Gene Expression Profiling in Humans Following Toxicant Exposure

David C. Christiani MD, MPH, SM

Professor of Medicine, Massachusetts General Hospital
Harvard Medical School
Professor of Occupational Medicine and Epidemiology
Harvard School of Public Health

Exploratory Study

Hypothesis

Exposure to particulate air pollutants (metal fume) induces systemic inflammation, which can be assessed in peripheral white blood cells by monitoring gene expression using microarrays pre- and post-exposure.

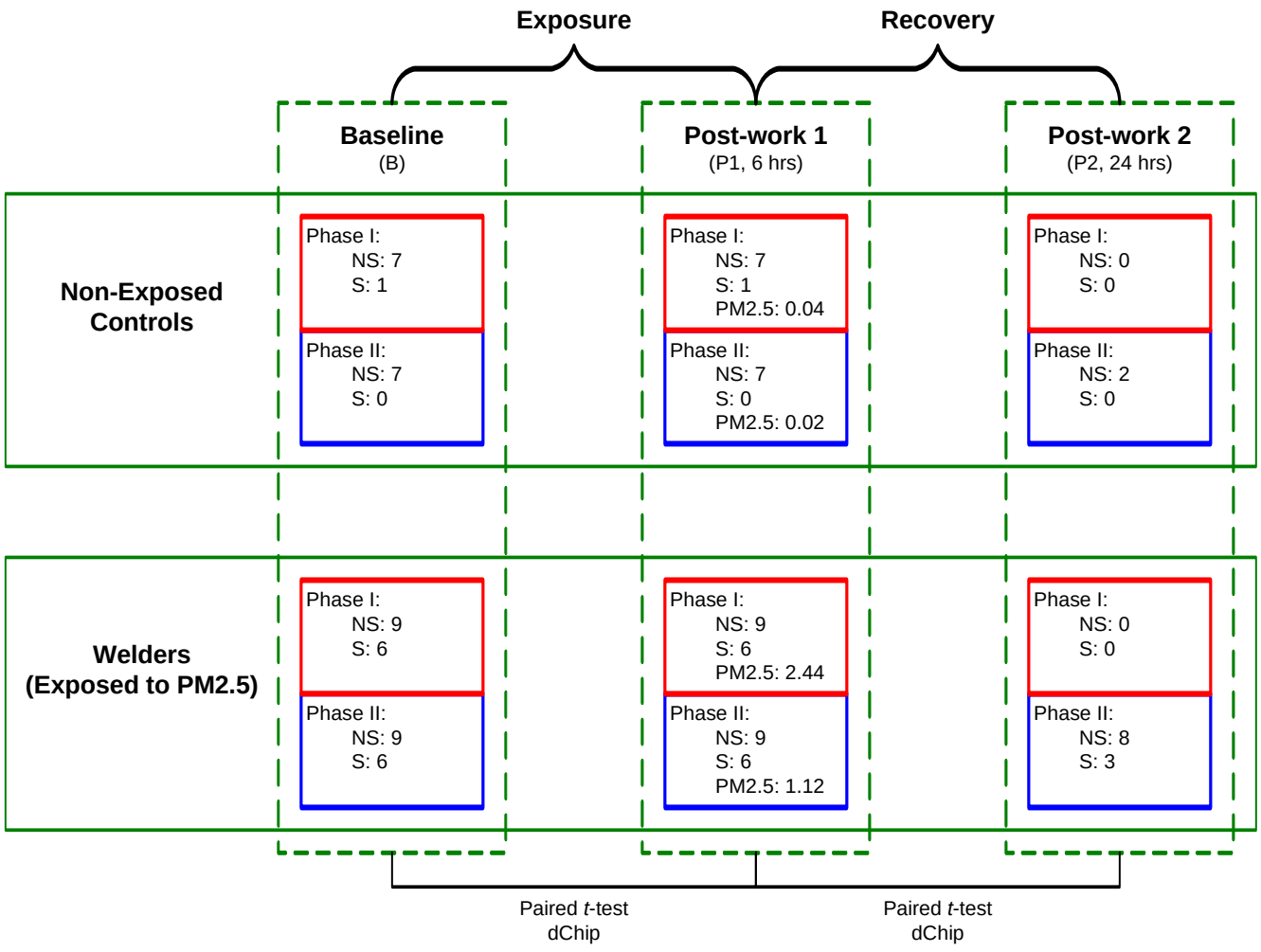
Exploratory Study

- **Phase I (2003)**
  - 22 subjects, including 7 non-exposed controls, 15 exposed (Welders)
- **Phase II (2004)**
  - 22 subjects, including 7 non-exposed, 15 exposed
  - 12 subjects (3 non-exposed and 9 exposed) participated in both studies.

