The use of biomarkers and genetic epidemiology

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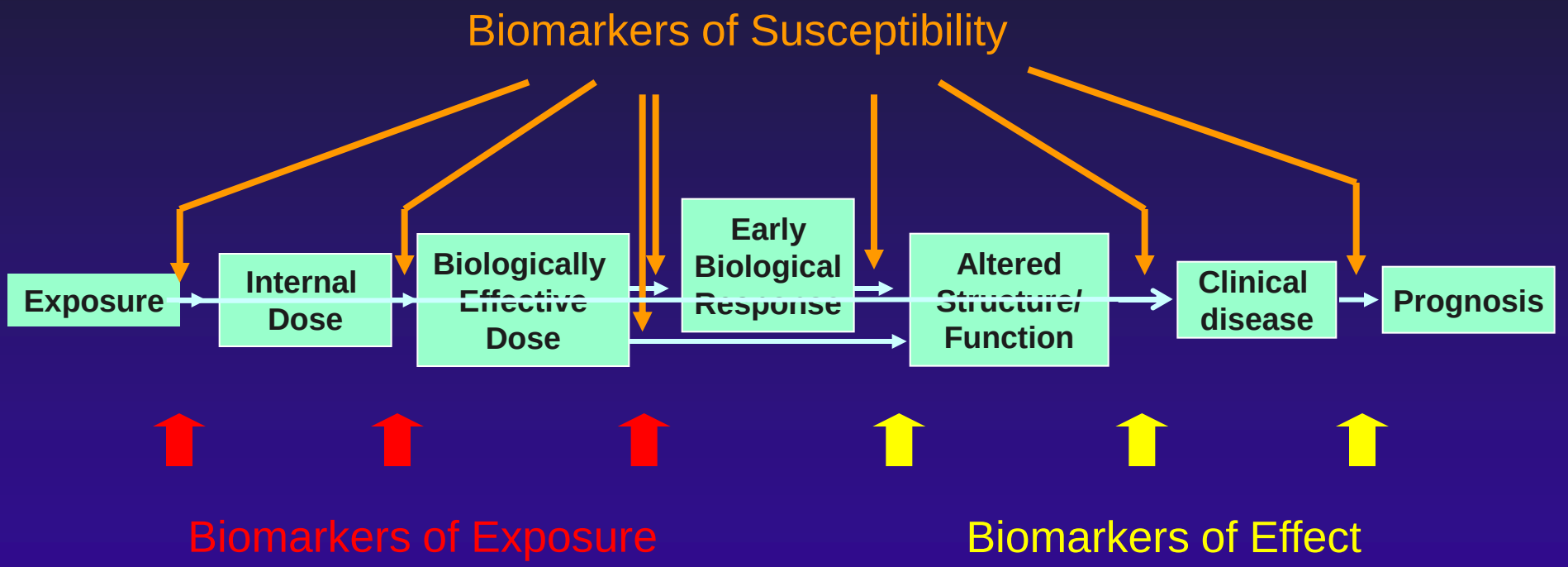
Biomarkers


Biomarkers of Susceptibility
Exposure
Internal Dose
Biologically Effective Dose
Early Biological Response
Altered Structure/ Function
Clinical disease
Prognosis
Biomarkers of Exposure
Biomarkers of Effect

Types of biomarker

- Biomarkers of exposure: Measurements that indicate that exposure to a chemical or chemical class has occurred, but do not provide knowledge of adverse effects at the level of the organism
- Biomarkers of effect: Measurements that indicate that both exposure and adverse effects have occurred
- Biomarkers of susceptibility: Measurements used to assess an organism's inherent or acquired limitation to cope with a chemical exposure

Some examples of biomarkers

- **Biomarkers of exposure**
  - Body burden
    - Exhaled breath
    - Blood or urinary levels
  - Internal dose
    - Blood metabolite levels
    - Urinary metabolite levels
    - Protein adducts
    - Plasma cholinesterase inhibition
  - Biologically-effective dose
    - DNA adducts
- **Biomarkers of effect**
  - Sister chromatid exchanges
  - Micronuclei
  - Chromosomal damage
  - Red cell cholinesterase inhibition
  - Urinary beta-2-microglobulin
- **Biomarkers of susceptibility**
  - Breathing rate
  - Genotype or phenotype
    - P450
    - Glutathione S-transferase
    - Epoxide hydrolase
    - DNA repair enzymes

These divisions are not exact (e.g. DNA adducts could be considered a biomarker of effect rather than of exposure, micronuclei could be considered a biomarker of exposure rather than of effect)

Source of materials and methodologies for assessment of biomarkers

MATERIAL

- Biofluids
  - urine, serum, saliva
  - bronchoalveolar lavage
- Cells
  - peripheral blood cells
  - cell culture
- Tissue
  - necropsy
  - biopsy

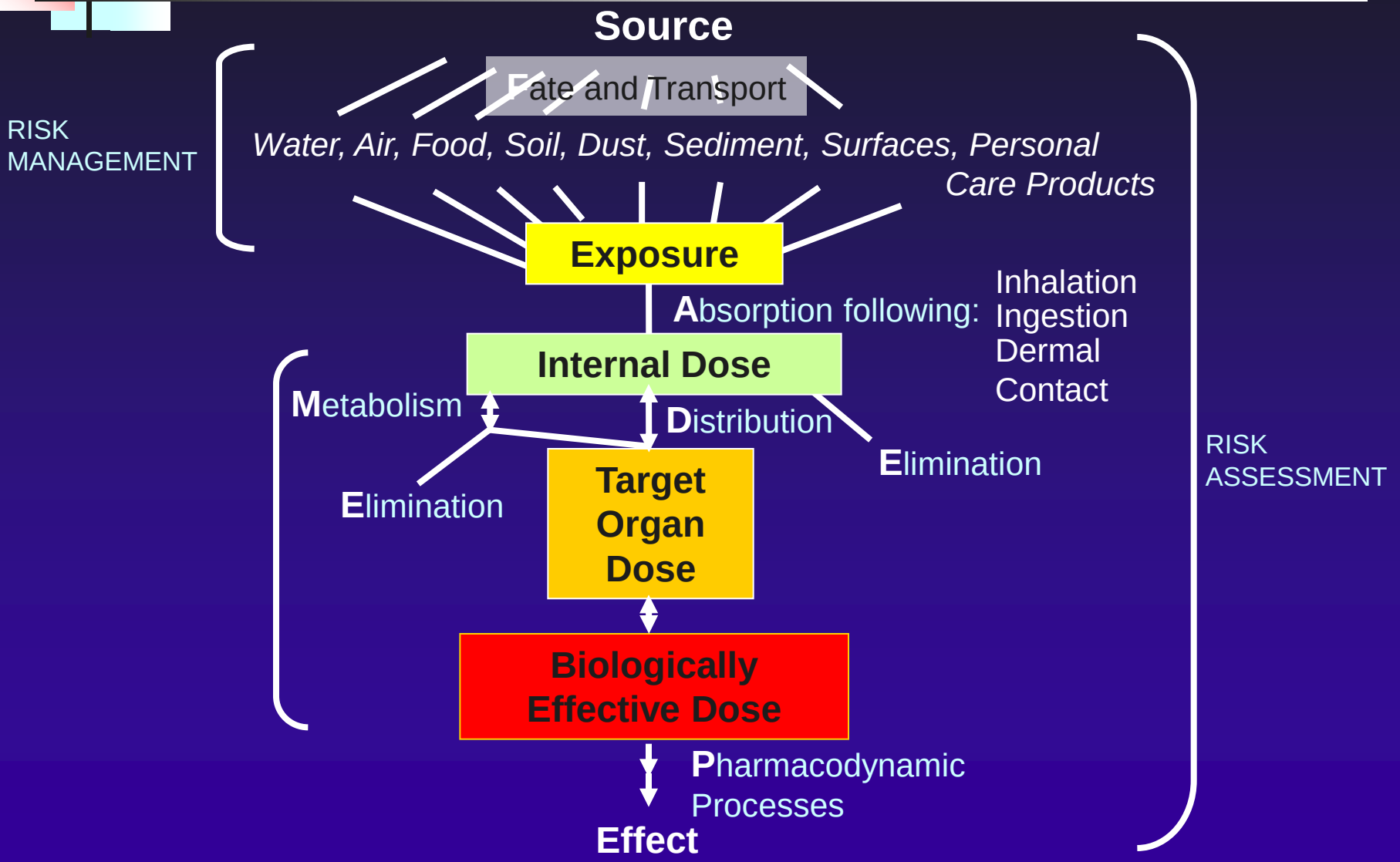
METHODOLOGY

- Chemical and biochemical analysis
- Biofluid MRS
- RT-PCR/Taq Man
- In situ hybridization
- MRI/MRS of tissue
- Microarrays
- 2-D gel electrophoresis
- Flow cytometry

Biomarkers of exposure

- The concentration of the substance of interest
- The concentration of a product of its biotransformation
- A biological (non-critical or incidental) effect of exposure, for example through interaction with a non-target molecule or cell
- A biomarker of exposure indicates that exposure has occurred, but does not provide knowledge of an adverse effect at the level of the organism

