National Cancer Institute

Studying Interactions Between Environmental Exposures and Genetic Variants: Examples and Lessons Learned

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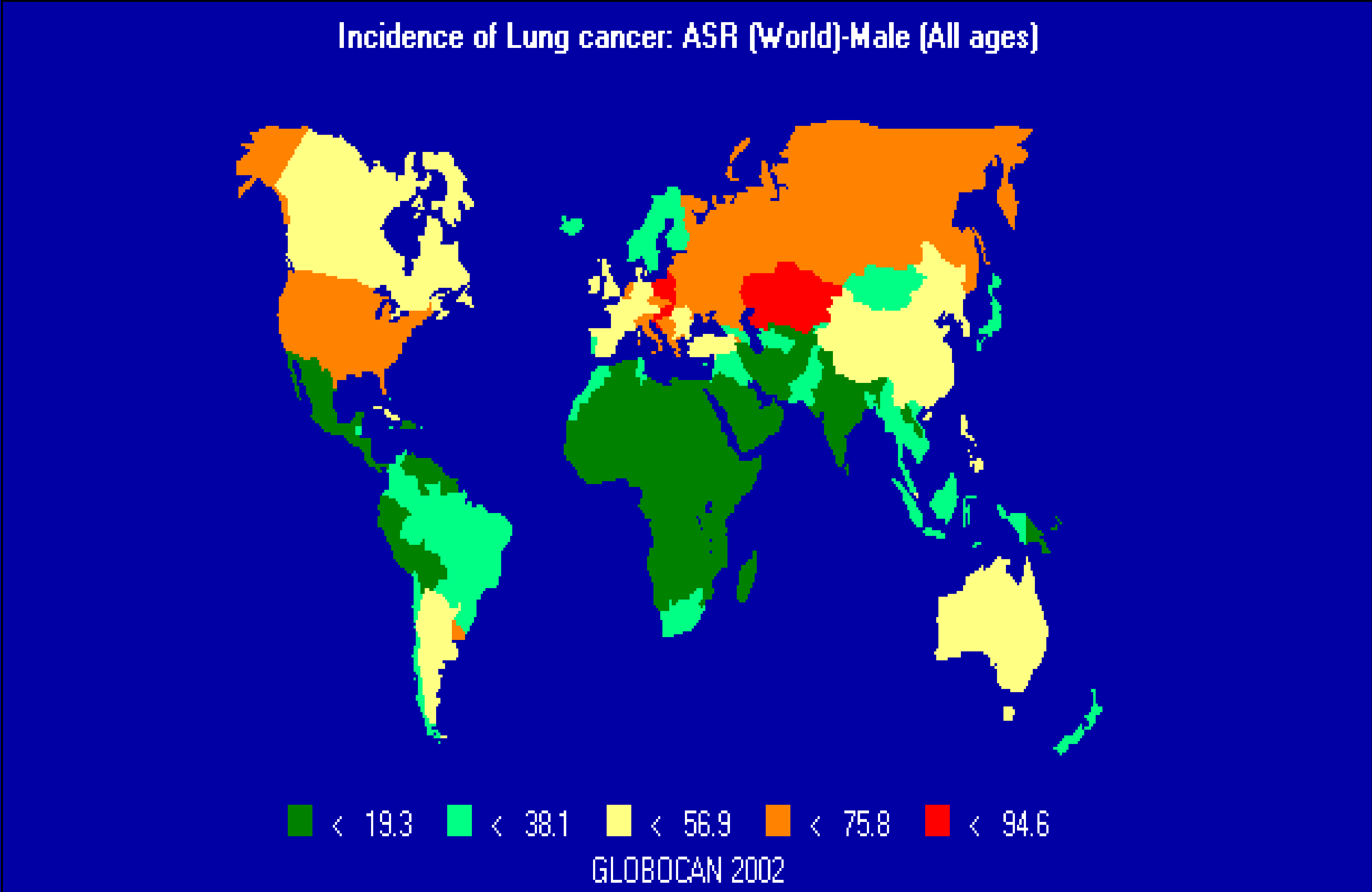
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

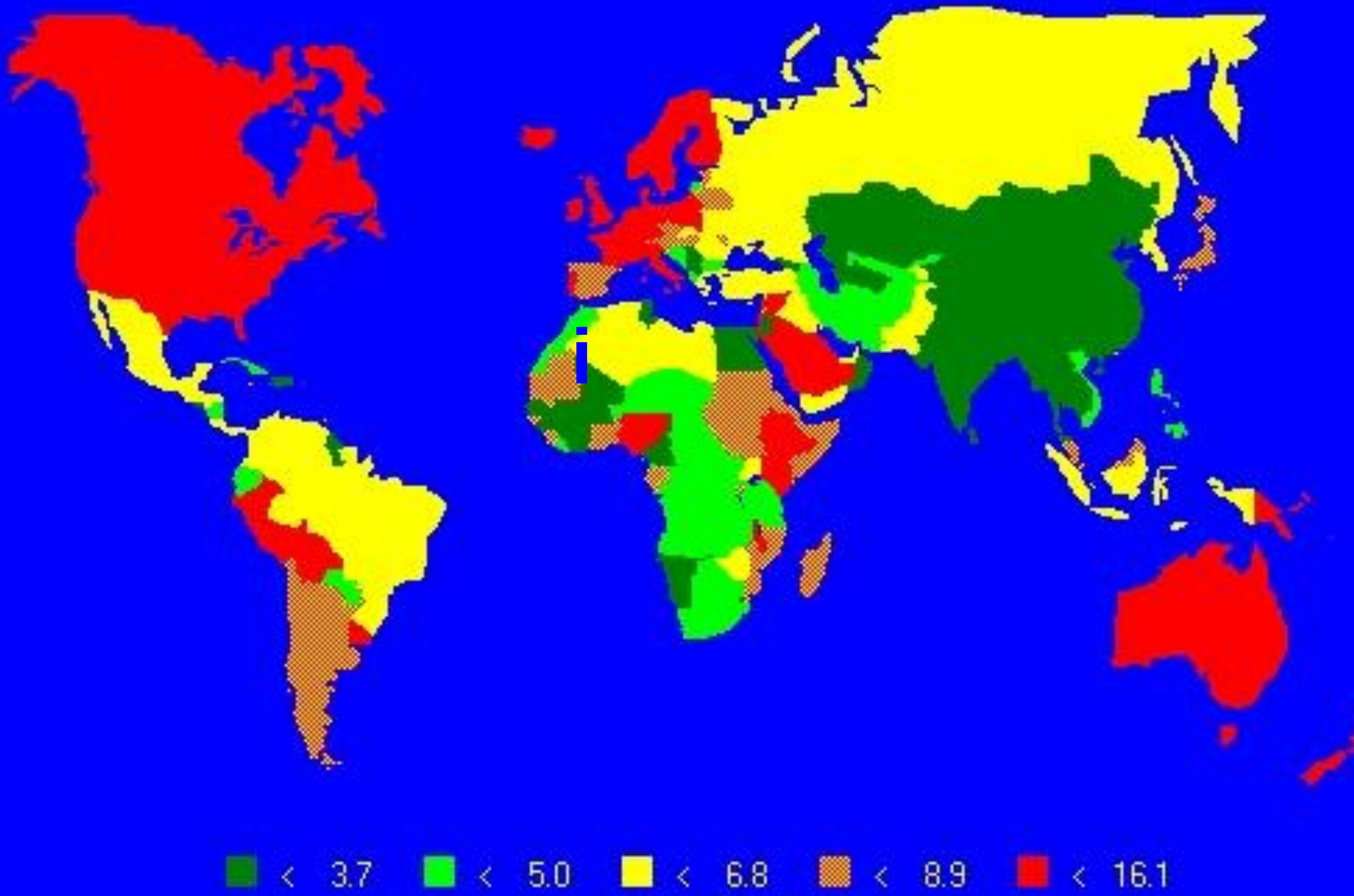
Overwhelming evidence that most cancer is caused by environmental exposures

- Geographic variation
- Migration studies
- Secular trends in fixed populations
- Analytic epidemiology
- Experimental models

