Application of Genomic Technologies in Assessing Gene-Environment Interactions: Implications for Occupational Health

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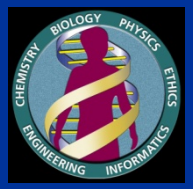
Disclosures

No disclosures to declare

In 2003 scientists in the Human Genome Project obtained the DNA sequence of the 3 billion base pairs making up the human genome



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What we learned

- The human genome is nearly the same (99.9%) in all people
- Only about 2% of the human genome contains genes, which are the instructions for making proteins
- Humans have an estimated 30,000 genes; the functions of more than half of them are unknown

Human Genome Project

Impacting many disciplines

Courtesy
U.S. Department of Energy
Human Genome Program

Global Carbon Cycles
Industrial Resources • Bioremediation
Evolutionary Biology • Biofuels • Agriculture • Forensics
Molecular and Nuclear Medicine • Health Risks

YGA 99-1133R

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National Research Council Report

Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment

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Toxicogenomics: Definition

- The application of genomic technologies (e.g., genetics, genome sequence analysis, gene expression profiling, proteomics, metabolomics, and related approaches) to study the adverse effects of environmentally-released chemicals and pharmaceutical agents on human health and the environment.

What can Toxicogenomics do?

- Provide insights into gene-environmental interactions and the response of biological pathways and networks to stress.
- Lead to information that is more discriminating, predictive, and sensitive than that currently used to evaluate toxic exposure or predict effects on human health.

