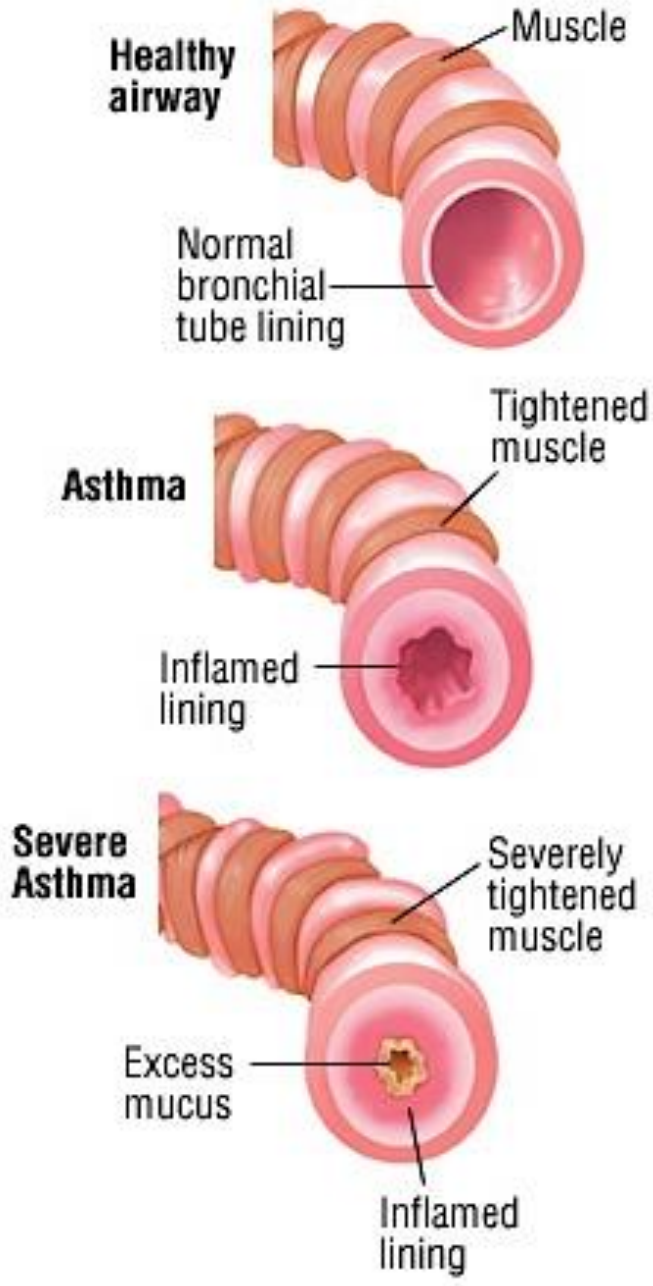
Whole Genome Sequencing of Bronchodilator Response in Minority Children with Asthma

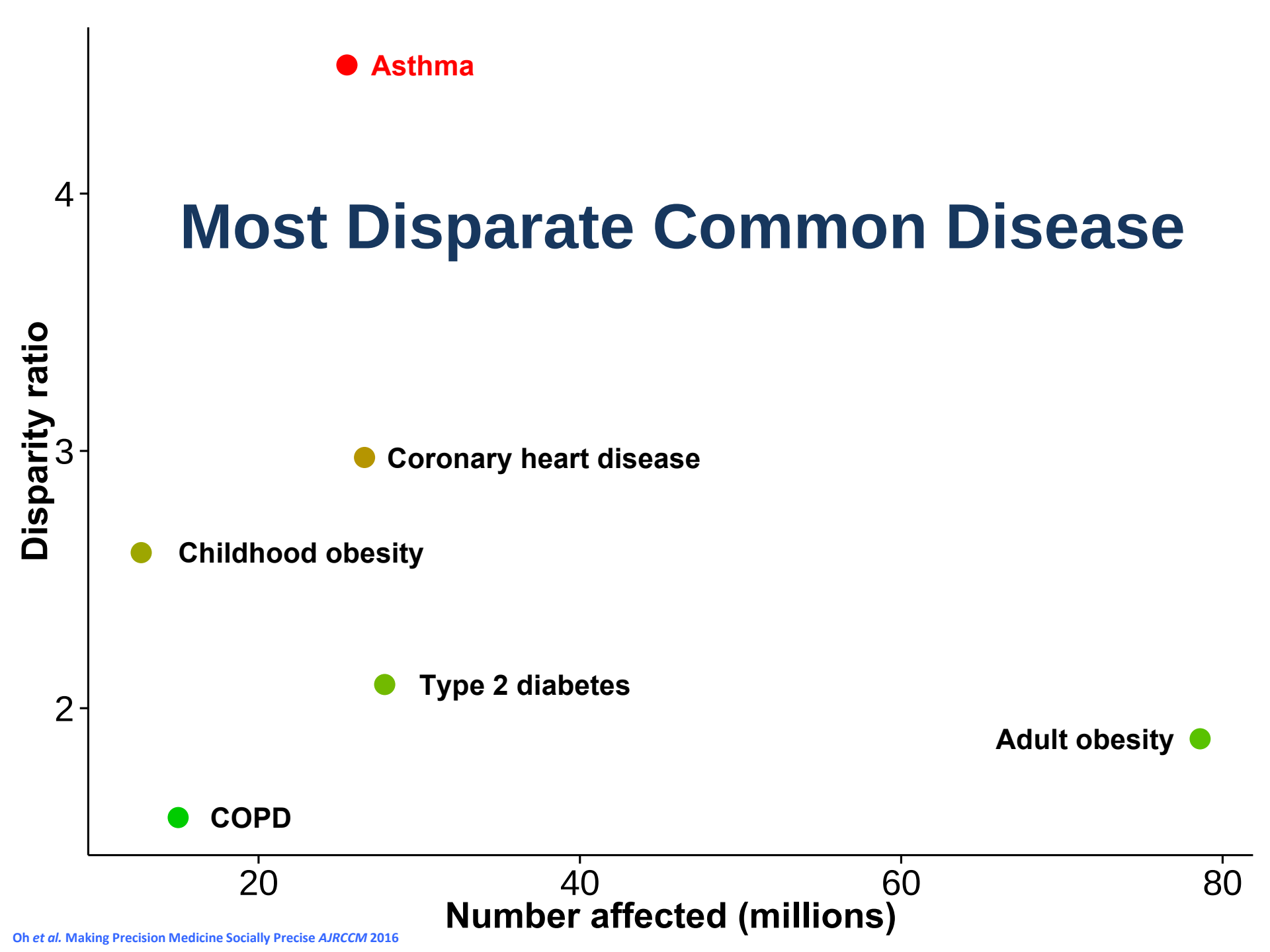
Marquitta White, MS, PhD
marquitta.white@ucsf.edu

UCSF Asthma Collaboratory
Asthma Translational Genomics Collaborative

What is asthma?

- Tightening of Airways
- Airway Remodeling
- Thick Mucous Production
- Acute and Chronic Phases
  - Wheezing
  - Coughing
  - Shortness of Breath

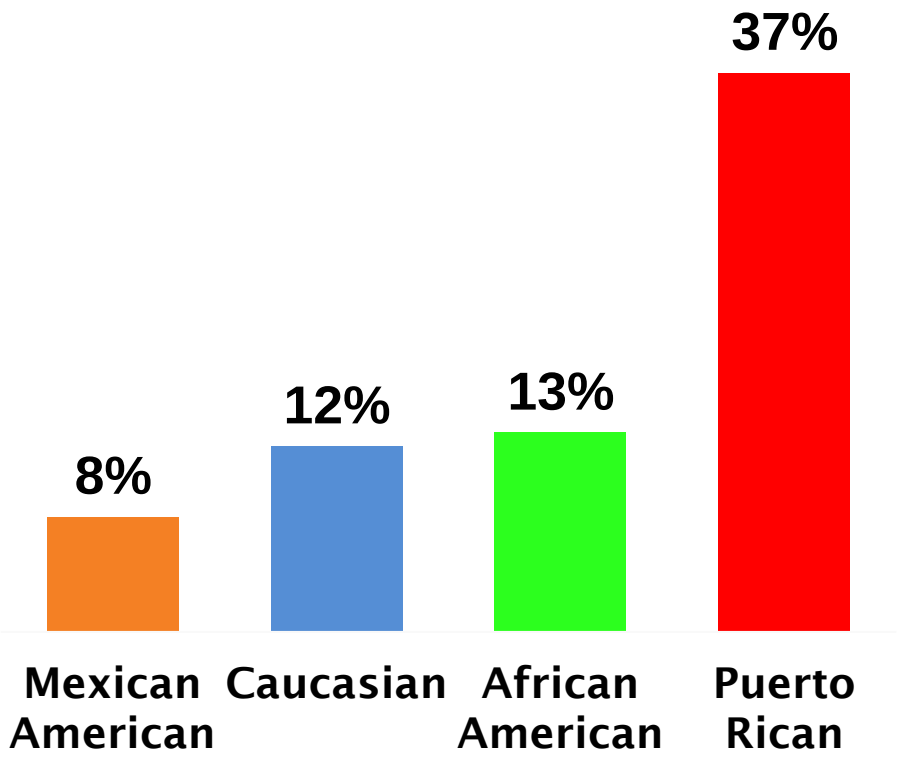




Oh *et al.* Making Precision Medicine Socially Precise *AJRCCM* 2016

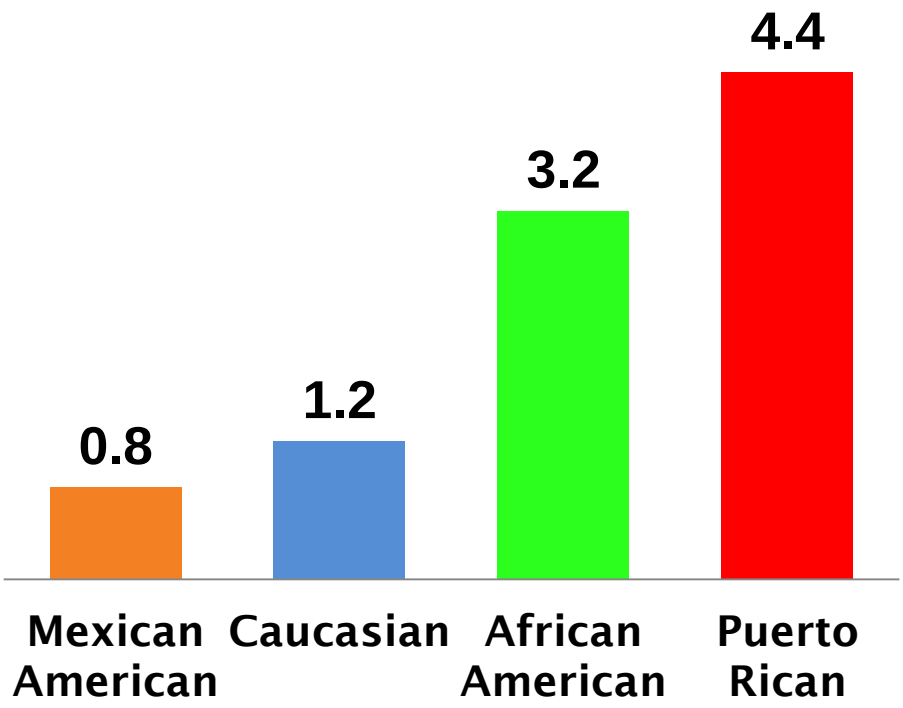
Asthma Health Disparty in the U.S.

