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Mechanistic Indicators of Childhood Asthma (MICA) Study

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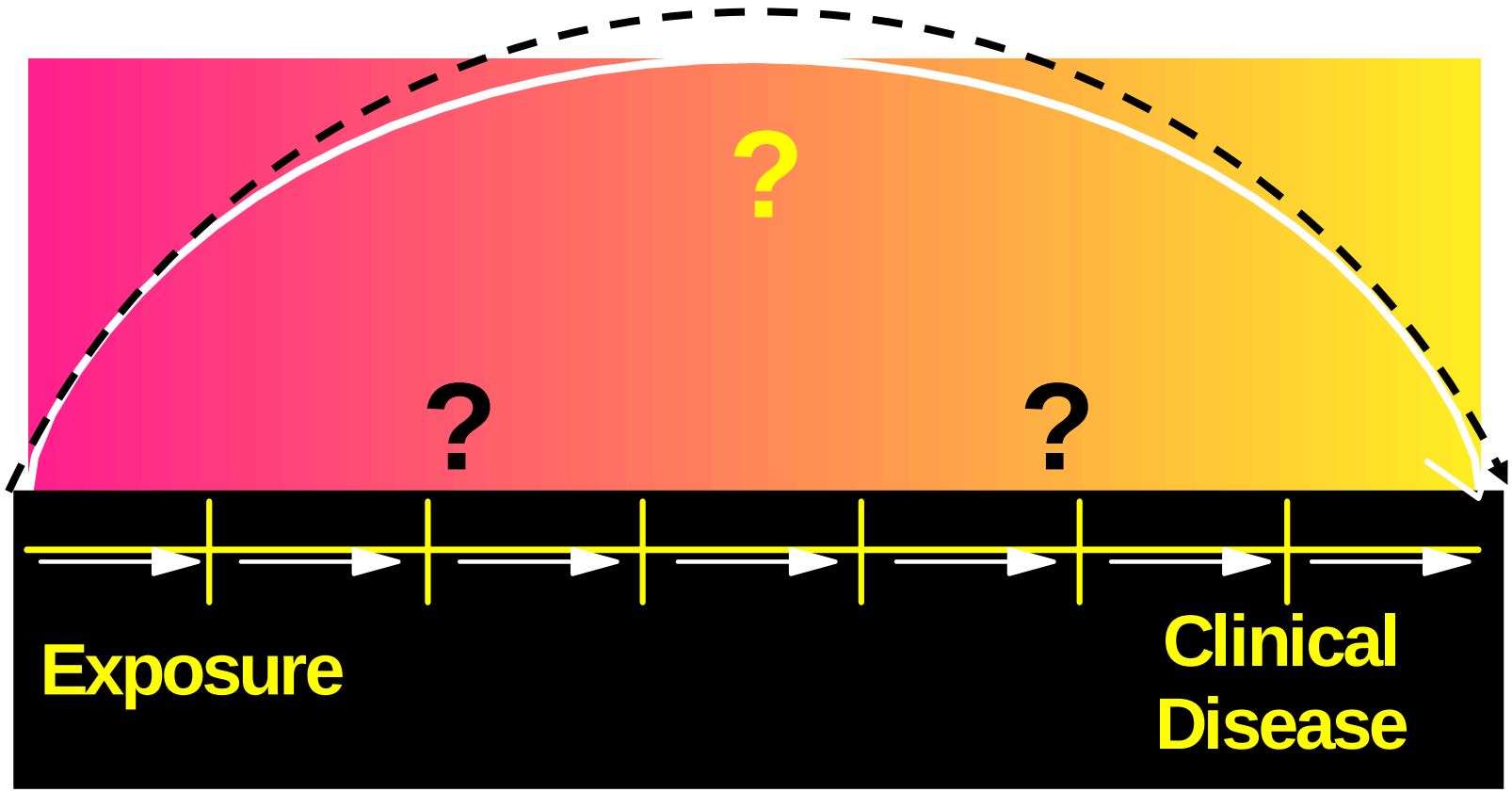
Mechanistic Indicators of Childhood Asthma (MICA)

- Apply state-of-the-art technologies to examine the interplay between environmental and genetic factors affecting asthma
- 100 asthmatic and 100 non-asthmatic children, ages 9-12 years (subset of Detroit Children's Health Study cohort)
- Collected multiple types of clinical, demographic, exposure, and gene expression data
- Consider markers of susceptibility, exposure, and effects to analyze and characterize combined risk factors that relate to asthma severity
- http://www.epa.gov/dears/studies.htm



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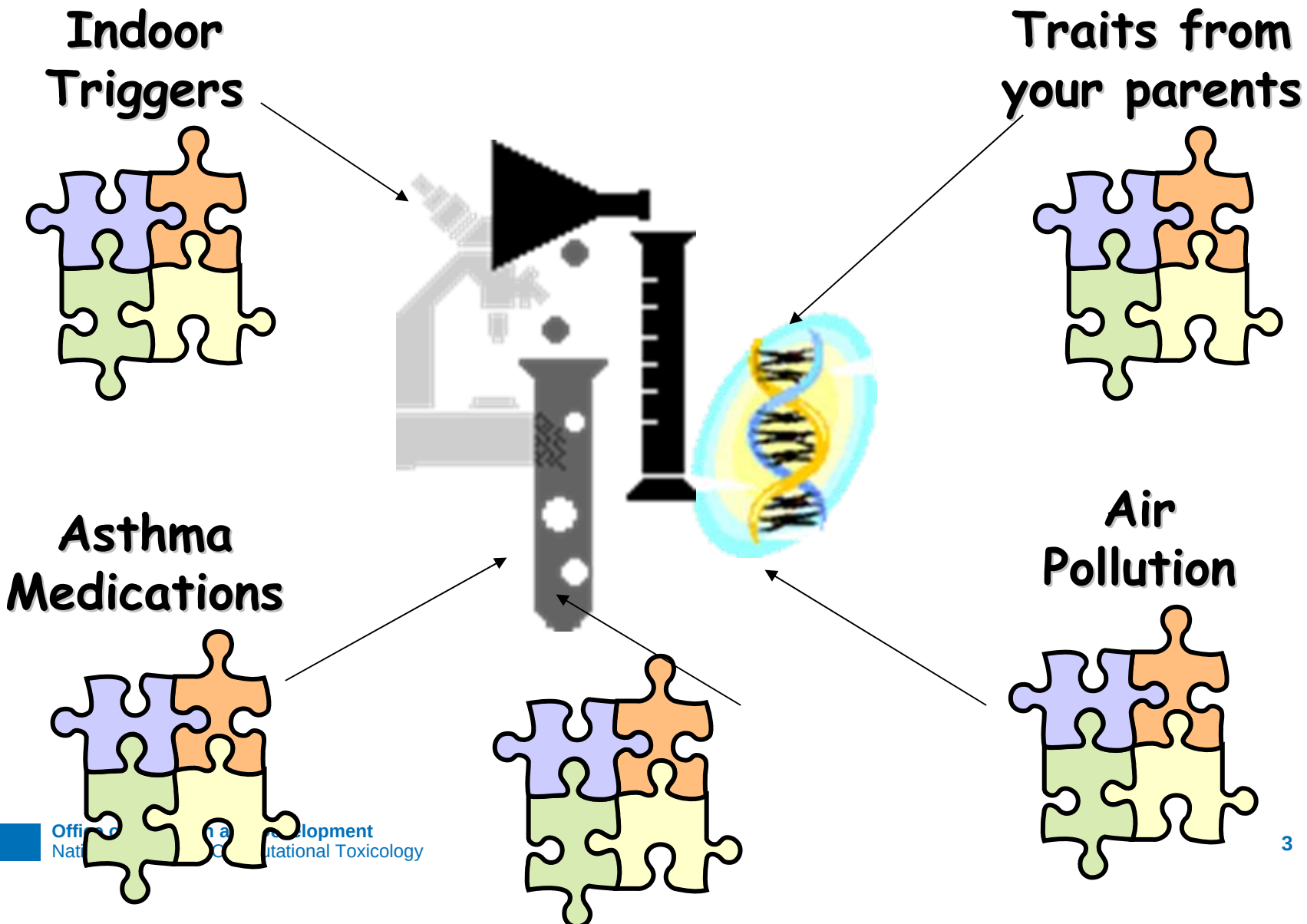
Slide taken from the **MICA** educational presentation used in our study in Detroit