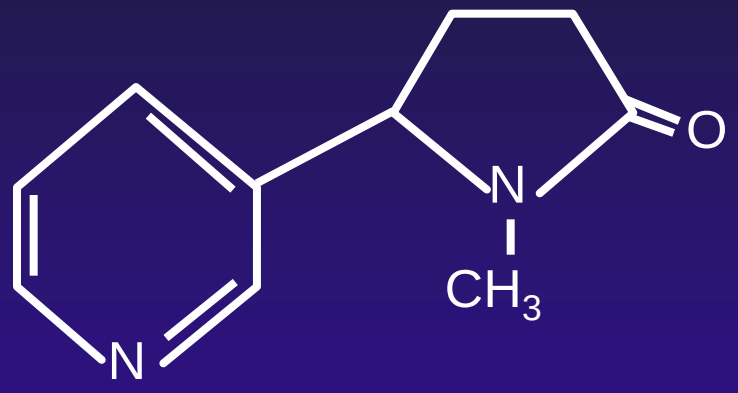
Exposure-effect continuum for environmental chemicals



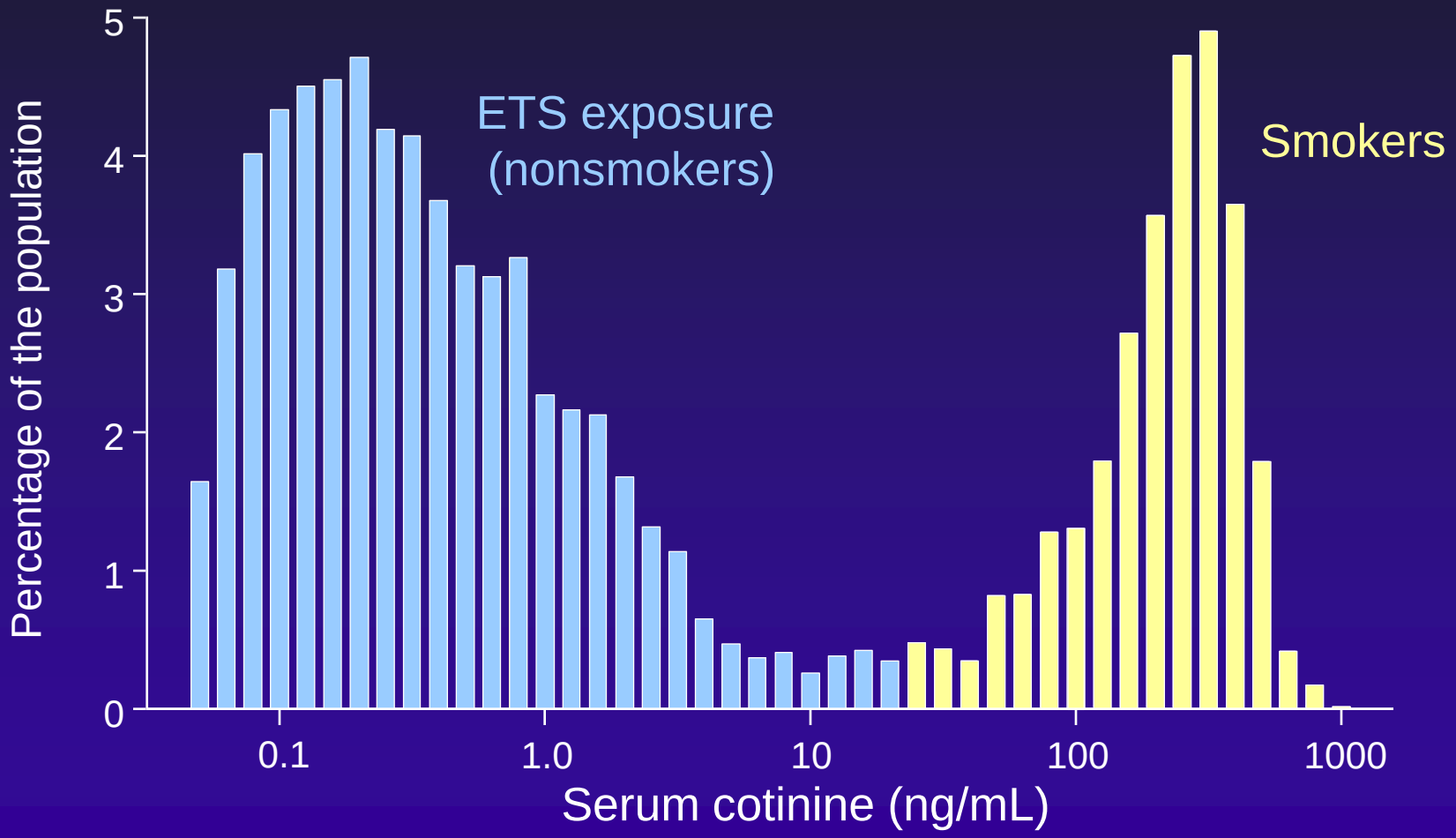
Angerer et al. Tox Sci **93**: 3-10 (2006)

Cotinine as a biomarker of exposure to cigarette smoke

- Cotinine is a metabolite of nicotine that tracks exposure to tobacco smoke
- In nonsmokers, tracks exposure to secondhand smoke



Exposure of the US population to tobacco smoke: Serum cotinine levels



NHANES III, 1988-1991

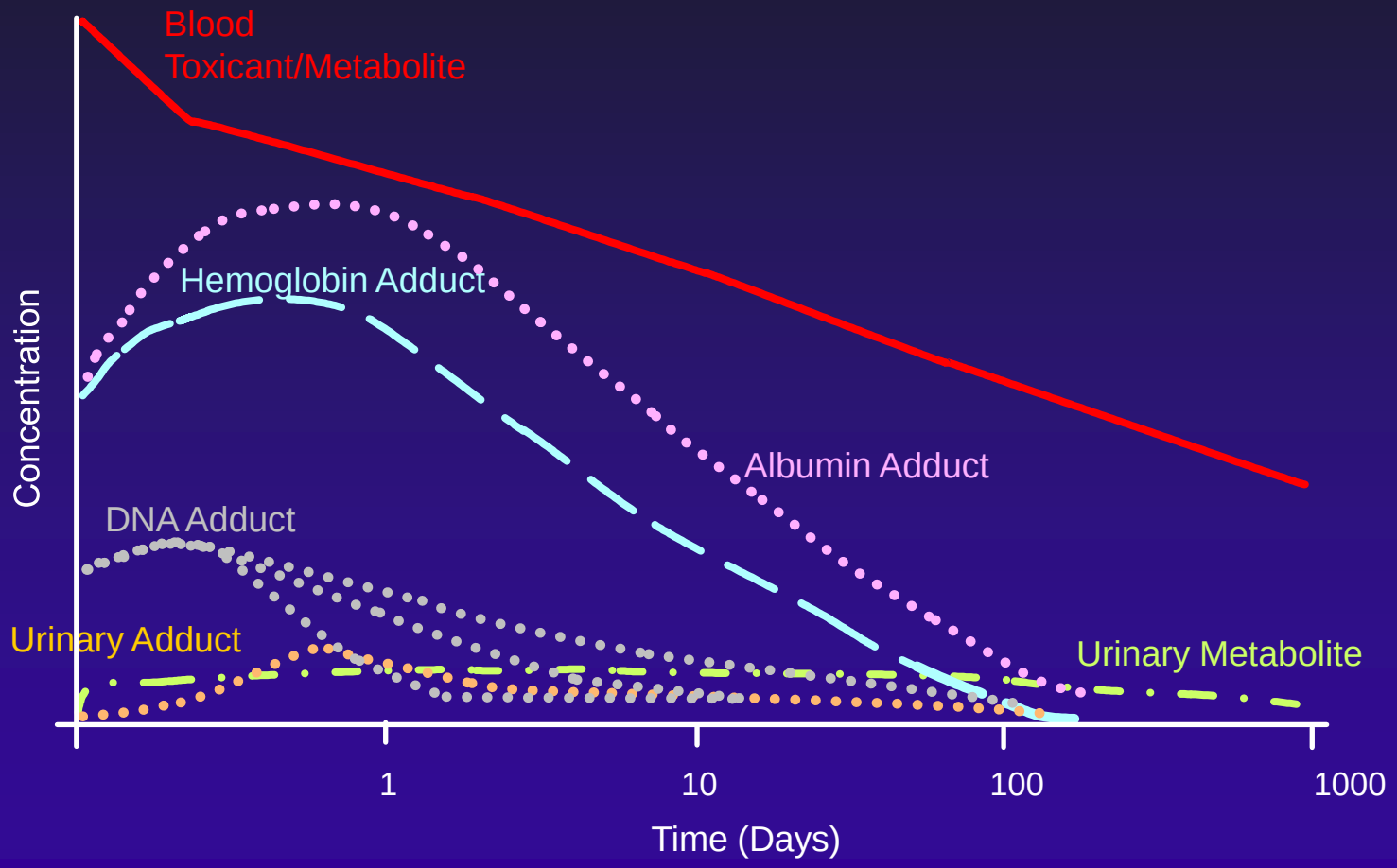
Biomarkers of exposure

Compound	Biomarker	Source
Styrene	Mandelic acid, Phenylglyoxylic acid	Urine
Benzene	Benzene Benzene metabolites	Blood, urine, breath Urine
Chloroform	Chloroform	Breath, blood
Dioxins	Dioxins	Blood Fat
PAHs	1-Hydroxypyrene	Urine
Heterocyclic aromatic amines	Parent compound and metabolites	Urine

Biomarkers of exposure

Compound	Biomarker	Source
Arsenic	Arsenic	Urine
Lead	Lead	Blood, bone
Nicotine	Cotinine	Urine
DDT	DDE	Serum
Nitrosating agents	N-nitroso-amino acids	urine

