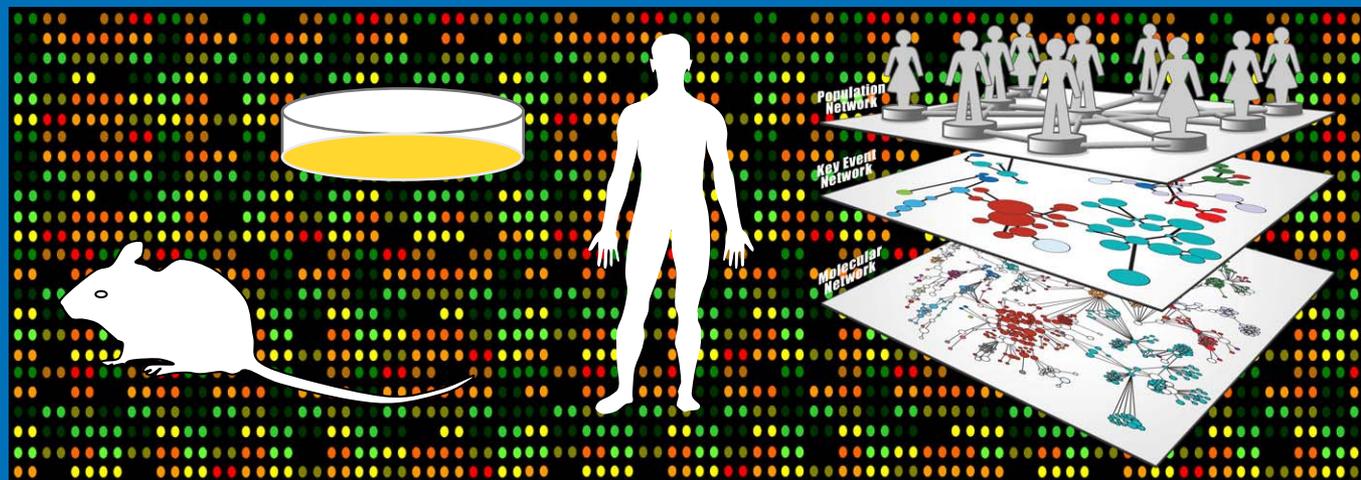


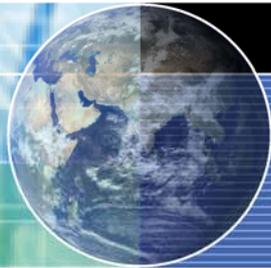
# From Gene Expression to Asthma Endotypes

## The Value of Integrating Multiple Data Sources

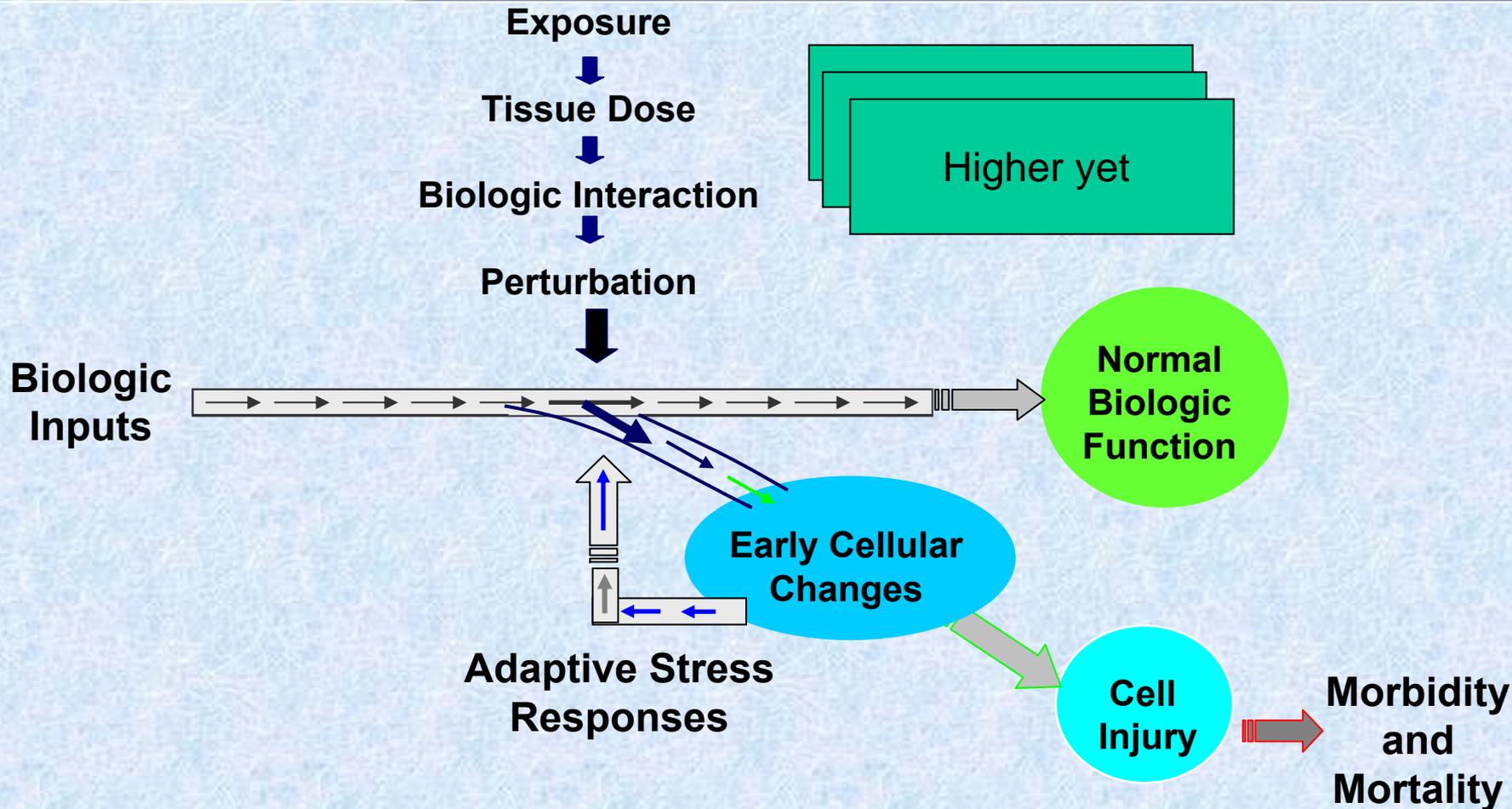
*Stephen W. Edwards  
SOT 2009 Annual Meeting*

*This talk does not necessarily reflect the views of the Environmental Protection Agency.*



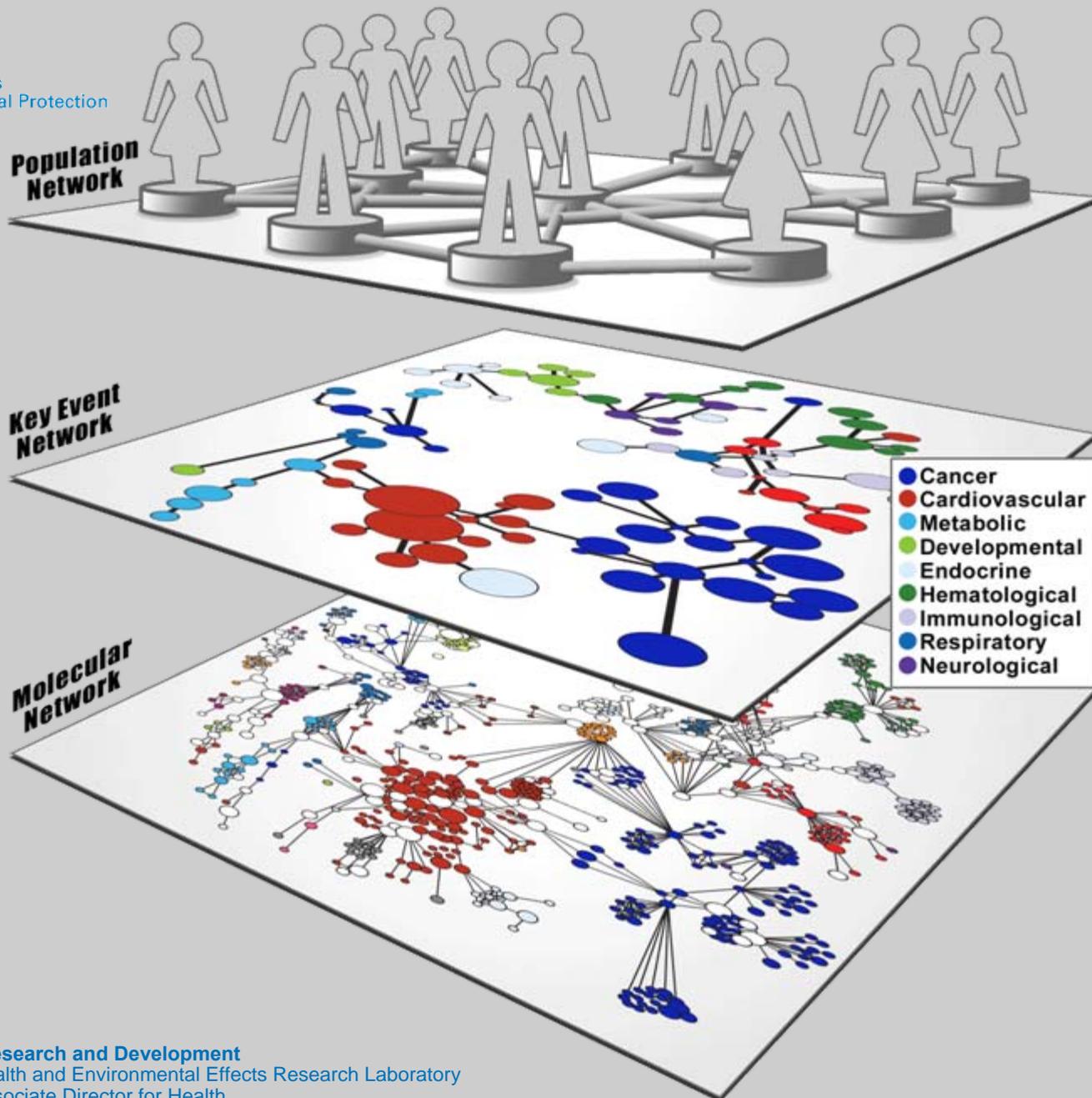


# A New Paradigm: Activation of Toxicity Pathways



Dan Krewski, et al.,  
*Tox Testing in the 21st Century*, NRC, June 2007.