Whole Blood Total RNA

- The extra cell fractionation procedure has a prolonged period prior to RNA stabilization, which caused significant *ex vivo* changes in gene expression profiling.
- Field survey - Not feasible to fractionate all blood samples in a timely fashion when all of the samples were collected within one day.

Study Design



Gene Alterations
-- Fold Change Analysis

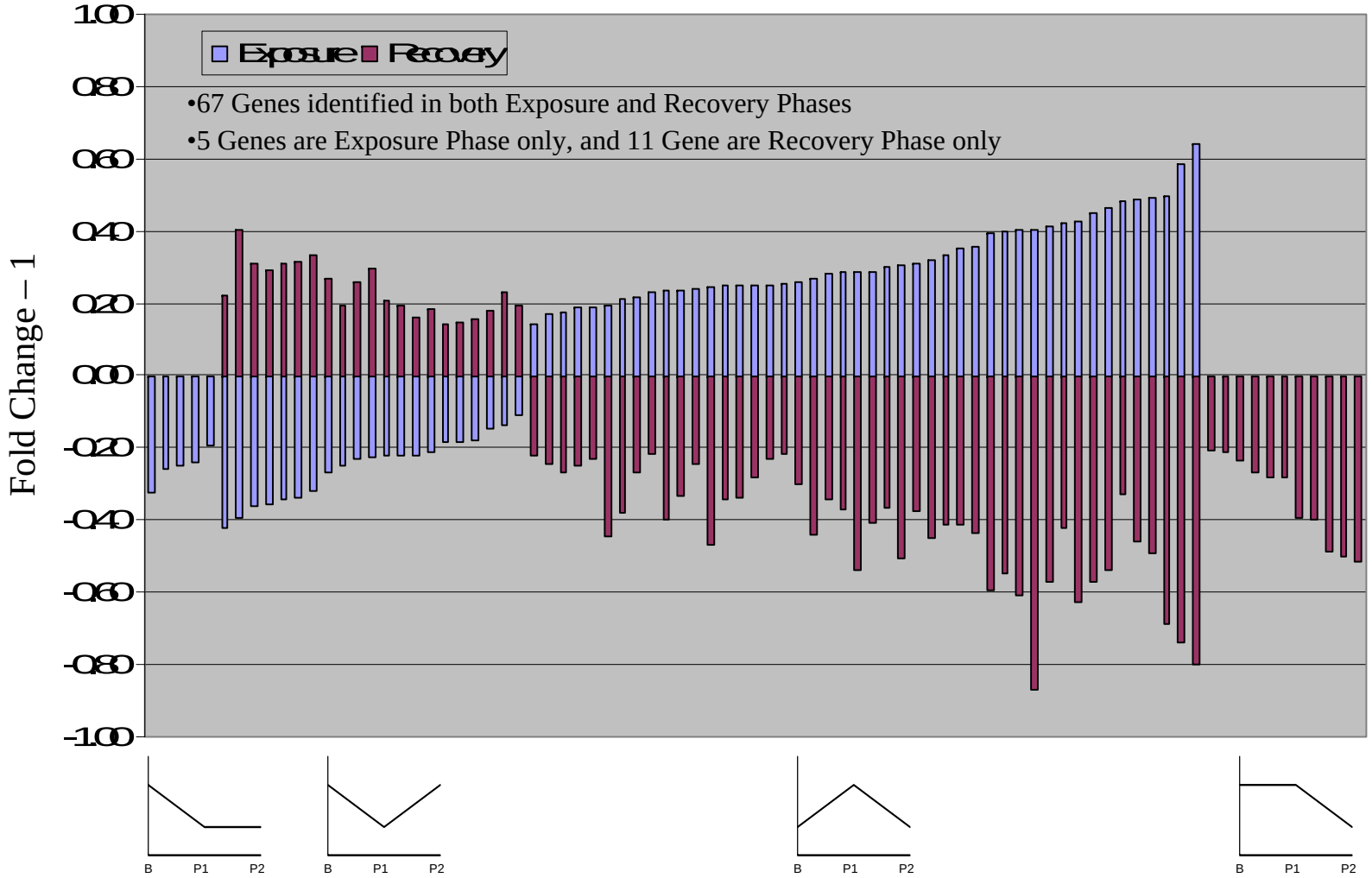
- **Genes expressed were different in the welder and control groups**
  - Expression changes of two-fold greater form the baseline to the post-exposure arrays and an absolute difference larger than 50.
- **Few genes found in each pair of arrays**
  - median number of genes is 20 with ranging from 1 to 123,

