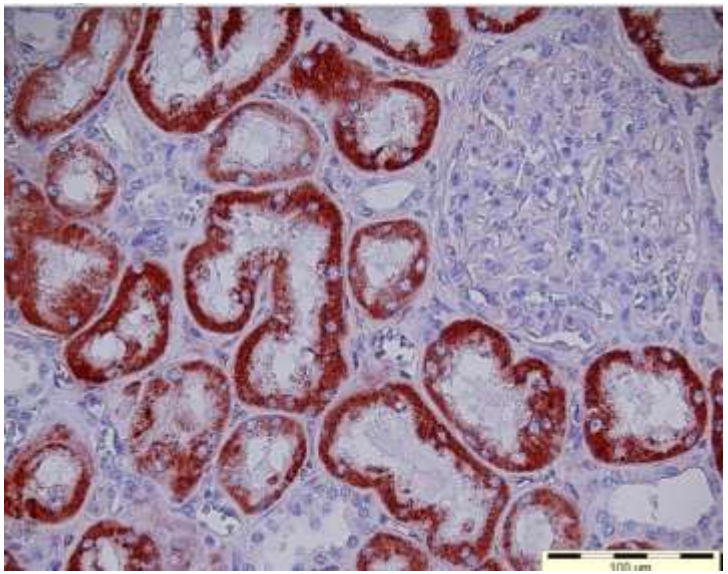
Current distribution suggests a past selective sweep in West and Central Sub-Subsharan Africa

Tzur et al 2010

Association is not Causation : Biological Mechanism



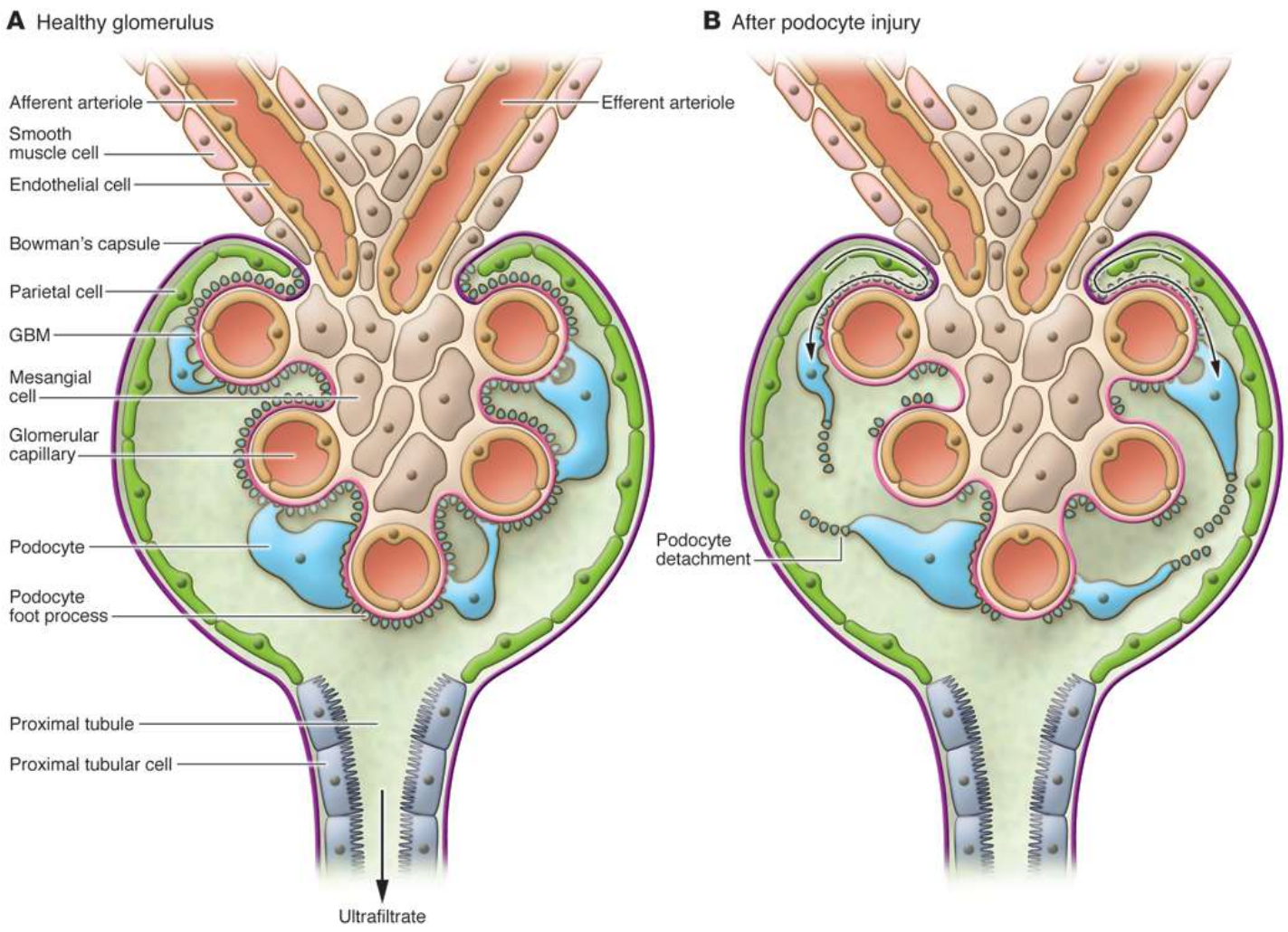
Biological Relationship between Parasite Killing and Kidney Disease?



Immunlocalization to Proximal Tubules of the Kidney unaffected by G1/G2 allelic state