THE NATIONAL ACADEMIES  
Advisers to the Nation on Science, Engineering, and Medicine





# Mechanistic Indicators of Childhood Asthma (MICA)

## **MICA-HSD**

*Jane Gallagher*

*Ed Hudgens*

Gina Andrews

Brooke Heidenfelder

Jeff Inmon

Mary Johnson

Danelle Lobdell

Pauline Mendola

Jim Prah

Scott Rhoney

Elizabeth Sams

## **DCHS**

Lucas Neas

Ann Williams

## **NCCT**

Elaine Hubal

David Reif

## **Administrative**

Walter Breyer

Edward Strubble

Mike Ray QA

Debra Walsh

Kay Williams

## **Nurses HSD**

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Deb Levin

Tracy. Montilla

## **IRB**

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Monica Nees

## **Contracts**

Robin Harris

Jennifer Hill

Lenora Hilliard

## **Student**

## **Contractors**

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Chrissy Lin

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Haluk Ozkaynak

Ron Williams

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Stephen Vesper

## **ETD**

Gary Hatch

Marsha Ward et al

Kay Crissman

McGee

## **Genomics core**

Susan Hester

Chris Corton

## **ADHIO**

Stephen Edwards

## **ECD**

David DeMarini

## **Region 5**

George Bollweg

Jackie Nwai

## **Michigan State**

## **U.**

J. Harkema

Lori Bramble

## **Johns Hopkins**

Robert Hamilton

John Wiseman

Carol Schultz

## **Mercy College**

H. El Fawal

## **Harvard University**

## **Rutgers University**

Tina Fain

## **UNC**

Stephen Rappaport

Suryamya W.

