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

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Respiratory diseases represent a public health challenge around the world!
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

Project 1:
Genetic Studies
- Association testing
- Admixture analysis
- GxE interaction [sex/gender effect]

Complex Trait [Asthma]

Sequence Polymorphism

Socio-cultural and exposure factors

Variations in Transcripts and Methylation

System Approaches [Network, Pathways] Regulatory Regions Resources

Project 2:
Gene expression studies
- RNA-seq,
- GEO/ArrayExpress

Project 3:
With H. Ji

- Genetic Studies
- Admixture analysis
- GxE interaction [sex/gender effect]
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 Society, 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

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
What are the risk factors of asthma?

- Air quality
- Obesity
- Stress

Unmeasured biological variables

- Genetics
- Socioeconomic status
- Health maintenance behaviors

Unmeasured environmental variables
Up to 60,000 trucks/day on major interstate
Candidate Gene Association Analysis
Common forms of DNA variation

Paternal Chromosome

```
ATTGGCCTAAACCCCCGATTAT
TAACCGGATTGGGGCTAATA
```

Maternal Chromosome

```
ATTGGCCTAACCAACCGATTAT
TAACCGGATTGGGTGCTAATA
```

All Information is contained in a single strand

Simplified Representation of an individual’s DNA Sequence (with SNP)

```
ATTGGCCTAAACCACCGATTAT
ATTGGCCTAAACCACCGATTAT
```

SNP

Insertion-Deletion (Indel)

Block Substitution

Inversion

Variable Number Tandem Repeat (VNTR)

Copy Number Variant (CNV)

Dots indicate that paternal segment does not exist on maternal chromosome (i.e., an indel)
Candidate gene association study design

Contingency Table

Allele 2 is associated with Phenotype

Marker A:
Allele 1 = 
Allele 2 =
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.

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SNP-based association: asthmatic vs. controls

Network analysis for European and African American Ancestry

- *RP focus genes* overlaid onto a global molecular network in IPA
Differences in Candidate Gene Association between European Ancestry and African American Asthmatic Children

Teseay M. Baye, Melinda Butsch Kovacic, Jocelyn M. Biagini Myers, Lisa J. Martin, Mark Lindsey, Tia L. Patterson, Hua He, Mark B. Ericksen, Jayanta Gupta, Anna M. Tsoros, Andrew Lindsley, Marc E. Rothenberg, Marsha Wills-Karp, N. Tony Eissa, Larry Borish, Gurjot K. Khurana Hershey

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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 provide valuable insights into the pathways that may predispose to asthma in individuals with European vs. African ancestry.
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

~3,000,000 common SNPs across genome
• Representing every gene

Regions of association

Chromosomal Location
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).
Asthma dbGaP

Caucasian (429 trios)

African-American (52 trios)

Hispanic (46 trios)

GWAS

SNP p-values

SNP p-values

SNP p-values

Selection Criteria

SNPs

SNPs

SNPs

Genes

Genes

Genes

Pathways

Pathways

Pathways

Networks

Networks

Networks

Commonality?

Commonality?

Commonality?
**Results**

**European American ancestry**

**African American ancestry**

**Hispanic American ancestry**

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

<table>
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<th>Genes</th>
<th>Chr</th>
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<th>African American</th>
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</tr>
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**RYR2**: Ryanodine receptor 2 [rs16835325]
Rank-based genome-wide analysis reveals the association of Ryanodine receptor-2 gene variants with childhood asthma among human populations


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 publicly 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, COXH13, ROBO2, RBPX1, PTPRO, NUP3, PDE1C, SEMA5A, and CTNNAL2) mapped by these SNPs were shared across populations. Ryanodine receptor 2 (RYR2, a striated muscle-related gene) showed the strongest association in European (p value = 2.55 x 10^-7) and was replicated in African (2.57 x 10^-3) and Hispanic (1.18 x 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
Admixed population and asthma
Example: African Americans
Example: African Americans

~20%

~80%
Why Ancestry?

Ancestry

Genetic testing
- Y-Chromosome
- Mitochondrial DNA

Forensic application
- Blood type uniparental
- STR, DNA profiling

Population structure
- Systematic ancestry differences
  - genetic drift [recent]
  - ancestral variation

Gene mapping
- Ancestry dependent risk factors
  - ancestry mapping

Provide clues about relatedness/differences and genetic risk factors
Schematics of population admixture

Local and global ancestry estimation

- Generation 0: 0 or 2 copies from population 1 at every marker
- Generation 1: 1 copy from population 1 at every marker
- Generation 2: 0, 1, or 2 copies from population 1 at a marker
- Generation 3: 0, 1, or 2 copies from population 1 at a marker

etc.
AncestrySNPminer

An online tool to retrieve and develop ancestry informative SNPs

AncestrySNPminer: workflow strategy

Input 1:
- Population 1
- Population 2
- Chromosome
- Chr position

Input 2 (Optional):
- Gene Info
- Functional Info
- Gene Ontology

International HapMap Project

Shared SNPs between populations

Filters
- CompM
- Delta
- Fst
- FIC
- SIC
- In

Spacing between markers

AIMs Panel

Local Database

Attributes

AncestrySNPminer: web interface
AncestrySNPminer

Demo

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

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 (liters)

African Ancestry (%)
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?
GWAS Trend: Large Cohorts → More Loci → Small Effects

Year = 2012
Median OR = 1.24
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