Role of Genomics and Environment in Childhood Asthma in African-Americans

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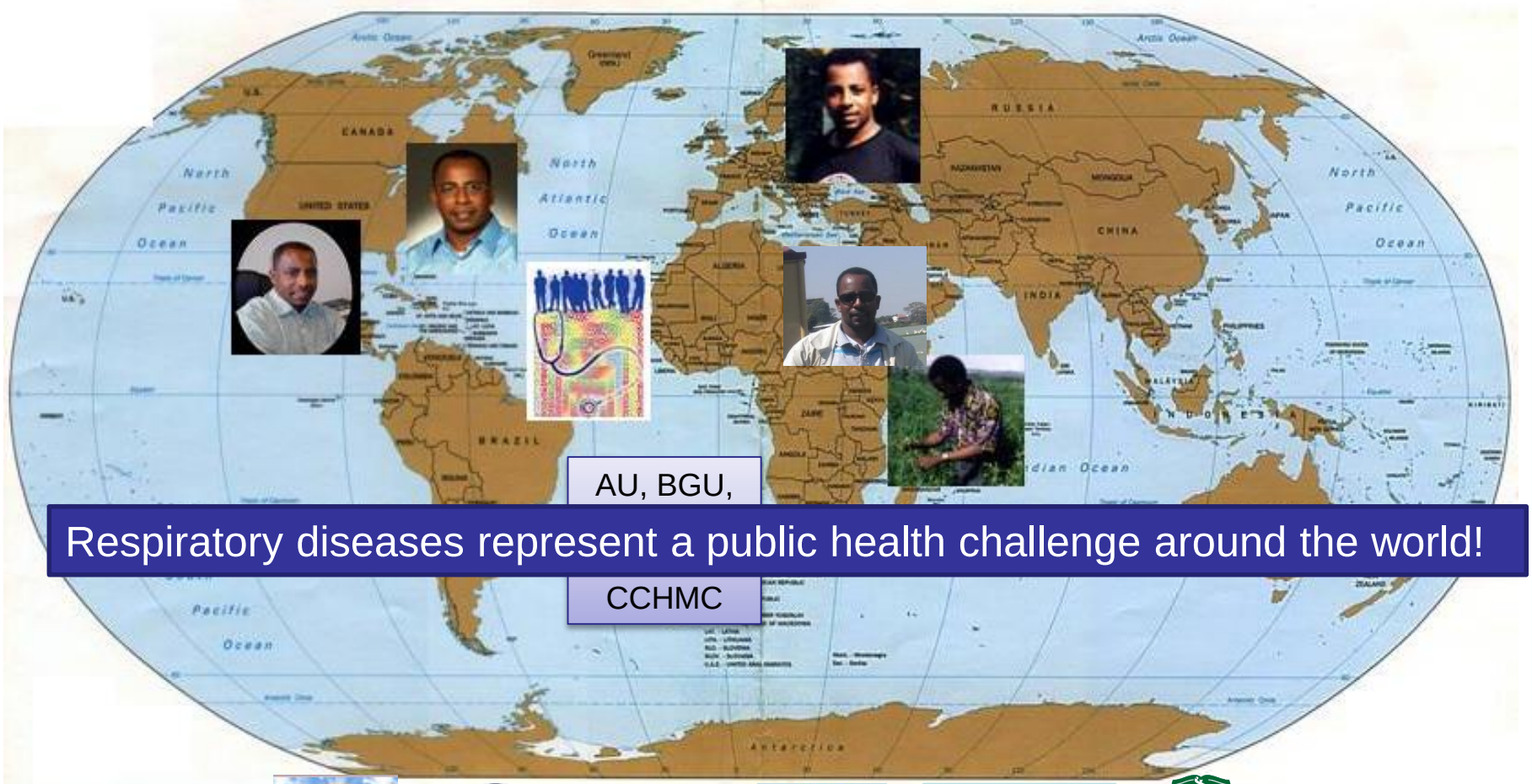

AU, BGU,

Respiratory diseases represent a public health challenge around the world!

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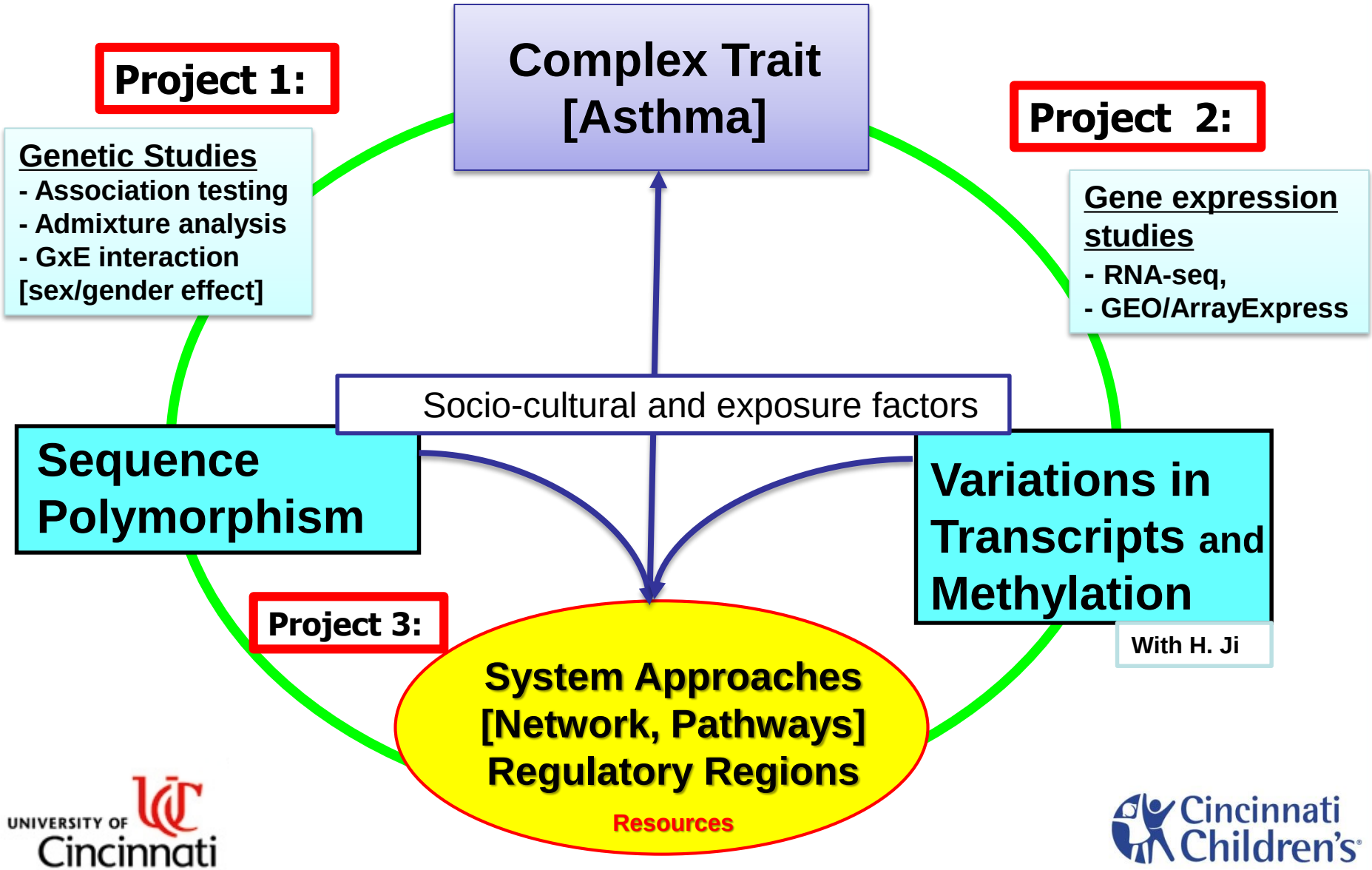
Outline

- Asthma prevalence within and across race
- Genetic determinants and racial differences in asthma
  - Candidate genes
  - Genome-wide
  - Admixture mapping
- Genetic ancestry and asthma-related genes
  - *AncestrySNPminer*…
- Role of environment in asthma [immigrant population]
- Concluding thoughts

Research Highlights



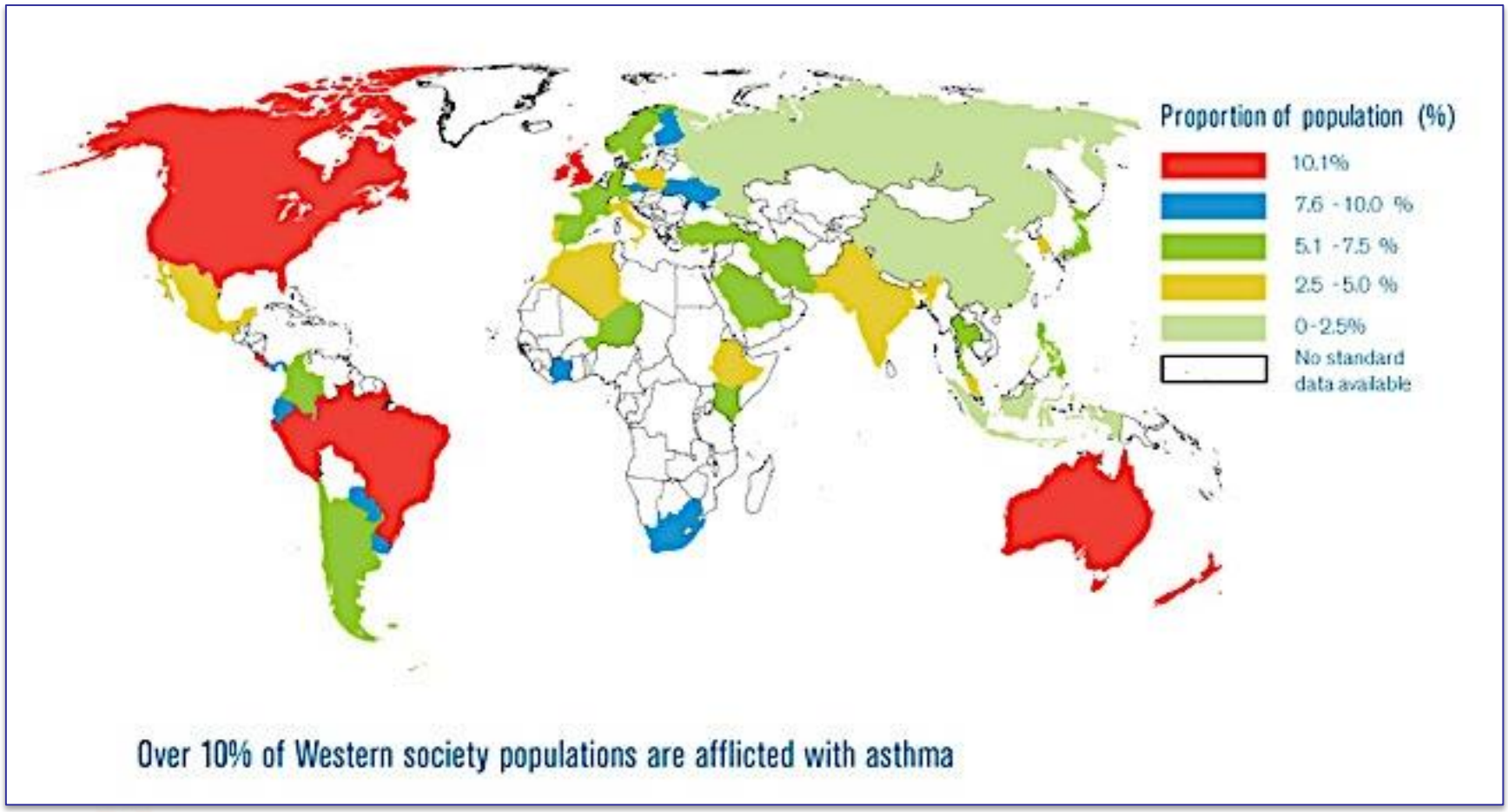
What is Asthma?

- Chronic disease of the airways that may cause:
  - Wheezing
  - Breathlessness
  - Chest tightness
  - Nighttime or early morning coughing
- Episodes are usually associated with widespread, but variable, airflow obstruction within the lung

Is asthma genetic or environment or both?

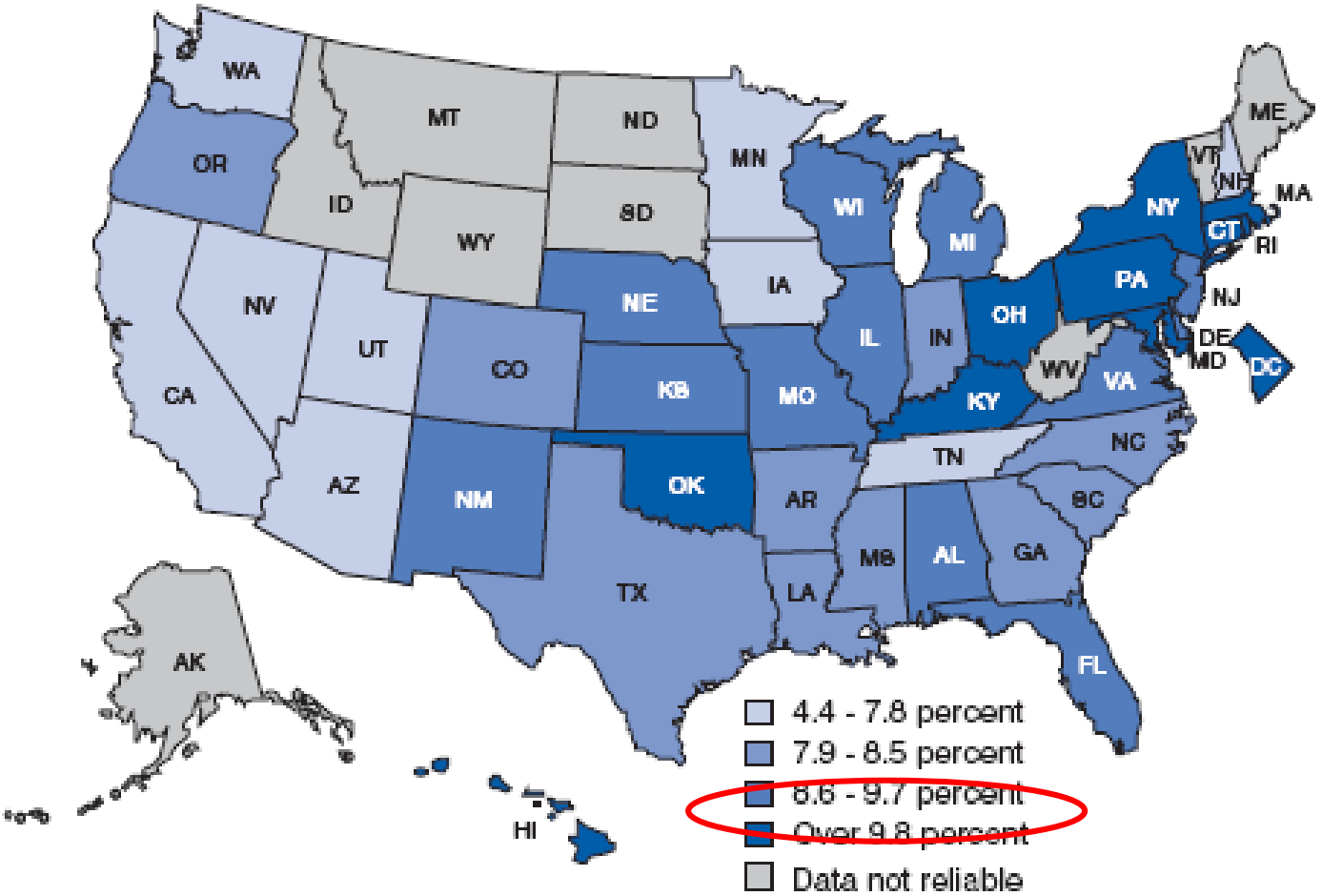
- Heritability estimates up to 79%
- Children with one asthmatic parent are **3-6 times** more likely to develop asthma than a child with two normal parents.
- Children with two asthmatic parents are **10 times** more likely to develop asthma than normal.
- Concordance rates in MZ twins are higher than DZ twins