Prevalence

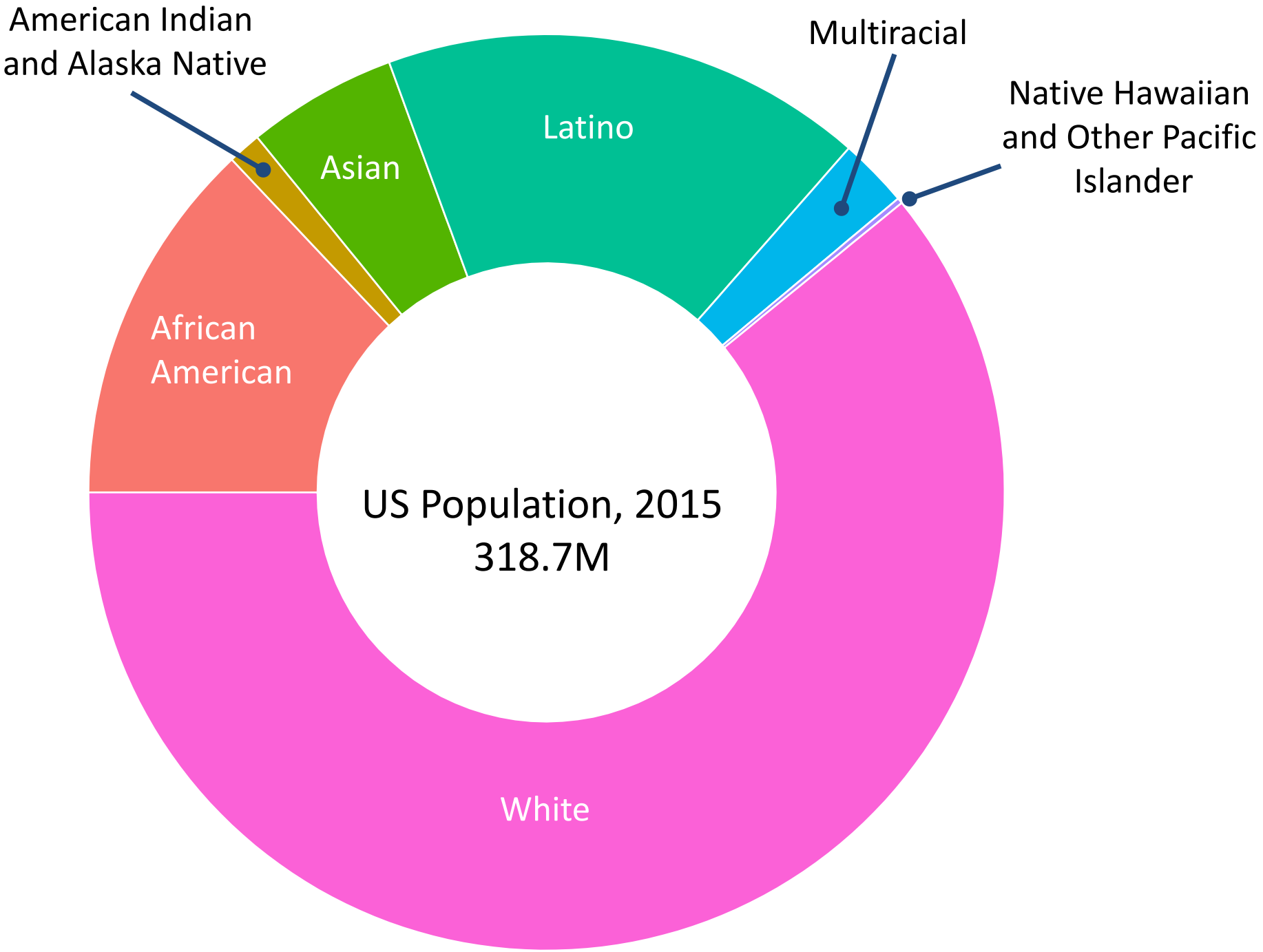


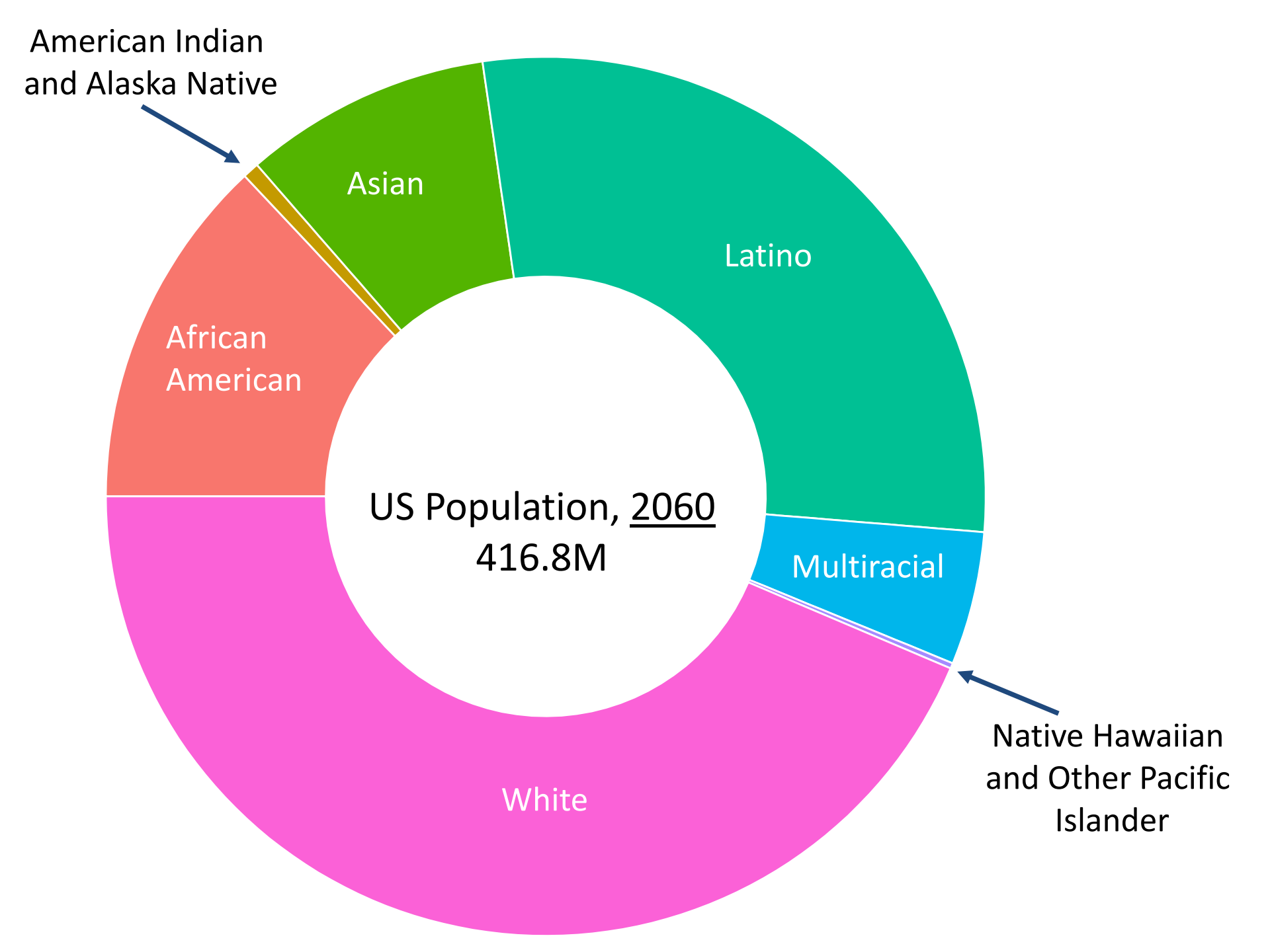
NHLBI Study of Latinos (SOL)
Barr et. ai., *AJRCCM* 2016