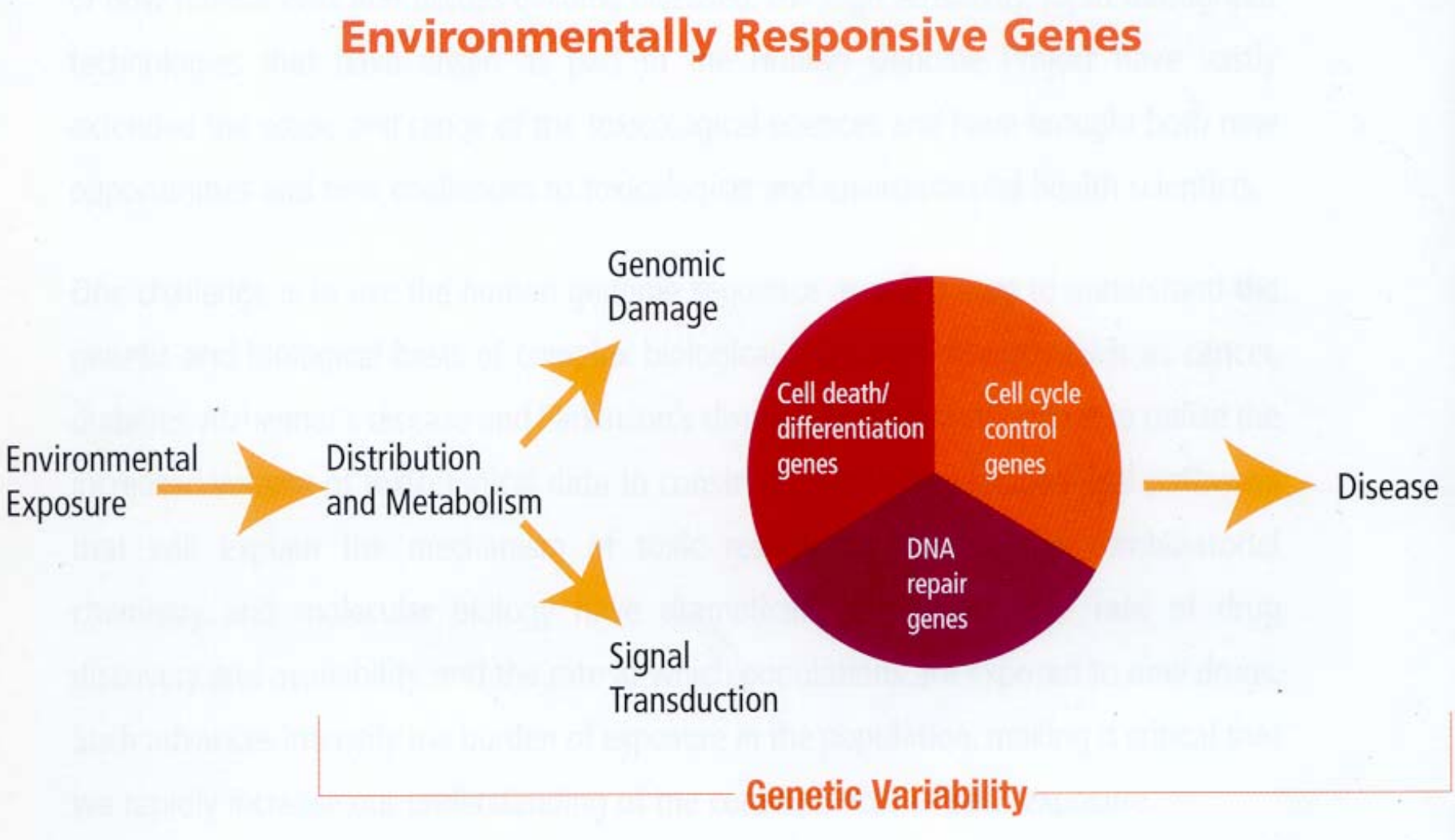
Assessing Toxicity

- Traditionally deals with phenotypic changes in an organism that result from exposure to chemical, biological or physical agents.
- These changes maybe reversible (e.g. transient skin reactions) or irreversible (e.g. cell death, mutation), leading to chronic diseases such as fibrosis or cancer.

Types of Studies

- Whole animal
- Isolated organ preparations
- Cell/tissue culture
- Evaluating exposure/dose
- Chronic exposures (Epi studies cohort; ca-co)
- Panel studies – exposure chambers, field labs

Molecular Epidemiology

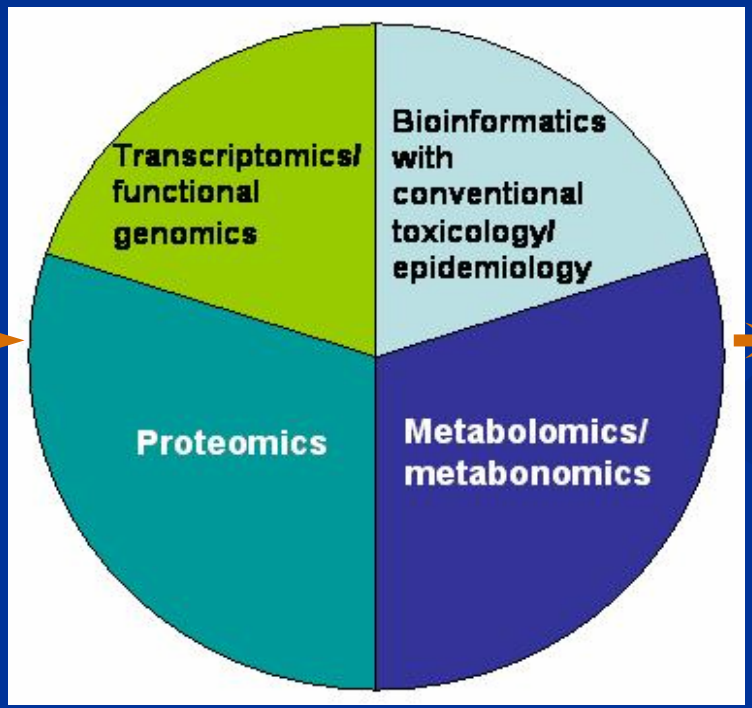


Sequencing & Genotyping Technologies

- Allow the simultaneous assessment of multiple variants across the whole genome, in large populations, rather than just single or several gene polymorphisms.
- GWAS now feasible for population studies – not hypothesis-driven, but provides opportunity for pathway analyses.

Toxicogenomics

Genomics



Signature pattern

(Novel biomarkers)

- Early diagnosis
- Predict susceptibility
- Monitor progression

Exposure-response integrated measures in disease development

Transcriptomics (or gene expression profiling)

- Measures RNA expression in a parallel assay system, usually using microarrays
- Array technology for transcriptomics has enabled the analysis of complex, multigene systems and their responses to environmental perturbations.

Transcriptomics

- Transcriptomics have also driven the growth of "systems biology" which brings together global measures to define biological states on the basis of integrated responses.
- Principle- genes exhibiting similar expression patterns my be related functionally and under common genetic control mechanisms.