Global burden of asthma



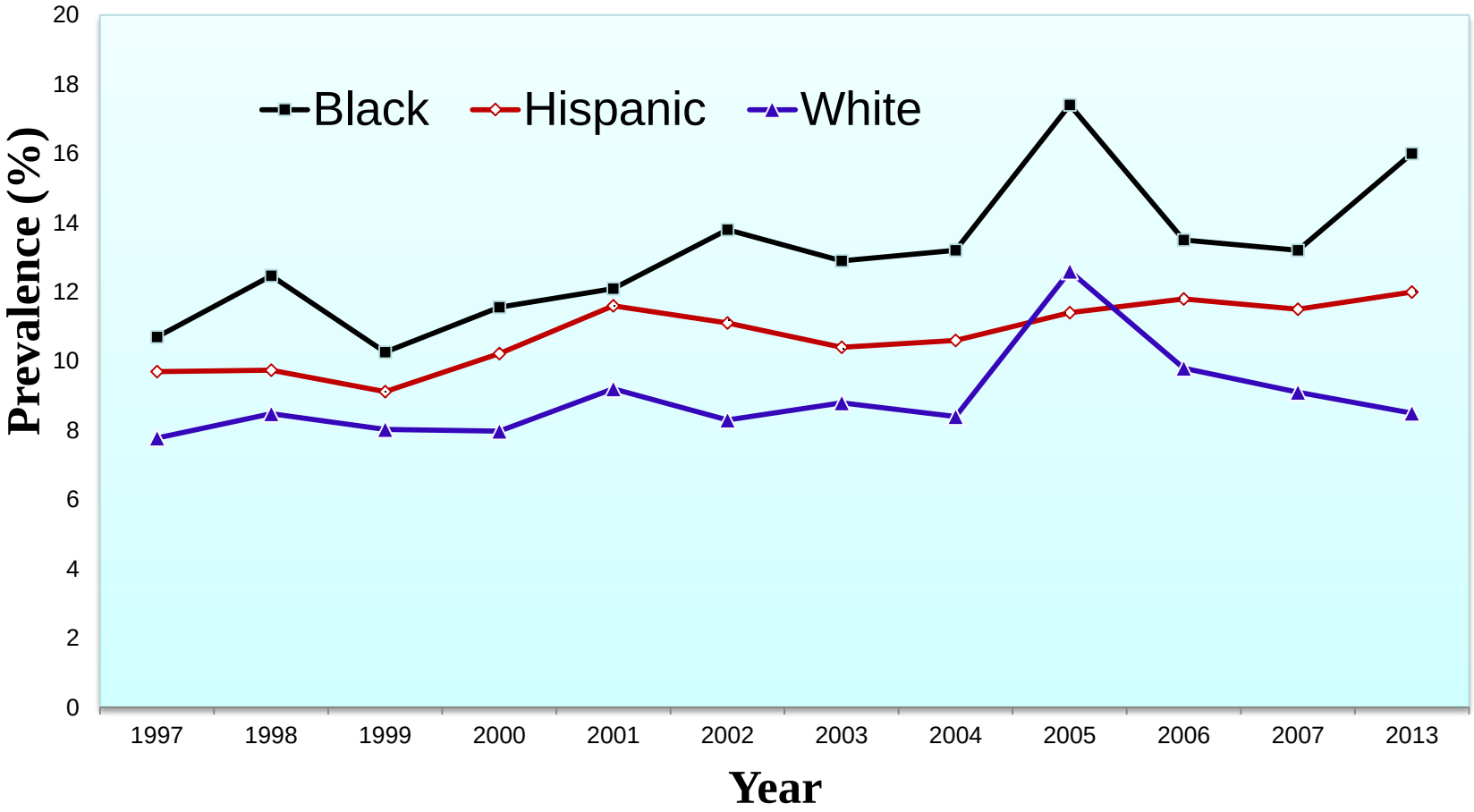
Over 10% of Western society populations are afflicted with asthma

Prevalence of asthma in the United States



Ohio Statewide Asthma Plan: Stated Goal- “Facilitate research on asthma in Ohio. Research translated to the clinic is critical to expand current knowledge and improve asthma health.”

Asthma Prevalence Differ by Race



Source: National Health Interview Survey; National Center for Health Statistics, *www.cdc.gov/nchs/nhis.htm*
The American Thoracic Socity, AJTCM, 2012

Asthma Prevalence Differ by Gender

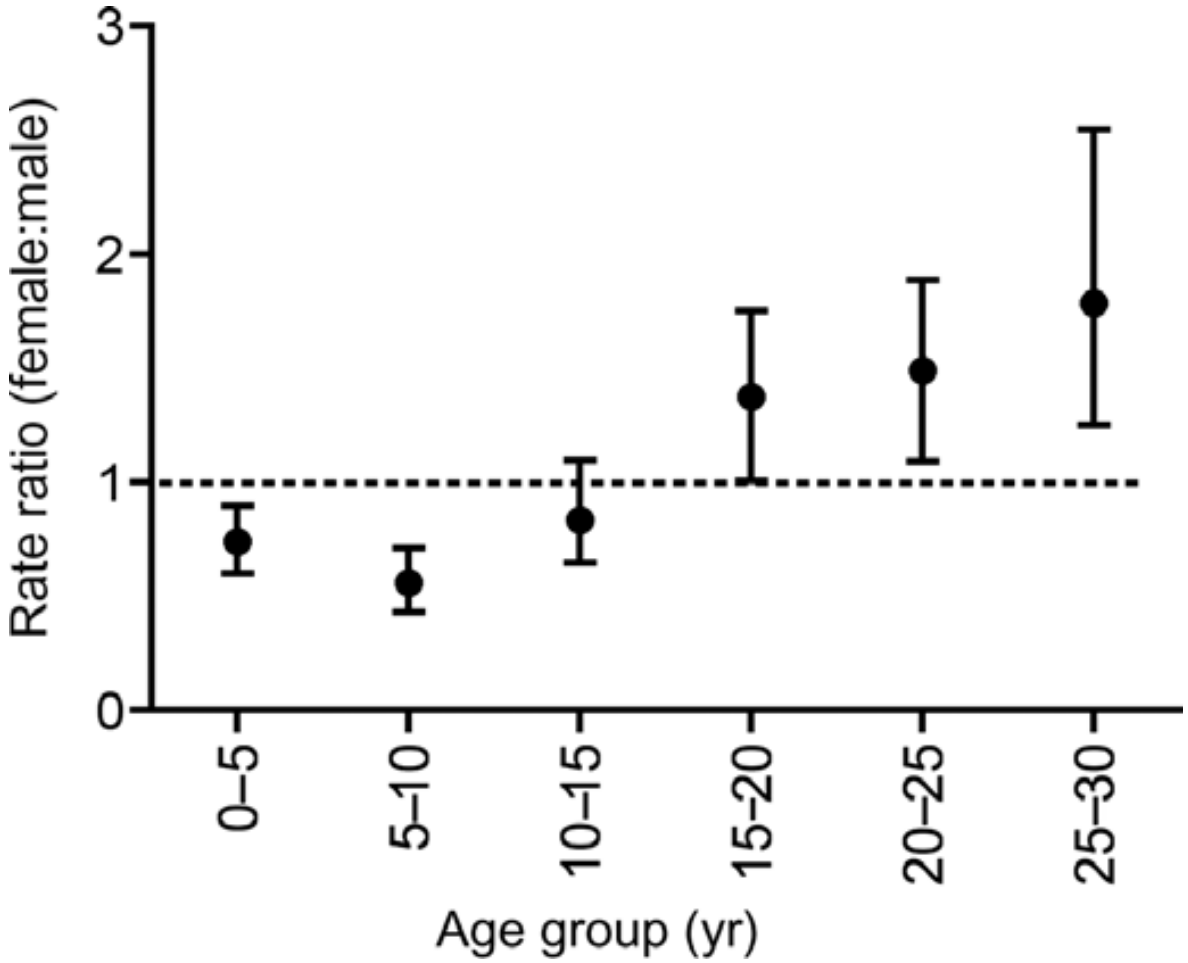
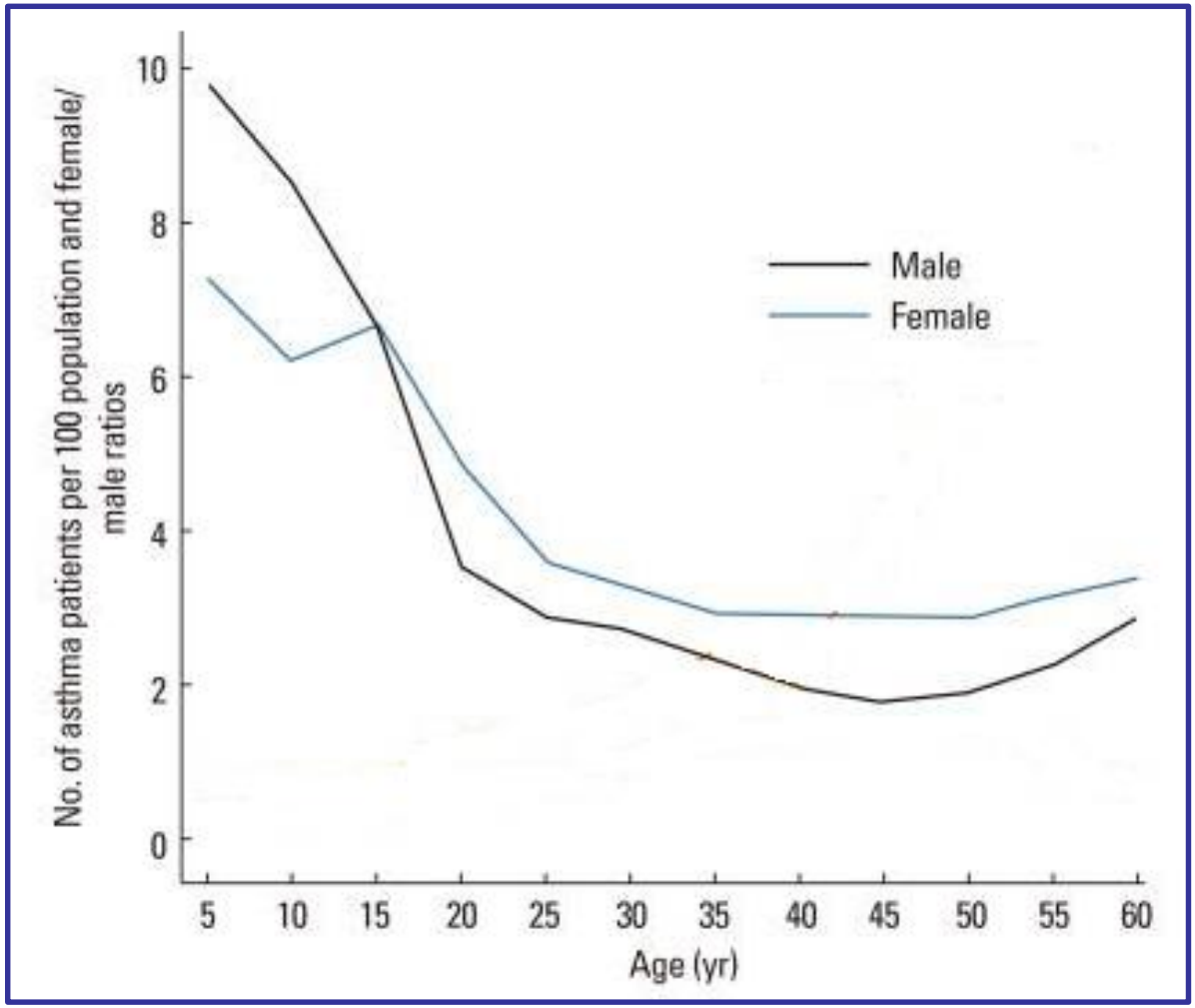


Figure . Female-to-male age-specific ratios for development of asthma. Bars represent 95% confidence intervals. Am J Respir Crit Care Med Vol 162. pp 68–74, 2000.