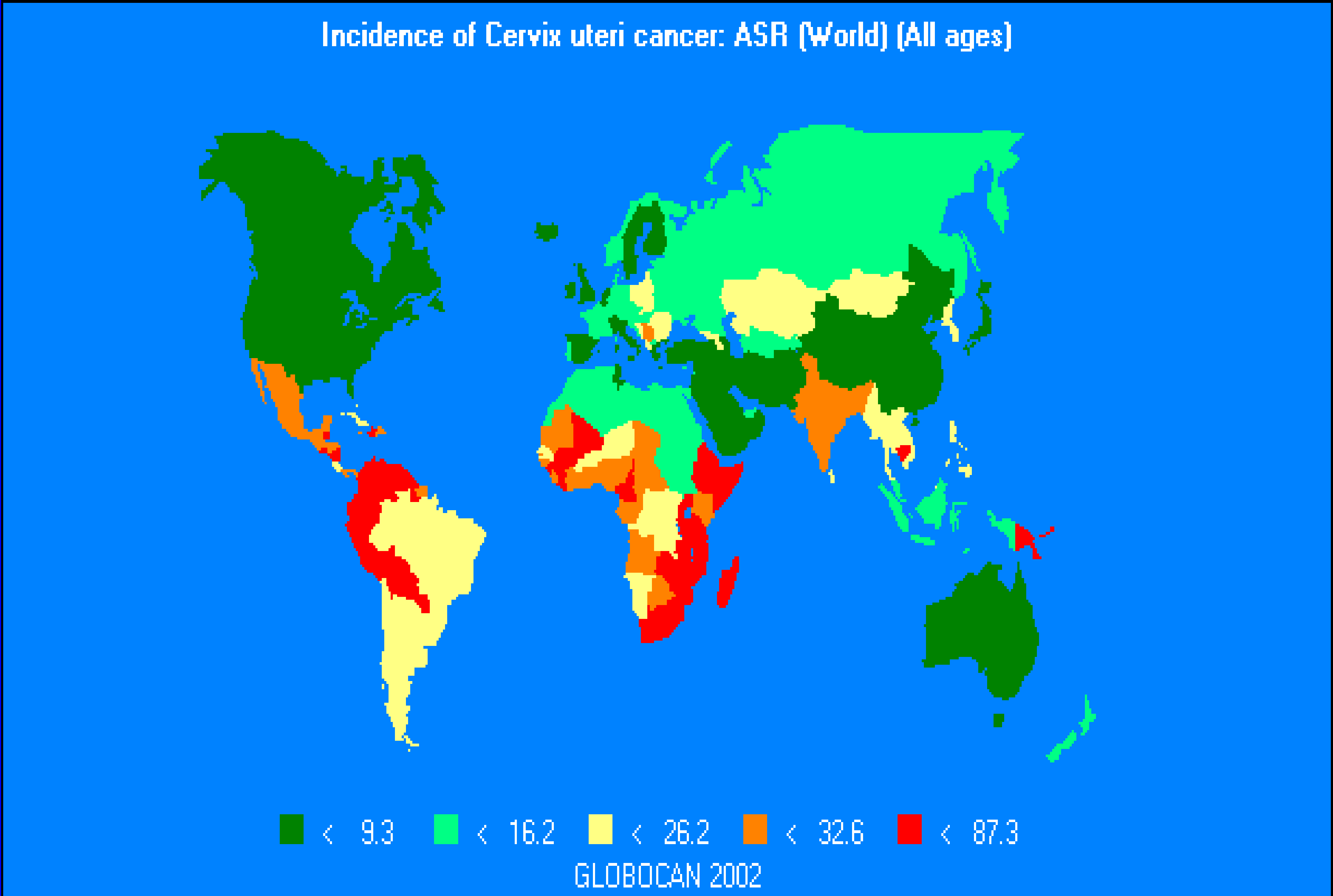
Incidence of Lung Cancer Among Males



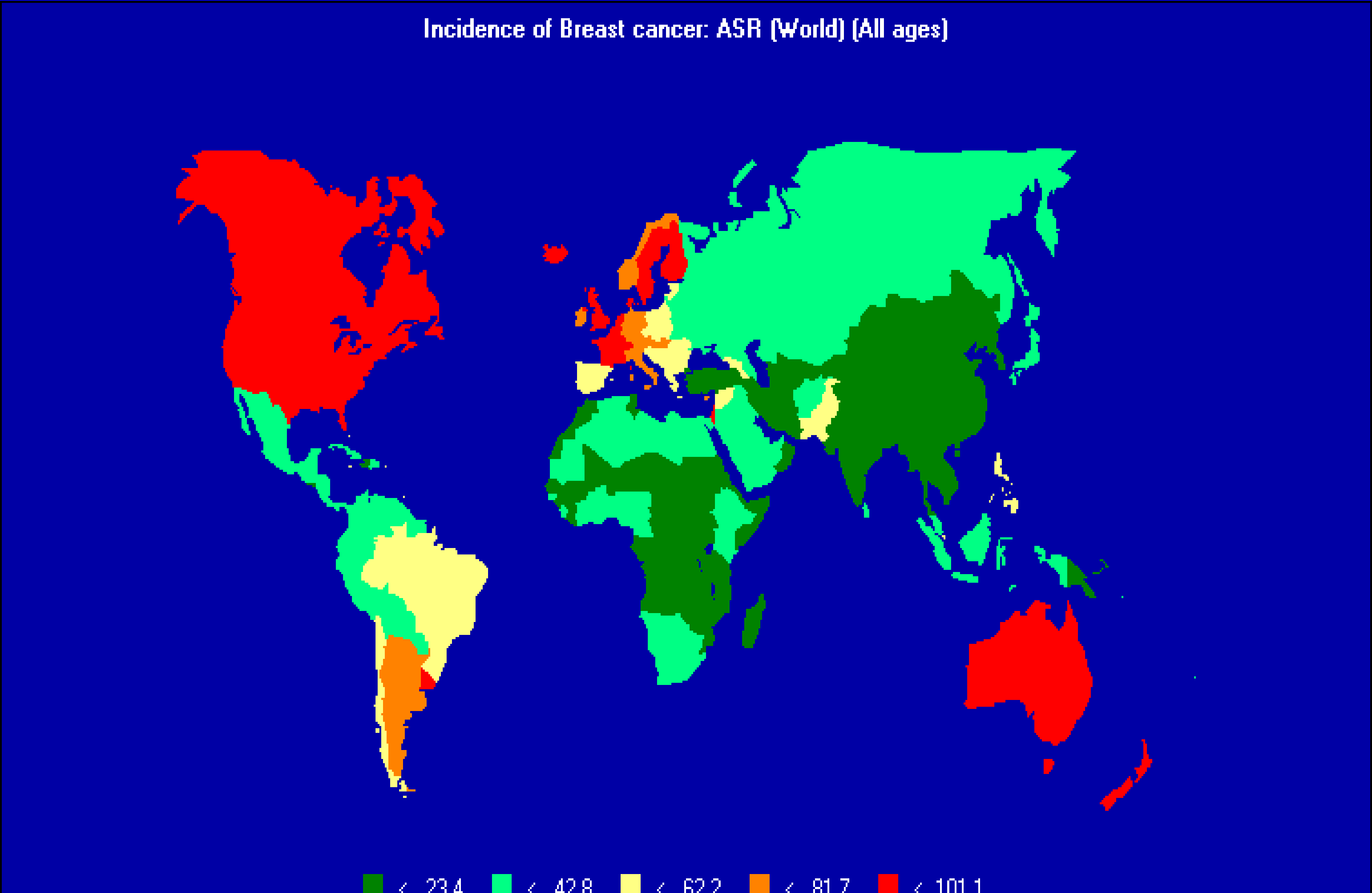
Incidence of Non-Hodgkin Lymphoma Among Men