MICA Study Design

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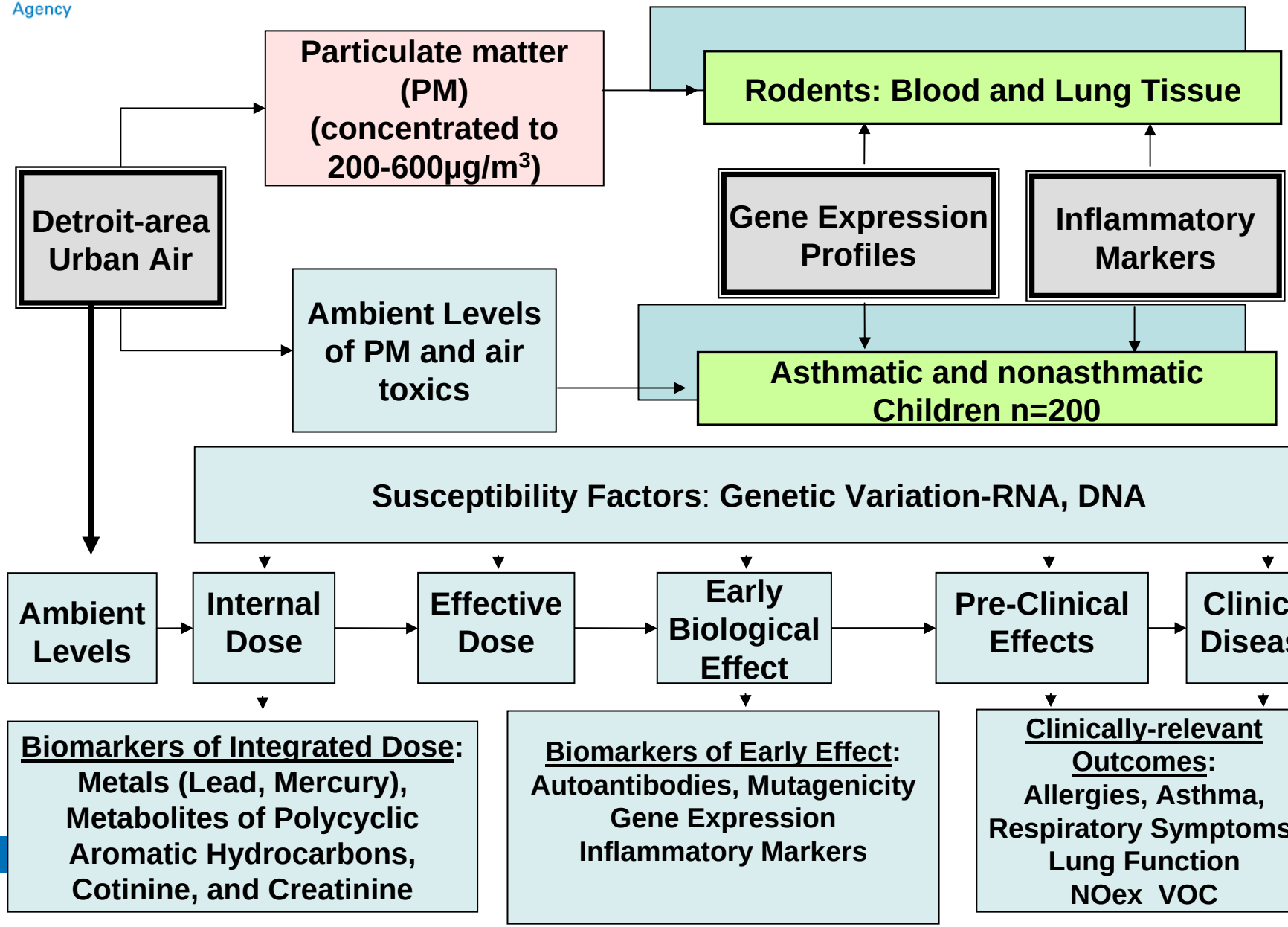
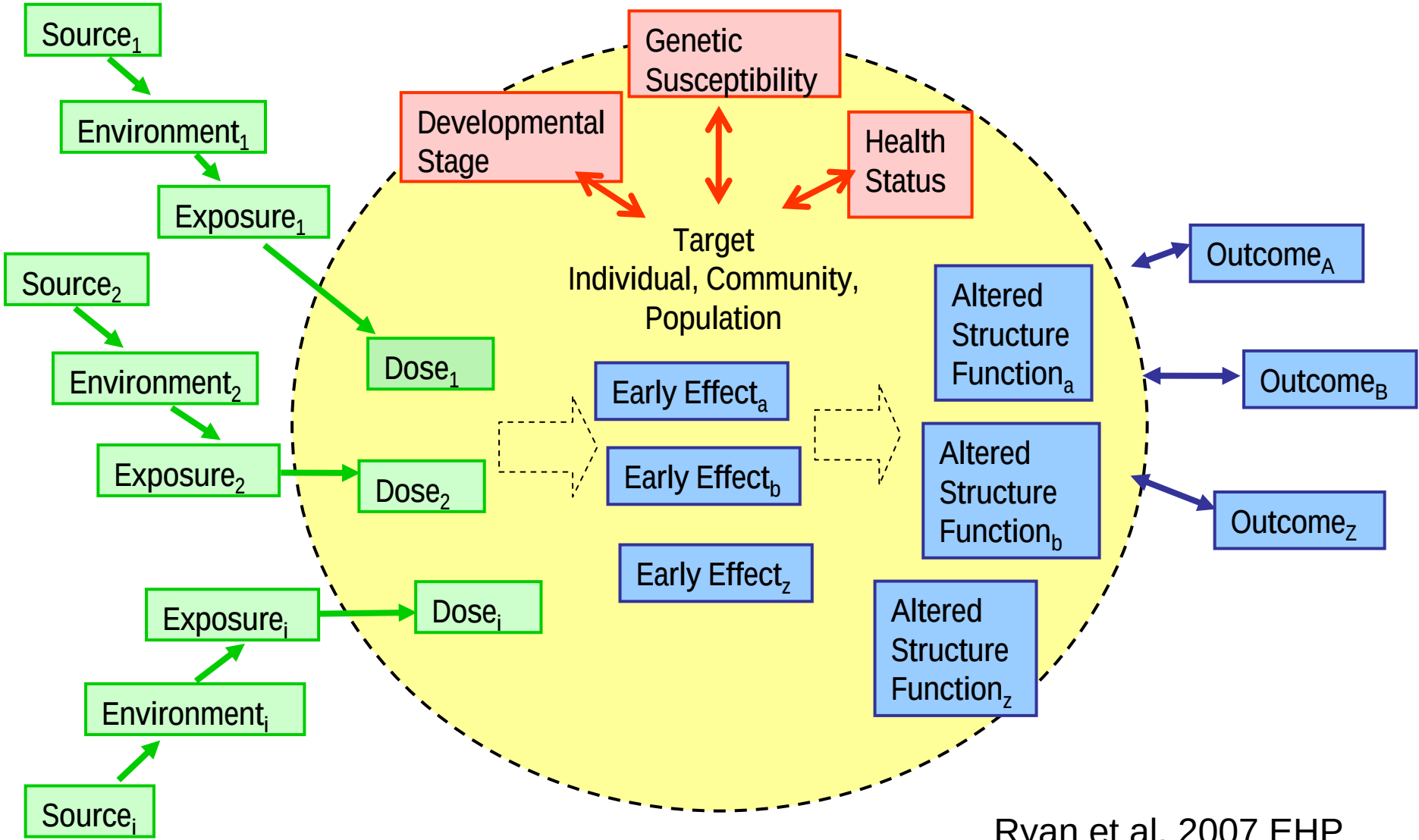
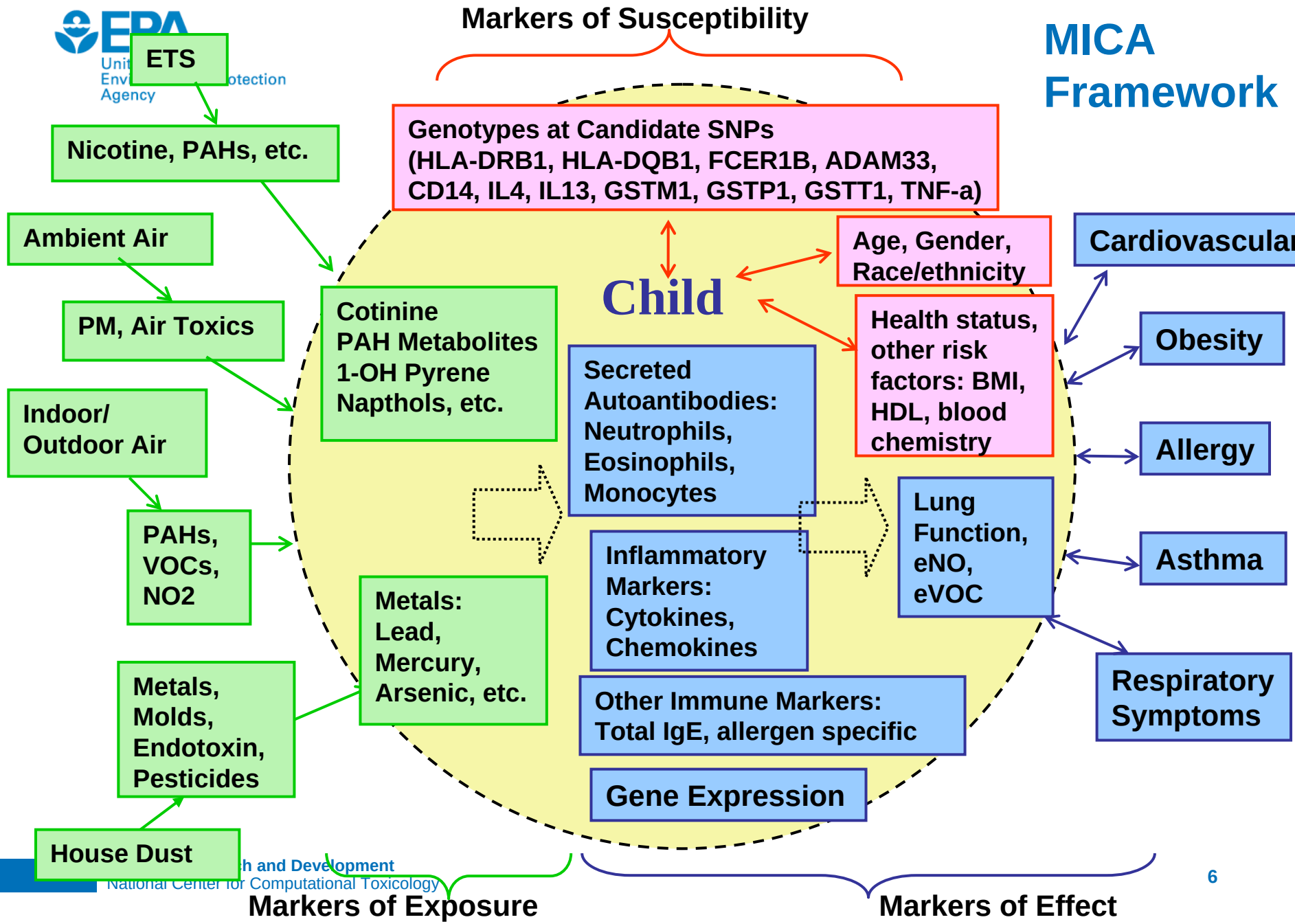