Mortality



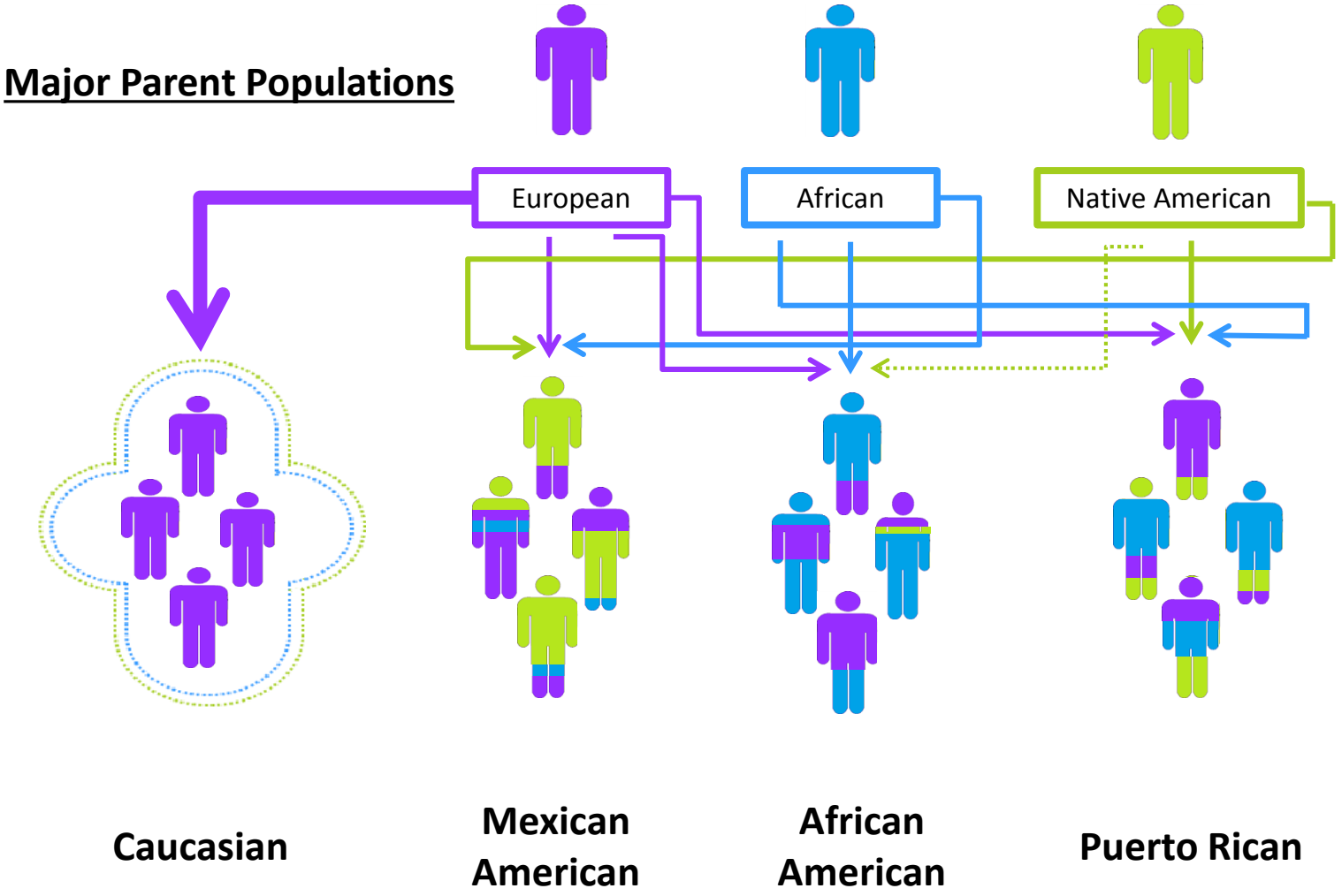
Akinbami L. CDC/NCHS


American Indian and Alaska Native
Asian
Latino
Multiracial
Native Hawaiian and Other Pacific Islander
African American
US Population, 2015
318.7M
White

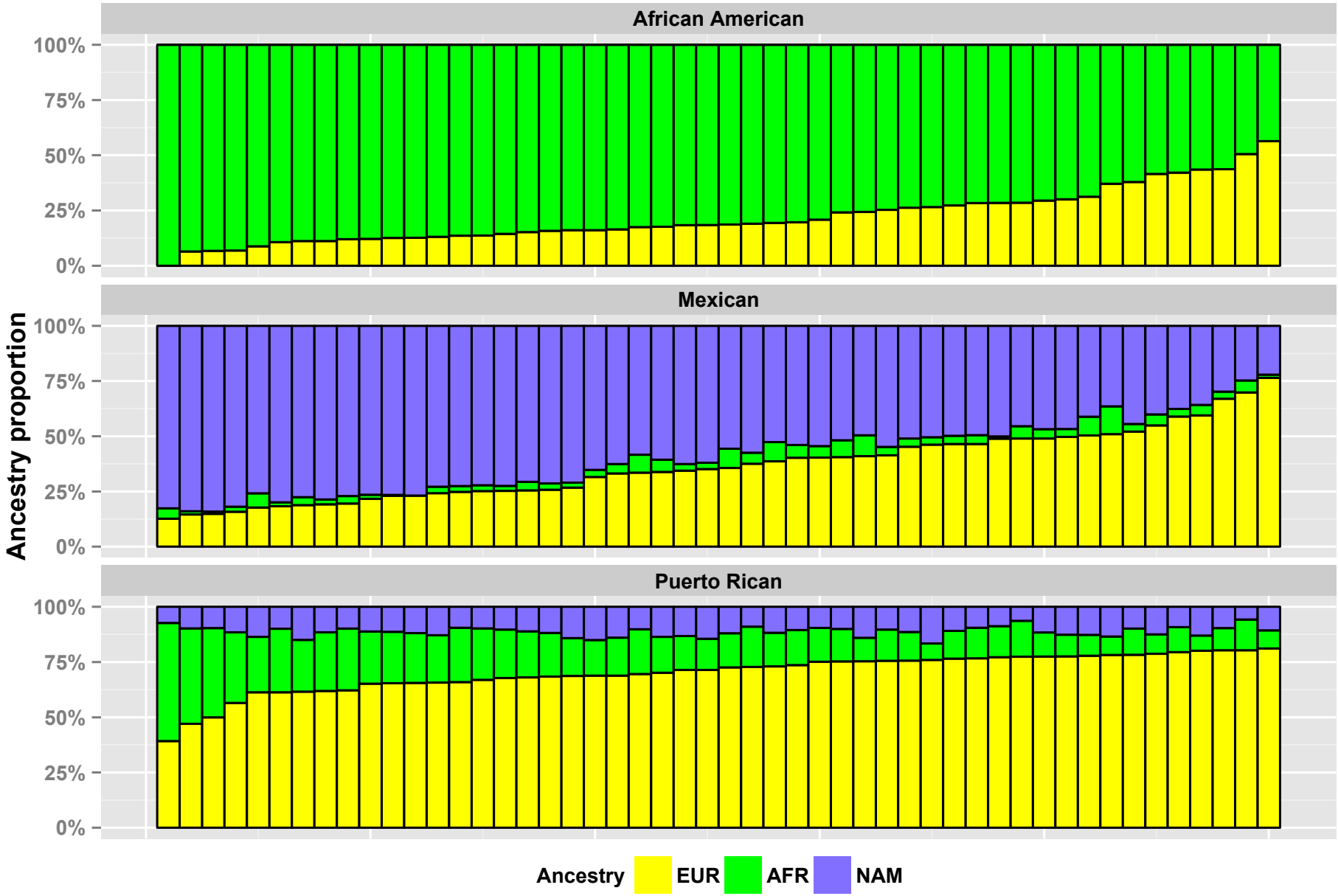

American Indian and Alaska Native
Asian
Latino
African American
US Population, 2060
416.8M
Multiracial
Native Hawaiian and Other Pacific Islander
White

Admixture, Ancestry and Health Disparities

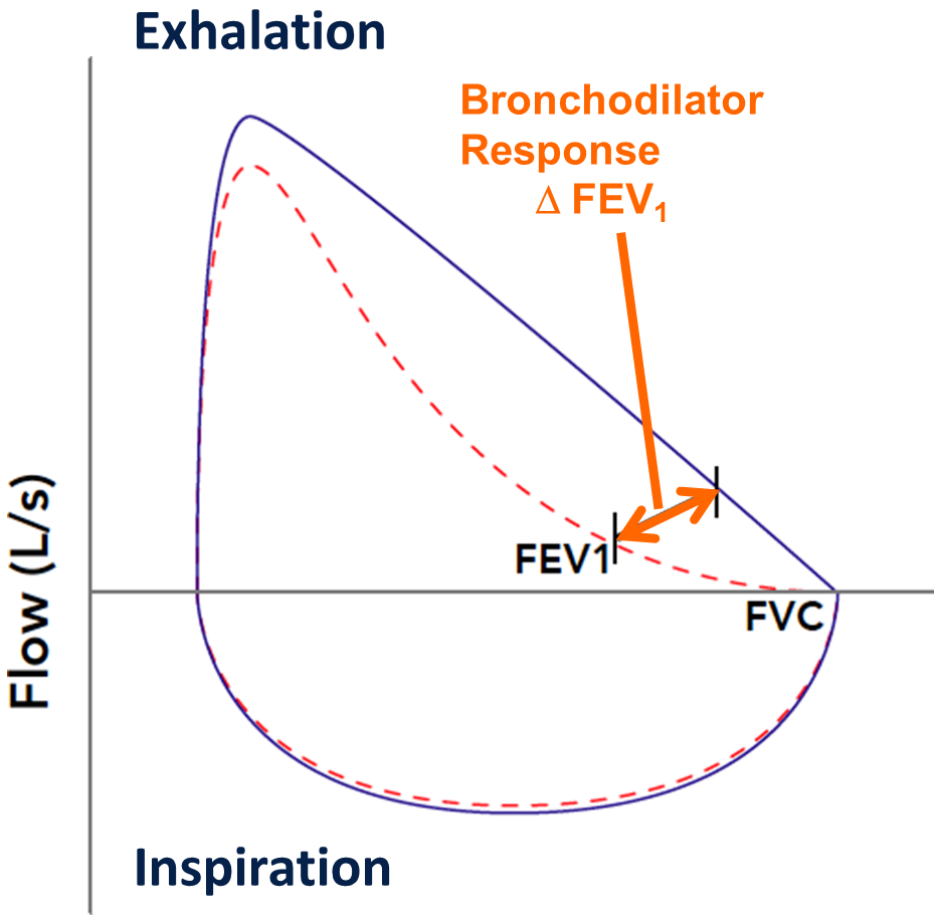
Admixture in the U.S.



