

Molecular epidemiology of occupational cancer

The example of bladder cancer

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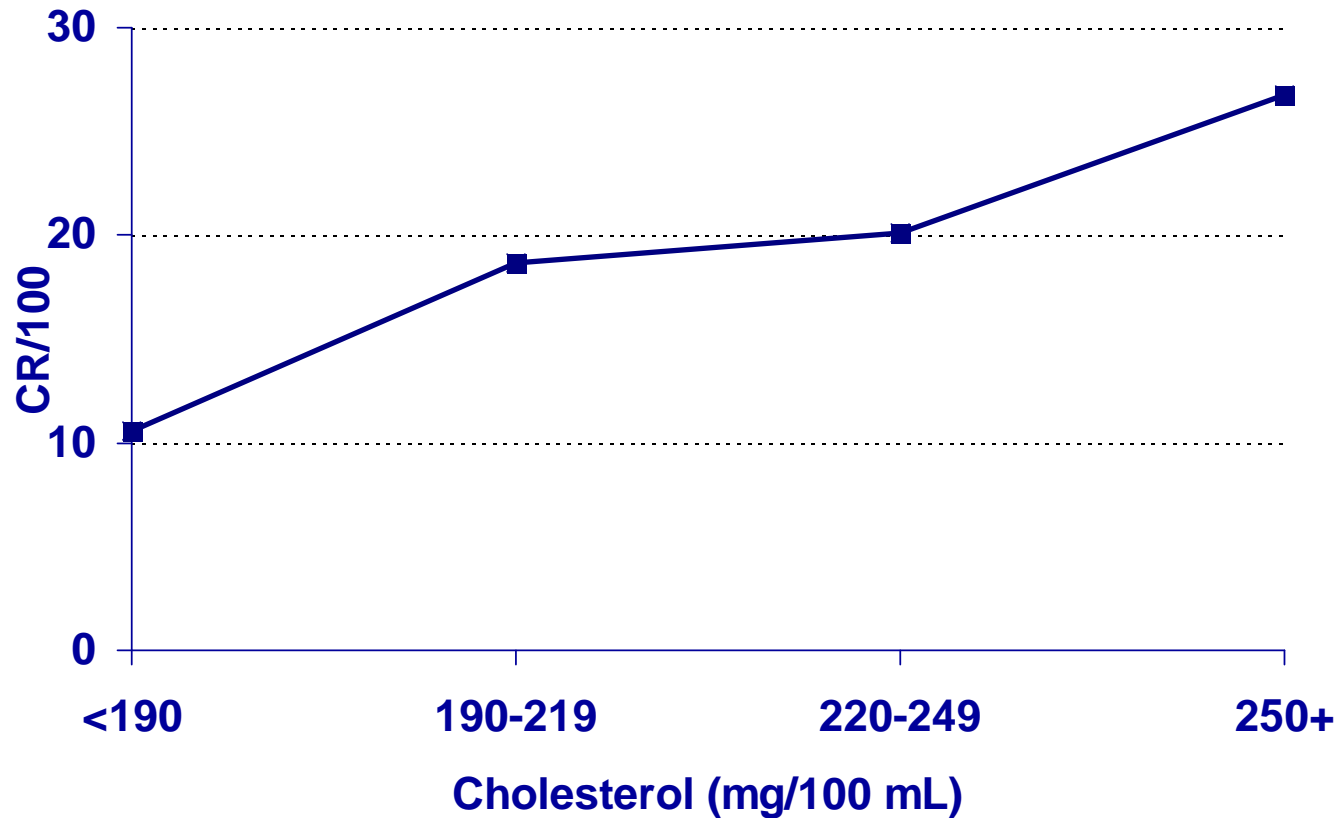
Reasons for increasing use of biomarkers in cancer epidemiology

- Limitations of traditional epidemiology
 - weak effects
 - complex diseases and exposure circumstances
- Technical developments
- Increasing knowledge on mechanisms

Biomarker

- Substance, structure or process that
 - can be measured in human body or products
 - may influence the incidence or outcome of disease
- Long tradition of use

Serum cholesterol and incidence of IHD among men in Framingham cohort 12 yr follow-up

