DIFFERENTIAL LUNG GENE EXPRESSION IN IMMUNOLOGICALLY-CHALLENGED RATS EXPOSED TO CONCENTRATED AIRBORNE PARTICULATES

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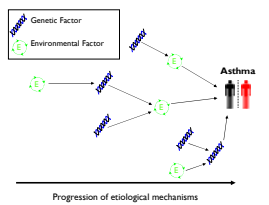
Abstract

Children residing in urbanized areas suffer disproportionately higher asthma-related morbidity and mortality. One explanation is that inner city children are exposed to higher levels of environmental asthma triggers such as airborne particulate matter. To elucidate gene-environment interactions conferring differential susceptibility in Detroit children, we first measured gene expression changes in 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, lung 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 numbers of differentially expressed genes. The KEGG biological pathways highlighted included "Cell communication," "Metabolism of xenobiotics by cytochrome P450," and several immunological signaling categories. Genes showing reduced expression in ovalbumin-challenged animals relative to controls included suppressor of cytokine signaling (SOCS) genes, which normally suppress the initiation of JAK-STAT inflammatory signals. Genes showing increased expression in ovalbumin-challenged animals included several members of the MAP kinase family, which promote release of asthma-associated cytokines, as well as CYP2B, which is necessary for proper xenobiotic metabolism and has demonstrated altered expression in response to inhaled pollutants. These data will inform the analysis of pathways and gene-environment interactions relevant to asthma.

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 airway hyper-reactivity.

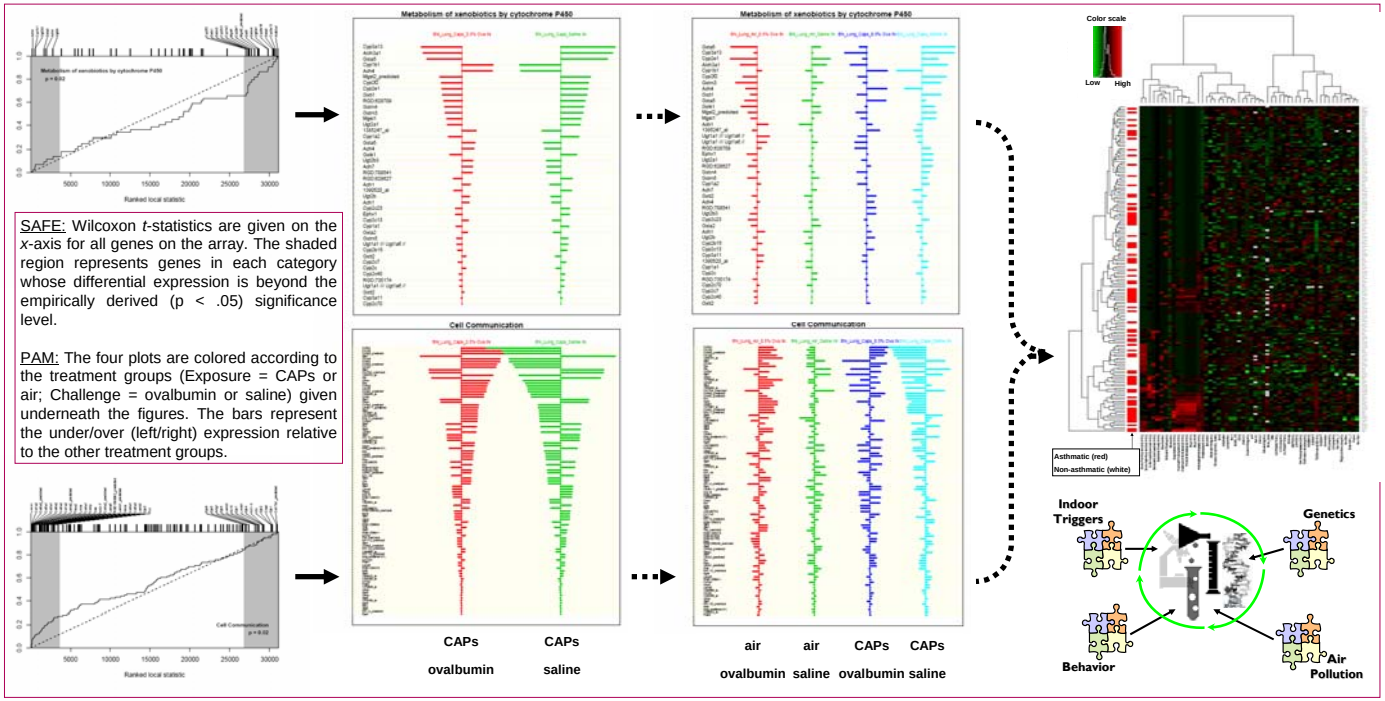
Approach

Animals: Male Brown Norway (BN) rats, aged 10-12 weeks, were assigned to one of four air/immunological experimental groups (*N*=16). Rats were free of pathogens and respiratory disease, and used in accordance with guidelines set forth by the Institutional Animal Care and Use Committee at Michigan State University. All groups were sensitized to chicken ovalbumin (OVA). Half of the animals were assigned to ambient-control air exposure groups, while the other half were assigned to CAPs exposure groups. Within both air exposure groups, four animals were challenged with saline vehicle (saline-control group), while another four were challenged with OVA by intranasal instillation (0.5% OVA in saline, 150 ul/nasal passage). The instillations were carried out first for 3 consecutive days, then once 10 days later. Rats were sacrificed two weeks after the first challenge. Lung sections were removed and immediately frozen in liquid nitrogen.

Measuring gene expression: Total RNA was isolated from the cranial lobe, using RNeasy Mini kits (Qiagen). RNA quality was checked using Nano LabChip kits (Agilent) and a Bioanalyzer. RNA was quantified on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies), then 3.8 ug of each sample was sent to Expression Analysis (Durham, NC) for cDNA target generation and hybridization to rat R230 2.0 whole genome arrays (Affymetrix).

Data analysis: The data were normalized by robust multiple-array averaging (RMA) [1]. The SAFE (Significance Analysis of Function and Expression) approach was used to highlight functionally related genes from among the 30,000+ probes measured on the array [2]. Centroid plots for each group were generated according to the PAM (Prediction Analysis of Microarrays) procedure [3]. All analyses were carried out using the R language [4].

Results



SAFE: Wilcoxon *t*-statistics are given on the *x*-axis for all genes on the array. The shaded region represents genes in each category whose differential expression is beyond the empirically derived ($p < .05$) significance level.

PAM: The four plots are colored according to the treatment groups (Exposure = CAPs or air; Challenge = ovalbumin or saline) given underneath the figures. The bars represent the under/over (left/right) expression relative to the other treatment groups.

Conclusions & Future Directions

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

The expression patterns altered by immune stimulation with ovalbumin included genes related to xenobiotic metabolism, cell communication, extracellular matrix (airway?) remodeling, and inflammation.

CAPs exposure may enhance allergens' effects (via airway remodeling, inflammation, and eosinophil or neutrophil hyperactivation).

Information on air quality, presence of known allergens, lung function, genetic susceptibility, and other intrinsic or environmental exposures must be integrated (see heatmap figure, above) to gain a comprehensive understanding of asthma etiology.

References

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