Occupational/environmental Cardio-pulmonary Disorders

- Active union membership of the Local 29, International Brotherhood of Boilermakers, Iron-Ship Builders, Blacksmiths and Forgers

Background

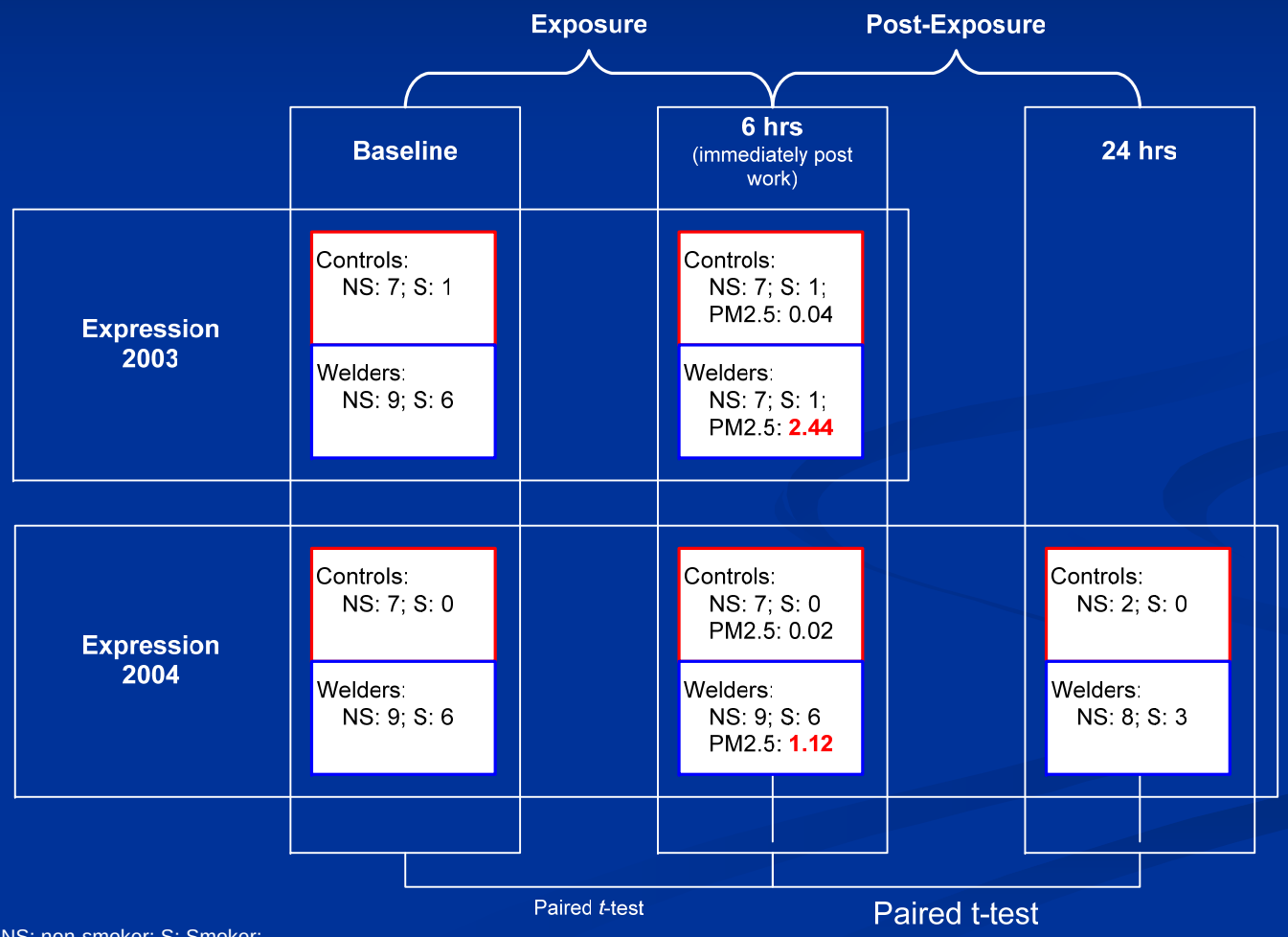
- Particulate exposure is associated with increases in morbidity and mortality from respiratory and cardiovascular diseases.
- Welding process generates high levels of metal fume containing respirable particles.
- Systemic alterations induced by inhaled particulates:
  - Change HRV
  - Release of inflammatory cytokines subsequent to pulmonary inflammation