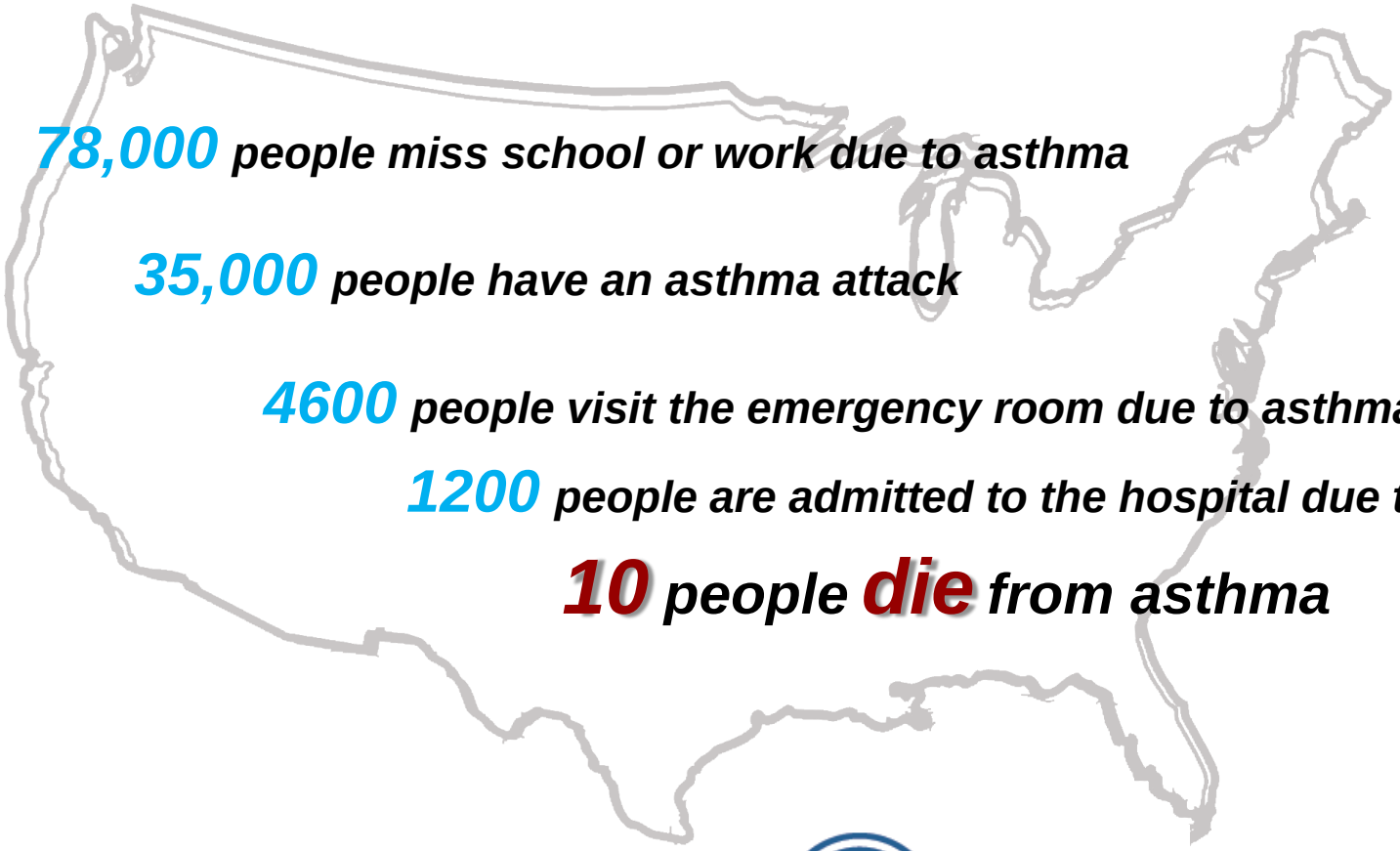
Gender "Flip-flop" in asthma prevalence



Allergy Asthma Immunol Res. 2011 April; 3(2): 74–80.

Asthma: Social and Economic Cost

Every day in America approximately…



78,000 ***people miss school or work due to asthma***

35,000 ***people have an asthma attack***

4600 ***people visit the emergency room due to asthma***

1200 ***people are admitted to the hospital due to asthma***

10 ***people*** ***die*** ***from asthma***

Economic cost: **56 Billion**

American Lung Association.
www.lungusa.org

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What are the risk factors of asthma?

Unmeasured biological variables

- Air quality
- Obesity
- stress

- Genetics
- Socioeconomic status
- Health maintenance behaviors

Unmeasured environmental variables

Up to 60,000 trucks/day on major interstate

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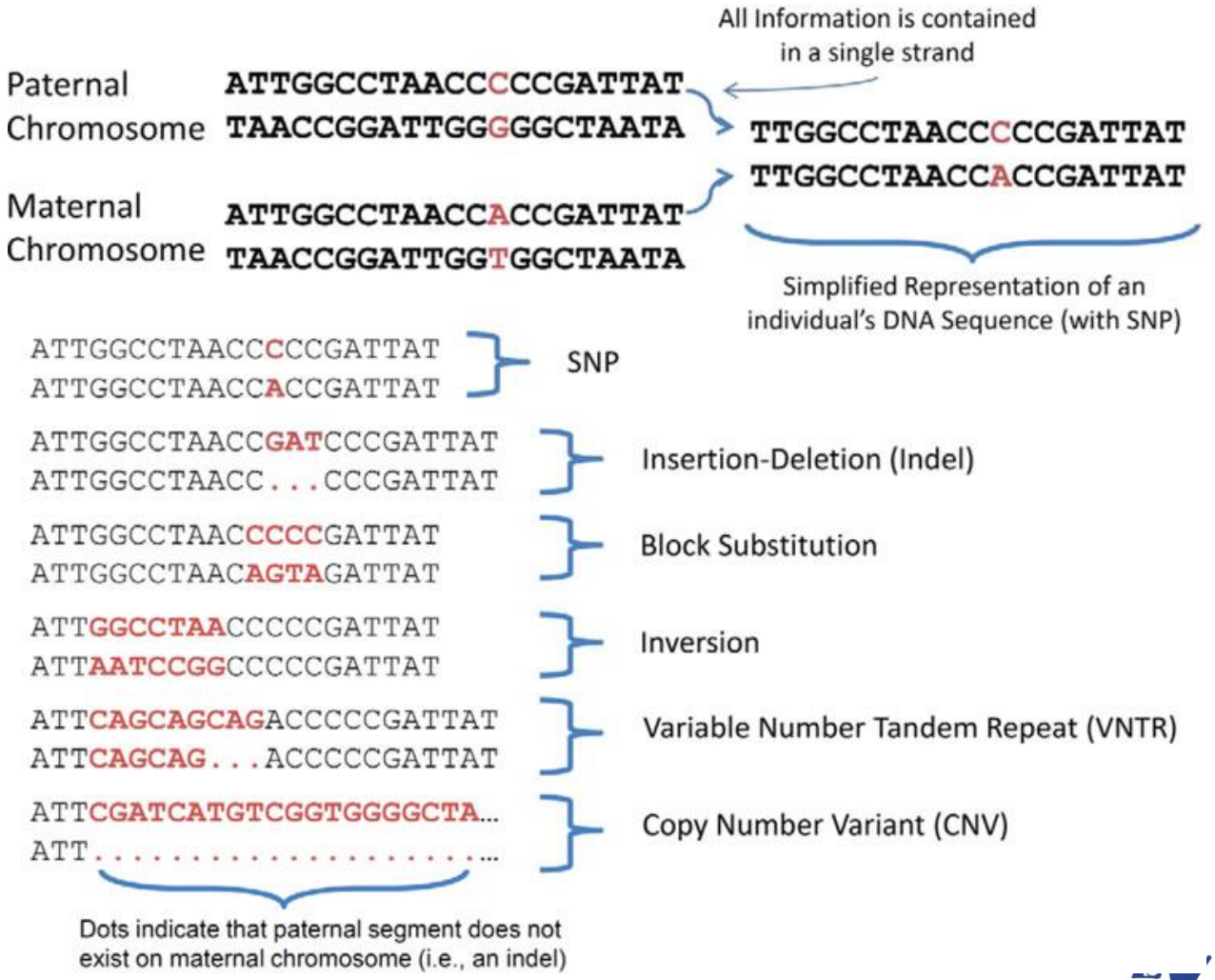
Candidate Gene Association Analysis

Common forms of DNA variation

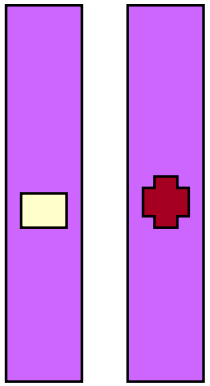


Candidate gene association study design

Control

Case
asthmatic

Allele 1

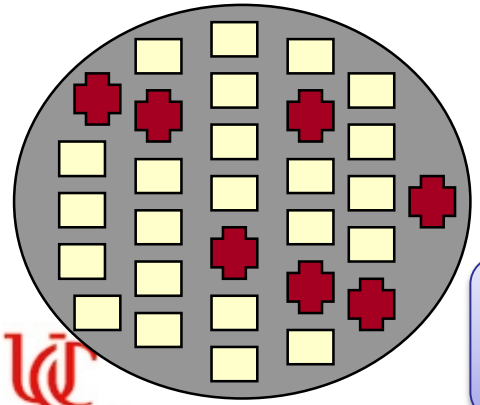


Allele 2

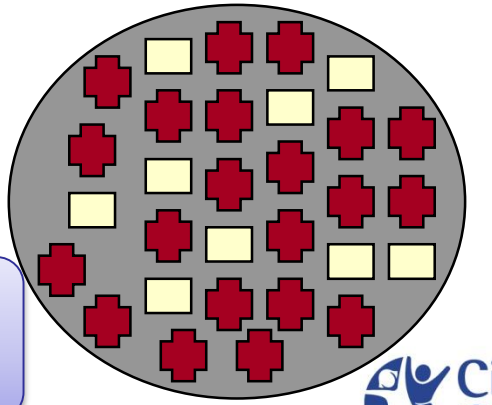
Marker A:

Allele 1 =

Allele 2 =

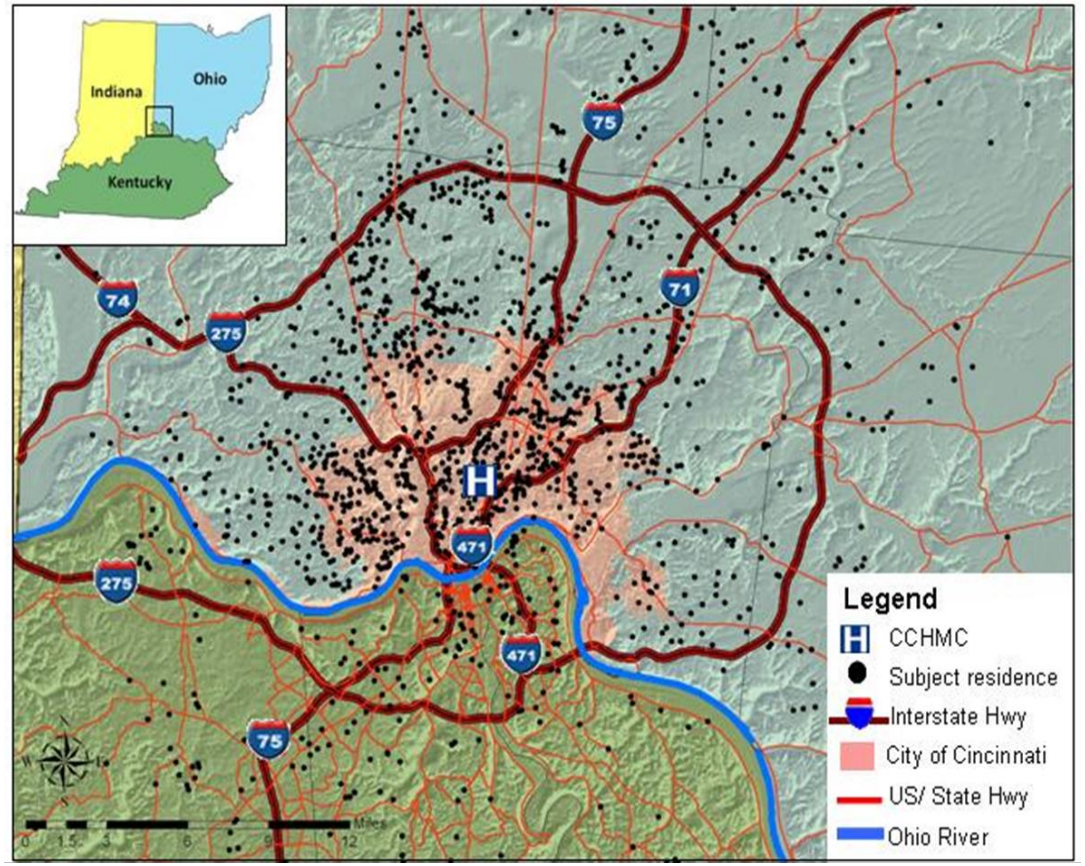


Allele 2 is associated with Phenotype



Contingency Table

The Greater Cincinnati Pediatric Clinic Repository (GCPCR)



- >7000 participants visiting CCHMC clinics with and without allergic diseases.
- Collect DNA, medical and exposure history, family medical history, SES, QOL, PFT and SPT results.
- Longitudinal design.

