National Cancer Institute

Susceptibility for Bladder Cancer: Historical Perspective and Lessons Learned

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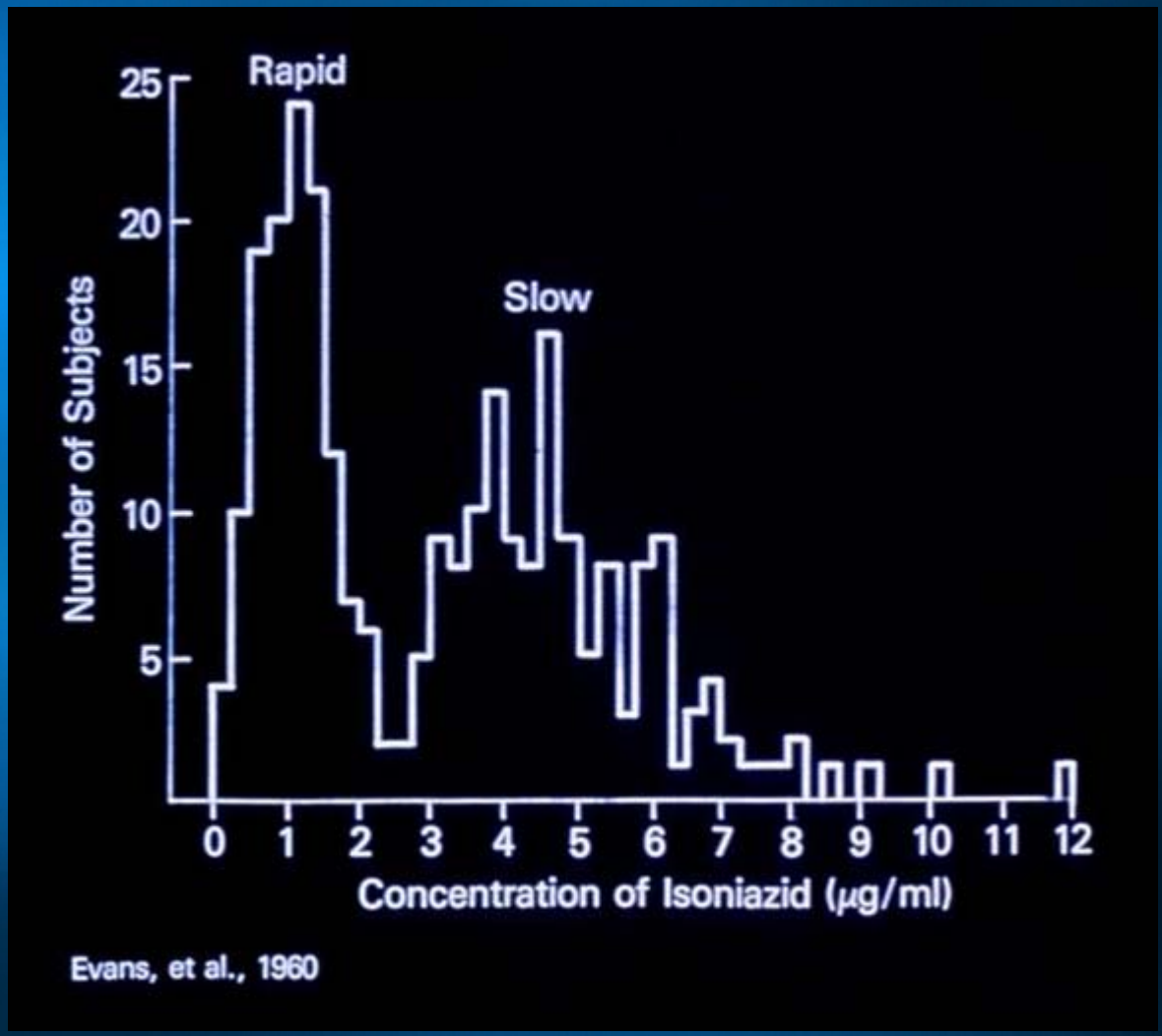
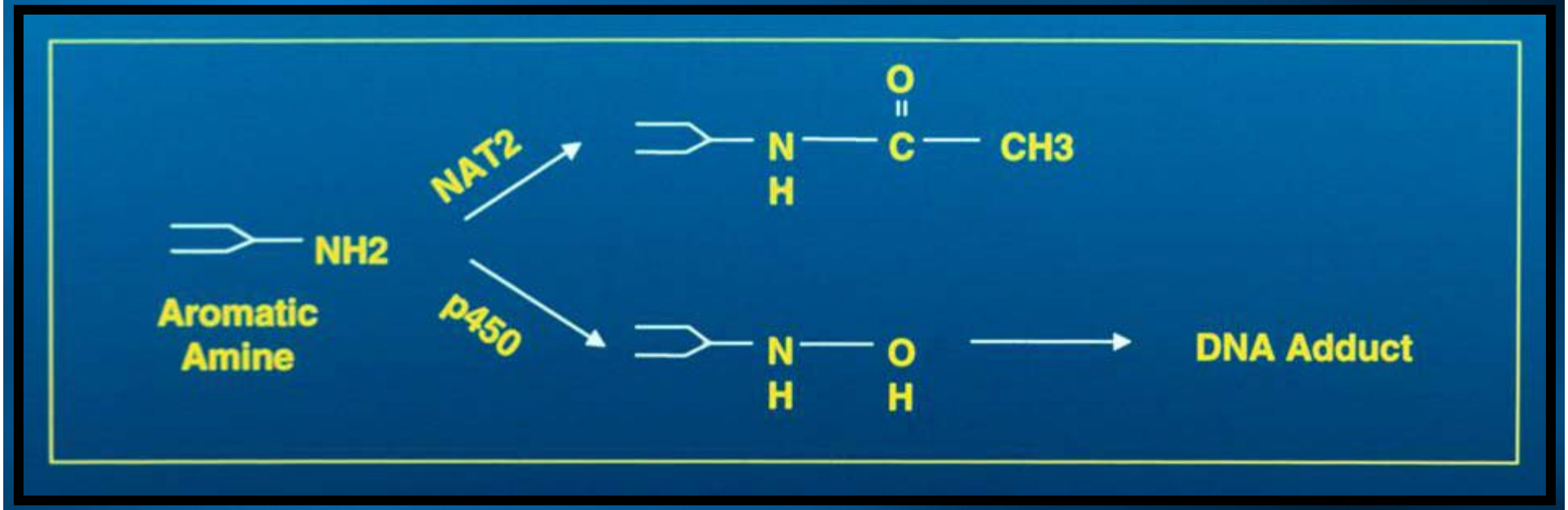
National Institutes of Health