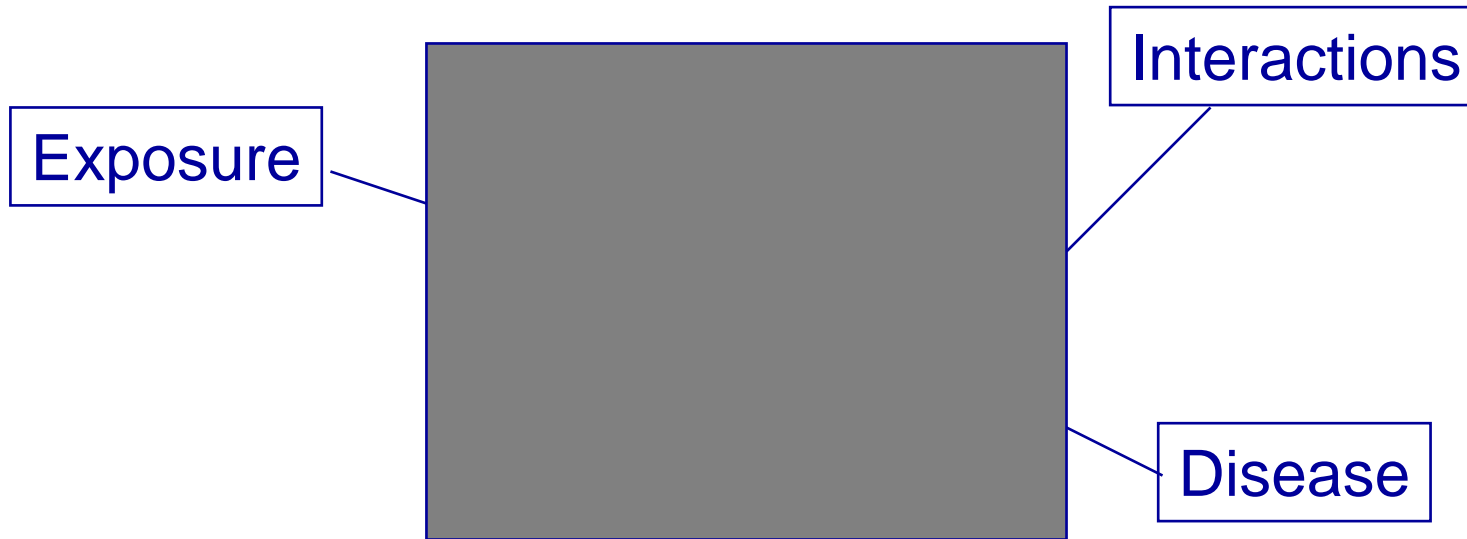


Truett et al., 1967

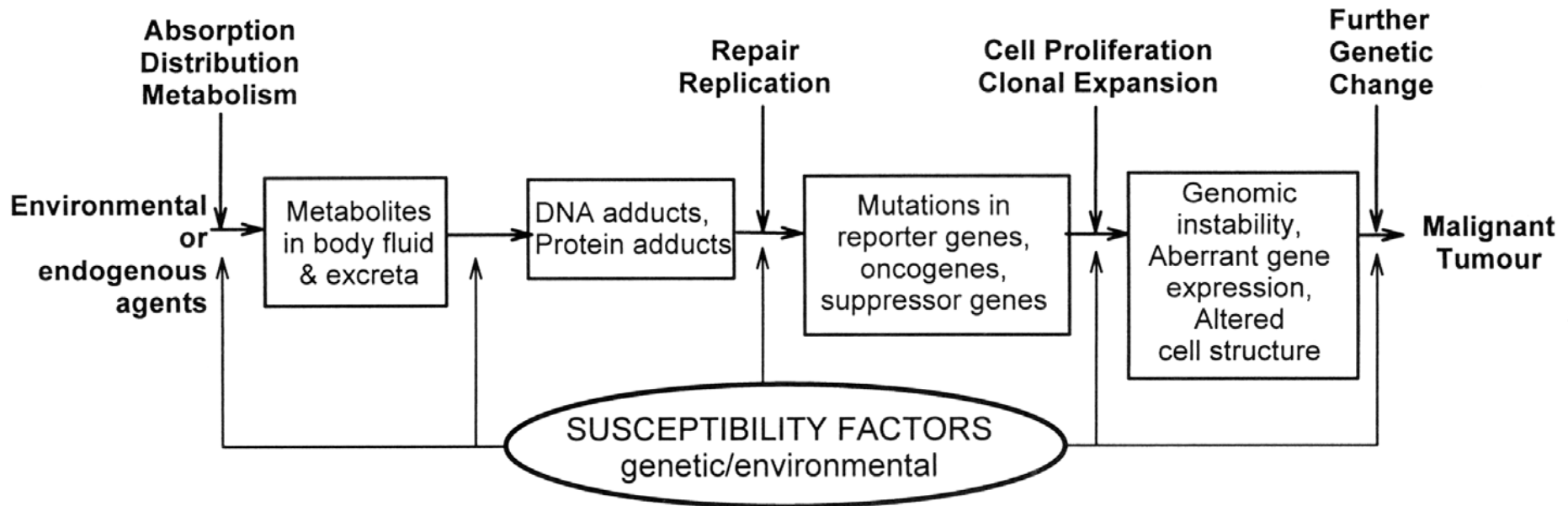
Marker, assay and measurement

- Marker: the variable to be measured
- Assay: the test aiming to measure the marker
- Measurement: an individual results of the value of the marker

'Black box' epidemiology



Opening the black box



Classes of biomarkers

<i>Class</i>	<i>Examples</i>
Exposure	DNA or protein adduct Viral DNA Urinary metabolite
Disease	Gene mutation Chromosomal aberration Epigenetic alteration
Susceptibility	Metabolic polymorphism DNA repair activity

Measuring exposure with biomarkers

- Aims
 - to measure biologically relevant exposure
 - to decrease exposure misclassification
- Problems
 - time relevance
 - susceptibility to confounding
 - dependence on disease process

What is exposure misclassification?

- To use sexual behavior to measure HIV infection status
- Problems
 - risky behaviors may not be validly reported
 - not all individuals reporting risky behaviors are HIV positive
 - individuals not reporting risky behaviors can be HIV positive

Successful application of exposure biomarkers

Aflatoxin and liver cancer

- Evidence of liver carcinogenicity from experimental studies
- Positive results from ecological studies (food surveys)
- No association in studies based on self-reported exposure
 - exposure misclassification?



Urinary AF biomarkers and liver cancer

- cohort of 18,000 men from Shanghai
- 7 year follow-up
- 22 cases of liver cancer and 140 controls
- analysis of urine samples

<i>Biomarker</i>	<i>Ca</i>	<i>Co</i>	<i>OR</i>	<i>95% CI</i>
No AF biomarker	9	87	1.0	Ref.
Any AF biomarker	13	53	2.4	1.0-5.9
AFB ₁ -N ⁷ -Gua	6	11	4.9	1.5-16

- results confirmed in 3 additional cohorts

Exposure biomarkers

Lessons from aflatoxin

- A single biomarker measurement might reduce substantially exposure misclassification
 - components of variability of biomarkers
 - time-relevance of exposure biomarkers
- The biomarker does not need to be very sophisticated

Ethylene oxide

- Intermediate chemical in production of glycol ethers, surfactants, etc.
- Medical instruments sterilization
- Food fumigation

Animal carcinogenicity data

<i>Species</i>	<i>Route</i>	<i>Results</i>
Mouse	subcutan.	sarcoma
	skin applic.	negative
	inhalation	lung, breast, lymphoma
Rat	oral	forestomach
	inhalation (2)	leukemia, brain

Epidemiological studies of chemical workers - LHP neoplasms

<i>Country</i>	<i>Process</i>	<i>N</i>	<i>Obs/Exp</i>
Sweden	ch/ox	733	7/2.2
USA	ch	767	3/3.0
Germany	ch/ox	2658	5/5.0
UK	mix	1471	5/2.3
USA	ch	1896	7/11.8
Italy	mix	637	5/0.5

Epidemiological studies of sterilization personnel - LHP neoplasms

<i>Country</i>	<i>N</i>	<i>Obs/Exp</i>
Sweden	2170	3/2.0
UK	1405	2/1.4
US	18254	36/33.8