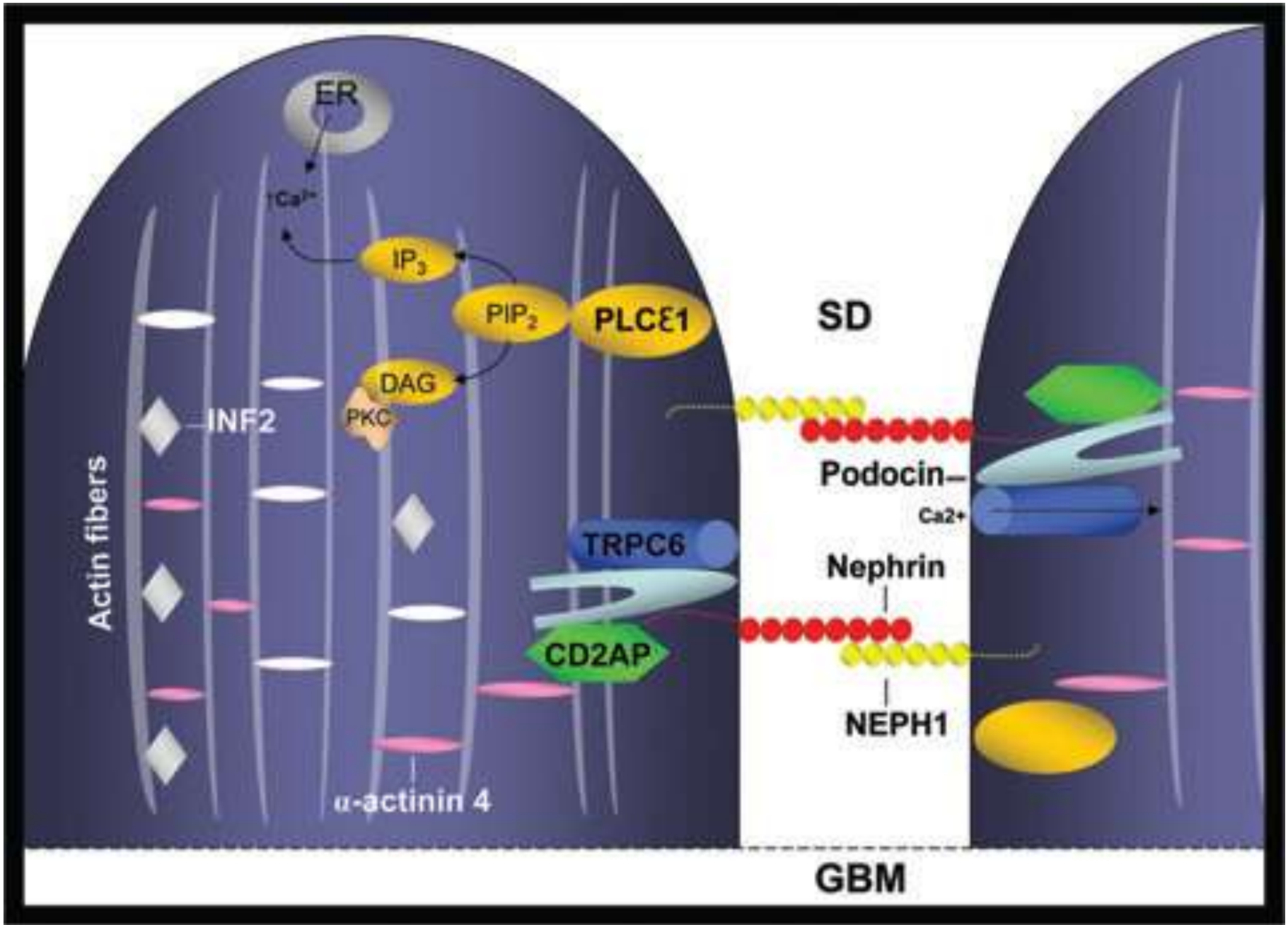
Genetics → Guides Research Plan

- ✓ **Recessive inheritance model, yet healthy null state in non-human and some humans suggests concentration dependent "gain of renal injury"**
- ✓ **Localization – Circulates in HDL3, immunolocalizes to monocytes/macrophages renal proximal tubules, and podocytes (unaffected by genotype)**
- ✓ **Circumstantial evidence favors circulating moeity and podocyte injury**



All 16 Reported Hereditary Steroid Resistant Nephrotic Syndromes with FSGS have been mapped to genes involved in podocyte integrity with many in which pathomechanism has been validated

Podocyte and slit-diaphragm proteins involved in hereditary nephrotic syndrome

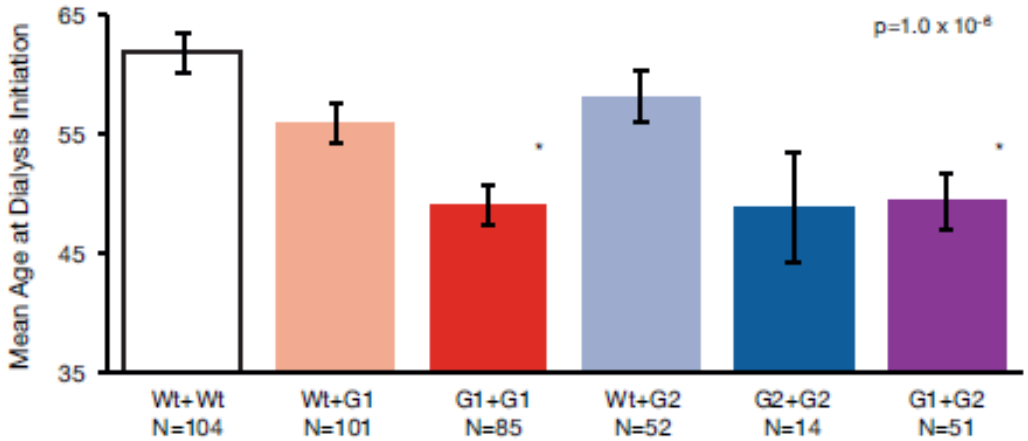


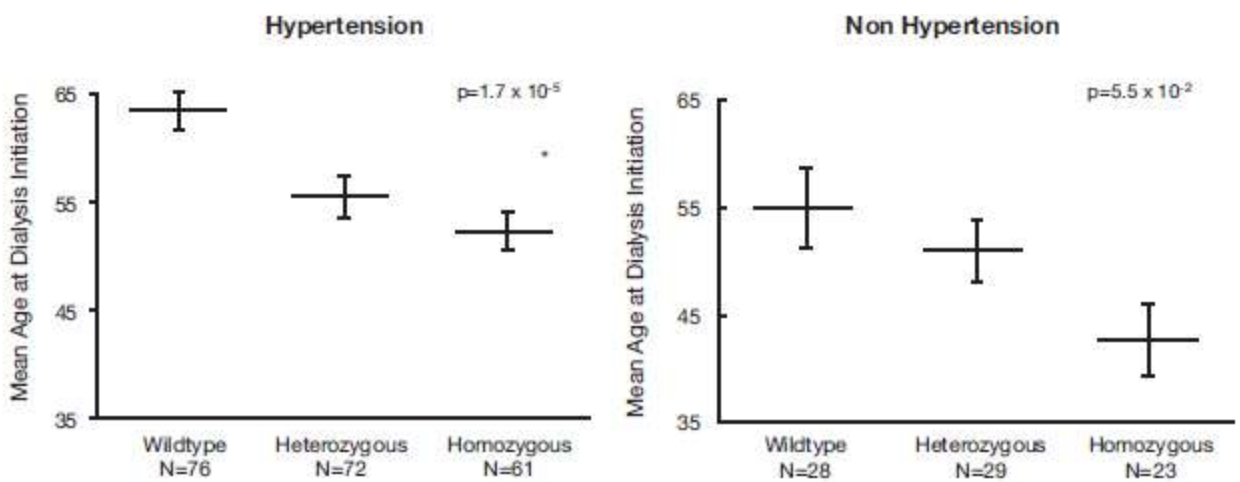
Clinical Significance before understanding Biology of Kidney Injury?