Global Ancestry

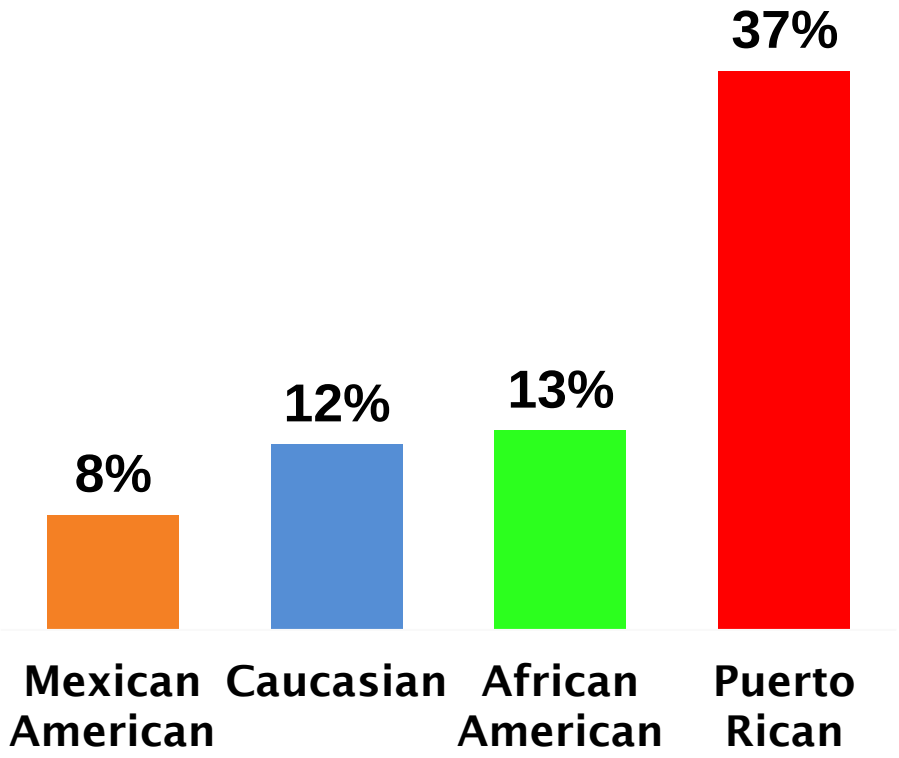


Albuterol


Exhalation
Bronchodilator Response
Δ FEV1
FEV1
FVC
Flow (L/s)
Inspiration

Flashback !!

Prevalence



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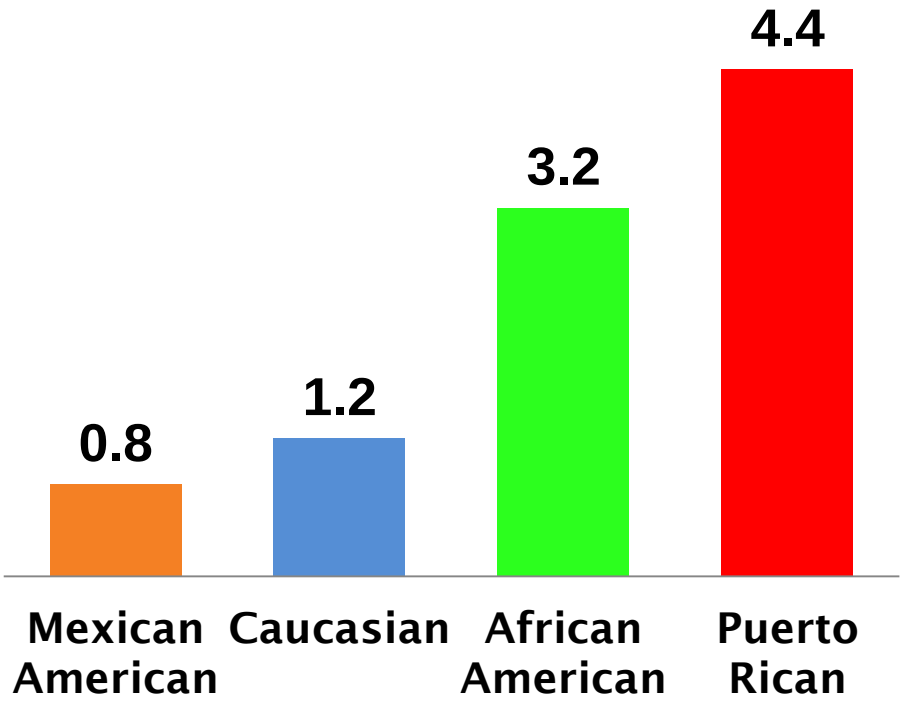
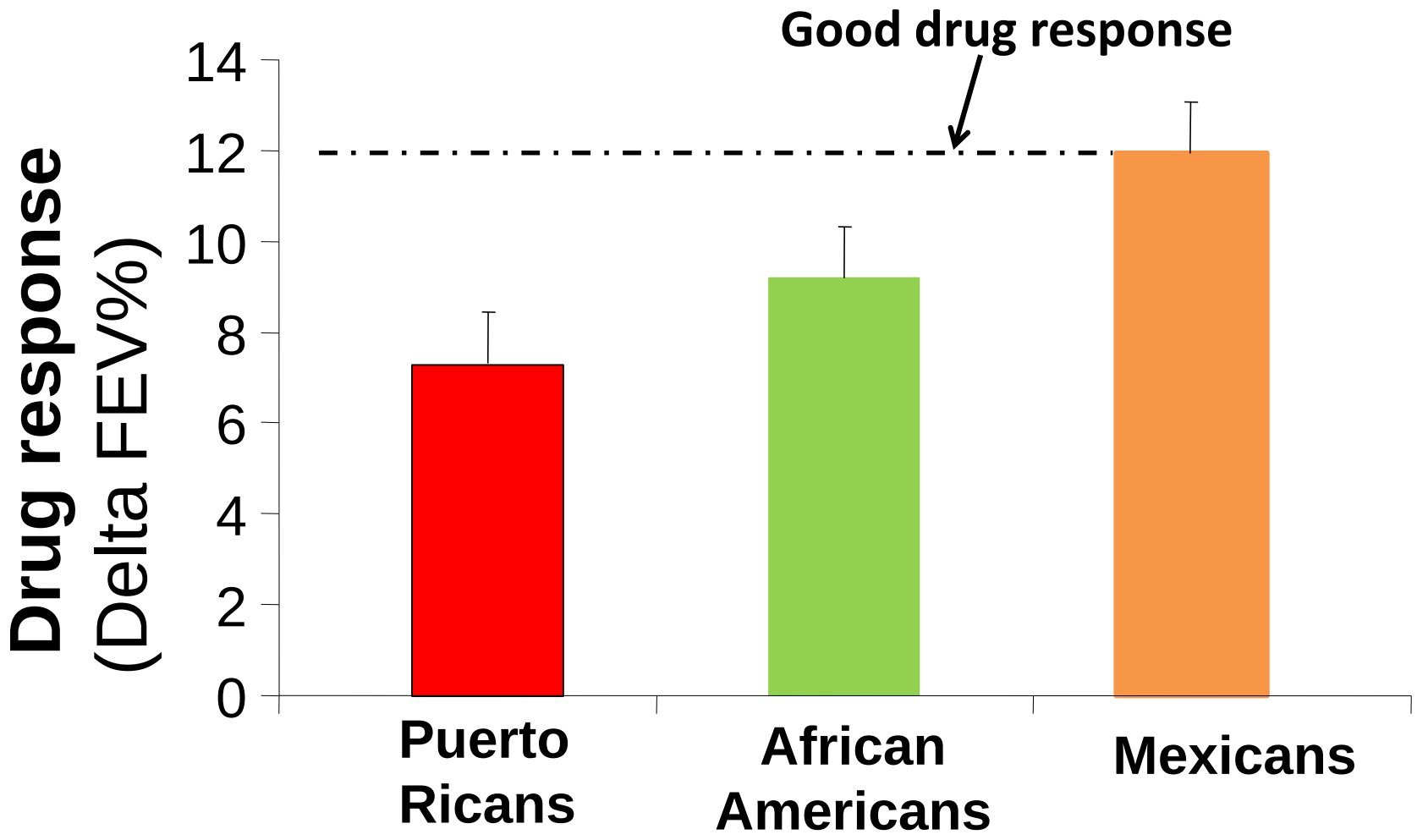


Akinbami L. CDC/NCHS

Variation in Drug Response may contribute to disparities



Naqvi et al. (2007) J. of Asthma

National Heart, Lung, and Blood Institute

Whole genome sequencing

1,500 minority children

Albuterol response

Study Populations: GALA & SAGE

1998 - present

Recruited > 10,000 Minority Children

Detailed Phenotype & Geocoded Data

Genomic

DNA (targeted, GWAS), RNA, methylation

Clinical

Drug response; neuroendocrine biomarkers; inflammatory cytokines; IgE; cotinine; medical history