Acetylation Polymorphism - Isoniazid Clearance from Plasma



Detoxification of Aromatic Amines by N-Acetylation

(Lower et al., EHP 1979)



NAT2 Slow Acetylation and Bladder Cancer Risk Among Workers Exposed to Aromatic Amines

1) Case-control studies in Europe and China
2) Cross-sectional study of dye workers in India

Case-Control Studies of Bladder Cancer and NAT2 Slow Acetylation Phenotype in Workers Exposed to Aromatic Amines

Year	Location	O.R.	(95% C.I.)
1982	England	18.6	(3.0-113.1)
1990	Poland	8.4	(2.0-34.0)

Bladder Cancer Risk in a Cohort of 1,972 Benzidine-Exposed Workers in China[a]

	Cases (n)	SIR[b]	95% C.I.
Entire Cohort	30	25	(17-36)
Exposure Level			
Low	3	5	(1-14)
Medium	19	36	(22-57)
High	8	158	(68-309) [c]

[a] Shanghai, Tianjin, Jilin
[b] Standardized Incidence Ratio using Shanghai Bladder Cancer Incidence Rates, adjusted for age: follow-up from 1972-1981
[c] Trend test, $p<0.01$

Nested Case-Control Study of *NAT2* Polymorphisms and Bladder Cancer Risk in China (Hayes et al., Carcinogenesis 1993)