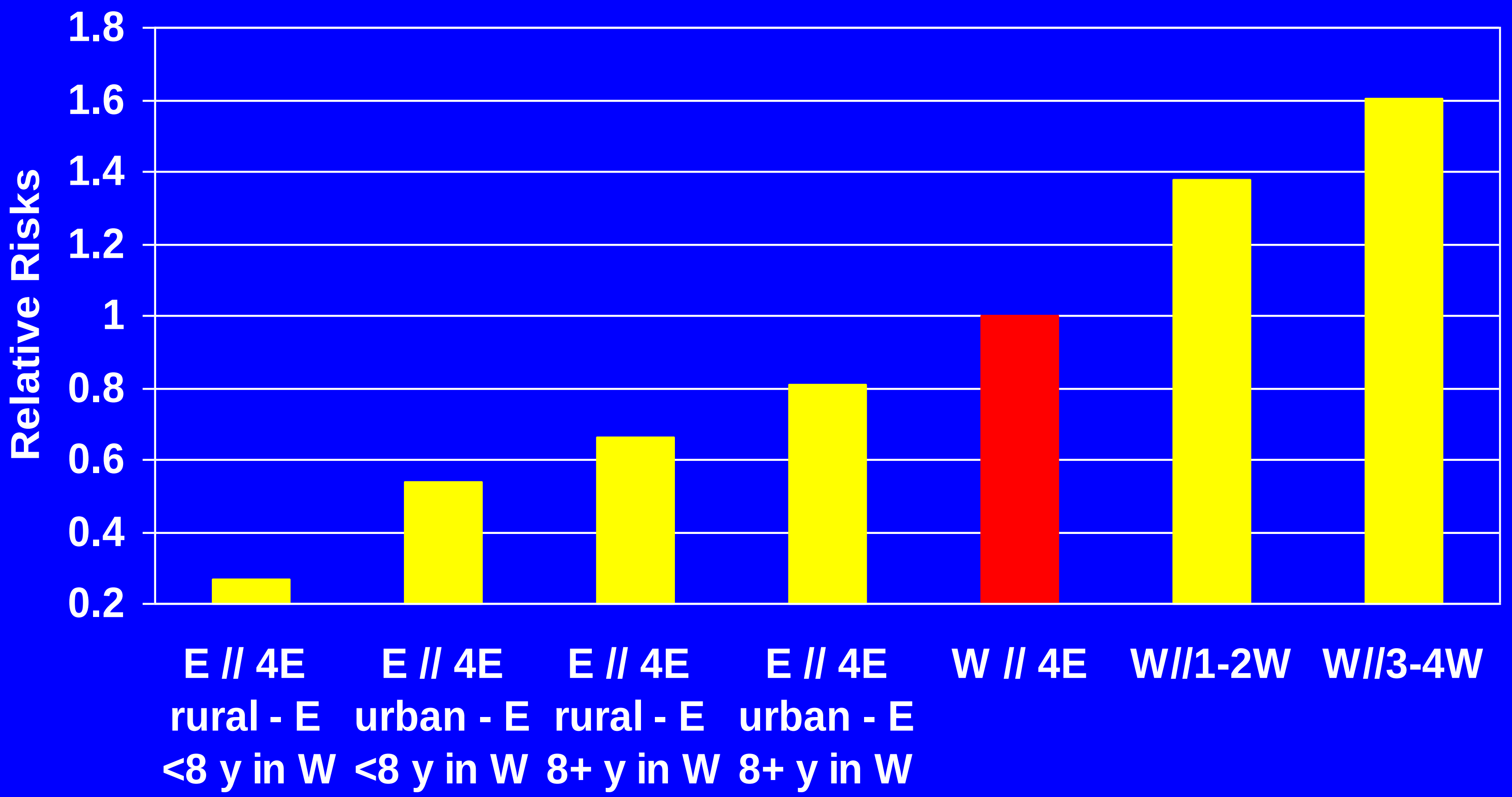
Incidence of Cervical Cancer



Incidence of Breast Cancer Among Women

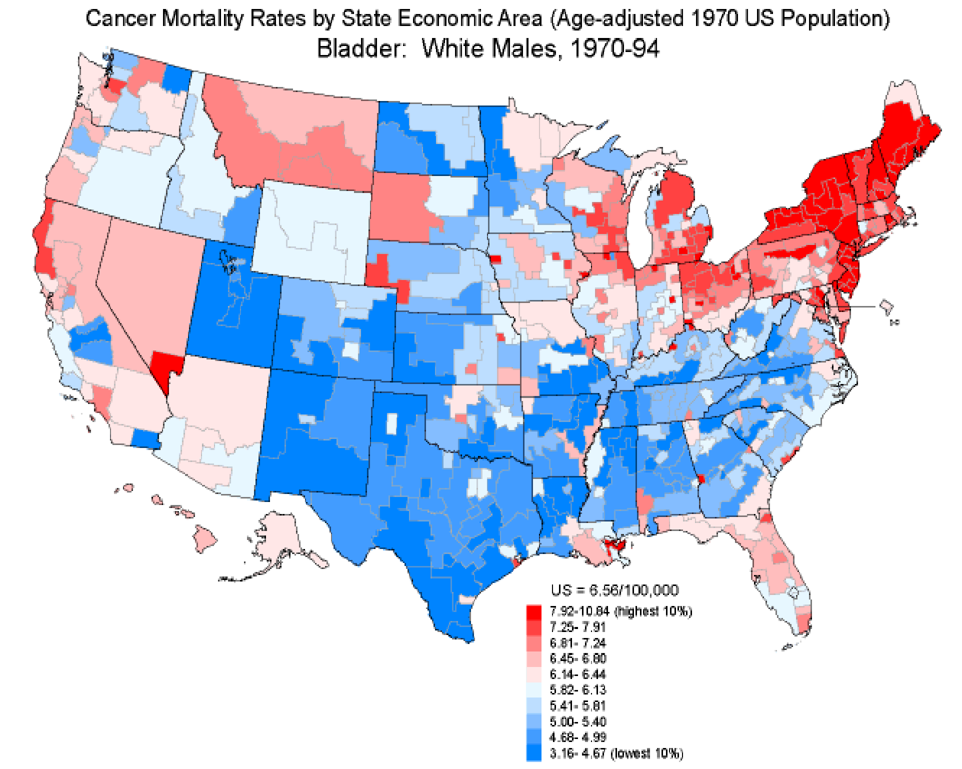


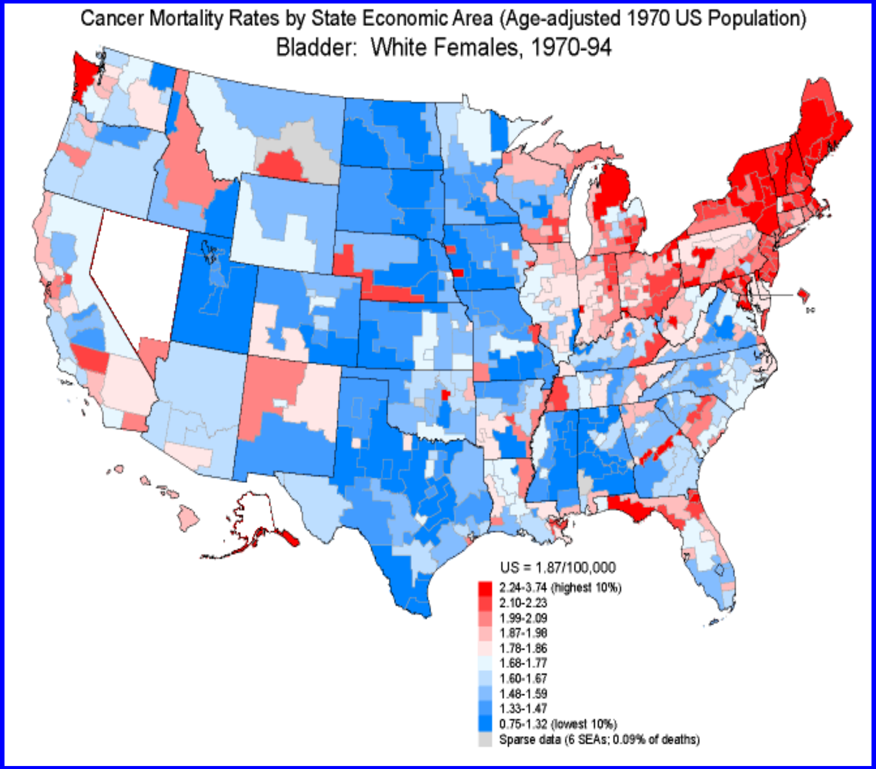
RRs of Breast Cancer in Asian-American Women by Migration History



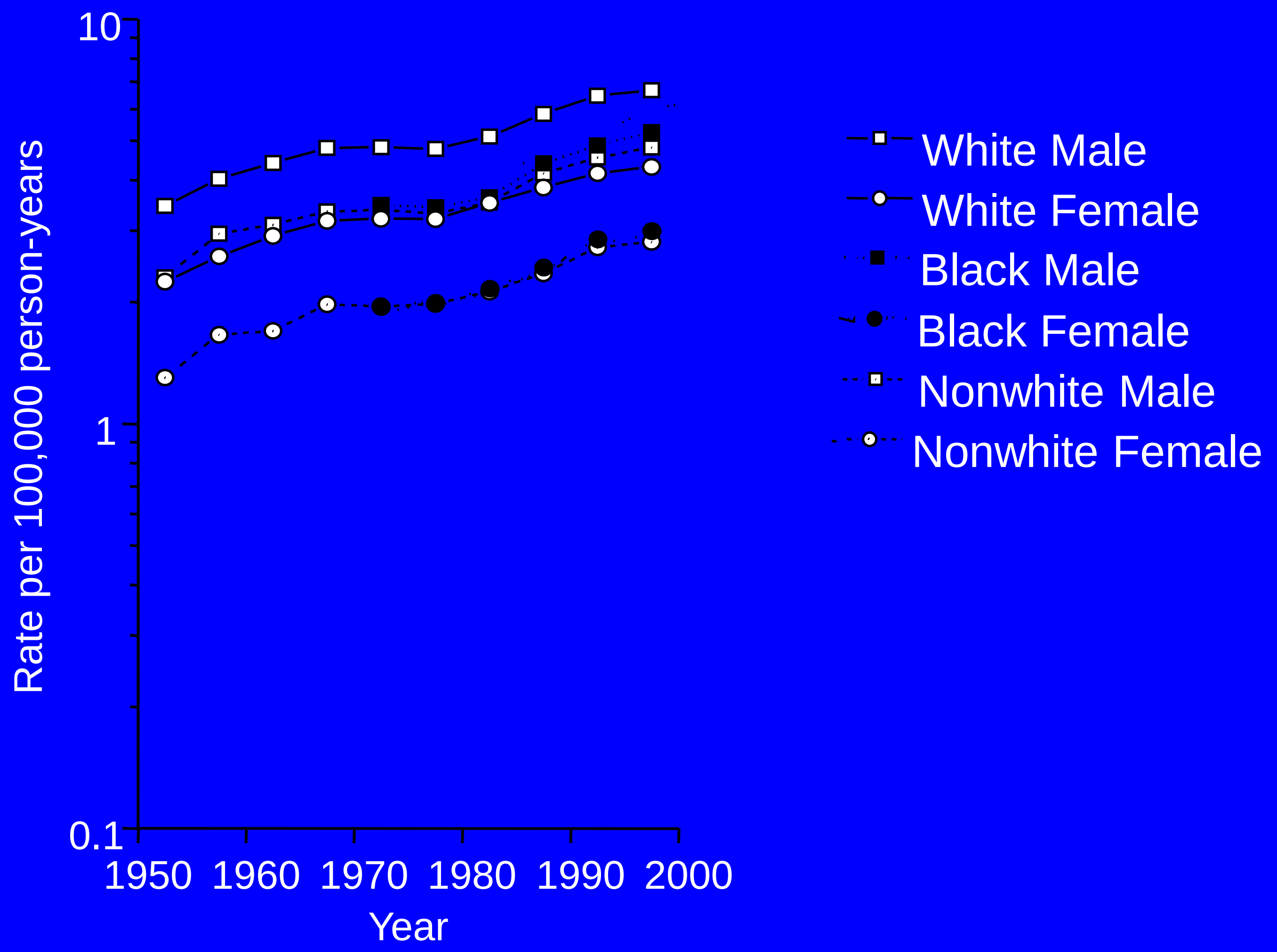
Ziegler, R. et al. JNCI 1993

Excess Bladder Cancer Mortality in Males and Females in New England





Non-Hodgkin Lymphoma Mortality Patterns in the U.S. by Race and Sex, 1950-54 to 1995-1999



Why study genetic modification of the fundamental forces that drive most cancer risk in most populations?

- Obtain mechanistic insight
- Clarify dose-response relationships, and more effectively evaluate low levels of risk
- Identify new environmental health hazards
- Develop more effective prevention and treatment strategies

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

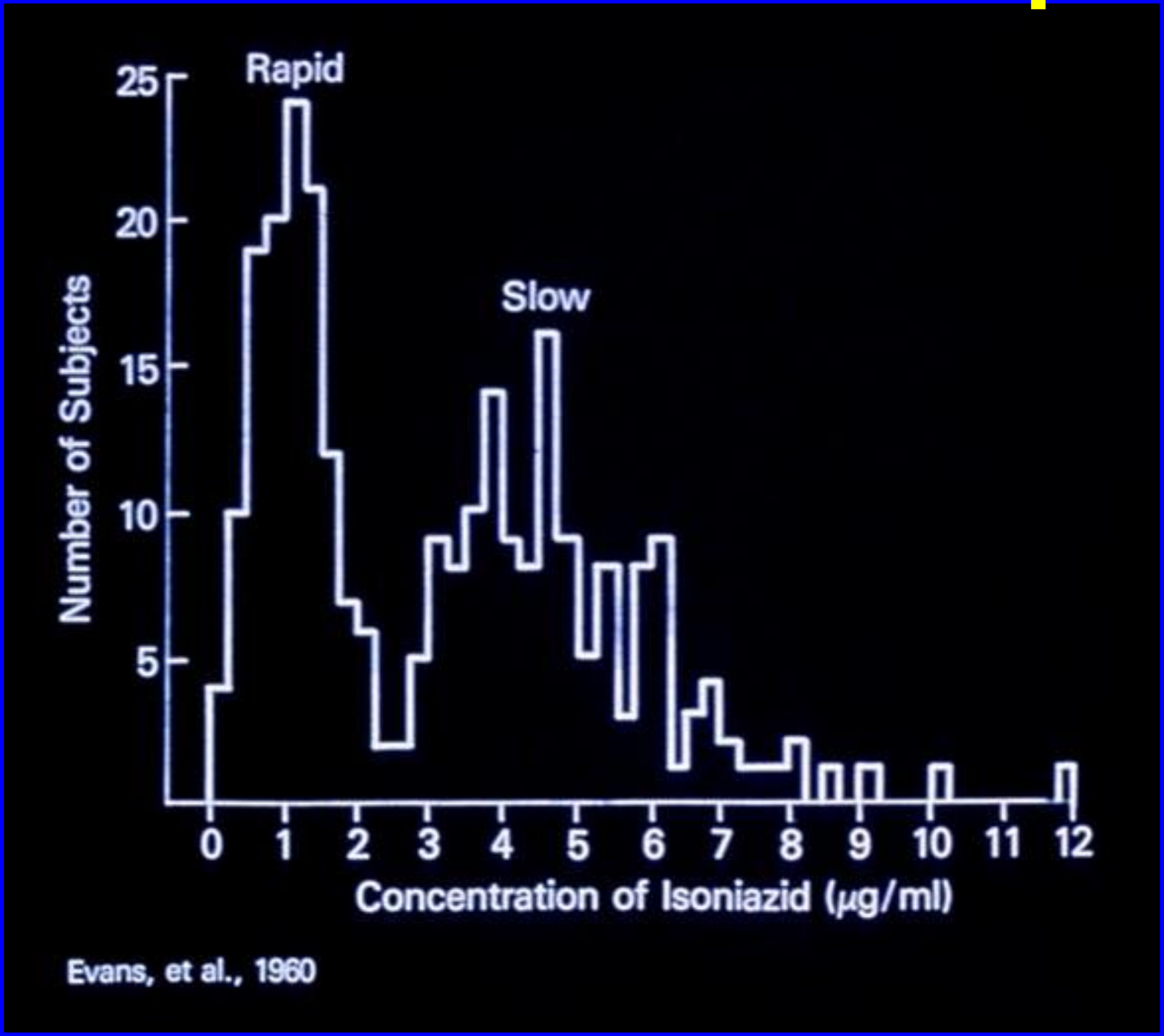
Data Resources	Response Rate	Specific Areas
CAPI	86%	Demographics Smoking Occupation/Environmental Family history Medical/Drugs
Blood/Buccal Cell	95%	Genetic Susceptibility Functional Assays
Diet Qx.	72%	Fluid intake Food Frequency Food Carcinogens
Urination Diary	60%	Urine pH Urinary freq
Toenails	77%	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
NAT2	Rapid/Intermediate	406	493	1.0		
	Slow	728	637	1.4	(1.2-1.7)	0.0002