Questionnaire

Exposures; behaviors; diet

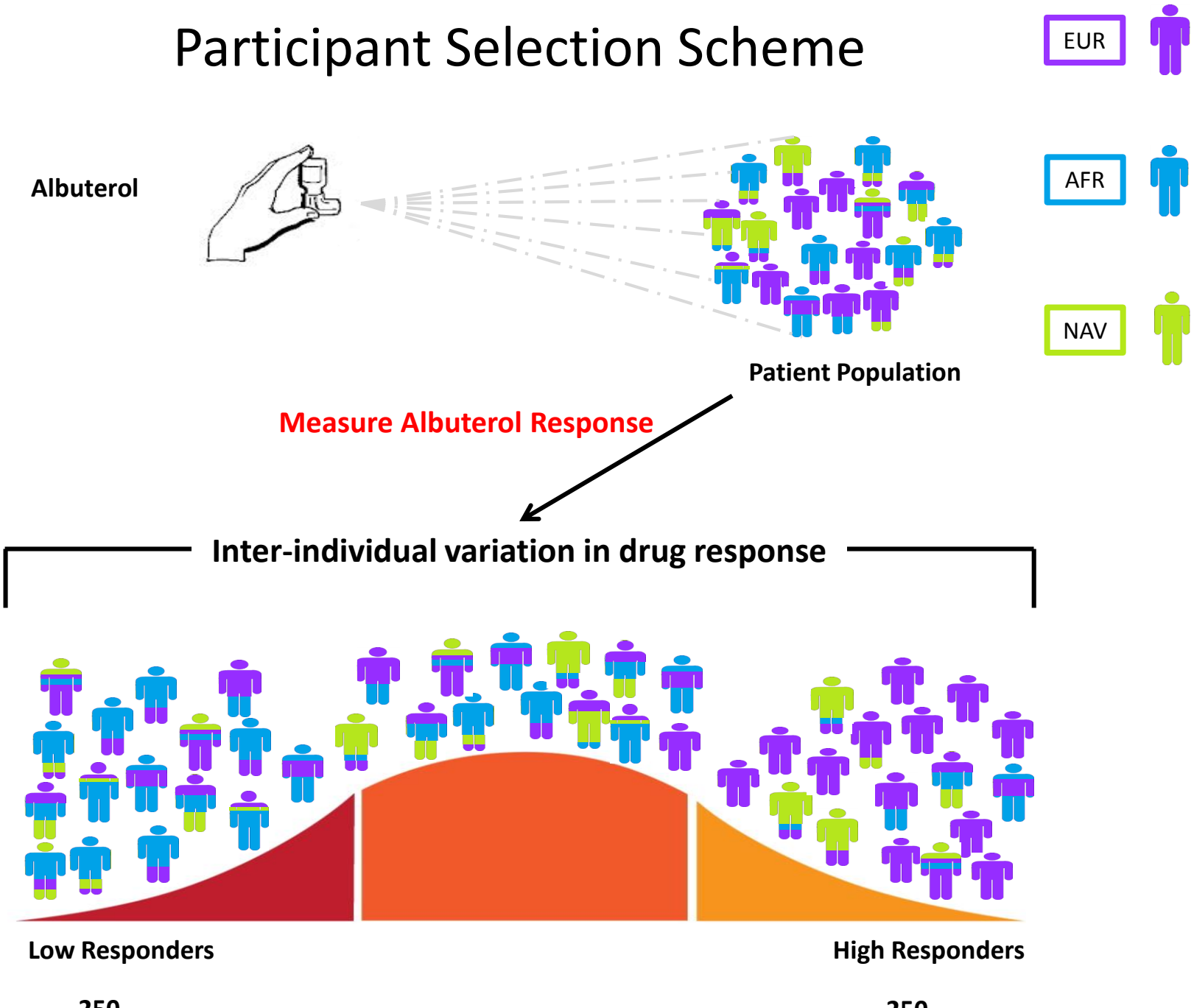
Socio-demographic

Perceived discrimination socioeconomic status; adverse childhood experiences

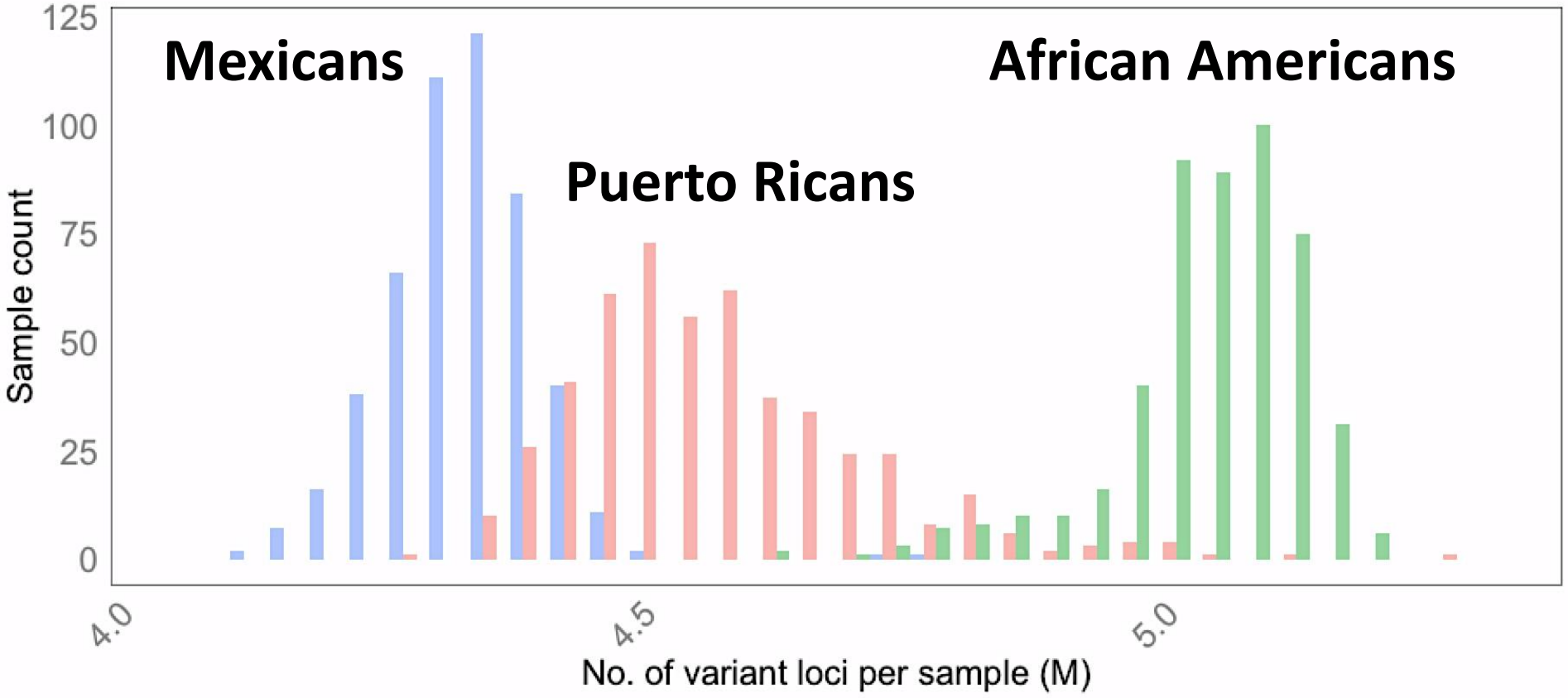
Built environment

Exposures linked to geocoded residential history (e.g., air pollution; neighborhood violence, Census tract characteristics); neighborhood deprivation; walkability

Participant Selection Scheme



4.1 – 5.3 million variants per sample



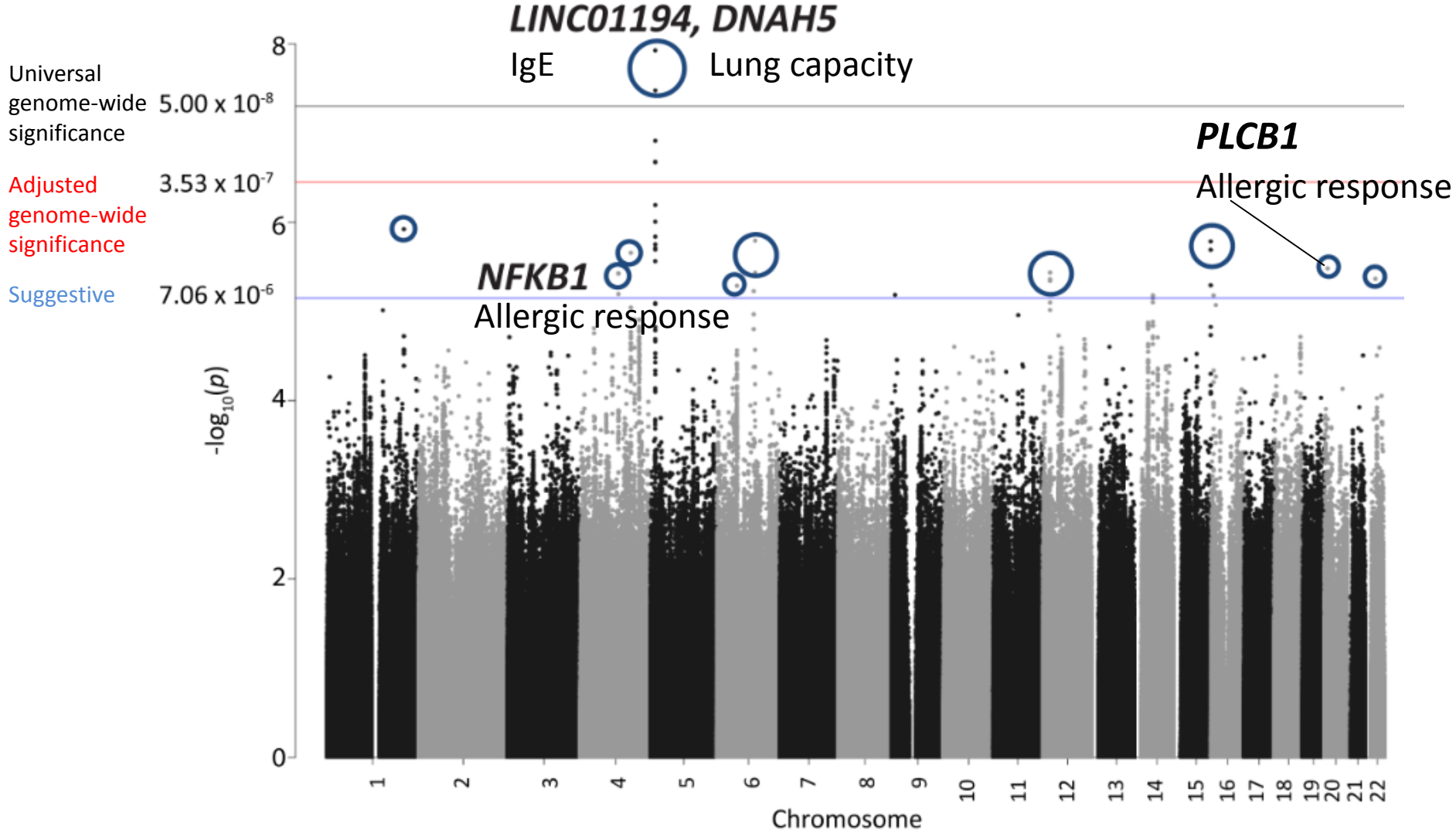
66 million loci jointly called in 1484 samples
(PASS FILTER)

Identifying BDR-associated loci

Common variant	Common and rare variant
Logistic regression	*SKAT-O, 1kb, 500bp increment*
PR	PR
MX	MX
AA	AA
Meta-analysis	Combined*

BDR = Genotype + Age + Sex + BMI category + 10 PCs (+ Local ancestries*)