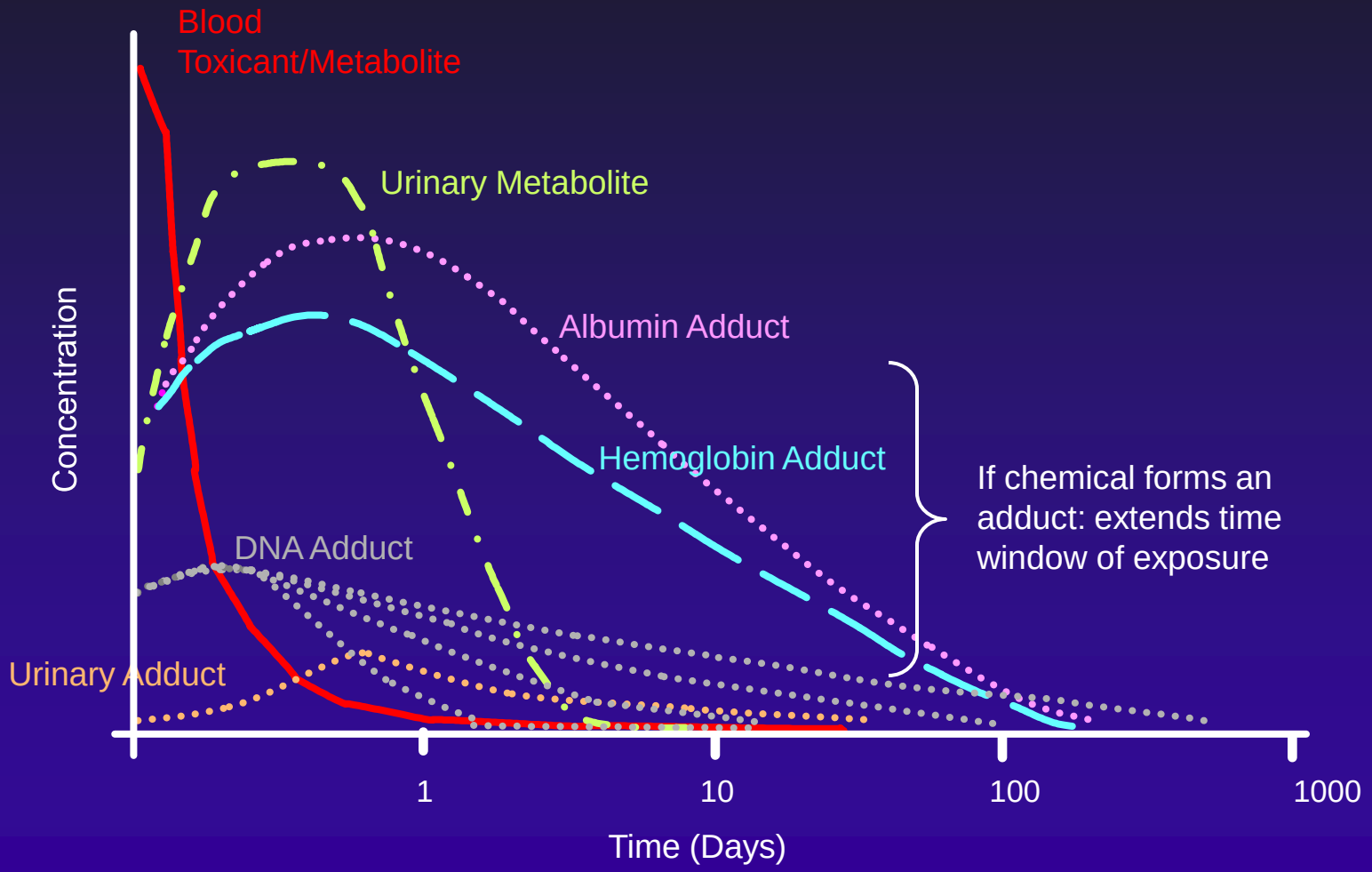
Post-exposure fate of a persistent chemical in blood and urine



Needham and Sexton. JEAEE **10**: 611-629 (2000)

Adapted from: Henderson et al. Crit Rev Toxicol **20**: 65-82 (1989)

Post-exposure fate of a non-persistent chemical in blood and urine



Needham and Sexton. JEAEE **10**: 611-629 (2000)

Adapted from: Henderson et al. Crit Rev Toxicol **20**: 65-82 (1989)

Potential value of biomarkers in exposure assessment

- Confirmation of more conventional approaches to exposure assessment
  - Corroborate estimates of exposure
  - Reduce misclassification of exposure
- Combined exposures to similar compounds
- Estimate of internal or target dose
  - Development and validation of PBPK models
- Bridge between studies in experimental animals and observations in humans
- Interindiviudal variation in exposure
  - Susceptible sub-populations

Potential limitations of biomarkers in exposure assessment

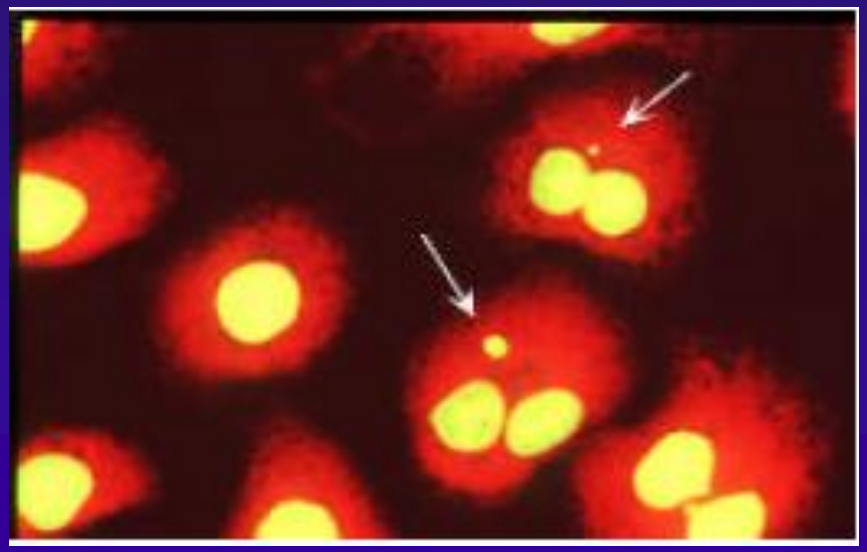
- Often restricted to recent exposures
- Intraindividual variation over time
- Relationship between biomarker and dose often unknown (importance of PK)
- Reduced study size relative to other exposure assessments
  - Need for individual sampling
  - Invasiveness of measure
  - Analytical issues
- Increased potential for confounding

Biomarkers of effect

- A measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease
- Biomarkers of effect are often a necessary, though usually not sufficient step in the pathological process towards disease

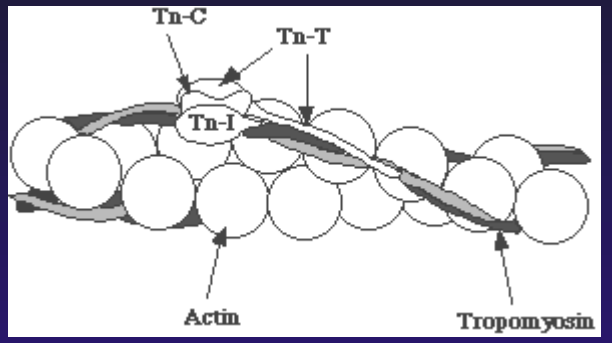
Example of a biomarker of genotoxicity

- Micronuclei (± centromere) formation in lymphocytes isolated from peripheral blood can be used for assessment of genotoxicity potential (cells are cultured for 3 days with cytochalasin B block of cytokinesis)

