Occupational Hygiene in the "Omic" Era; Implications and Consequences

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Voorbij de grenzen van de arbeidshygiene
14e symposium NVvA

Conclusion

- Clinical applications? Likely
- Etiological research? Likely
- Occupational Hygiene? No, but maybe indirect

Outline

- What is 'OMICS'
  - Examples of applications (Clinical, Toxicogenomics)
  - Future direction
- Implications and consequences for Occupational Hygiene?
- Will not address:
  - Assay specific problems
  - Multiple comparison problems (False-positive findings)

What is 'OMICS'

- The study on a global basis, the level, activities, regulations and interactions of:
  - Genes (Genomics)
  - mRNA transcripts (transcriptomics)
  - Proteins (Proteomics)
  - Metabolites (Metabolomics)

Not new….

- Breakthroughs in platform development
  - Throughput
  - Ease of quantification
  - Costs

Proteomics

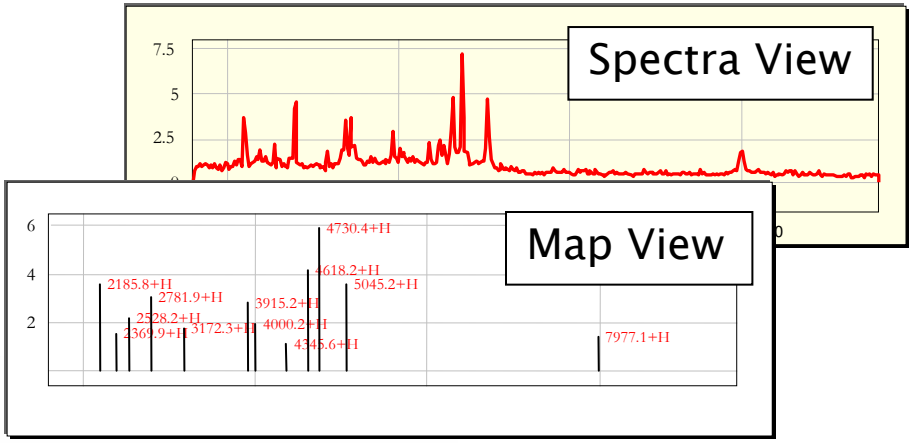
- The study of the **proteome**, the complete set of proteins produced by a species, using the technologies of large-scale protein separation and identification (Tissue, Cell, Serum, Urine)

- Provides information regarding the proteome's dynamic and rapid changes.
  - Disease (endogenous factors)
  - exogenous exposure

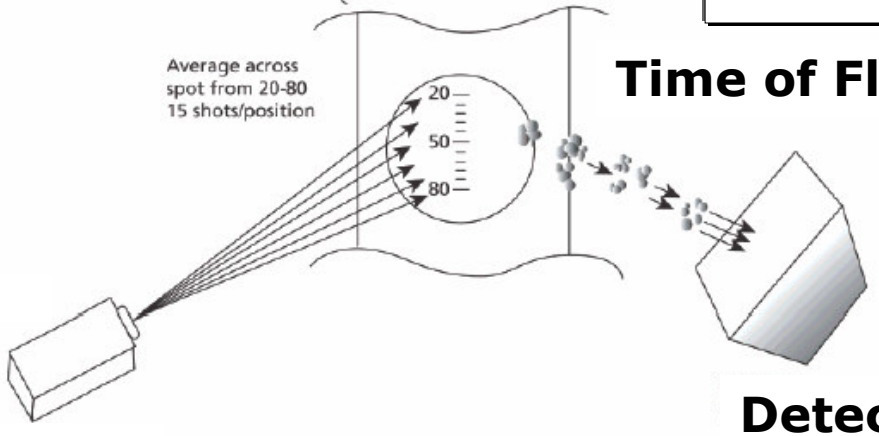
- These changes include:
  - DNA alterations, mRNA splicing, temporal and functional regulation of gene expression, and post-translational modifications.

How Does It Work?