Asthma candidate genes study

- 52 asthma Candidate genes

  - 507 SNPS [most *tagSNPs*)

- European and African American ancestry

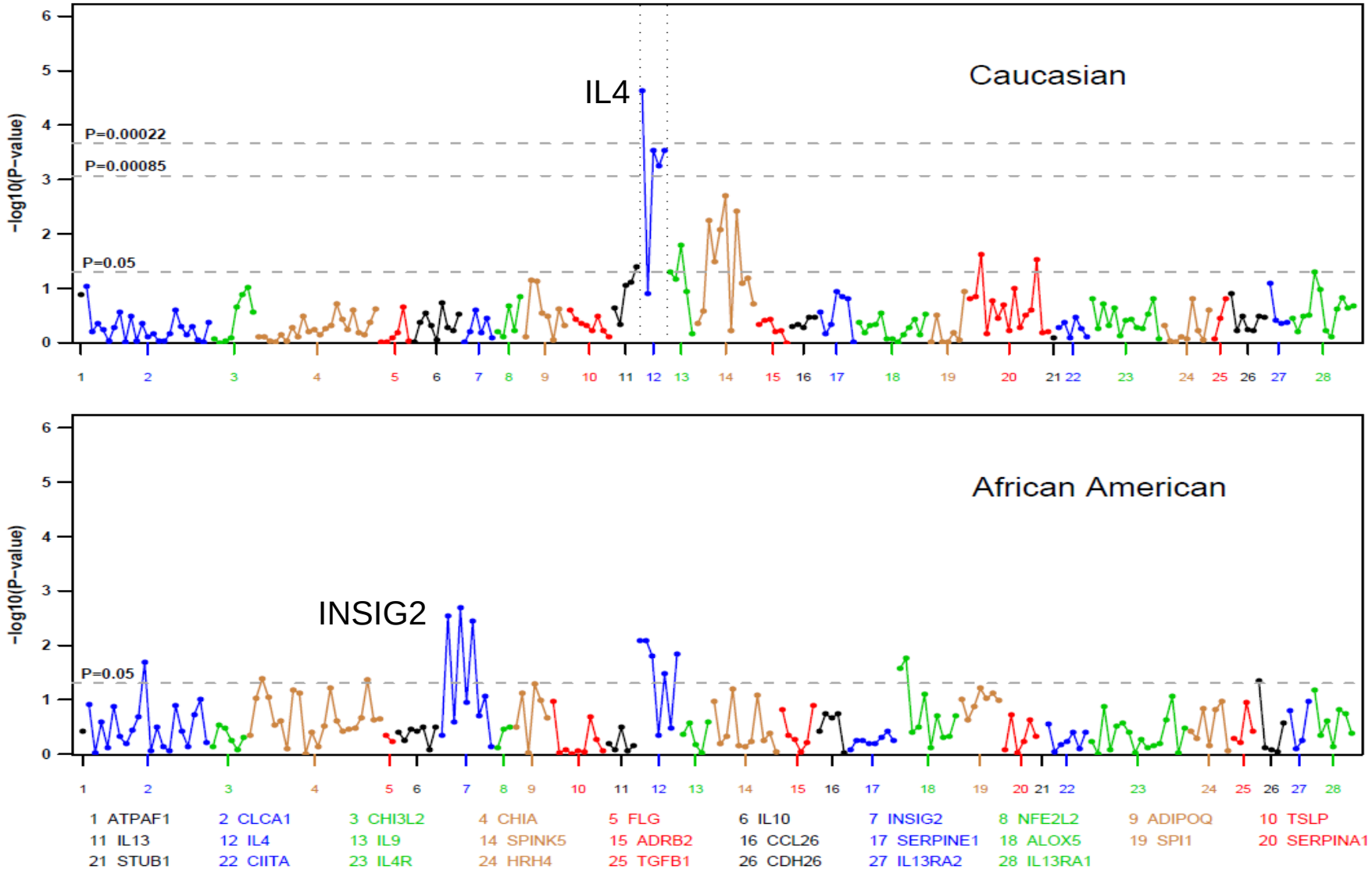
- Greater Cincinnati Pediatric Clinic Repository (GCPCR)

  - well-characterized primarily asthma cohort

  - most living in/near Greater Cincinnati, Ohio.

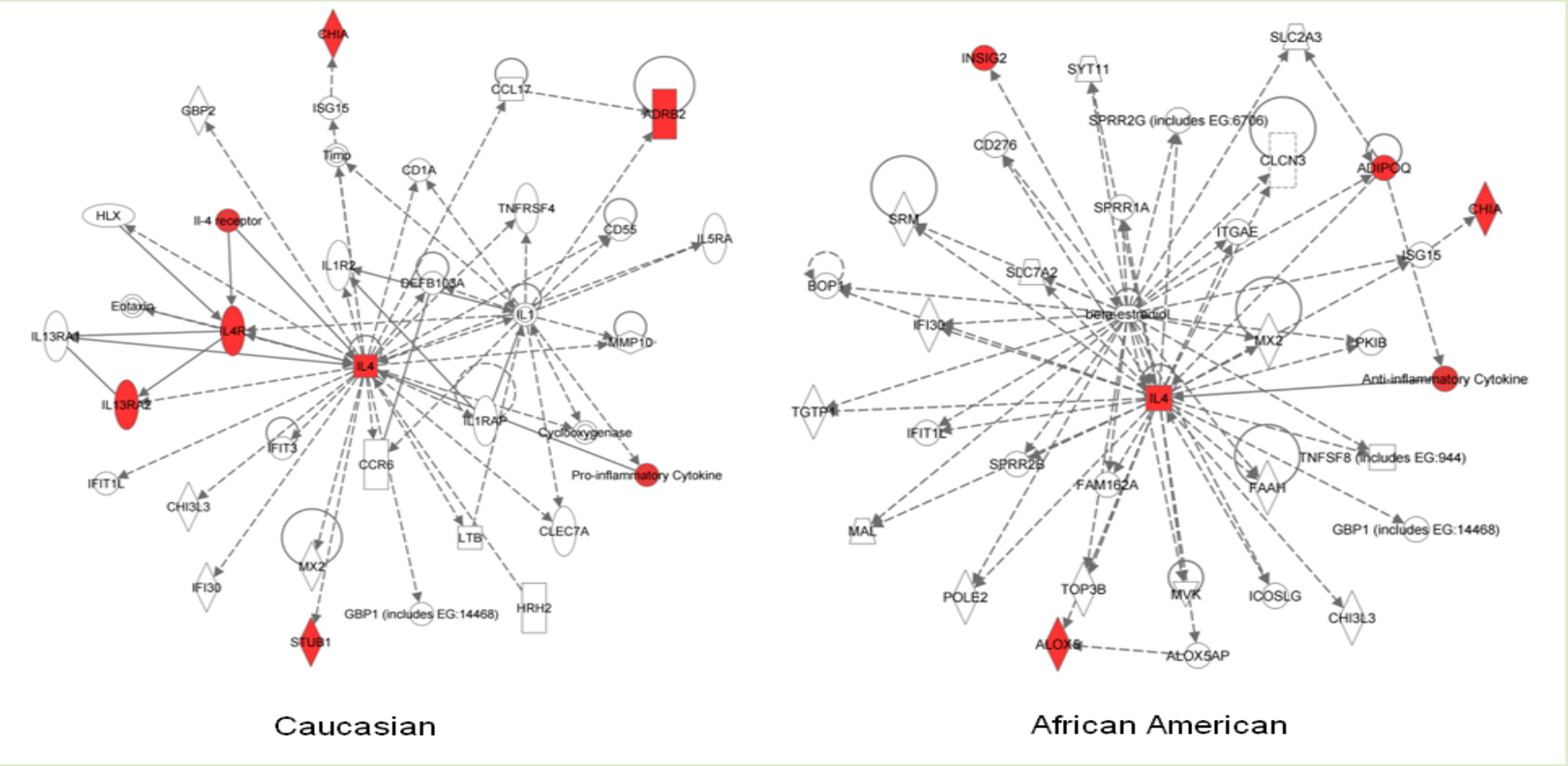
ADCY2	ADIPOQ	EGFR	IL-9
DNAH5	ADRB2	FLG	INSIG2
DYNC1I1	ALOX5	GSTP1	KIAA0494
DYNC1I2	ATG5	HRH4	LTC4S
KIF3A	ATG7	IFIH1/MDA 5	NFE2L2
KIFC2	ATPAF1	SHIP1	SERPINA2
PDE4B	CARD15	IL-10	SERPINE1
PLAU	CCL26	IL-13	SPI1
SERPINA1	CDH26	IL-13RA1	SPINK5
SERPINB3	CHI3L2	IL-13RA2	STUB1
SPRR2B	CHIA	IL-17A	SYK
	CIITA	IL-4	TGFB1
	CLCA1	IL-4R	TSLP
	DDX58/R IG-I		

SNP-based association: asthmatic vs. controls



PLoS One. 2011; 6(2): e16522.

Network analysis for European and African American Ancestry



- ***RP focus genes*** **overlaid onto a global molecular network in IPA**

OPEN ACCESS Freely available online

PLoS one

Differences in Candidate Gene Association between European Ancestry and African American Asthmatic Children

Tesfaye M. Baye[1], Melinda Butsch Kovacic[1], Jocelyn M. Biagini Myers[1], Lisa J. Martin[1], Mark Lindsey[1], Tia L. Patterson[1], Hua He[1], Mark B. Ericksen[1], Jayanta Gupta[1], Anna M. Tsoras[1], Andrew Lindsley[1], Marc E. Rothenberg[1], Marsha Wills-Karp[1], N. Tony Eissa[2], Larry Borish[3], Gurjit K. Khurana Hershey[1]*

1 Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, United States of America, 2 Department of Medicine, Baylor College of Medicine, Houston, Texas, United States of America, 3 Department of Medicine, University of Virginia, Charlottesville, Virginia, United States of America

Abstract

Background: Candidate gene case-control studies have identified several single nucleotide polymorphisms (SNPs) that are associated with asthma susceptibility. Most of these studies have been restricted to evaluations of specific SNPs within a single gene and within populations from European ancestry. Recently, there is increasing interest in understanding racial differences in genetic risk associated with childhood asthma. Our aim was to compare association patterns of asthma candidate genes between children of European and African ancestry.

Methodology/Principal Findings: Using a custom-designed Illumina SNP array, we genotyped 1,485 children within the Greater Cincinnati Pediatric Clinic Repository and Cincinnati Genomic Control Cohort for 259 SNPs in 28 genes and evaluated their associations with asthma. We identified 14 SNPs located in 6 genes that were significantly associated (p-values <0.05) with childhood asthma in African Americans. Among Caucasians, 13 SNPs in 5 genes were associated with childhood asthma. Two SNPs in IL4 were associated with asthma in both races (p-values <0.05). Gene-gene interaction studies identified race specific sets of genes that best discriminate between asthmatic children and non-allergic controls.

Conclusions/Significance: We identified IL4 as having a role in asthma susceptibility in both African American and Caucasian children. However, while IL4 SNPs were associated with asthma in asthmatic children with European and African ancestry, the relative contributions of the most replicated asthma-associated SNPs varied by ancestry. These data provides valuable insights into the pathways that may predispose to asthma in individuals with European vs. African ancestry.

PLoS One. 2011 6(2):e16522

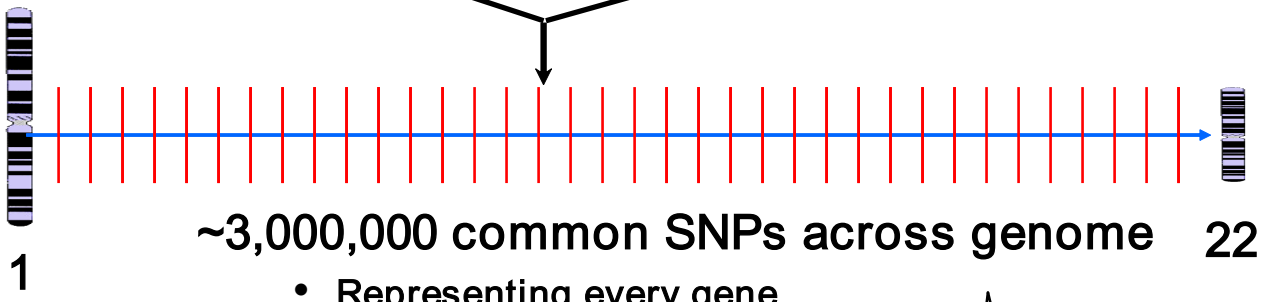
Focusing on one (or few genes) may miss an obvious signal!!!