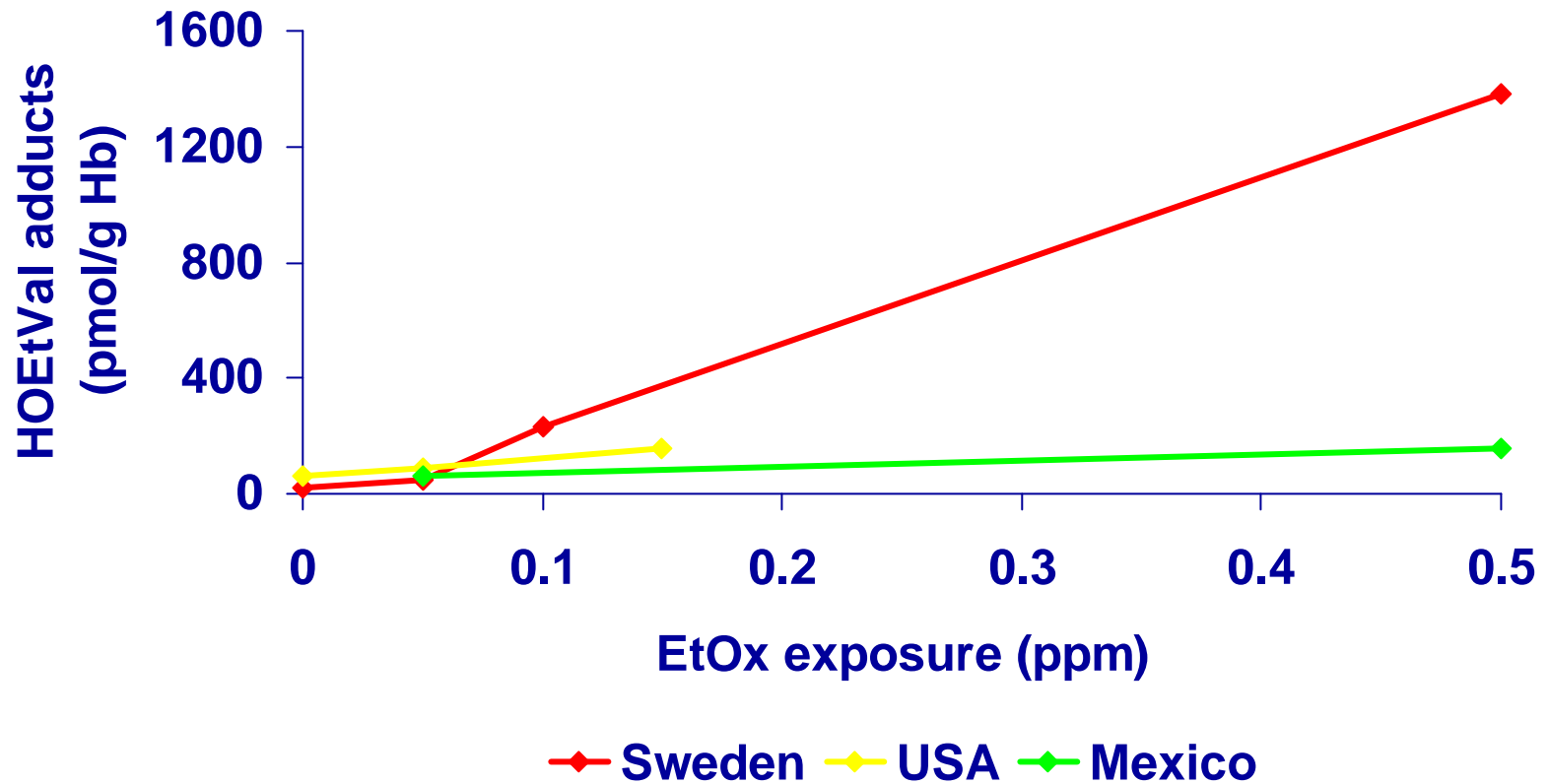
Dose-response analysis in US cohort

RR for 45 ppm-yrs: 1.20 (95% CI 1.05-1.38)

Ethylene oxide Hb adducts

- *N*-2(hydroxyethyl) adducts at N-terminal of His and Val
- Studies of exposed workers from Netherlands, Sweden, US, Mexico
- Same adducts found in exposed rats and mice, with dose-response

Hb adducts: dose-response



Mechanistic considerations in overall evaluation of ethylene oxide carcinogenicity

- dose-related increase of same Hb adducts in rodents and exposed humans
- dose-related increase of CA, SCE and MN in exposed humans
- similar malignancies in rats and humans
- gene mutations in rodents
- strong mutagen and clastogen in all systems

Measuring disease with biomarkers

- Aims
 - to study early events (follow-up, intervention, mechanisms, etc.)
 - to increase specificity and sensitivity in the definition of disease
- Problems
 - timing of disease (incidence or prevalence)
 - predictive value of effect marker

Bladder cancer

An example of gene-environment
interaction in occupational cancer

Nat Rothman, NCI, Bethesda,
USA

Paolo Boffetta, IARC, Lyon,
France

Spanish Bladder Cancer Study

- Hospital-based case-control study (1998-2001).
- 18 hospitals in 5 regions.
- Controls matched on region, age, gender and ethnicity.
- Participation rates: 85% cases and 88% controls.



	Cases	Controls
	1,219	1,271
With DNA	↓ 98%	↓ 93%
	1,188	1,173



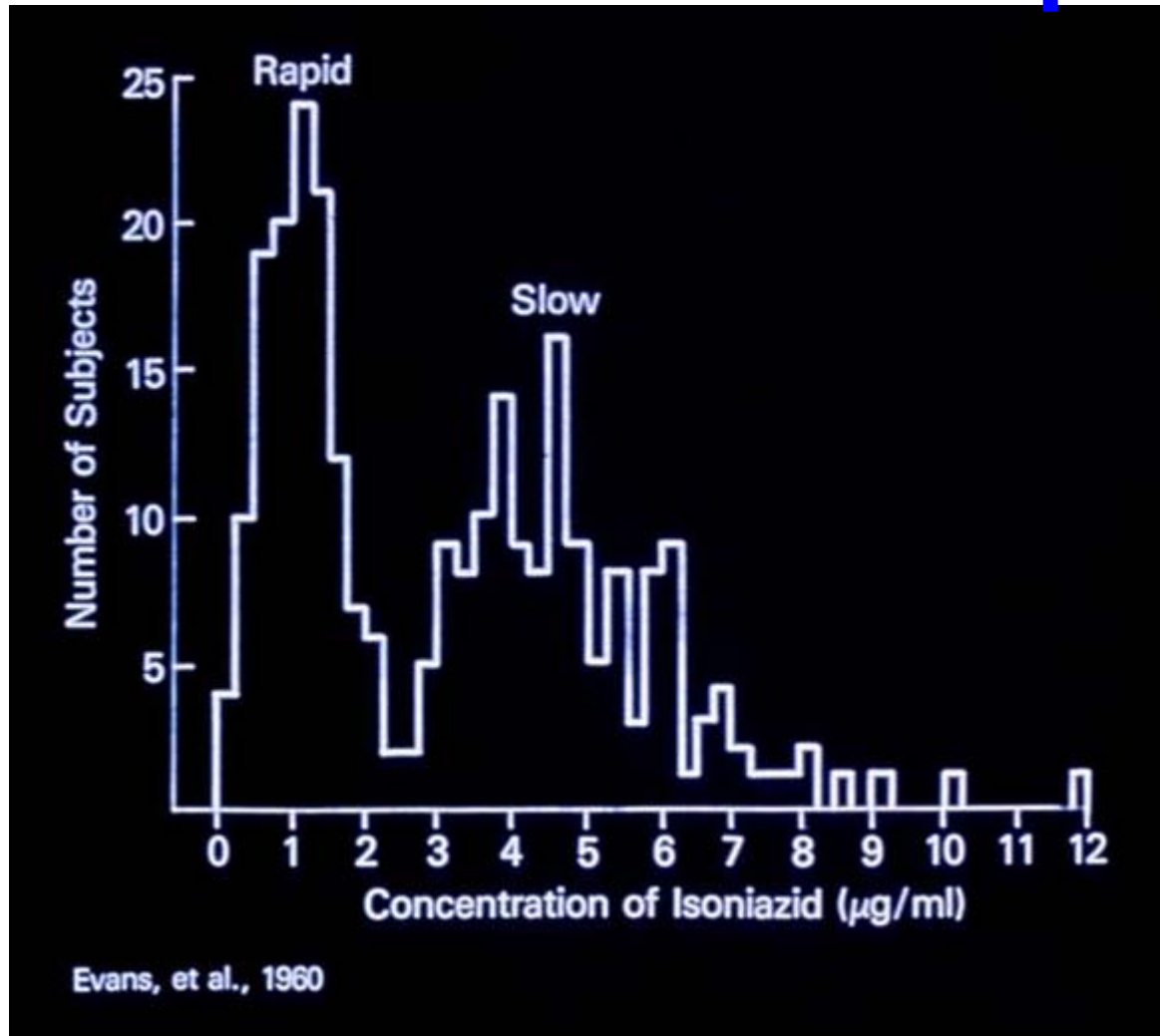
Data Collection

<u>Data Resources</u>	<u>Response Rate</u>	<u>Specific Areas</u>
CAPI	86%	Demographics Smoking Occupation/Environmental Family history Medical/Drugs
Blood/Buccal Cell	95%	Genetic Susceptibility Functional Assays
Diet Qx.	72%	Fluid intake Food Frequency Food Carcinogens
Urination Diary	60%	Urine pH Urinary freq
Toenails	77%	Arsenic/Selenium
Hair dye Qx.	85%	Hair Dye