## **SWRI**

David Caaman

## **RTI**

Frank Weber

Peter Groshe

## **Westat**

Kurt Patrizi

Andrea Ware

## **Henry Ford Health**

## **System**

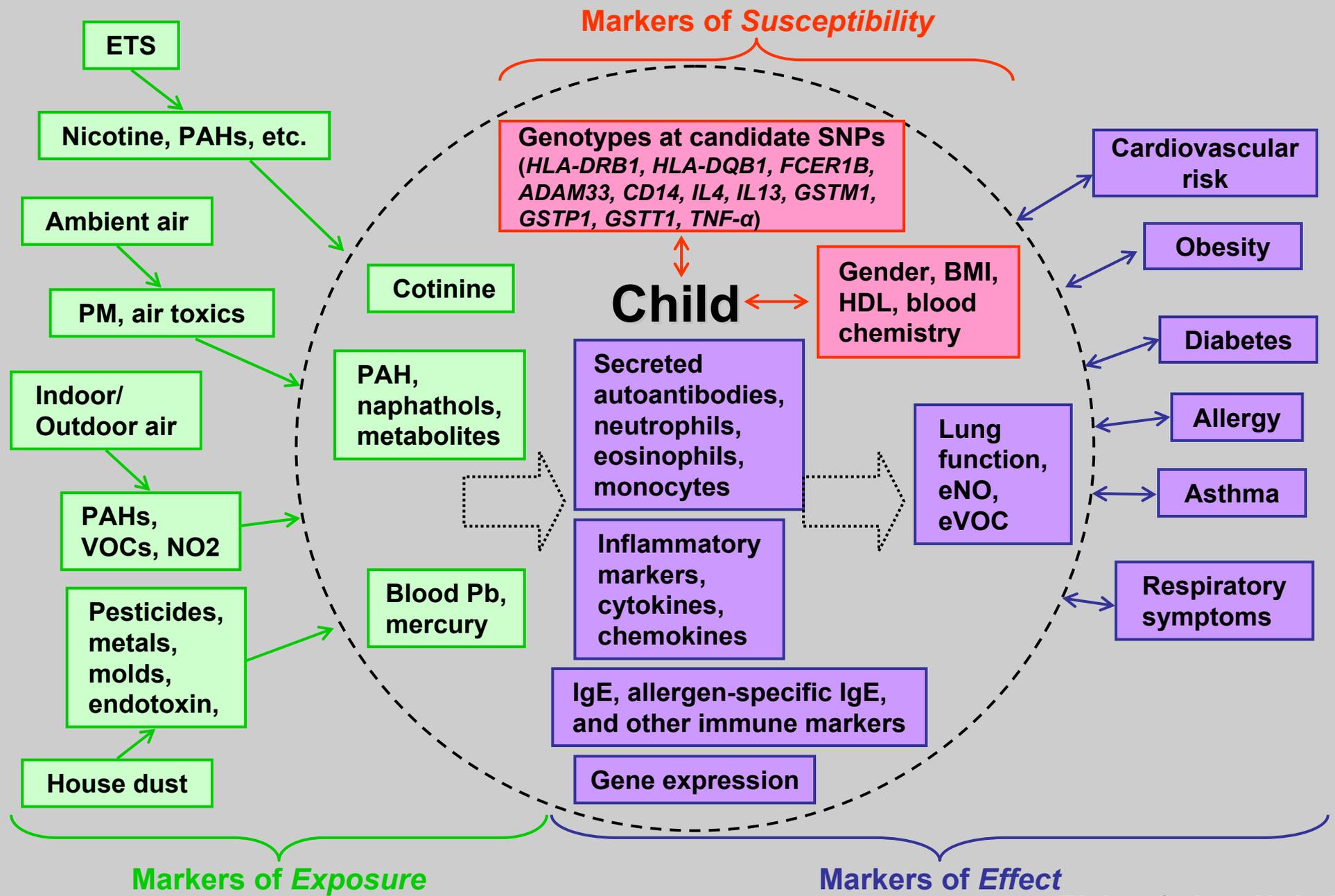
Clinical and Lab

## **Expression Analysis**

Wendell Jones

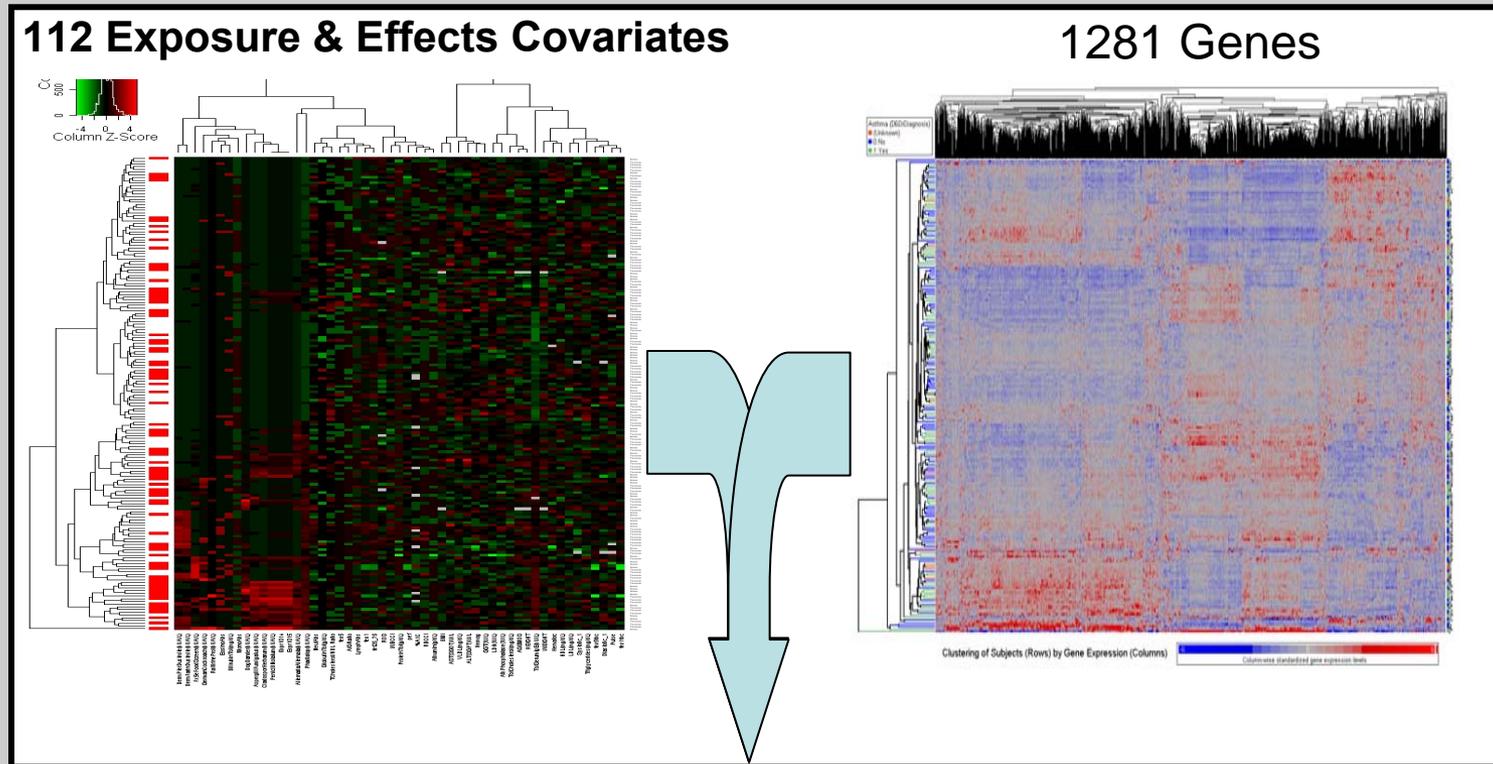
Stephen Siefert

# Mechanistic Indicators of Childhood Asthma (MICA)



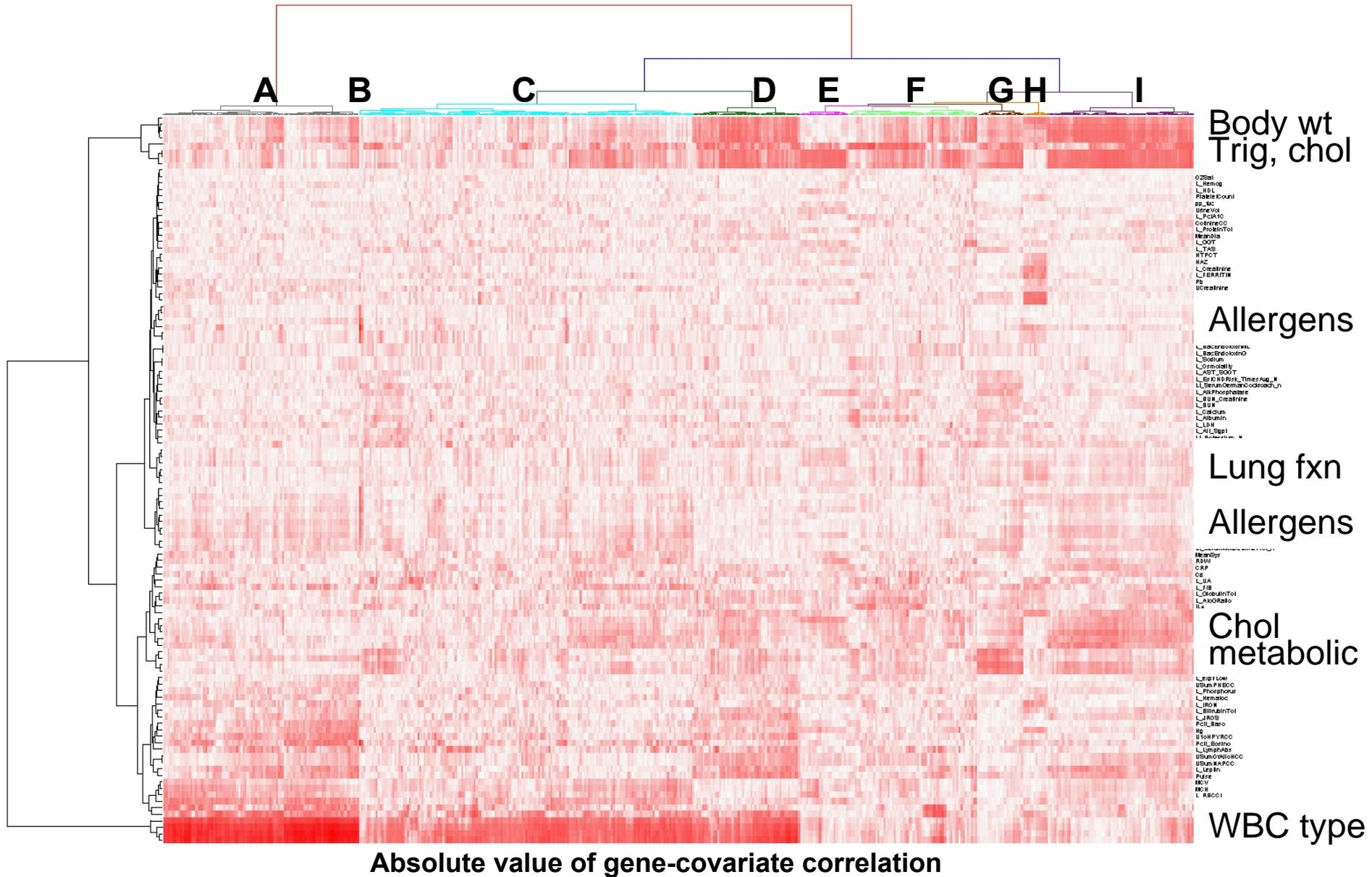
# We can leverage MICA covariate information for the gene expression analysis

193 people  