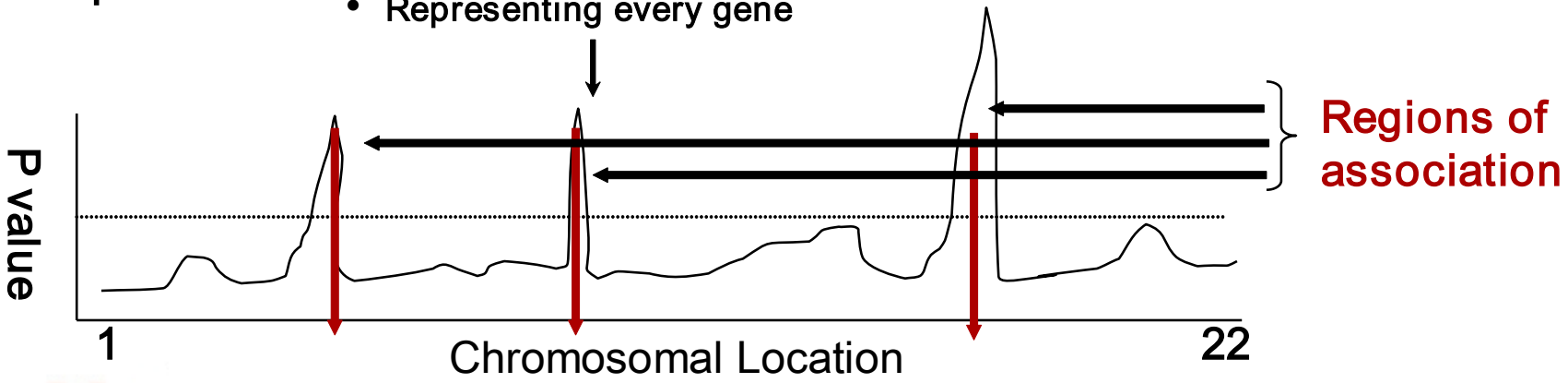
Genome-Wide Association Analysis

Genome-wide association

Disease Population
N=500

Matched Control Population
N=500



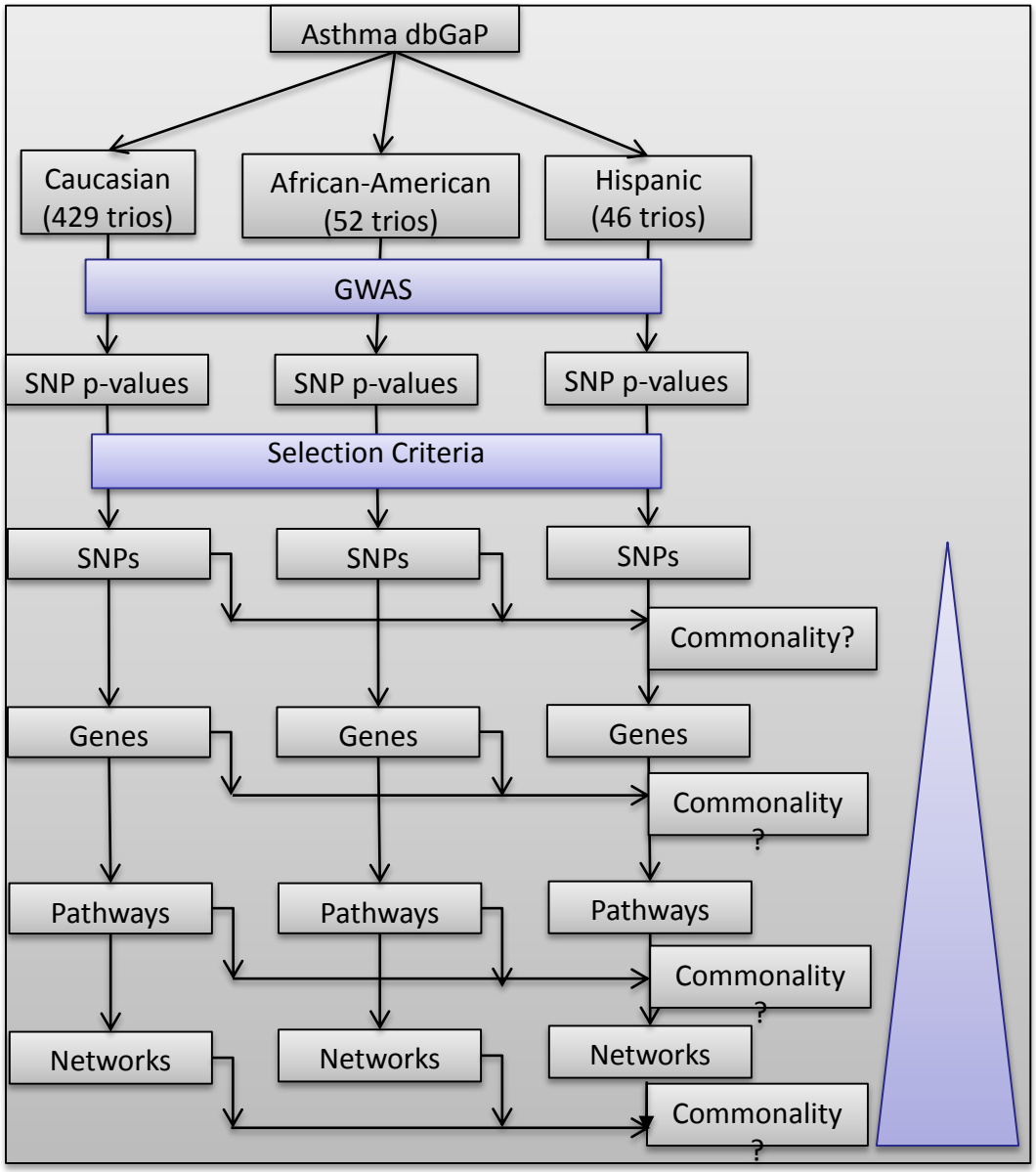


Study population

Asthma dbGaP data:

- Childhood Asthma Management Program (CAMP) and the Childhood Asthma Research and Education (CARE) Network.
- 527 affected offspring trios from European, African, and Hispanic American
- 851,248 SNPs shared by all study populations (AA, EA, HA).

Work flow diagram



Results

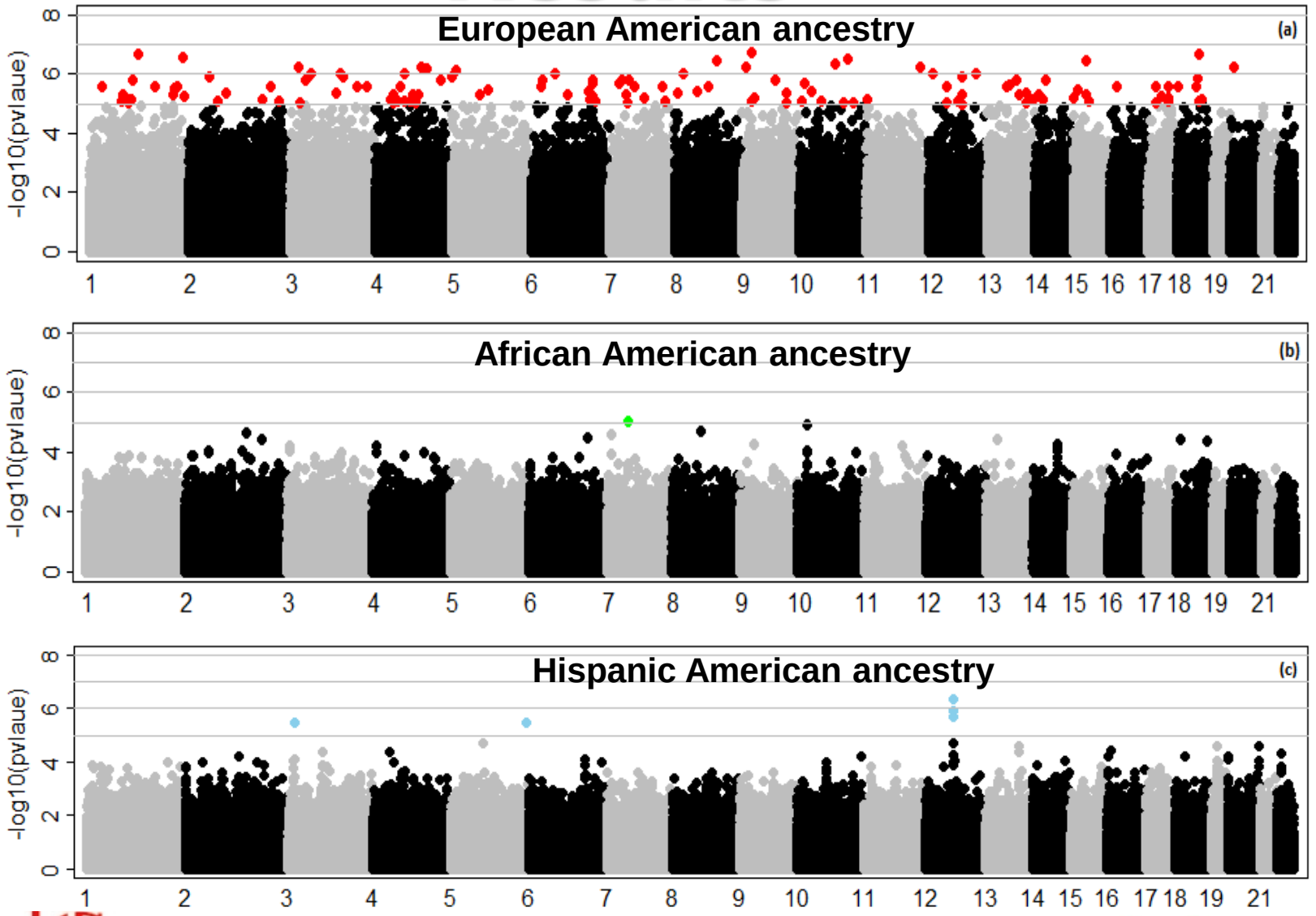


Figure 2: dbGaP data: Manhattan plots from TDTs for (a) European (b) African, (c) Hispanic Americans ancestry

11 genes shared by the top 1,000 SNPs

Genes	Chr	p-values		
		European American	African American	Hispanic American
RYR2	1	2.55×10^{-7}	2.57×10^{-4}	1.18×10^{-3}
CSMD1	8	4.23×10^{-6}	4.15×10^{-4}	4.18×10^{-4}
CDH13	16	2.01×10^{-5}	2.31×10^{-4}	1.60×10^{-3}
ROBO2	3	2.01×10^{-5}	3.93×10^{-4}	1.36×10^{-3}
RBFOX1	16	2.21×10^{-5}	1.76×10^{-3}	3.86×10^{-5}
PTPRD	9	2.38×10^{-5}	2.08×10^{-4}	3.12×10^{-4}
NPAS3	14	3.74×10^{-5}	1.58×10^{-3}	1.02×10^{-3}
PDE1C	7	9.62×10^{-5}	9.41×10^{-4}	1.32×10^{-3}
SEMA5A	5	1.08×10^{-4}	4.03×10^{-4}	1.34×10^{-3}
CTNNA2	2	1.83×10^{-4}	1.28×10^{-3}	1.57×10^{-3}
PDE4D	5	1.83×10^{-4}	4.02×10^{-4}	1.62×10^{-3}

RYR2: Ryanodine receptor 2 [rs16835325]

change the outcome®

Ding et al. Human Genomics 2013, 7:16
http://www.humgenomics.com/content/7/1/16

Human Genomics

PRIMARY RESEARCH Open Access

Rank-based genome-wide analysis reveals the association of Ryanodine receptor-2 gene variants with childhood asthma among human populations

Highly accessed

Lili Ding[1], Tilahun Abebe[2], Joseph Beyene[3], Russell A Wilke[4], Arnon Goldberg[5], Jessica G Woo[1], Lisa J Martin[1], Marc E Rothenberg[1], Marepalli Rao[6], Gurjit K Khurana Hershey[1], Ranajit Chakraborty[7] and Tesfaye B Mersha[1*]

Abstract

Background: The standard approach to determine unique or shared genetic factors across populations is to identify risk alleles in one population and investigate replication in others. However, since populations differ in DNA sequence information, allele frequencies, effect sizes, and linkage disequilibrium patterns, SNP association using a uniform stringent threshold on *p* values may not be reproducible across populations. Here, we developed rank-based methods to investigate shared or population-specific loci and pathways for childhood asthma across individuals of diverse ancestry. We performed genome-wide association studies on 859,790 SNPs genotyped in 527 affected offspring trios of European, African, and Hispanic ancestry using publically available asthma database in the Genotypes and Phenotypes database.

Results: Rank-based analyses showed that there are shared genetic factors for asthma across populations, more at the gene and pathway levels than at the SNP level. Although the top 1,000 SNPs were not shared, 11 genes (*RYR2*, *PDE4D*, *CSMD1*, *CDH13*, *ROBO2*, *RBFOX1*, *PTPRD*, *NPAS3*, *PDE1C*, *SEMA5A*, and *CTNNA2*) mapped by these SNPs were shared across populations. Ryanodine receptor 2 (*RYR2*, a statin response-related gene) showed the strongest association in European (p value $= 2.55 \times 10^{-7}$) and was replicated in African (2.57×10^{-4}) and Hispanic (1.18×10^{-3}) Americans. Imputation analyses based on the 1000 Genomes Project uncovered additional *RYR2* variants associated with asthma. Network and functional ontology analyses revealed that *RYR2* is an integral part of dermatological or allergic disorder biological networks, specifically in the functional classes involving inflammatory, eosinophilic, and respiratory diseases.

Conclusion: Our rank-based genome-wide analysis revealed for the first time an association of *RYR2* variants with asthma and replicated previously discovered *PDE4D* asthma gene across human populations. The replication of top-ranked asthma genes across populations suggests that such loci are less likely to be false positives and could indicate true associations. Variants that are associated with asthma across populations could be used to identify individuals who are at high risk for asthma regardless of genetic ancestry.

Keywords: Asthma, GWAS, Ancestry, Trans-ancestral analysis, Rank analysis, Imputation, dbGaP, 1000 Genomes project, Networks/pathways, *RYR2*