Sample analysis and Spectra Acquisition using SELDI-TOF



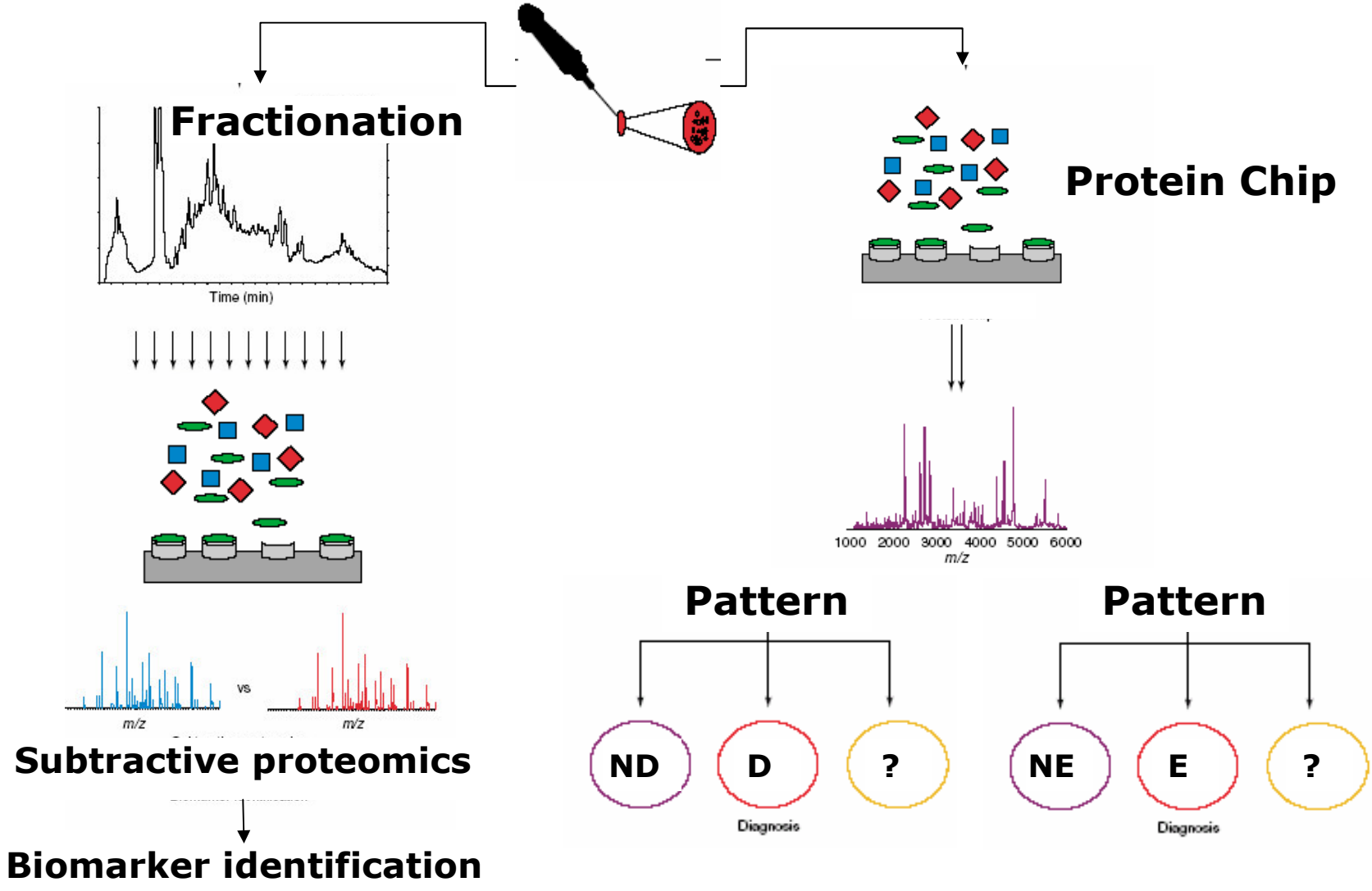
Time of Flight



Laser

Detector plate

Biomarker Discovery or Diagnostic Proteomics



Diagnostic Proteomics

- Seminal paper on Ovarian Cancer (Petricoin et al., 2002)
  - Cluster pattern segregated cancer from non-cancer with a sensitivity of 100% and specificity of 95%, and positive predictive value of 94%.
- However, results have not been reproduced in other studies concerns about the black box approach
- Expensive platform

Biomarker Discovery Toxicogenomics

Identification of proteomic markers by SELDI-TOF MS in serum from workers exposed to benzene

Vermeulen et al., 2005 Submitted

Benzene study in Tianjin, 2000-2001

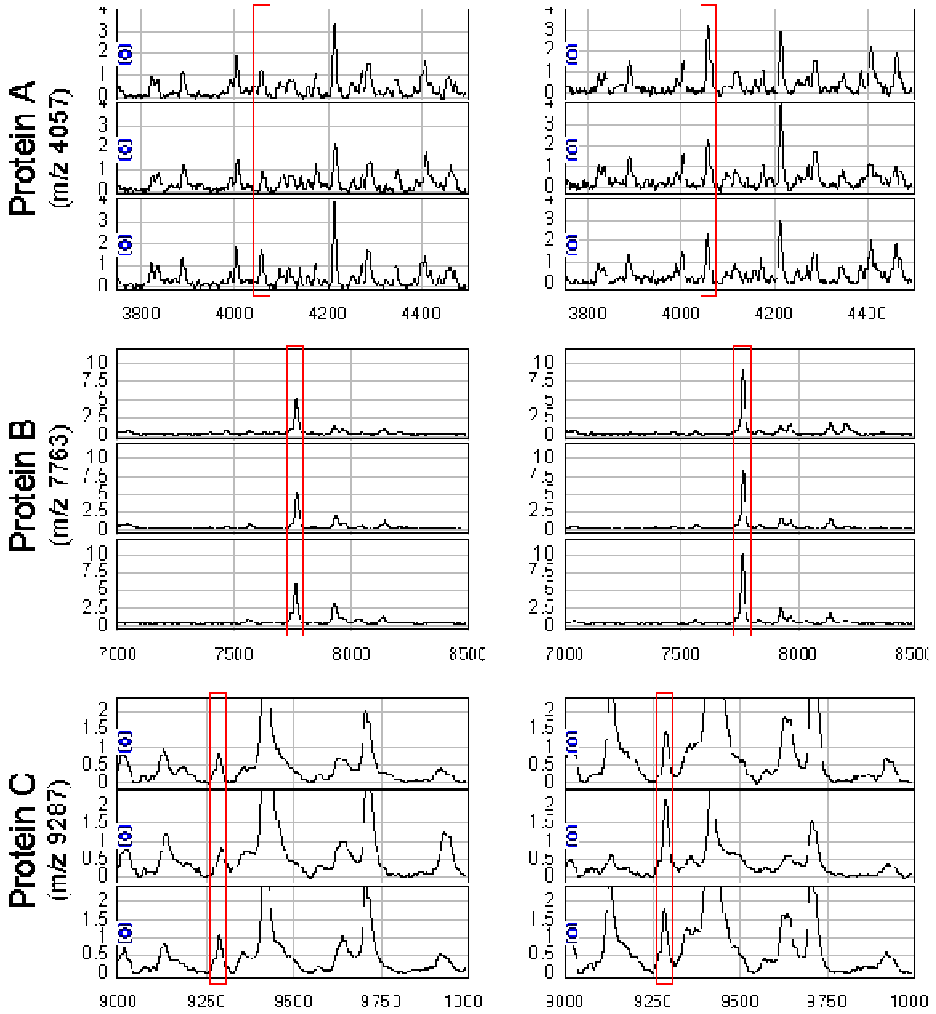
- 250 healthy shoe manufacturing workers from two factories with benzene exposure
- 140 healthy age sex matched unexposed controls in clothes factories

Materials and Methods

- Two sequential studies of 10 Exposed-Control pairs
- Serum samples were fractionated and three fractions were selected for profiling on three types of ProteinChip® array surfaces (WCX, H50 and IMAC-Cu).
- Patterns of protein expression were detected by surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS)