Exploratory Study

--Hypothesis

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

Gene Expression Profiling in Humans Following Toxicant Exposure



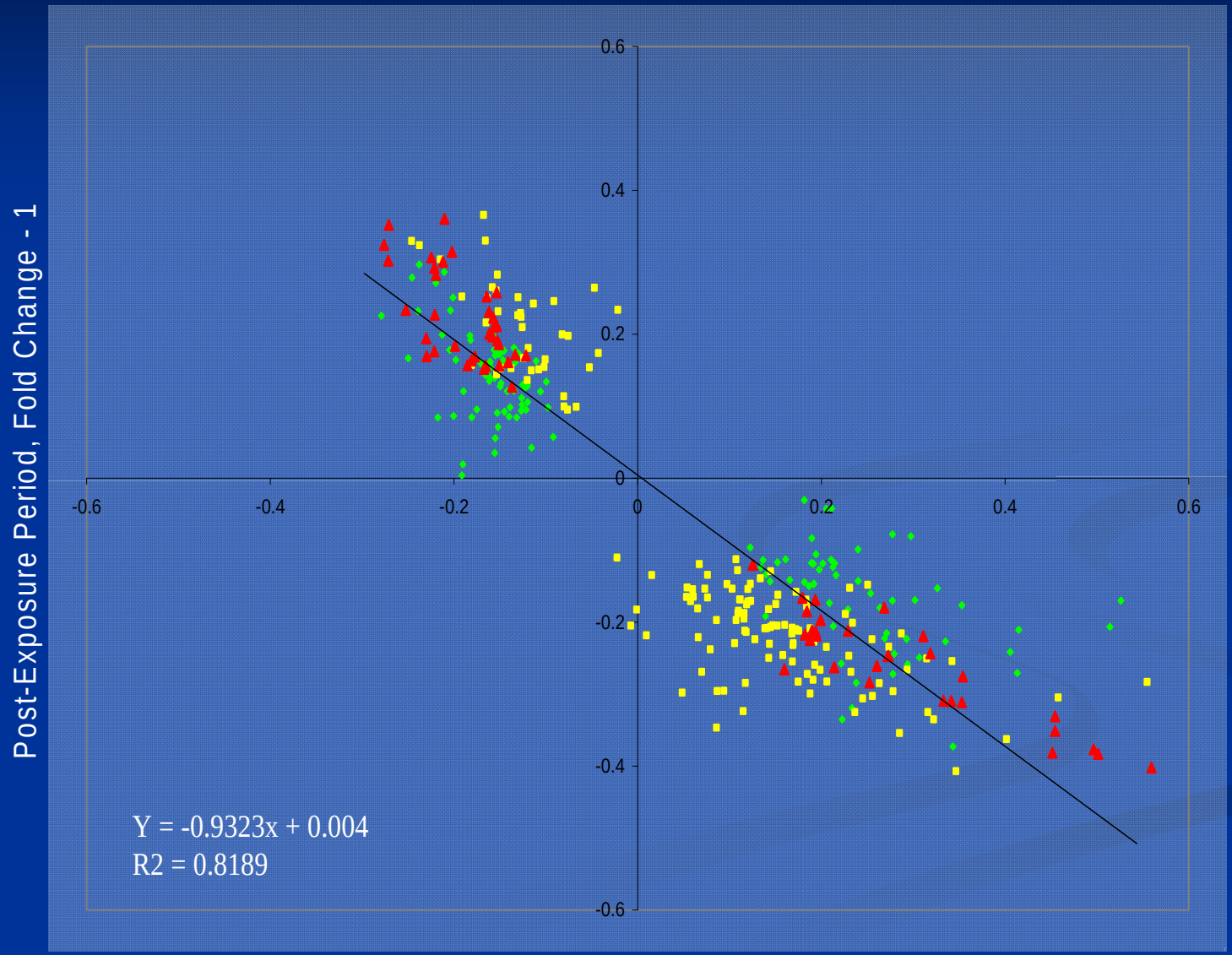
NS: non-smoker; S: Smoker;

PM2.5: average exposure between 6 hrs time point and baseline

Microarray Hybridization

- **Microarray Core Facility of Dana-Farber Cancer Institute/ Harvard Cancer Center**
- **Affymetrix U133A GeneChips** (22,215 probe sets)
- **Batch Analysis** (baseline and post-shift samples)
- **Normalization and calculation of expression values** (dChip Analyzer 2006)
- **The Detection Call** (Affymetrix MAS 5)
  - the dichotomous data of absolute gene expression status, which was either expressed (Present Call) or non-expressed (Absent Call).