Biomarker Framework

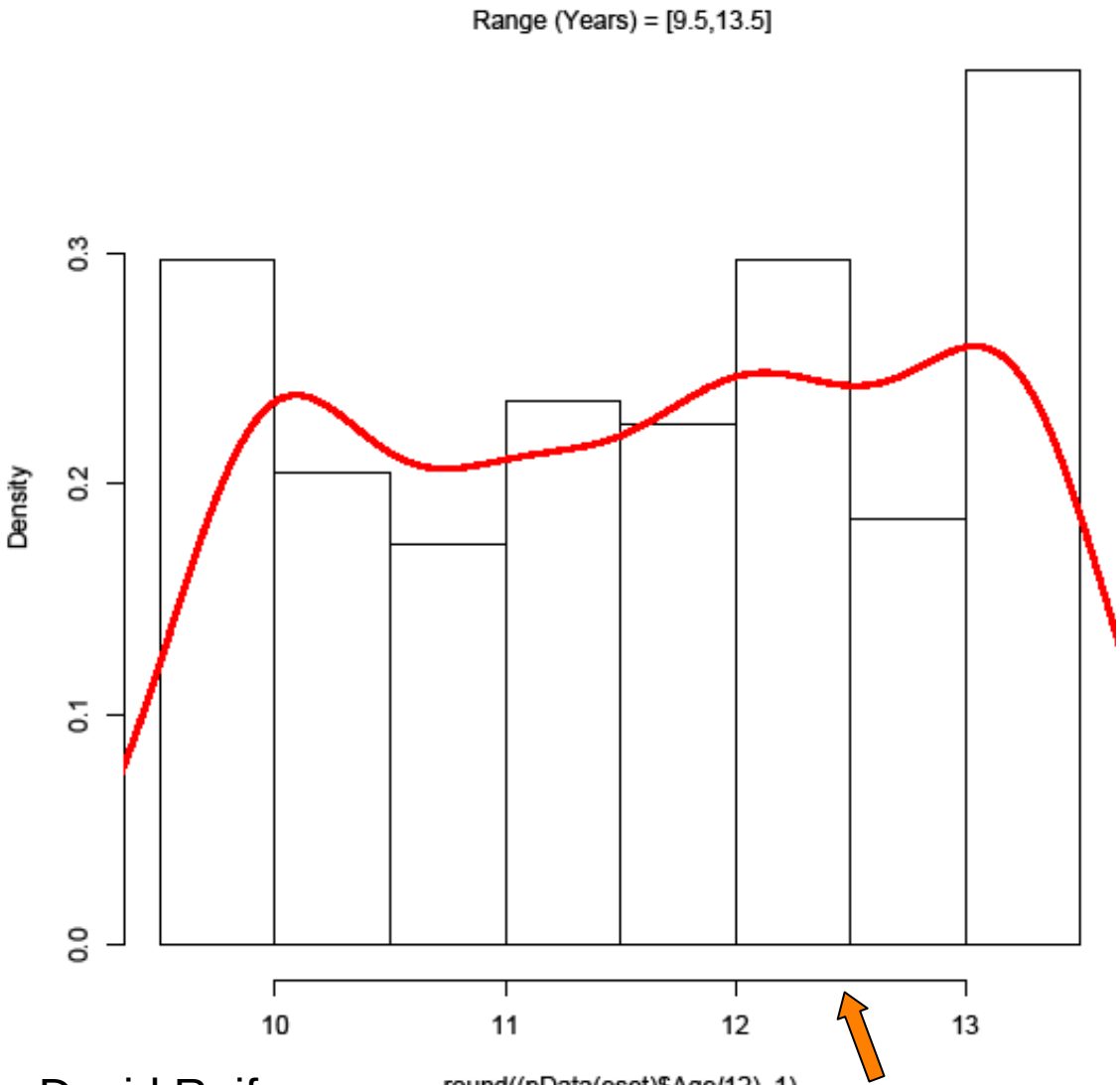


Ryan et al. 2007 EHP

MICA Framework



Characteristics of our MICA Study Sample



Study sample includes both boys and girls

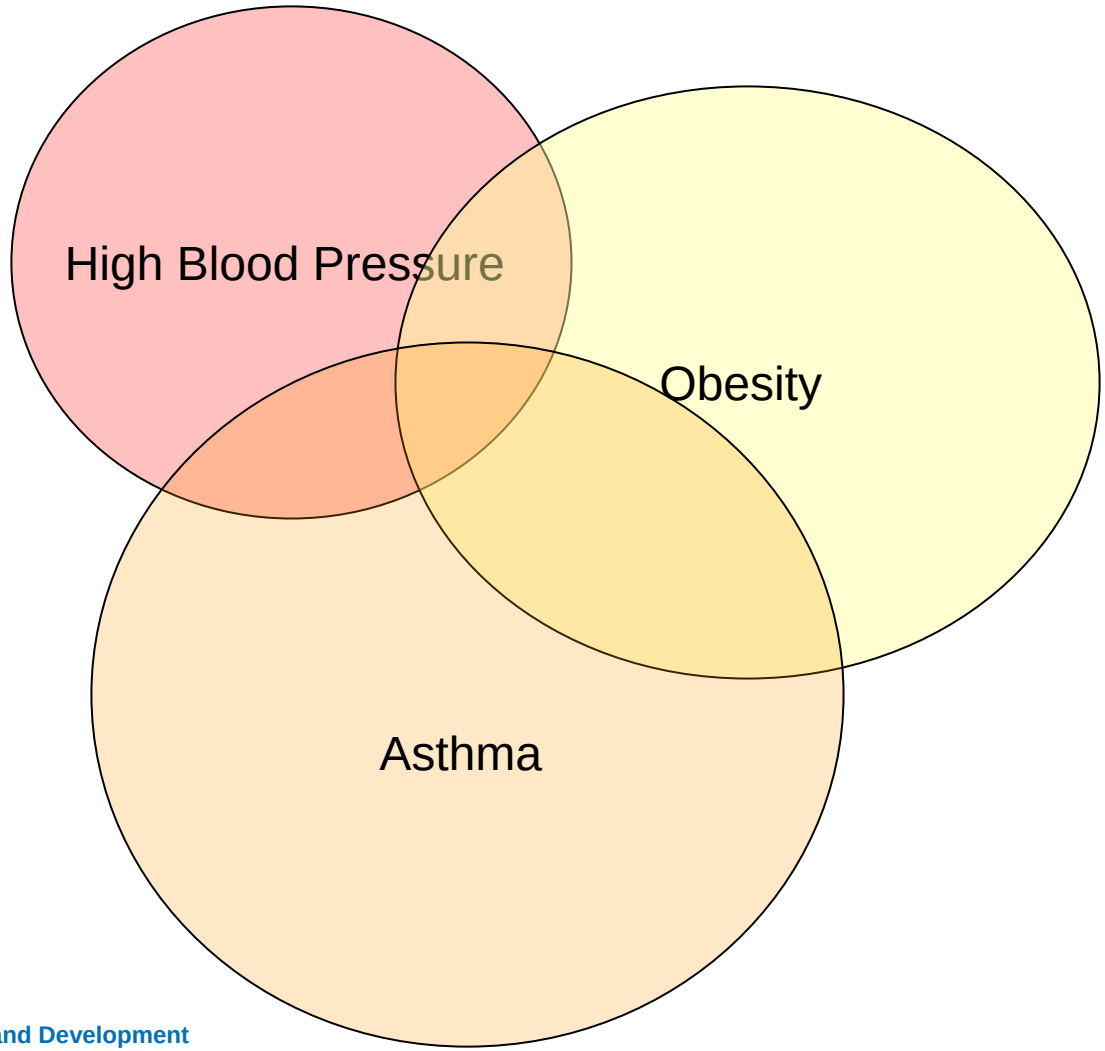
Study sample is predominantly African-American (>80%)

Study sample includes children diagnosed as **asthmatic** and **non-asthmatic**

Distribution of age across all categories is fairly uniform

Average age at menarche (nation-wide)

MICA Childhood Study Multiple Risk Factors



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Collaborative NCCT-MICA Goals

- The objectives of the NCCT include advancement of a systems approach to evaluate complex relationships between
  - environmental factors
  - physiological biomarkers
  - health outcomes.
- NCCT collaborating to apply advanced statistical and machine learning methods to evaluate biomarker data collected in MICA
- Contribution of the NCCT component:
  - analyze genetic and gene expression data
  - use a systems biology approach to put data into framework for evaluating ecogenetics

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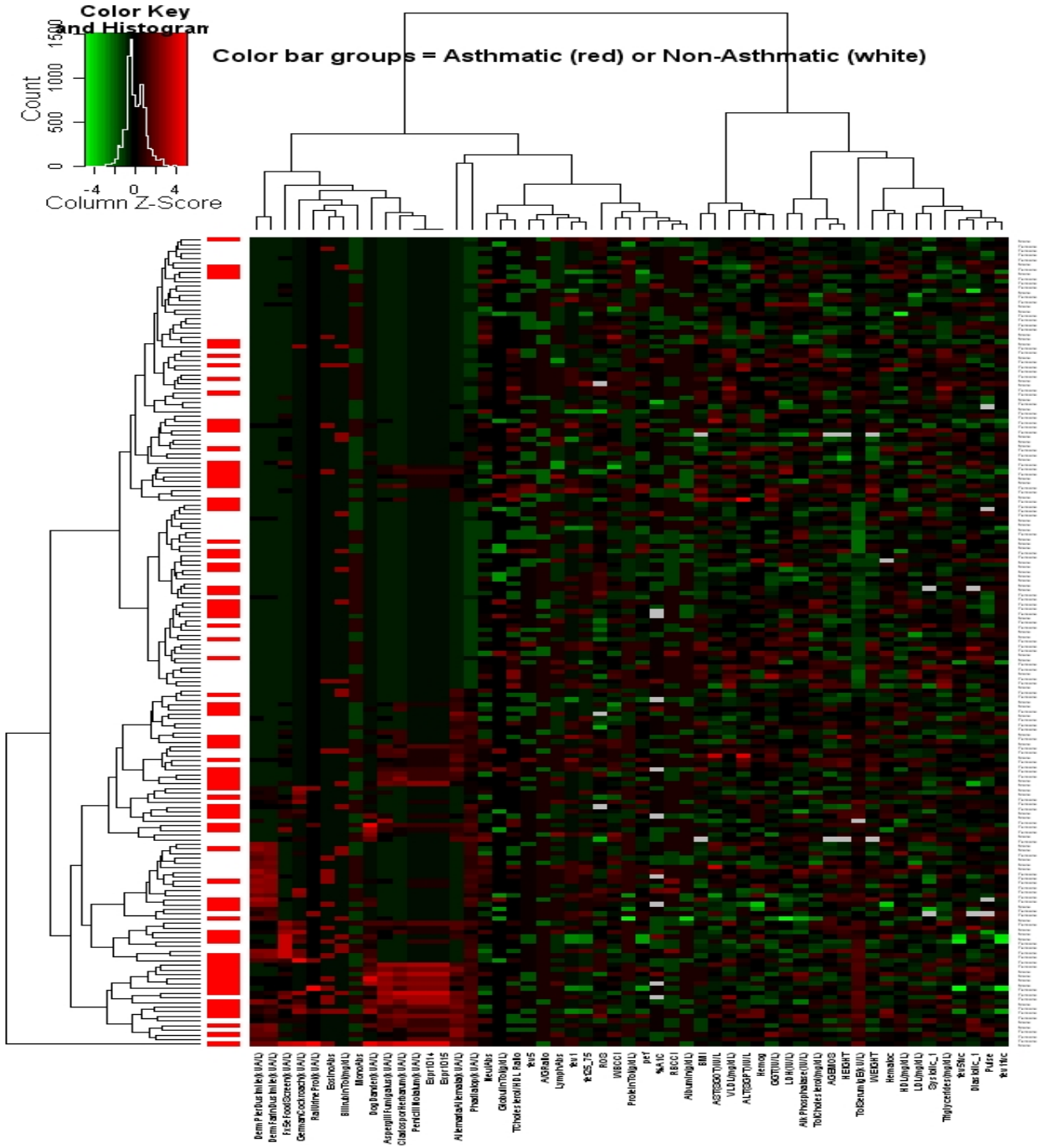
Data Analysis Methods