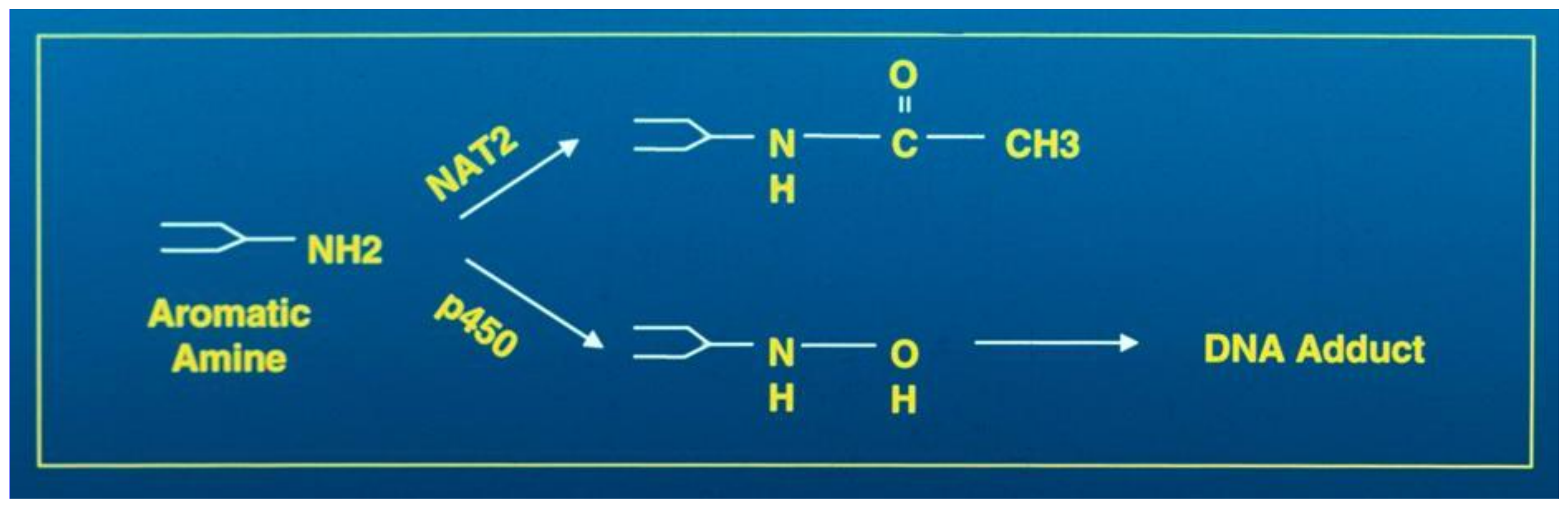
Garcia-Closas M et al. *Lancet,* 2005

Acetylation polymorphism - Isoniazid clearance from plasma



Detoxification of Aromatic Amines by N-Acetylation

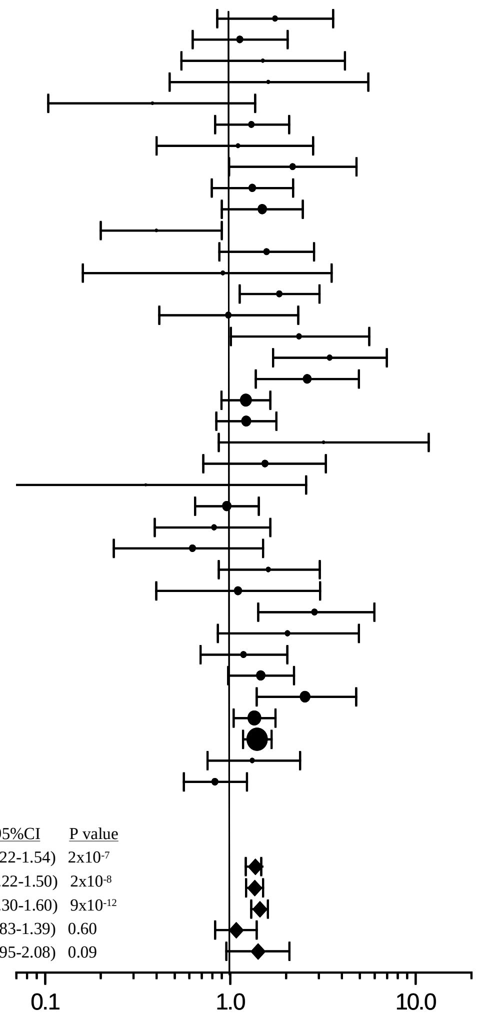
(Lower et al., EHP 1979)



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

Study	Year	Country	Cases
Lower	1979	Denmark	71
Lower	1979	Sweden	115
Lower	1979	USA	34
Woodhouse	1982	UK	30
Miller	1983	USA	26
Evans	1983	UK	100
Cartwright	1984	Portugese	47
Hanssen	1985	Germany	105
Ladero	1985	Spain	130
Mommsen	1985	UK	228
Karakaya	1986	Turkey	23
Kaisary	1987	UK	98
Horai	1989	Japan	51
Roots	1989	Germany	102
Lee	1994	Korea	98
Ishizu	1995	Japan	71
Dewan	1995	India	77
Risch	1995	UK	189
Brockmoller	1996	Germany	374
Okkels	1997	Denmark	254
Su	1998	Taiwan	27
Peluso	1998	Italy	114
Taylor (Black)	1998	USA	15
Taylor (Whites)	1998	USA	215
Hsieh	1999	Taiwan	74
Kim	2000	Korea	112
Jaskula-Sztul	2001	Poland	56
Kontani	2001	Japan	149
Giannakopoulos	2002	Greece	89
Hao	2004	China	69
Mittal	2004	India	101
Hung	2004	Italy	201
Tsukino	2004	Japan	325
Gu	2005	USA	504
Garcia-Closas	2005	Spain	1134
McGrath (NHS)	2006	USA	63
McGrath (HPFS)	2006	USA	124

	Cases	OR 95%CI	P value
All studies (N=36)	5594	1.37 (1.22-1.54)	$2x10^{-7}$
Studies of Caucasian (N=24)	4403	1.35 (1.22-1.50)	$2x10^{-8}$
Europe (N=18)	3437	1.44 (1.30-1.60)	$9x10^{-12}$
USA (N=6)	966	1.07 (0.83-1.39)	0.60
Studies of Asians (N=9)	975	1.41 (0.95-2.08)	0.09



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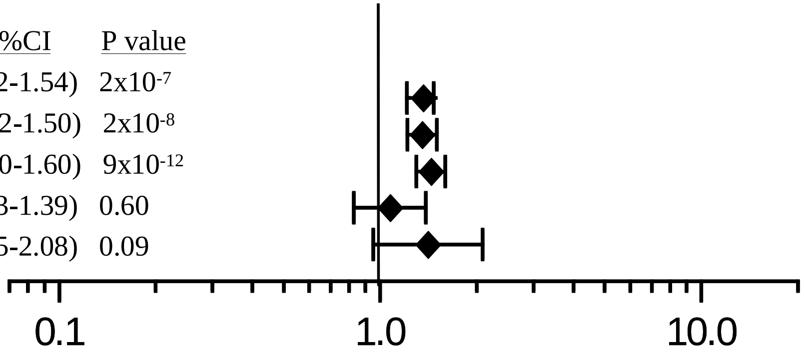