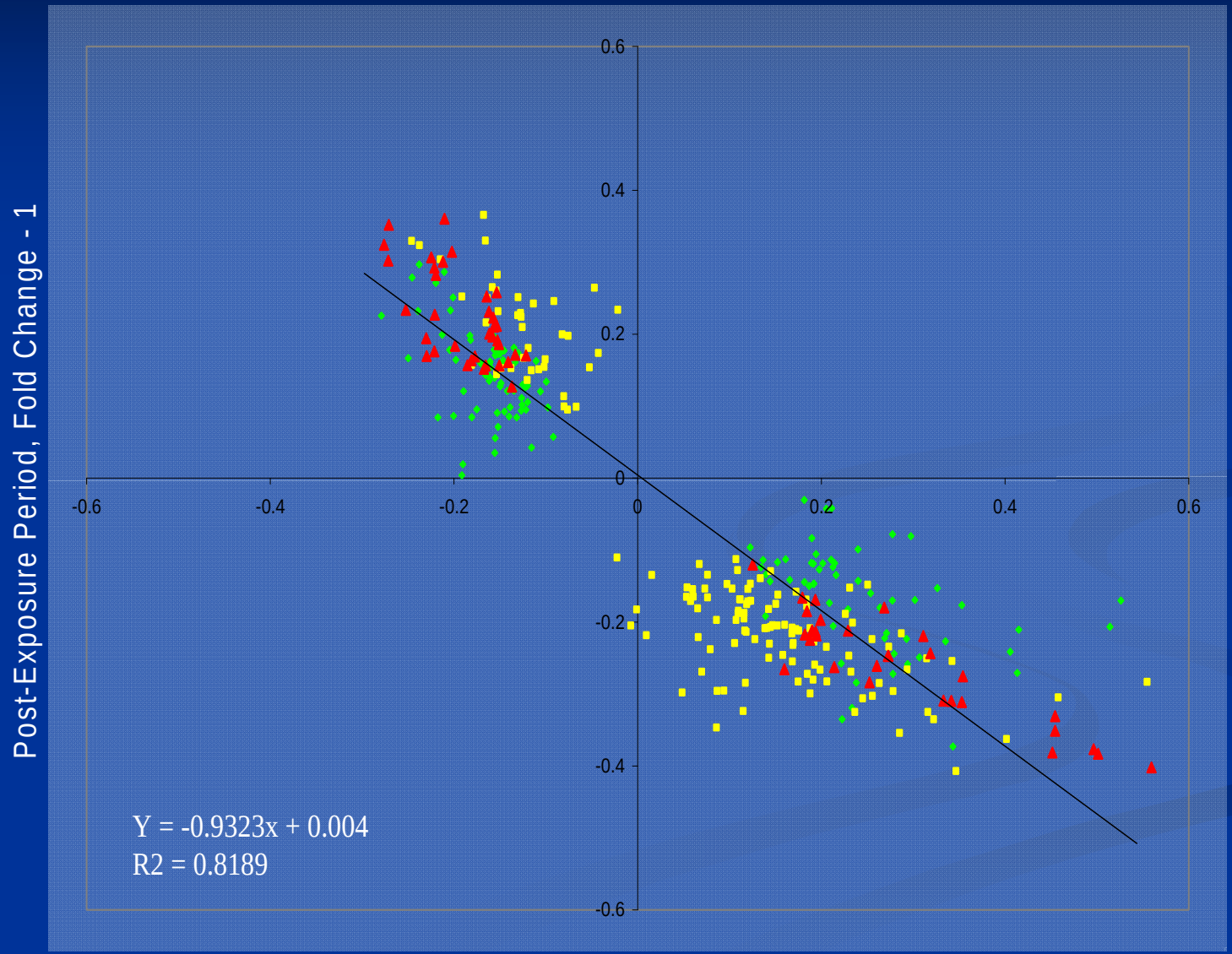
Acute metal exposure can cause transient gene expression alterations in whole blood total RNA



Results

- Sixty-five genes changed significantly from baseline in both the exposure period (6 hrs.) and the post-exposure period (24 hrs.) with opposite directions of expression changes.
- ***Red triangles*** represent genes significantly different from baseline expression in both the exposure and post-exposure periods; ***Green diamonds*** represent genes that tested significantly only in the exposure period; ***Yellow squares*** represent genes tested significantly only in the post-exposure period.

Acute metal exposure can cause transient gene expression alterations in whole blood total RNA



Results - 1

- The observation of genes clustered in the functional pathways related to inflammatory and immune responses support the hypothesis that metal particulate induces systemic inflammation.

- Using toxicogenomic techniques in epidemiologic studies of human exposure effects is feasible.

Wang et. al., Env Health Perspectives, 2005

Results - 2

- Acute metal exposure causes transient gene expression alterations in whole blood total RNA using a repeated measure design.
- The time course of expression alterations was consistent with the results of wbc counts, which rose after exposure.
- Smoking alters expression profiles in the whole blood total RNA and is a confounding factor.
- At lower exposure levels, non-smokers had larger short-term fold changes than did smokers, and these changes were related to metal fume exposure level.