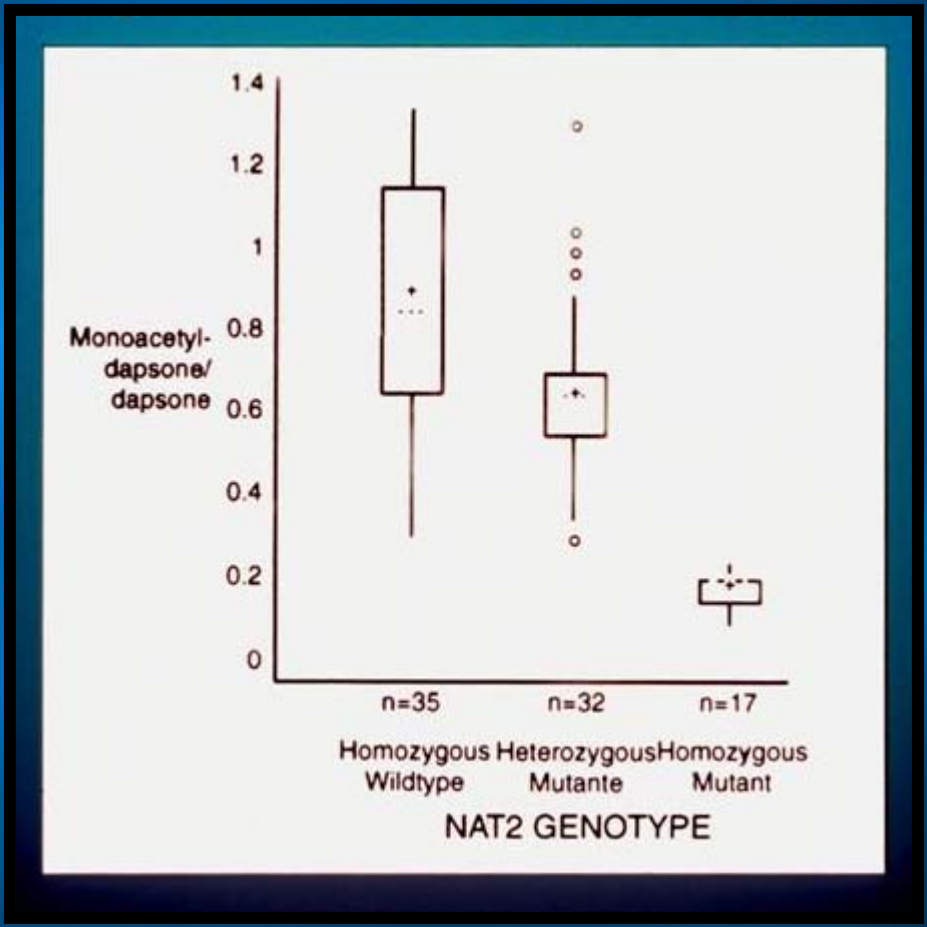
- **Benzidine-Exposed Cases: 38**
- **Benzidine-Exposed Controls: 43**
  Matched for age, city I(Shanghai, Tianjin)
- **Phenotype: Dapsone serum metabolites analyzed by HPLC**
- **Genotype: *NAT2* alleles analyzed from leukocyte DNA by PCR**

Case-Control Studies of Bladder Cancer and NAT2 Slow Acetylation Phenotype in Workers Exposed to Aromatic Amines

Year	Location	O.R.	(95% C.I.)
1982	England	18.6	(3.0-113.1)
1990	Poland	8.4	(2.0-34.0)
1993	China	0.4	(0.1-1.3)

Acetylation Activity, By *NAT2* Genotype

(Rothman et al., Pharmacogenetics, 1993)



Each genotype different from the other at p< .0001

NAT2 Slow Acetylation Genotype and Bladder Cancer Risk in Asia: Benzidine-Exposed Workers vs. General Population

	OR	95% CI
Benzidine-exposed workers (China, n=38 cases)	0.3	(0.1-1.3)
General population (Taiwan, Japan: 4 studies, n=251 cases)	2.3	(1.4-3.8)

Cross-Sectional Study of Urothelial Cell DNA Adducts Among Benzidine-Exposed Workers in India