Top 10 loci from trans-ethnic meta-analysis



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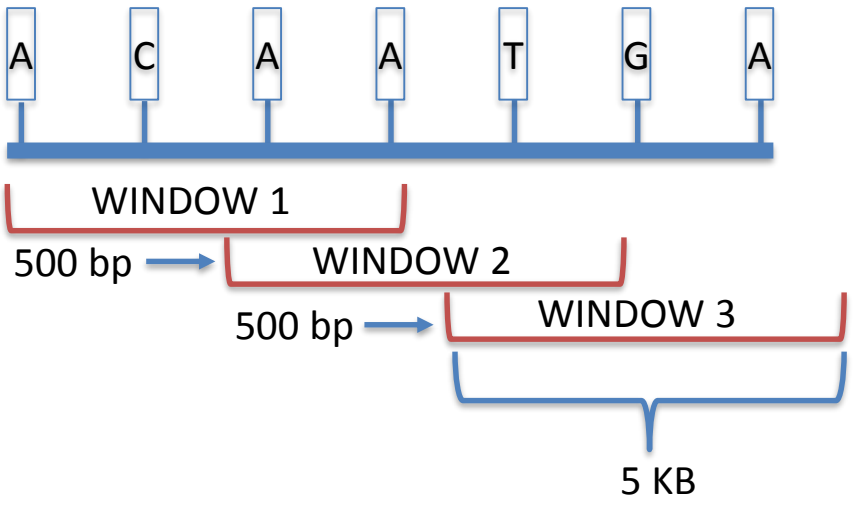
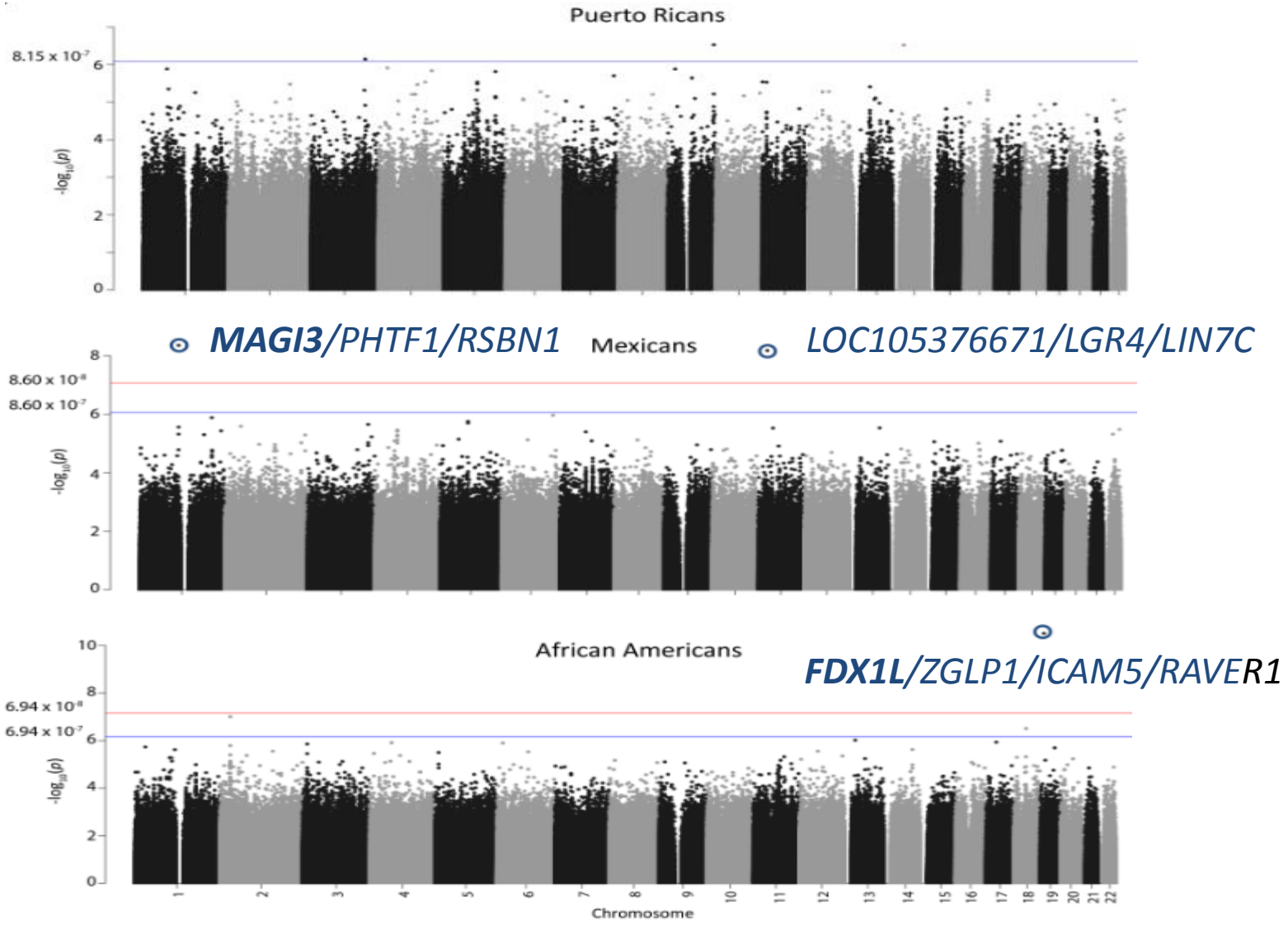
Rare Variant Analyses

Method : SKAT-O

- Data Collapsing Method
- Computationally feasible for WGS data
- Optimized for variants with differing effect sizes and directions



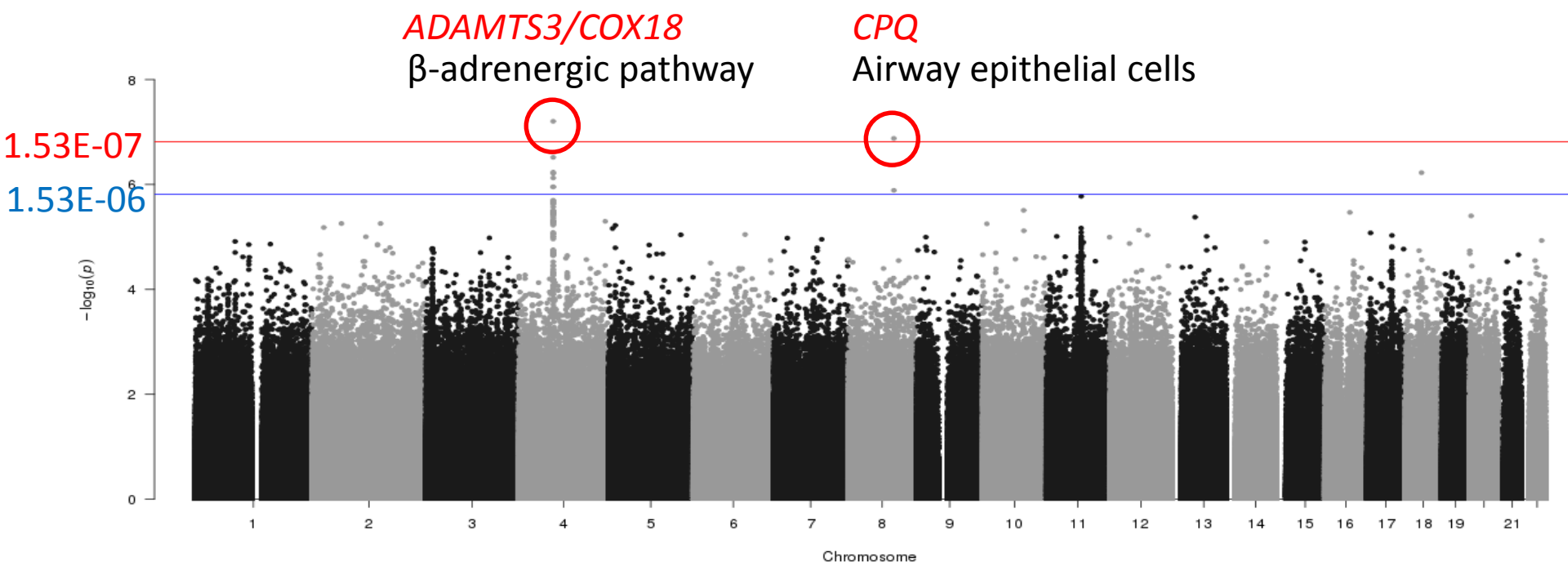
Population-specific BDR association



Adjusted genome-wide significance

1kb window, 500bp increment

BDR association shared across populations



BDR ~ GT + age + sex + BMI.cat + 10PC + local.AFR + local.NAM

1kb window, 500bp increment

Adjusted genome-wide significance

Analysis strategy

Common variant

Logistic regression

PR

MX

AA

Meta-analysis

Common and rare variant

SKAT-O, 1kb, 500bp increment

PR

MX

AA

Combined

Replication of 27 associated common variants

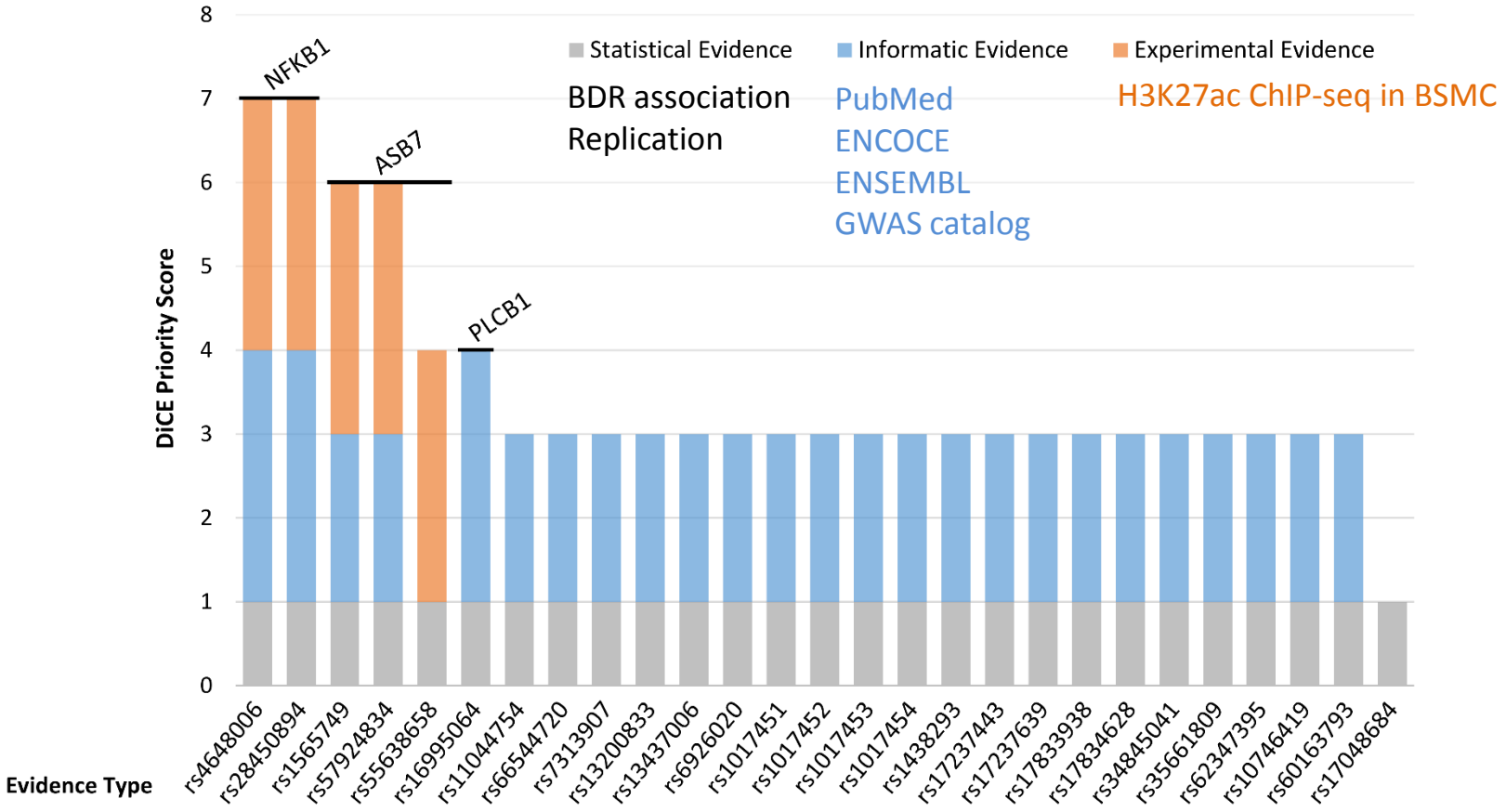
GALA I (108 PR, 202 MX), SAGE I (141 AA), HPR (414 PR), SAPPHIRE (1,022 AA), CHOP (281 AA)