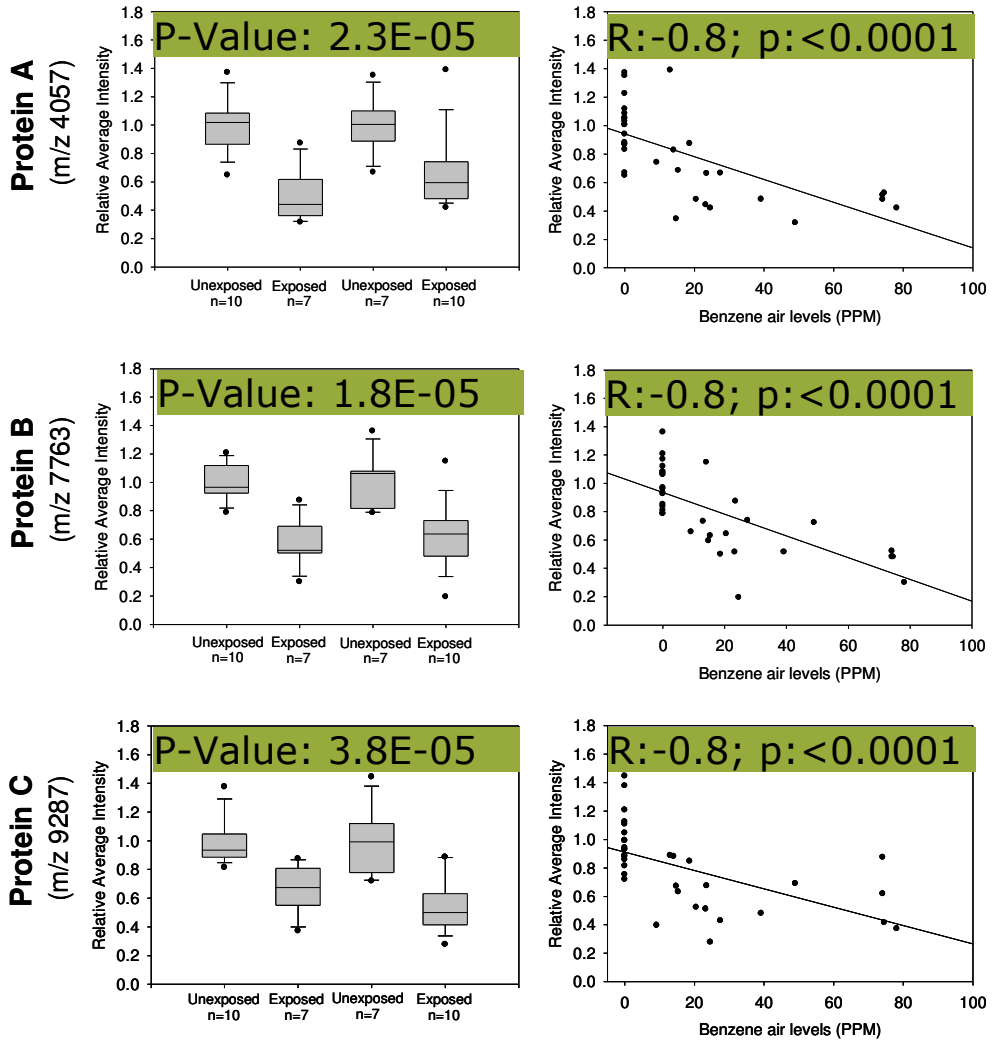
Initial Screening Phase

- Univariate statistical analyses revealed 18 peaks that exhibited significant differences ($p<0.01$) between exposed and non-exposed subjects
- The M/Z of these protein markers ranged from ~4.0kDa to ~73kDa



Replication Phase

- 3 Peaks were confirmed
- All three proteins showed a high correlation with benzene exposure (r > 0.75)
- Additional regression analyses showed that smoking, recent infections, sex, age and cell counts were not significantly associated with levels of any of the 3 proteins



Identification of Protein Markers

- Proteins are platelet derived CXC-chemokines
  - Modulating inflammation
  - Regulating chemotaxis
  - Influencing hematopoiesis
  - Stimulating cell proliferation
  - Chemoprotective of normal hematopoietic progenitors

Implications for exposure assessment

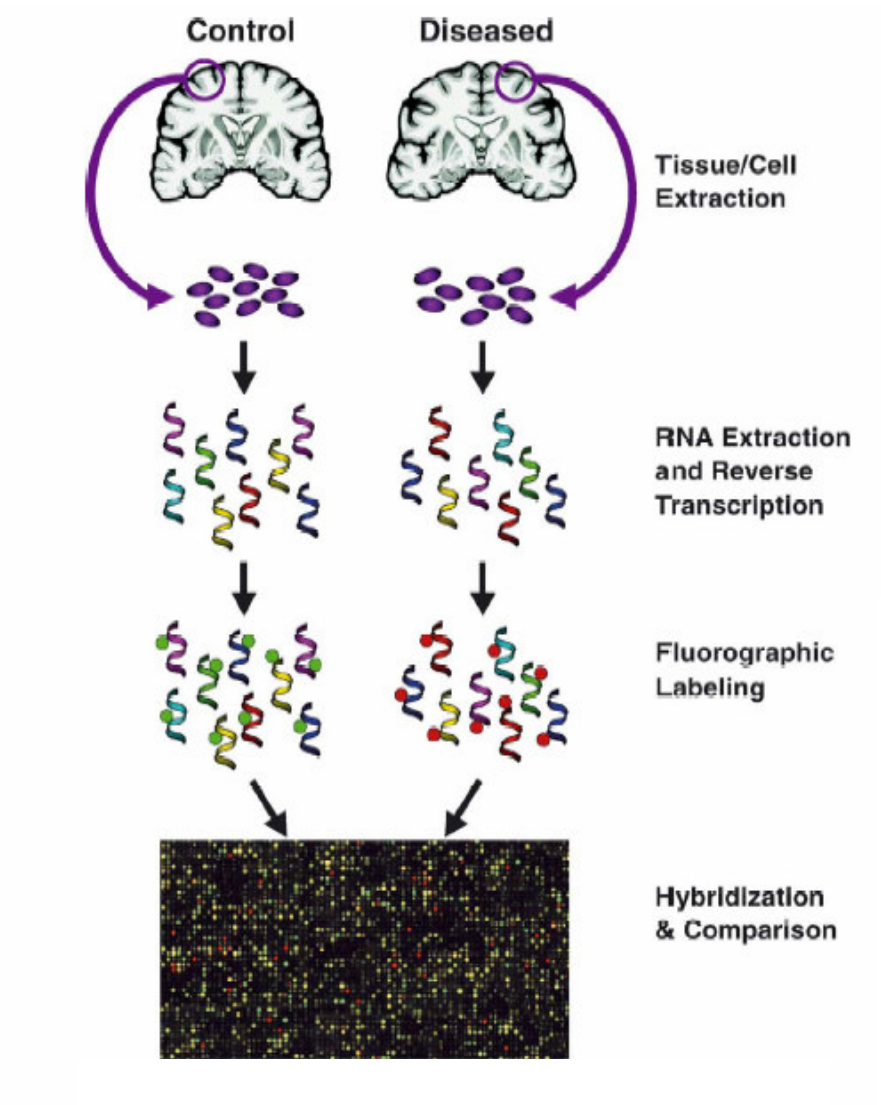
- 'Proof of Principle' exercise suggest that proteomics is capable of identifying differences in protein expression due to environmental factors

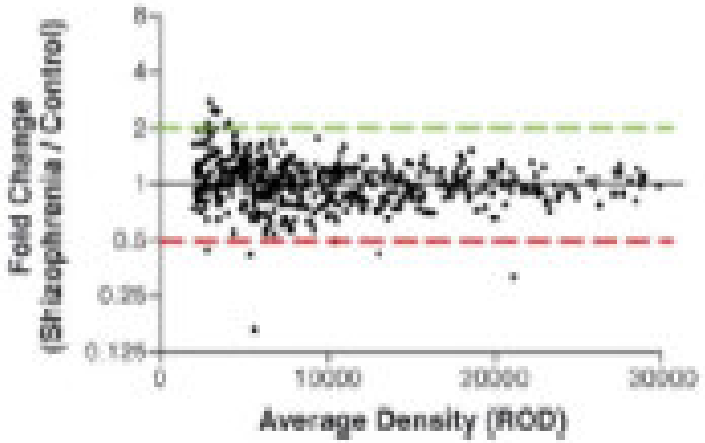
- but:
  - Marker not specific to exposure but to effect
  - Most likely a short-term marker
  - Expensive

Transcriptomics

- The study of the **transcriptome**, the complete set of RNA transcripts produced by the genome at any one time.
  - Its the dynamic link between the genome, the proteome and the cellular phenotype

How Does it Work?