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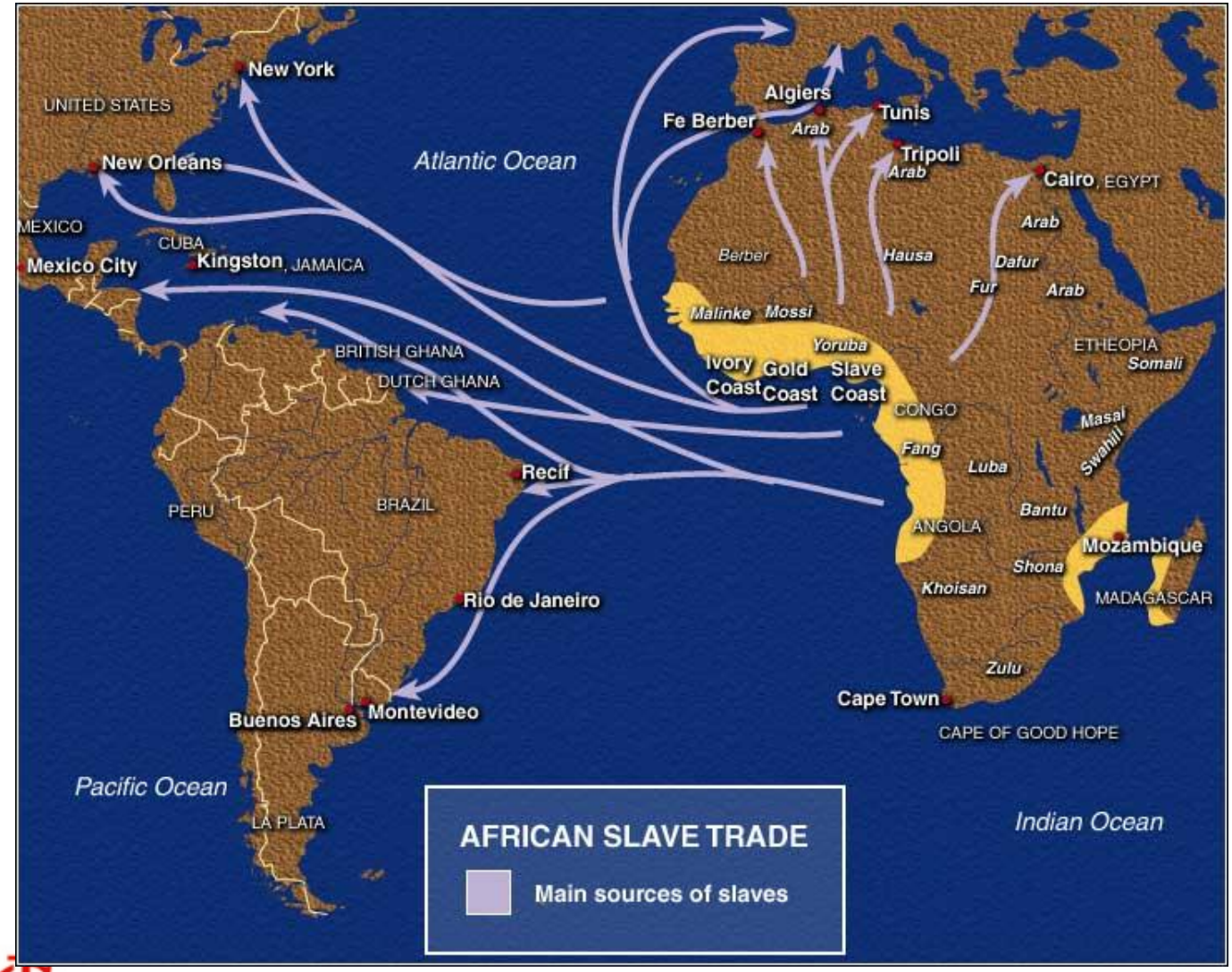
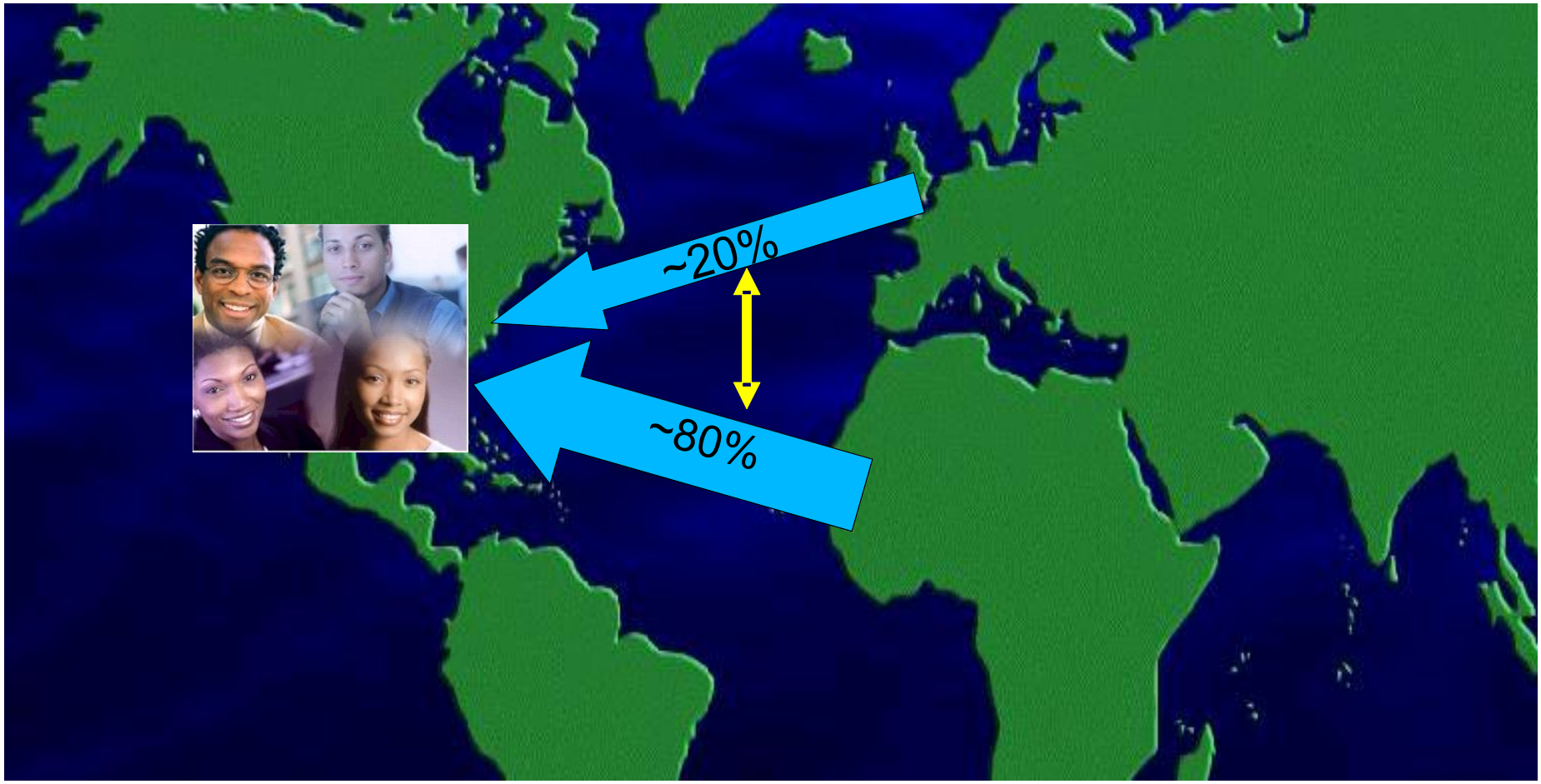
Admixed population and asthma

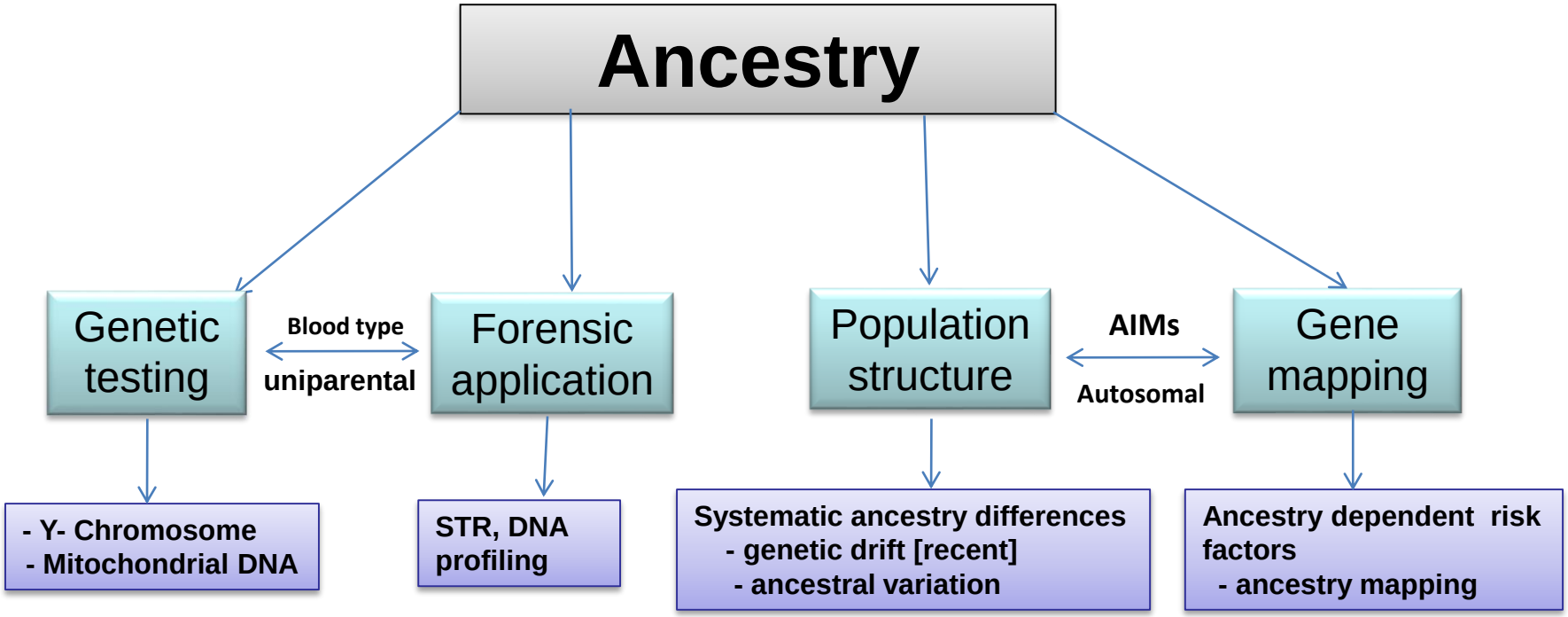
Example: African Americans



Example: African Americans

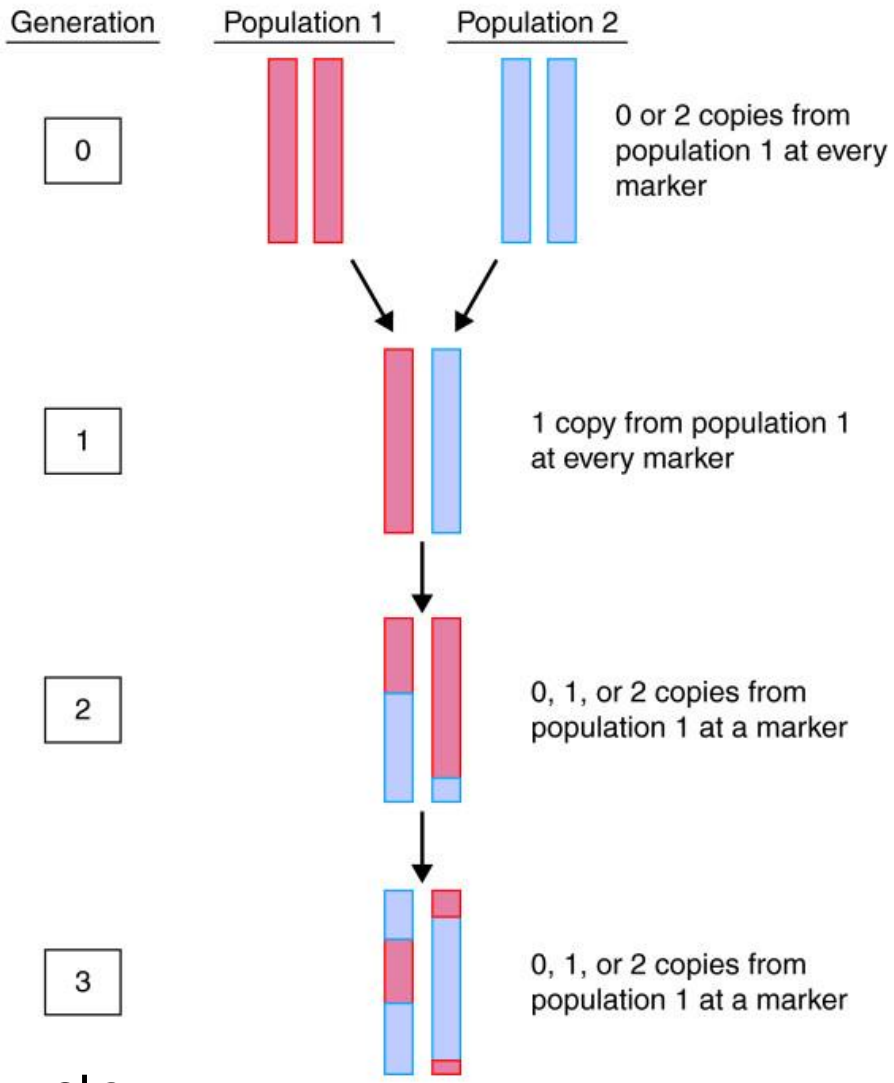


Why Ancestry?



Provide clues about relatedness/differences and genetic risk factors

Schematics of population admixture



etc.

