

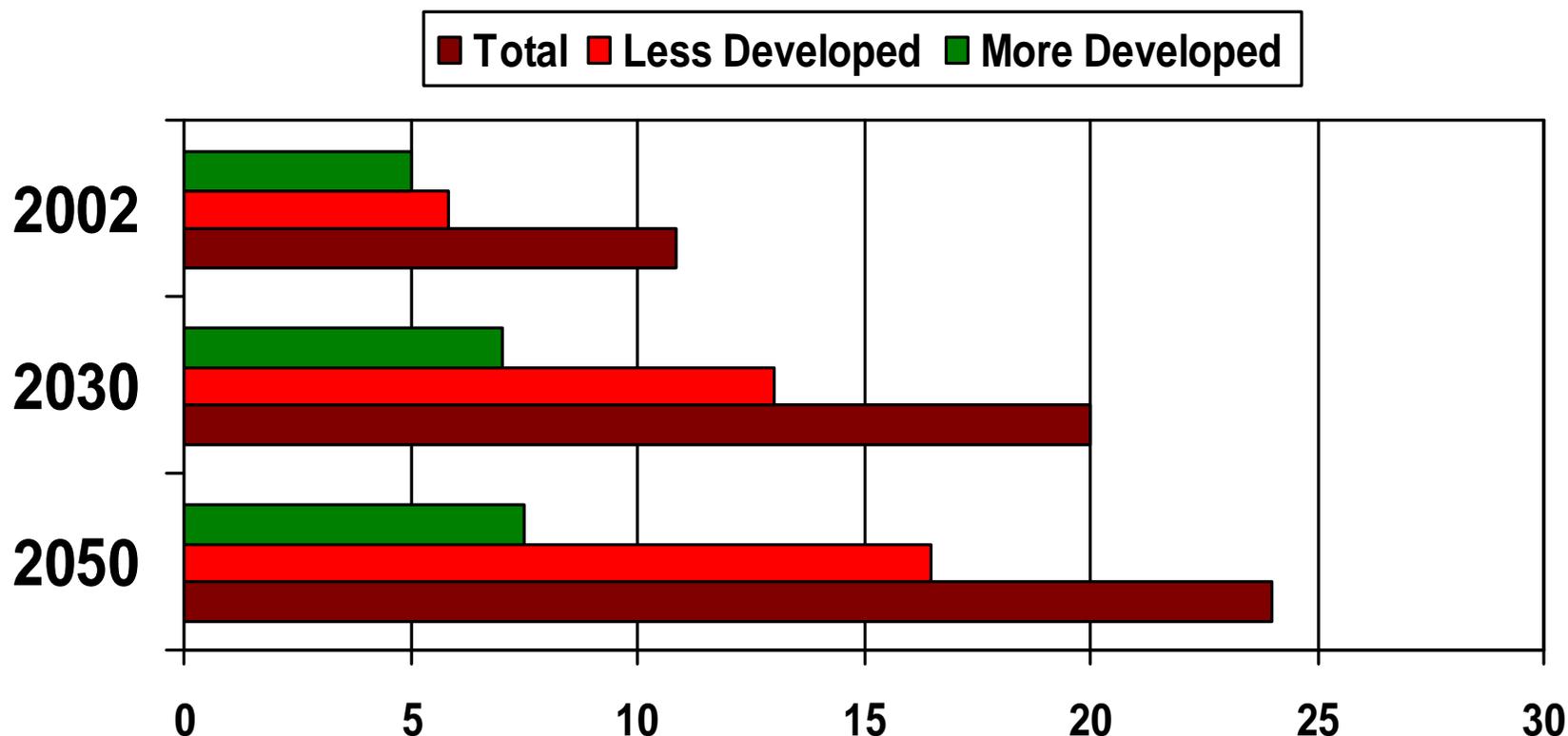


Complementing the genome with its “exposome”

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The Growing Global Cancer Burden



In 2008 : 12.4 million new cases; 7.6 million deaths

Environment and cancer prevention

- One third of cancers are preventable – the most cost-effective response (Action against Cancer: European Partnership)
- But the majority (~90%) of cancers have an environmental cause, so the potential for prevention is much higher; “aetiology gap”
- Increased research on causes of cancer e.g.
 - Diet and metabolism
 - Environmental chemicals

Importance of environmental exposure assessment

- Most major common diseases have an environmental aetiology
- Currently exposure measurement is problematic in many areas, leading to misclassification
- Large prospective cohort studies (e.g. UK Biobank) are predicated on the availability of accurate exposure assessment
- Exposure biomarkers can contribute to several areas in addition to elucidating disease aetiology

Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology



- Wild CP (2005) Cancer Epidemiology, Biomarkers and Prevention, 14: 1847-1850.
- Wild CP (2009) Mutagenesis 24: 117-125.

Uca Pugnax, the male Fiddler Crab

What is the “exposome”?

- “At its most complete, the exposome encompasses life-course environmental exposures (including lifestyle factors) from the prenatal period onwards”
- A comprehensive measurement of all exposure events (exogenous and endogenous) from conception to death

Challenges in characterising the “exposome”

- **Scale and complexity:** characterisation of life-course environmental exposures, including lifestyle, nutrition, occupation etc., as well as endogenous events at different target sites within the body
- **Dynamic:** Unlike the genome, the “exposome” changes over time – possibility of critical windows of exposure e.g. in early life
- *However, even partial characterisation can bring major benefits*

Aim and Approaches

Exposure x Time » “exposome”

- Tools
 - Laboratory technology
 - Cohorts/biobanks
- Skills
 - Inter-disciplinary
 - Epidemiology; biostatistics; laboratory sciences; bioinformatics
- Co-operation
 - International co-ordination (funding and science)
 - Integration with other initiatives

Advances in exposure assessment

- Biomarkers
- Geographic information systems
- Personal and environmental monitoring
- Increasingly sophisticated questionnaires

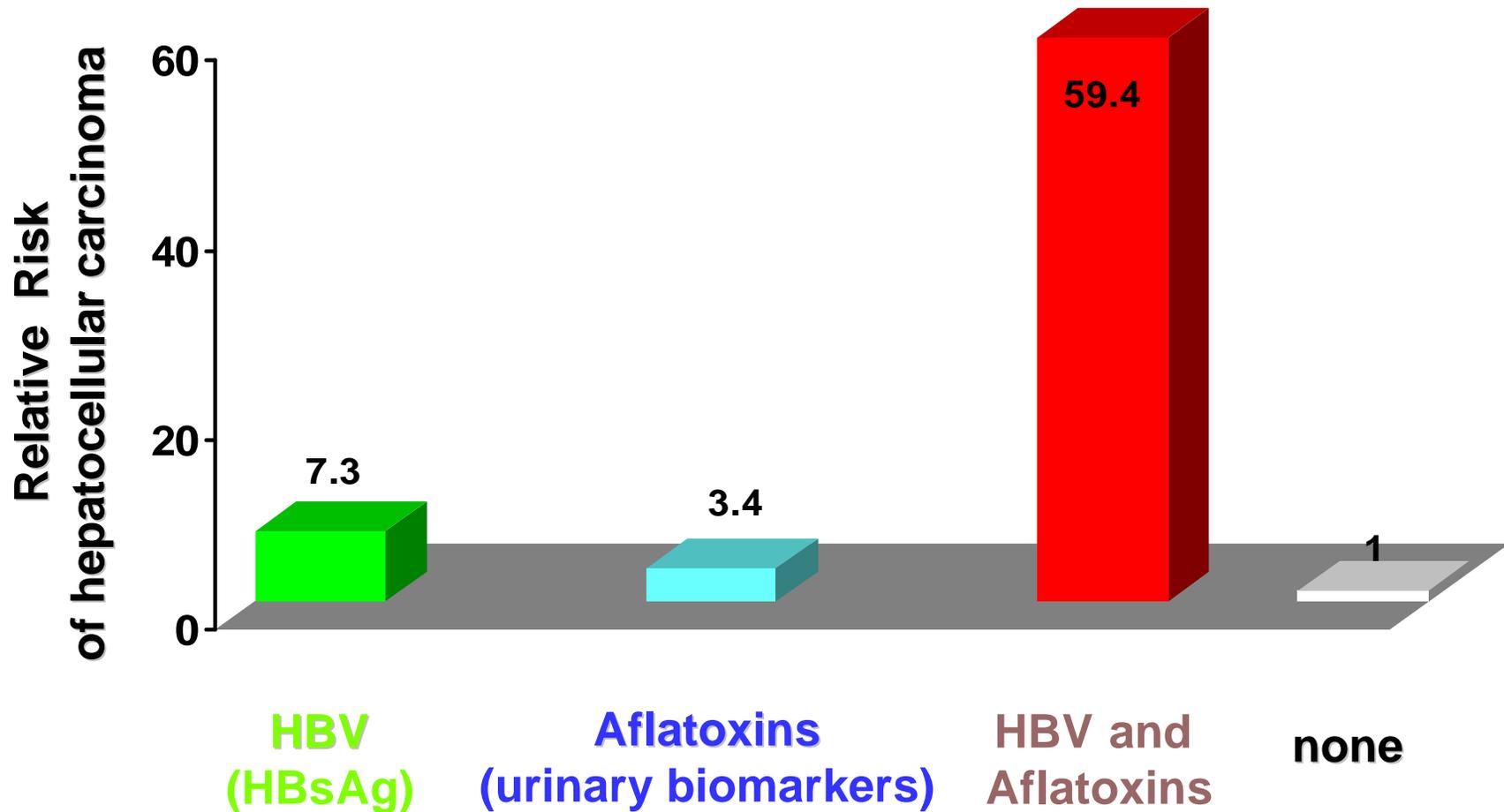
Exposure biomarkers – what do they promise?