- *Clinical epidemiology*
- *Kidney transplantation*
- *Threshold for ART in HIV infection*
- *Hypertension management in African Americans*

Clinical Epidemiology

Age of onset of Renal Replacement



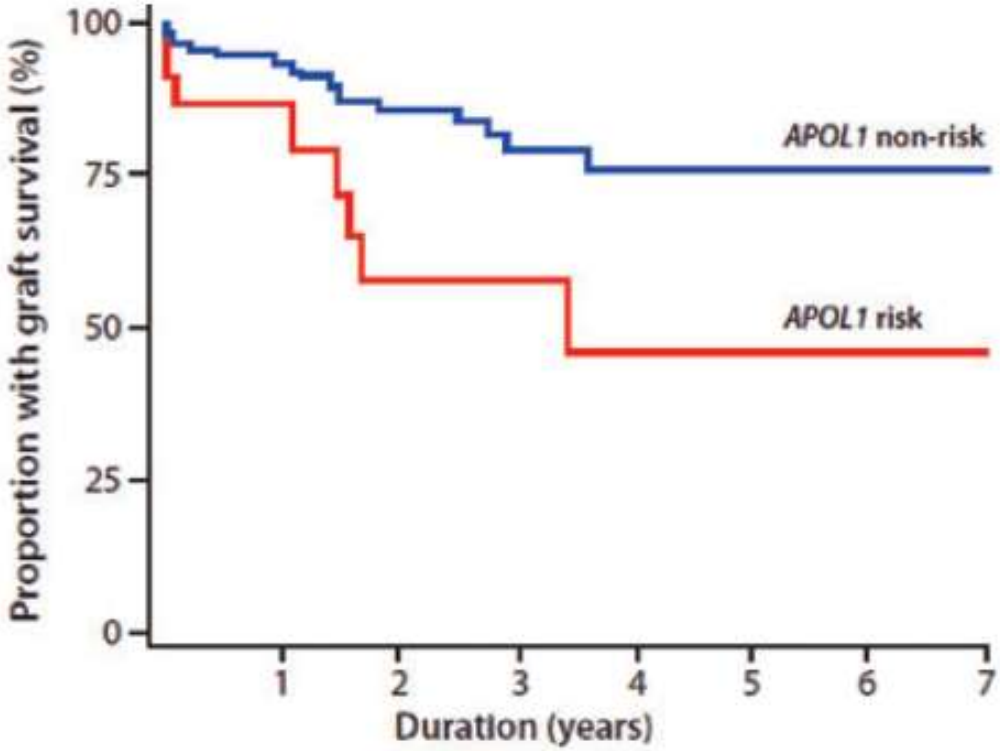


Kanji et al. 2010

American Journal of Transplantation
doi: 10.1111/j.1600-6143.2011.03513.x

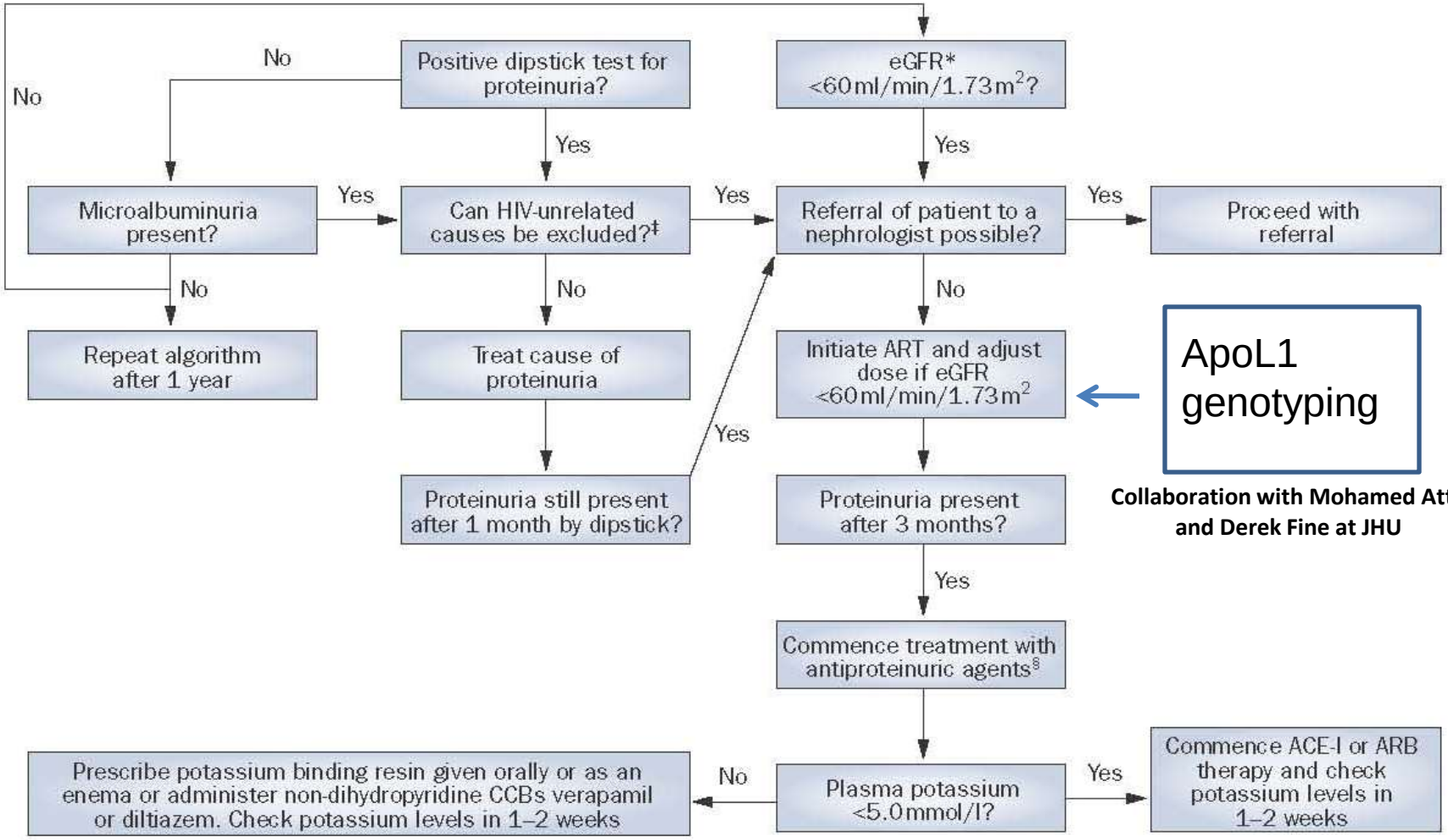
A. M. Reeves-Daniel et al. 2011

The *APOL1* Gene and Allograft Survival after Kidney Transplantation



Risk seems to travel with the donor kidney genotype

Algorithm for screening of kidney disease upon diagnosis of HIV



ApoL1 genotyping

Collaboration with Mohamed Atta and Derek Fine at JHU

Fabian J, Naicker S. Nat Rev Nephrol (2009) 5: 591-8

TAKE HOME MESSAGES

- Evolutionary adaptation followed by environmental change can give rise to common variants for common disease
- Association is not causation
- Value of imputation from Tag SNPs to stronger possibly causal variants (other examples: T1DM, PD)
- It's not all genetics

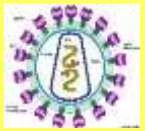
GENETIC RISK

APOL1 genetic susceptibility (risk homozygotes)