NAT2 slow acetylation, tobacco use, and bladder cancer risk

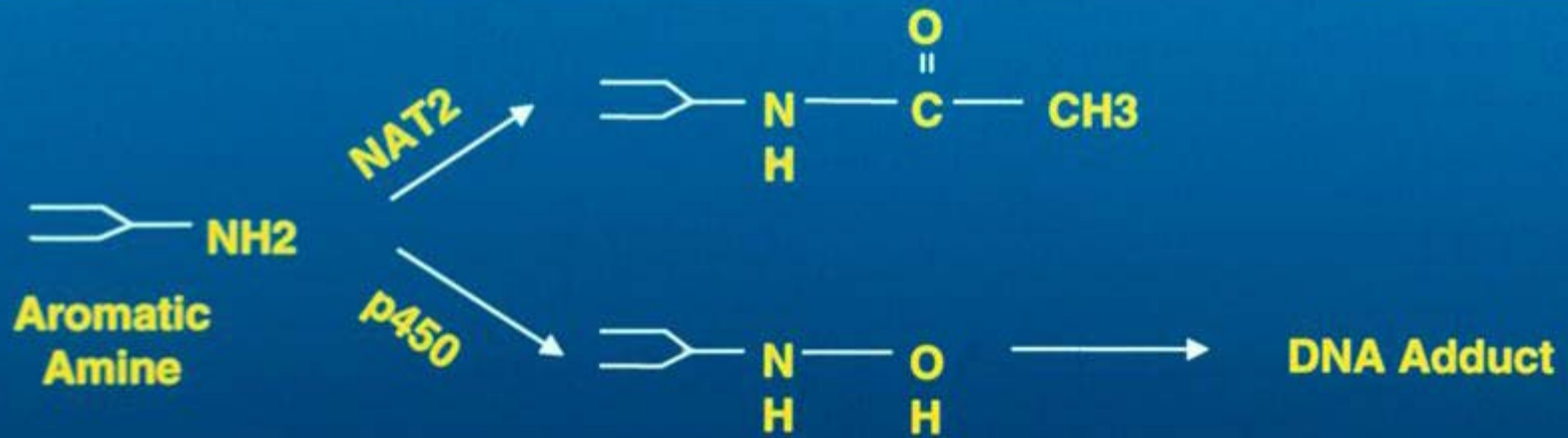
Genotype		Cases	Controls	OR	95%CI	p-value
<i>NAT2</i>	Rapid/Intermediate	406	493	1.0		
	Slow	728	637	1.4	(1.2-1.7)	0.0002