Perform ChIP-seq to identify H3K27ac peaks in bronchial smooth muscle cells

Prioritize associated loci for further analyses

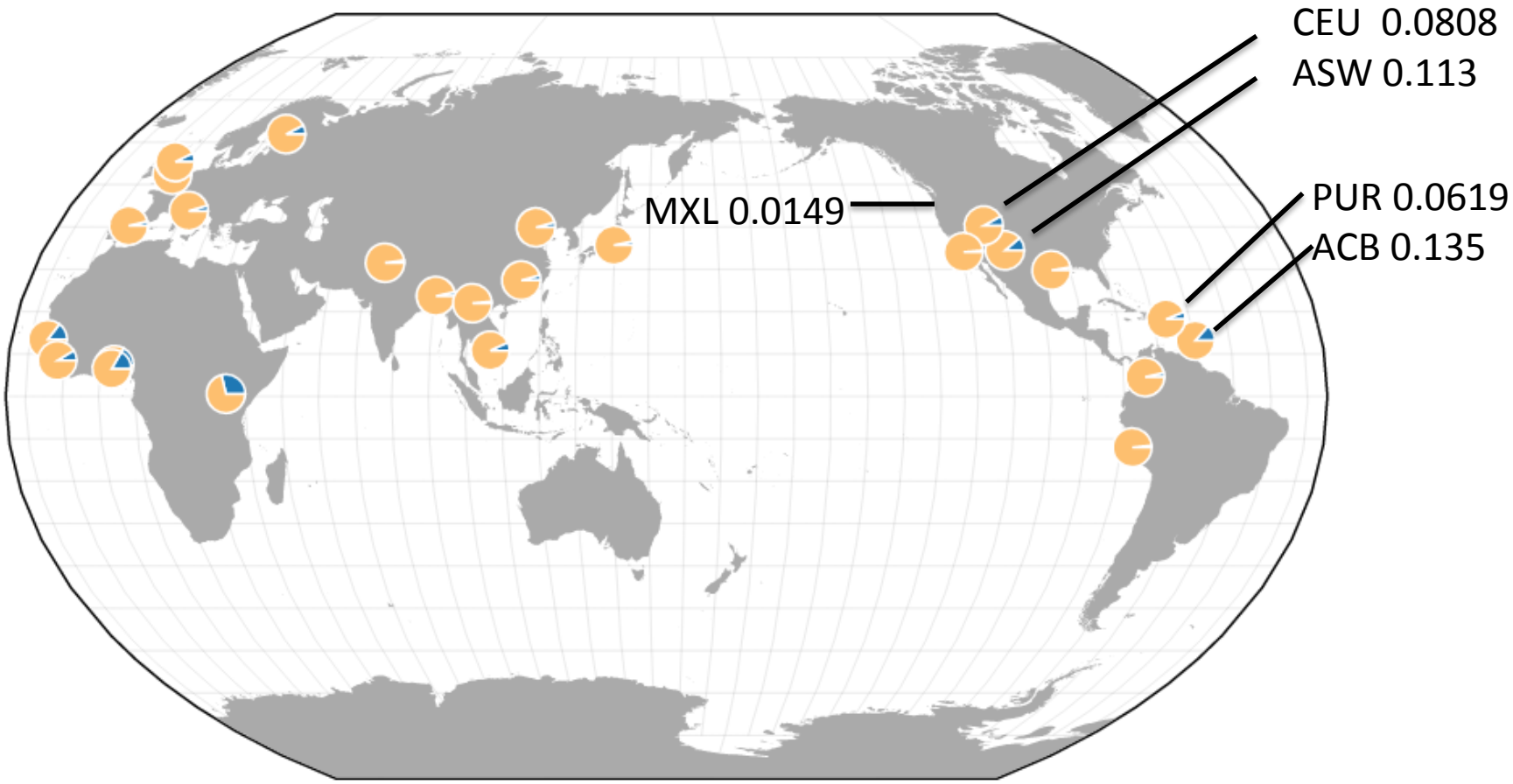
DiCE Priority Score

Diverse Convergent Evidence for variant prioritization



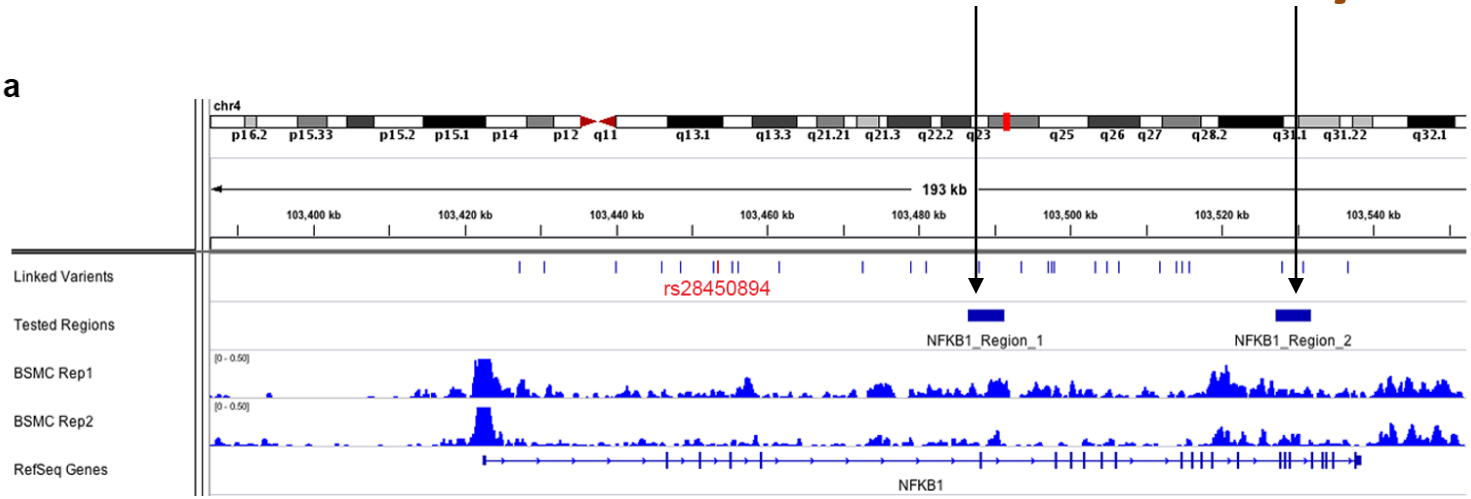
NFKB1 low BDR allele tracks with African ancestry

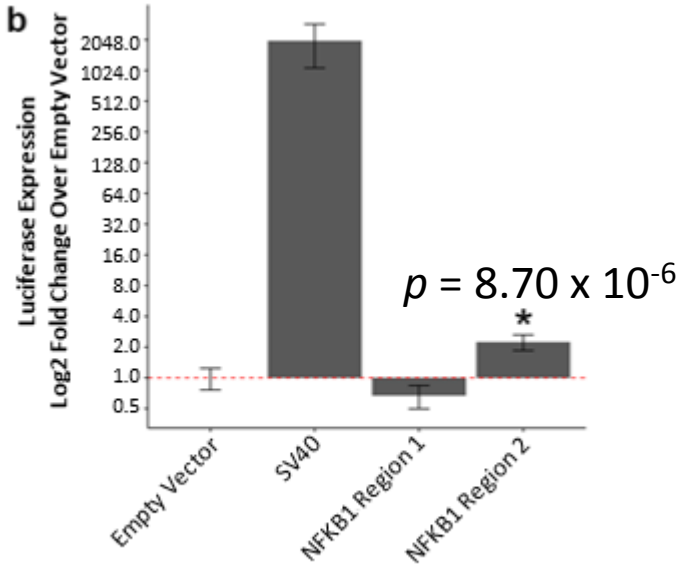
rs28450894 T/C



The minor allele is the low BDR allele

Potential enhancer activity





RNA-seq

- Do BDR-associated SNPs in *NFKB1 region* (rs28450894) regulate gene expression of *NFKB1* or neighboring genes?