SECOND HIT

Gene – Gene interactions

Gene – Environment interactions



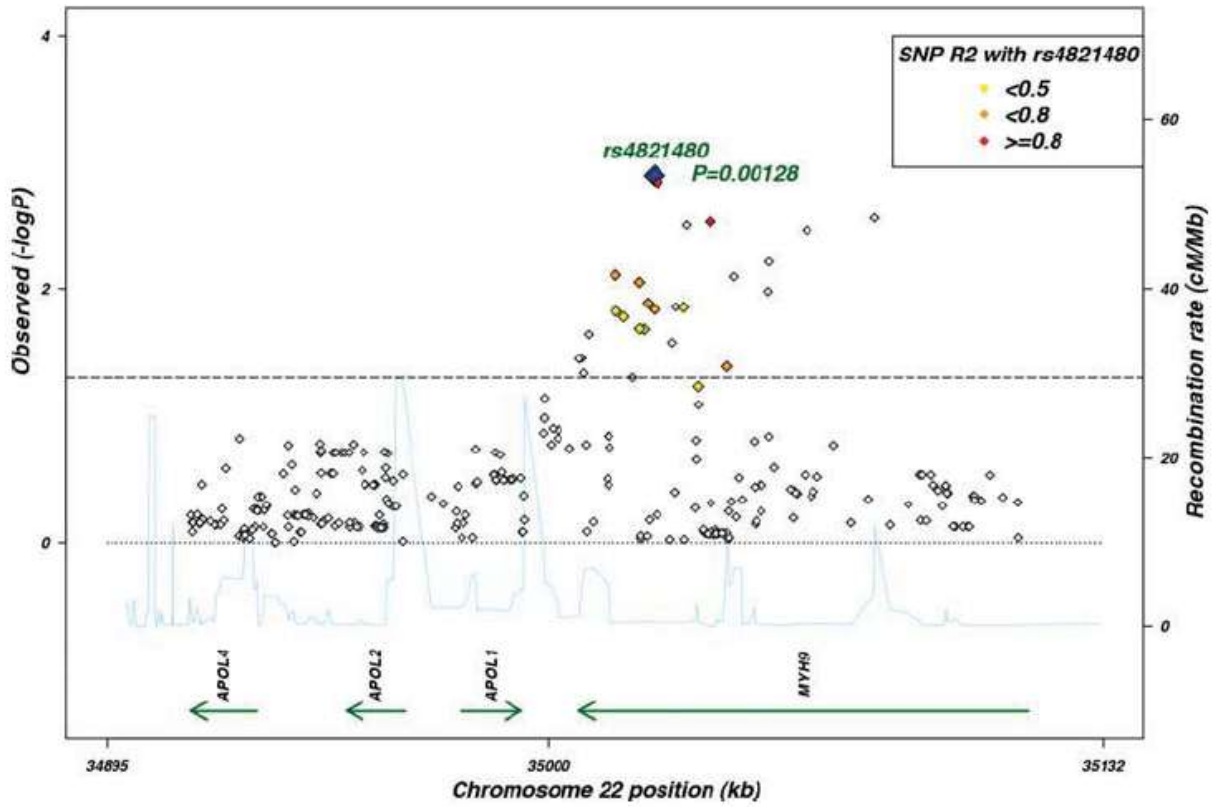
Chronic Kidney Disease
Secondary Hypertension

10-12% African Americans: 4% lifetime risk of CKD rises to 50% with HIV

Human Molecular Genetics, 2011, Vol. 20, No. 12 **2450–2456**
doi:10.1093/hmg/ddr118
Advance Access published on March 23, 2011

The *MYH9/APOL1* region and chronic kidney disease in European-Americans

Conall M. O'Seaghdha[1,2], Rulan S. Parekh[3,4], Shih-Jen Hwang[1], Man Li[4], Anna Köttgen[4,9], Josef Coresh[4,5,6], Qiong Yang[7], Caroline S. Fox[1,8,*,†] and W.H. Linda Kao[4,5,6,*,†]



Claim that in Europens, MYH9 variants tag yet another causative unrelated to G1 and G2 of APOL1 and not in either MYH9 or APOL1

Differential Effects of *MYH9* and *APOL1* Risk Variants on *FRMD3* Association with Diabetic ESRD in African Americans

Barry I. Freedman[1]*, Carl D. Langefeld[2]*, Lingyi Lu[2], Jasmin Divers[2], Mary E. Comeau[2], Jeffrey B. Kopp[3], Cheryl A. Winkler[4], George W. Nelson[5], Randall C. Johnson[5,6], Nicholette D. Palmer[7,8,9], Pamela J. Hicks[7,8,9], Meredith A. Bostrom[7,8,9], Jessica N. Cooke[8,9,10], Caitrin W. McDonough[8,9,10], Donald W. Bowden[7,8,9,11]*

Table 4. Combined chromosome 22-*FRMD3* interaction analysis.

		T2DM-ESRD Cases Only		NDNN Controls Only		Cases vs. Controls	c22-risk homozygotes	non-c22 risk homozygotes	c22 risk homozygotes vs. non-risk	
Marker	Gene Interaction	P-Value Additive	OR (95% CI)	P-Value Additive	OR (95% CI)	Homogeneity P-Value	OR (95% CI)	OR (95% CI)	Homogeneity P-Value*	Classic Logit P-Value**
rs2378658	*MYH9*	9.5E-6	0.69 (0.59,0.82)	9.2E-1	1.01 (0.85,1.20)	5.7E-3	0.83 (0.67,1.03)	1.25 (1.06,1.46)	2.9E-3	4.0E-3
	APOL1	4.5E-3	0.75 (0.61,0.91)	1.9E-1	1.17 (0.93,1.49)	3.6E-3	0.74 (0.55,1.0)	1.13 (0.98,1.31)	1.3E-2	2.6E-2
rs1535753	*MYH9*	1.7E-5	0.70 (0.60,0.82)	8.2E-1	1.02 (0.86,1.21)	5.3E-3	0.81 (0.66,1.01)	1.24 (1.06,1.46)	1.8E-3	2.4E-3
	APOL1	6.0E-3	0.76 (0.62,0.92)	1.4E-1	1.20 (0.94,1.52)	3.1E-3	0.72 (0.53,0.98)	1.12 (0.97,1.29)	1.0E-2	1.6E-2
rs1535752	*MYH9*	5.0E-4	0.69 (0.56,0.85)	6.1E-1	1.06 (0.84,1.34)	1.2E-2	0.80 (0.61,1.07)	1.27 (1.03,1.57)	1.1E-2	1.3E-2
rs942283	*MYH9*	2.3E-6	0.68 (0.57,0.79)	8.7E-1	1.01 (0.85,1.20)	2.8E-3	0.80 (0.65,0.99)	1.25 (1.07,1.47)	1.0E-3	1.4E-3
	APOL1	3.6E-3	0.74 (0.61,0.91)	1.7E-1	1.18 (0.93,1.5)	2.9E-3	0.72 (0.53,0.97)	1.12 (0.97,1.29)	9.8E-3	1.9E-2
rs942280	*MYH9*	9.3E-7	0.67 (0.57,0.78)	8.1E-1	1.02 (0.86,1.21)	1.6E-3	0.80 (0.65,0.99)	1.28 (1.09,1.51)	4.8E-4	7.0E-4
	APOL1	1.2E-3	0.72 (0.59,0.88)	1.6E-1	1.18 (0.93,1.5)	1.3E-3	0.71 (0.52,0.95)	1.15 (0.99,1.33)	4.4E-3	6.7E-3
rs942278	*MYH9*	1.5E-5	0.70 (0.59,0.82)	7.7E-1	1.03 (0.86,1.22)	4.2E-3	0.85 (0.69,1.06)	1.30 (1.11,1.53)	1.6E-3	2.3E-3
	APOL1	6.5E-3	0.76 (0.62,0.93)	1.3E-1	1.20 (0.95,1.52)	3.0E-3	0.76 (0.56,1.03)	1.17 (1.01,1.35)	1.2E-2	1.9E-2
rs10867977	*MYH9*	1.7E-4	0.67 (0.54,0.83)	7.8E-1	1.03 (0.82,1.30)	1.2E-2	0.85 (0.64,1.13)	1.31 (1.06,1.62)	1.6E-2	2.2E-2