Acetylation polymorphism - Isoniazid clearance from plasma

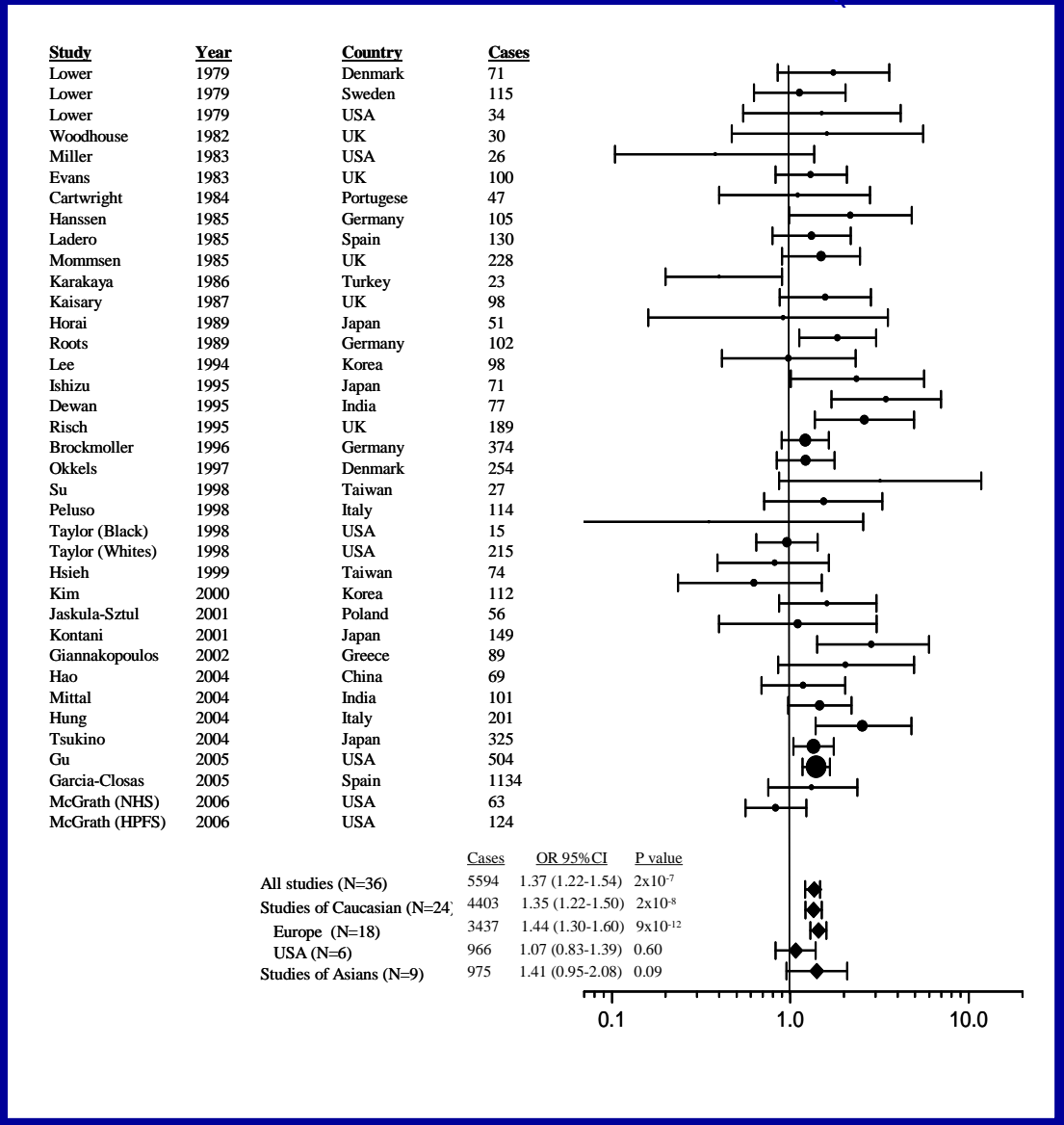


Detoxification of Aromatic Amines by N-Acetylation

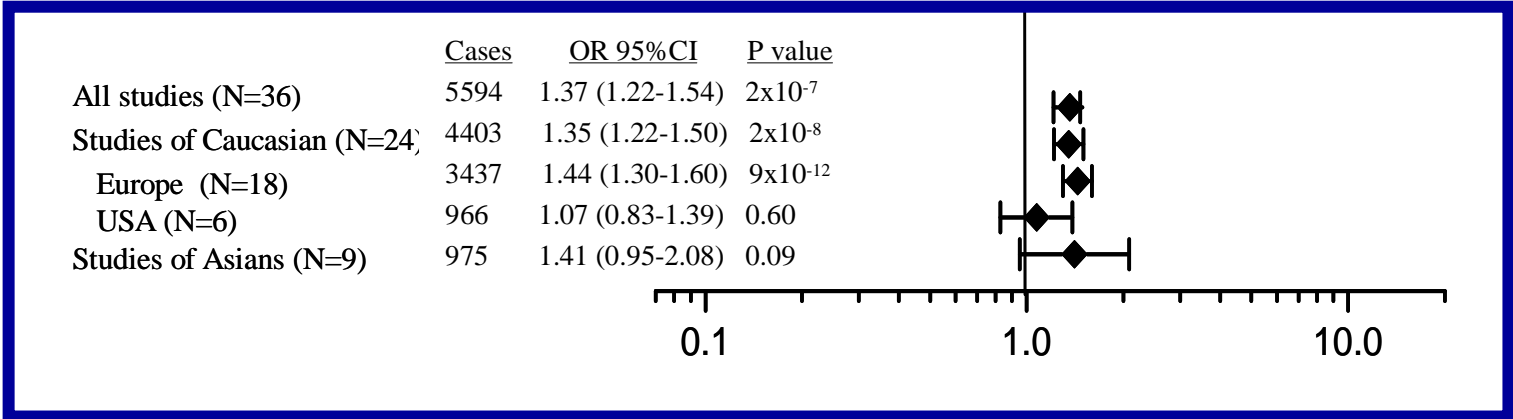
(Lower et al., EHP 1979)



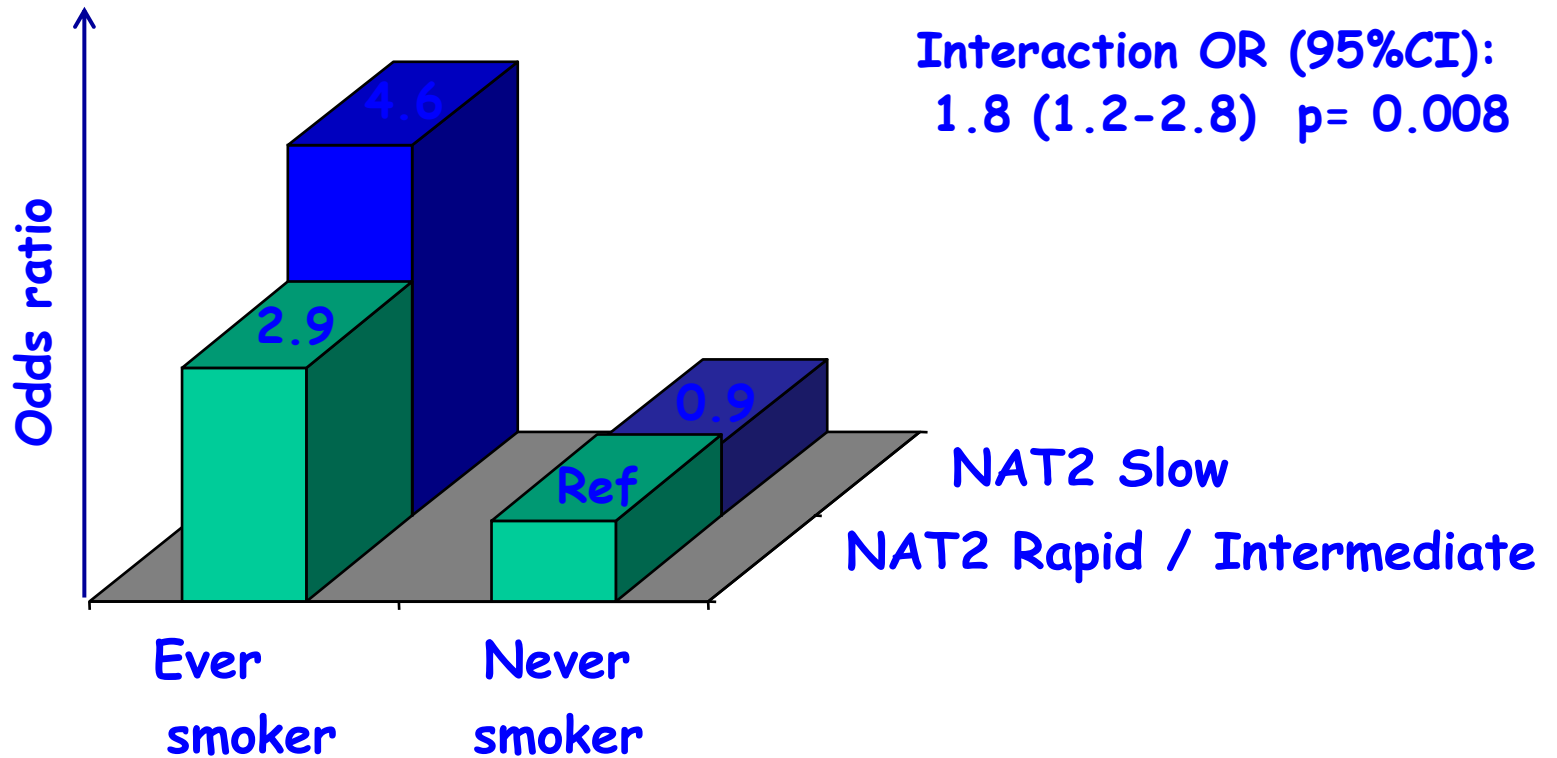
Meta-Analysis of Case-Control Studies of NAT2 Slow Acetylation and Bladder Cancer (Rothman et al., IJG, 2007)



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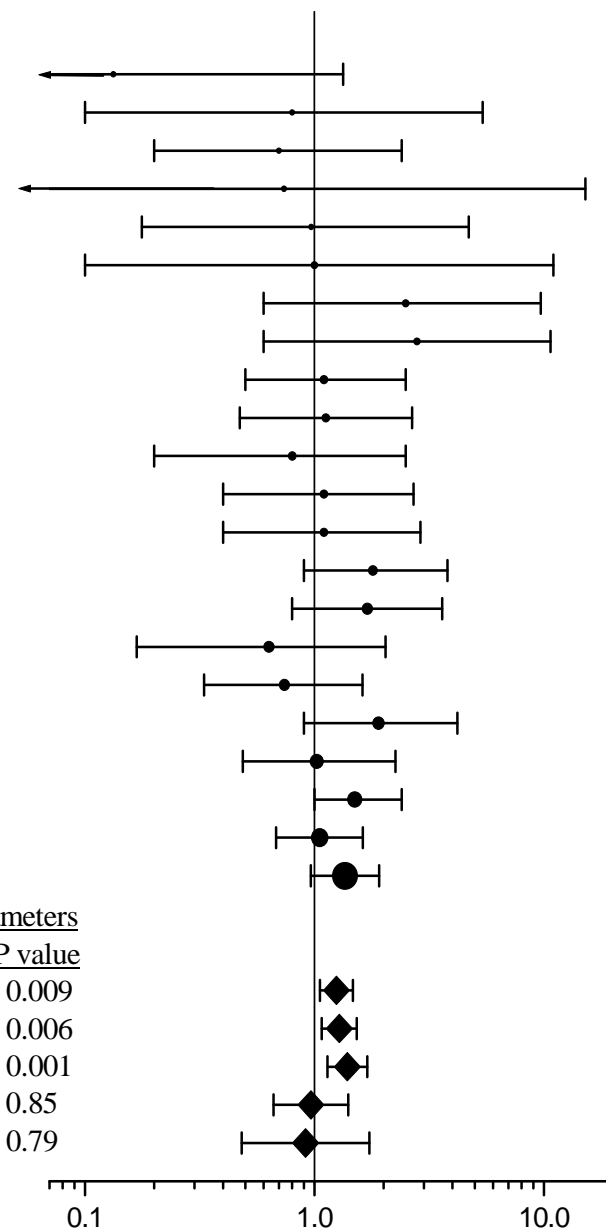