- Traditional statistics: linear regression, logistic regression, ANOVA, linear discriminant analysis.
- Machine learning: recursive partitioning trees, bootstrap aggregation (bagging) techniques, evolutionary computation-optimized classifiers, multifactor dimensionality reduction, random forests.
- Bioinformatics: protein interaction databases, knowledge (literature) mining tools, biological pathway database and inference software.
- Graphical approaches: cluster diagrams, expression “heat” maps, dendrograms, overlaid scatter plots (both exploratory and summary), distributional “violin” plots, regression plots.



MICA: Physiological Markers/ Health Status

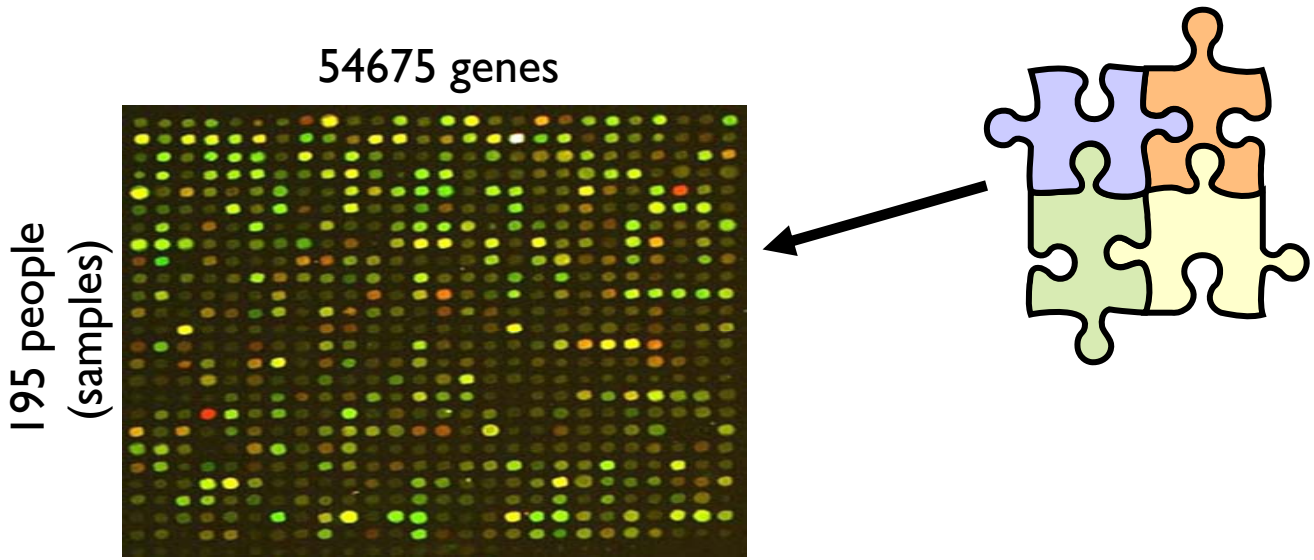
Red – Asthmic

White – Non Asthmatic

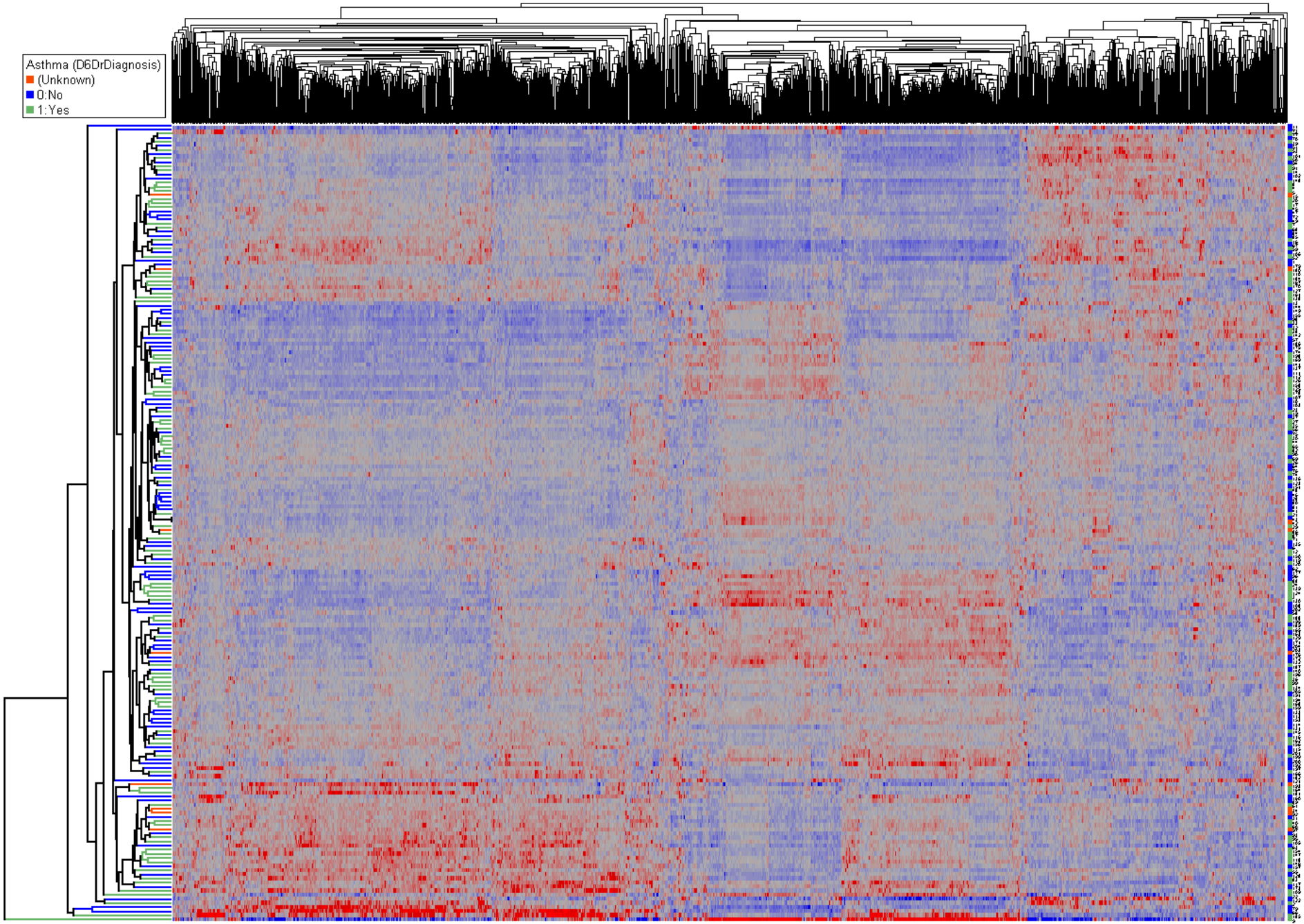
Gene expression measured using oligonucleotide microarrays

{gene expression}

Genetics



IMPORTANT: Total RNA was taken from whole blood samples in the absence of any deliberate experimental perturbation.



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Clustering of Subjects (Rows) by Gene Expression (Columns)

-6 0 6

Column-wise standardized gene expression levels

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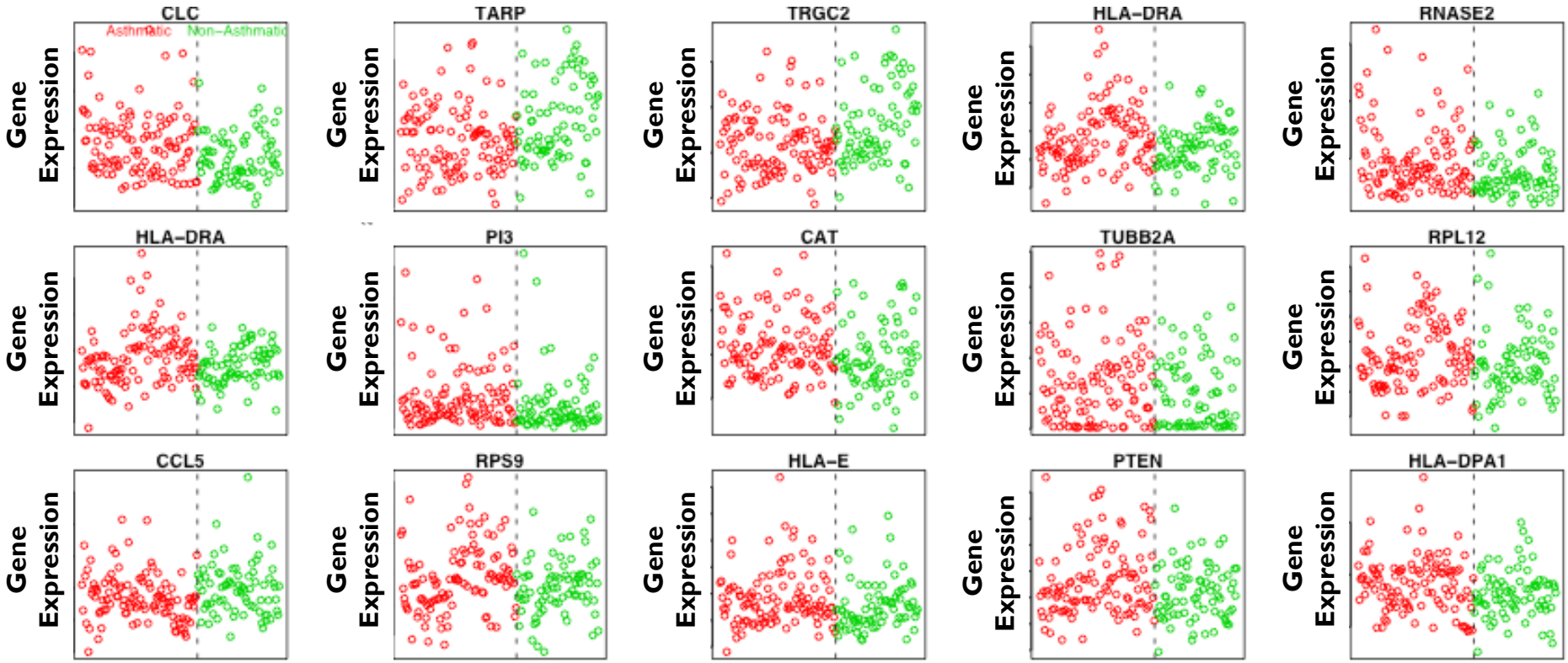
Can Gene Expression Distinguish Subtypes of Asthmatics?