Diagnostic Transcriptomics

- Seminal paper: Paik et al., NEJM, 2004
  - Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer
- Concerns about reproducibility
- Expensive

Biomarker Discovery Toxicogenomics

Discovery of Novel Biomarkers by Microarray Analysis of Peripheral Blood Mononuclear Cell Gene Expression in Benzene-Exposed Workers

Forrest et al., EHP 2005

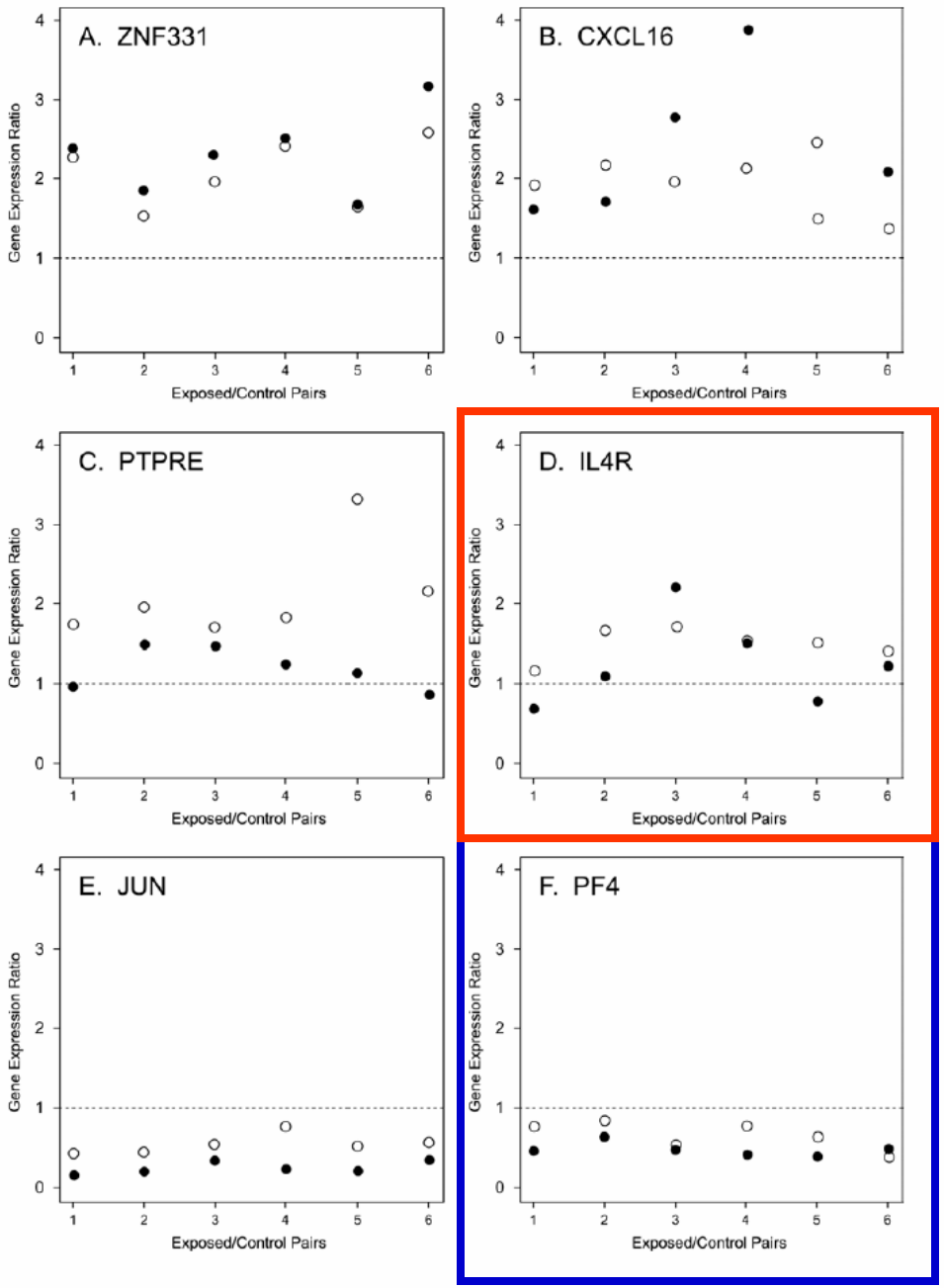
Materials and Methods

- PBMC RNA was stabilized in the field and analysed using a comprehensive human array, GeneChip set (>39,000 unique transcripts; 6 exposed-control pairs)

- Confirmation with real-time PCR (~13 exposed-control pairs)

Results

- 29 Known genes were identified that were highly likely to be differentially expressed
- 6 Genes were selected for confirmation by real-time PCR (*CXCL16, ZNF331, JUN* and *PF4)*
- Finding was confirmed with RT-PCR in a larger dataset from 28 subjects

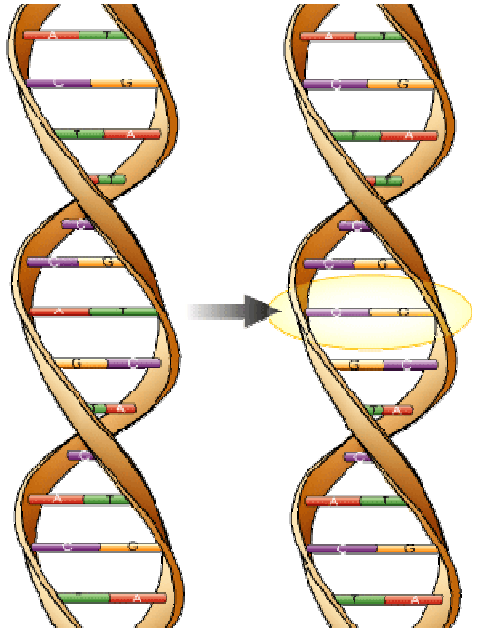


Implications and Consequences *Proteomics & Transcriptomics*

- Indirect:
  - Provides insight in the mechanism of action
  - Provides insight in characterizing the functional significance of genetic polymorphisms
  - Might change risk assessment
- Direct:
  - Might provide biomarkers of individual integrated biological response
    - Personalized exposure dosimeters
    - Define exposure by biologically relevant responses