1,592 T2DM-ESRD Discovery and Replication cases versus 1,671 Discovery and Replication non-diabetic non-nephropathy (NDNN) controls (* Homogeneity P-Value refers to the P-Value from the test for homogeneity of the odds ratio; ** Two-locus logistic regression interaction analysis).

doi:10.1371/journal.pgen.1002150.t004

Lancet 2010; 375: 1287–95

Renal Medicine 1

Genetic kidney diseases

Friedhelm Hildebrandt

TABLE 1: PRINCIPLES

TABLE 2: SINGLE GENE GLOMERULAR DISEASE

TABLES 3: CYSTIC, INTERSITIAL AND TUMOROUS KIDNEY DISEASE

TABLE 4: RENAL TUBULE AND METABOLIC DISEASE

TABLE 5: NEPHROLITHIASIS

TABLE 6: CAKUT SYNDROMES

Amy J McKnight, Diane Currie and Alexander P Maxwell

J Pathol 2010; 220: 198–216

Table 4. Online resources for renal genetics

Autosomal dominant polycystic kidney disease [mutation database (PKD1/PKD2)]	http://pkdb.mayo.edu/cgi-bin/mutations.cgi
Autosomal recessive polycystic kidney disease (mutation database (ARPKD/PKHD1)]	http://www.humgen.rwth-aachen.de
CDDB (Collecting Duct DataBase)	http://cddb.nhlbi.nih.gov/cddb
CORGI (Centralized Online Renal Genetics Initiative)	http://www.qub.ac.uk/neph-res/CORGI
EuReGene (European Renal Genome Project)	http://www.euregene.org
GENECURE (GENomic stratEgies for treatment and prevention of Cardiovascular death in Uraemia and end-stage REnal disease)	http://www.genecure.eu
GUDMAP (GenitoUrinary Development Molecular Anatomy Project)	http://www.gudmap.org
KGDB (human Kidney Gene DataBase)	http://www.urogene.org/kgdb
Kidney development database (collation of studies relating to kidney development)	http://golgi.ana.ed.ac.uk/kidhome.html
Predictions (EU-funded PREvention of DIabetic ComplicaTIONS)	http://www.rzuser.uni-heidelberg.de/~jb5/aboutproject.htm
ReGeNet (the REnal GEnome NETwork project)	http://www.regenet.eu
Renal genes (aims to provide information on nephronophthisis, nephrotic syndrome and urinary tract malformations)	http://www.renalgenes.org

J Pathol 2010; 220: 198–216

Living donor kidney transplantation in patients with hereditary nephropathies

Patrick Niaudet

Nat. Rev. Nephrol. 6, 736–743; 2010

Key points

- Living donor kidney transplantation is often presented as the best option for patients awaiting renal transplantation, but patients whose renal failure is the result of an inherited disease might not be suitable candidates for living related transplantation
- The possible occurrence of the same disease in the related living donor should be excluded before living donor transplantation is performed in a patient with a hereditary nephropathy
- For living donor transplantation to be suitable for a particular patient, the risk of graft loss should not be higher than it would be if the patient received a graft from a deceased donor
- Individuals with autosomal dominant polycystic kidney disease requiring renal transplantation should only receive a kidney from a relative if the disease has been excluded in that relative; if imaging data is equivocal, the potential living related donor should undergo molecular testing
- Female heterozygotes for X-linked Alport syndrome who are considering donating a kidney should be informed about the long-term increased risk of renal dysfunction associated with kidney donation
- Living donor kidney transplantation is contraindicated in patients with atypical hemolytic uremic syndrome because of the high risk of recurrence and the associated high risk of graft loss

Table 1 | Autosomal recessive diseases that progress to end-stage renal disease

Disease	Gene	Gene product	Is living related donor transplantation appropriate?
Congenital nephrotic syndrome (Finnish type)	*NPHS1*	Nephrin	Yes
Autosomal recessive SRNS	*NPHS2*	Podocin	Yes—after genetic testing to exclude the Arg229Gln (p.R229Q) variant in the donor
Autosomal recessive SRNS	*NPHS3* (*PLCE1*)	Phospholipase Cε	Yes
Pierson syndrome	*LAMB2*	Laminin β2	Yes
Schimke's immuno-osseous dystrophy	*SMARCAL1*	HepA-related protein	Yes
Nephronophthisis	*NPHP1* to *NPHP9*	Nephrocystines 1 to 9	Yes
Cystinosis	*CNTS*	Lysosomal cystine transporter	Yes
ARPKD	*PKHD1*	Fibrocystin	Yes
Alport syndrome	*COL4A3*, *COL4A4*	α3 and α4 type IV collagen	Yes
Primary hyperoxaluria	*AGXT*	Alanine glyoxylate aminotransferase	No
Atypical HUS	*CFH*, *CFHR1*, *CFHR3*, *CD46*	Complement factor H, complement factor H-related proteins 1 and 3	No

Abbreviations: ARPKD, autosomal recessive polycystic kidney disease; HUS, hemolytic uremic syndrome; SRNS, steroid-resistant nephrotic syndrome.

Nat. Rev. Nephrol. 6, 736–743; 2010

Table 2 | Autosomal dominant diseases that progress to end-stage renal disease

Disease	Gene	Gene product	Is living related donor transplantation appropriate?
ADPKD type 1	*PKD1*	Polycystin 1	Yes—for unaffected relatives
ADPKD type 2	*PKD2*	Polycystin 2	Yes—for unaffected relatives
Atypical HUS	*CFH*, *CFHR1*, *CFHR3*, *CFB*, *CFI*, *C3*	Complement proteins	No

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; HUS, hemolytic uremic syndrome.

Nat. Rev. Nephrol. 6, 736–743; 2010

Table 3 | X-linked diseases that progress to end-stage renal disease

Disease	Gene	Gene product	Is living donor related transplantation appropriate?
Alport syndrome	*COL4A5*	α5 type IV collagen	Yes—for unaffected males or females and for heterozygous females without proteinuria, aged >45 years and after information is provided on the long-term risks of renal disease after donation
Fabry disease	*GLA*	α-Galactosidase	A risk of renal dysfunction exists in heterozygous female donors; living related transplantation is possible in donors who do not have the mutation

Nat. Rev. Nephrol. 6, 736–743; 2010

Box 1 | Ultrasonographic diagnostic criteria for testing individuals at risk of ADPKD[12]

- In at-risk individuals aged 15–39 years, the presence of three or more cysts (unilateral or bilateral) can establish a diagnosis of ADPKD
- In at-risk individuals aged 40–59 years, the presence of at least two cysts in each kidney can establish a diagnosis of ADPKD
- In at-risk individuals aged ≥60 years, at least four cysts should be present in each kidney to establish a diagnosis of ADPKD
- In at-risk individuals aged ≥40 years, the presence of fewer than two cysts excludes a diagnosis of ADPKD
- In at-risk individuals aged 30–39 years, the absence of cysts excludes a diagnosis of ADPKD in 98% of cases
- In at-risk individuals aged <30 years, a negative renal ultrasound scan does not exclude a diagnosis of ADPKD; other imaging techniques such as computed tomography scanning and magnetic resonance imaging may be useful and molecular genetic testing might be needed

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease.