Proteomics

- The study of collections of proteins in living systems.
- Since the same proteins may exist in multiple modified and variant forms, proteomes are complex than the genomes and transcriptomes.

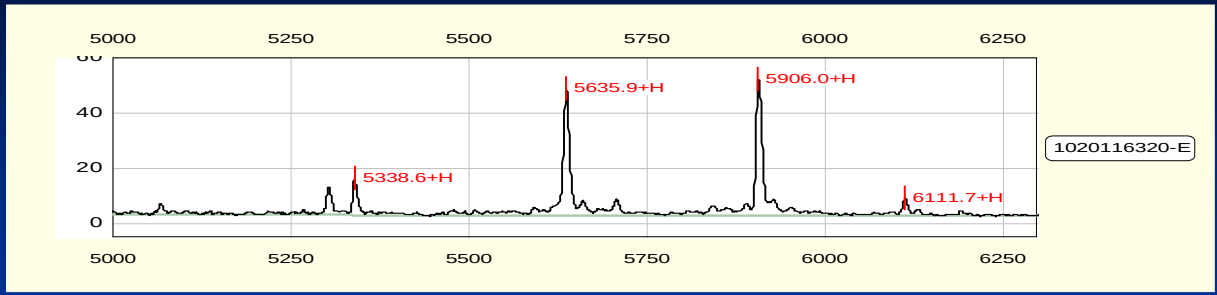
Proteomics

- Use mass spectrometry (MS) and microarray technologies to resolve and identify the components of complex protein mixtures, to identify and map protein modifications, to characterize protein functional associations, and to compare proteomic changes quantitatively in different biological states.

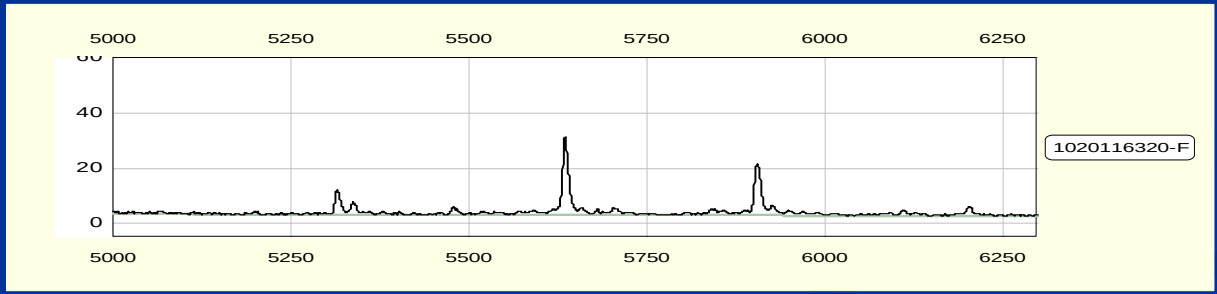
Proteomic Profiling in the Sera of Workers Occupationally Exposed to Arsenic and Lead

- Metal workers: 46 male smelter workers with higher urine As and/or blood Pb
- Controls: 45 age matched male officers
- Surface-Enhanced Laser Desorption/Ionization Time-Of-Flight Mass Spectrometer (SELDI-TOF-MS)
- The WCX2 (weak cationic exchange) ProteinChip