- **Defining etiology**
 - Improved exposure assessment – reduced misclassification
 - Identifying susceptible individuals or subgroups – heterogeneous response to exposure
 - Contributing to biological plausibility

Exposure biomarkers – what do they promise?

- **Evaluating Interventions**
 - Primary and secondary prevention
 - Bio-monitoring e.g. occupational setting
- **Hazard and Risk Assessment**
 - Mechanistic data (e.g. IARC Monographs)
 - Extrapolation from animal to human (reducing uncertainties)
 - Pharmacokinetic-based models

Interaction between HBV infection and aflatoxins in hepatocellular carcinoma



International Agency for Research on Cancer

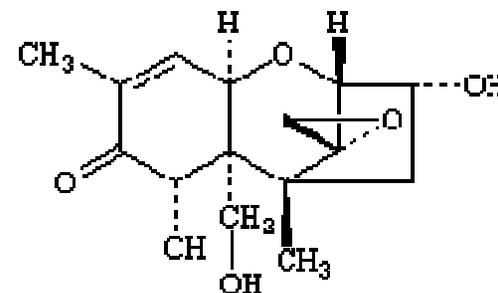


adapted from Qian et al, CEBP 1994, following Ross et al., Lancet 1992

Validation and application

- A plea for validation – difficult to find support for , but essential for progress
- An integral part of method development should be the consideration of throughput, cost and applicability to biobank samples

Deoxynivalenol ("vomitoxin")

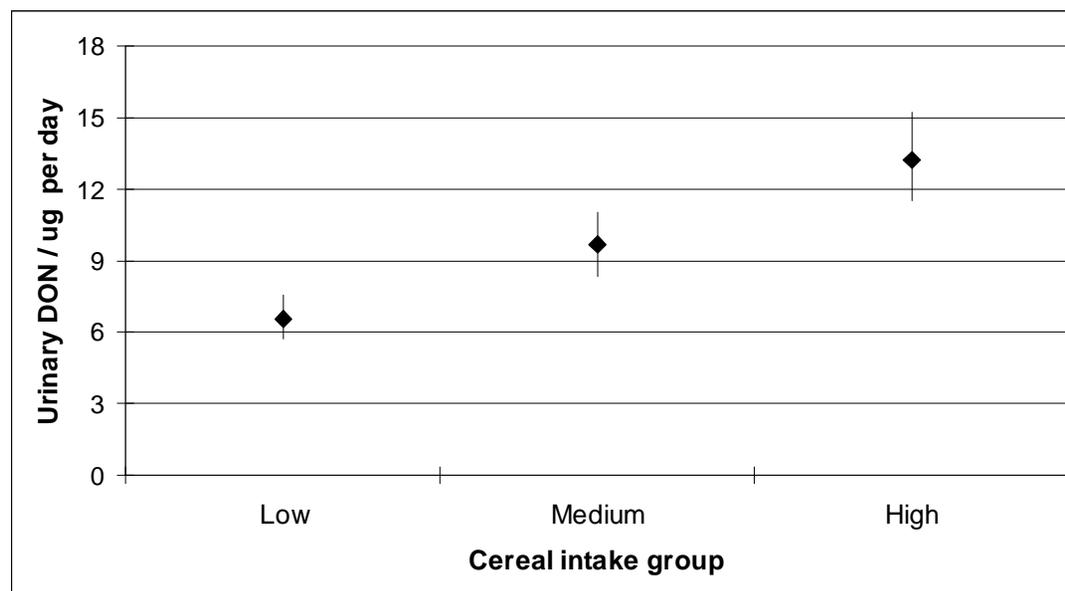


- Produced by *Fusaria spp*; common contaminant of cereals
- Vomiting, feed refusal, weight loss, immuno-modulation in animals and induces IgA nephropathy in mice
- Alters cell signalling and cytokine expression (e.g. MAPK)
- Linked to GI poisoning in China and India
- Urinary biomarker applied to the UK National Diet and Nutrition Survey samples

DON exposure in relation to cereal consumption

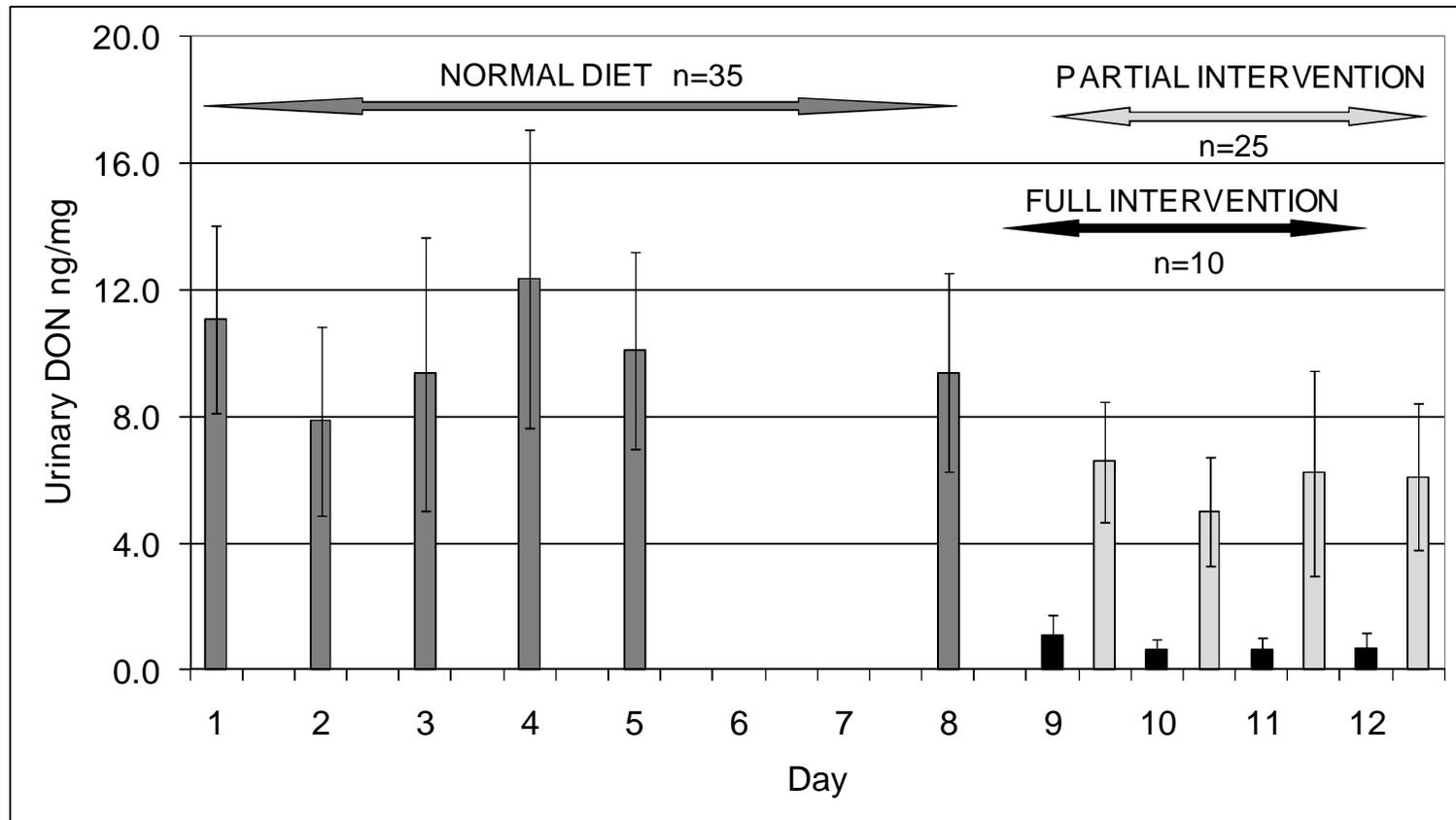
Group	Cereal Intake g/day (range)	DON $\mu\text{g}/\text{day}$ Mean (range)
Low	107 (88-125)	6.6 (5.8-7.6)
Medium	179 (162-195)	9.6 (8.4-11.0)
High	300 (276-325)	13.1 (11.5-15.1)