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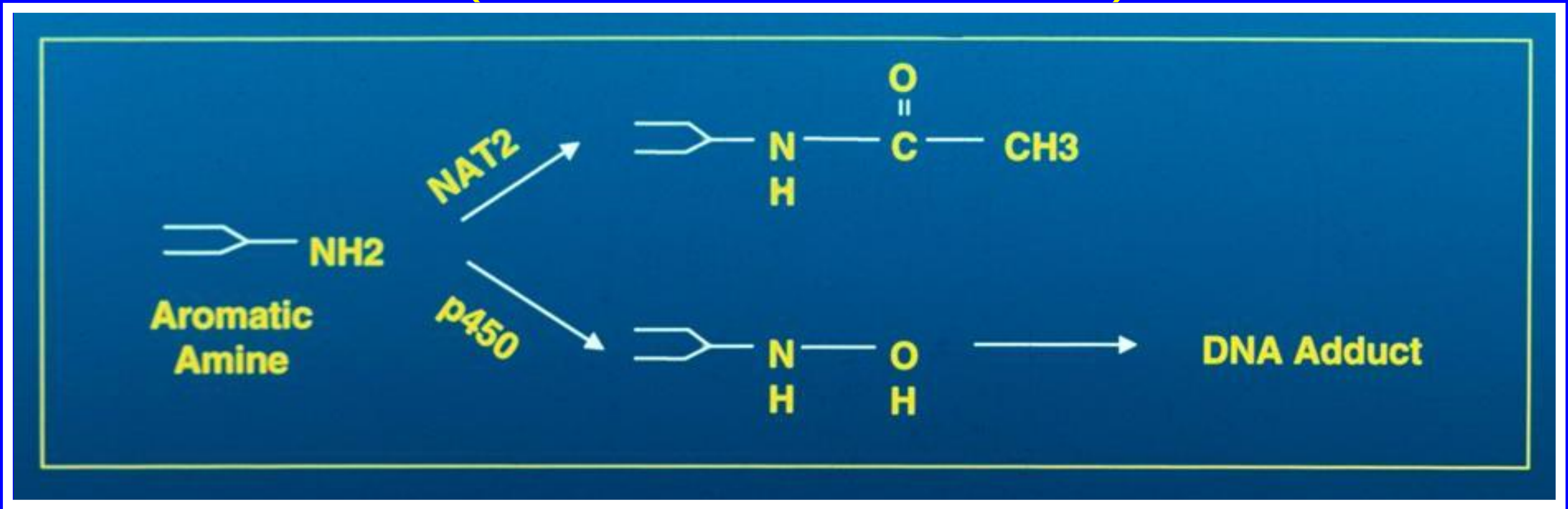
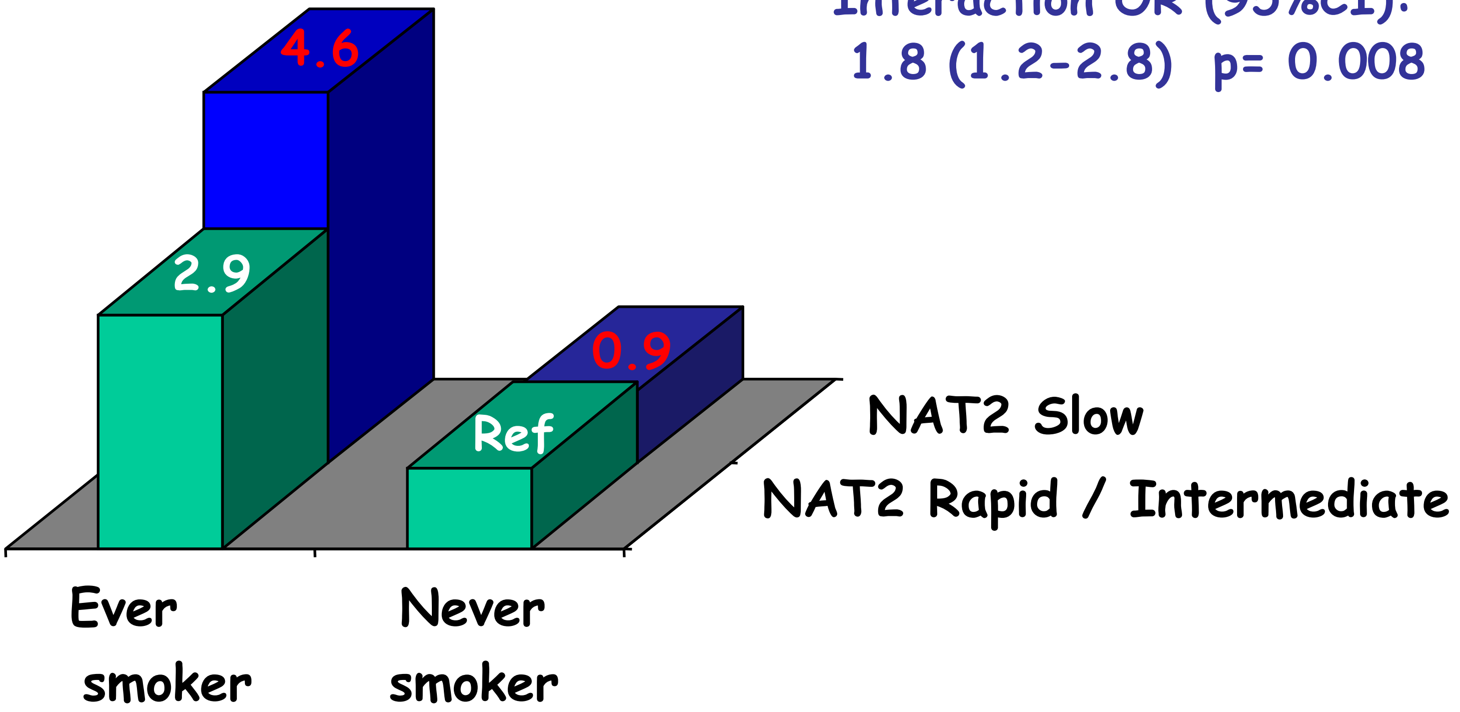


Multiplicative interaction between *NAT2* and cigarette smoking: Spanish study



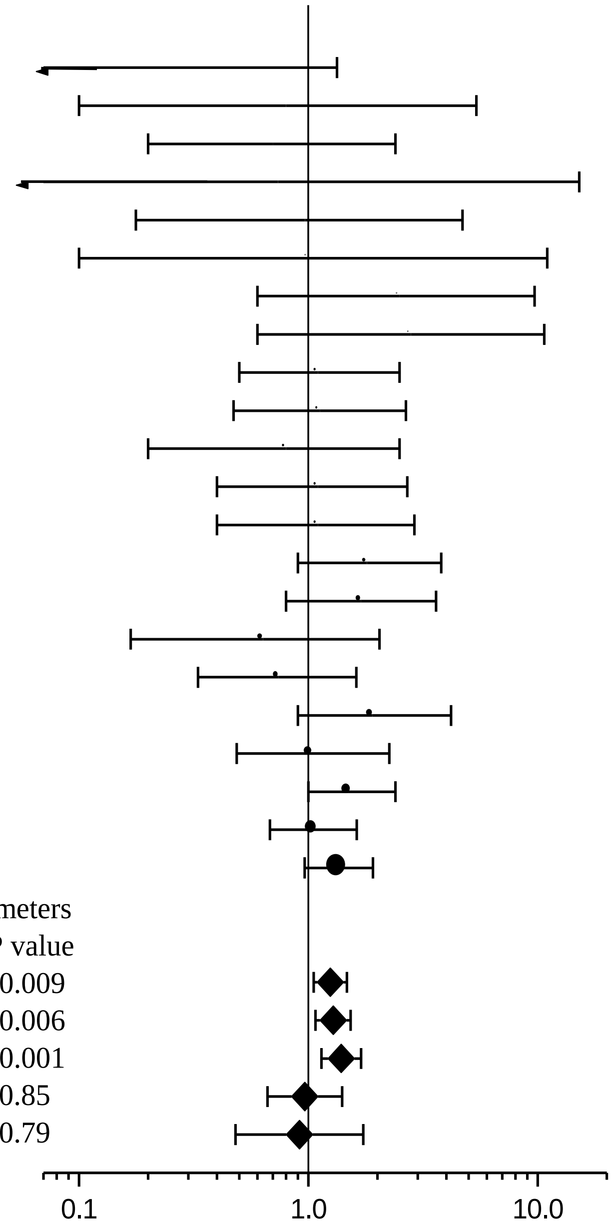
Interaction OR (95%CI):
1.8 (1.2-2.8) p= 0.008

Garcia-Closas M et al. *The Lancet*, 2005

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

Study		Country	Cases
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

	Cases	Interaction parameters OR 95% CI	P value
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



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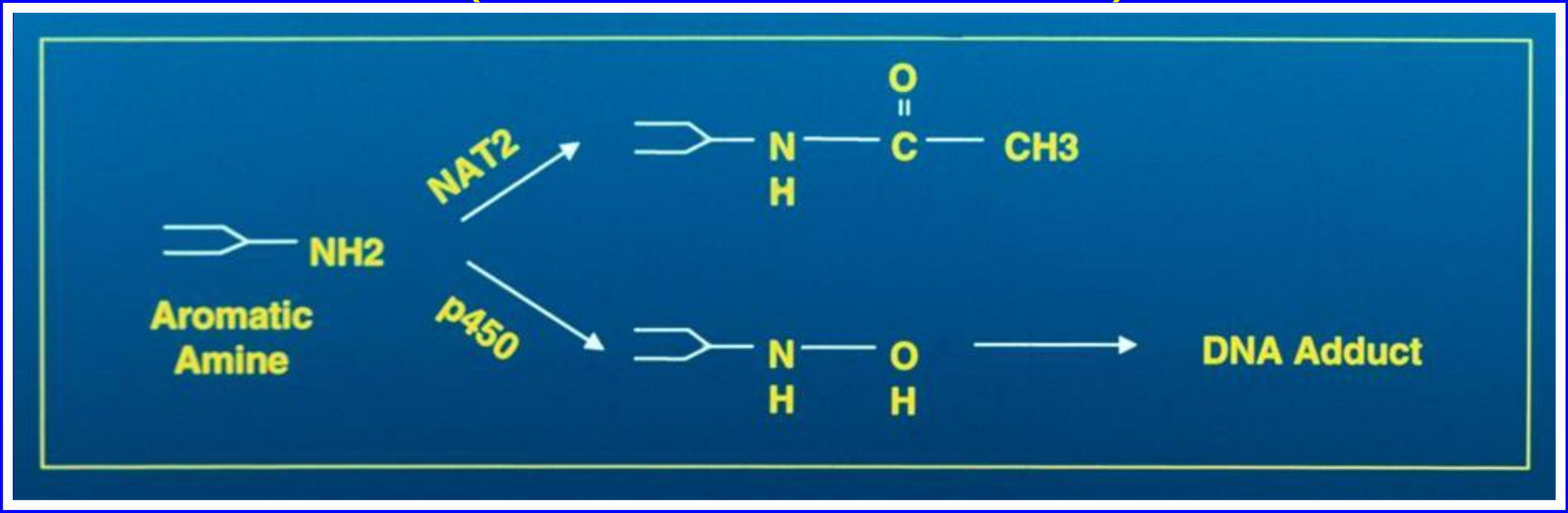
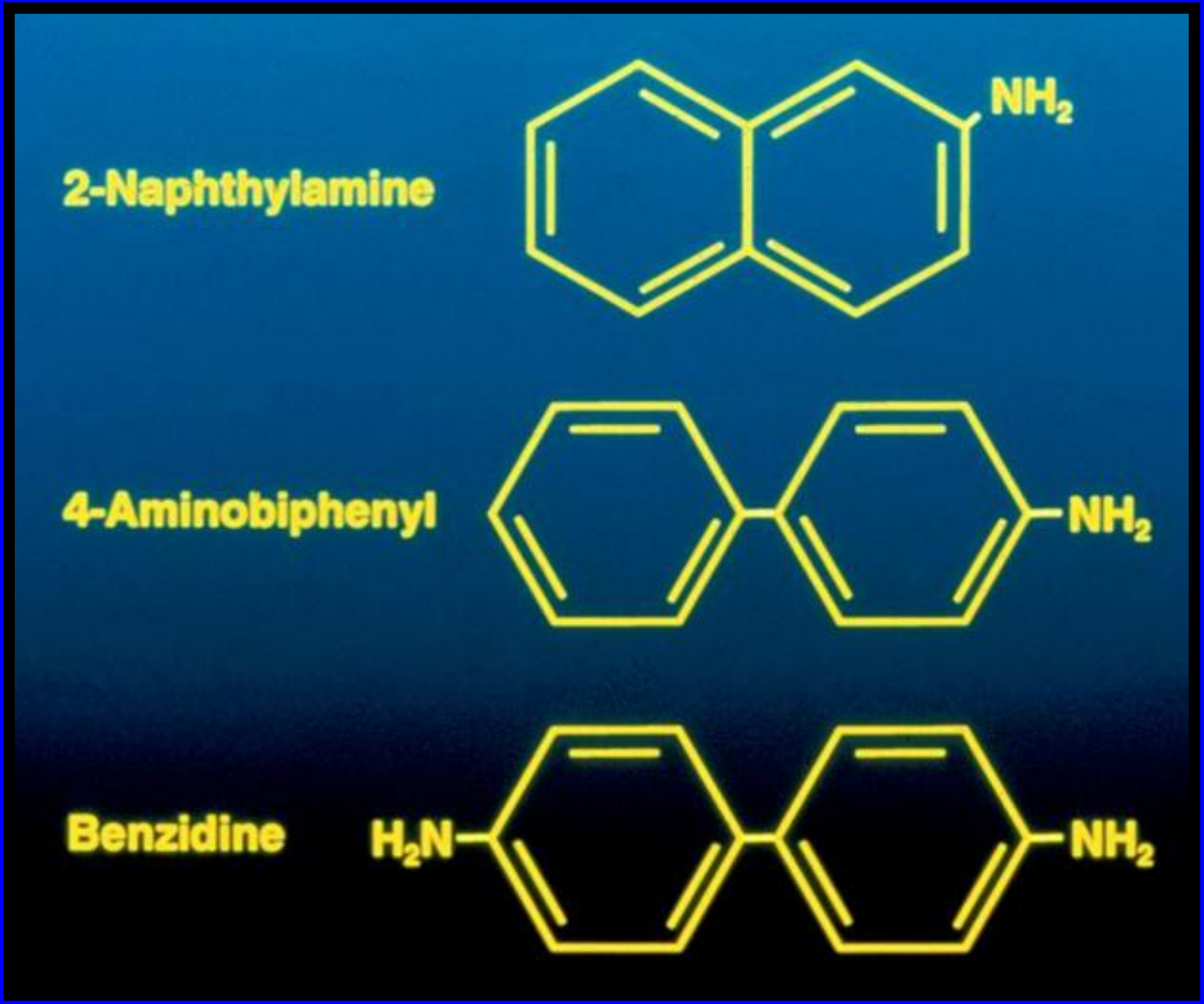