- To identify subtypes, first applying an unbiased (i.e. without knowledge of asthma status) analysis to assess association between gene expression data and information on clinical, demographic, and exposure indicators.
- Next, select only gene expression probe sets that are significantly correlated with at least one of the demographic, clinical, or exposure indicators.
  - This filtering method prevents selecting only genes whose expression is associated with broadly-defined, imperfect asthma diagnoses.
- Examination of the genes differentiating asthma subtypes in this context highlights mechanistic genomic etiologies underlying the disease. These include subtypes of asthma characterized by
  - Patterns of gene expression associated with immune over-stimulation and household allergy exposures
  - Combinations of genomic biomarkers with demographic factors such as gender

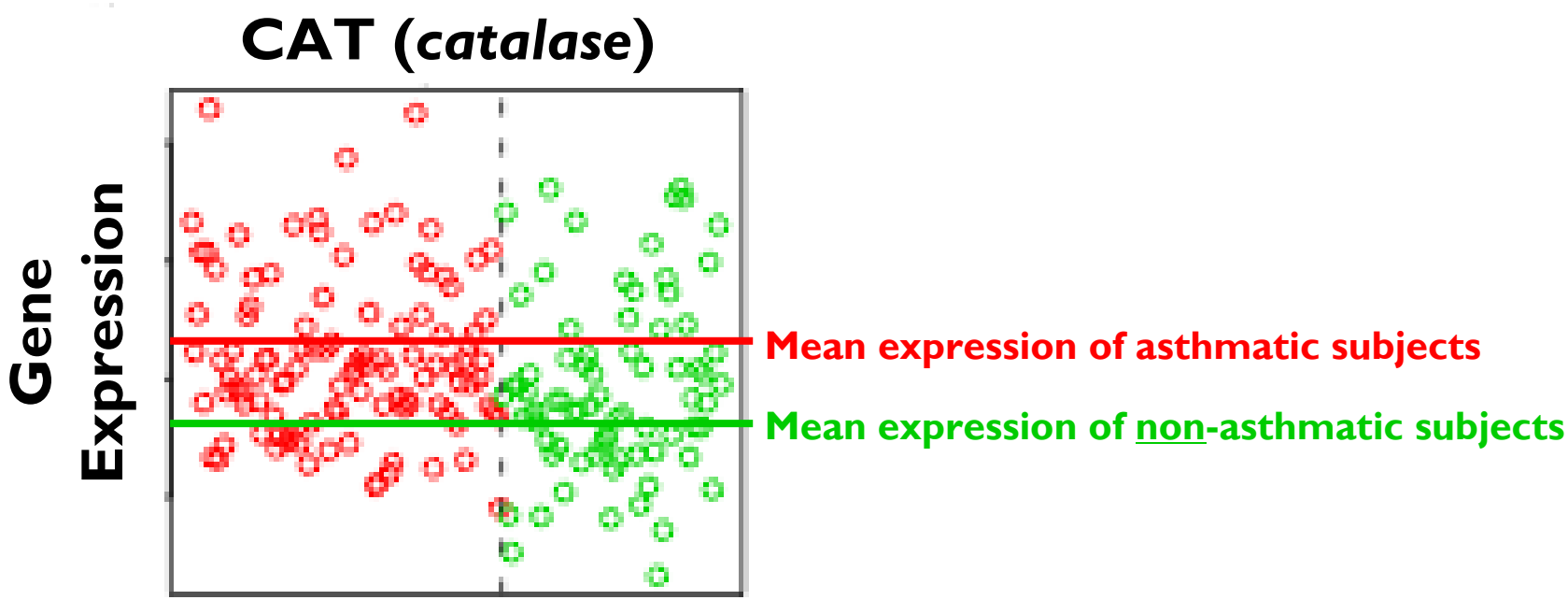
Why not just do the usual "here are some main-effect genes that discriminate asthmatics versus non-asthmatics"?



Does **not** address gene expression profiles associated with subtypes of asthma

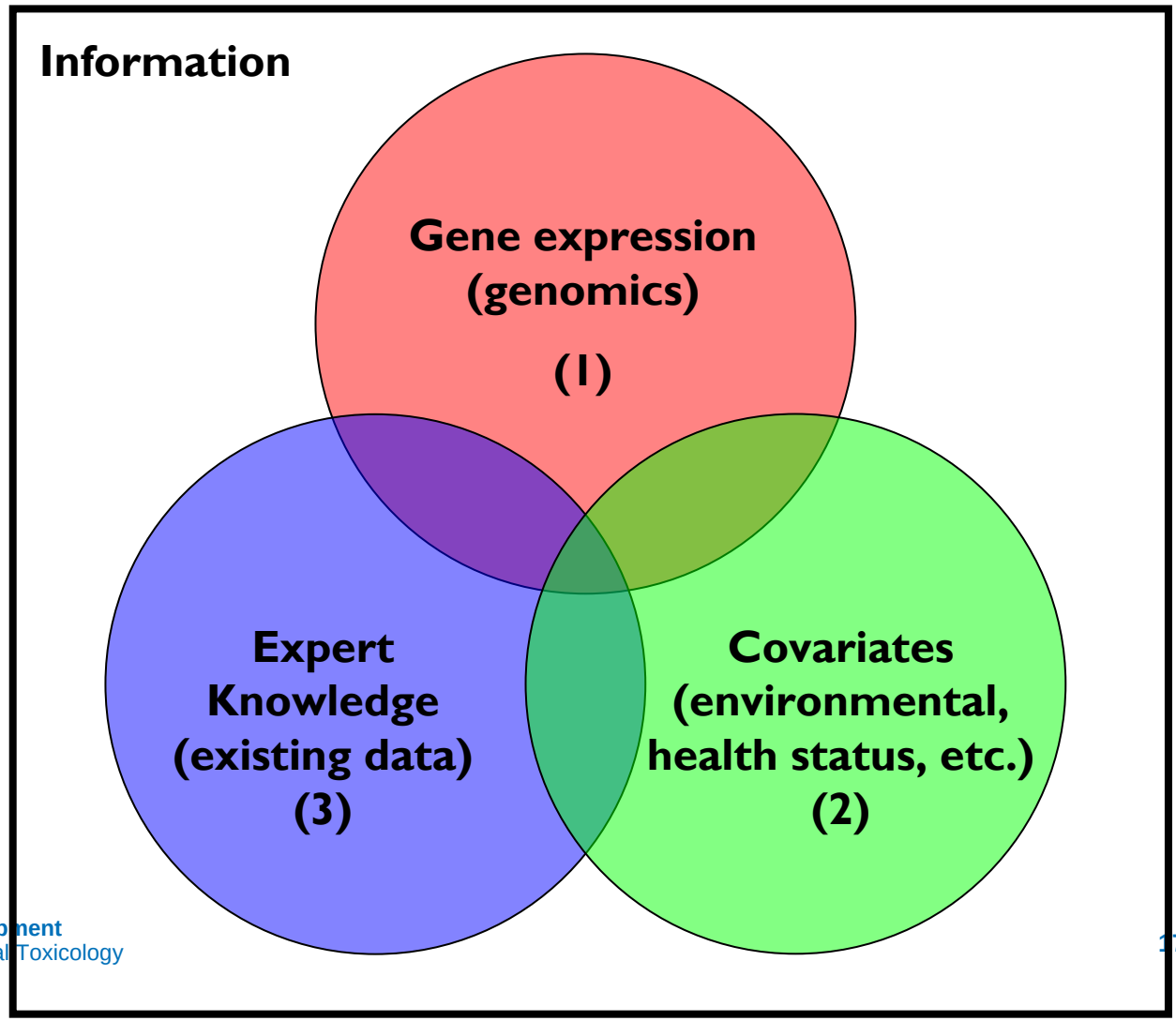
Does **not** link gene expression with other biomarkers or covariates (Where is the context?)

Why not just do the usual "here are some main-effect genes that discriminate asthmatics versus non-asthmatics"?