(samples)



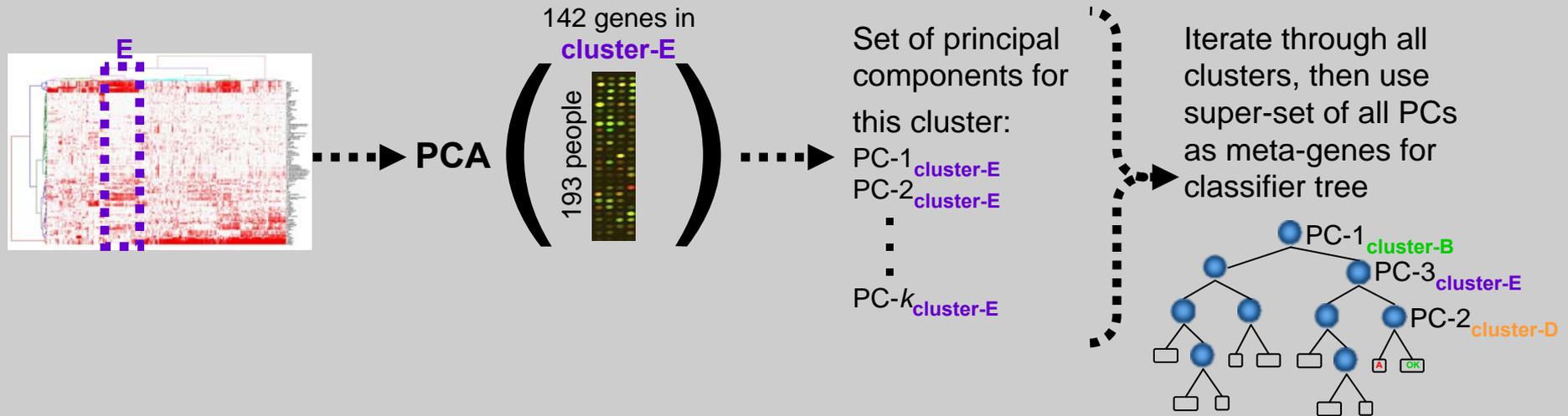
- Can we
  - Identify asthma endotypes?
  - Define mechanism for each endotype?
  - Identify key events?
  - Establish human bioindicators in blood?

# Finding context for gene expression patterns

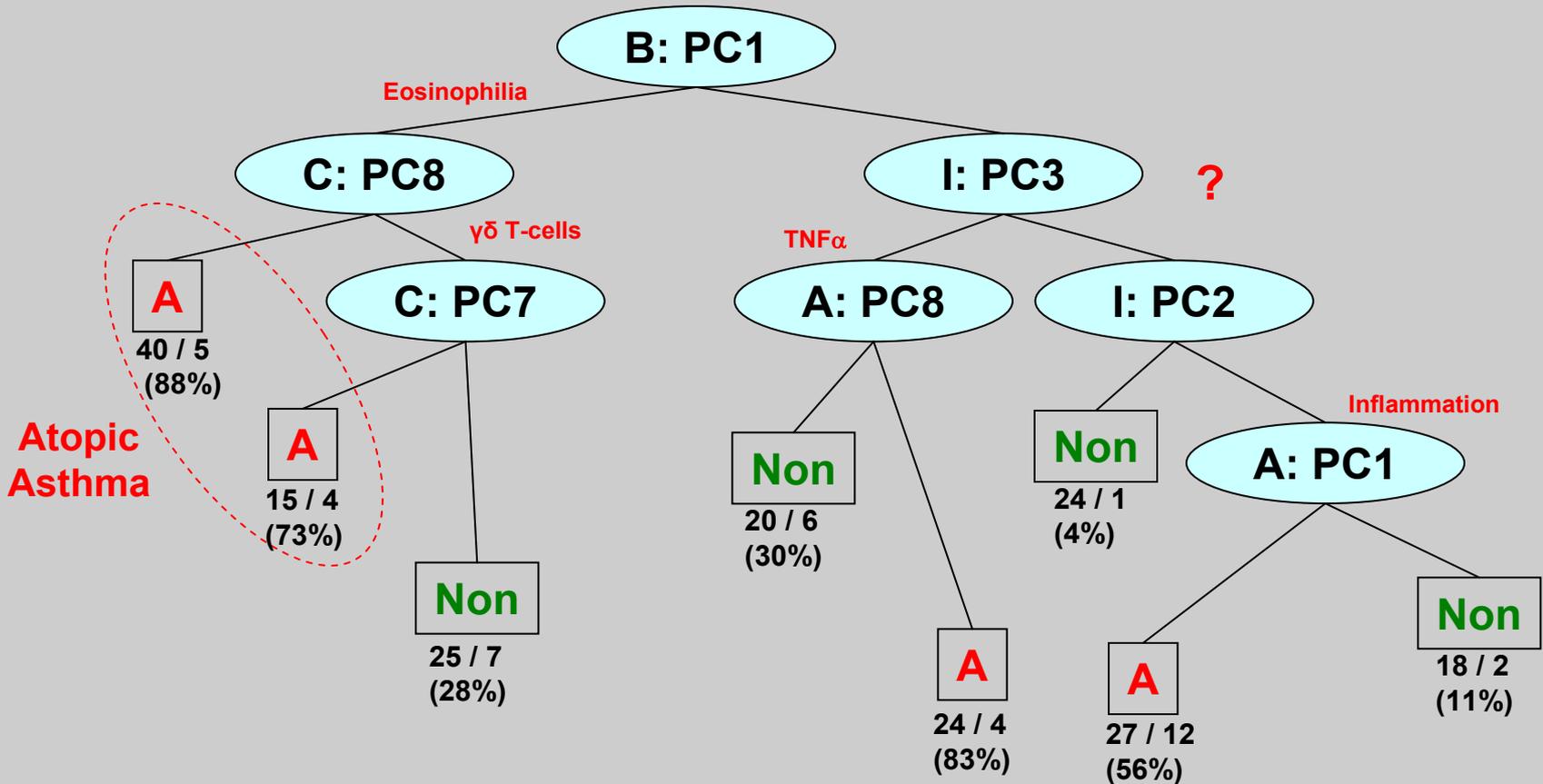


# From gene expression to differing asthma endotypes

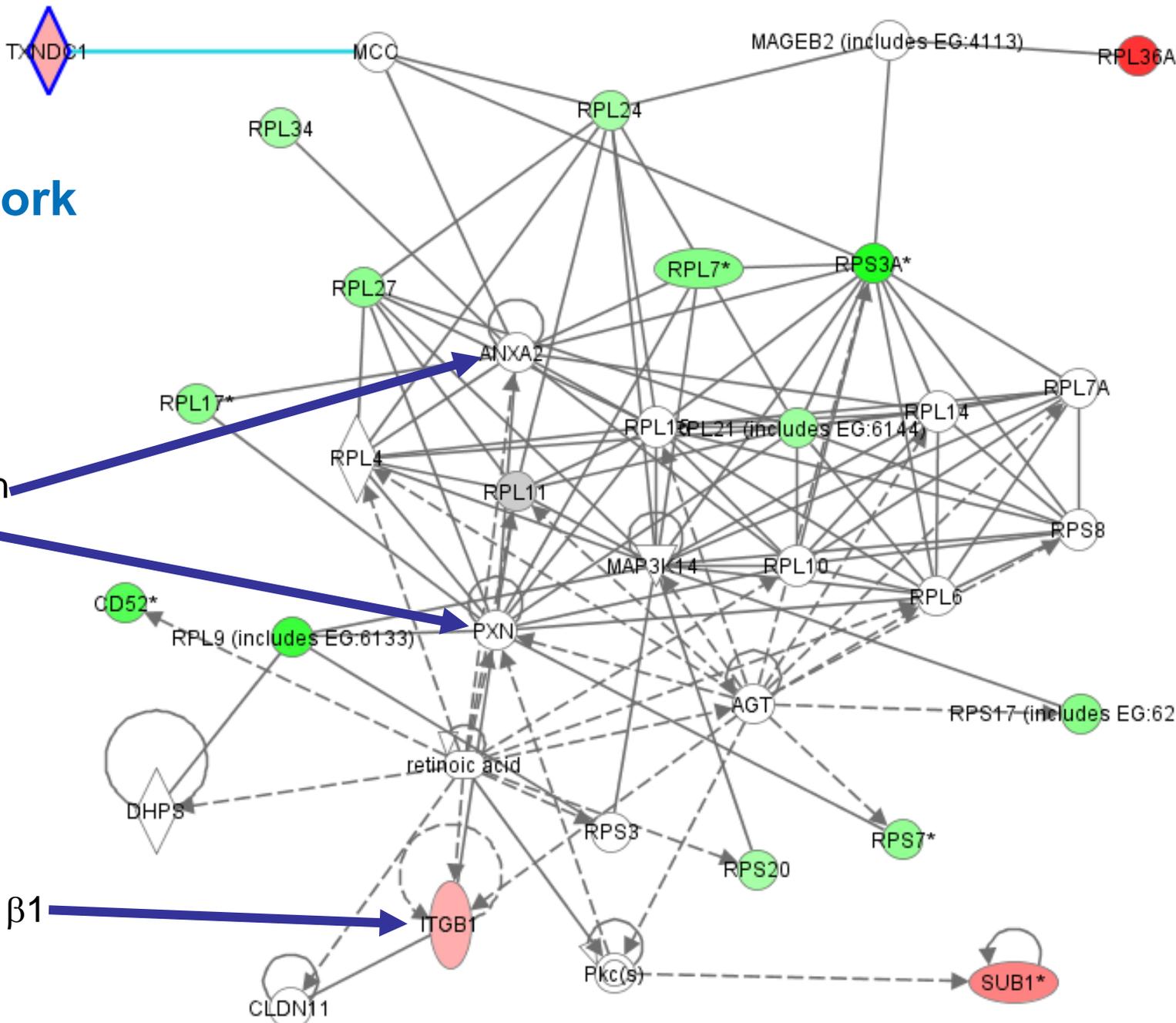
What if we derive meta-genes from covariate clusters (or subclusters)?