Carcinogenic Aromatic Amines



NAT2 Genotype and Bladder Cancer Risk, By Aromatic Amine exposure

- Benzidine-exposed workers:

  OR = **0.3**, 95% CI: 0.1-1.0

  Non-smokers:

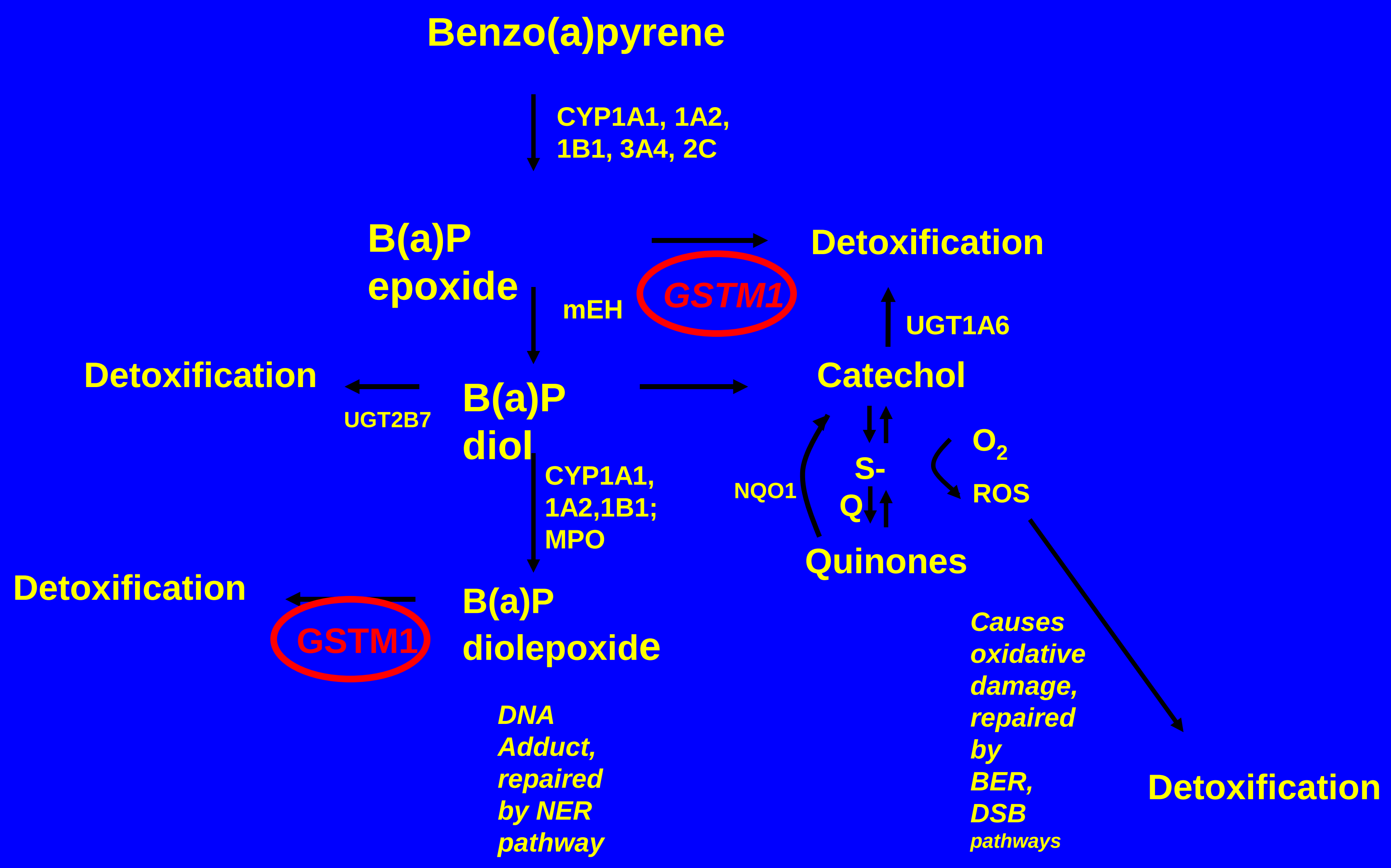
  OR = **0.9**, 95%CI: 0.6-1.3

- Smokers:

  OR = **1.6**, 95%CI: 1.3-1.9

(Hayes et al., Carcinogenesis 1993; Garcia-Closas et al., Lancet 2005; Carreon et al., IJC, 2006; Rothman et al., IJE, 2007)

Role of GSTM1 in Benzo(a)pyrene Metabolism



GSTM1 null genotype and bladder cancer risk in the Spanish study

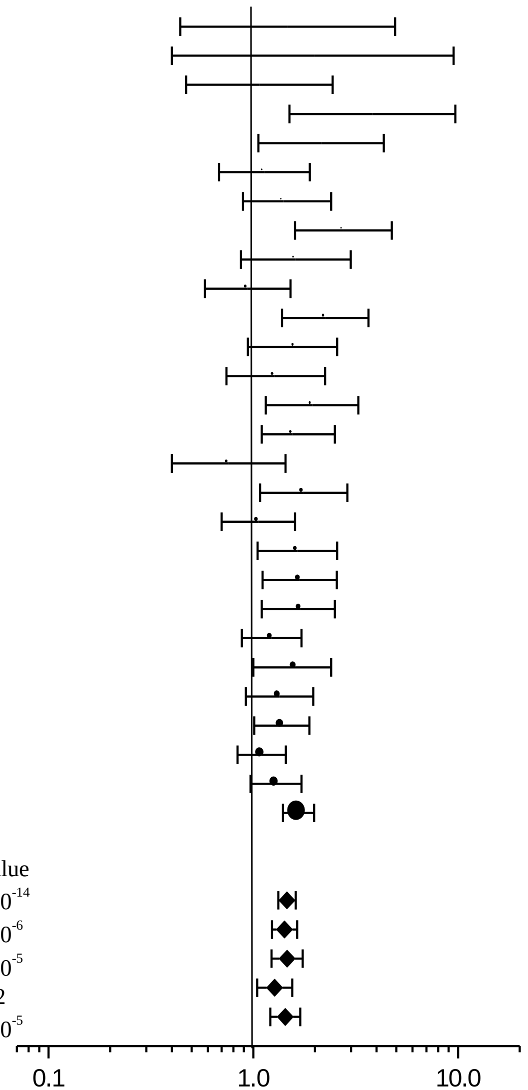
Genotype		Cases	Controls	OR	95%CI	p-value
GSTM1	**+/+**	**70**	**107**	**1.0**		
	+/-	**352**	**454**	**1.2**	**(0.8-1.7)**	**0.38**
	-/-	**716**	**571**	**1.9**	**(1.4-2.7)**	**0.0002**

Garcia-Closas M et al. *Lancet,* 2005

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

Study		Country	Cases
Romkes	Unpublished	USA	13
Bell (blacks)	1993	USA	16
Heckbert	1992	USA	29
Daly	1993	UK	53
Mungan	2000	Netherlands	61
Šalagovic	1999	Slovakia	76
Lin (whites)	1994	USA	89
Georgiou	2000	Greece	89
Chern	1994	UK	95
Zhong	1993	UK	97
Aktas	2001	Turkey	103
Srivastava	2004	India	106
Moore	2004	Argentina	114
Törüner	2001	Turkey	121
Jeong	2003	Korea	126
Peluso	2000	Italy	130
Steinhoff	2000	Germany	135
Schnakenberg	2000	Germany	157
Kang	1999	Korea	174
Hung	2004	Italy	201
Bell (whites)	1993	USA	213
Kim	2002	Korea	216
Lee	2002	Korea	232
Okkels	1996	Denmark	234
Tsukino	2004	Japan	325
Karagas	2005	USA	354
Brockmöller	1996	Germany	374
García-Closas	Current	Spain	1139