Zhai et al, Biometals, 2005

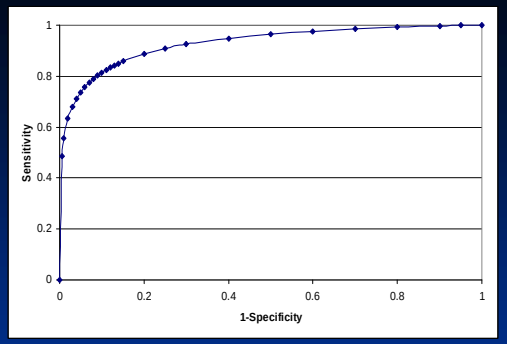


A. Metal worker

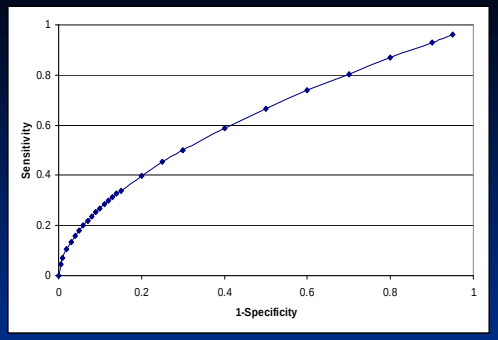


B. Control

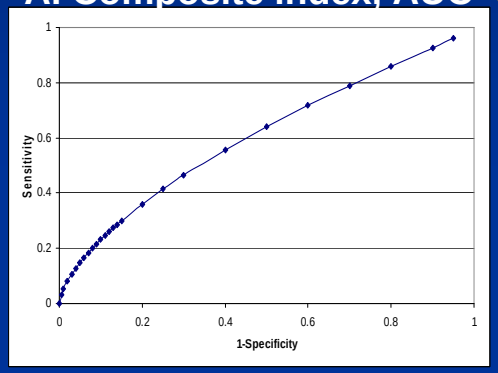
Serum protein profiles of metal worker and control.
Abscissa- m/z in Daltons; Ordinate-relative intensity



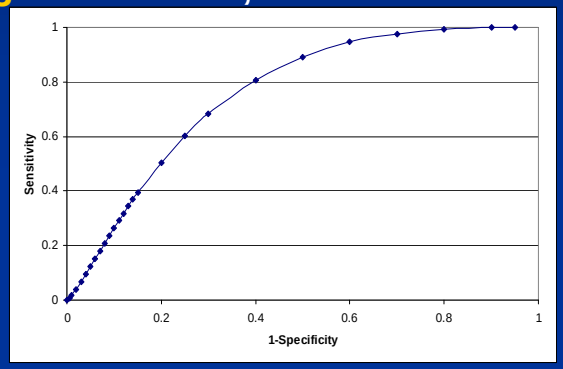
A. Composite index, AUC=0.93



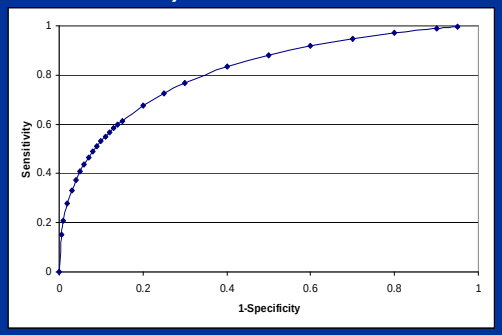
B. M2097, AUC=0.63



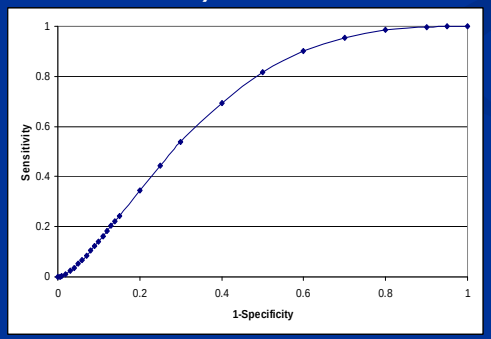
C. M2953, AUC=0.61



D. M3941, AUC=0.76



E. M5338, AUC=0.82



F. M5639, AUC=0.69

Multiple serum proteomic markers could achieve higher sensitivity and specificity than individual marker for detection of metal mixture exposure

ROC curve analysis of the 5 selected protein markers (B, C, D, E, F) and composite index (A) *Zhai et al, Biometals, 2005*

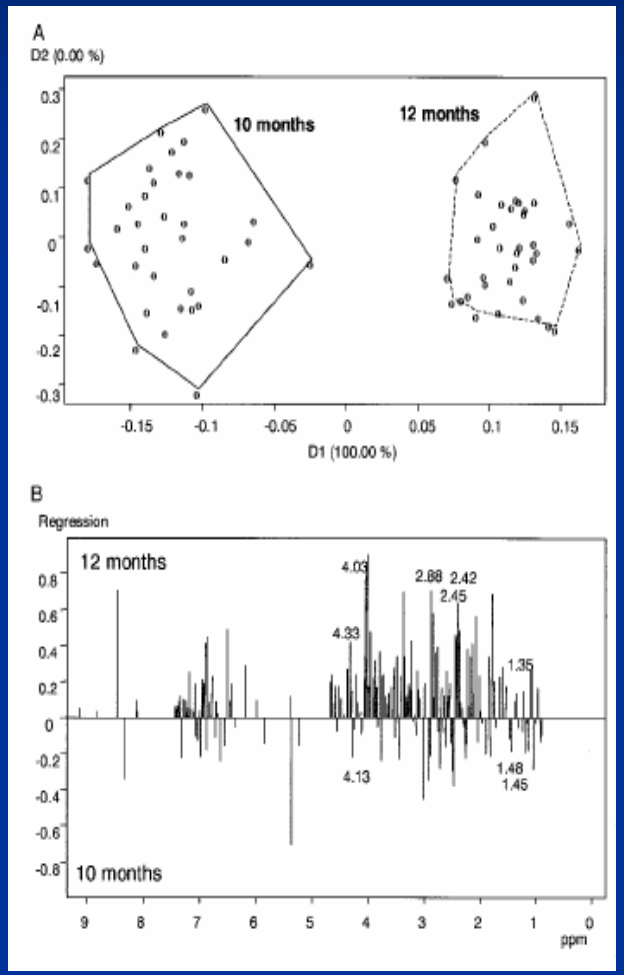
Metabonomics (or metabolomics)