Toxicogenetics / Genomics

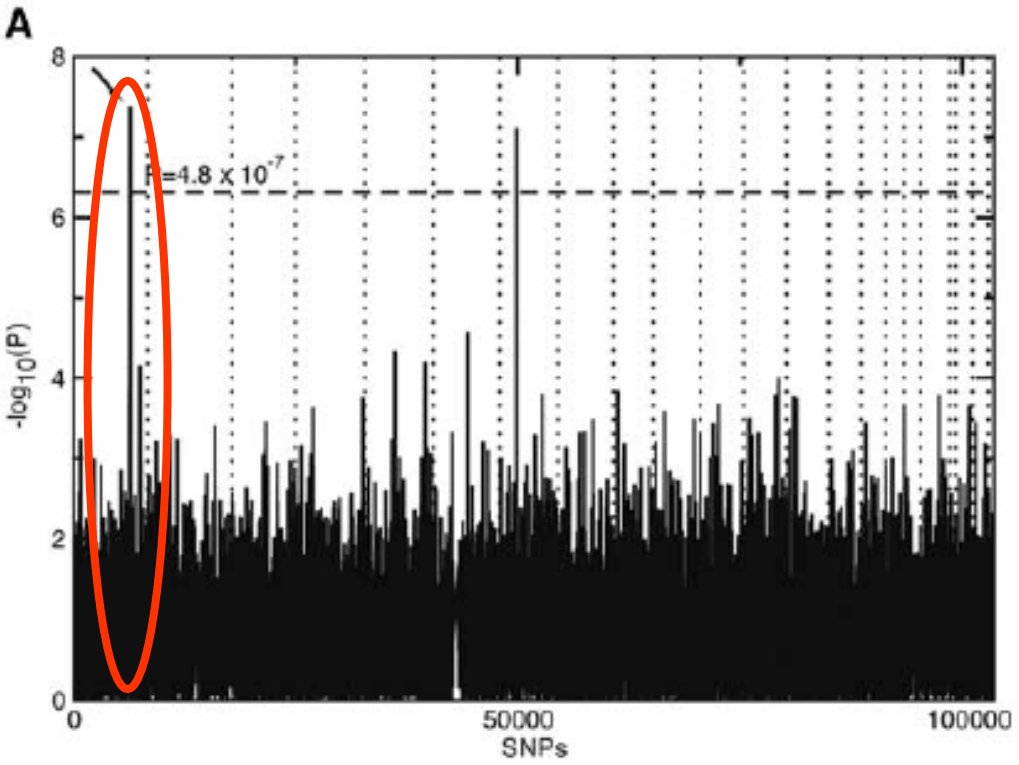
- Study of the influence of hereditary factors on the effects of potentially toxic substances on individual organisms.
  - Single-Nucleotide Polymorphism (SNP) analyses
  - Haplotype analyses (Pattern of SNPs on a block)
  - Genechips (whole genome scans)



Human Genome Project

Clinical Genomics

Polymorphism in Age-Related Macular Degeneration



Compliment factor H gene (CFH)

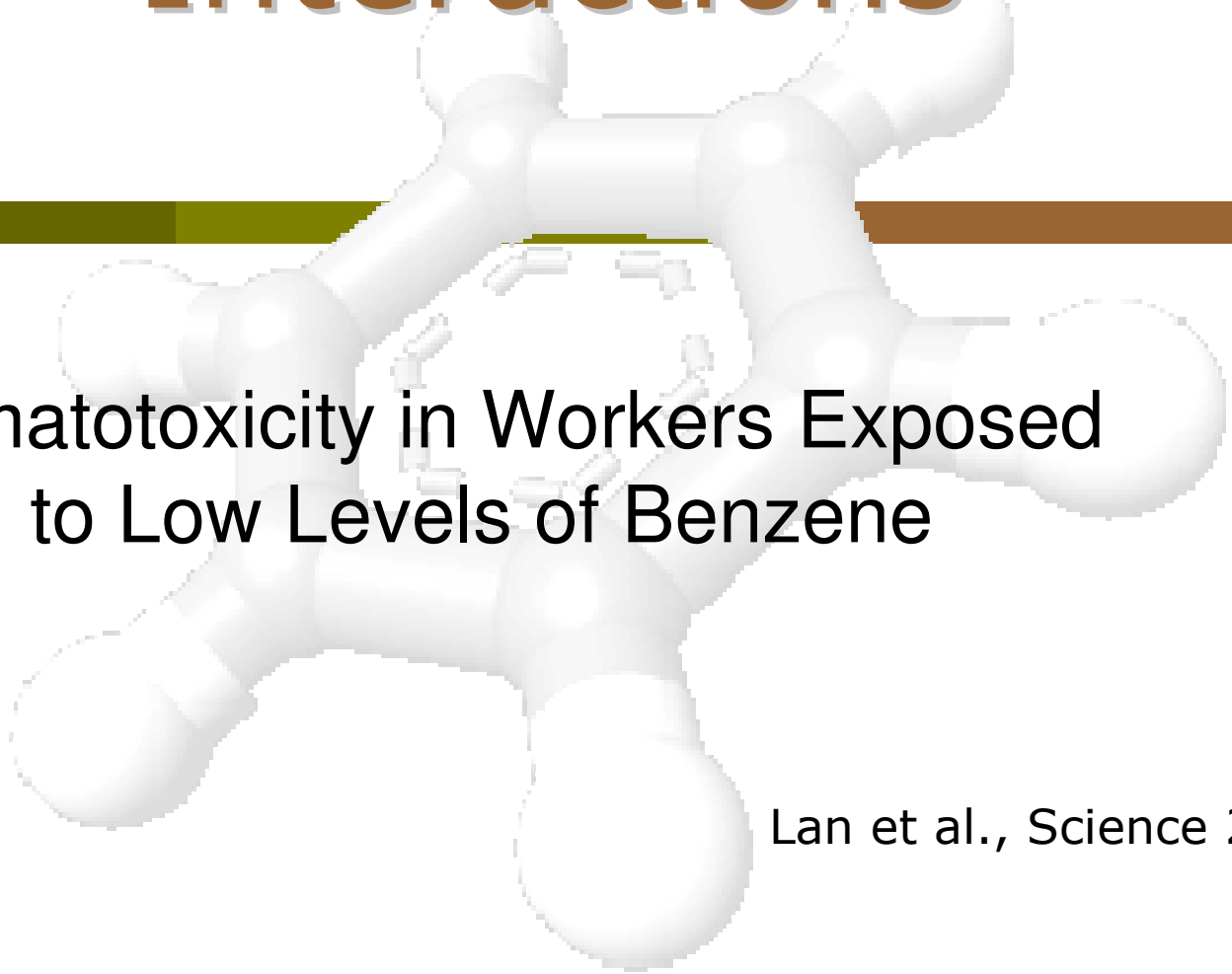
Y402H	OR	PAR
Heterozygous	4.6 (2.0 - 11)	0.70
Homozygous	7.4 (2.9 - 19)	0.23

Klein et al., Science 2005

Need to Distinguish

- *High-penetrance genes (Mutation)*:
  - High-risk very uncommon in the normal population (*BRCA1 and BRCA2 – Breast Cancer)*
- *Low-penetrance genes (Polymorphism)*:
  - Low-risk but more common in the normal population (metabolic genes)