Reliance on group means **ignores** the complexity of asthma etiology

The ultimate goal is to glean mechanistic information regarding asthma subtypes



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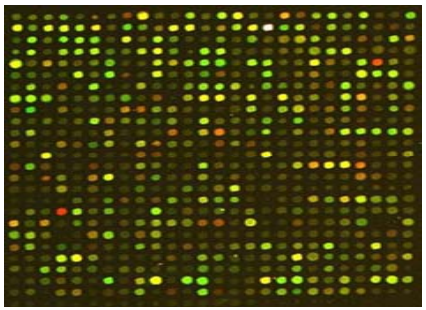
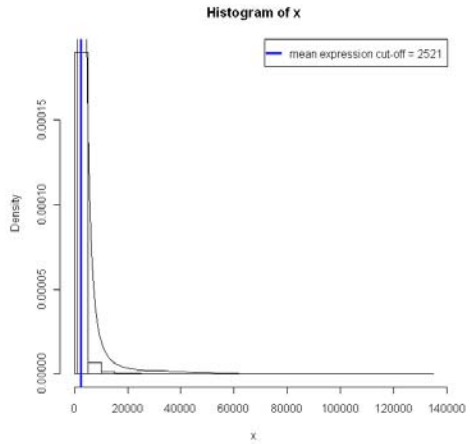
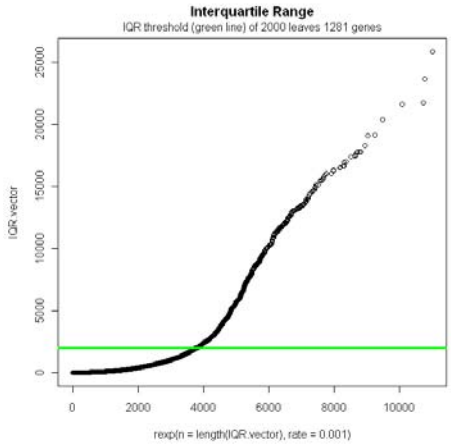
Analysis pipeline for the gene expression data

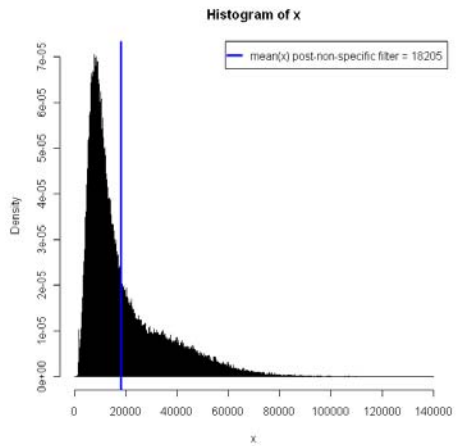


Gender adjustment

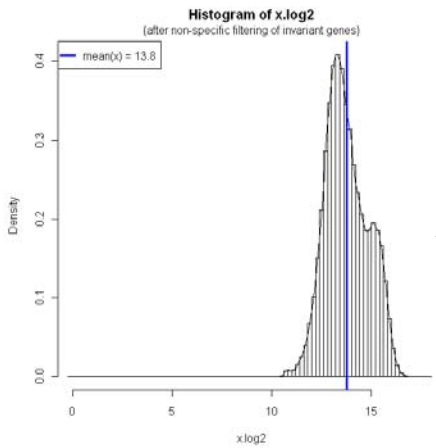


IQR filtering

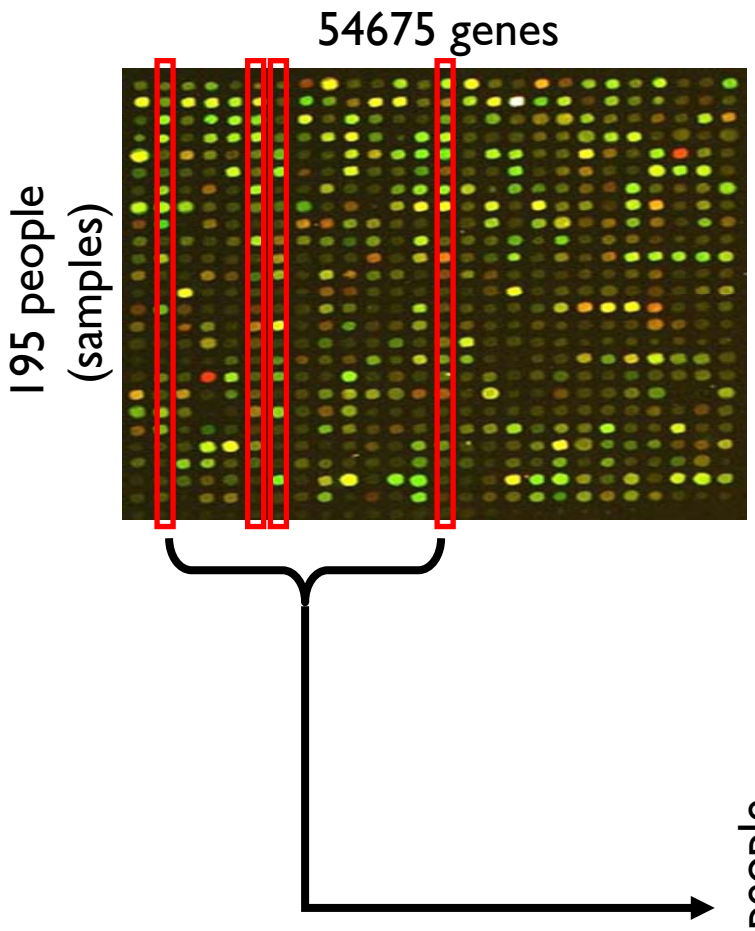




Log_2 transformation



How do we leverage MICA covariate information for the gene expression analysis?



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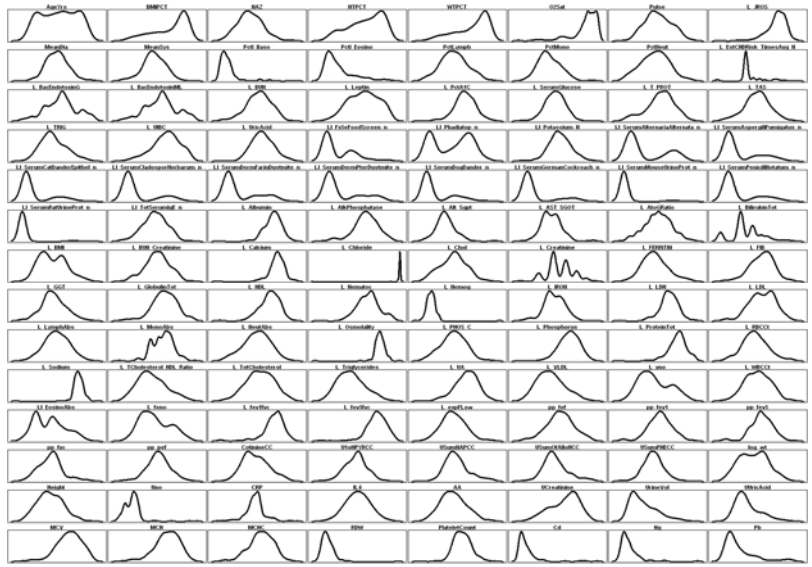
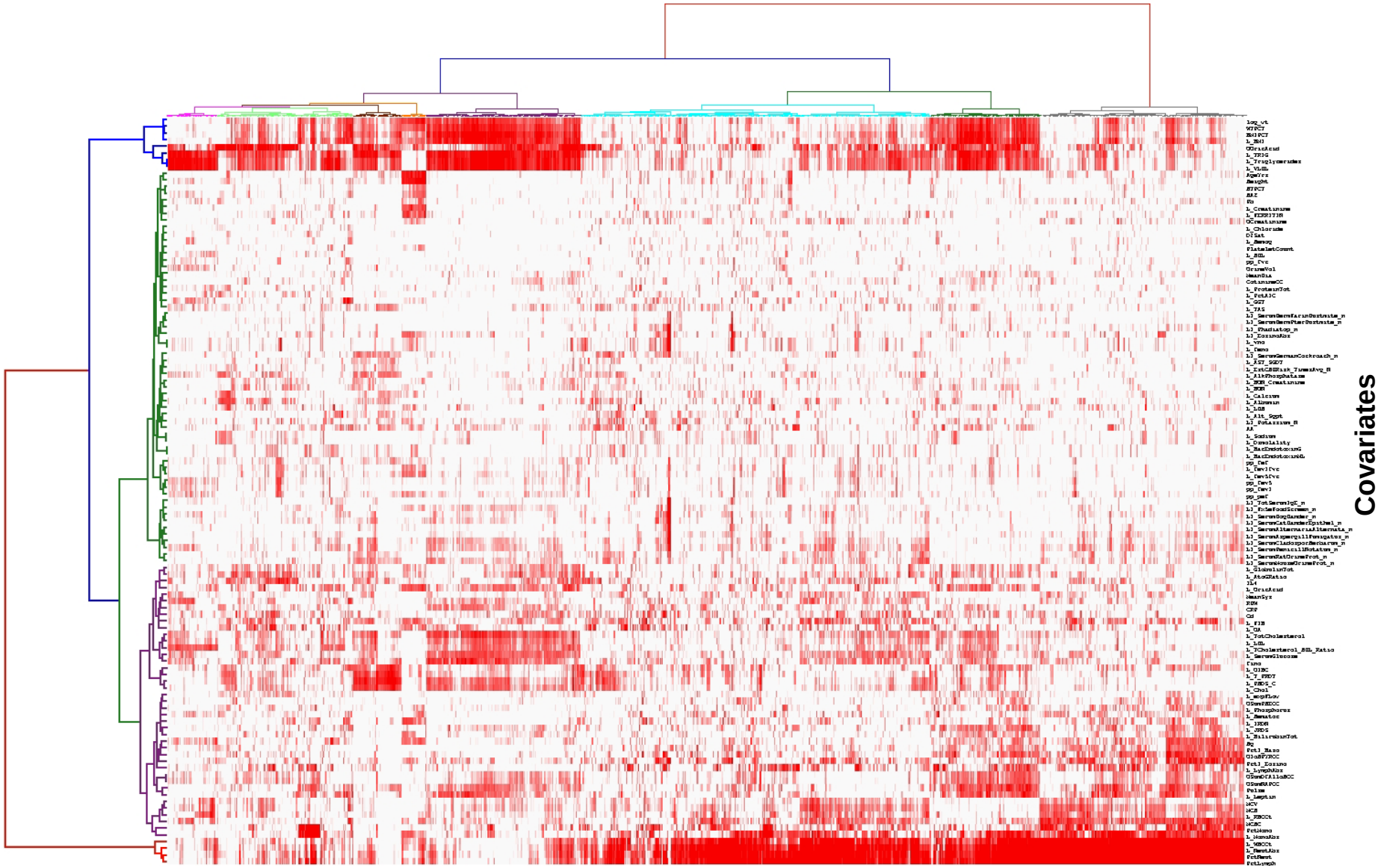


Results in a list of genes having significant correlation with at least one MICA covariate

Are MICA covariates reflective of underlying gene expression patterns?