Multiplicative interaction between *NAT2* and cigarette smoking: Spanish study



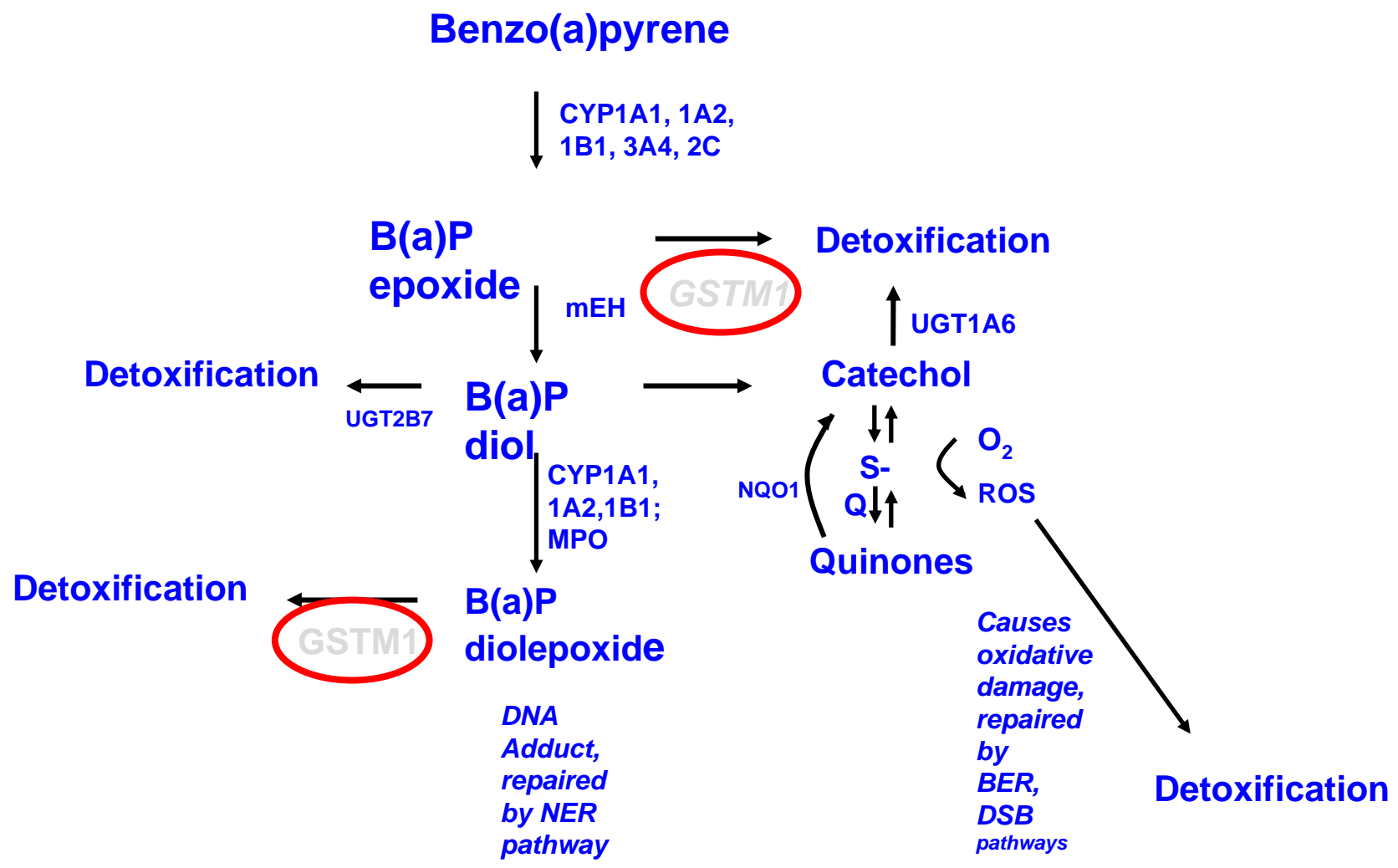
Meta-Analysis of Case-Only Studies of NAT2 Slow Acetylation, Tobacco Use, and Bladder Cancer

<u>Study</u>		<u>Country</u>	<u>Cases</u>
Karakaya	1986	Turkey	23
Miller	1983	USA	26
Ishizu	1995	Japan	47
Horai	1989	Japan	50
Jaskula-Sztul	2001	Poland	59
Lower	1979	Denmark	67
Dewan	1995	India	77
Romkes	Unpublished	UK	91
Kaisary	1987	UK	98
Mittal	2004	India	101
Roots	1989	Germany	101
Hanssen	1985	Germany	105
Ladero	1985	Spain	130
Mommsen	1985	UK	149
Risch	1995	UK	178
Hung	2004	Italy	201
Taylor (whites)	1998	USA	215
Okkels	1997	Denmark	253
Tsukino	2004	Japan	325
Brockmöller	1996	Germany	374
Gu	2005	USA	502
García-Closas	Current	Spain	1134



	<u>Cases</u>	<u>OR</u>	<u>95% CI</u>	<u>P value</u>
All studies (N=22)	4306	1.2	(1.1-1.5)	0.009
Studies of white populations (N=16)	3683	1.3	(1.1-1.5)	0.006
Europe (N=13)	2940	1.4	(1.1-1.7)	0.001
USA (N=3)	743	1.0	(0.7-1.4)	0.85
Studies of Asian populations (N=3)	422	0.9	(0.5-1.7)	0.79