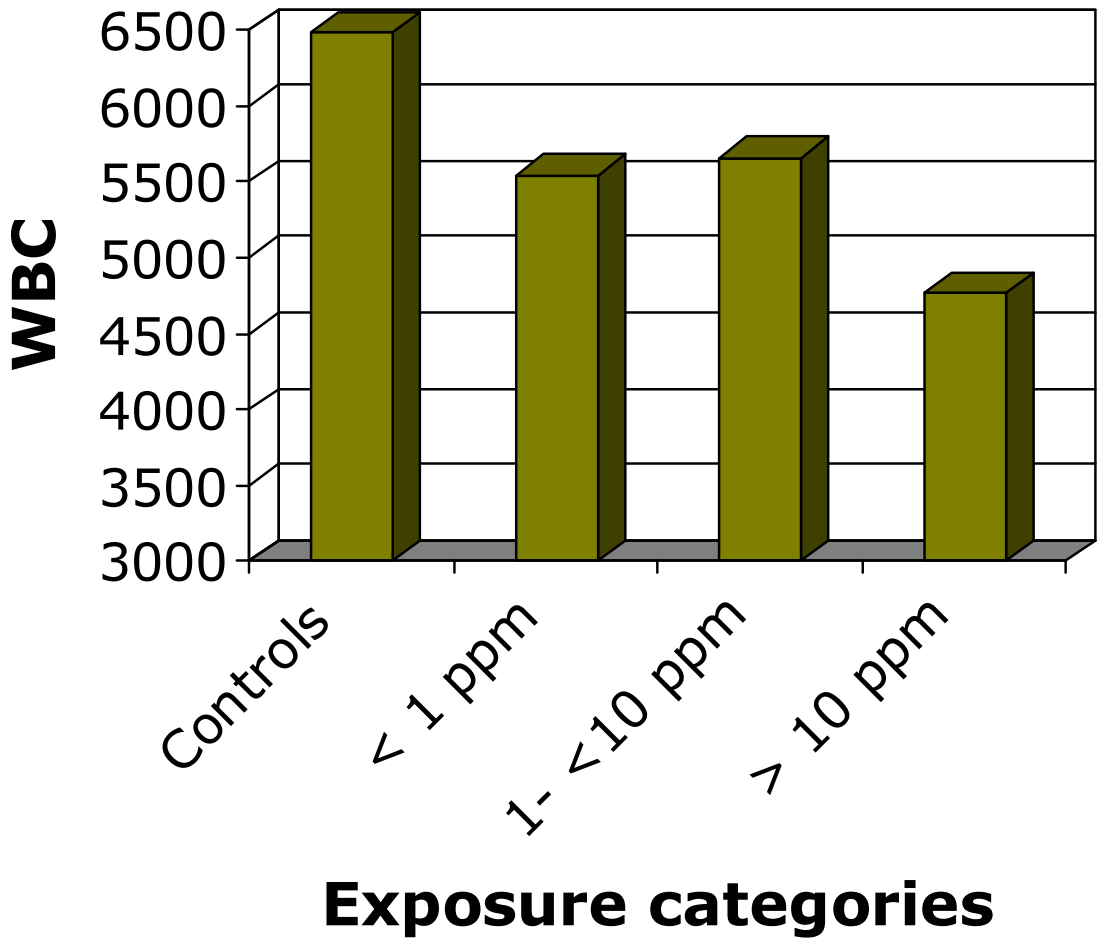
Gene-Environment Interactions



Hematotoxicity in Workers Exposed to Low Levels of Benzene

Lan et al., Science 2004

WBC Count and Benzene Exposure in Previous Month



Controls (n=140)

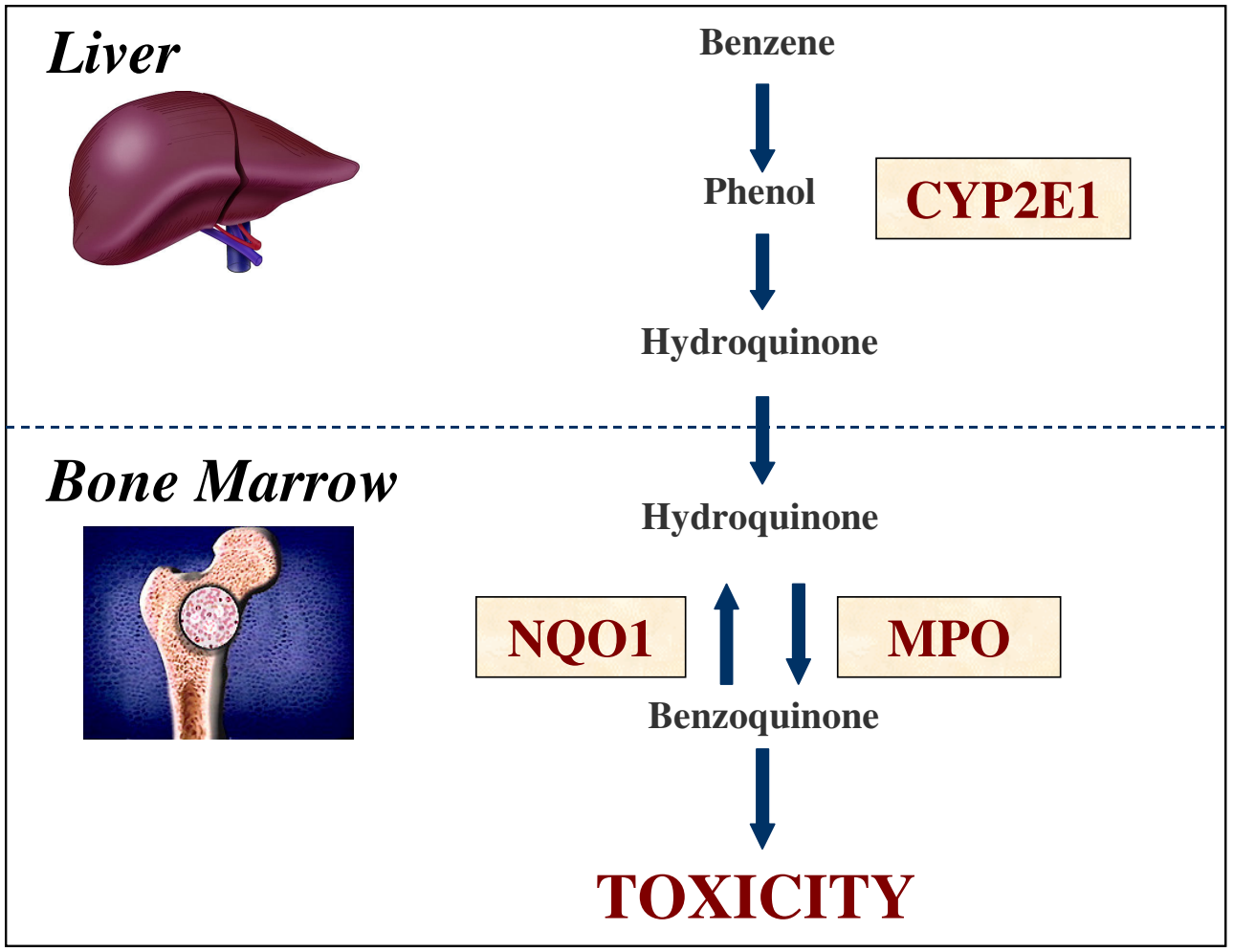
<1 ppm (n=109)

1-10 ppm (n=110)