# Endotypes of Childhood Asthma



Legend:  
**A** = Predicted asthmatic  
**Non** = predicted non-asthmatic  
 Subjects in group / subjects mis-classified  
 (% asthmatics in group)



# I:PC3 Top Network

Annexin  
Paxillin

Integrin  $\beta$ 1

# I:PC2 Top 2 Networks

## Key Hubs

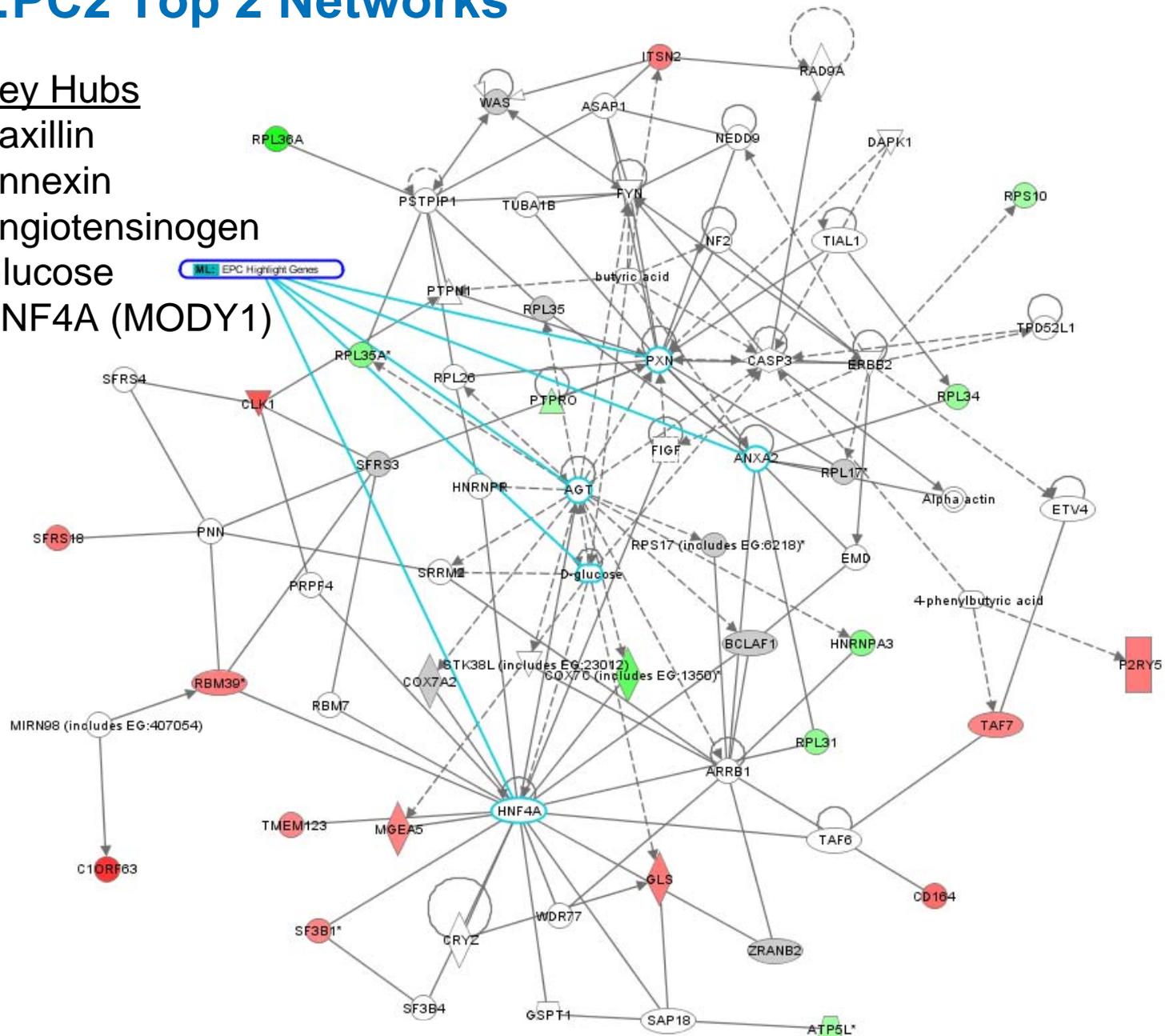
Paxillin

Annexin

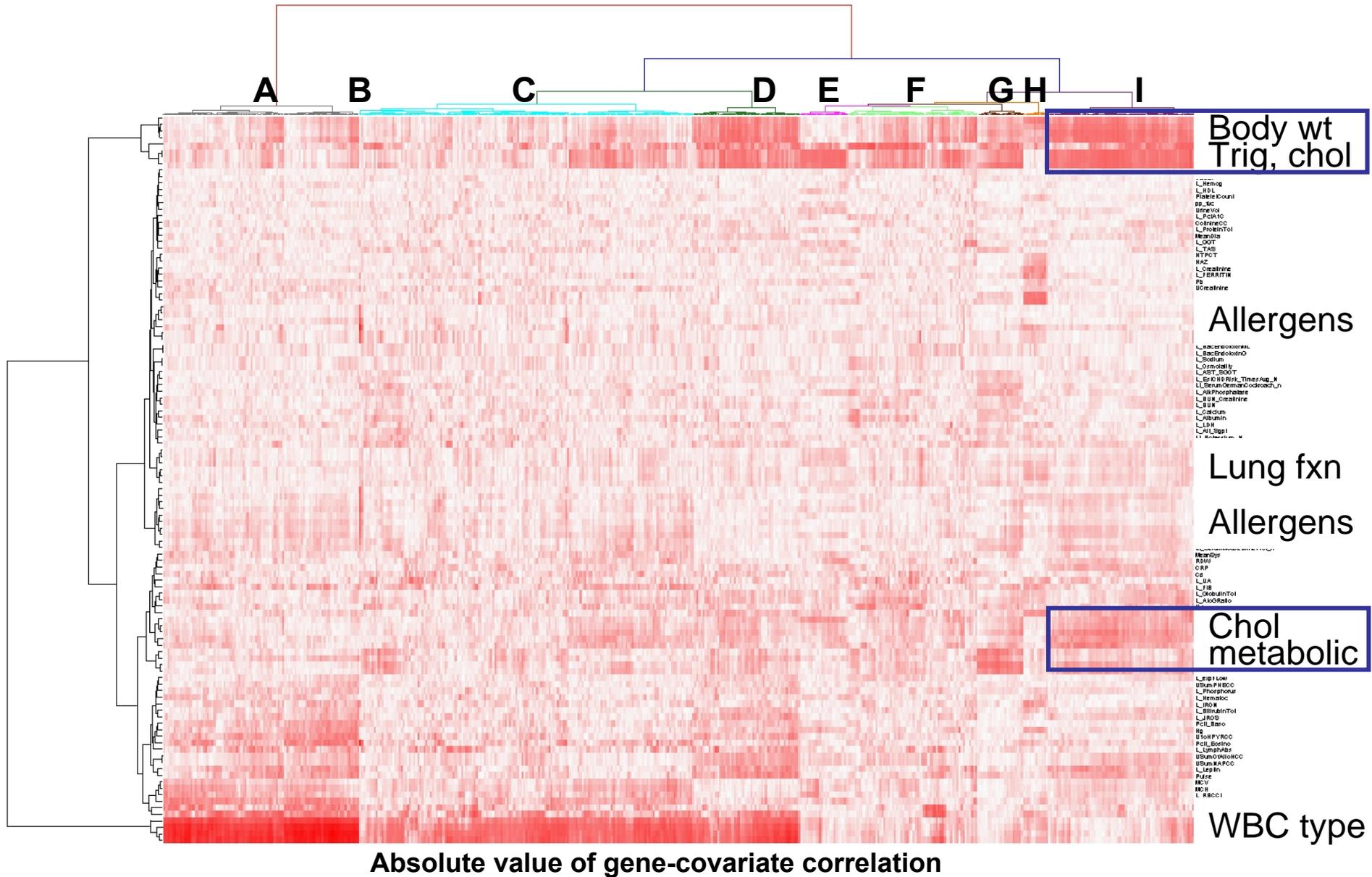
Angiotensinogen

Glucose

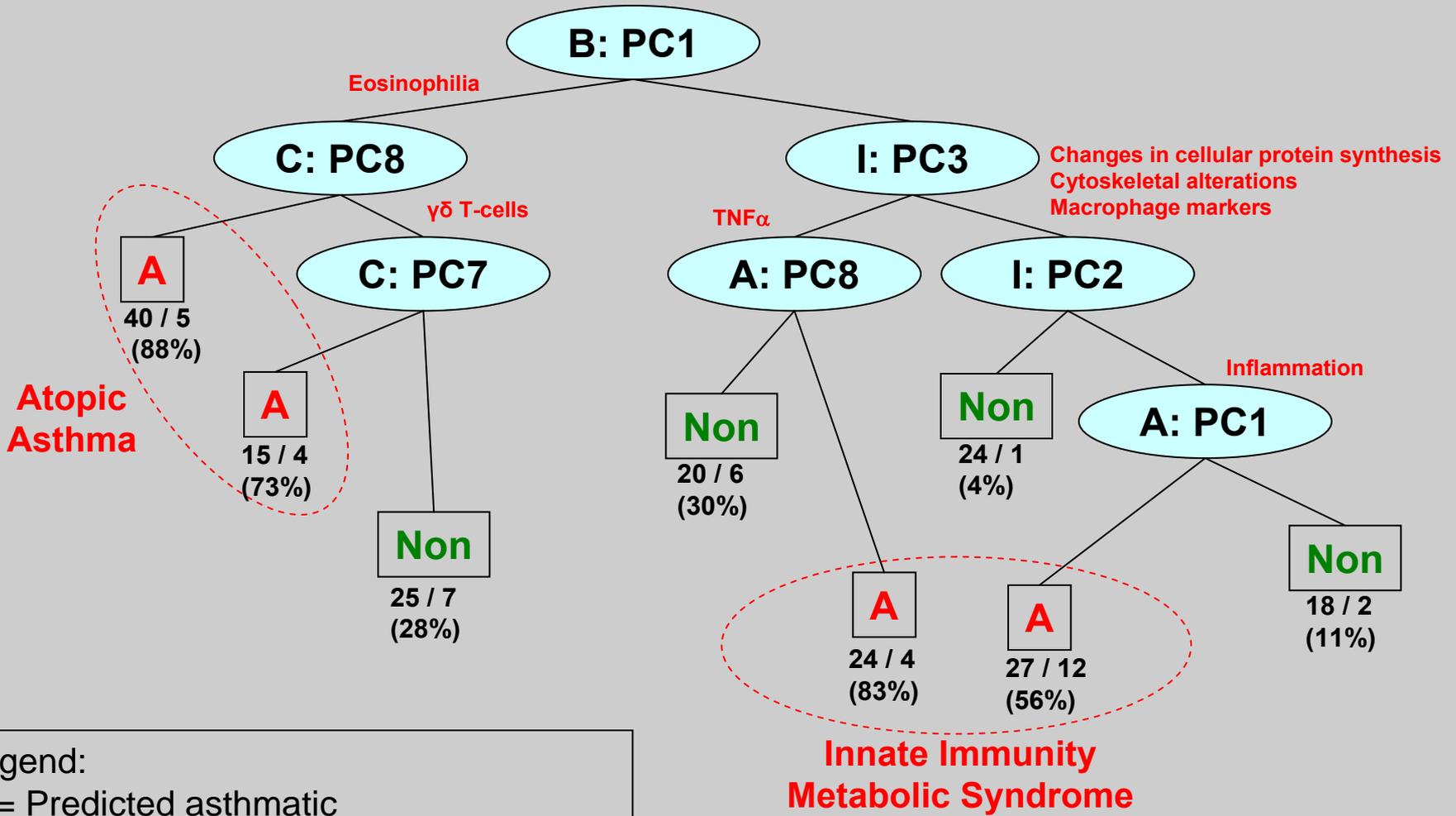
HNF4A (MODY1)



# Finding context for gene expression patterns

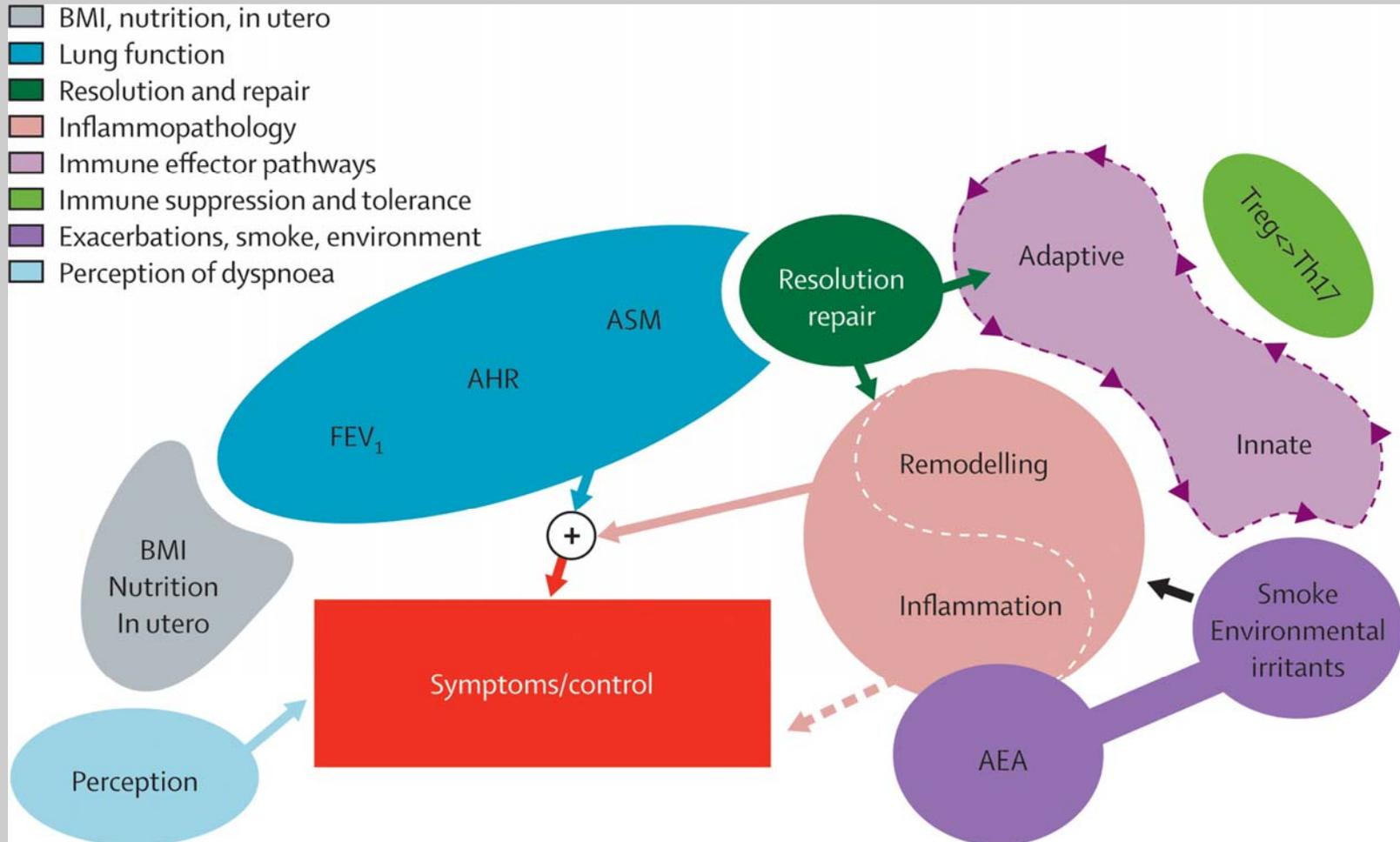


# Endotypes of Childhood Asthma



Legend:  
**A** = Predicted asthmatic  
**Non** = predicted non-asthmatic  
 Subjects in group / subjects mis-classified  
 (% asthmatics in group)