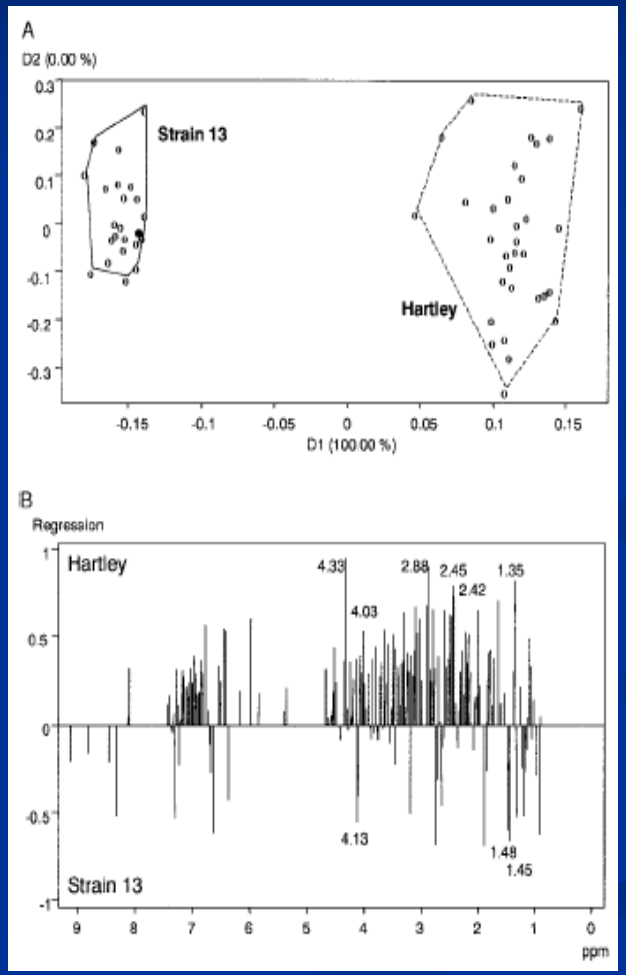
- The study of small molecule components of biological systems, which are the products of metabolic processes.
- Since metabolites reflect the activities of RNA's proteins and genes that encode them, metabonomics allow functional assessment of diseases, drug and chemical toxicity.

Metabonomics

- Use NMR spectroscopy and mass spectrometry MS, and can assess simultaneously dozens to thousands of compounds in biofluids such as urine or in cell and tissue extracts.
- Attractive potential for using urine, saliva, sputum as non-invasive media for analyses of biomarkers of exposure, early responses and early disease detection.

Identification of disease- and nutrient-related metabolic fingerprints in osteoarthritic Guinea pigs.





Principal component discriminant analysis (PCDA) score plots visualizing disease-related differences in NMR spectra.

Lamers et al. J Nutr. 2003

Conclusions

New "omic" techinologies generally described generally as "Toxicogenomics" have the potential for moving environmental health sciences and pharmacology into a new stage of discovery. However, we are only at the threshold and a new initiative on the scale of the Human Genome Project (e.g. a Human Toxicogenomic Initiative) is needed to realize this potential. **(NAS/NRC report, 10/9/2007)**

Research Team

- Recruitment
  - Andrea Shafer
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- Laboratory
  - Li Su
  - Ema Rodrigues
  - Ed Fox
- Data management and data analysis
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  - Sal Mucci
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  - Janna Frelich

- Doctoral Students
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  - Jenn Cavallari
- Post-doctoral Fellows/Res Scientists
  - Mike Wang
  - Rihong Zhai
- HSPH collaborators
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  - Xihong Lin
  - Donna Neuberg