**DON was detected
in 296/300 (98.7%)
of the urines**

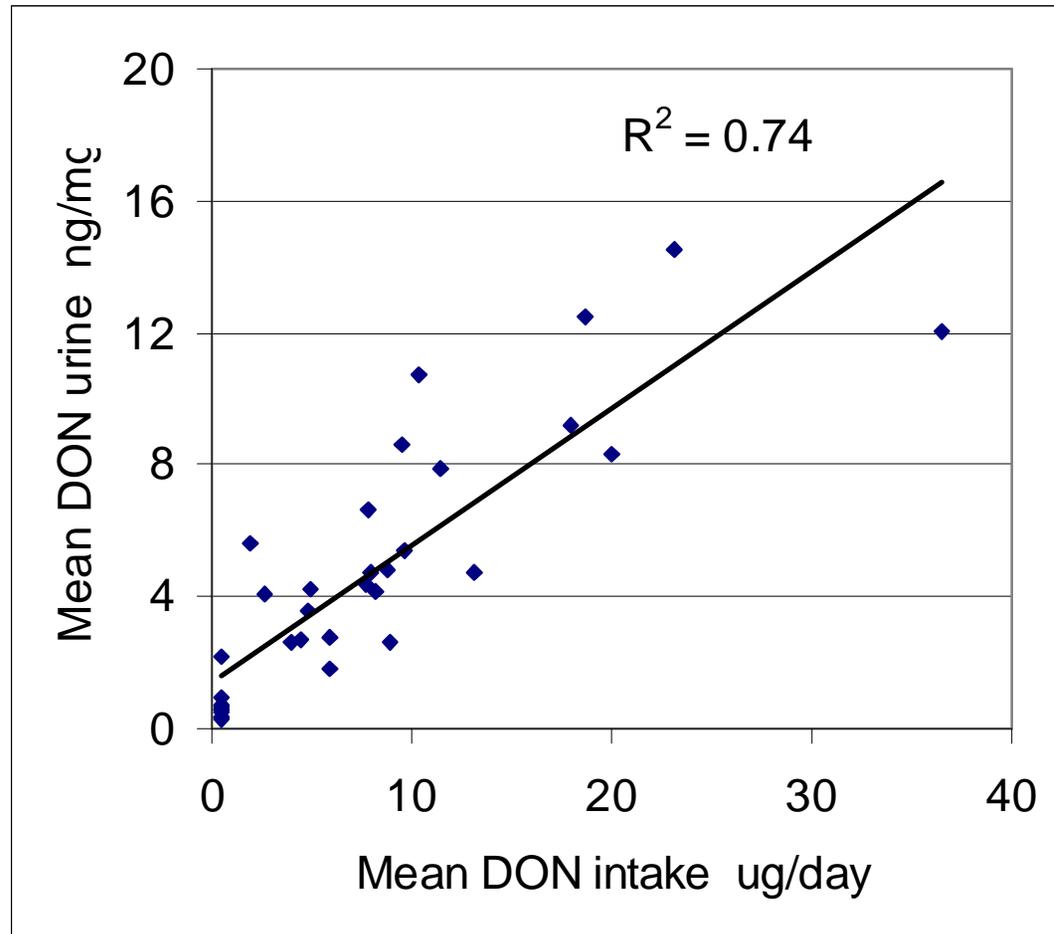


Data are adjusted for sex, age and BMI; p for trend <0.001 , adjusted $R^2 = 0.182$.

Intervention study to reduce DON exposure by avoidance of cereals



Correlation between DON intake and urinary excretion at the individual level



Validation requirements

- Analytical performance
- Dose-response relationship between exposure and biomarker
- Intra-individual variation over time

Biomarker validation

- Recommendation
 - Ø Priority to funding biomarker development and validation under a structured programme of priority exposures

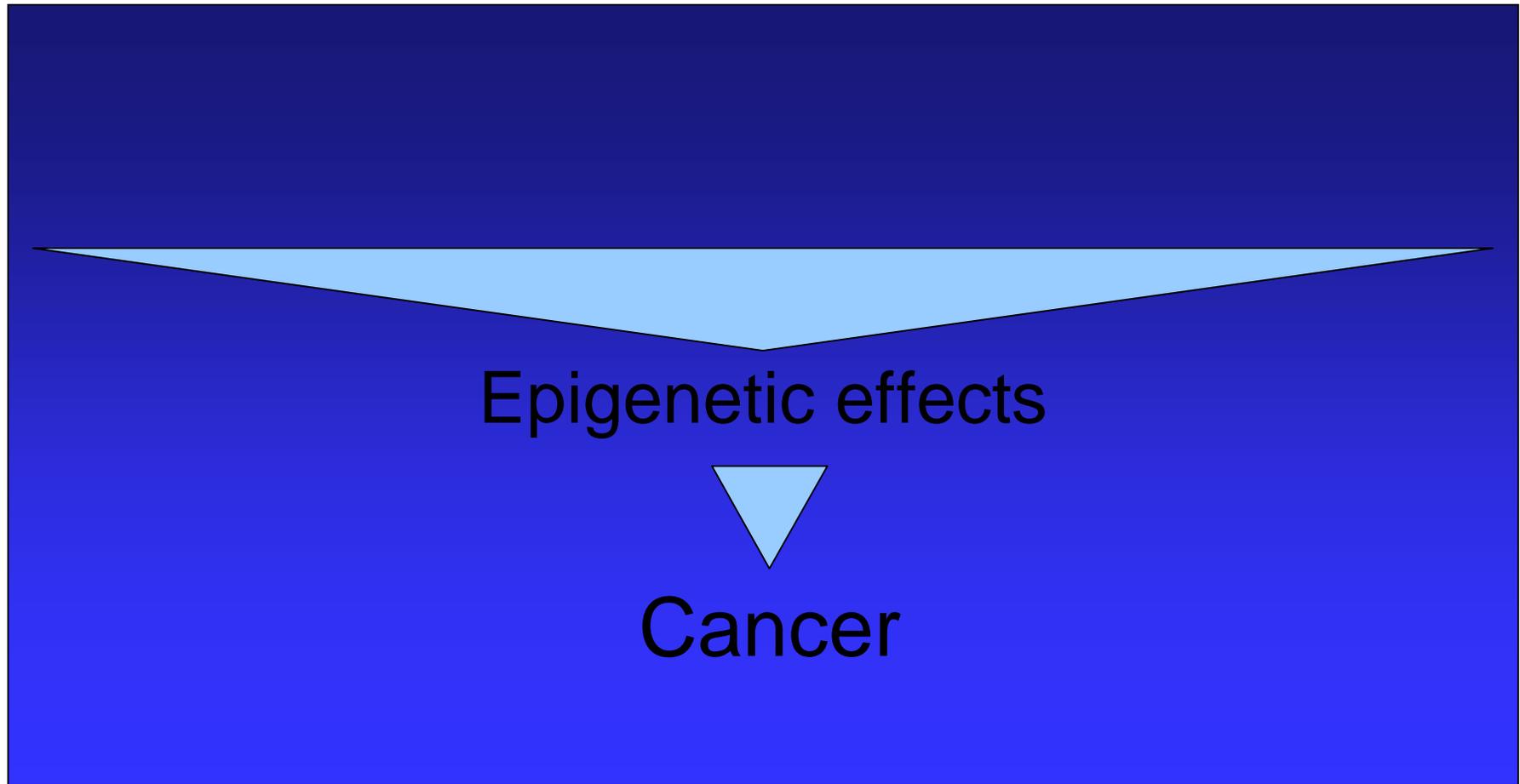
Complementary emphasis in exposure biomarkers