	Cases	OR 95% CI	P value
All studies (N=28)	5072	1.5 (1.3-1.6)	$2x10^{-14}$
Studies of white populations(N=18)	3539	1.4 (1.2-1.6)	$1x10^{-6}$
Europe (N=13)	2841	1.5 (1.2-1.7)	$2x10^{-5}$
USA (N=5)	698	1.3 (1.1-1.5)	0.02
Studies of Asian populations (N=6)	1073	1.4 (1.2-1.7)	$3x10^{-5}$



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

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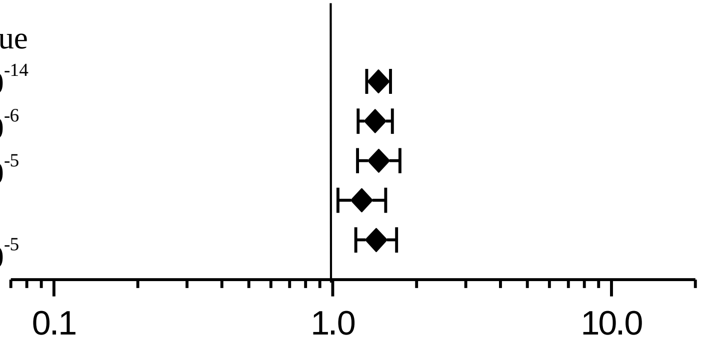
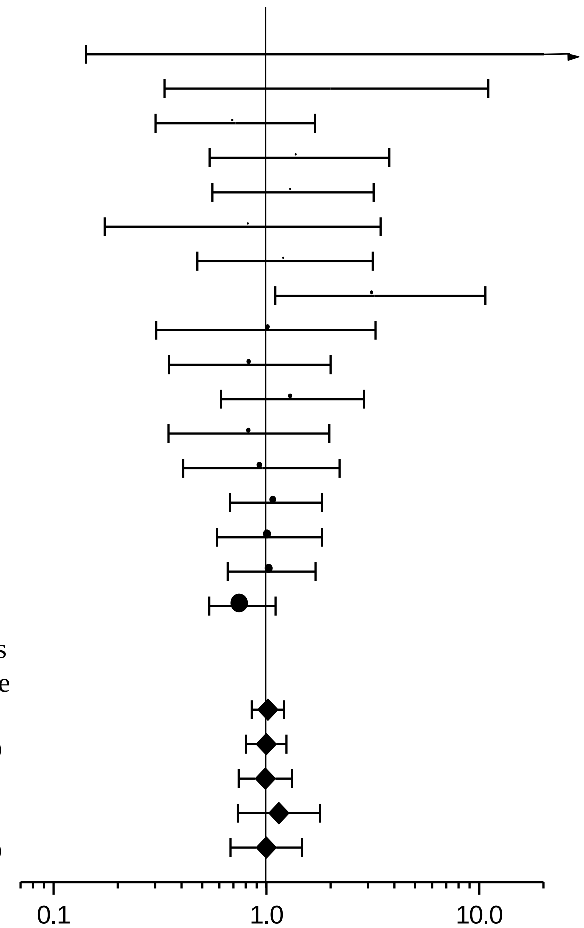


Case-Only Meta-Analysis of *GSTM1* Null Genotype, Smoking, and Bladder Cancer Risk

Study		Country	Cases
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
Brockmöller	1996	Germany	374
García-Closas	Current	Spain	1139

		Interaction parameters	
	Cases	OR 95% CI	P value
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



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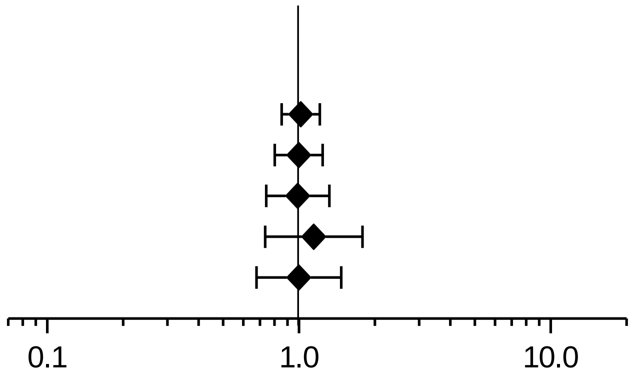


Exposure assessment implications & wish list

1) Improved biomonitoring of tobacco constituents, data on intraindividual variation, repeat samples

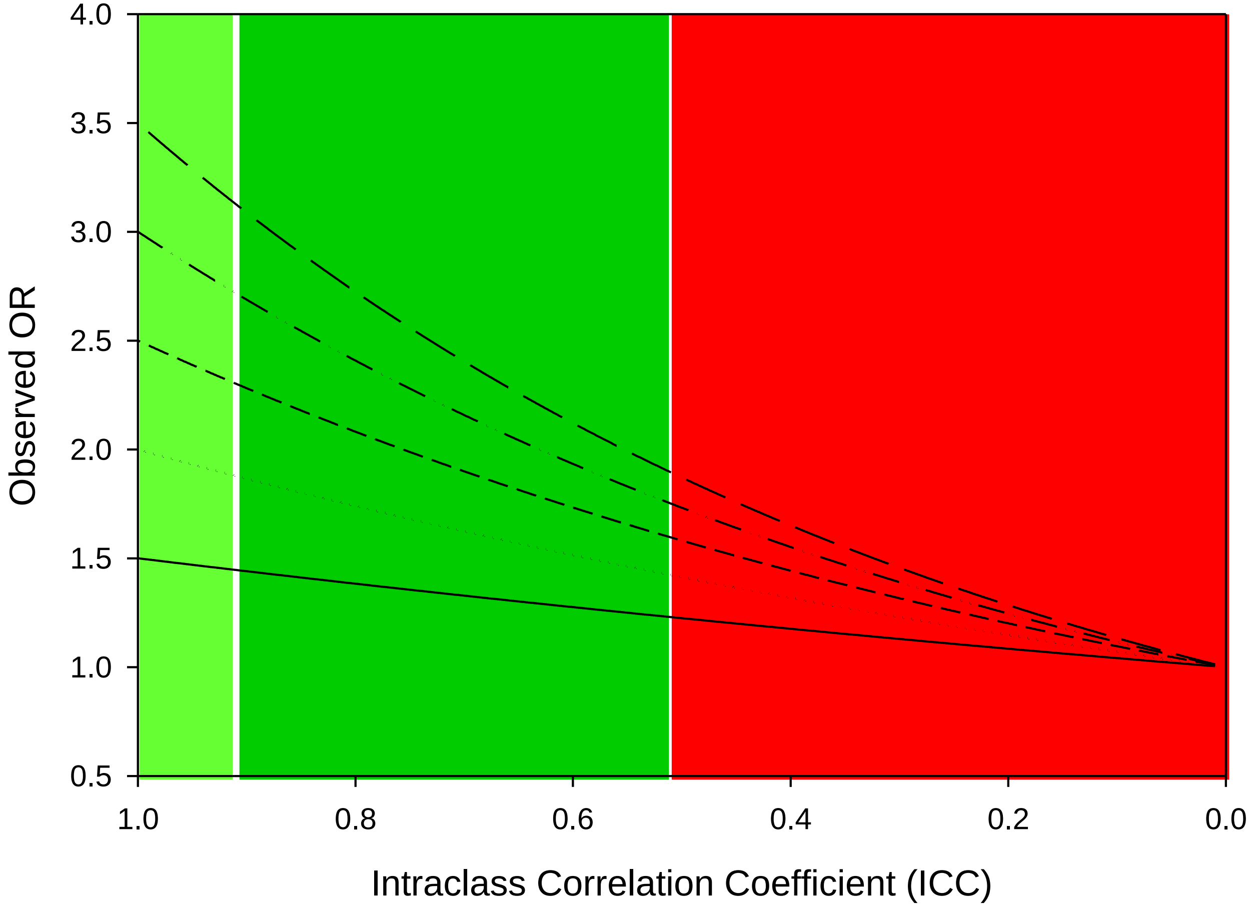
2) Broad "omic" approach to explore potential exogenous and endogenous factors that might interact with GSTM1

Fundamental metric of measurement error – the Intraclass Correlation Coefficient (ICC)

<u>True between subject variation</u>

True between subject variation + all unwanted sources of variation (e.g., intraindividual and analytic variance)

Impact of the ICC on the Observed Odds Ratio (OR) Given True OR for Disease of 1.5, 2.0, 2.5, 3.0 and 3.5.



Exposure and genotype misclassification

Sensitivity: probability of correctly identifying exposed/susceptible subjects.

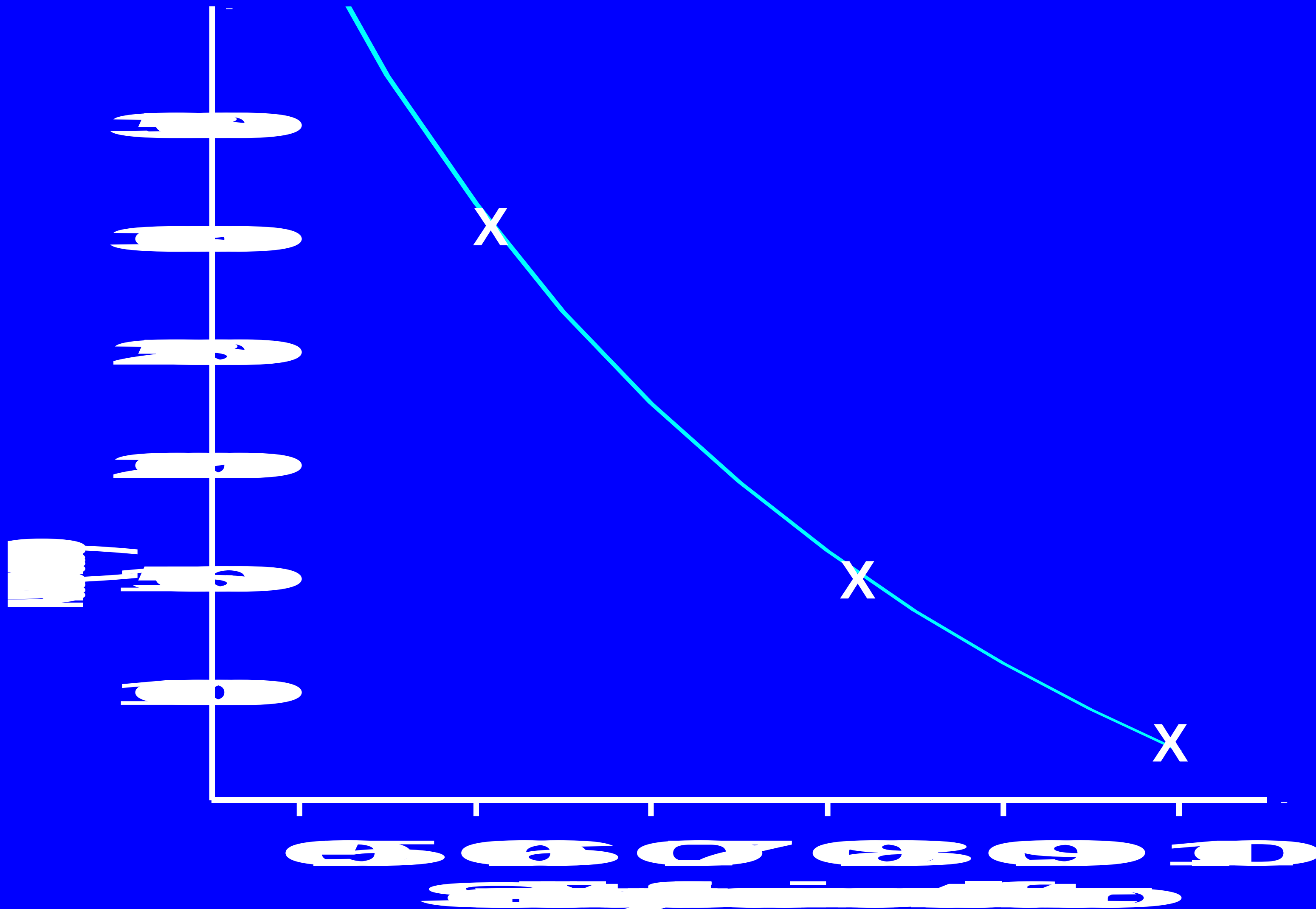
Specificity: probability of correctly identifying unexposed/non-susceptible subjects.