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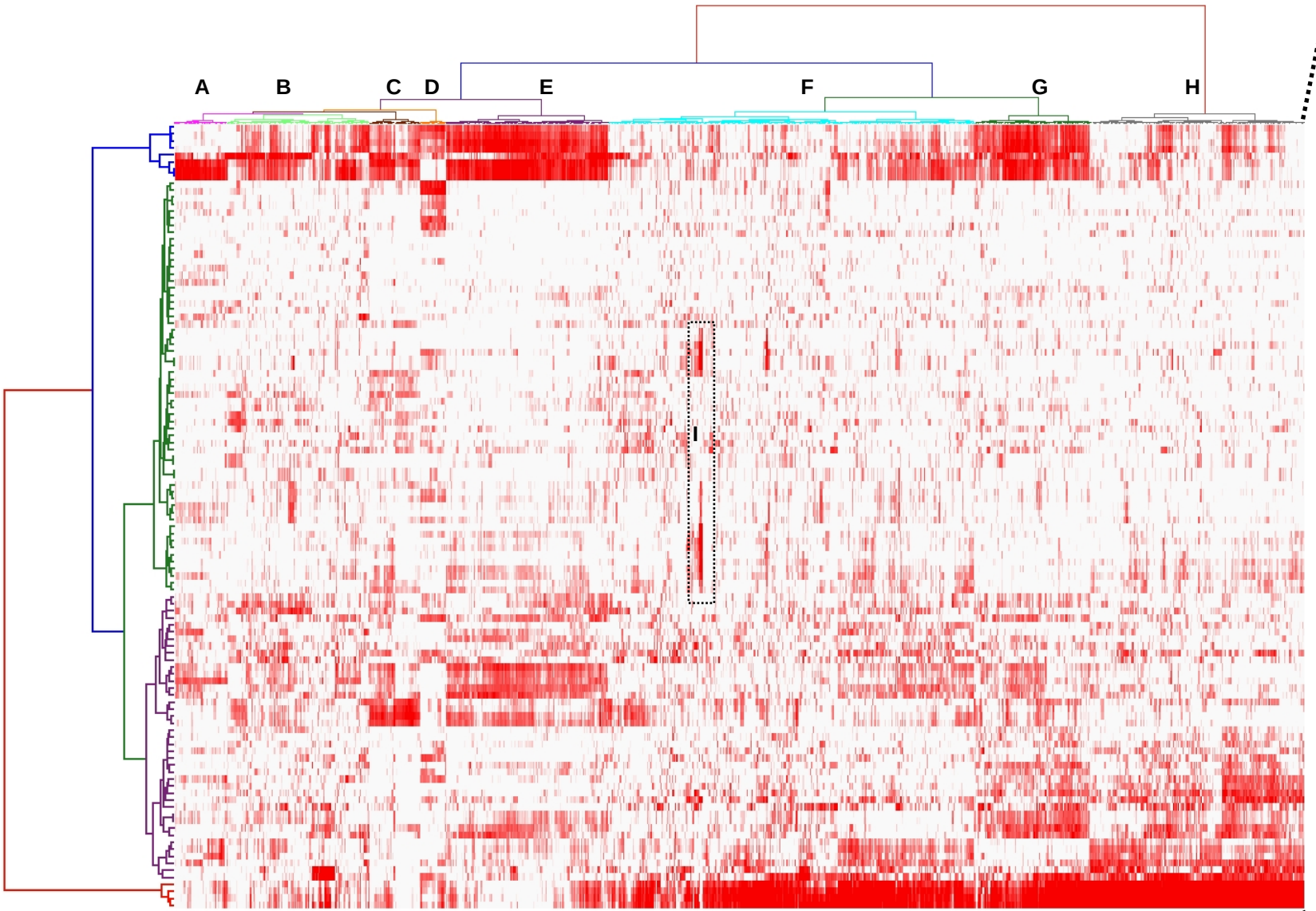
Finding context for gene expression patterns



Absolute value of gene-covariate correlation

0.0 — 1.0

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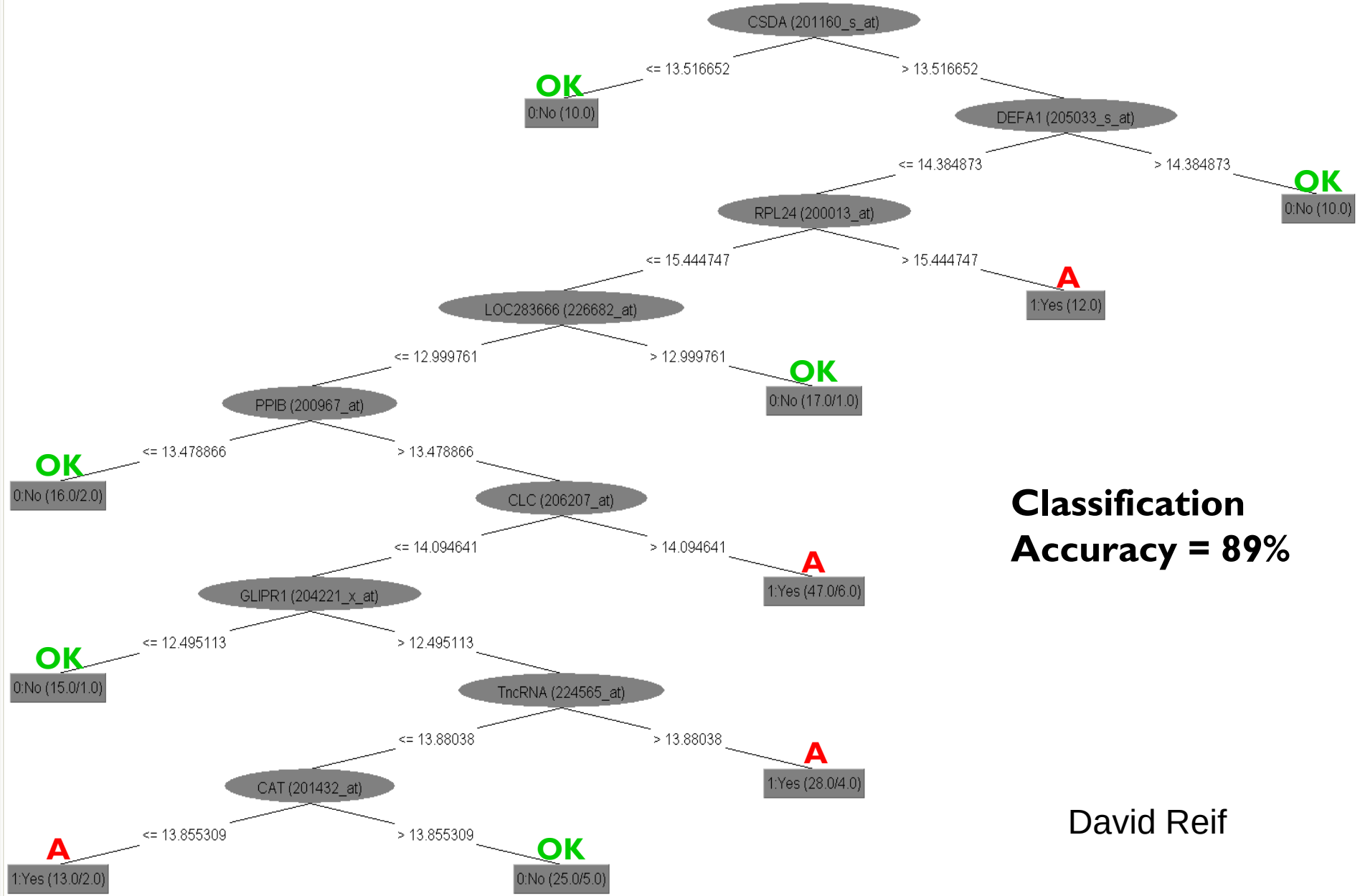
Can we discriminate between subtypes of asthma?



Classification Accuracy = 89%

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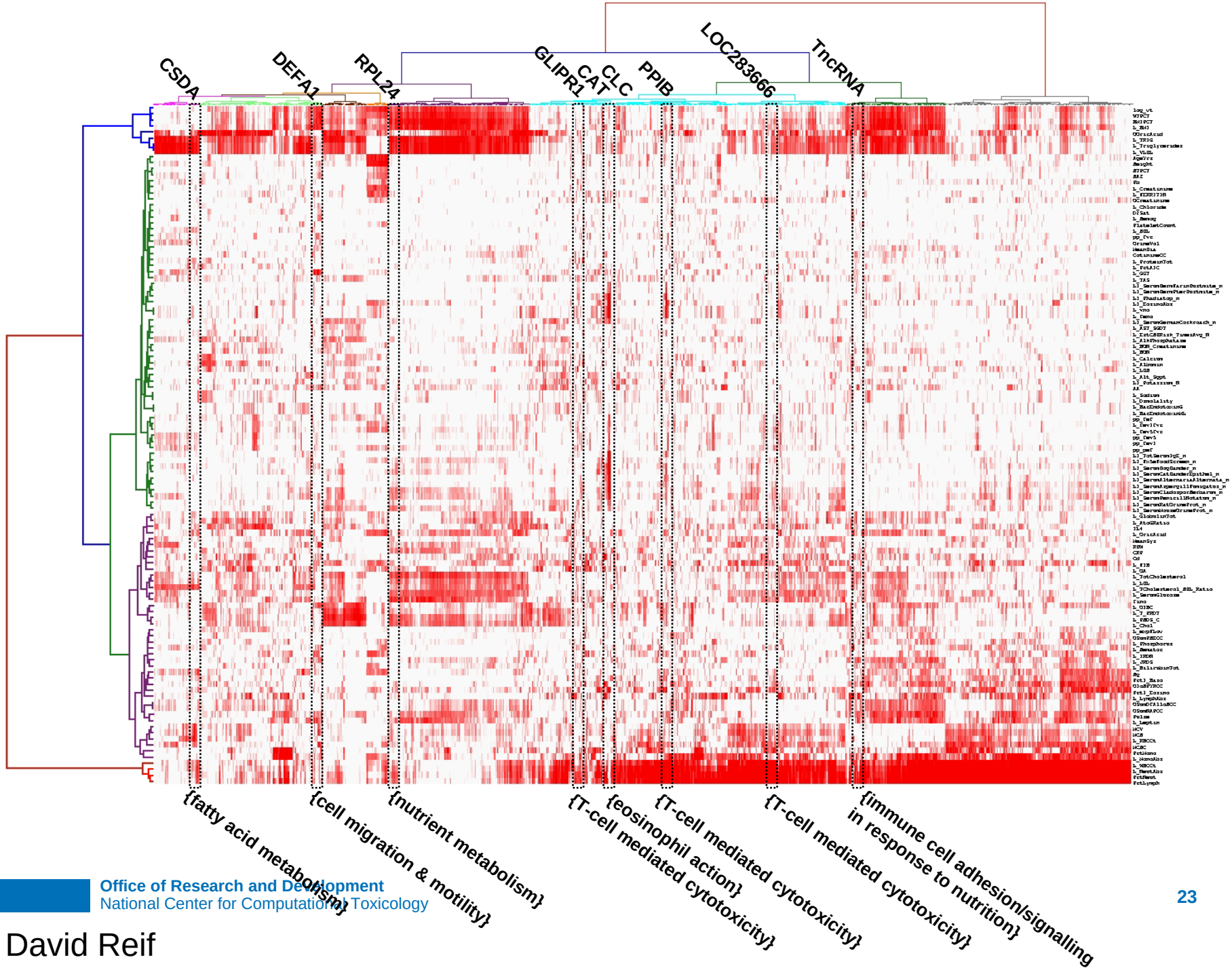
CSDA
DEFA1
RPL24
GLIPR1
CAT
CLC
PPIB
LOC283666
TncRNA
{fatty acid metabolism}
{cell migration & motility}
{nutrient metabolism}
{T-cell mediated cytotoxicity}
{eosinophil action}
{T-cell mediated cytotoxicity}
{T-cell mediated cytotoxicity}
{immune cell adhesion/signalling in response to nutrition}