Paired *t* Tests

	Subject	Smoking Status	Test	No. of Genes (P <0.05)	Down regulated Genes (%)
Phase I					
Controls	7	NS	P1 vs. B	7	0.714
Welders	15	All	P1 vs. B	101	0.812
	9	NS	P1 vs. B	126	0.944
	6	S	P1 vs. B	65	0.415
Phase II					
Controls	7	NS	P1 vs. B	0	-
Welders	15	All	P1 vs. B	127	0.283
	9	NS	P1 vs. B	174	0.471
	8	NS	P2 vs. P1	311	0.871
	8	NS	P2 vs. B	60	1.000
	6	S	P1 vs. B	24	0.458

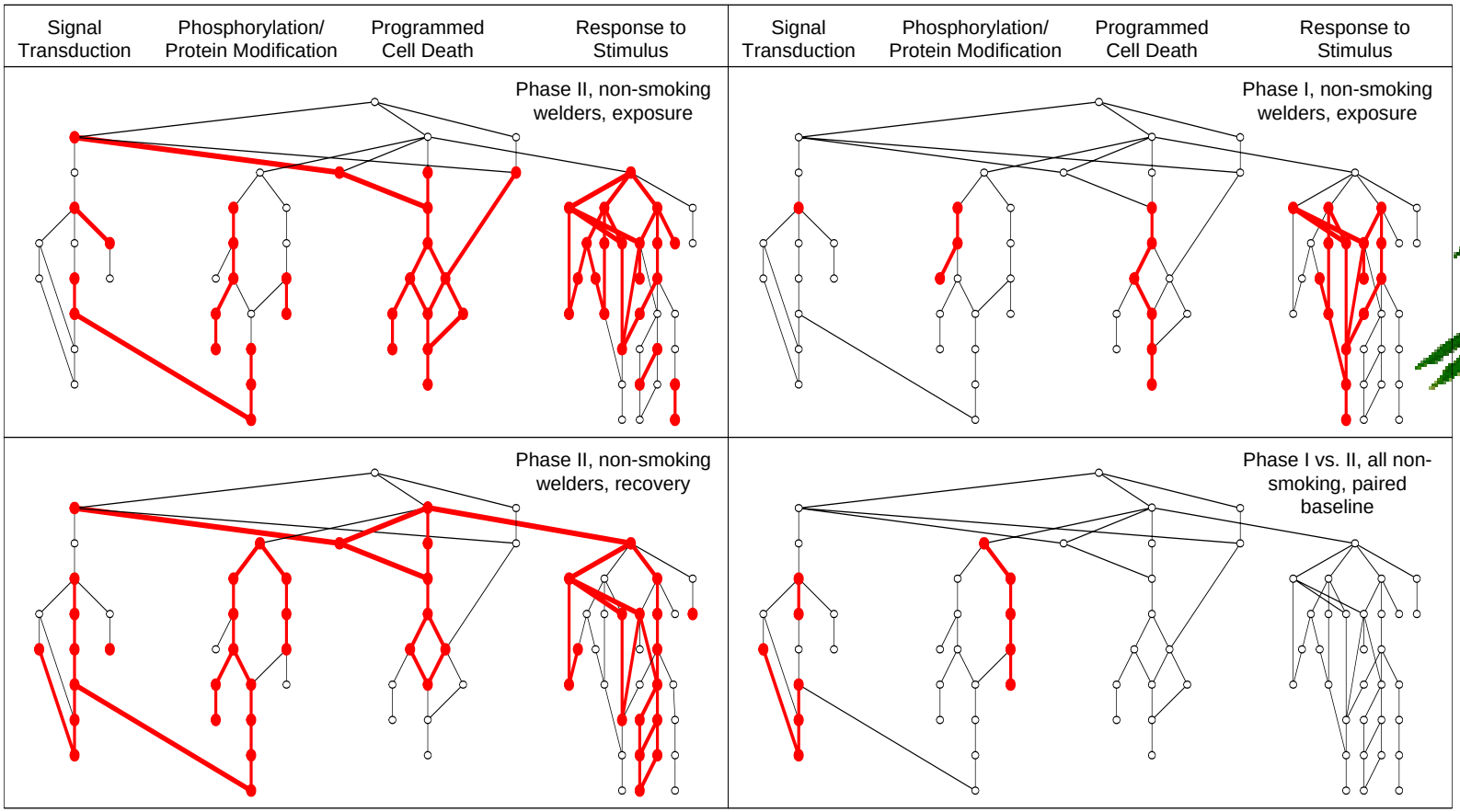
Genes with at least 50% Present Calls (dChip) in each group of paired *t* tests
B: Baseline; P1: Post-work 1; P2: Post-work 2