- First generation exposure biomarkers tended to focus on a classical mutagen – carcinogen model of carcinogenesis (*metabolites, adducts, chromosomal alterations, somatic mutations*)
- Further advances are expected with a new generation of tools to measure these endpoints (e.g. advances in mass spectrometry, lab-on-a-chip)

A new generation of tools for exposure assessment – *emergence from new knowledge of mechanisms*

- Epigenetic changes (promoter methylation, histone acetylation, microRNA)
- Altered gene, protein or metabolite levels (“omics” technologies) - pathways

Diverse exposures, common pathways

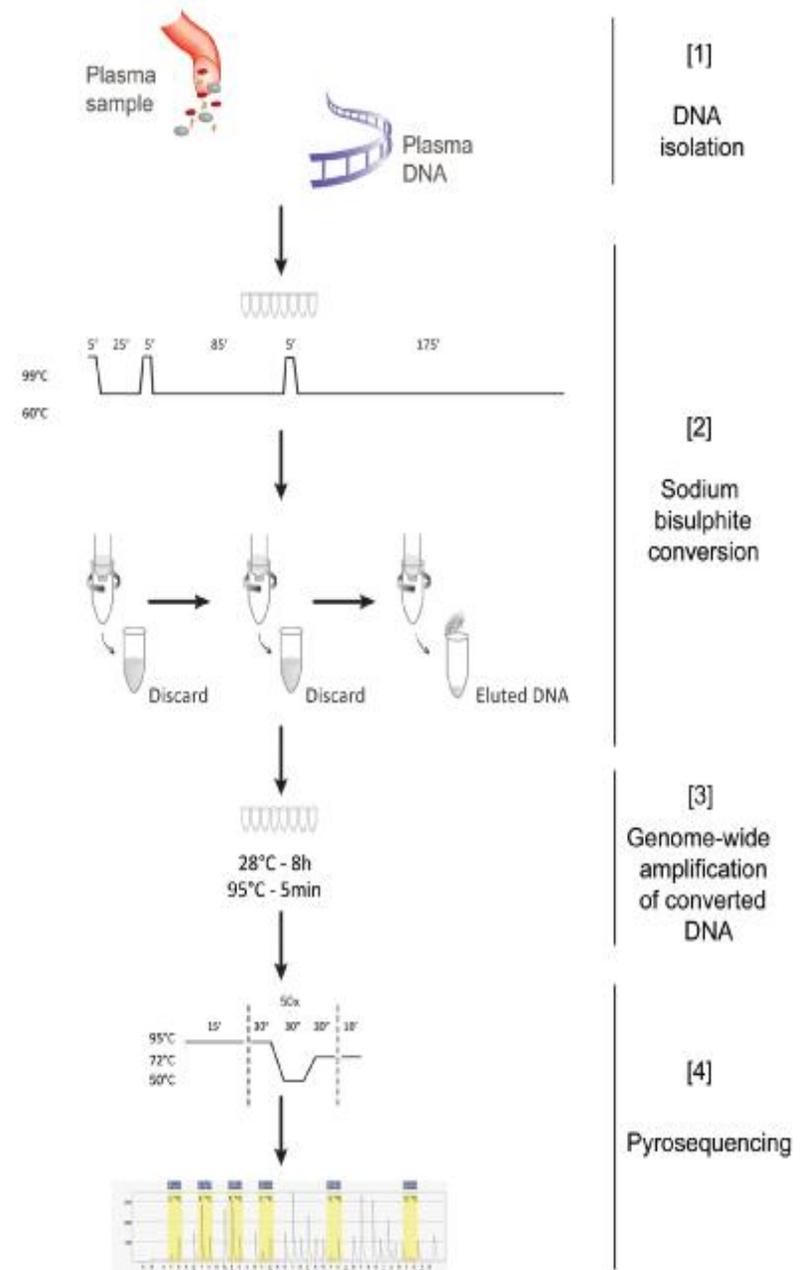


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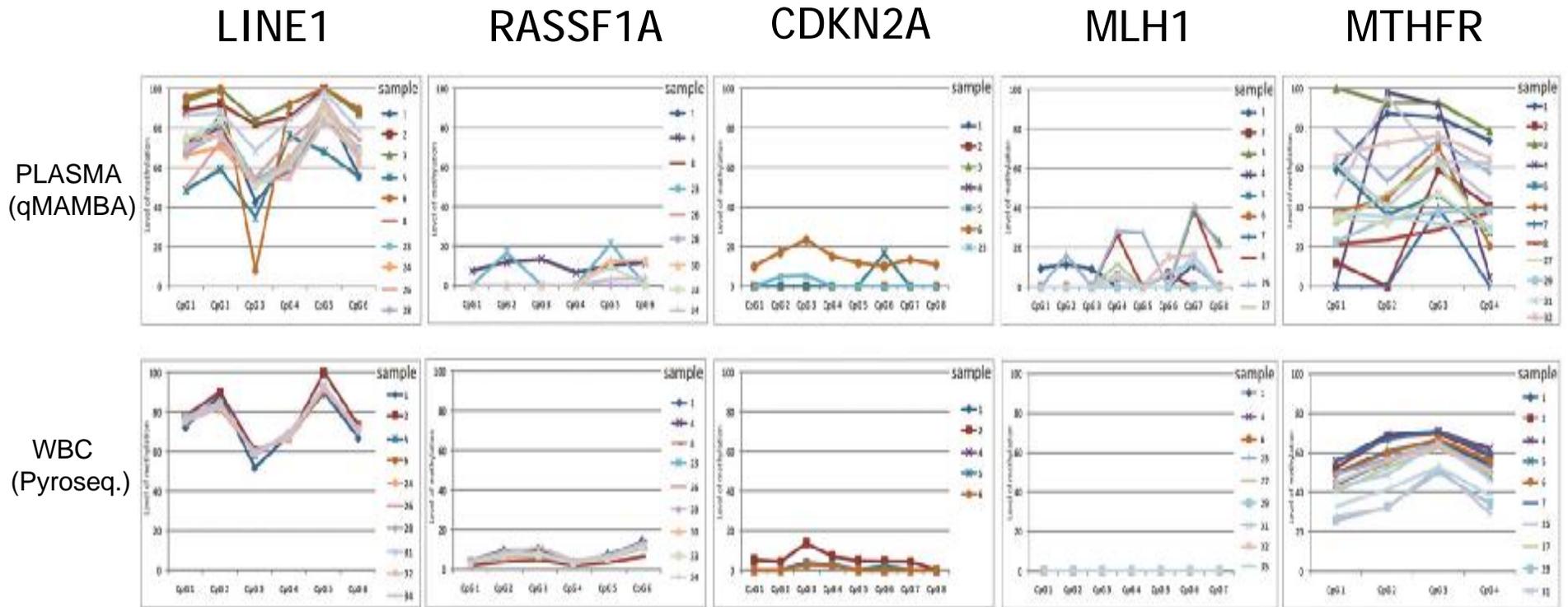


Epigenetic biomarkers – applicability to population studies

Quantitative analysis
of DNA methylation
after whole bisulfite
amplification of a
minute amount of
DNA from body fluids
(*Vaissiere et al.,
Epigenetics, 2009*)



Quantitative profiles of DNA methylation in plasma DNA samples



DNA methylation levels of 4 cancer-associated genes and repetitive elements (LINE1) in plasma DNA samples (as analyzed by qMAMBA, upper panels) and corresponding white blood cells (as analyzed by pyrosequencing, lower panels) of different cancer patients (from EPIC cohort)

Epigenetic biomarkers – applicability to population studies

- Detection of stable miRNAs in plasma and serum – differences by disease status (*Mitchell et al., PNAS 105: 10513, 2008; Chen et al., Cell Res., 18: 997, 2008*)
 - Cell and tissue specific expression
 - Stable in biological fluids such as plasma and serum
 - PCR based assays available
 - Profiling a small number may provide discrimination
 - Genetic variations in miRNA processing genes and in miRNA binding sites may confer genetic susceptibility
 - **Functional information is vital**

Can “omics” help improve exposure assessment?