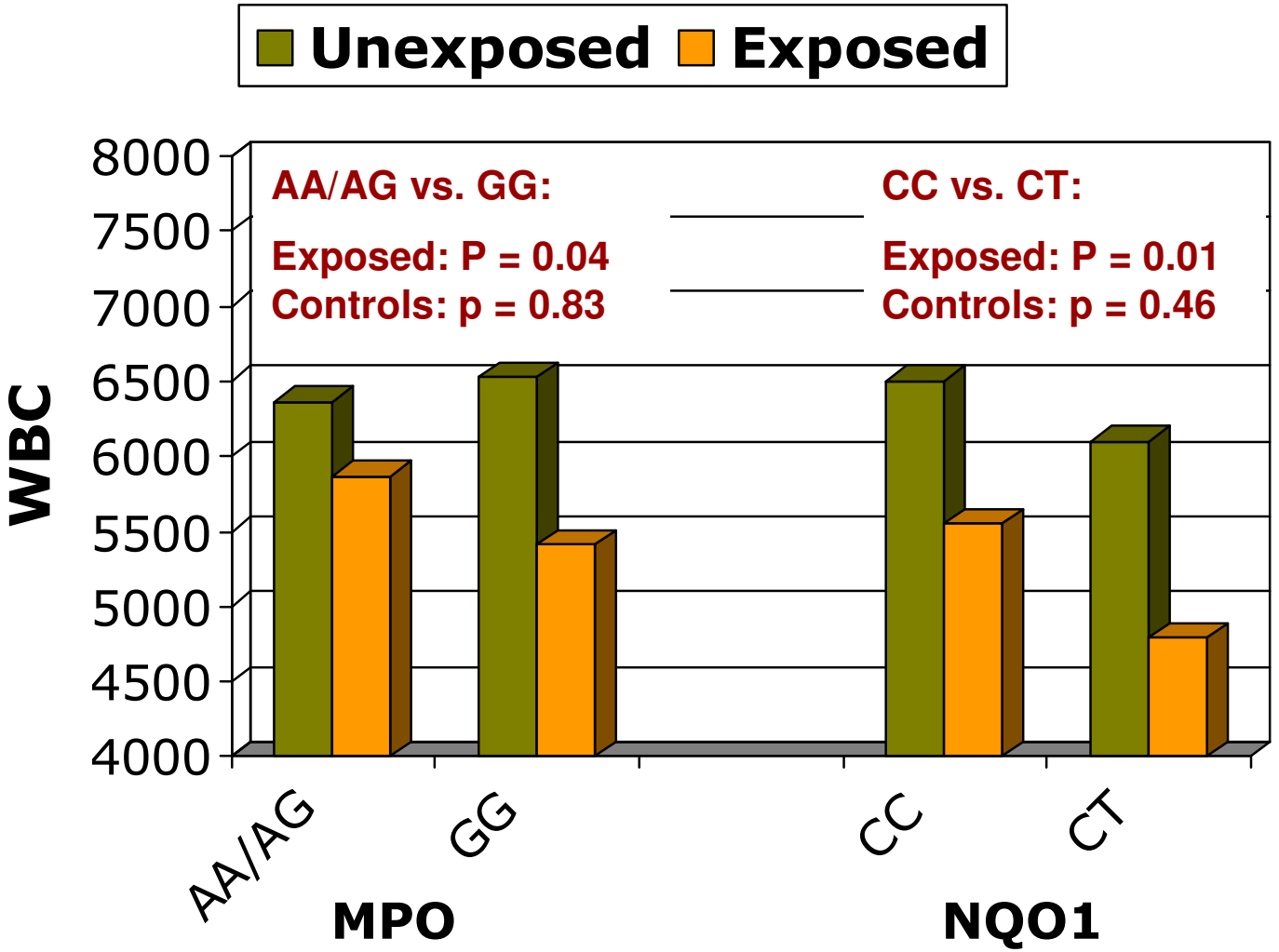
>10 ppm (n=31)

(P trend<0.0001; <1ppm vs control: p<0.0001)

Genetic Susceptibility for Benzene Toxicity (Candidate Gene Approach)



MPO and NQO1 Polymorphism for WBC Toxicity in Workers Exposed to Benzene and Controls



Conclusions

- Exposure to < 1 ppm benzene associated with decrease in WBCs, granulocytes, lymphocytes, CD4+ cells, CD4/CD8 ratio, B cells
- Genetically defined subgroups with greater sensitivity to benzene probably exist
- Raises additional concerns about health effects of exposure to benzene at current standard

Lan et al., Science 2004

Implications and Consequences *Genomics*

- Indirect:
  - Provides insight in the mechanism of action
  - Identify genetically defined subgroups with greater sensitivity to exogenous exposures
  - Might change risk assessment
- Direct:
  - Genetic screening in the workplace
    - Pre-placement
    - Targeted intervention

Fact or Fiction?

Dear

Thank you for attending our final selection panel and pre-employment medical examination which, as you know, involved an obligatory DNA analysis. I regret having to inform you that although you made a strong impact at interview you have been unsuccessful in your application on the basis of predicted genetic susceptibilities.

Your DNA profile showed that although you have the potential for innovation and creativity, features which were apparent from your initial interview, you have at the same time a high risk of developing a manic-depressive psychosis which would seriously impair your performance, and in the position to be filled, potentially put colleagues at risk. As well as this, however, you have a genetic susceptibility to develop liver disease on exposure to several metals, which are found throughout our premises. I regret that, on the basis of these results, we cannot offer you employment but wish you success in the future.

Yours faithfully,

Gene-Environment Interaction and Genetic Screening - Ethics

Employee

Possible Advantages

- Avoidance of future ill-health
- Awareness of a special need to use precautions
- Avoidance of wasted time and effort in training for an unfiltered career

Possible disadvantages

- Anxiety about future health and security of job
- Risk of not being employed in a desirable job

Employer

Possible Advantages

- Less sickness absence and labor turnover
- Chance to target the deployment of control measured