Phase II: Gene Expression Alterations in Different Periods



Functional Clustering

-- Biological Process of Gene Ontology

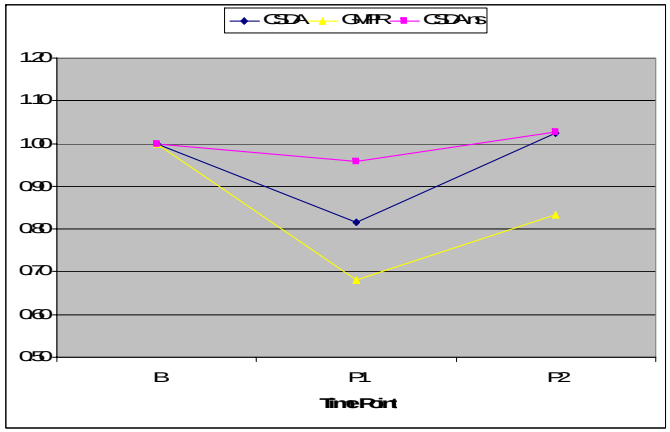


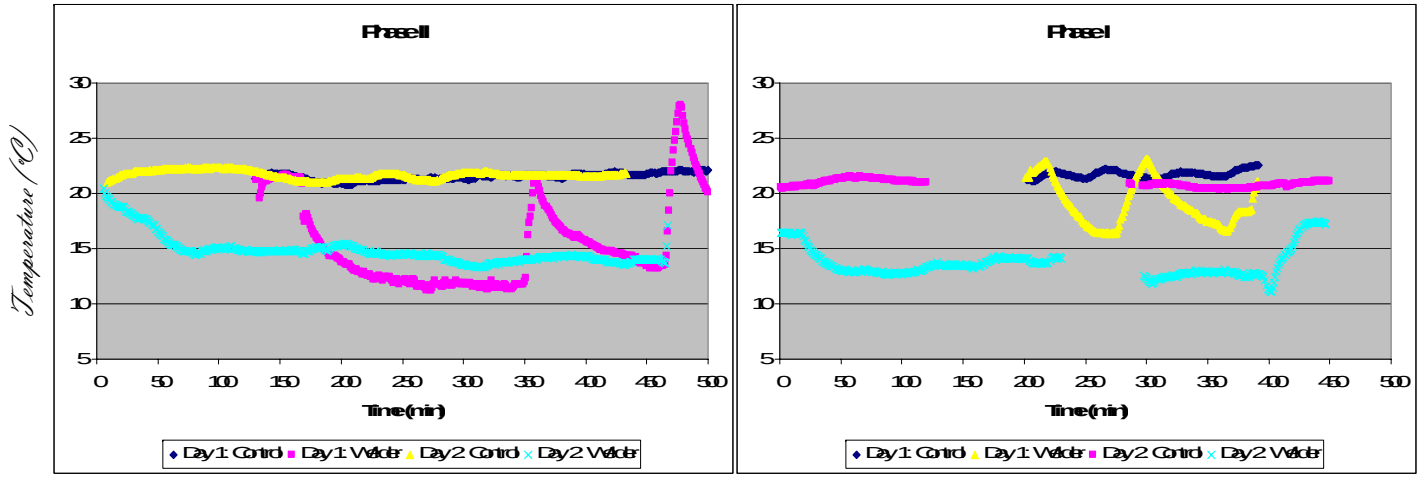
From Function Clustering to Gene Identification

GO Term: 9409, response to cold

A change in state or activity of an organism (in terms of movement, secretion, gene expression, enzyme production, etc.) in response to temperatures below the optimal temperature for that organism.