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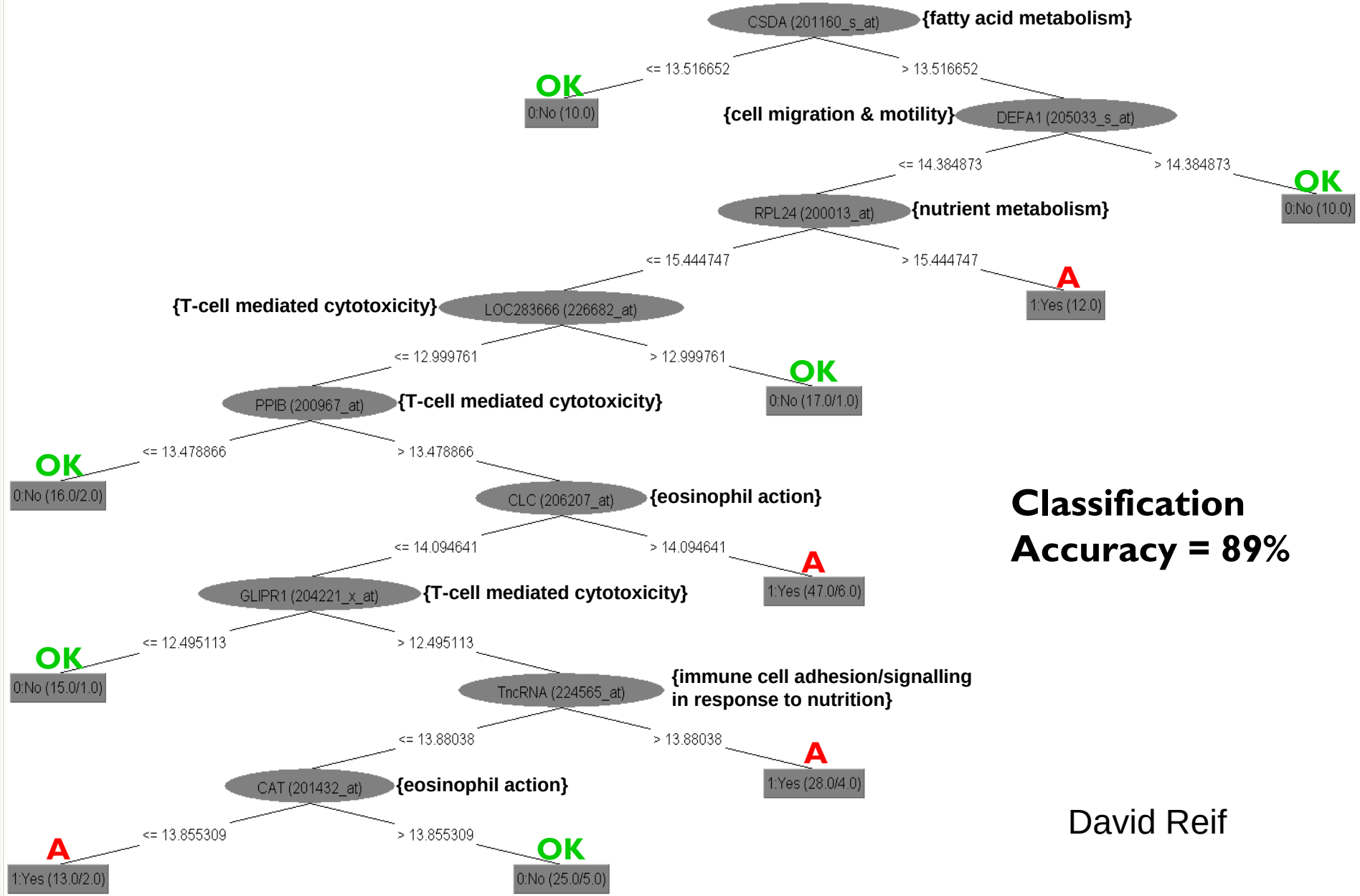
Can we leverage covariate information to put gene expression asthma classifier results in context?



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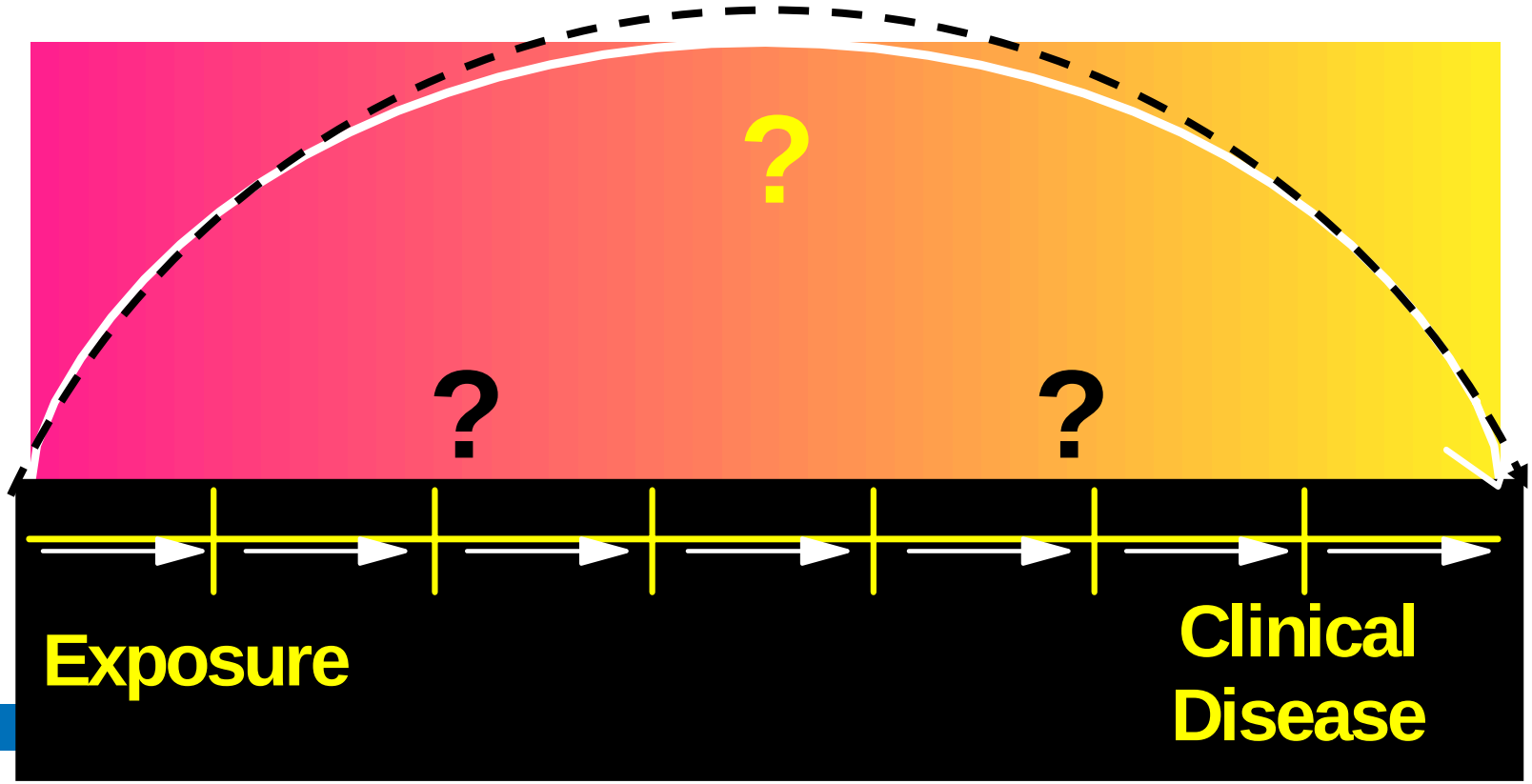
Summary

- Integration of diverse set of exposure, effects and susceptibility measures
- High-data content technologies, elucidating the genetic and environmental basis for toxicity and disease



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Acknowledgements

- **Jane Gallagher**, Human Studies Division, NHEERL
- **Stephen Edwards**, National Human and Environmental Effects Laboratory
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- **Wendell Jones**, Expression Analysis
- **John Wambaugh**, National Center for Computational Toxicology

Disclaimer

Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official Agency policy.