- Do specific exposures, or categories of exposure, alter the expression of specific groups of genes, proteins or metabolites (“exposure fingerprint”)?
- How do such alterations relate to dose?
- How stable are the alterations over time?
- How do potential confounding factors affect the association between exposure and “omics” biomarkers

Transcriptomics and exposure assessment

(see *Wild CP, Mutagenesis 24: 117-125, 2009*)

- **Smoking** – *Lampe et al., CEBP, 13: 445-453, 2004;*
van Leeuwen et al., Carcinogenesis, 28: 691-697, 2007
- **Benzene** - *Forrest et al., EHP 113: 801, 2005*
- **Arsenic** – *Fry et al., PLoS Genet., 3: 2180-2189, 2007;*
Wu et al., 111: 1429-1438, 2003
- **Metal fumes** – *Wang et al., Env. Health Persp., 113:*
233-241, 2005
- **Air pollution** – *van Leeuwen et al., Mutat. Res., 600:*
12-22, 2006

Metabonomics and population studies

- Connects molecular events to those at the macro level
- Applicable to blood and urine samples
- LC-mass spectrometry methodology affordable and of requisite throughput
- Demonstrated applicability to studies of diet
(*Solanky et al., Anal. Biochem., 323: 197-204, 2003;*
Holmes et al., Nature, 453: 396-400, 2008)

Problems in comparisons of “omics” data in poorly designed studies

See Potter JD Trends in Genetics, 19: 690-695, 2003

- Unmeasured confounding by lack of information on age, sex and other exposures
- Bias through differences in sample processing
- Selection bias through sampling procedures
- High costs leading to one-off or small-scale studies

Next-Generation DNA Sequencing and molecular epidemiology

(Pleasance et al., Nature Jan 14, 2010)

- Massively parallel sequencing techniques promise the capacity to paint a genome-wide portrait of mutation in human cancer
- Tumour (small cell lung) and normal cell lines derived from same individual
- 22,910 somatically acquired substitutions consistent with tobacco exposure
- The smoking history of the patient is not recorded

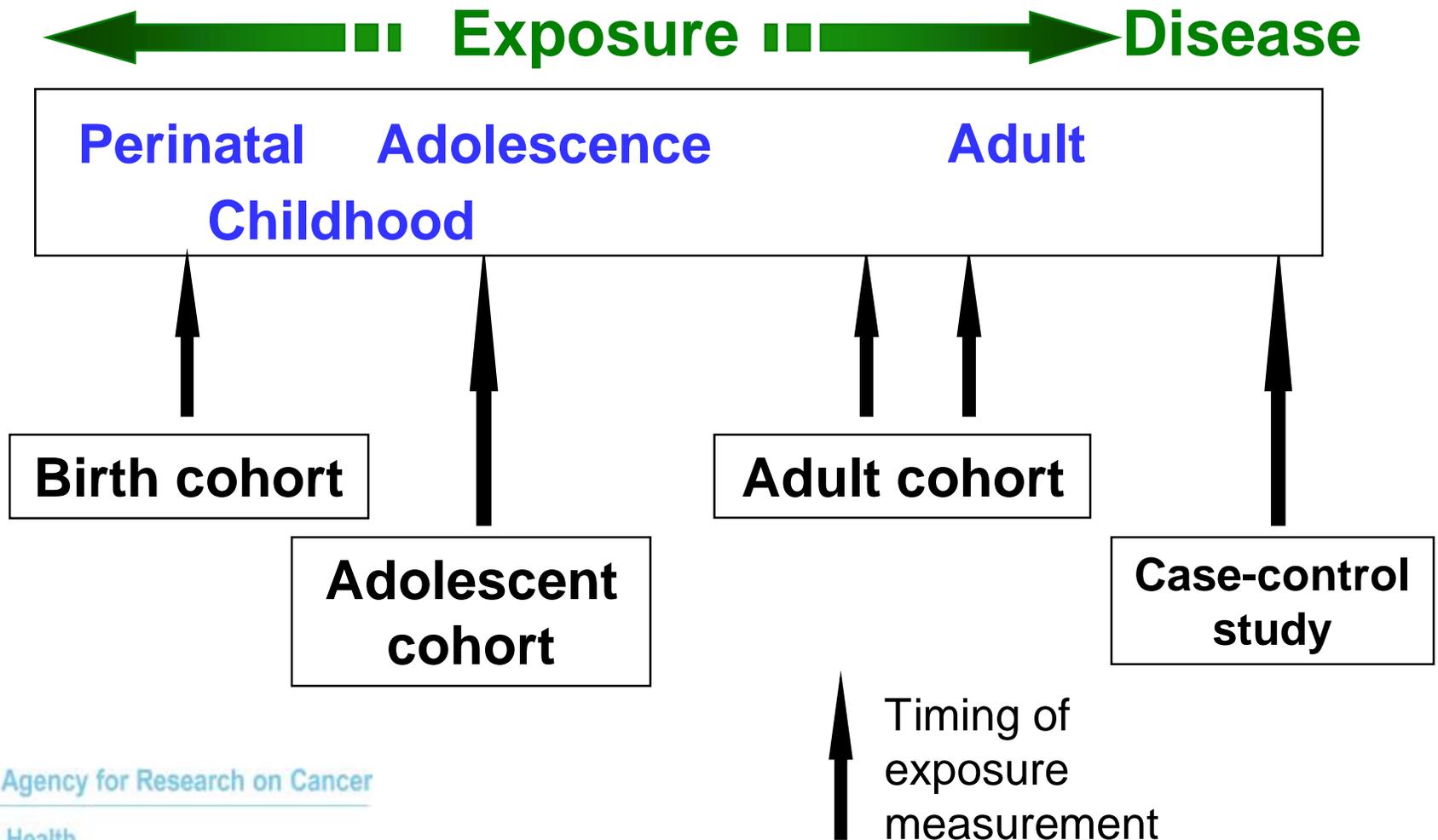
Next-generation biomarkers and epidemiology

- Recommendation
 - Ø Priority on exploring how next-generation biomarkers reflect exposure and reveal relevant disease mechanisms

Early life exposure and cancer risk

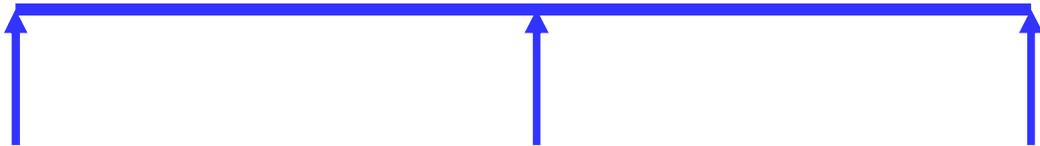
- Observational studies linking early life exposures (or intergenerational effects) to disease later in life
- Foetal programming; epigenetic remodelling; adaptive response
- Vulnerability of children to environmental exposures (*Wild and Kleinjans, Cancer Epi. Bio. Prev., 2003*)

Temporal application of exposure biomarkers in cancer epidemiology



Longitudinal study of aflatoxin exposure and child growth in Benin

Subjects: 200 children, aged 16-37 months from four villages, two high, two low aflatoxin exposure

Time:	February	May/June	October
Survey:	1	2	3
			
Serum AF-alb:	X	X	X
Anthropometry:	X	X	X
Questionnaire:	X	X	X

Longitudinal Study of Aflatoxin Exposure and Child Growth in Benin

Gong et al., *Environ. Health Perspec.* (2004) 112, 1334-1338

Aflatoxin Exposure Group	Mean AF-alb over 8 months Height increase (cm)	
	Unadjusted	Adjusted ^a
lower quartile	4.9 (4.5,5.3) ^{*,c}	5.9 (5.2,6.6)
mid-lower quartile	4.4 (4.1,4.7) ^{**}	5.3 (4.8,5.9)
mid-upper quartile	4.1 (3.8,4.5) ^{**}	4.8 (4.4,5.2)
upper quartile	4.1 (3.8,4.5) ^{**}	4.2 (3.9,4.6)

200 children, aged 16-37 months followed over 8 months

^aAdjusted for age, height, weaning status, mothers SES and village.