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%.

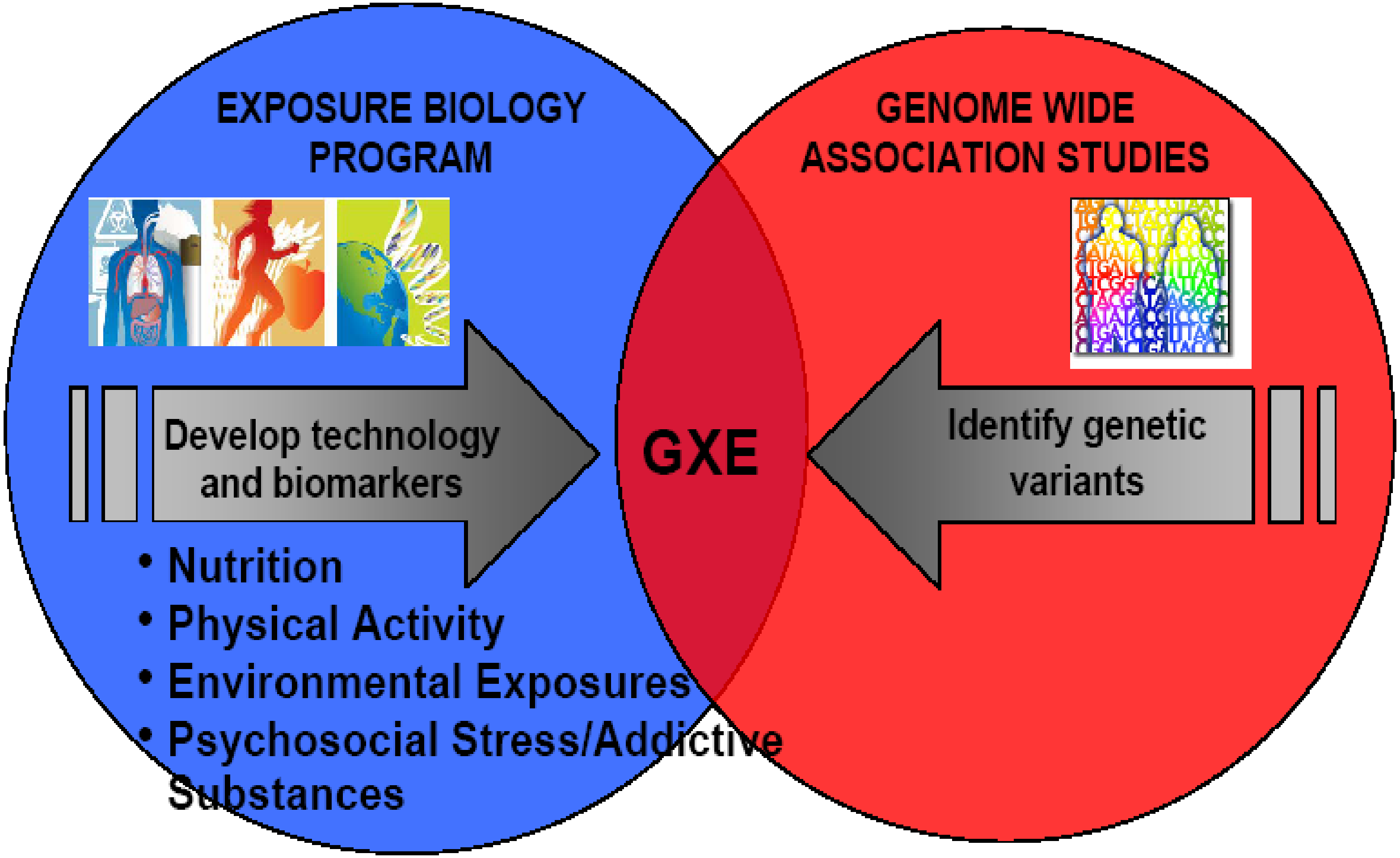
Effect of exposure assessment sensitivity on sample size: P(E)=50%, P(G)=50%



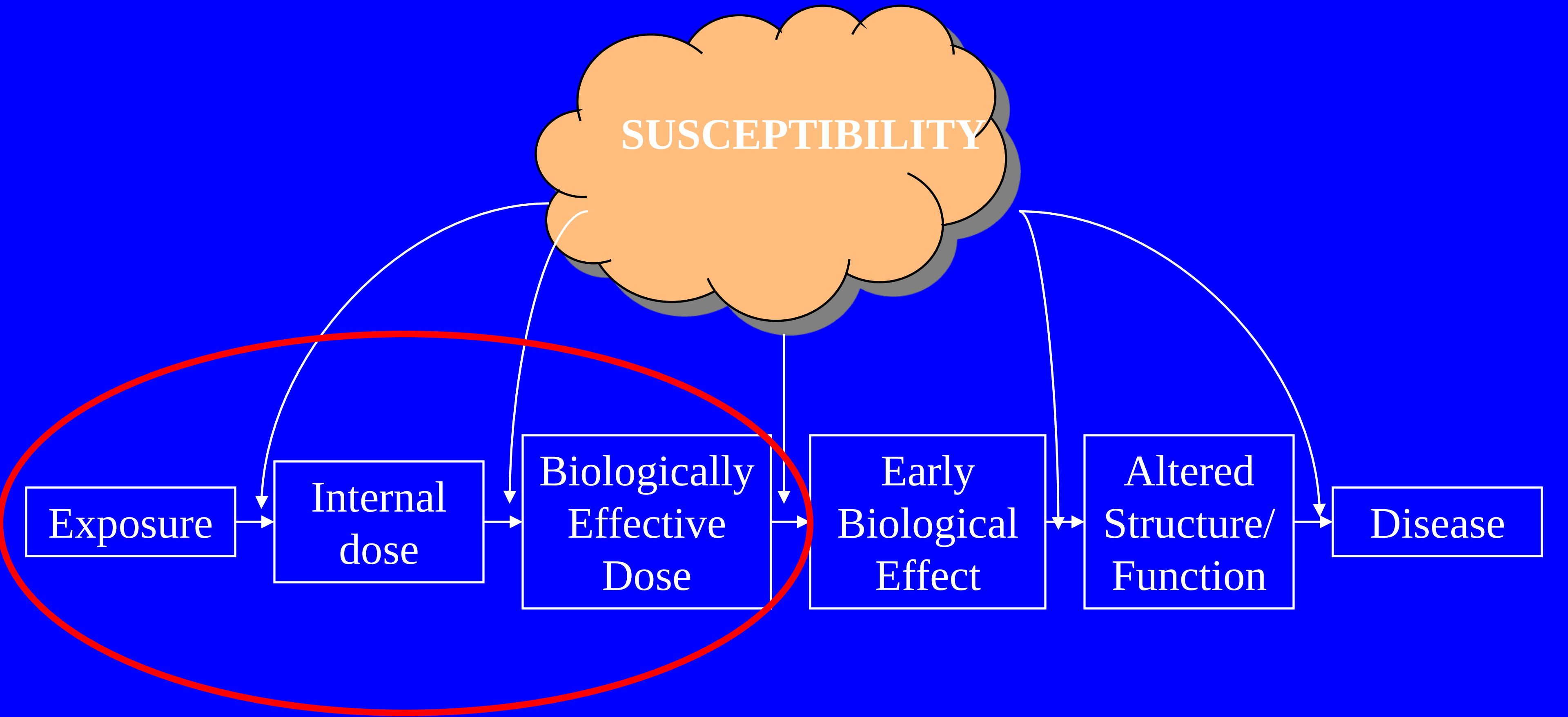
Where is “Big Science” and exposure assessment?

Genes, Environment, and Health Initiative:

Exposure Biology Program



Exposure assessment



New technology – features

- Real-time monitoring
- Wearable (miniature/nanotechnolog

- Cell phone technology/GPS enabled
  locate exposure in time/space
- Multiple exposures

Tools for diet and physical activity

- Dietary assessment using cell phones &digital imaging

- Physical activity -- cell phones with accelerometers GPS

Sensors for chemical exposures

- Wearable nanosensor array for real-time monitoring of diesel and gasoline exhaust

- Enzyme-based wearable environmental sensor badge for personal exposure assessment– pesticides, ozone, volatile organic compounds (VOC), heavy metals

Stress

- Wireless skin patch sensors to detect and transmit addiction and psychostress data (measures alcohol, skin temp and conductance, respiration and subject location (via GPS)

- A portable salivary biosensor of psychosocial stress

Biomonitoring

- Bringing new nanotechnology to bear
- Streamlining sample preparation via “Lab on a chip”
- Adduct-omics

Future research needs and opportunities

1) High quality, well-designed studies with appropriate control groups, and state-of-the-art genomic analysis

2) Continuing need to develop and validate new exposure assessment tools and methods, and incorporate into new and ongoing epidemiological studies

3) Tremendous scientific opportunities

Acknowledgments – Spanish Bladder Cancer Study

NATIONAL CANCER INSTITUTE

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