Nat. Rev. Nephrol. 6, 736–743; 2010

HEREDITARY INTERSTITIAL KIDNEY DISEASE - CLINICAL APPROACH

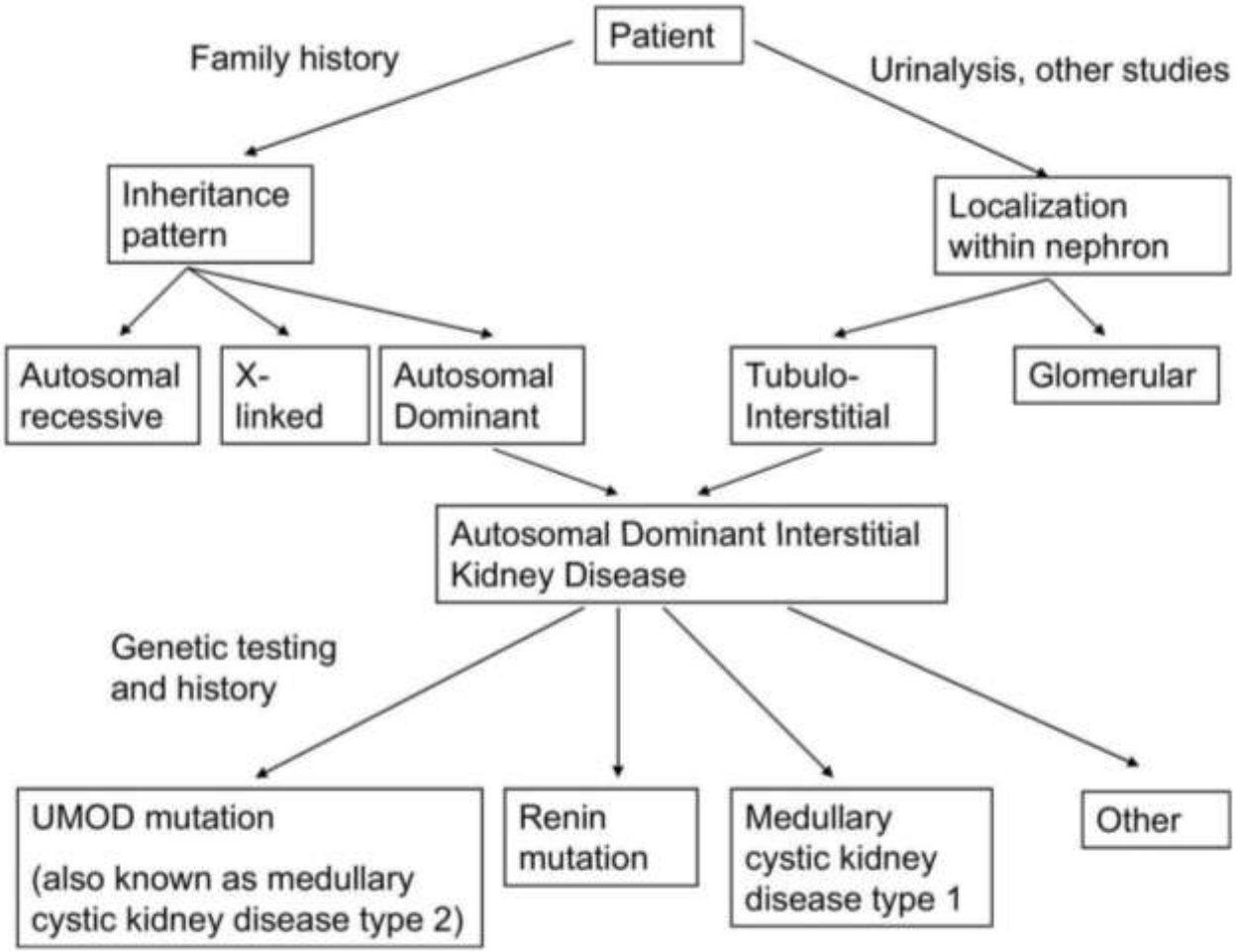
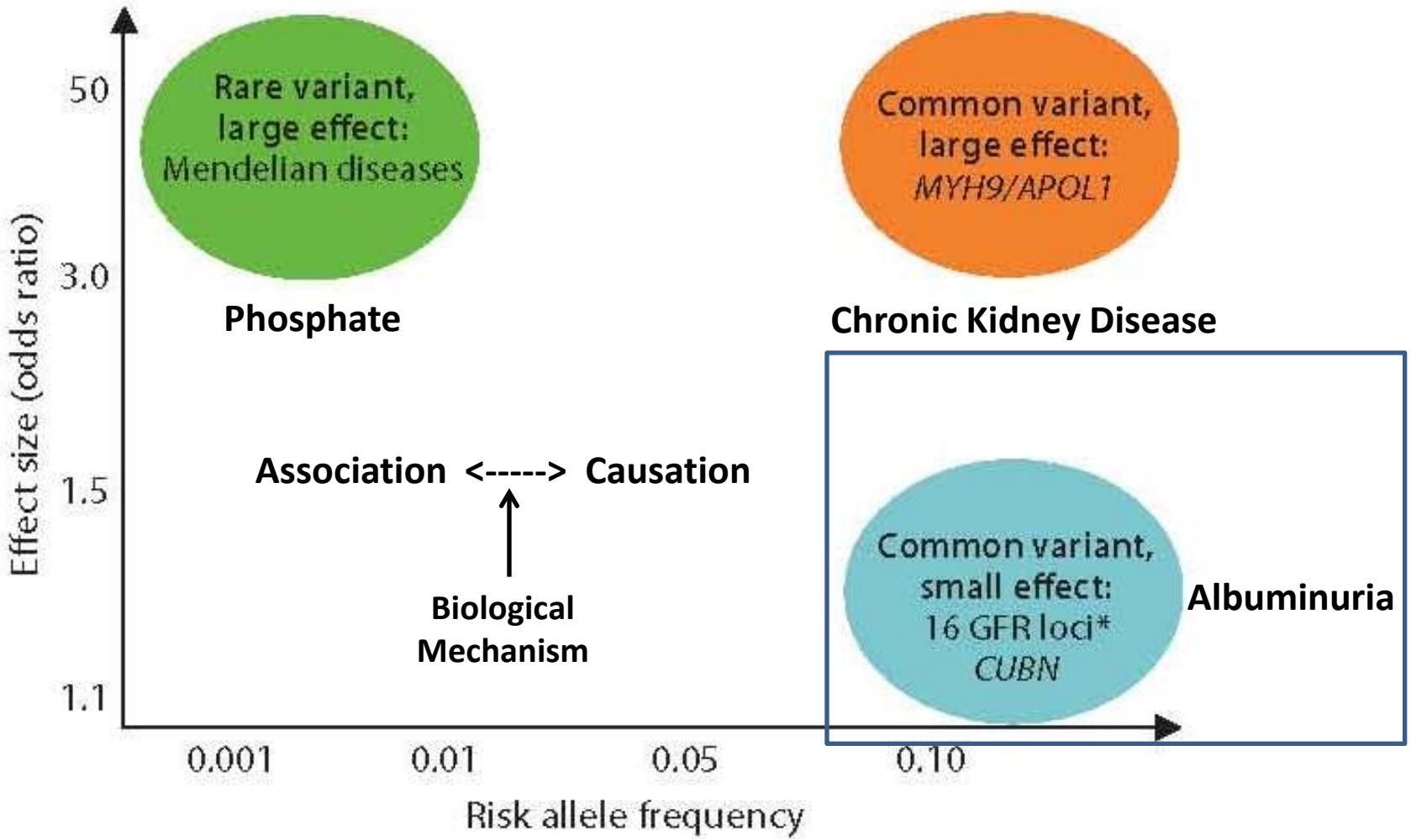