^cData labelled * are significantly different to **.

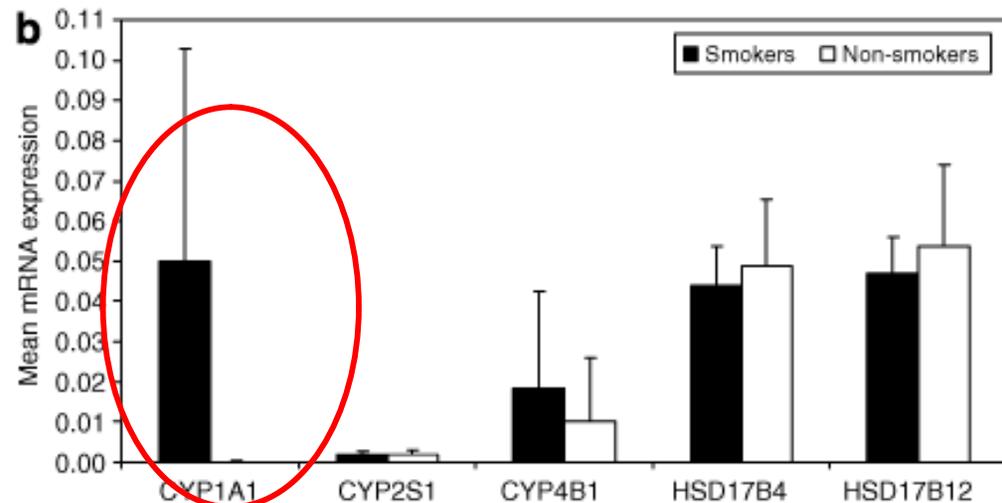
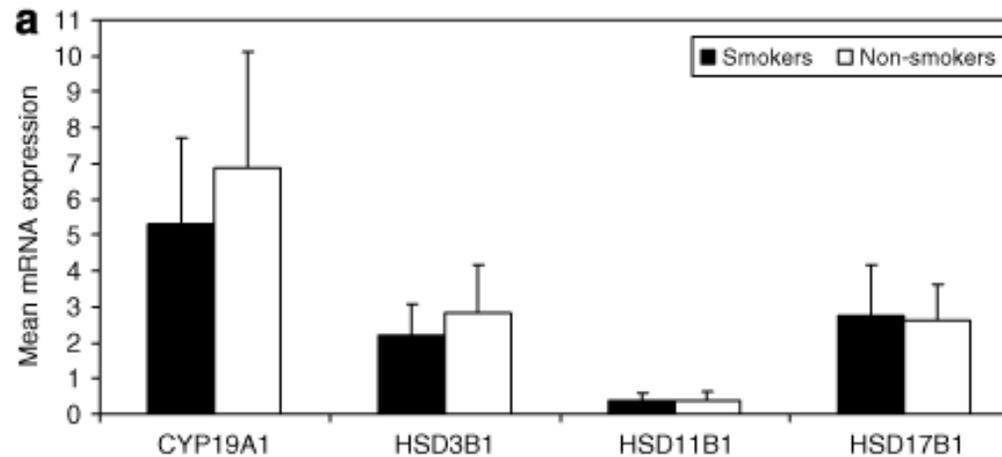
Activation of inflammation/NF- κ B signalling in infants born to arsenic-exposed mothers

Fry et al., PLoS Genetics, 3: 2180-2189, 2007

- 32 pregnant women in Thailand in high and low areas of arsenic exposure
- Toenail analysis of arsenic; cord blood for microarray gene expression
- Expression signatures highly predictive of prenatal arsenic exposure; genes related to stress, inflammation, metal exposure and apoptosis

Gene expression in the placentas of cigarette-smoking mothers

Huuskonen et al., Clin. Pharmacol. Ther., 2008



Early life exposure and cancer risk

- Mechanism-based biomarkers to relate exposure to disease – a necessity?

Cohorts to cover life course

- Rich resource of cohorts (with biobanks) internationally spanning exposures from *in utero* to adult life
- Many cohorts exist but struggle for long-term support
- New cohorts may be envisaged to fill gaps
 - in age range
 - In geographic distribution (most cohorts in high resource countries)

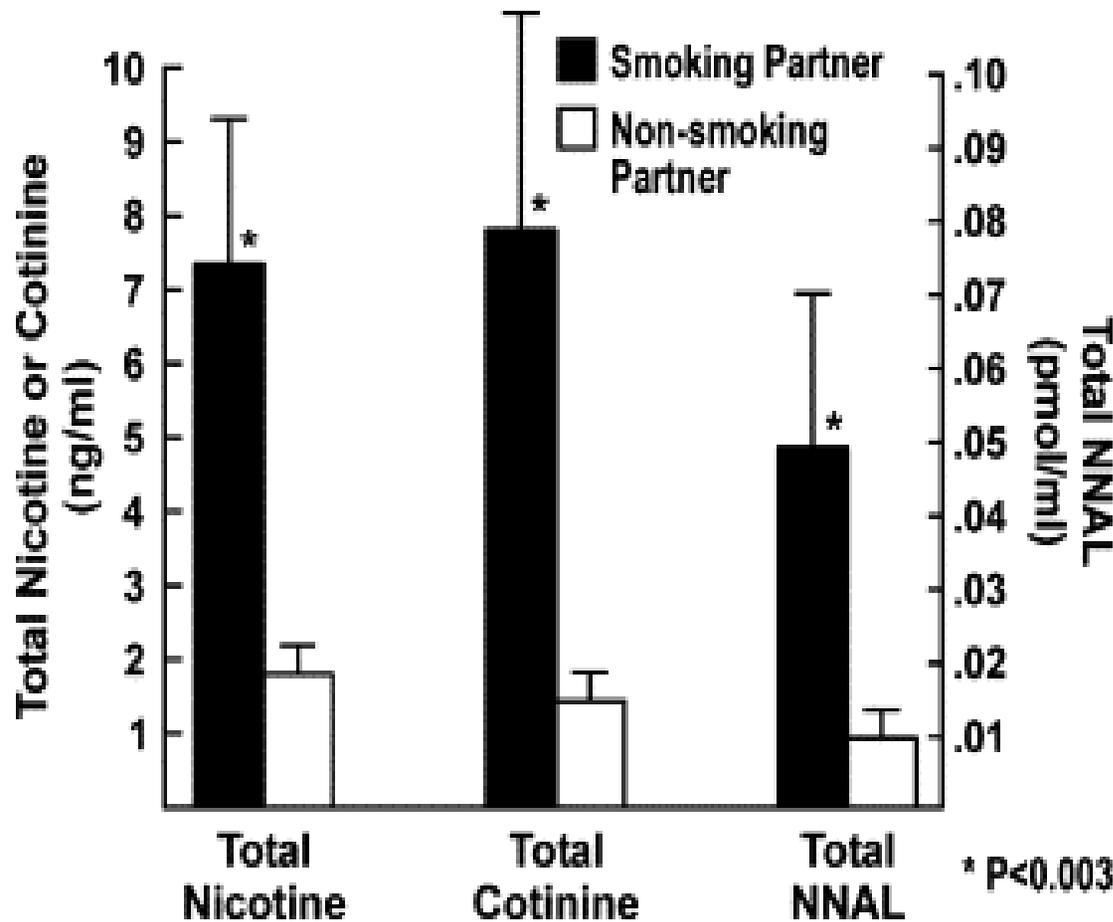
Cohort studies to cover life-course

- Recommendation:
 - Ø Infrastructure support to a network of recognized “lifecourse cohorts” which include biological banks
 - Ø Consider the possibility of support for repeat measurements
 - Ø Comprehensive review of current networks and initiatives