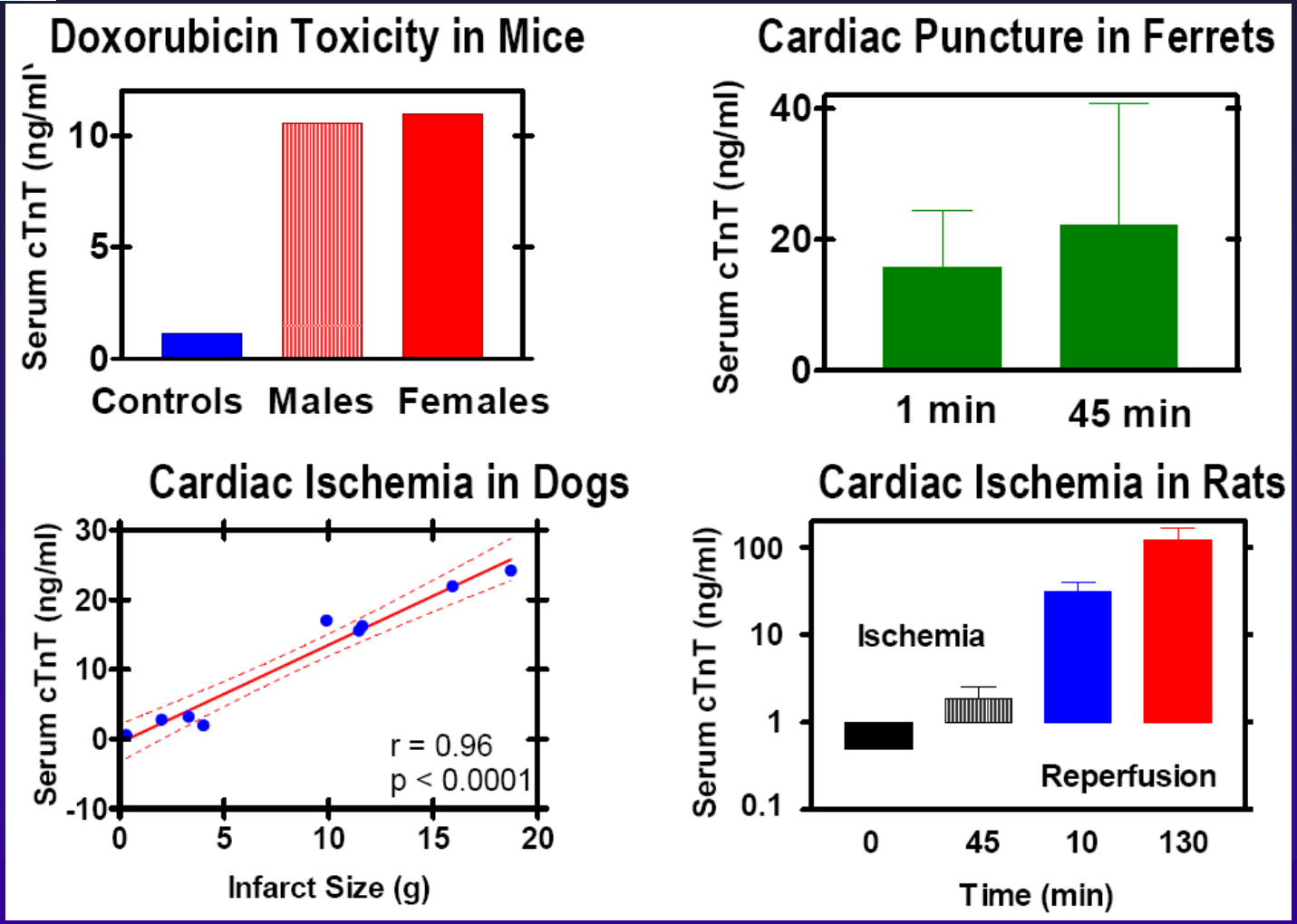


Cardiac troponin

- Gold standard biomarker of myocardial injury in man
- Myofibrillar protein regulating contraction that leeks from injured cardiac cells
- 3 subunits: C binds Ca^2, I inhibits actin and myosin interaction at low [Ca^2+], T binds tropomyosin

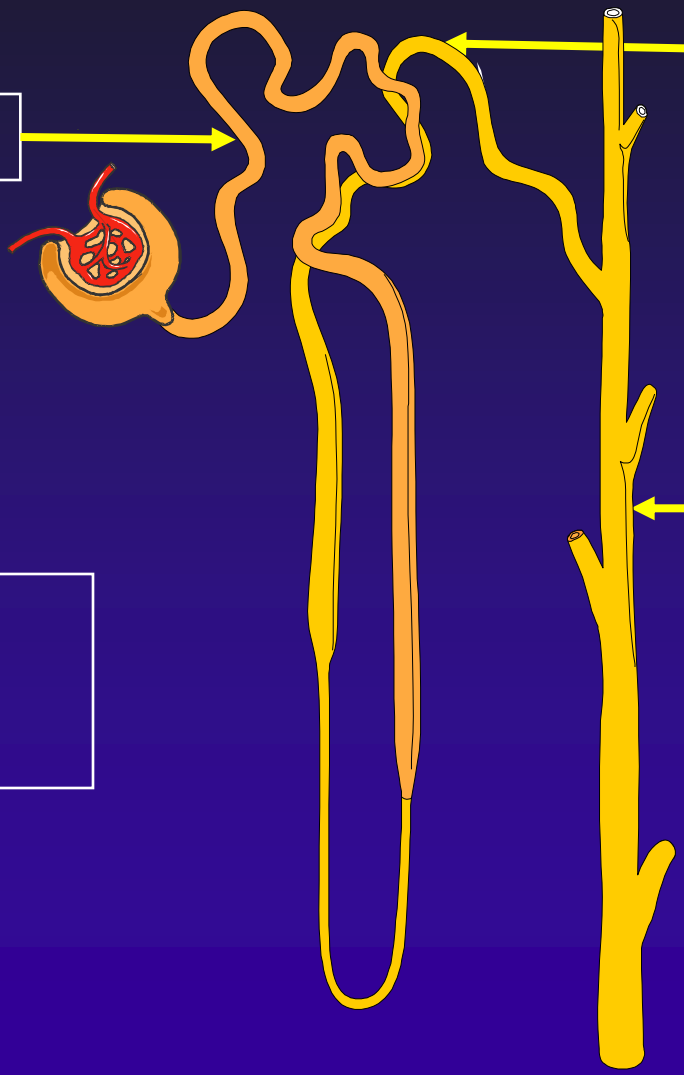


Use of cTnT in experimental animals



O'Brien et al, 1997

Proposed biomarkers of renal toxicity (HESI)



Proximal Tubule

α-GST

Distal Tubule

μ-GST

Collecting Duct

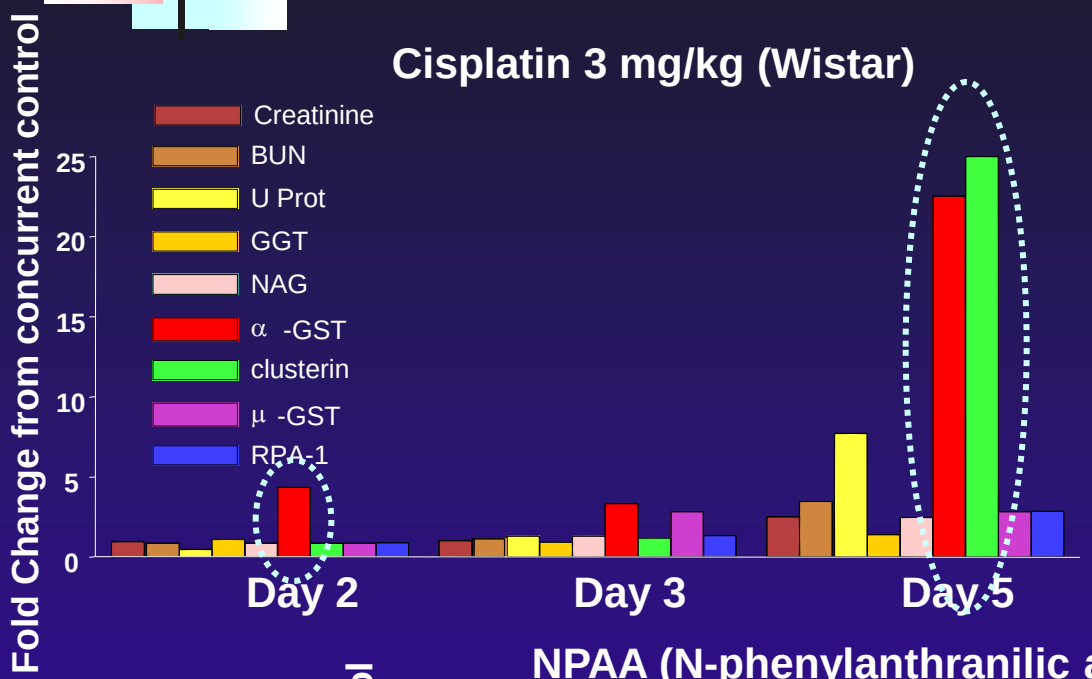
RPA-1

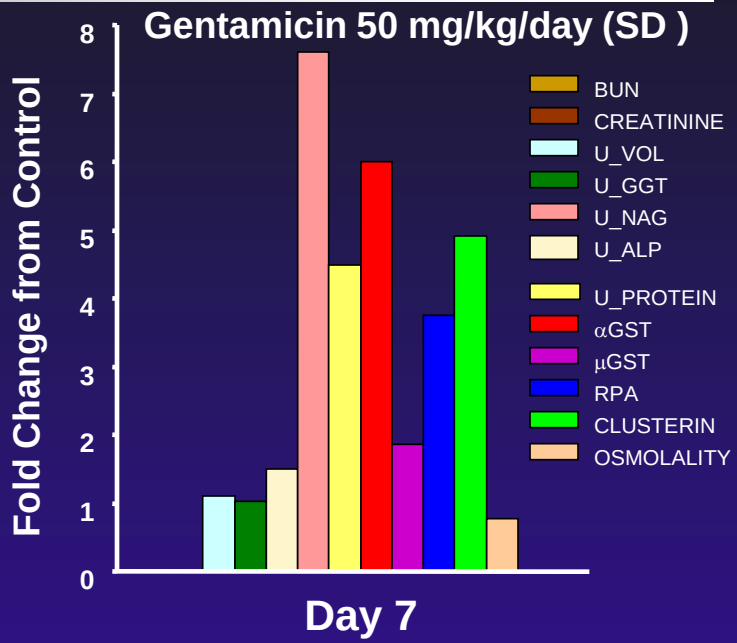
(renal papillary antigen-1)