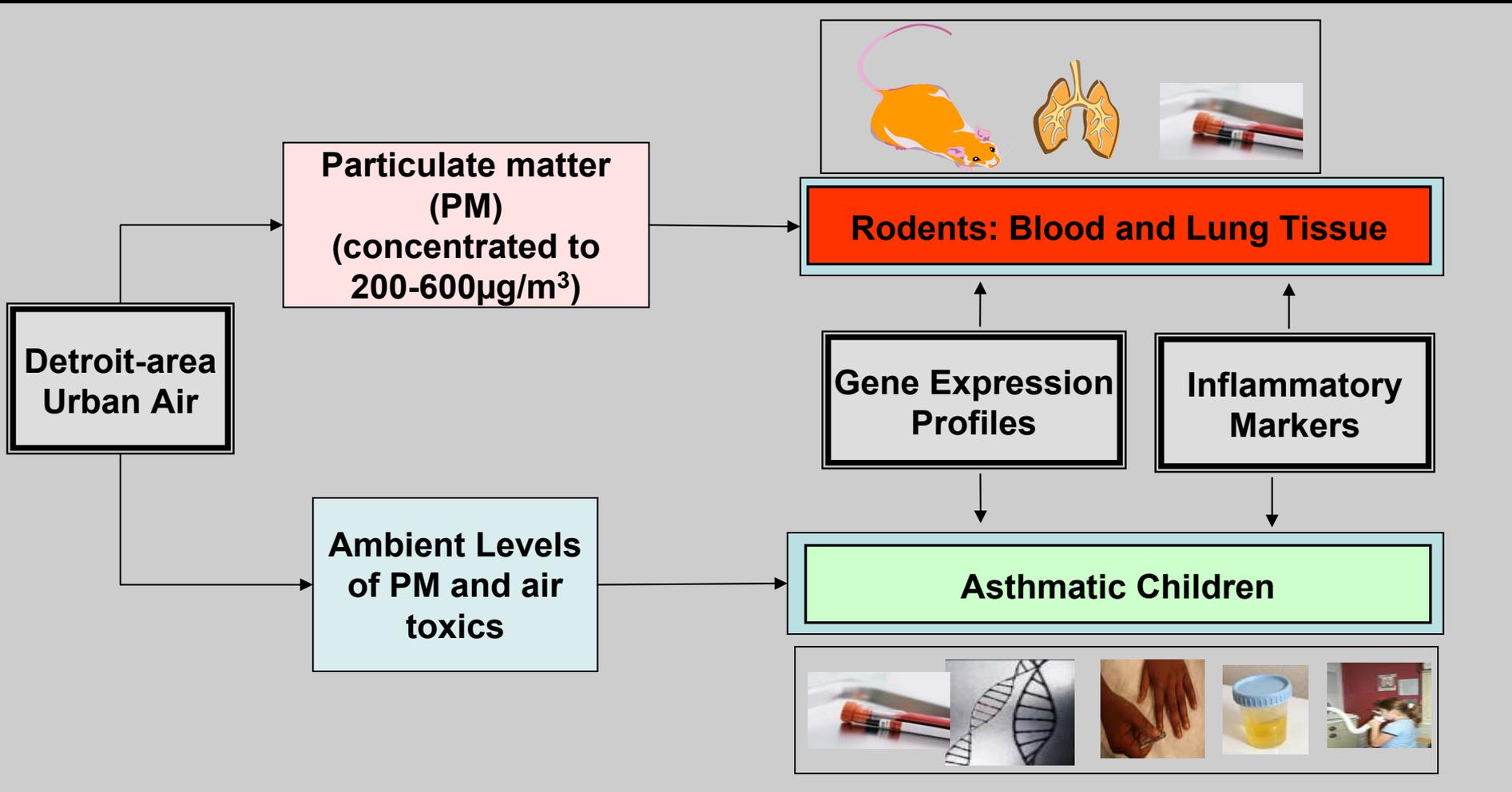
# Previously Proposed Asthma Endotypes



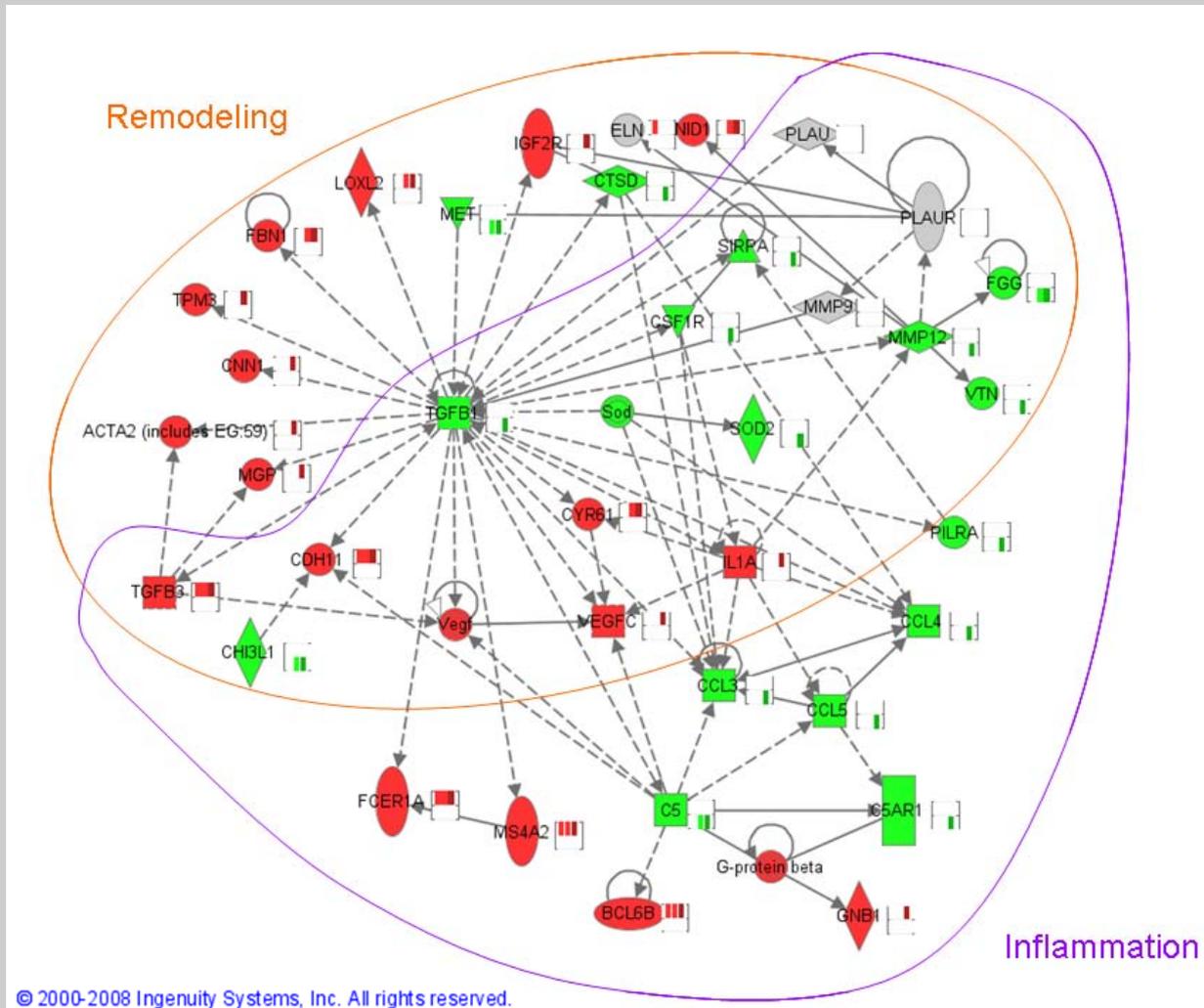
## Conclusions

- Identify asthma endotypes
  - Identified 2-4 potential asthma endotypes which are consistent with previously proposed classifications
  - These endotypes could help to better define susceptible subpopulations for risk assessment of air pollutants
- Define mechanism for each endotype
  - Data suggests that asthma linked with metabolic syndrome is mediated by innate immune responses potentially via priming or activation signals from adipose
  - Molecular mechanisms are currently under investigation
- Identify key events
  - More work is needed
- Establish human bioindicators in blood
  - Monitoring the “activation state” of circulating monocytes and neutrophils may serve as a good bioindicator for non-atopic asthma