Cross-Sectional Study of Benzidine-DNA Adduct Formation in Urothelial Cells

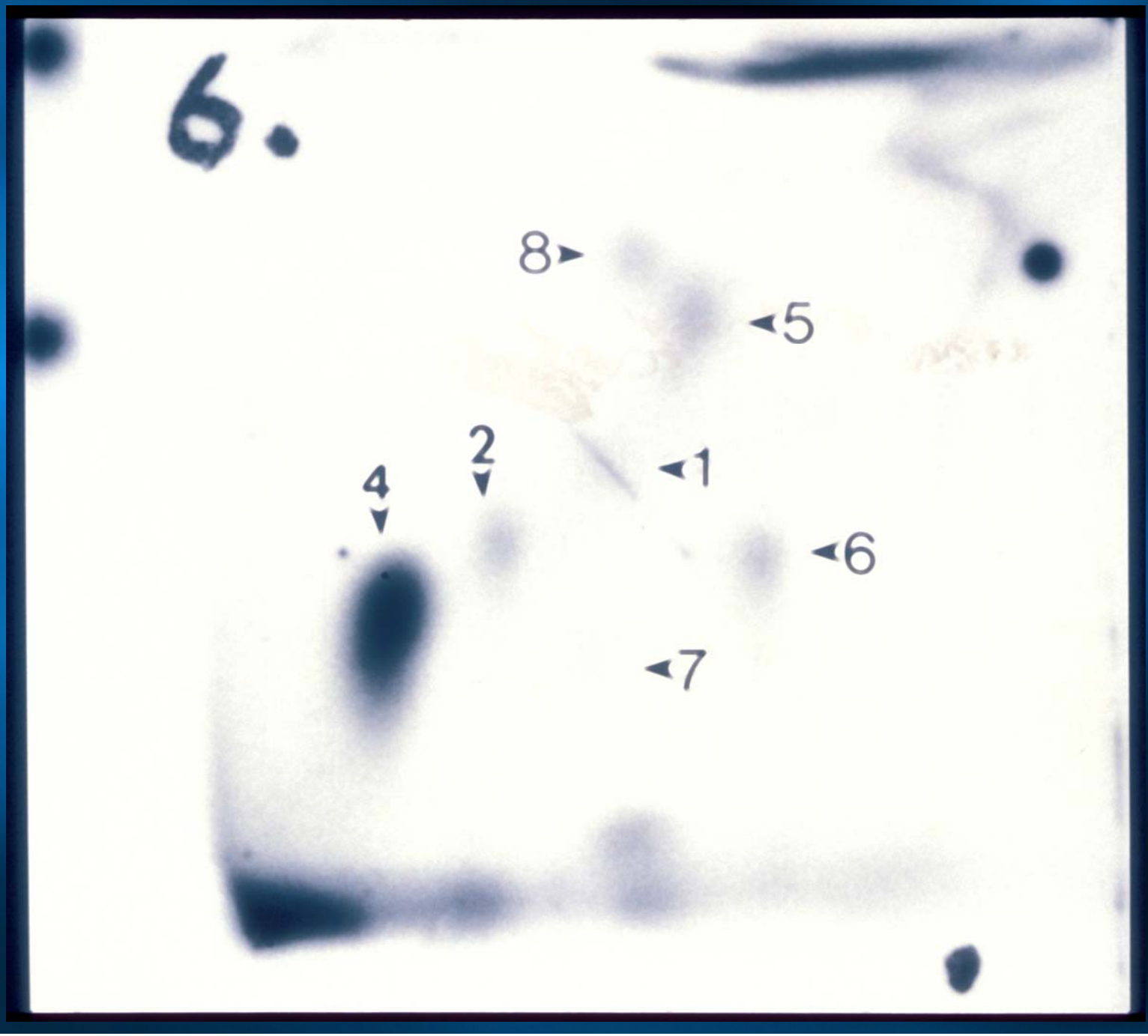
- 15 workers exposed to Benzidine
- 18 workers exposed to Benzidine-based dyes
- 15 unexposed controls matched on gender, age, and smoking status
- Adduct analysis by ^{32}P-post labeling in exfoliated urothelial cells

Demographic Characteristics and Benzidine Metabolite Levels, By Exposure Group

(Rothman et al., PNAS 1996)

	EXPOSURE		
	Controls (n=15)	Benzidine-Dye (n=18)	Benzidine (n=15)
	mean (sd)	mean (sd)	mean (sd)
Age (years)	26.5 (5.1)	23.8 (3.7)	23.3 (4.1)
Bidi Use (#/day)	2.2 (3.7)	3.2 (4.4)	2.2 (4.1)
Total Benzidine Metabolites [a] (ng/umol cr)	0.0 (0.0)	2.7 (5.6)	46.5 (30.5)