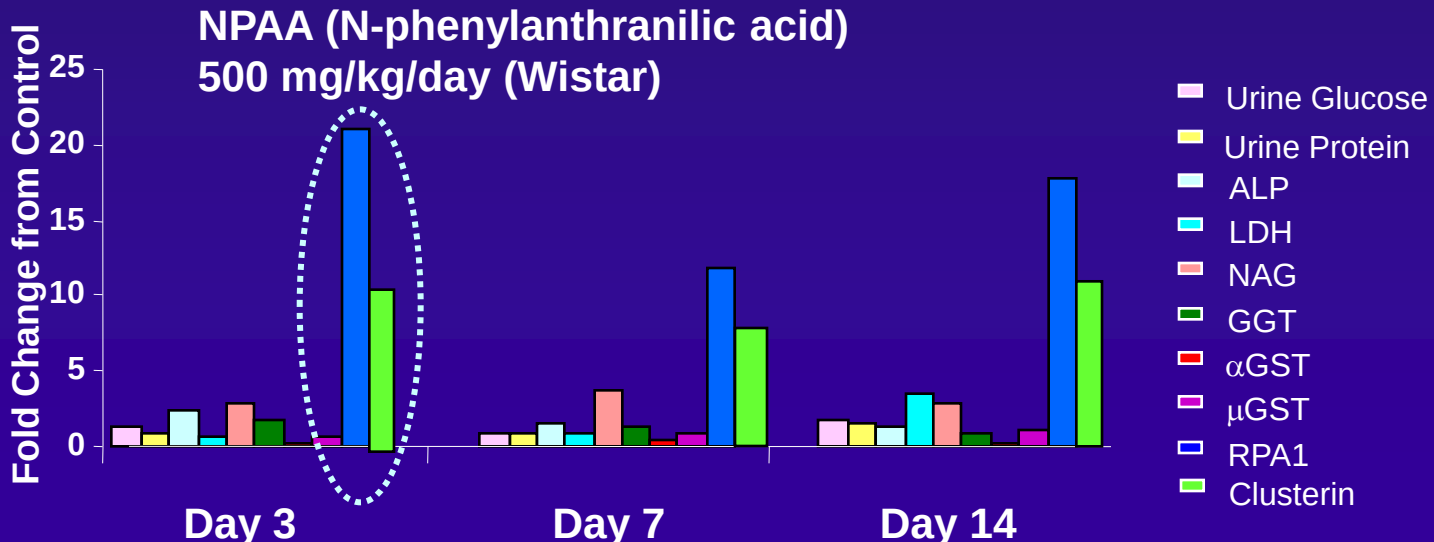
No specific nephronal location

Clusterin

Relative performance of biomarkers for renal toxicity







HESI

Potential value of biomarkers of effect

- Understanding mode or mechanism of action and its human relevance
- Definition of nature and shape of dose-response relationship
- Extrapolation from experimental doses to levels of human exposure and possible dose transitions
- Mechanistic basis of “thresholds” or points of departure in dose-response curve

Potential limitations of biomarkers of effect

- Uncertainty in qualitative and quantitative relationship between biomarker and toxic response
  - Specificity of response
- Temporal relationship between exposure of relevance and measurement of biomarker of effect
- Possible confounding
- Inter-species extrapolation

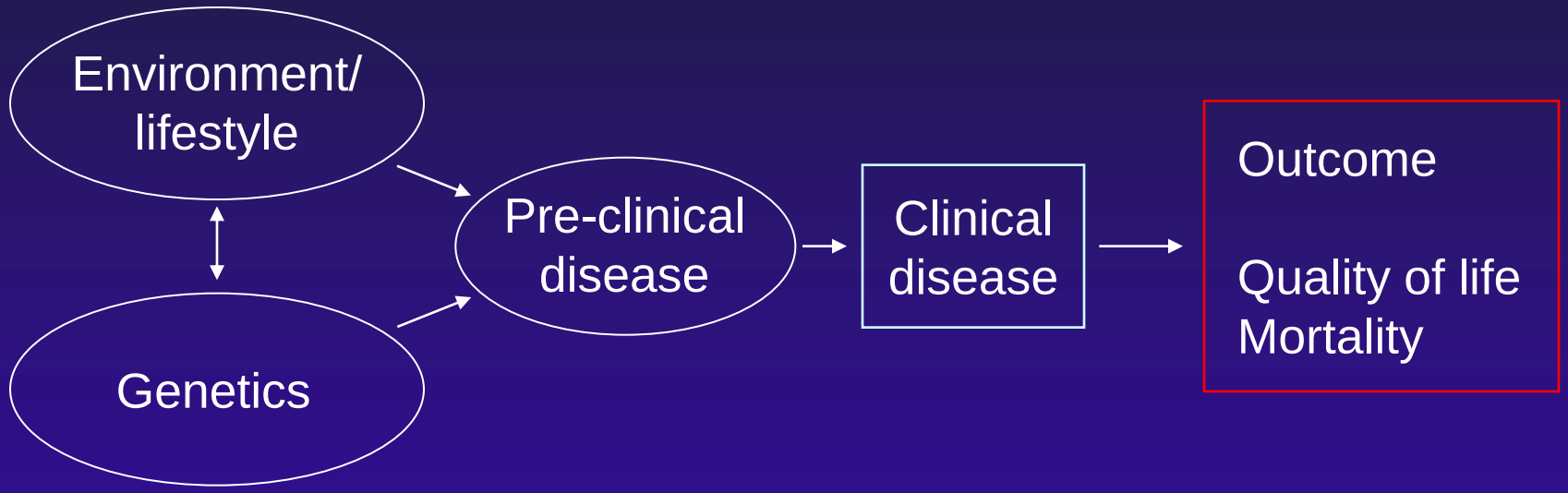
Biomarkers of susceptibility

- Biomarkers of susceptibility should reflect individual susceptible to disease
- Such biomarkers are usually genetically determined but may also be acquired
- Biomarkers of susceptibility are independent of environmental exposure
- Biomarkers of susceptibility may affect either internal dose or toxicant response

Genetic epidemiology

- Gene-environment interactions
  - Where environment represents a chemical exposure
- Interaction (effect modification) occurs when the estimate of effect of exposure (environment) depends on the level of another factor (gene) within the population
- Interaction is distinct from confounding (or selection or information bias), but rather a real difference in the effect of exposure in various subgroups that may be of considerable interest

Gene-environment interaction



Gene-environment interactions

- Some genetic factors and environmental exposures can cause disease independently
  - Genetic defect (BRCA 1,2) causes breast cancer
  - Exposure to oestradiol causes breast cancer
- Some responses occur only with combination of certain genetic factors and environmental exposures
  - Asthma? Autoimmune diseases?
- Some genetic factors modulate extent of internal exposure
  - GST polymorphisms regulate activation/inactivation of some solvents
- Some genetic factors modulate response to environmental exposures
  - hERG polymorphisms determine QTc prolongation by some drugs
- Environmental exposures can modulate genetic disease
  - Lead exposure exacerbates severity of acute intermittent porphyria

Examples of polymorphisms affecting susceptibility

- Variants of several P450 genes associated with increased lung cancer risk in smokers
  - e.g. *CYP1A1* - 10% of Caucasians have one such variant
  - Another variant present only in African-Americans
- Deletion of one of glutathione S-transferase genes (*GSTM1*) associated with increased risk of bladder and lung cancer from exposure to several toxic substances (e.g., PAHs, aflatoxin)
  - 50% of Caucasians carry deletion

Example: Cancer (SMRs)

Cancer	Japan	Not U.S. Born	U.S. Born	U.S. Cauc
Stomach (M)	100	72	38	17
Intestine (F)	100	218	209	483
Breast (F)	100	166	136	591

(MacMahon B, Pugh TF. Epidemiology: Principles and Methods . Boston: Little, Brown and Co, 1970:178.)

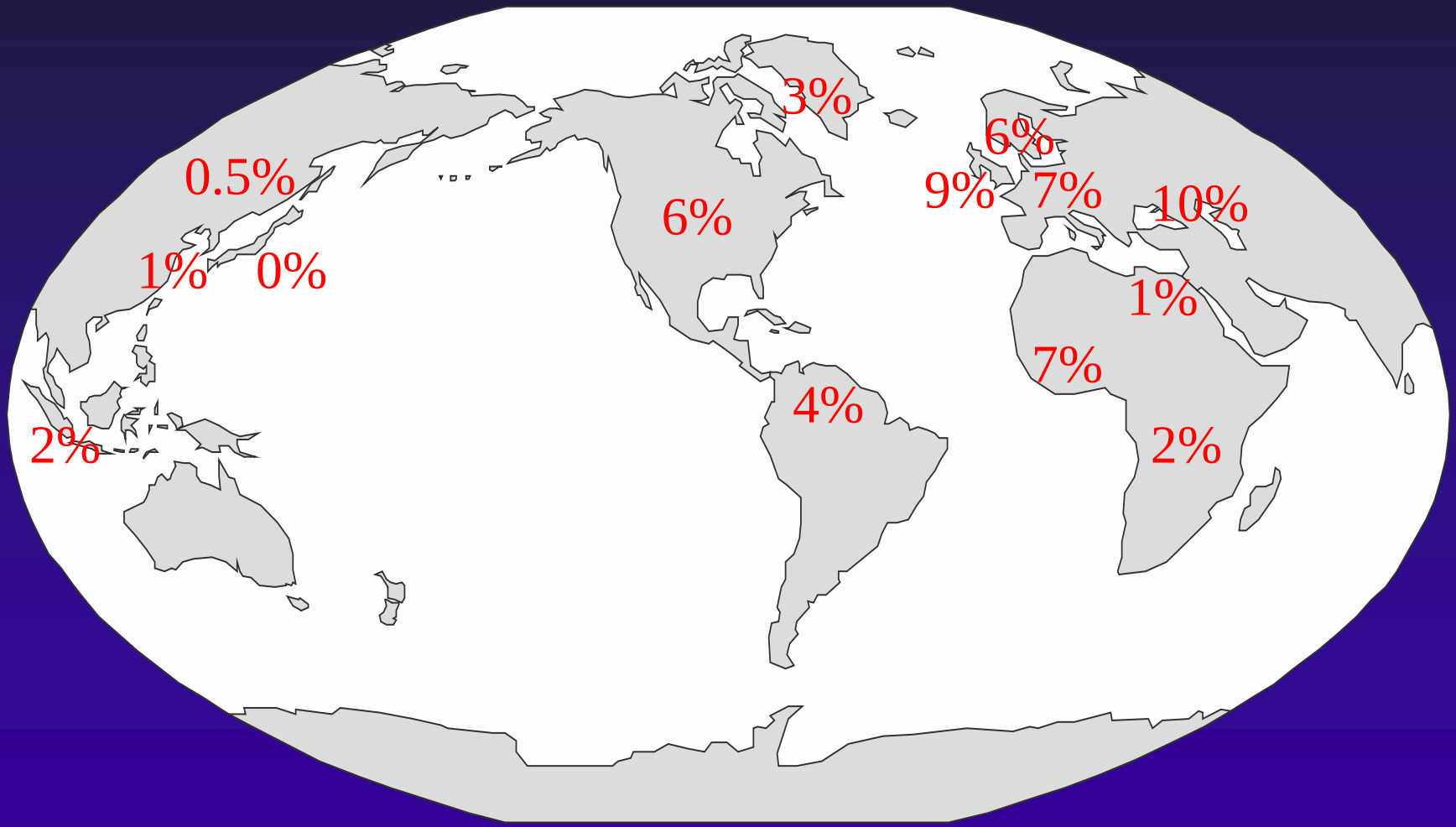
Genetic polymorphisms: environmental susceptibility