# MICA Study Design

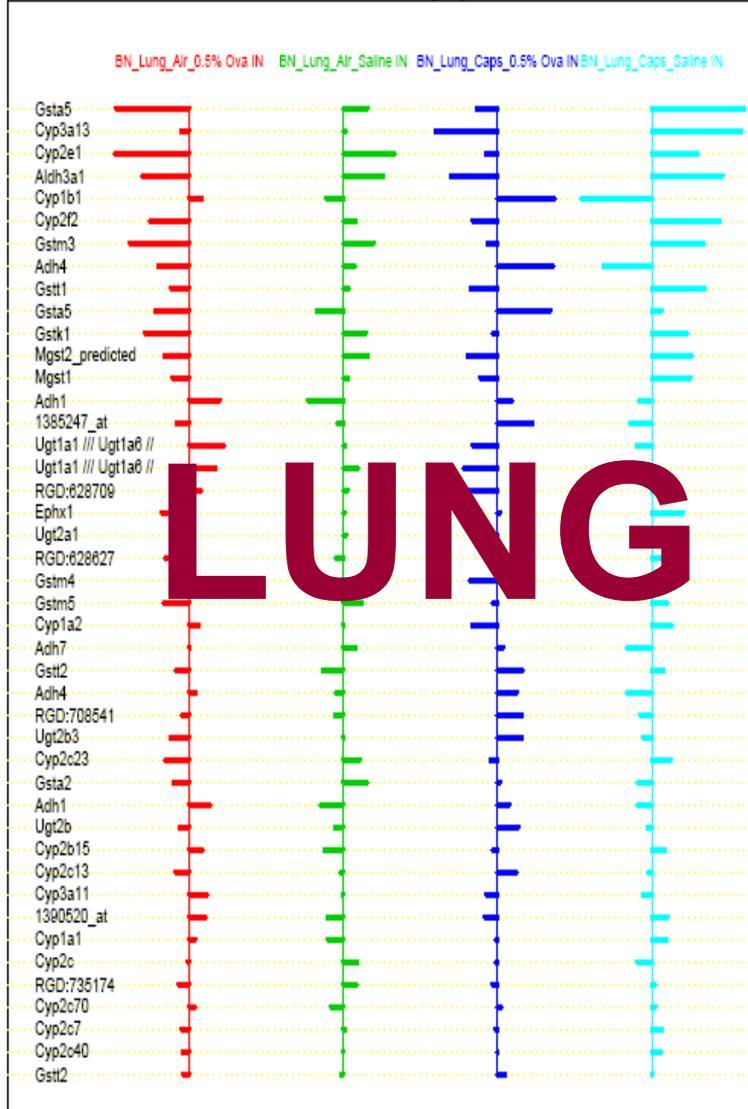


# Gene expression in the lung implicates inflammation and remodeling in response to CAPs + ovalbumin

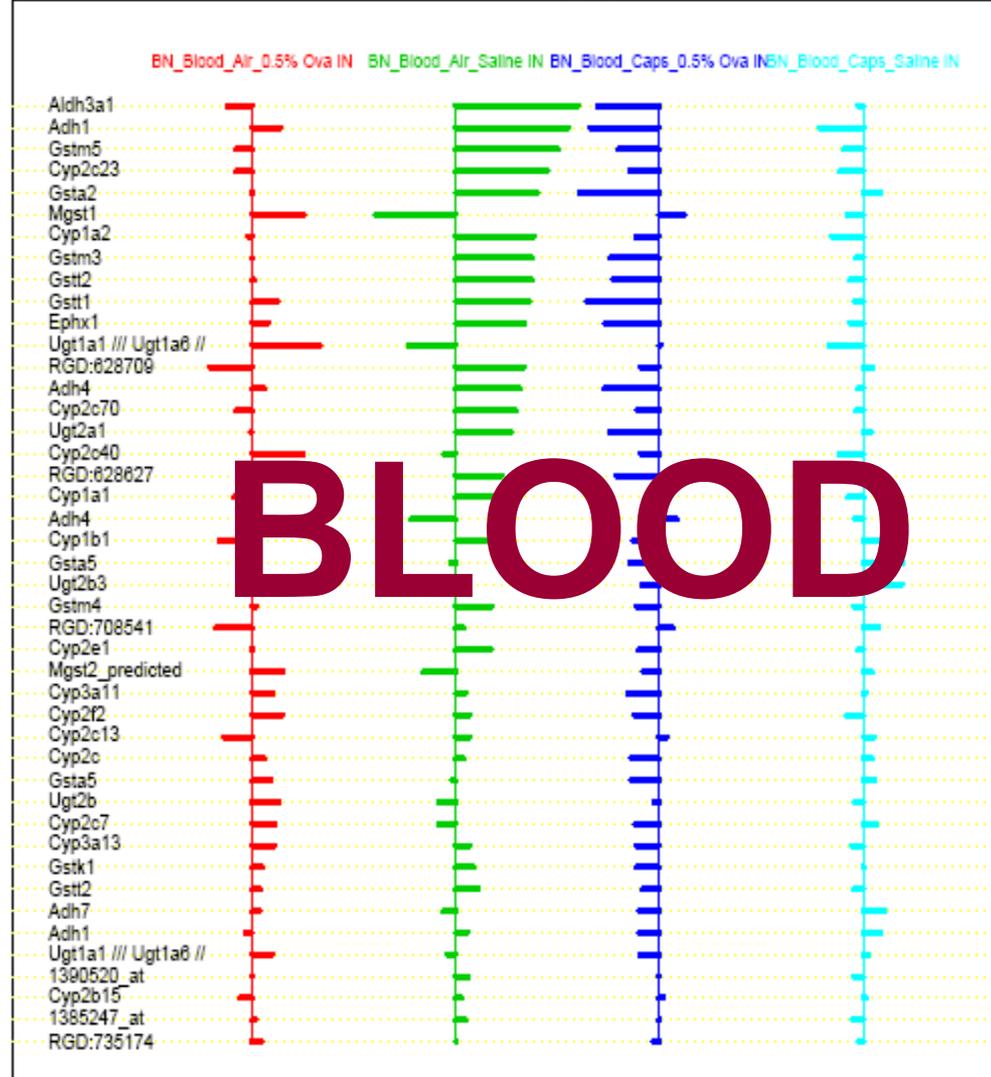


# Lung vs. Blood comparison in rodents is ongoing

Metabolism of xenobiotics by cytochrome P450



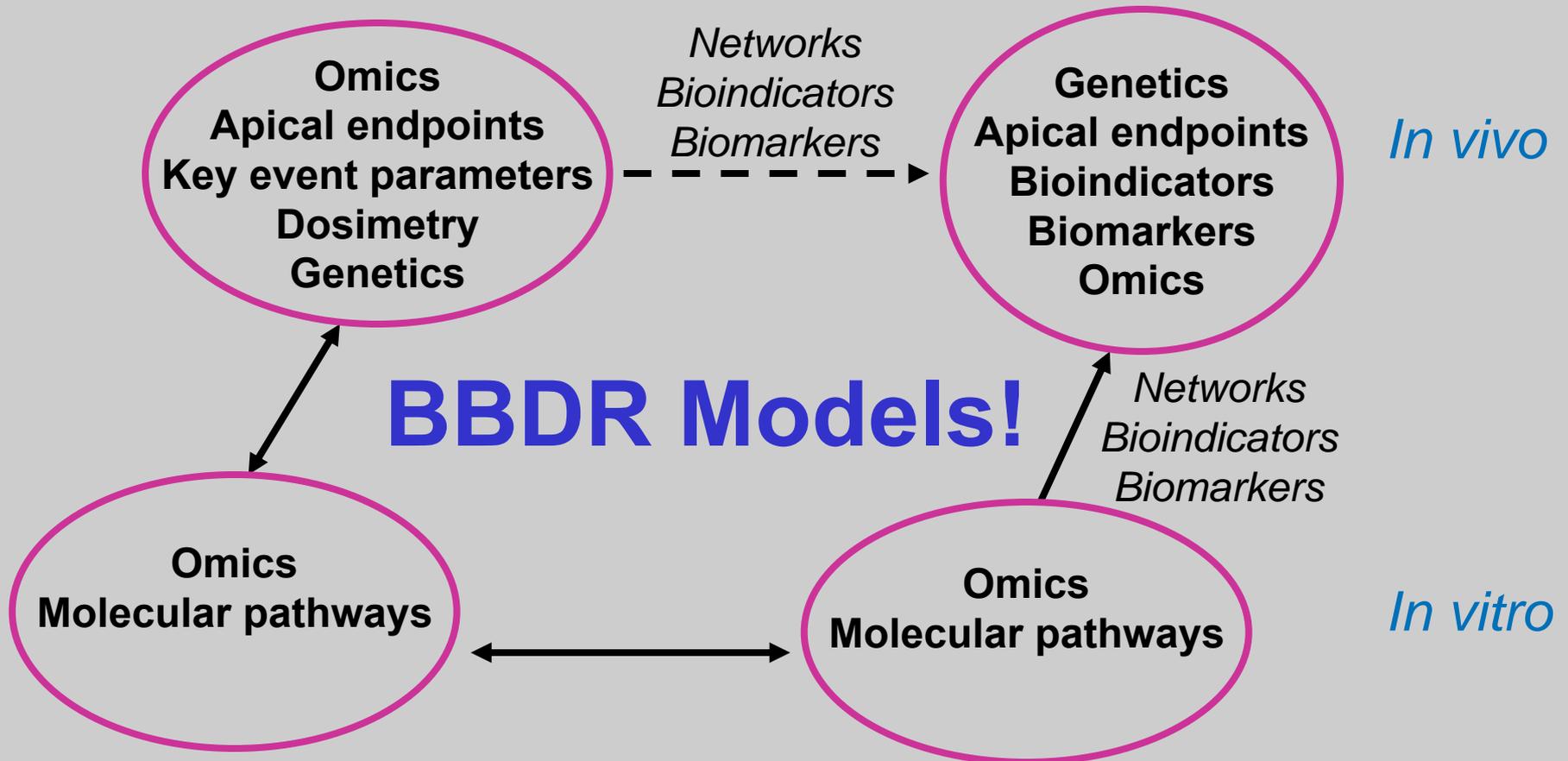
Metabolism of xenobiotics by cytochrome P450