Figure 1. Approach to the diagnosis of inherited interstitial kidney disease.

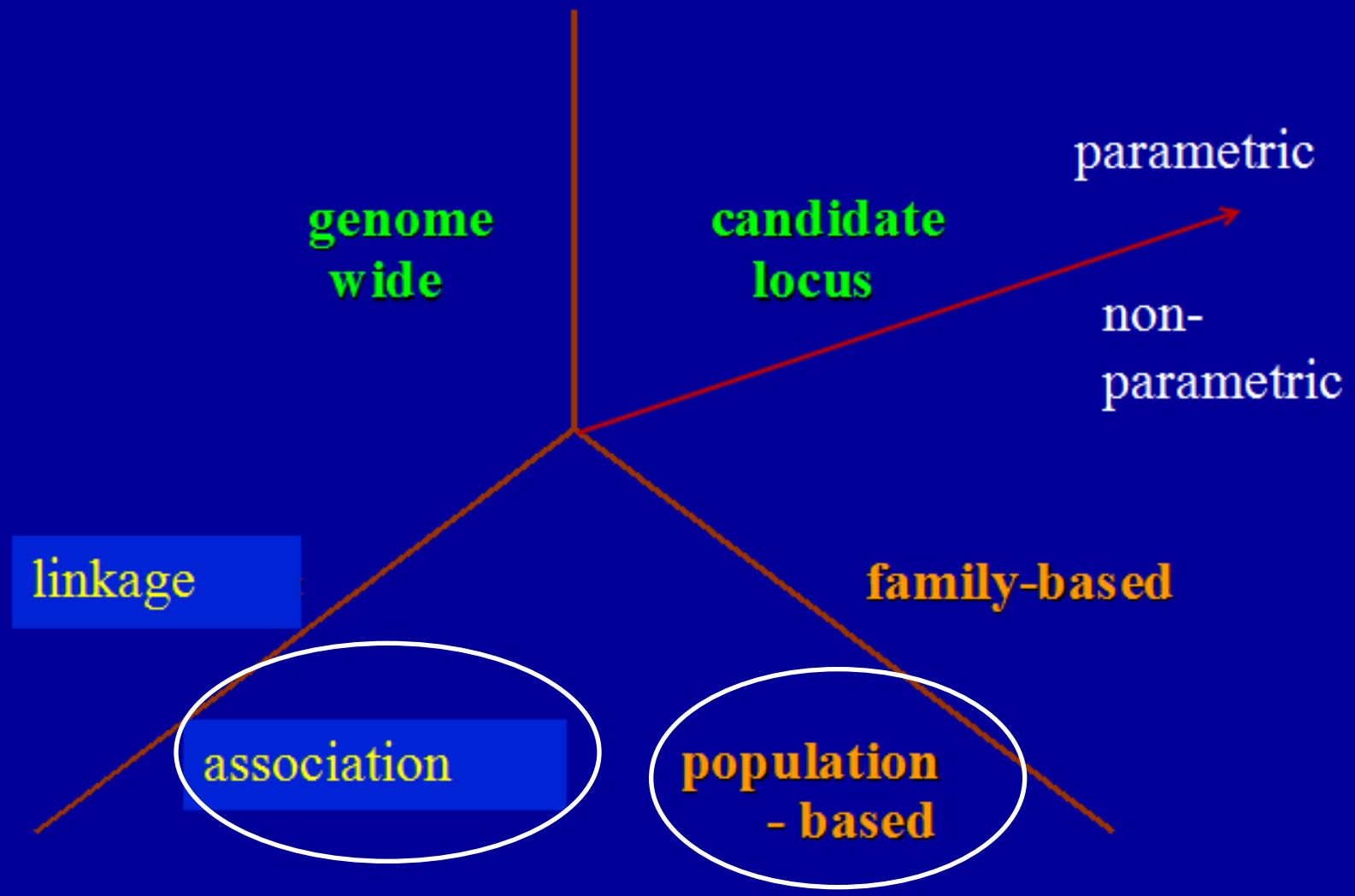
Bleyer et al. Seminars in Nephrology 2010

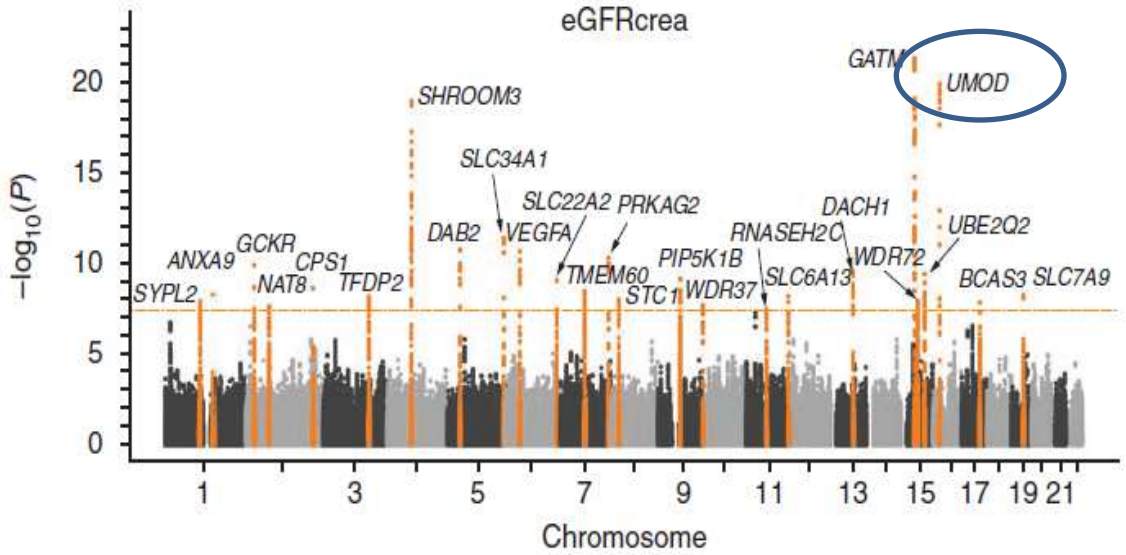
Relation of effect size and risk allele frequency of DNA sequence variants associated with kidney function and disease risk phenotypes