- Many genetic polymorphisms identified affect response to xenobiotics
  - Altered kinetics or dynamics
  - e.g. genes affecting metabolism, detoxication, DNA repair, receptors, cell cycle control, etc.
  - Many genes increase risk from exposure, but some have protective effect
- Environmental Genome Project has identified ~ 600 genes with alleles potentially exhibiting differential responses to environmental exposures

Genetic polymorphisms of some P450 enzymes

Enzyme	Major mutation	Effect	% of PM
CYP1B1	$Val_{432} \rightarrow Leu$	Defective enzyme	~10%
CYP2A6	$Leu_{160} \rightarrow His$	Defective enzyme	<1%
CYP2C9	$Arg_{144} \rightarrow Cys$	Altered specificity	~5%
CYP2C19	Cryptic splice site in exon 5	No enzyme	3%
CYP2D6	Mutation at intron 4/ exon 5 $\rightarrow$ stop codon	No enzyme	8%
CYP2B6	Several	Reduced enzyme	~75%
CYP3A5	Mutation in intron 3 $\rightarrow$ stop codon	Reduced enzyme	~70%

Distribution of PMs for CYP2D6



Case Study: N-acetyltransferases (NAT)

- Two genes, NAT1 and NAT2
- Catalytic transfer of acetyl group from donor (Acetyl-Co-A) to substrate (amine)
- Both are polymorphic, with multiple different allelic variants
- Phenotype associated with ‘slow’ acetylation

Gene-environment interactions

Polymorphism (NAT2)

Slow acetylation	Fast acetylation
Bladder cancer relative risk associated with smoking = **8**	Bladder cancer relative risk associated with smoking = **1.5**

Interaction - effect of smoking is different in subjects with a specific polymorphic gene

- Better understanding of the effects of established toxicants
- Uncover low levels of risk previously masked by genetic heterogeneity

NB: Data are for illustrative purposes only

Modulation of DNA adducts in smokers by genetic polymorphisms

DNA adducts in relation to Genotype			
	- / slow	***+ / fast***	***p***
GSTM1	1.30	1.03	0.05
GSTT1	1.28	1.12	0.41
NAT1	1.58	1.11	0.05
NAT2	1.29	1.03	0.06

Combinations of 3 genotypes

$GSTM1^{-}$, $NAT1^{slow}$, $NAT2^{slow}$	2.03
$GSTM1^{+}$, $NAT1^{fast}$, $NAT2^{fast}$	0.91

Godschalk *et al* (2001)

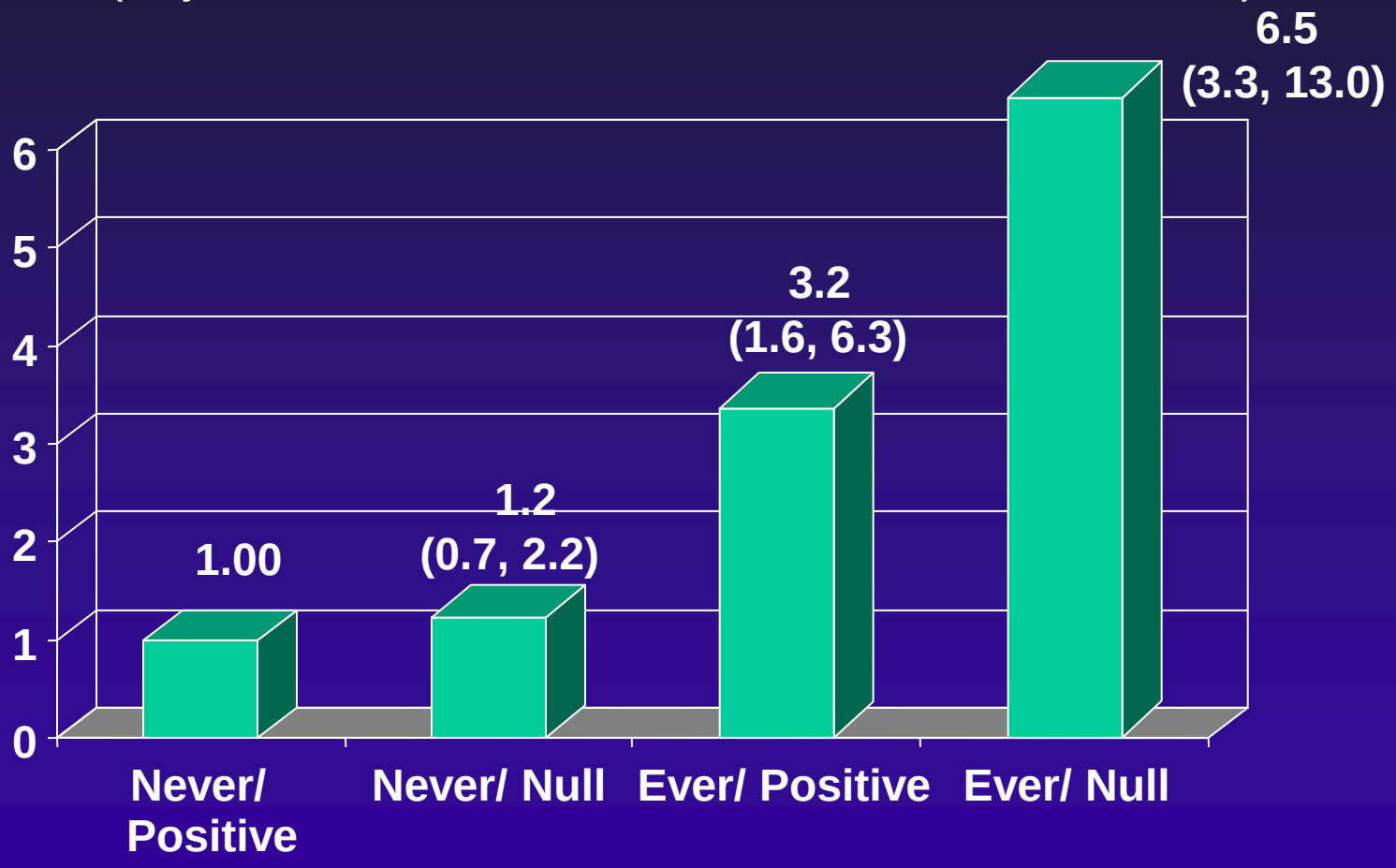
Variability and toxic response: GST and tobacco smoke

- Glutathione S-transferase (GST)
  - Metabolic enzyme
  - Participates in metabolic detoxication of benzo(a)pyrene
  - GST null donors are more sensitive to the induction of chromosomal aberrations due to tobacco smoke
  - GST null also has been linked to increased risk of lung and bladder cancer in smokers
  - Knowledge of genotype may impact behaviour
  - Biomarker of susceptibility

Norppa (2003). *Mut Res*

Interactions between smoking and GSTM1

(Adjusted odds ratios and 95% confidence intervals)



Stucker *et al.* (1999), *Int Epidmiol Assoc*

Gene-environment interaction and lung cancer risk

Adduct level	GSTM1	OR
Low	present	1.0
Low	null	2.3
High	present	6.9
High	null	16.2

Tang et al., Carcinogenesis 1998

Carcinogen-DNA adducts and cancer risk

Study design:	Type of cancer	Risk	Reference
Cohort (smokers only)	Lung	1.22	H. Bak, poster Porvoo
Cohort (current smokers)	Lung	2.98	Perera et al, 2002
Case-control	Bladder	1.9	Benhamou et al, 2003
Case-control	Breast (PAH)	1.97	Rundle et al, 2002
Case-control	Breast (PhIP)	4.03	Zhu et al, 2003