More than exposure assessment....
biomarkers and biological plausibility

- Proof exposure, biological evidence
- Demonstration of a plausible mechanism

Demonstration of exposure – environmental tobacco smoke



Nicotine/Cotinine
Urinary TSNA
4-ABP-Hb
Urinary mutagenicity

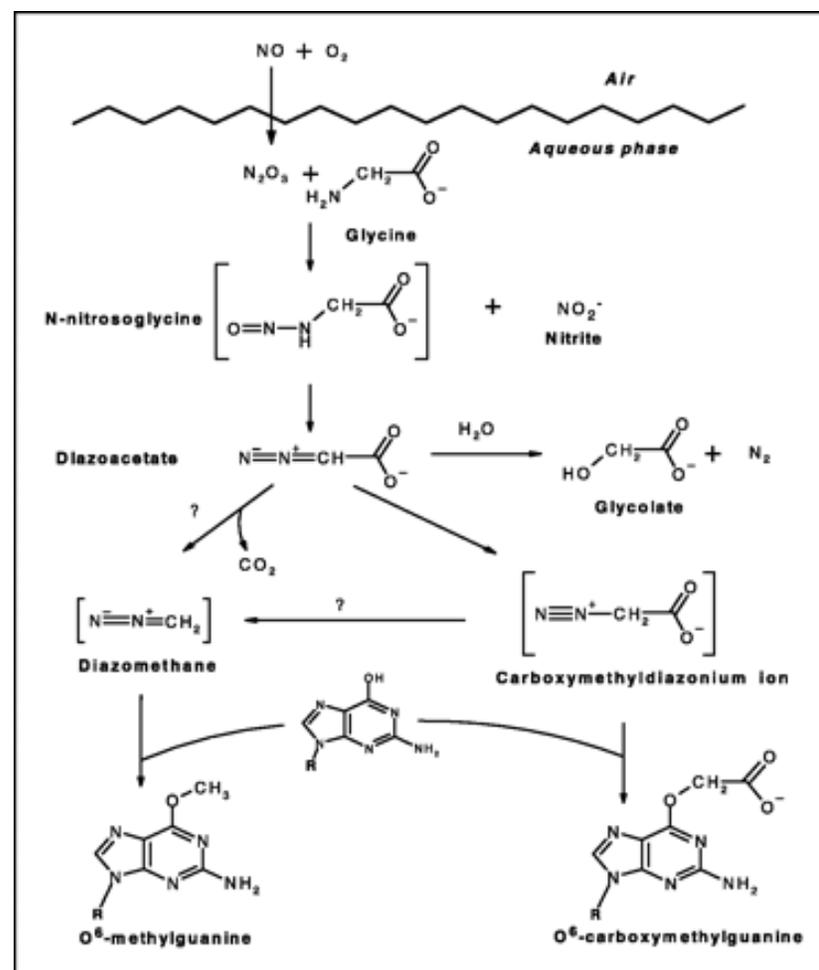
*Demonstration
of exposure
and plausibility
of association
with disease*

Red Meat and Colon Cancer Risk: biomarkers and biological plausibility

- Red and processed meat is associated with increased colorectal cancer (CRC); one hypothesis is that this is due to heterocyclic amines (HCA)
- However, white meat contains HCA but is not associated with CRC risk
- Studies of the N-acetyl gene required for HCA activation and CRC are equivocal
- Red but not white meat stimulates endogenous intestinal N-nitrosation in humans

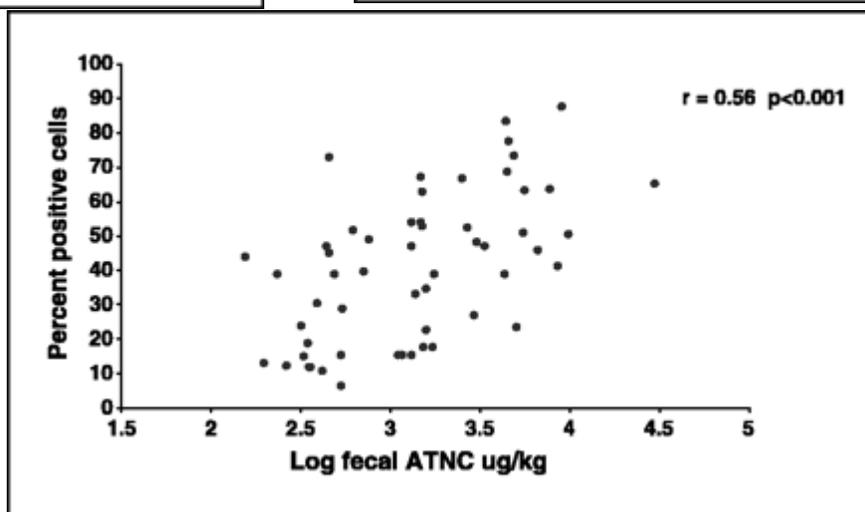
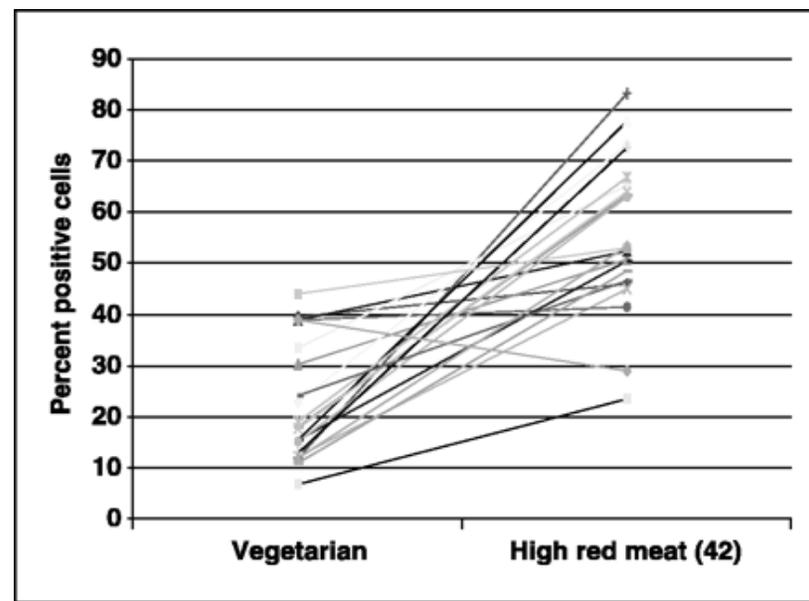
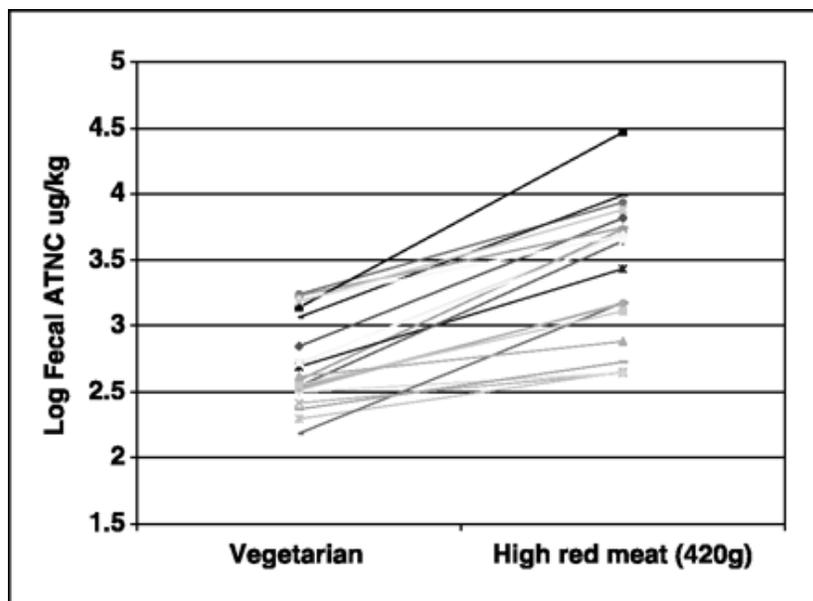
Red Meat and Colon Cancer Risk: biomarkers and biological plausibility

- Volunteers in metabolic suite
- Fed high (420g) red meat, vegetarian and high red meat, high-fibre diets for 15 days in randomized cross-over trial
- Tested whether total faecal N-nitroso compounds and O⁶-carboxymethylguanine adducts in colon DNA were associated with red meat diet