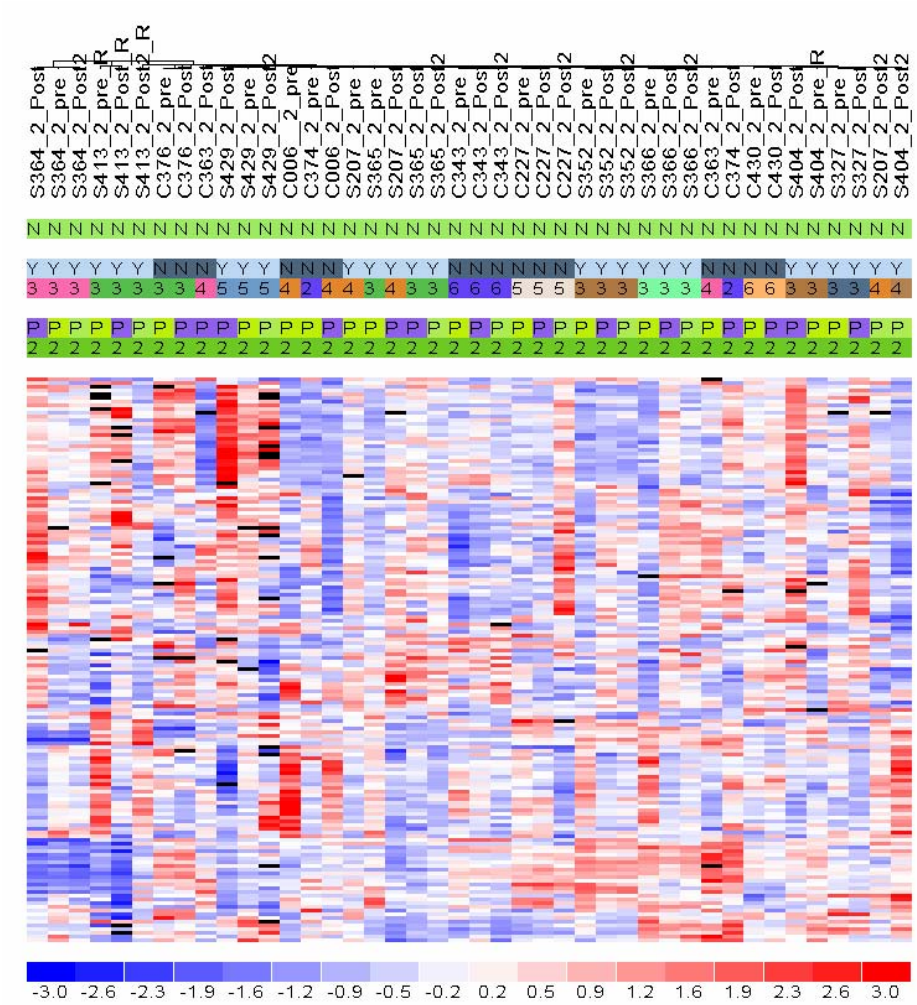
Three human genes annotated in this category. Expressions of two genes, ***CSDA*** and ***GMPR***, were significantly altered in response to metal particulate exposure.