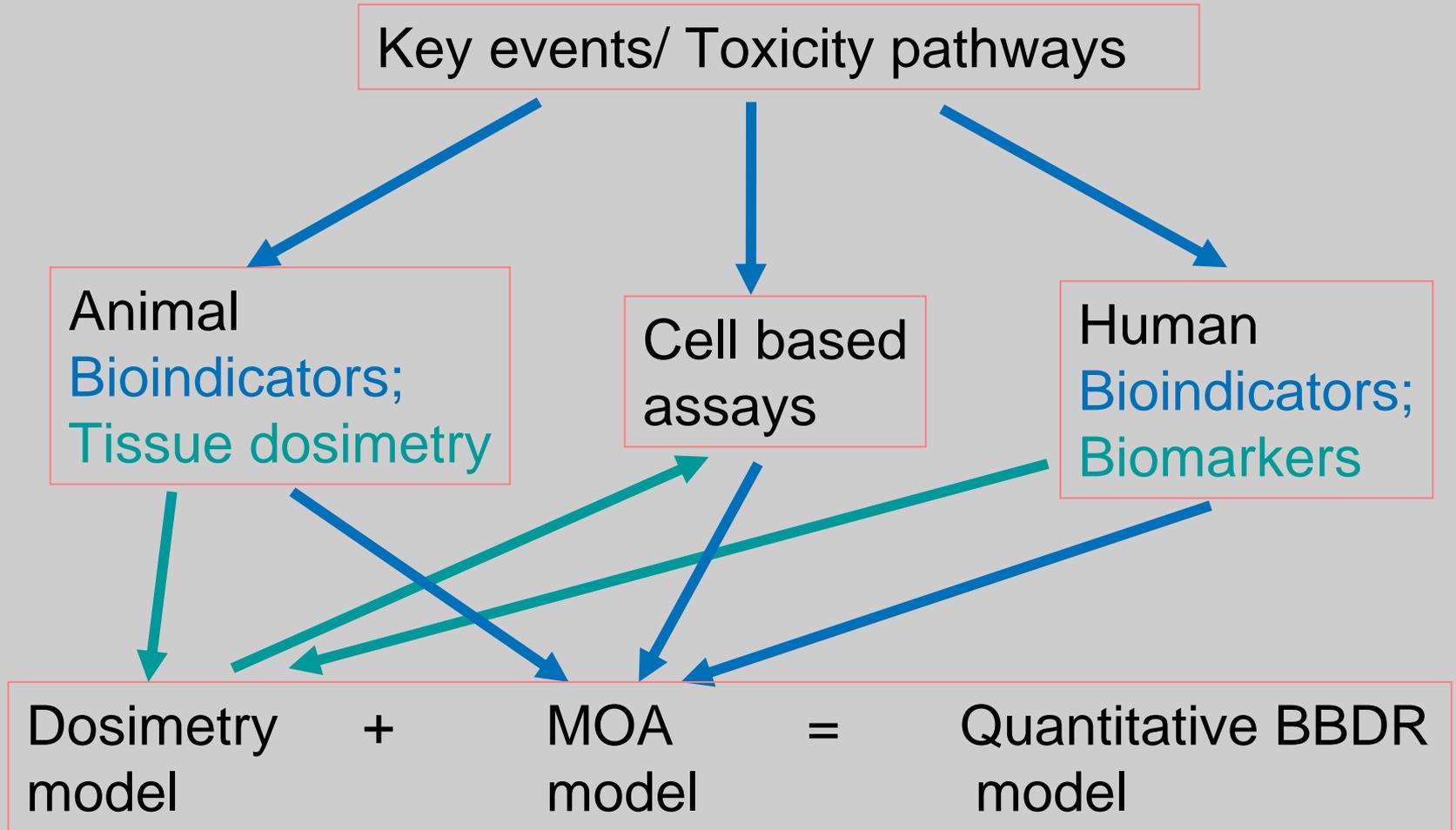
# Integrated Systems Toxicology

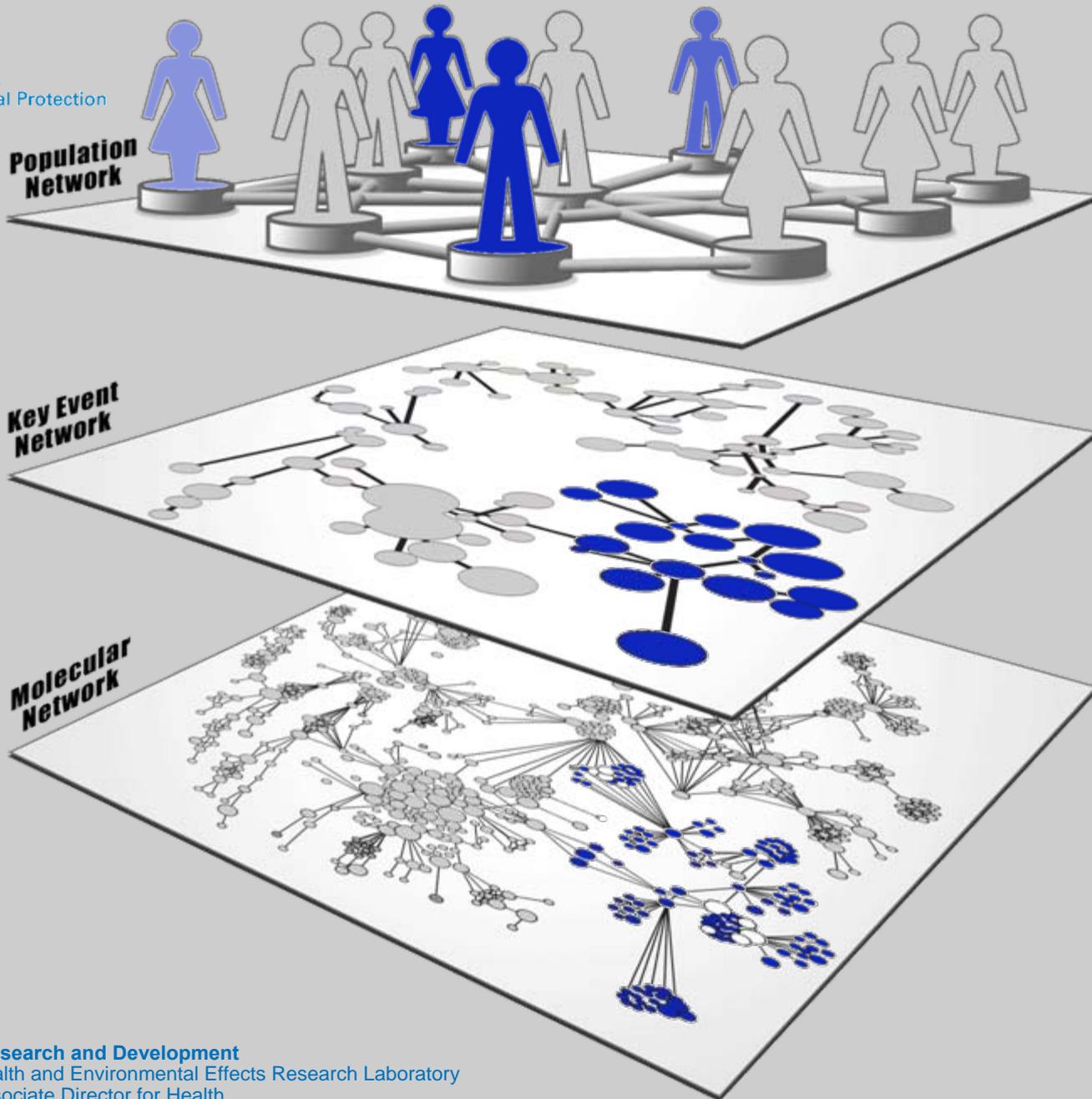
Model Organism

Target Organism



# Toxicity Pathways & BBDR Modeling

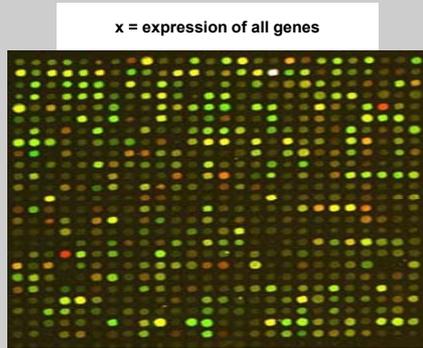




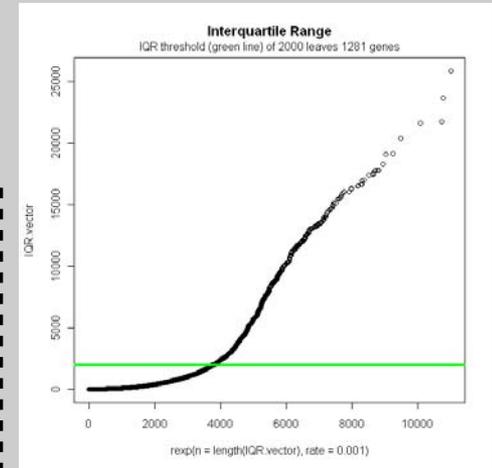
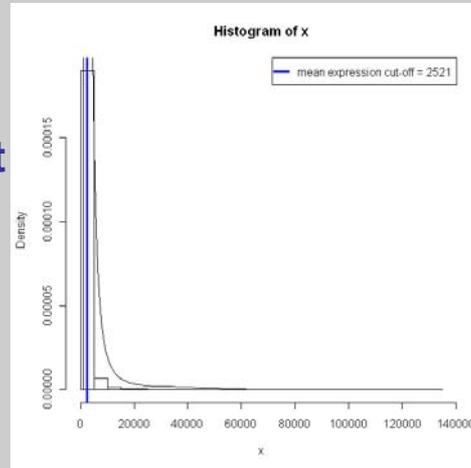
# Acknowledgements

- **NHEERL Human Studies Division**
  - Jane Gallagher
  - Brooke Heidenfelder
  - Ed Hudgens
  - Lucas Neas
  - Mary Johnson
  - Ann Williams
- **National Center for Computational Toxicology**
  - David Reif
  - Elaine Cohen Hubal
- **Expression Analysis**
  - Wendell Jones

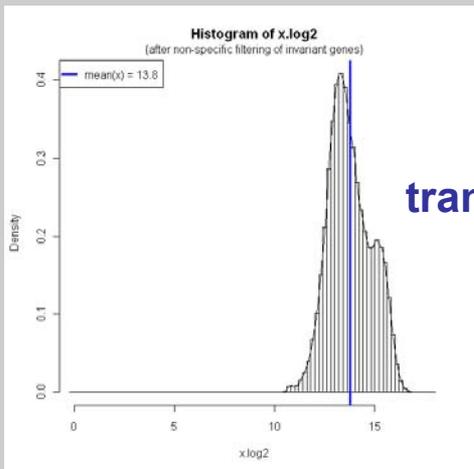
# Analysis pipeline for the gene expression data



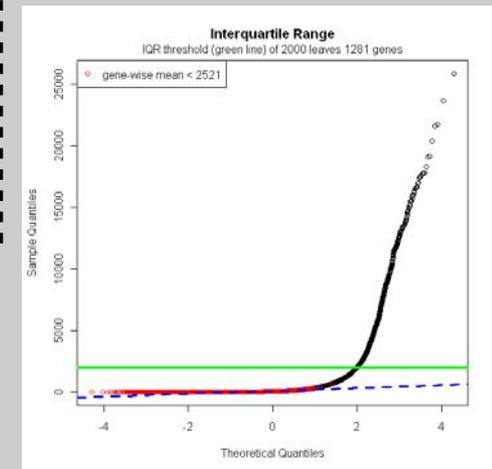
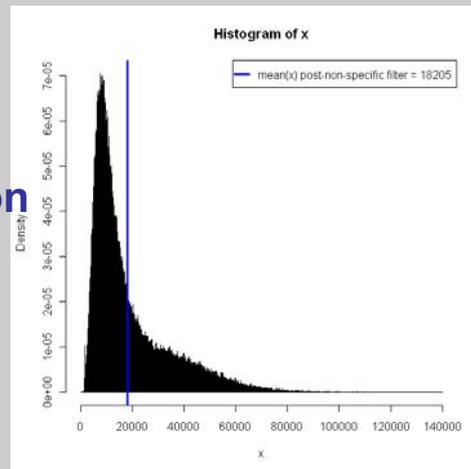
**Gender adjustment**

**IQR filtering**

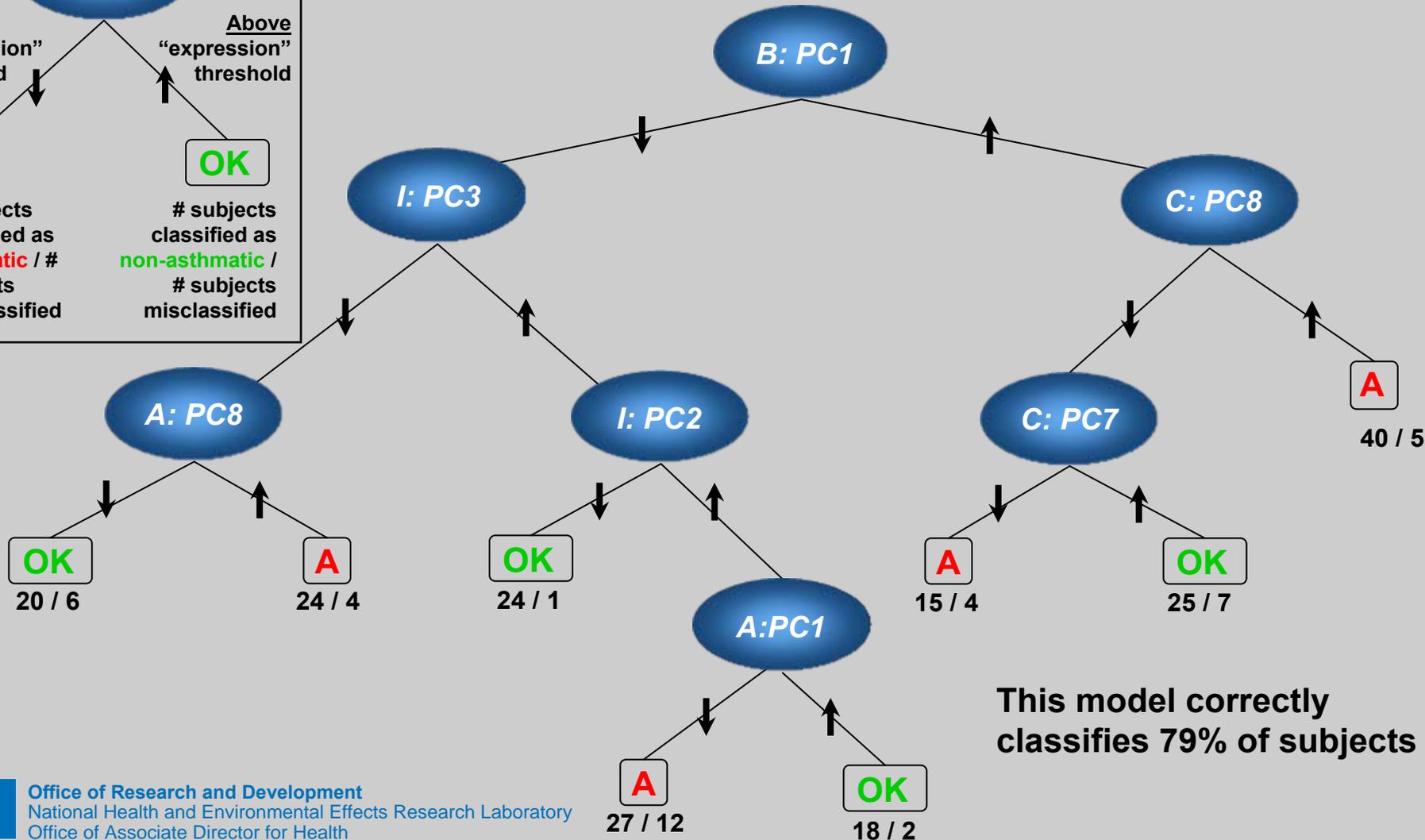
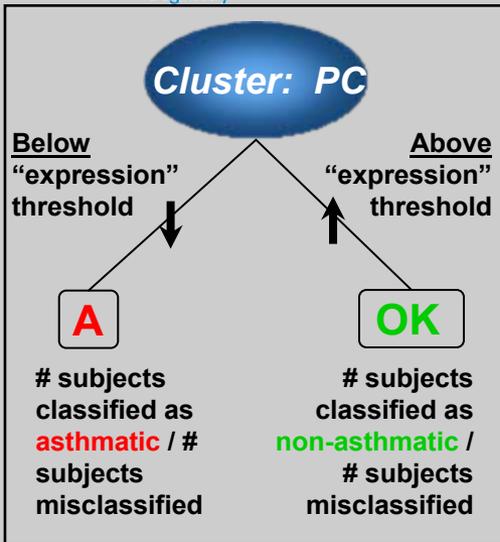
**Log<sub>2</sub> transformation**

**IQR filtering**



# Can we discriminate subtypes of asthma? (Unscaled covariance matrix)



# Previously Proposed Asthma Endotypes

- Atopic vs. non-atopic asthma
  - No resolved asthmatics included in this study
- By symptoms
  - Day-time vs. nocturnal symptoms
  - Limitation of activity
  - Need for rescue drugs & treatment responses
  - Lung function
- By inflammatory cells found in sputum - Douwes (2002), Thorax 57:643
  - Eosinophilic
  - Neutrophilic
  - Mixed (both neutrophils & eosinophils found)
  - Paucigranulocytic (few or no granulocytes found)
- Other clinical determinants
  - Obesity
  - Nutrition
  - Prenatal stress
  - Tobacco smoke
  - Environmental air pollutants