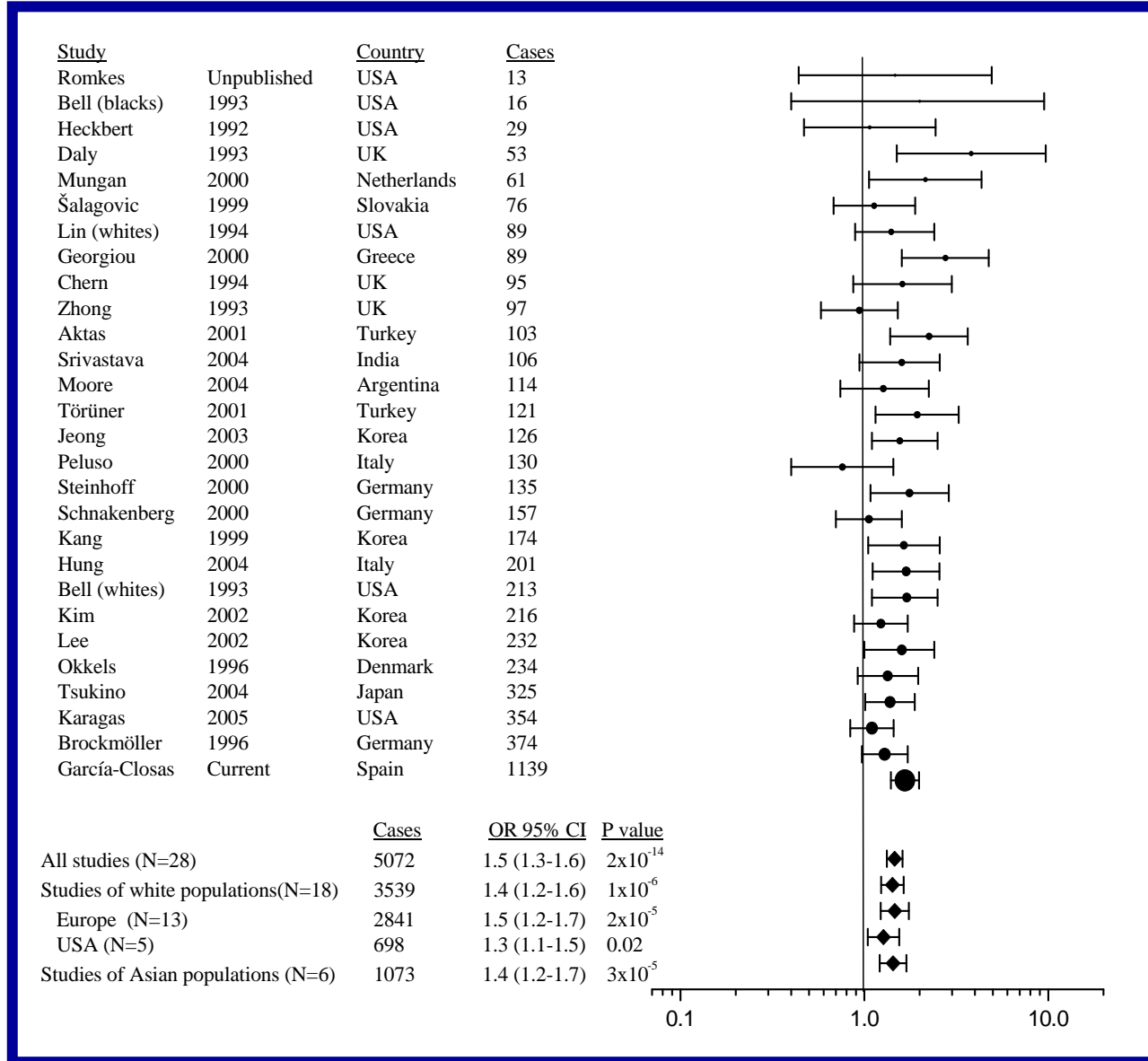
Role of GSTM1 in Benzo(a)pyrene Metabolism



GSTM1 null genotype and bladder cancer risk in the Spanish study

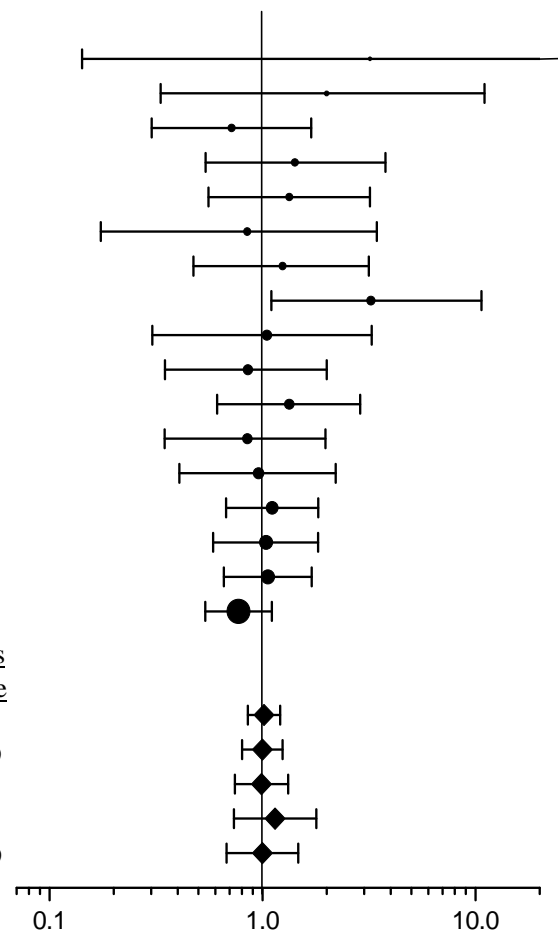
Genotype		Cases	Controls	OR	95%CI	p-value
<i>GSTM1</i>	+/+	70	107	1.0		
	+/-	352	454	1.2	(0.8-1.7)	0.38
	-/-	716	571	1.9	(1.4-2.7)	0.0002

Meta-Analysis of *GSTM1* Null Genotype and Bladder Cancer



Case-Only Meta-Analysis of *GSTM1* Null Genotype, Smoking, and Bladder Cancer Risk

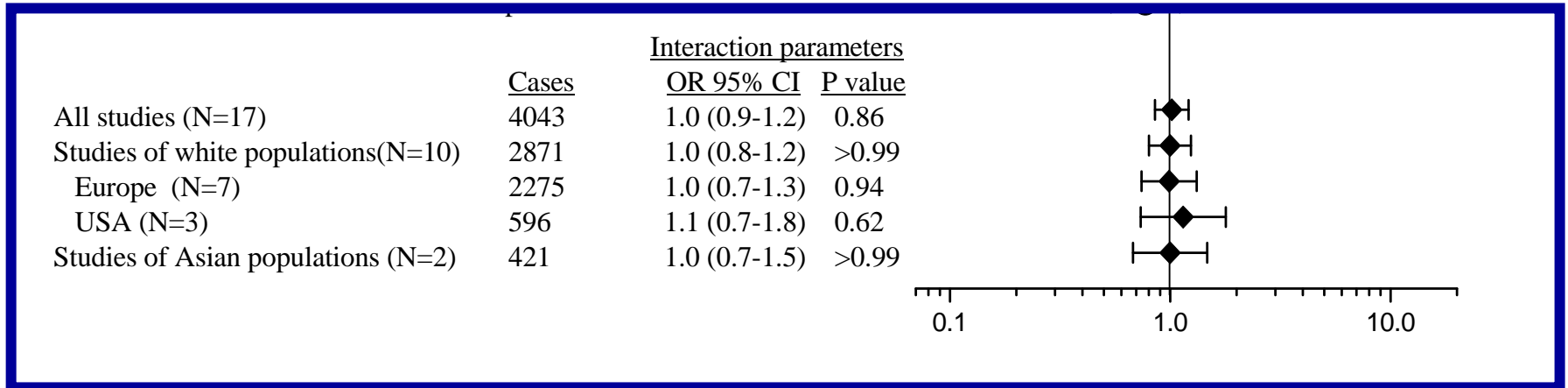
<u>Study</u>		<u>Country</u>	<u>Cases</u>
Heckbert	1992	USA	29
Daly	1993	UK	51
Aktas	2001	Turkey	103
Srivastava	2004	India	106
Moore	2004	Argentina	106
Chern	1994	UK	109
Törüner	2001	Turkey	111
Peluso	2000	Italy	148
Hung	2004	Italy	201
Lee	2002	Korea	203
Bell (whites)	1993	USA	213
Kang	1999	Korea	218
Okkels	1996	Denmark	253
Tsukino	2004	Japan	325
Karangas	2005	USA	354
Brockmüller	1996	Germany	374
García-Closas	Current	Spain	1139