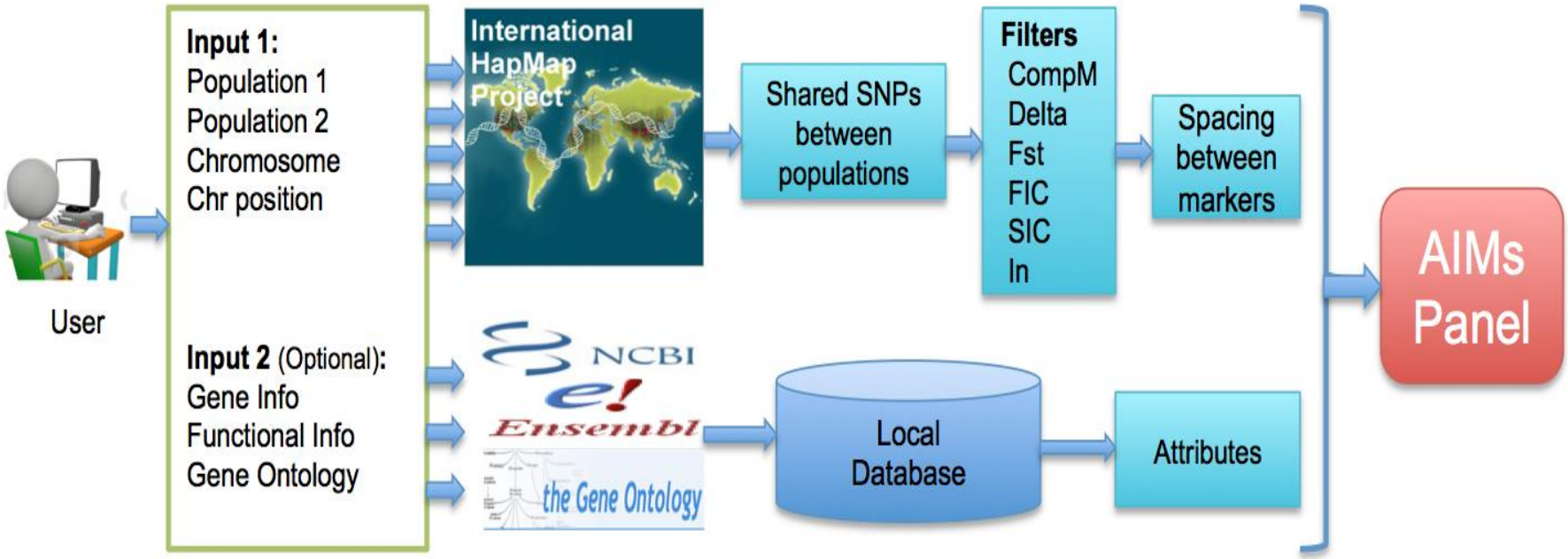
Local and global ancestry estimation

AncestrySNPminer

An online tool to retrieve and develop ancestry informative SNPs

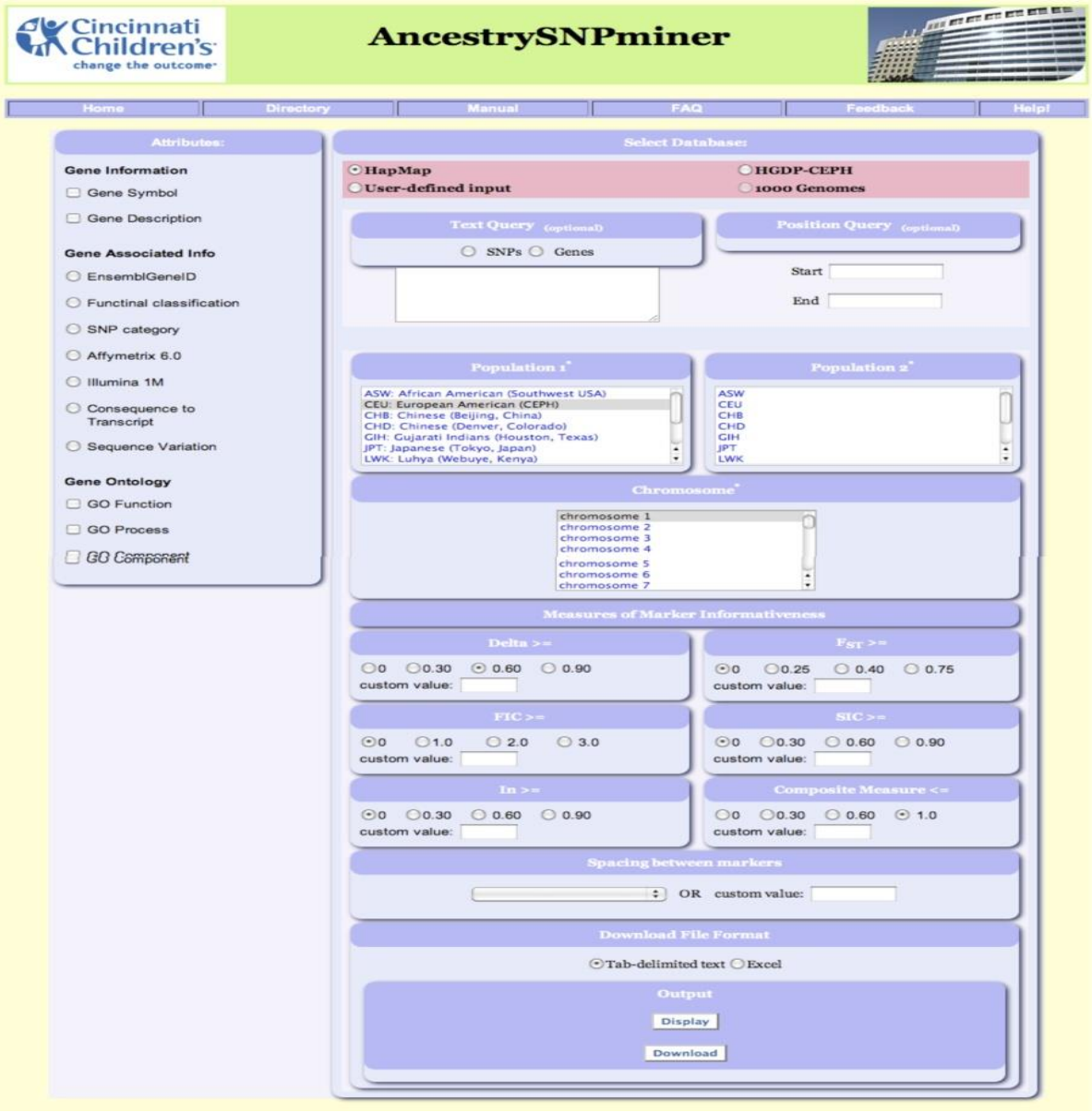
Genomics, 2012 Jul;100(1):57-63.

AncestrySNPminer: workflow strategy



Genomics, 2012 Jul;100(1):57-63.

AncestrySNPminer: web interface



AncestrySNPminer

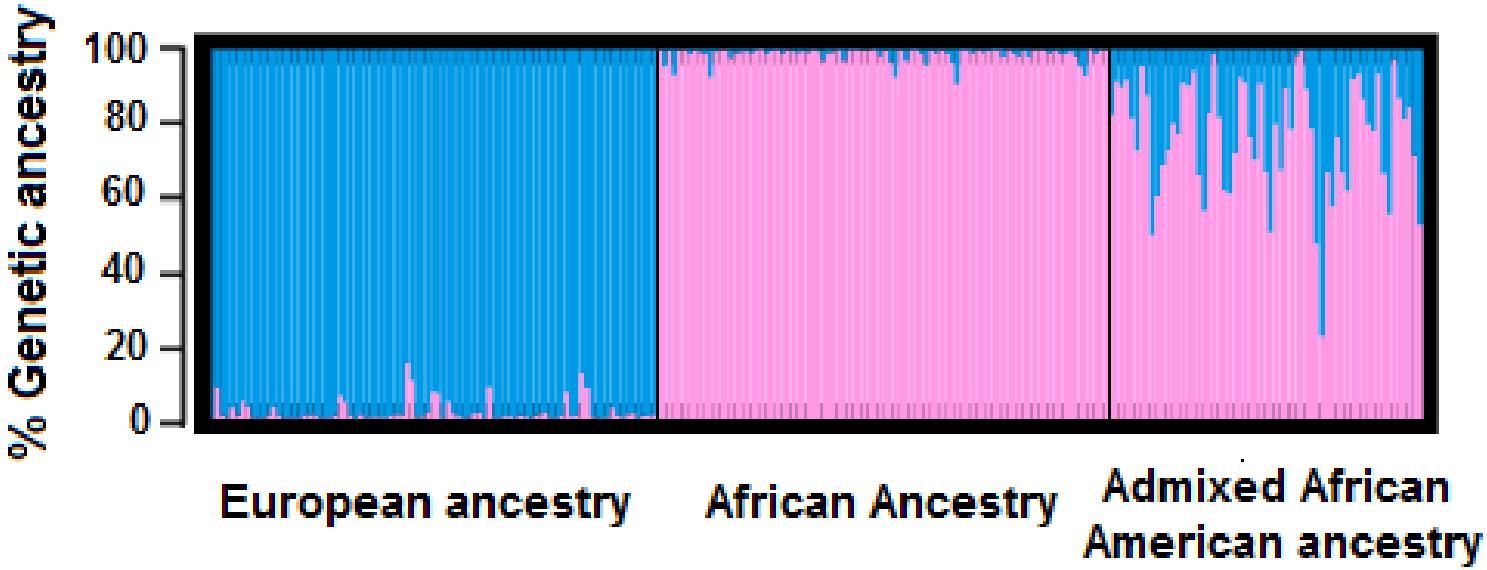
Demo

https://research.cchmc.org/mershalab/AncestrySNPminer/home.php

Genomics, 2012 Jul;100(1):57-63.

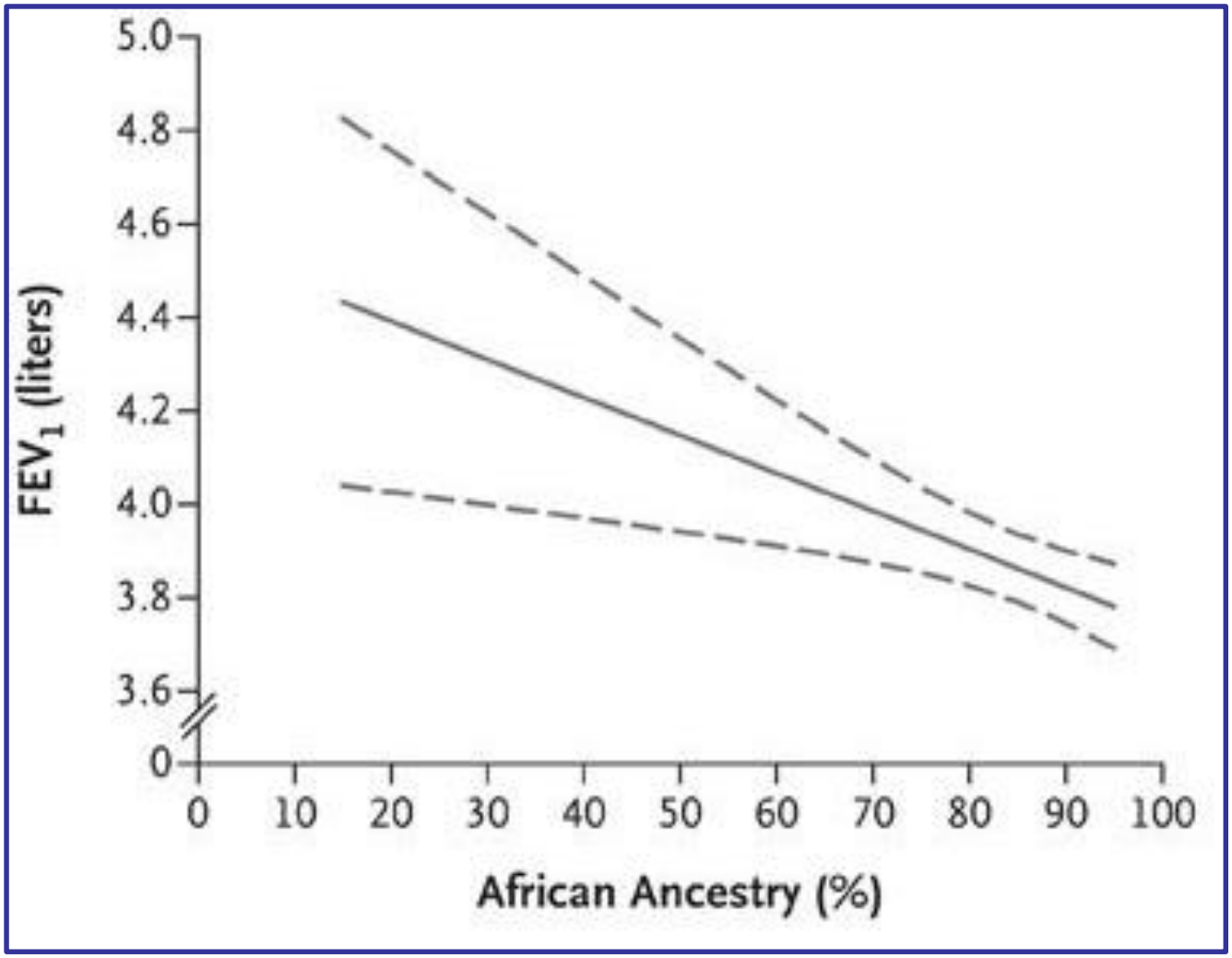
Ancestry proportion of GCPCR cohort

- AAs have heterogeneous origins with varying degrees of European ancestry



- Each vertical bar represents 1 subject. For each AA subject, the proportion of European (green) and African ancestry (red) are displayed
- Self-reported racial groups may misclassify population as shown here!

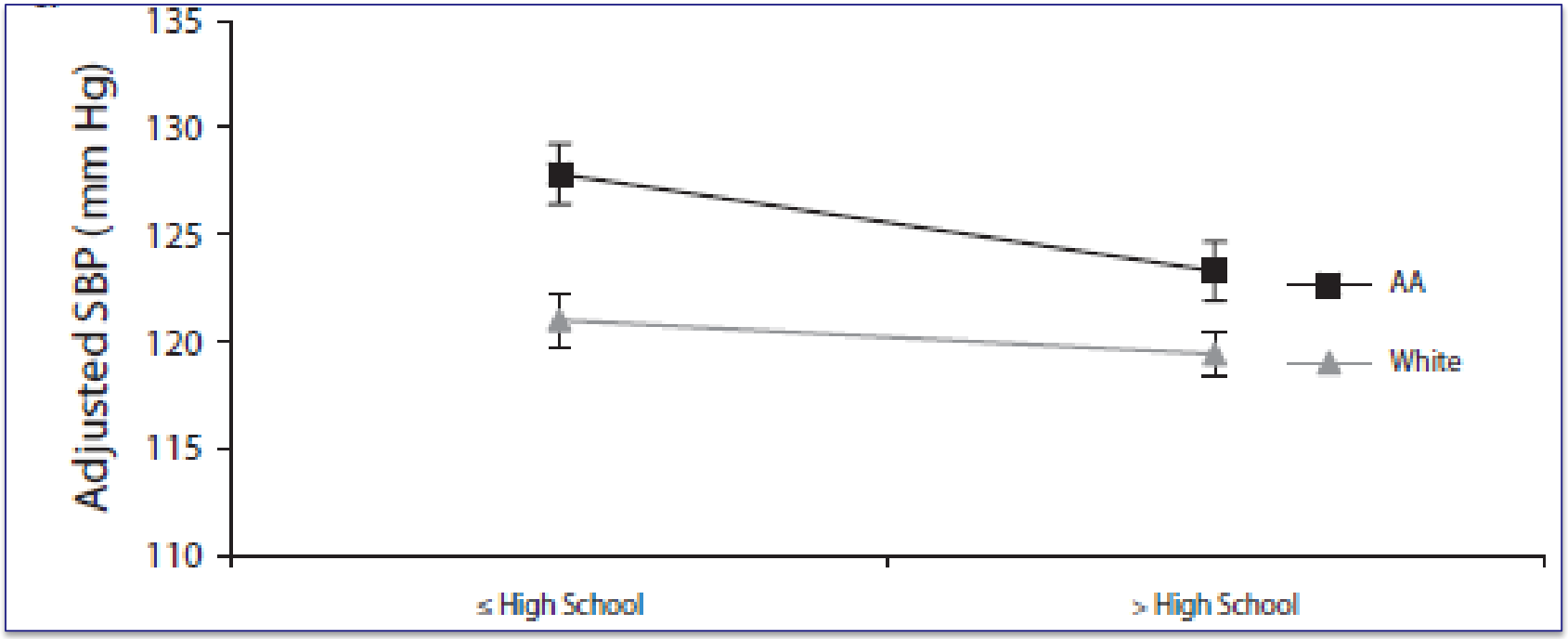
Lung function as a function of African ancestry