Lewin et al., Cancer Res 2006

Red Meat and Colon Cancer Risk: biomarkers and biological plausibility



More than exposure assessment.... *biomarkers and intervention studies*

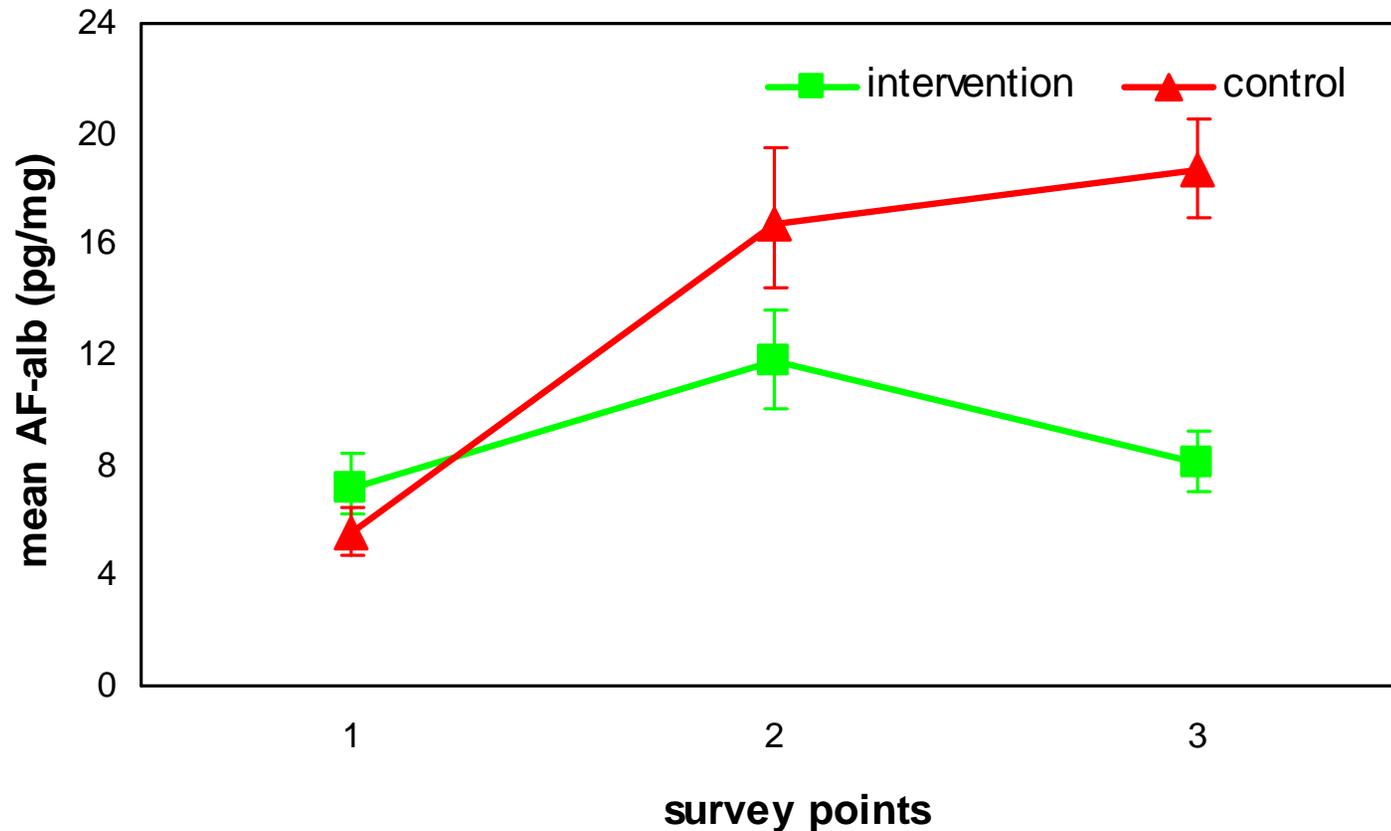
- Proof of concept for modifying exposure-disease relationship (*e.g. anti-oxidants, induction of detoxification enzymes, avoidance of exposure*)
- Surrogate (earlier) outcome



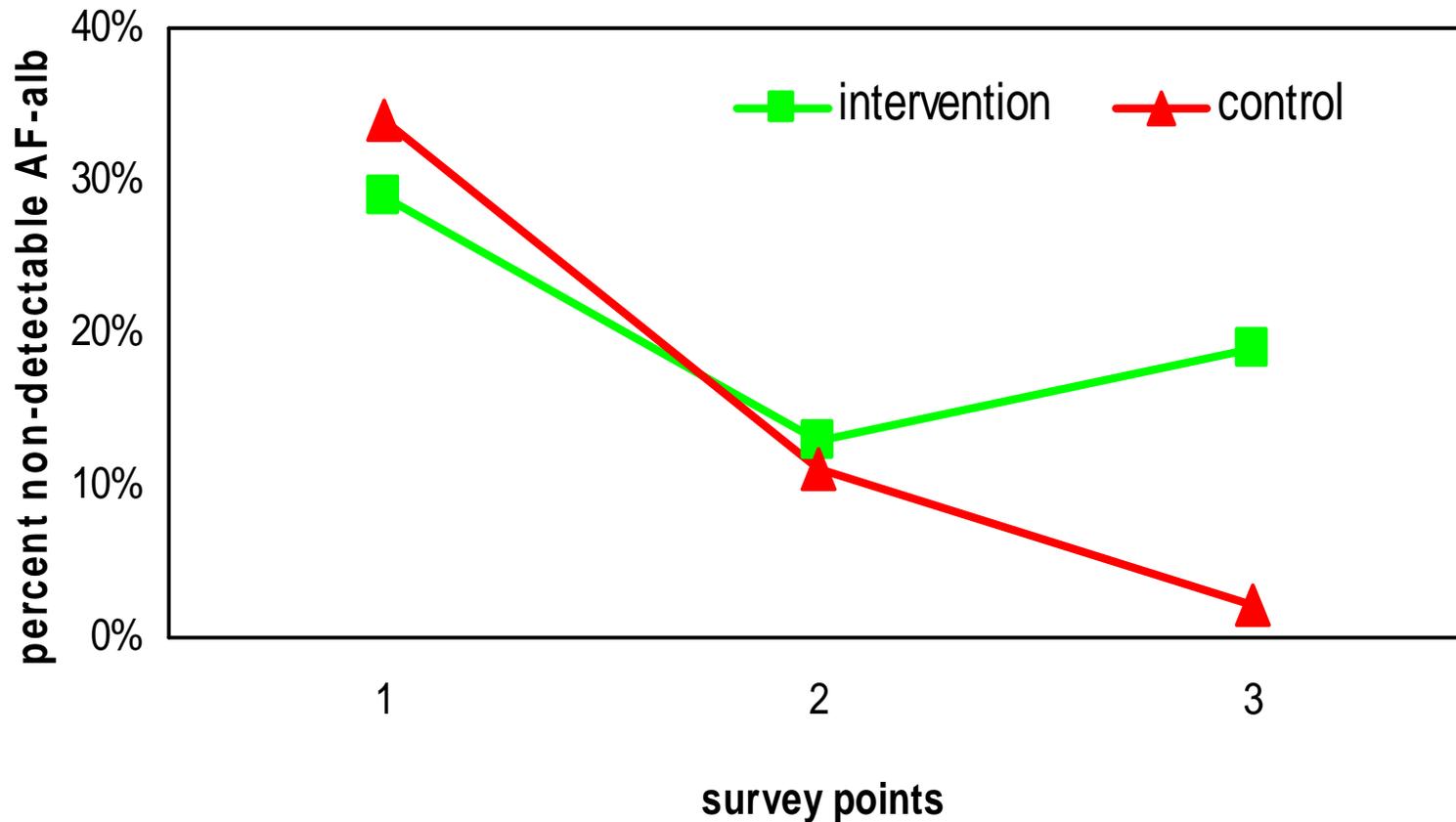




Mean levels of AF-alb are reduced in individuals following intervention



Intervention increases the number of individuals with non-detectable blood AF-alb



“Common soil” of mechanistic research



A plea for two-way translation

Summary – 1

- Ø Invest in development and validation of exposure biomarkers to complement genetic analysis in epidemiological studies
- Ø Encourage application of new methodologies (e.g. “omics”) and knowledge of mechanisms (e.g. epigenetics) to population-based research
- Ø Consider infrastructure support to key prospective cohort studies in relation to coverage of lifecourse and geography

Summary – 2

- Ø Prioritize studies of biological plausibility in establishing aetiology, particularly in cases of modest risk elevation such as diet and cancer
- Ø Explore the integration of biomarkers into proof-of-principle intervention studies
- Ø Train a new generation of multi-lingual researchers able to operate in an interdisciplinary environment

Acknowledgements

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