Conclusion: Carcinogen-DNA adducts appear to be a risk indicator for cancer, especially in smokers

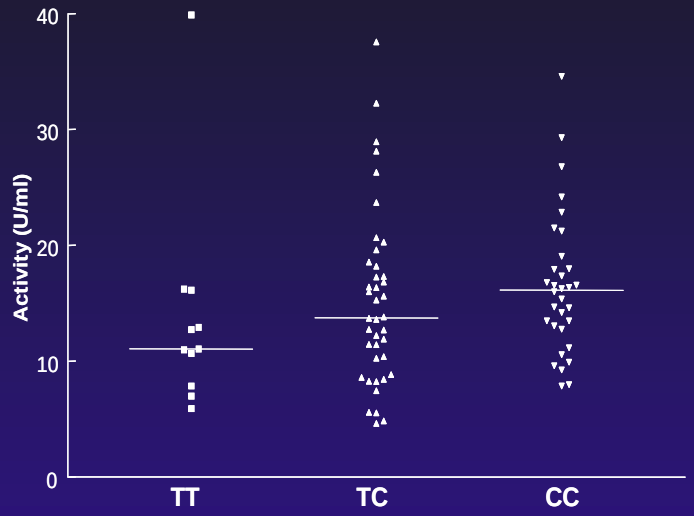
Functional and non-functional polymorphisms

- Functional polymorphism - a change in the DNA sequence results in a change in the expression or function of the protein, e.g. introduction of a premature termination codon
- Non-functional polymorphism - a change in the DNA sequence that has no effect on the expression or activity of the protein, e.g. change in DNA sequence within an intron

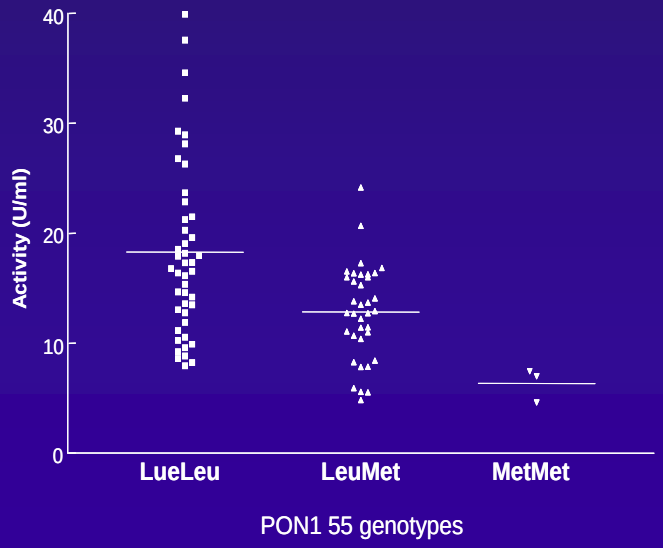
Importance of understanding functional consequence of a polymorphism (PON1)

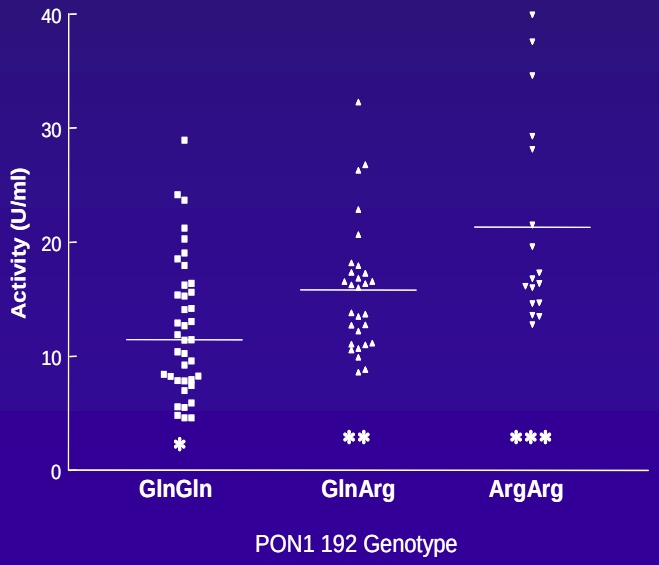
- Cherry *et al* (2002). *Lancet* **359**, 763-764.
  - Cases were more likely than referents to have at least one R allele at position 192 (Gln to Arg substitution); odds ratio 1.93 (95% CI 1.24-3.01)
  - More likely to to have diazoxonase activity below normal median (1.77, 1.18-2.67)
  - "Our results support the hypothesis that organophosphates contribute to the reported ill health of people who dip sheep"
- Mackness *et al* (2003). *Pharmacogenetics* **13**, 81-88.
  - Farmers reporting chronic ill health due to organophosphate exposure have a higher proportion of the PON1-192R polymorphism
  - This was associated with lower rates of diazoxon hydrolysis than the controls
  - "…their ill health may be explained by a lower ability to detoxify diazoxon"

Interindividual variability in PON1 activity towards diazoxon



QQ QR RR





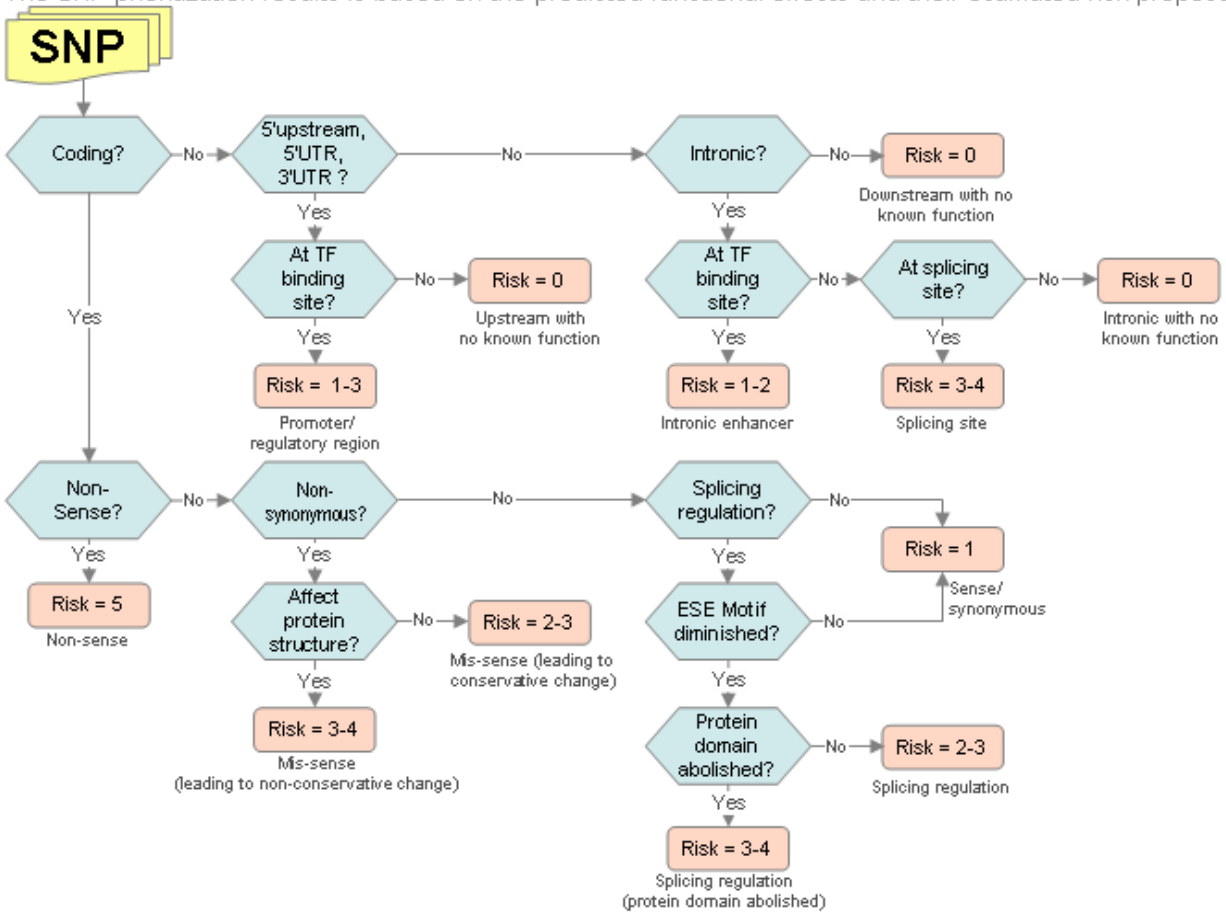
SNP selection for association studies

Regulatory / Functional SNPs -

Decision Tree

The SNP prioritization results is based on the predicted functional effects and their estimated risk proposed by Tabor *et al.* in Nat Rev Genet.

Yuan (2006)



Ranking	Risk Definition
0	No effect
1	Very low
2	Low
3	Medium
4	High
5	Very High

Risk Ranking: ?