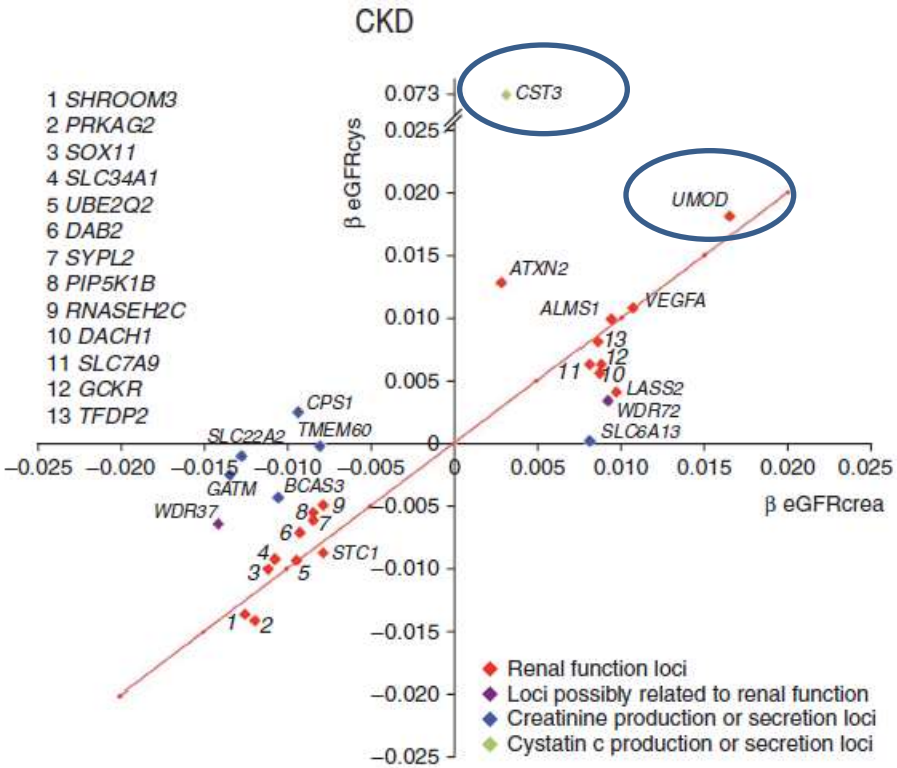


Adapted from Kidney and Blood Pressure Research 2011:34:225-234

Disease Gene Mapping

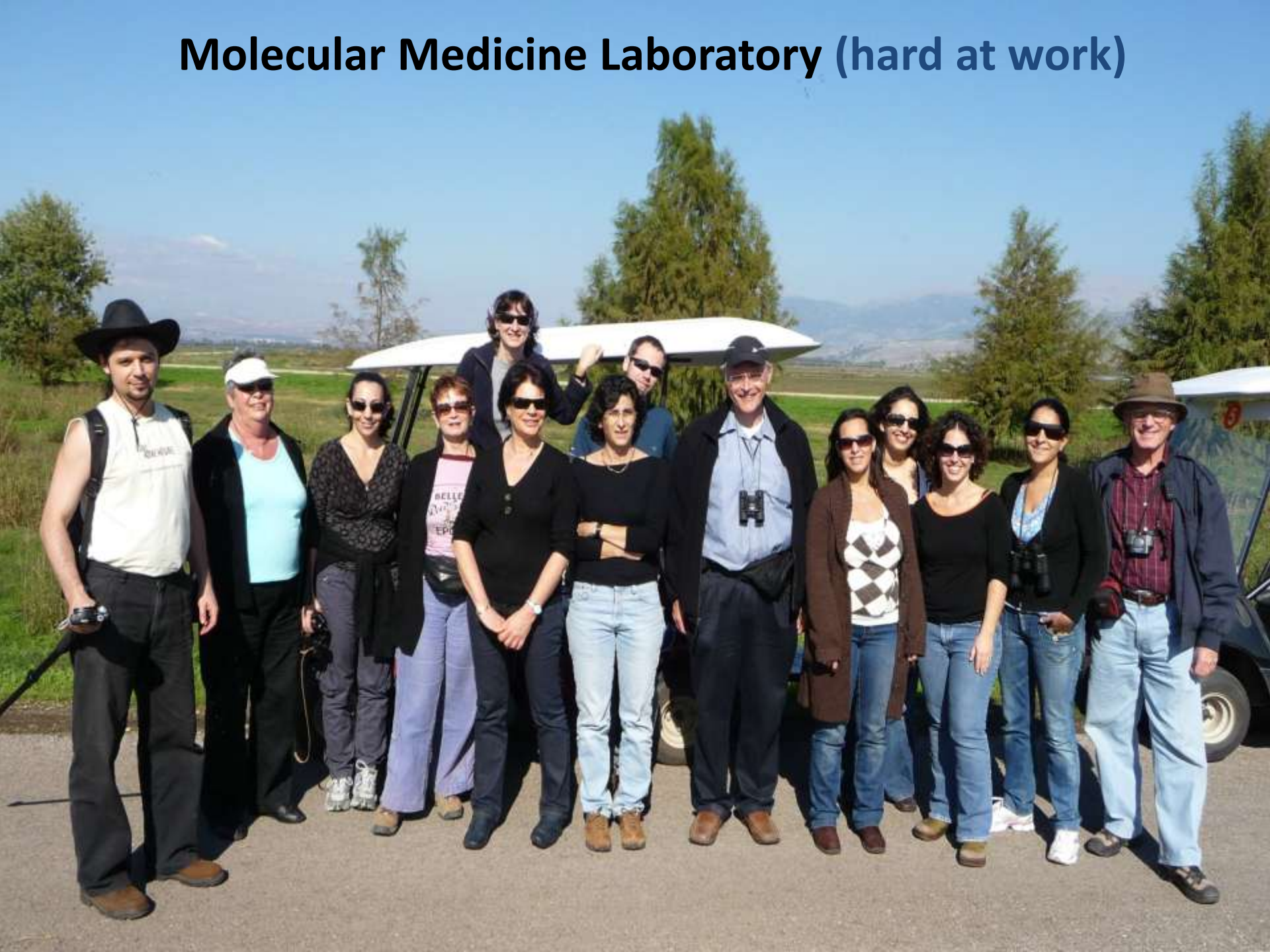






Kottgen et. al 2010

Molecular Medicine Laboratory (hard at work)



Looking for Answers

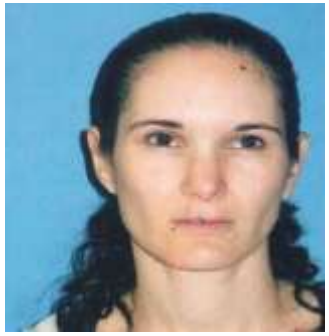
Guennady Yudkovsky

Shay Tzur

Daniella Magen

Sara Selig

Tali Shemer



Ayala Ofir

Doron Behar

Neil Bradman

Walter Wasser

Saharon Rosset

Ayele Tarekegn

Alan Templeton

Yonas Hailisellasie

Sivan Bercovici

Dan Geiger

Liran Shlush


Köszönöm
Szépen
Thanks
شُكْرًا جزيلا
Grazie
תודה
Shukran
Gracias
Merci