Interaction parameters

	<u>Cases</u>	<u>OR 95% CI</u>	<u>P value</u>
All studies (N=17)	4043	1.0 (0.9-1.2)	0.86
Studies of white populations(N=10)	2871	1.0 (0.8-1.2)	>0.99
Europe (N=7)	2275	1.0 (0.7-1.3)	0.94
USA (N=3)	596	1.1 (0.7-1.8)	0.62
Studies of Asian populations (N=2)	421	1.0 (0.7-1.5)	>0.99

Case-Only Meta-Analysis of GSTM1 Null Genotype, Smoking, and Bladder Cancer Risk





Bladder Cancer

Excellent model to evaluate susceptibility and gene-environment interactions

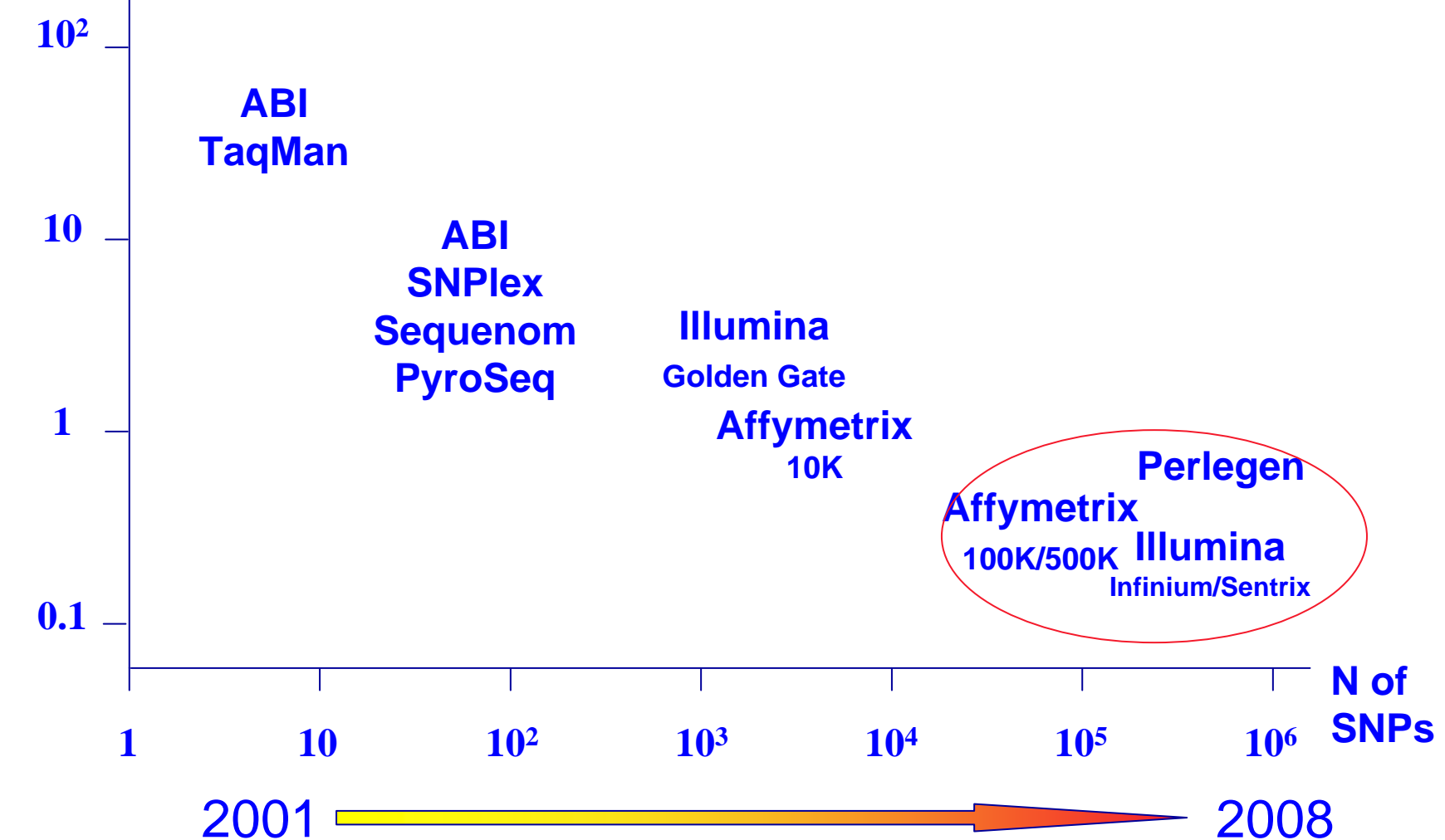
- **Major causes well described**
 - Tobacco smoking**
 - Occupational exposures (aromatic amines, diesel)**
 - Water contaminants (chlorinated by-products, arsenic)**
- **Good understanding of genetic variation in carcinogen metabolism**
- **Familial association not well explained**
- **Important etiologic and clinical applications of genomic data (e.g., recurrence, survival)**

Approaches to studying genetic susceptibility

- **Family studies**
- **Hypothesis-driven candidate gene studies**
- **Agnostic genome-wide scan studies**

Progress in genotyping technology leads to genome-wide scans

Cost per genotype
Cents
(USD)





Ongoing bladder cancer genome-wide scans

- **NCI - Spanish Bladder Cancer Study**
(1M SNP, 400K CNV Illumina chip)

New England Bladder Cancer Study

Several cohort studies

- **MD Anderson**
- **deCODE** (8q24 association)

Exposure and genotype misclassification

Sensitivity: probability of correctly identifying exposed/susceptible subjects.

Specificity: probability of correctly identifying unexposed/non-susceptible subjects.

Effect of exposure and genotype misclassification on sample size to detect an interaction*

Exposure accuracy Sensitivity	Gene accuracy Sensitivity	No. of cases for 80% power
100%	100%	718
80%	100%	1,600
100%	95%	900
80%	95%	2,044

* Interaction model: $OR_G = 2.0$; $OR_E = 2.0$; $OR_{GE} = 8.0$
 $P(E)=50\%$; $P(G)=50\%$
Genotype and exposure assessment specificity = 100%.

Conclusions

- Biomarkers offer new opportunities to overcome some of the limitations of epidemiology
- Assess sources of bias (misclassification etc.) and confounding
- Assess improvement over traditional methods (if available)
- Apply a sound study design