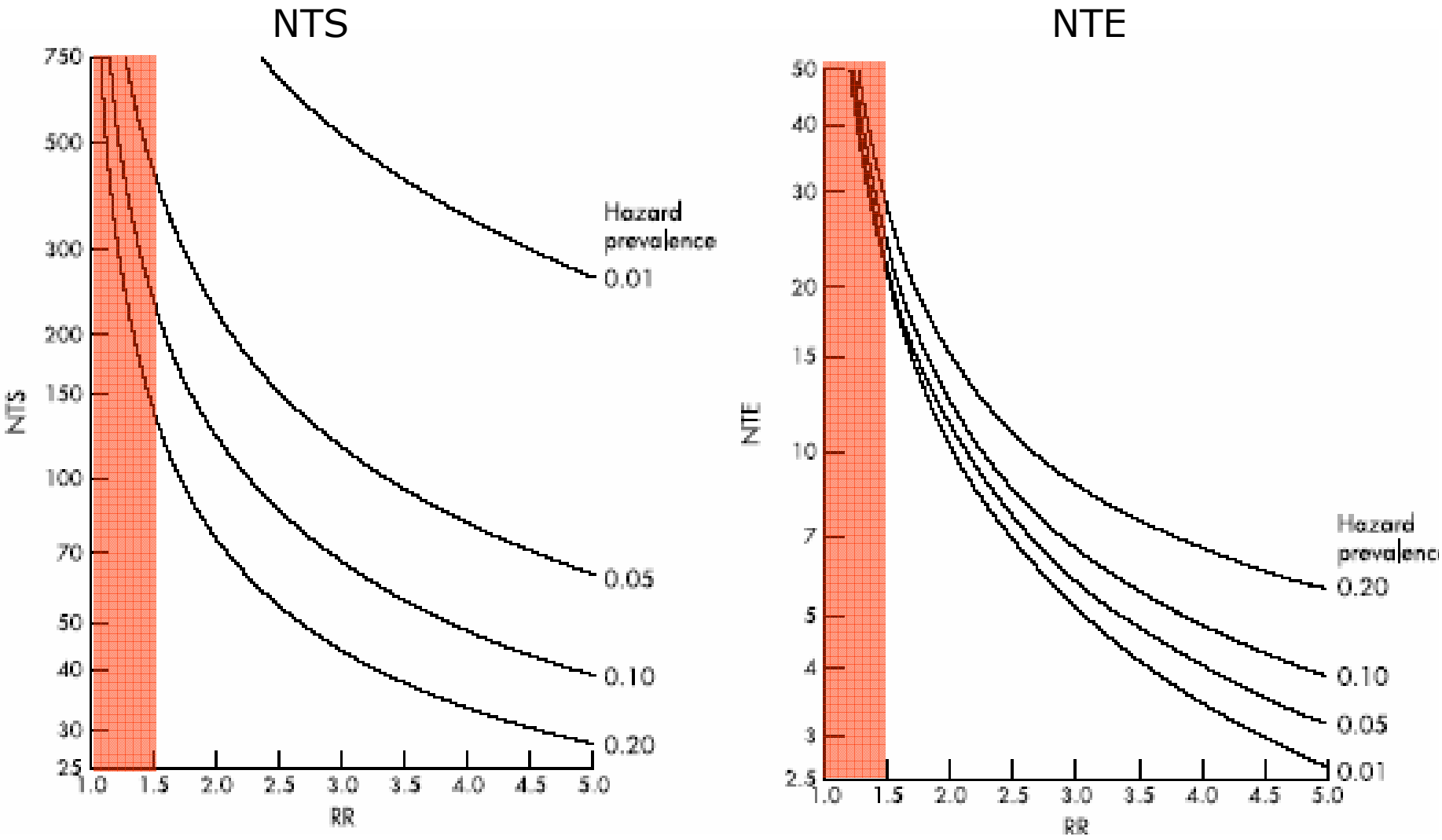
Possible disadvantages

- Direct and indirect cost
- Cost of excluding and replacing applicants

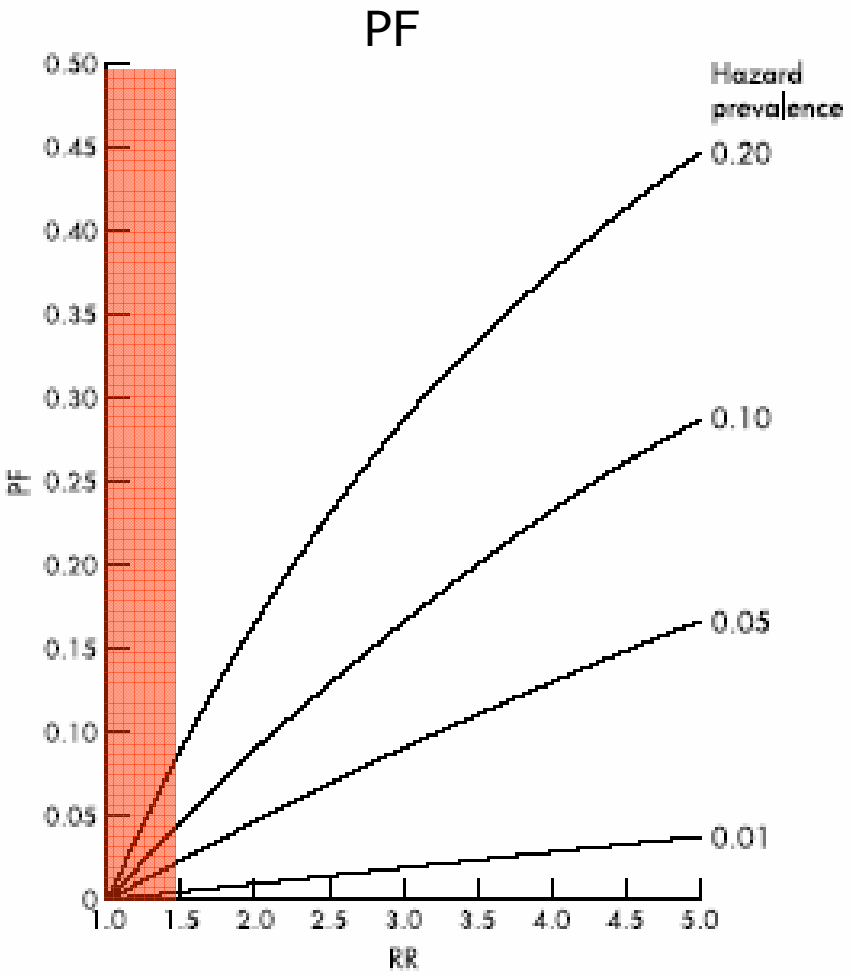
Gene-Environment Interaction and Genetic Screening - Science

- Pre-placement screening
  - Number needed to screen to prevent a case (NTS)
  - Number excluded to prevent a case (NTE)
  - Proportion of adverse outcomes that can be prevented by the screening program (PF)
- The PF, NTS, and NTE, depend on the prevalence of the prognostic indicator and the relative risk (RR)
- Calculations take no account of the importance, severity, or longevity of the health outcome

Gene-Environment Interaction and Genetic Screening - Science



Gene-Environment Interaction and Genetic Screening - Science



Let's consider

- Bakers and ✂-amylase sensitizatior (Nieuwenhuizen et al., 1999)

Screening

- Atopy RR 3.0
- NTS: 77
- NTE: 28
- PF: 0.4

Exposure reduction

- Exposure reduction (High – Low)
- PF:0.22

Gene-Environment Interaction and Genetic Screening - Science

- Protecting the more susceptible

- Let's consider
  - Workers exposed to PAHs (Cumulative risk 10%)
  - Screening for GSTM1 (RR 1.34)
  - Prevalence genotype 50%
  - Risk reduction of 50%

  - With screening: Exposure reduction for 30 workers to prevent 1 cancer
  - Without screening: Exposure reduction for 35 workers to prevent 1 cancer

Gene-Environment Interaction and Genetic Screening - Science

- For **low-penetrant genes**, screening for genetic variants in order to identify high-risk subgroups in the population is not a practical and useful strategy for pre-placement or to focus exposure reduction efforts

- However, ethical or economic consequences need to be considered as well

Implications and Consequences of Omics

- Clinical applications? Likely
- Etiological research? Likely
- Occupational Hygiene? No, but maybe indirect

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Leading America to Better Health, Safety and Well-Being

Bedankt!

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