FastSNP

Sample size requirement for gene-environment interaction studies

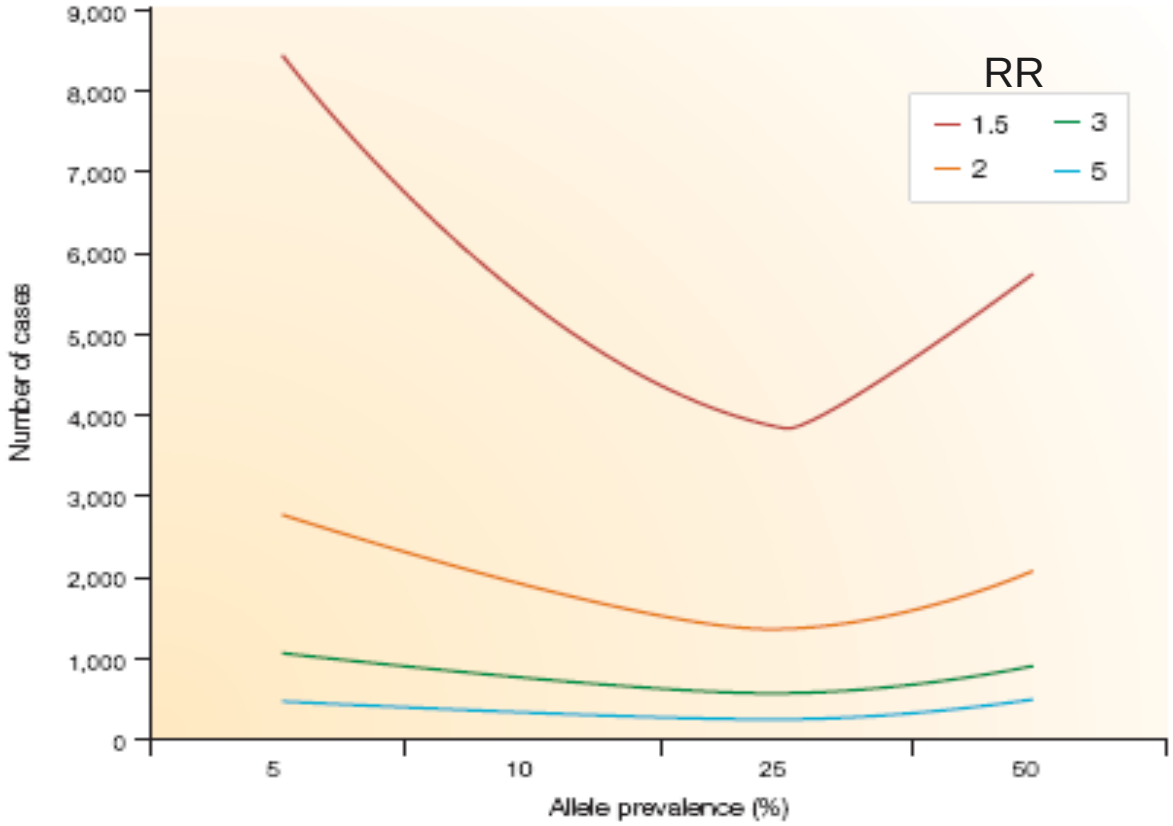


Figure 2 | **Number of cases needed to detect a range of multiplicative interactions, according to allele prevalence.** The model assumes the following: a dominant genetic model, a dichotomous exposure prevalence of 10%, a relative risk for a genotype of 1.5, a relative risk for exposure of 1.5 and a 1:1 case:control ratio. As the graph shows, thousands of cases and controls are needed to detect interactions with relative risks of 1.5 and 2. Calculations were carried out using Quanto Beta version 0.5 (REF. 13).

Hunter (2005)

Breast cancer case-only analysis of the effect of tobacco smoke exposure

Genetic Polymorphism		Never (N)	Passive (N)	Former (N)	Current (N)
CYP 1B1	Any Val	41	52	44	55
	Leu/Leu	21	39	17	13
	ORi (95%CI)	1	0.69 (0.35-1.37)	1.33 (0.59-2.96)	2.32 (1.00-5.38)
SULT1A1	Arg/Arg	36	51	38	25
	Any His	26	40	23	43
	ORi (95%CI)	1	1.08 (0.55-2.11)	0.79 (0.37-1.68)	2.55 (1.21-5.36)
COMT	VaL/Val	22	26	19	18
	Any Met	40	65	42	50
	ORi (95%CI)	1	1.26 (0.62-2.57)	1.07 (0.49-2.35)	1.42 (0.65-3.13)

(Nov, 2003)

Development of novel biomarkers

- Unique opportunities exist for the development of new biomarkers on the basis of genomics, proteomics, metabonomics, etc
- Ideally they should be mechanistically-related
- Initial development in animal models, and using a combination of *in vitro* and *in vivo* approaches
- Critical to the application of biomarkers is their validation

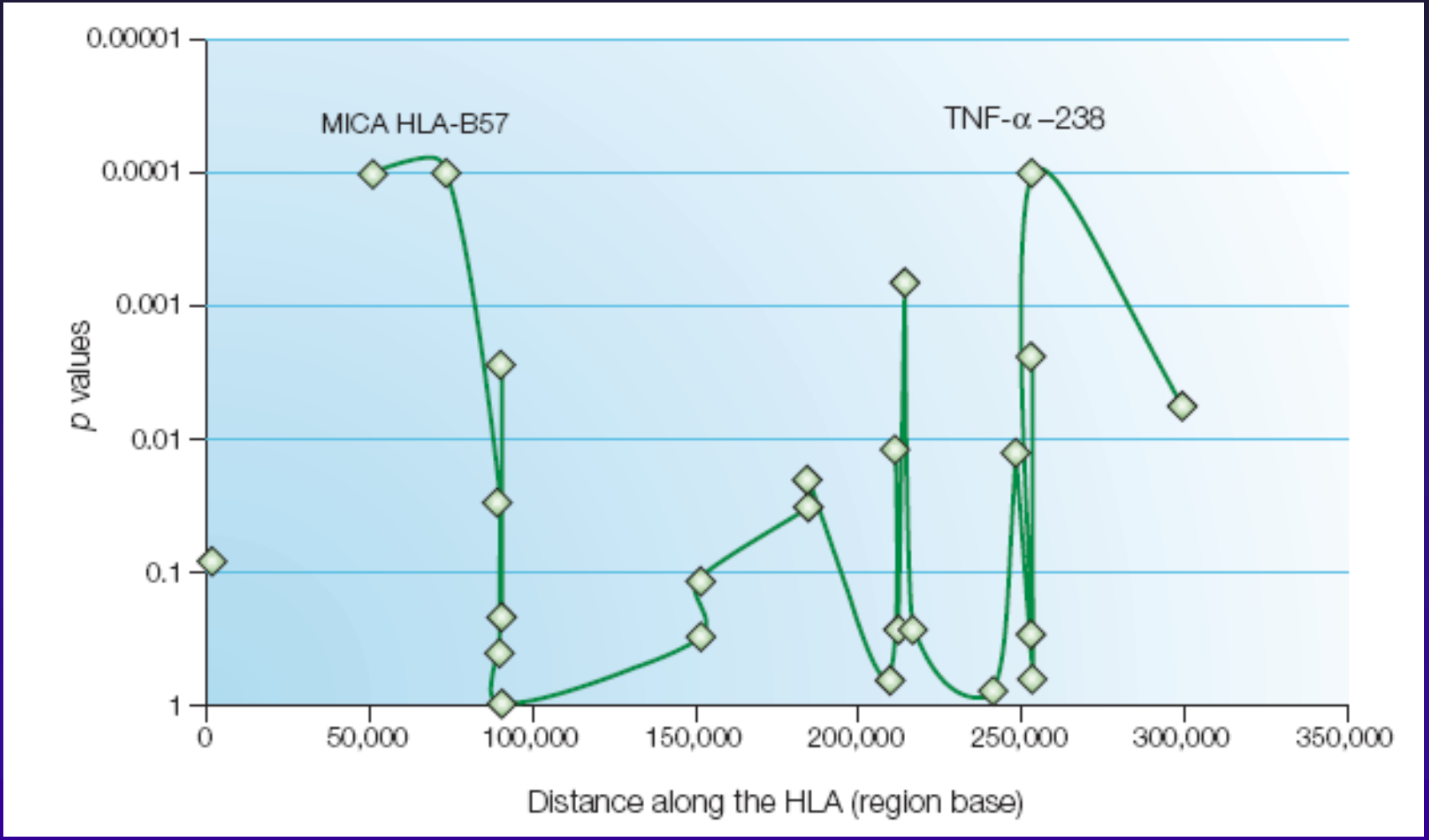
Expression platforms

- Transcriptomics
  - Microarrays
- Proteomics
  - Chromatography, 2D gel electrophoresis, mass spectrometry
  - Protein arrays, yeast 2-hybrid screen
- Metabonomics
  - Chromatography, nuclear magnetic resonance (NMR), mass spectrometry

-Omics in the search for novel biomarkers

- “Top down”, rather than “bottom up”, approach
- Possible identification of completely novel biomarkers
- Biomarkers comprising “suites” of analytes?
- Knowledge of the human genome informs choice and development of biomarkers
- Validation is a major issue
  - Relevance, specificity, reproducibility, …..

Genetic markers in the HLA-B region associated with abacavir hypersensitivity



Roses (2002)

Biomarkers in epidemiology

- Genetic factors can contribute to disease risk from environmental chemicals
- Identified effects to date have generally been relatively modest
- Need for improved exposure assessment
- Statistical study design issues when exploring multiple polymorphisms
- Potential application of biomarkers of exposure and of effect
- “New generation” of biomarkers under development – how should they be validated

Further information

- Smolders R et al (2009). *J. Toxicol. Environ. Health, Part B*,**12**:107-123
- Wagner JA (2008). *Annu. Rev. Pharmacol. Toxicol.* **48**:631-651
- Altar CA et al (2008). *Clin. Pharmac. Ther.* **83**:368-471