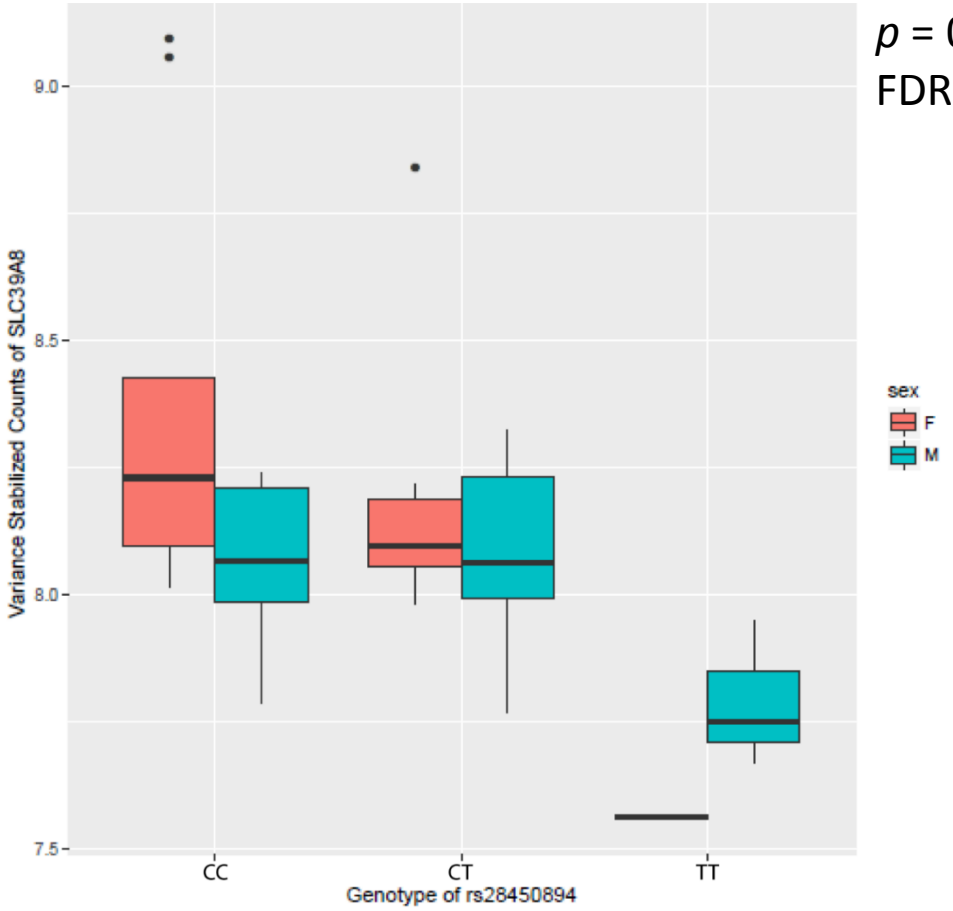
RNA-seq samples

- 39 African American samples
- Whole blood
- Genes within 1Mb of rs28450894 (13 genes)

BDR	No. of copies of low BDR-associated allele at rs28450894	No. of samples
High	0	8
	1	8
	2	1
Low	0	10
	1	9
	2	3
Total		**39**

150 bp paired-end, HiSeq4000, 4 lanes

eQTL for *SLC39A8*



p = 0.0066

FDR-adjusted p =0.0856

Gene expression = Genotypes + Age + Sex + Batch + GC + Genotype * Sex

SLC39A8 Expression

Gene Information

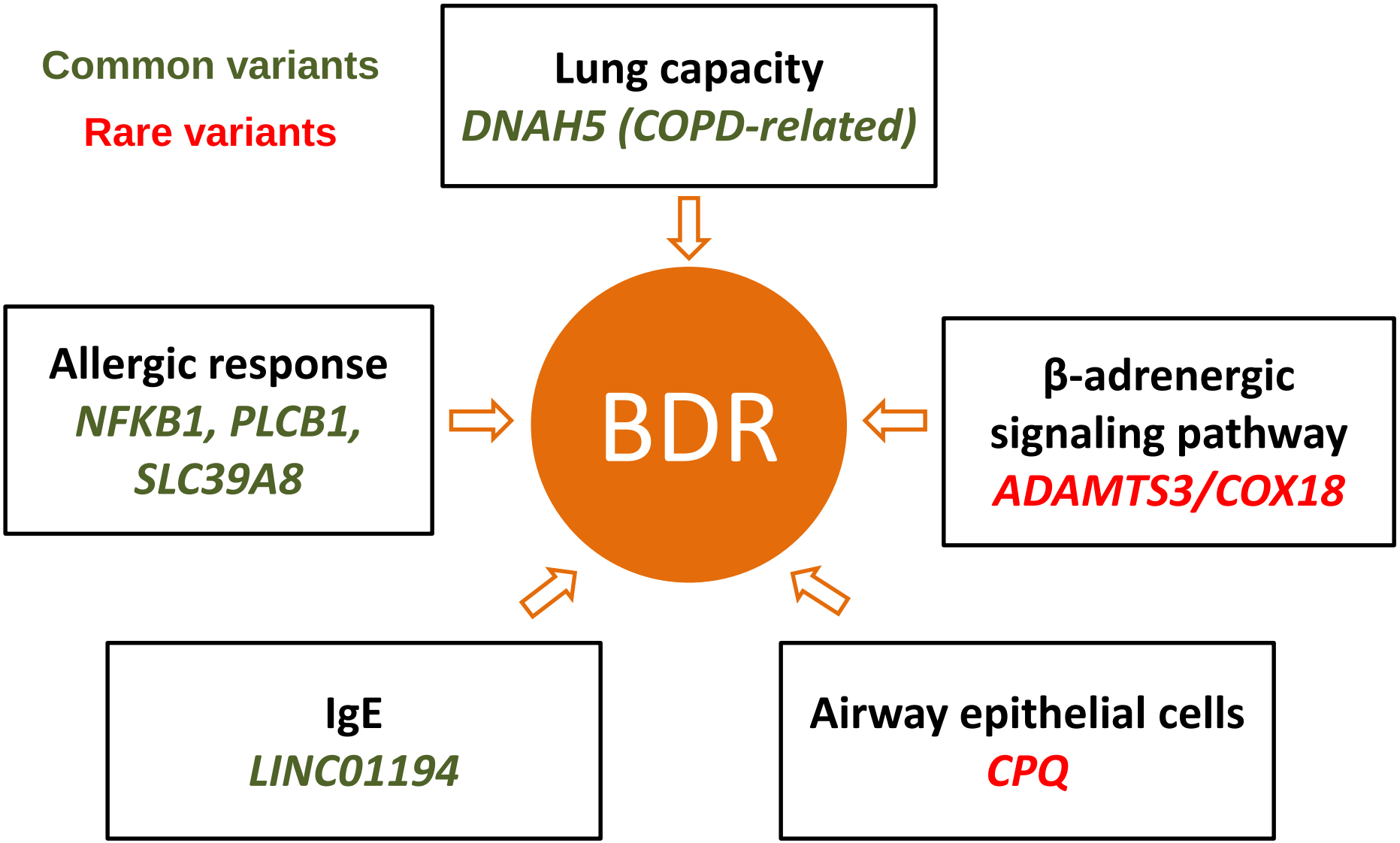
SLC39A8

- Transmembrane Zinc and Manganese transporter
- Mutations cause CDG2N (congenital disorder)
- SLC39A8 is also a regulator of IFN-γ

Potential Impact

- IFN-γ is a biomarker of severe asthma and is associated with asthma exacerbations
- *SLC39A8* may play a critical role in regulating intracellular zinc during inflammatory stress

BDR association summary



Summary

- Identified population-specific and shared BDR association
- Common variant analysis
  - Functionally validated *NFKB1* locus despite lack of statistical replication
  - Discovered potential impact of *SLC39A8* expression
- Common and rare variant analysis
  - Lack of WGS data for replication

Challenges and opportunities

- Sample size and power
- Understudied population
- Tools development
  - WGS analysis
  - Rare variant analysis
  - Admixed population

Future work

- Manuscript in revision
- Gene / Pathway-based analyses
- Ancestry-specific gene expression using RNA-seq data

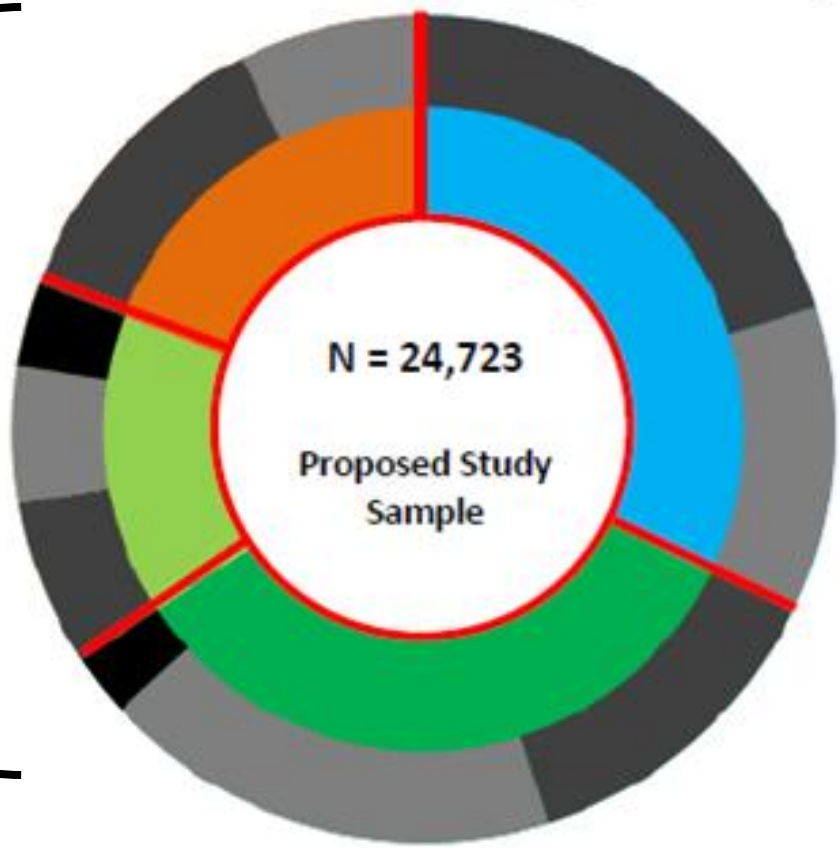
INCREASED SAMPLE SIZE and INCREASED REPLICATION RESOURCES

- NHLBI X01 Mechanism

Next Frontier in Asthma Genetics

Figure 1. Distribution of Asthma Status by Racial/Ethnic Group in Proposed Study Population

NHLBI X01
N= 18,000



European American
African American
Mexican American/ Other Latino
Puerto Rican

Asthma Control
Asthma Case
Parents (Trio Studies)

† Inner ring represents % of each racial/ethnic group in the study cohort

‡ Outer ring represents % of individuals of individuals with varying disease states within each racial/ethnic group

Team Science

UCSF

Esteban Burchard
Angel Mak
Kevin Keys
Zachary Szpiech
Ryan Hernandez
Noah Zaitlen
Dara Torgerson
Donglei Hu
Scott Huntsman
Sam Oh
Celeste Eng
Sandra Salazar
Walter Eckalbar
Nadav Ahituv

NEW YORK GENOME CENTER

Soren Germer
Karen Bunting
Robert Darnell
NYGC team

Hospital Universitario N.S. de Candelaria

María Pino-Yanes

NIH National Heart, Lung, and Blood Institute

TOPMed

TOPMed team

National Jewish Health®

Max Siebold