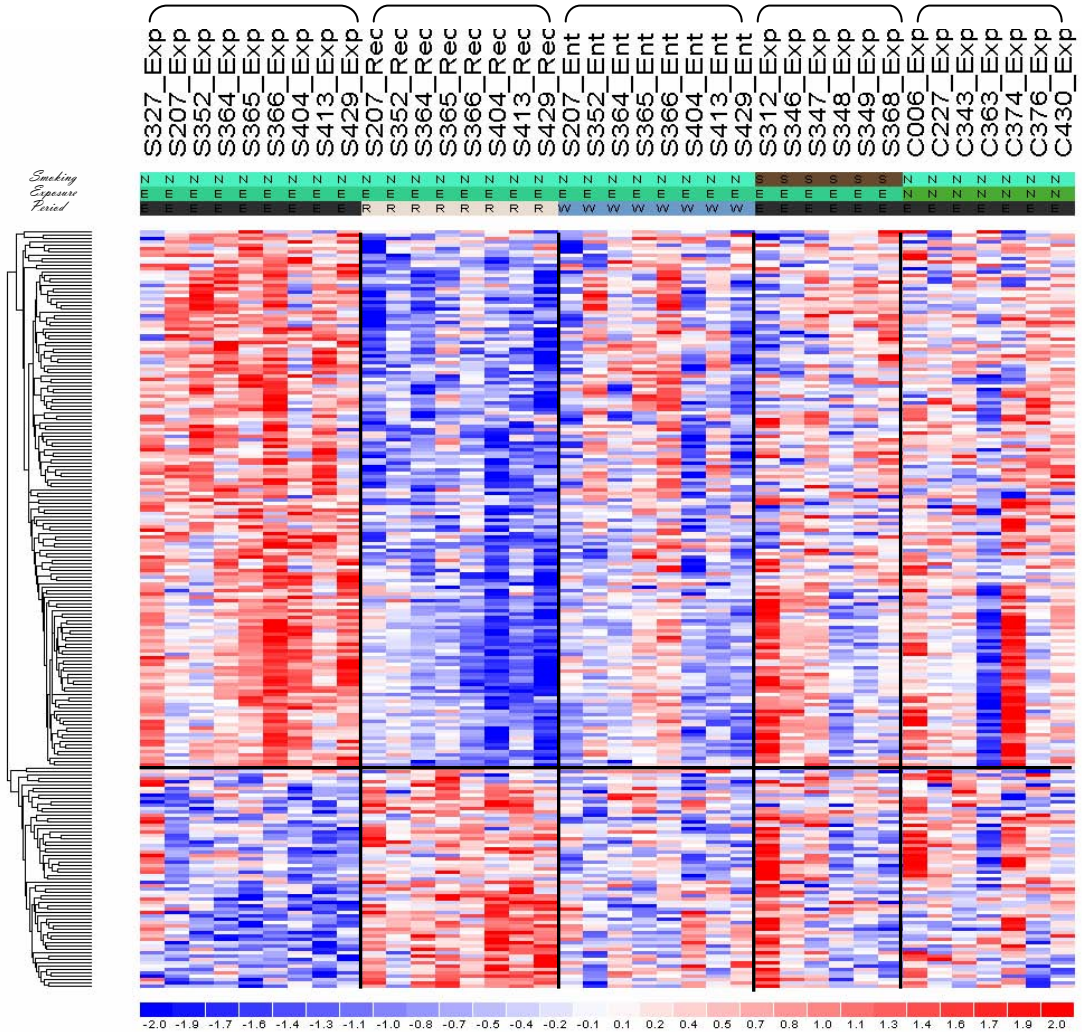
Hierarchical Clustering

Genes from Paired *t*-Test (Non-smoking Welder, P1 vs. B)



Phase II arrays

Hierarchical Clustering



ANOVA Analysis (dChip)

- Subject: 8 non-smoking welders
- Factor: Time points (B, P1, and P2)
- Results (p<0.05): 226 genes P Calls > 50%

Summary

- Transient gene expression alterations induced by short-term metal-particulate exposure genes can be detected in whole blood total RNA using a repeated measure design.
- The observation of genes clustered in the functional pathways related to inflammatory and immune responses support the hypothesis that metal particulate induces systemic inflammation.

Summary

- The time course of expression alterations was consistent with the results of blood cell counts.
- Smoking may alter expression profiles in the whole blood total RNA and is a confounding factor.

Relevant Issues

- Repeated measure design (self-control) and genomic technologies (Transcriptomics, Proteomics).
- The dichotomous data of absolute gene expression status verse continuous expression values in the data mining of the microarray data.
- Multiple Test Adjustment:
  - Genes expressed are interrelated;
  - Function annotations are not accurate, and are not completed;
  - There are multiple probe sets targeting to one gene.

Microarray for Epidemiology
-- Applicable, Feasible, Expensive

Key issues (less controlled conditions):

- Hypothesis
- Study design
  - Including self-paired samples
- Sampling strategy
- Data mining
  - Incorporating the dichotomous data of absolute gene expression status with continuous expression values
  - Multiple Test Adjustment:
    - Genes are interrelated;
    - Function annotations are not accurate, and are not completed;
    - There are multiple probe sets targeting to one gene.
  - Functional annotation integration

Microarray for Immunotoxicity

- What is the target tissue for microarray study?
- What types of the expected changes in gene expressions, long-term or short-term?
- Is it possible to collect self-paired samples?
- How to minimize the in vitro variations?
- What is strategy for data mining?

Acknowledgements

HSPH EH

Zhaoxi (Michael) Wang

Jee Young Kim, Jiu Chiuan Chen, Ema Rodrigues, Paul Boyce, Li Su, Wei Zhou

DFCI

Donna Neuburg, Cheng Li

Ed Fox, Maura Berkeley, Pamela Mole

HSPH BIOSTAT

LJ Wei, Xin Lu

Grants

NIEHS T32 ES07069

NIH ES00986 & CA074386