COMPARING GENE EXPRESSION PATTERNS IN BLOOD AND LUNG TISSUE OF IMMUNOLOGICALLY-CHALLENGED RATS EXPOSED TO CONCENTRATED AIRBORNE PARTICULATES

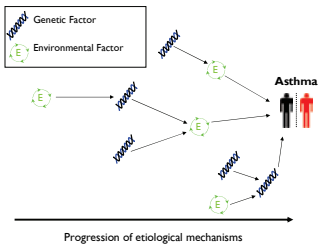
D.M. Reif[1], B.L. Heidenfelder[2], E.Cohen Hubal[1], J.R. Harkema[3], J.E. Gallagher[2]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA
[2]Human Studies Division, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA
[3]Department of Pathobiology and Diagnostic Intervention, Michigan State University, East Lansing, MI, USA

1. Abstract

Children residing in urbanized areas suffer disproportionately higher asthma-related morbidity and mortality, possibly due to higher levels of environmental asthma triggers such as airborne particulate matter. As part of a larger study of gene-environment interactions conferring differential asthma susceptibility in Detroit children, we explored gene expression patterns in blood and lung tissue from sensitized rats exposed to concentrated airborne particulates (CAPs) [PM 2.5]. Brown Norway rats were sensitized with ovalbumin, then immunologically challenged with either saline or ovalbumin before chamber exposure to CAPs. To measure gene expression differences between saline (control) and ovalbumin (challenged) animals in the presence of CAPs, both lung and blood RNA was isolated and hybridized to Affymetrix rat whole genome chips. A structured permutation approach was used to highlight knowledge-based gene annotative categories exhibiting unexpectedly high ($p < 0.05$) numbers of differentially expressed genes. These GO and KEGG categories included "Cell communication," "Metabolism of xenobiotics," and several immunological signaling categories. Because gene expression is tissue-specific, we did not observe a high concordance between expression changes of particular genes in blood versus lung. However, our approach does suggest that while blood and lung respond to environmental challenges through a unique set of genes, these differentially expressed genes can be grouped into common functional collections. These data will help guide the analysis of pathways and functional gene categories relevant to asthma.

2. Introduction

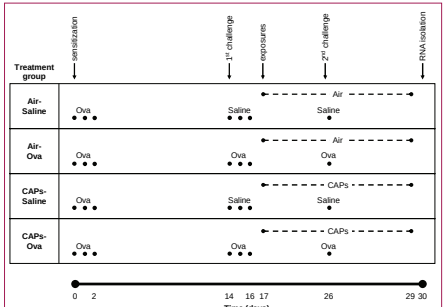


For complex diseases such as asthma, both genetic and environmental factors play important etiological roles.

The Mechanistic Indicators of Childhood Asthma (MICA) study is integrating multiple biomarker and exposure measures to elucidate disease mechanisms.

The animal studies highlight gene expression signatures characteristic of biological pathways that can be used to inform analysis of human gene expression data in the context of air quality, presence of known allergens, genetic susceptibility, and other intrinsic or environmental exposures.

3. Methods



(A)

Experimental design and treatment schedule: The initial ovalbumin (Ova) sensitizations and first Ova or Saline challenges were administered intranasally over three consecutive days (0-2 and 14-16, respectively). Animals were exposed to concentrated airborne particulates (CAPs) or normal laboratory air (Air) on days 17-29. A second Ova or Saline challenge was administered intranasally on day 26. RNA was isolated from lung sections or blood taken on day 30.

(B)

SAFE: Ranked local statistics (comparing treatment groups) are given on the *x*-axis for all genes on the array. Tick marks show the location of gene-wise statistics within a category. The shaded region represents genes in each category whose differential expression is beyond the significance level ($p < 0.05$) of the empirically derived null distribution.

(C)

Centroid plots: The four plots are colored according to the treatment groups (Exposure = CAPs or air; Challenge = ovalbumin or saline) given above each vertical centroid lines. The bars represent the under/over (left/right) expression relative to the other treatment groups.

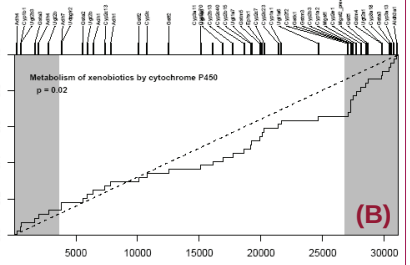
4. Results

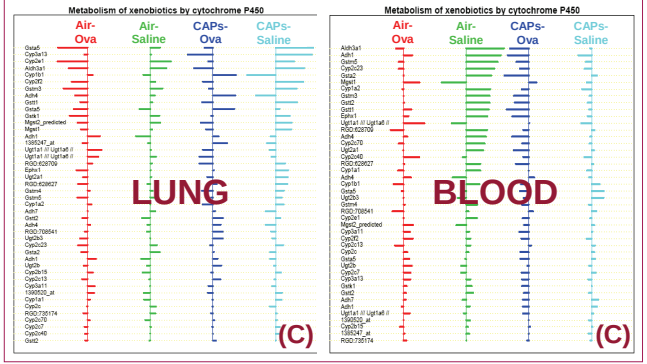
All analyses were carried out using the R language.

The gene expression data for all four treatment groups were normalized by robust multiple-array averaging (RMA).

(B) The SAFE (Significance Analysis of Function and Expression) approach was used to highlight differentially expressed genes within functionally related categories from among the 30,000+ probes measured on the array.

(C) Centroid plots for each group were generated according to a modified PAM (Prediction Analysis of Microarrays) procedure.



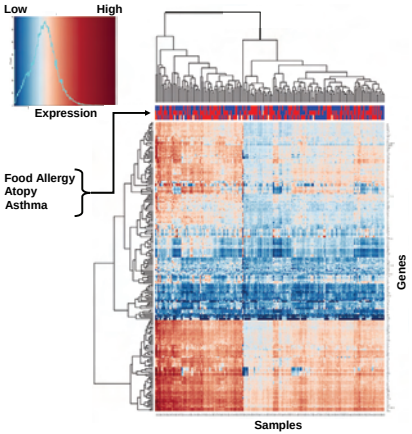


5. Conclusions & Next Steps

CAPs exposure alone (without OVA challenge) resembles gene expression patterns of normal air. Immunological challenge drastically alters the genetic response to airborne particulate matter.

While the particular genes altered by *exposure-challenge* groups varied by tissue, differentially expressed genes in both tissues are related as members of functional groups involving xenobiotic metabolism, cell communication, extracellular matrix remodeling, and inflammation.

Can we use the mechanistic insight gleaned from the animal studies to inform gene selection for human studies?
- -Highlighting pathways (signaling, biochemical, etc.)
- -Known demographic modifiers (race, gender, BMI, etc.)
- -Exposure information (mold, smoking, air quality, etc.)



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