FEV1= Forced Expiratory Volume

Kumar R et al. N Engl J Med 2010;363:321-330

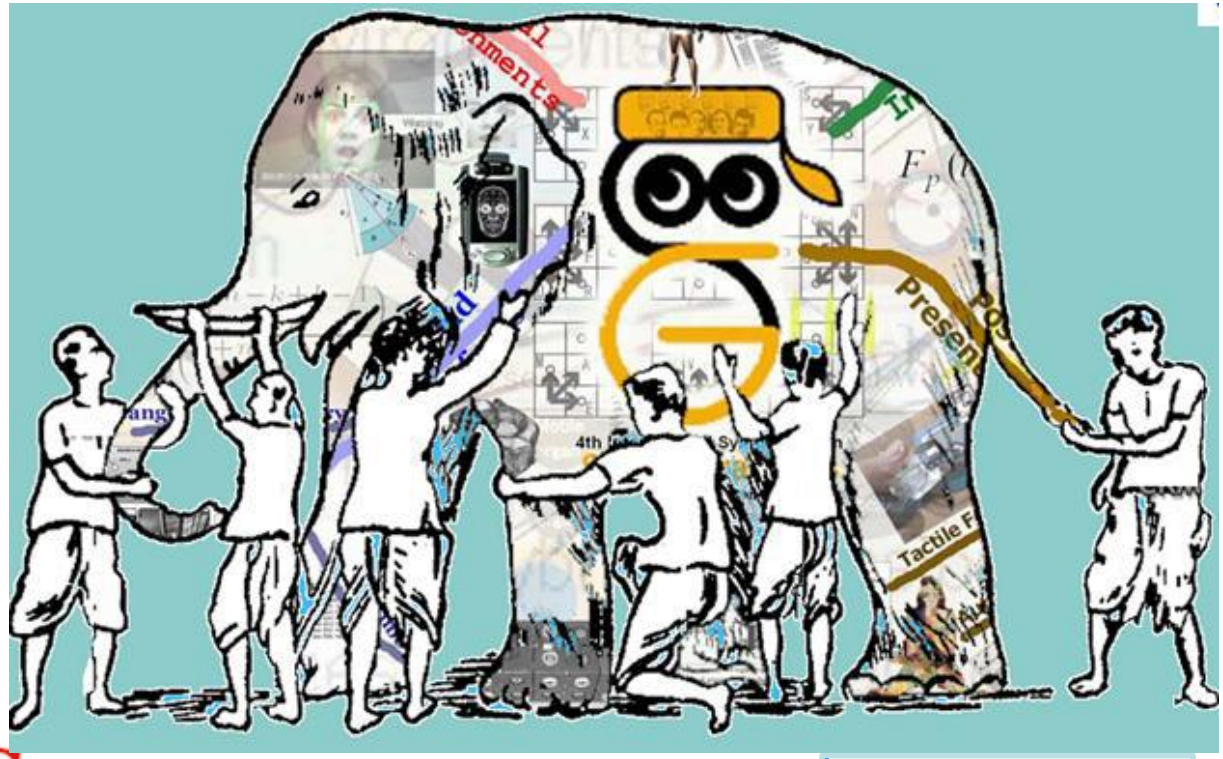
Interaction between race and education



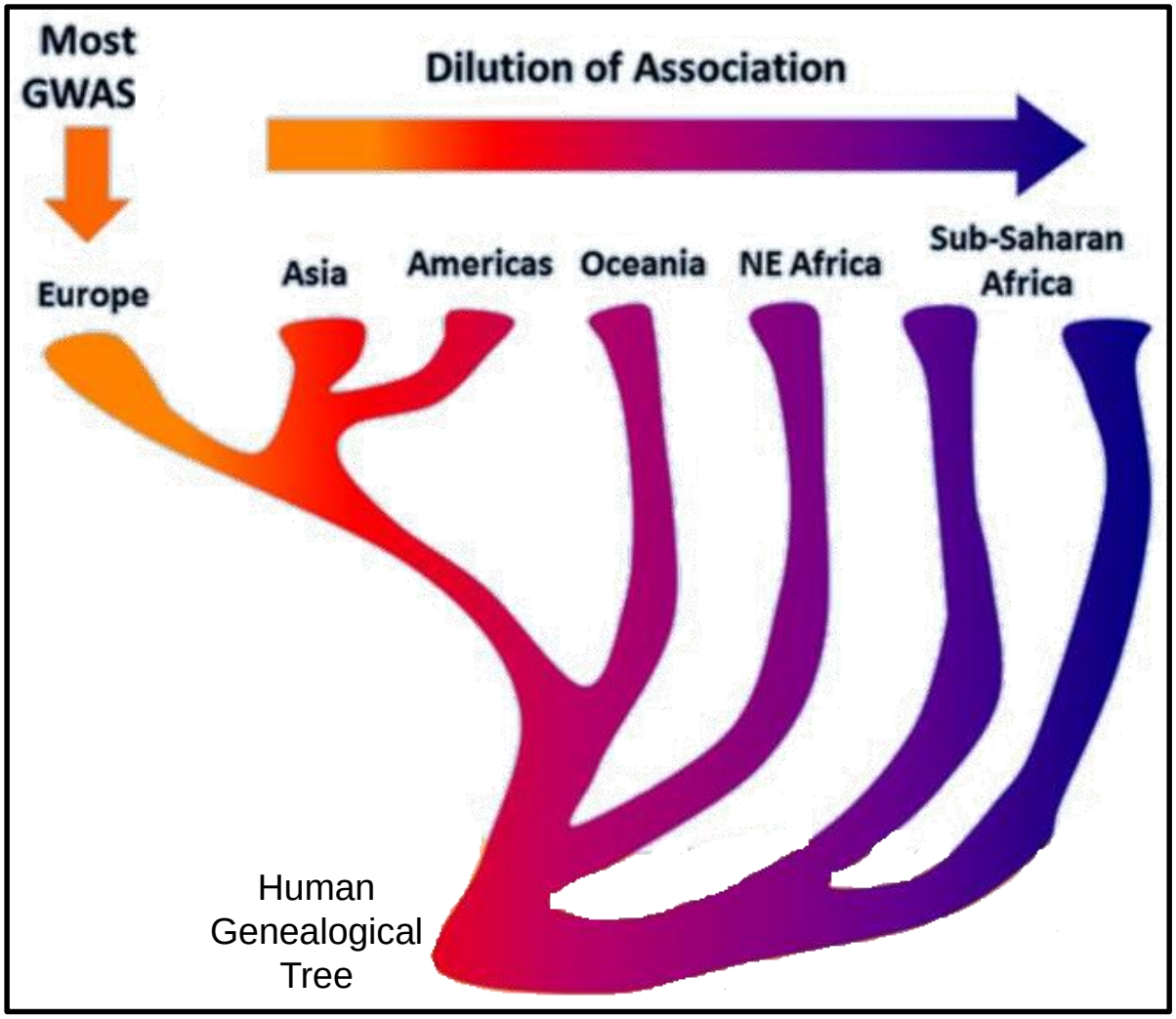
American Journal of Public Health 2012: e1–e7

Challenges in asthma etiology?

- Extreme heterogeneous phenotype, population variation
- Phenotyping is based on highly subjective measures
- Genetics study is based on European reference SNP panel

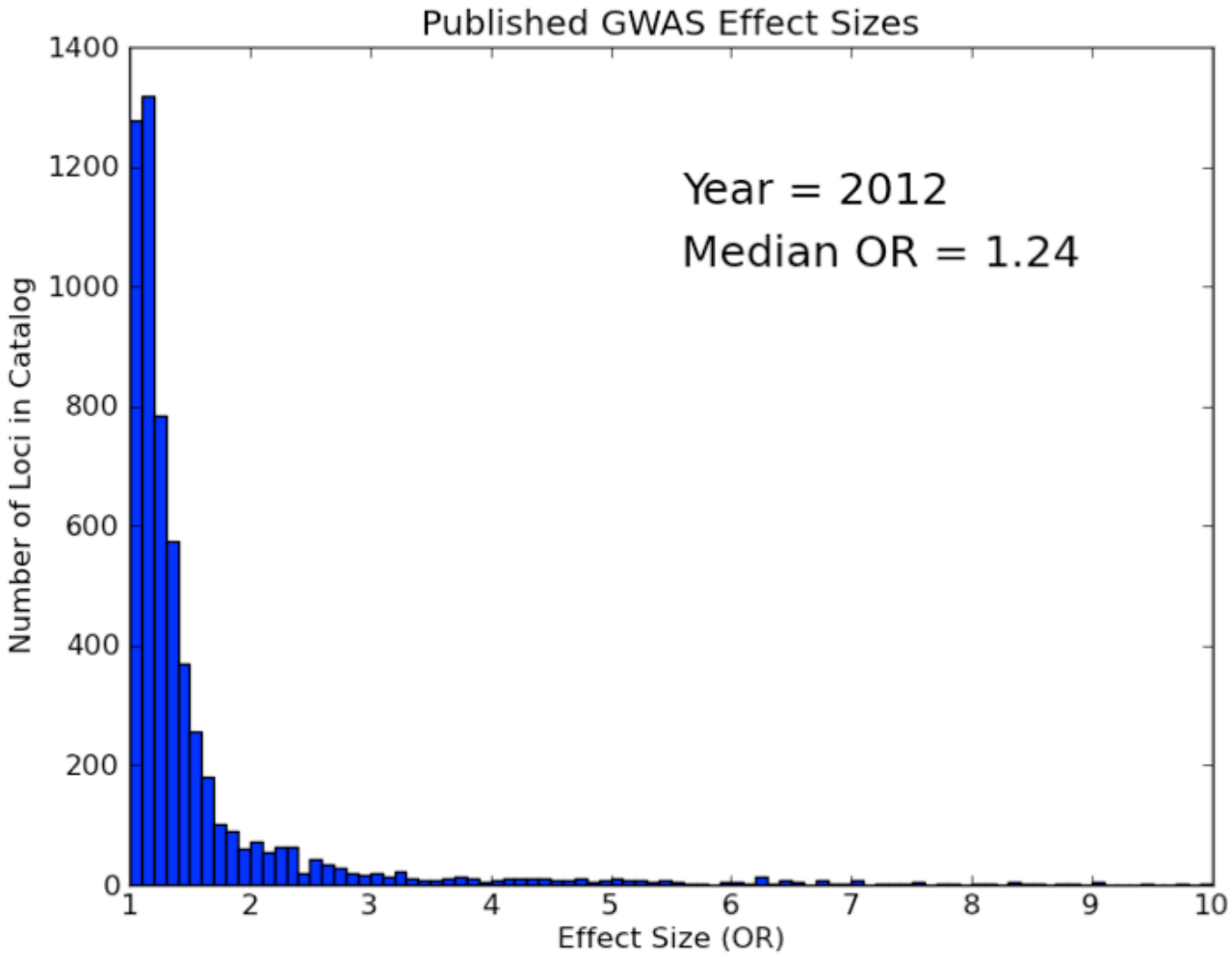


To what extent can we extrapolate European ancestry based GWASs to African Americans?



PLoS Biol 11(9): e1001660, 2013
http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1001660

GWAS Trend: Large Cohorts → More Loci → Small Effects

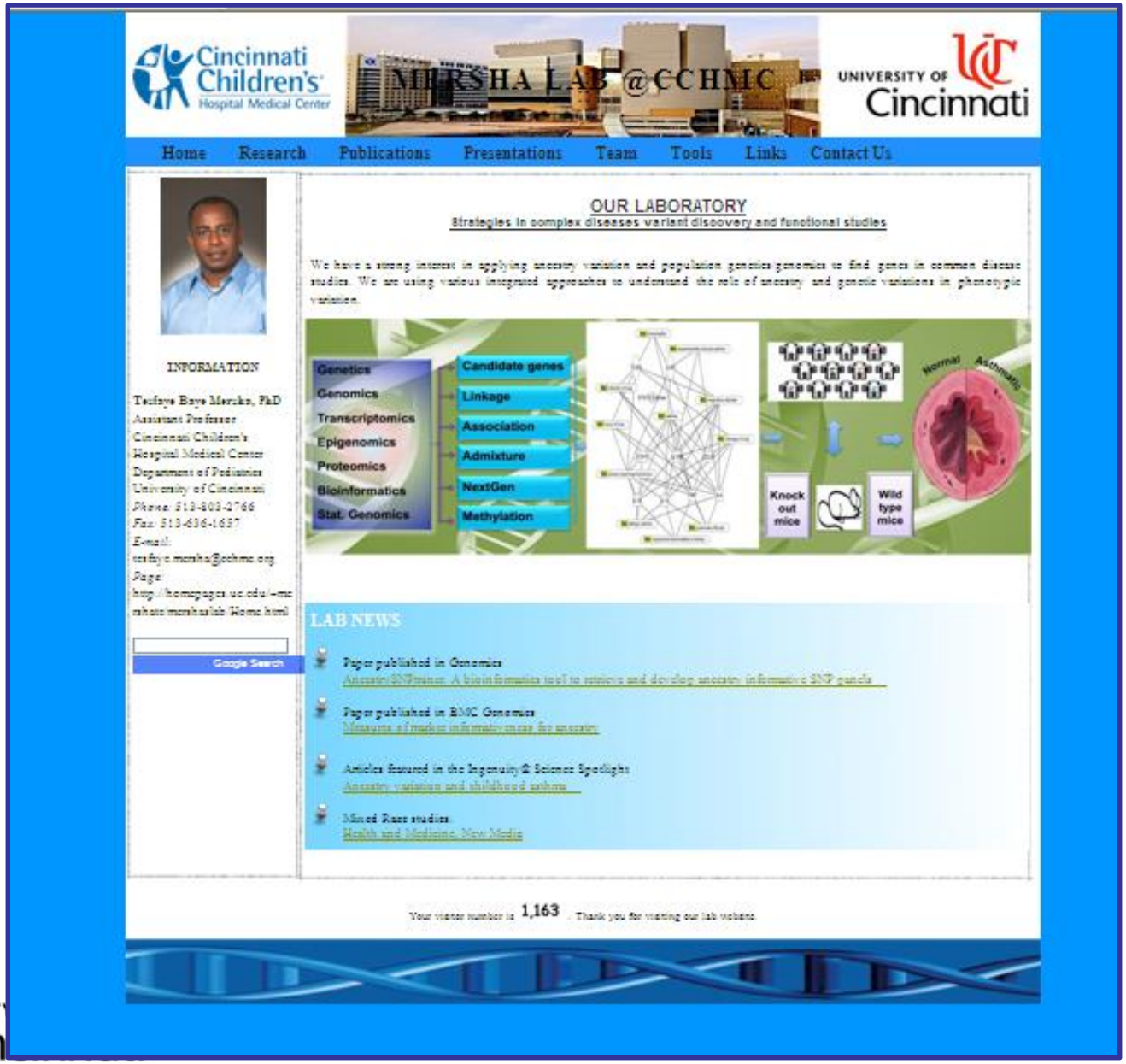


Conclusion/Future Directions

- Difference in disease prevalence could be genetics, environment or both.
- Population vary in allele frequency and genotype frequency
  - **Population-specific reference panel/WGS**
- **Data mining and integration across domains**
  - Genetics, gene expression, epigenetics
- **Dissect non-genetic factors that correlate with ancestry**
  - Socio-cultural and environmental exposure factors

"I still don't have all the answers, but I'm beginning to ask the right questions."

https://research.cchmc.org/mershalab/Home.html



Thank you