[a] benzidine + N-acetylation + N, N`-diacetylbenzidine (ng/umol cr)


6.
8
5
2
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4
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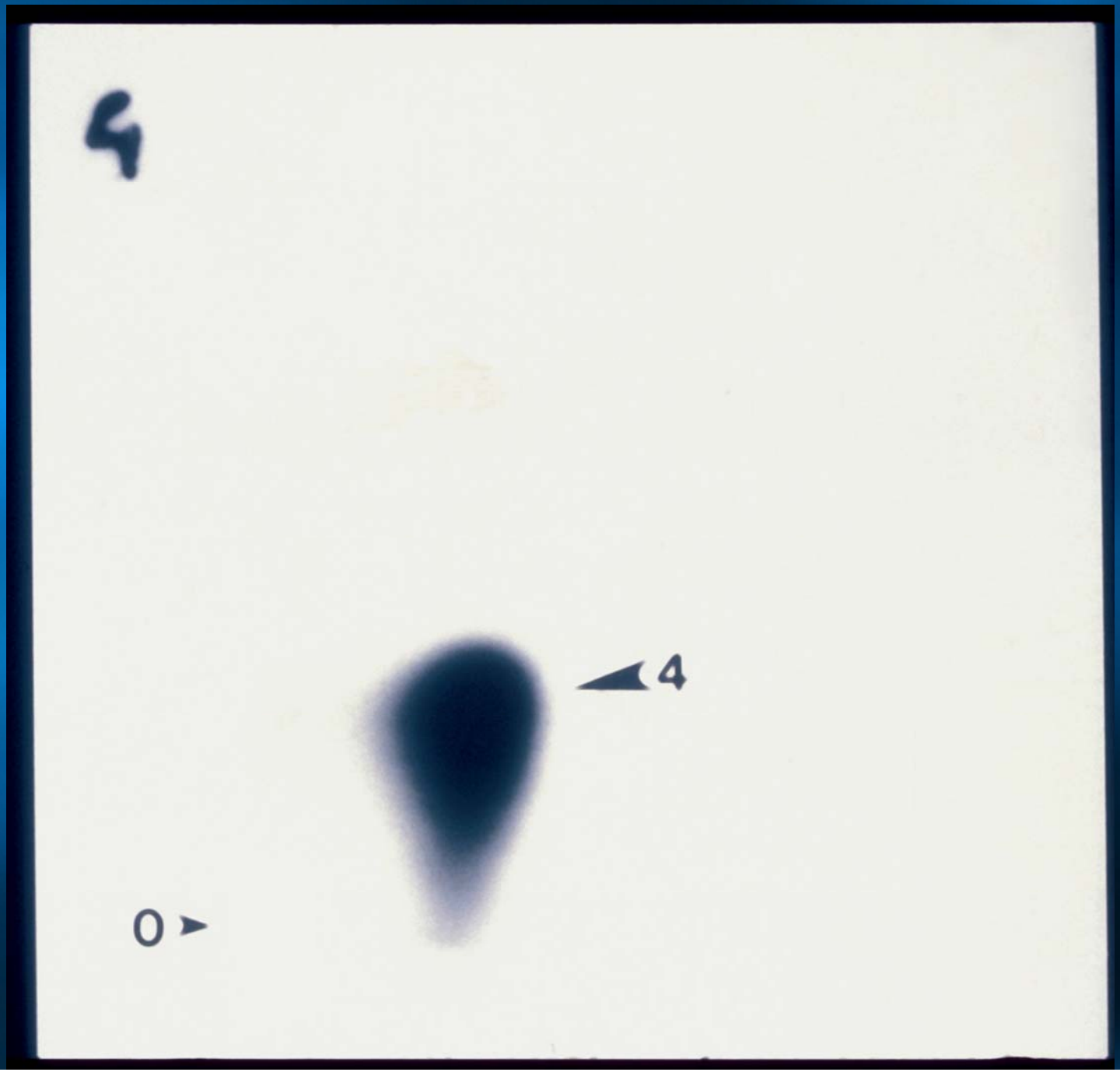
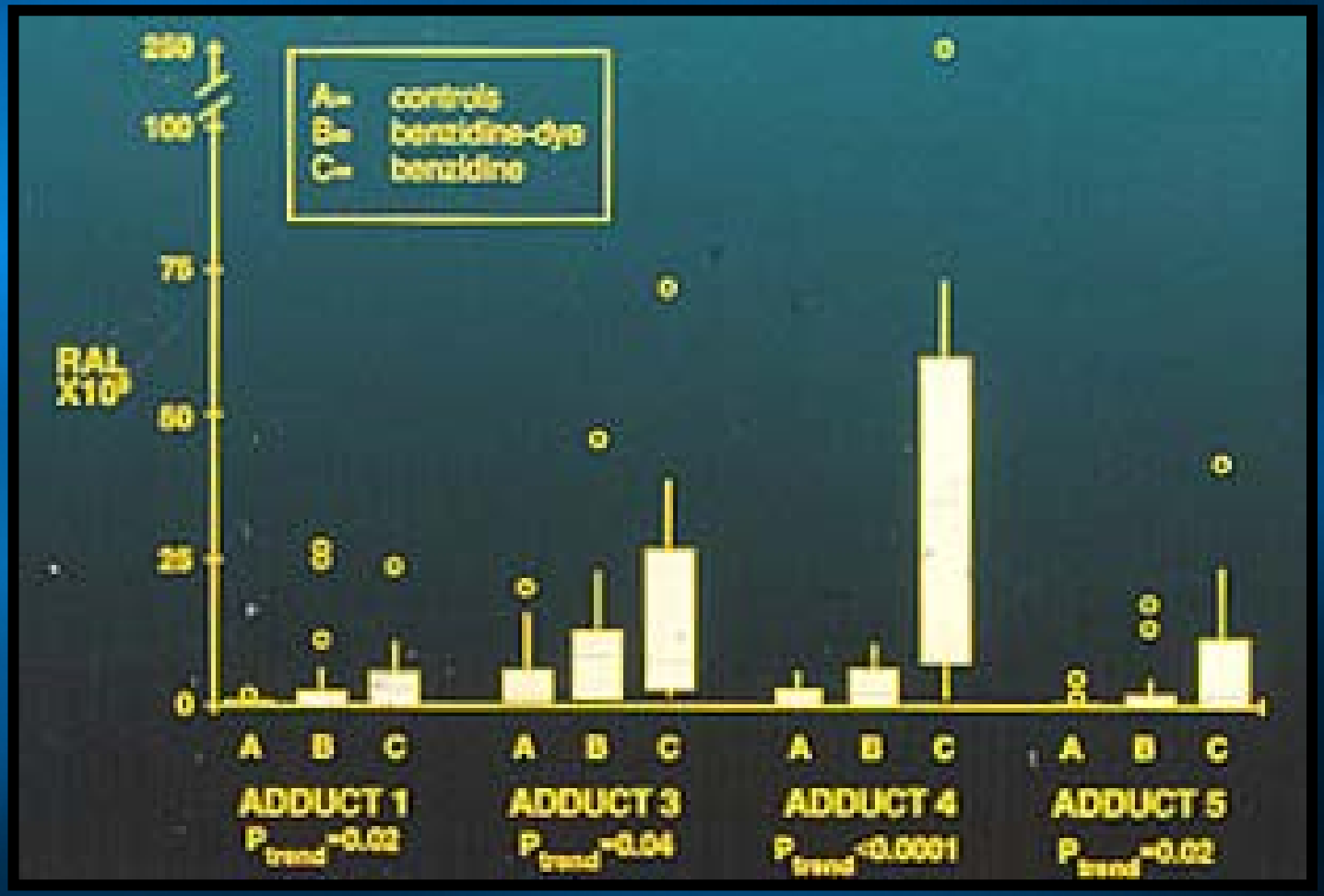
7


4
4
0

DNA Adducts by ^{32}P-Post Labeling in Urothelial Cells of Benzidine-Exposed Workers (Rothman et al., PNAS, 1996)



Cross-Sectional Study of Benzidine-DNA Adduct Formation in Urothelial Cells

- Predominant adduct in benzidine-exposed workers was N-(Deoxyguanosin-8-yl)-N'-Acetylbenzidine
- Evidence that for benzidine, N-acetylation is an activation rather than a detoxification process

New Nested Case-Control Study of NAT2 Phenotype and Genotype and Bladder Cancer Risk in China Benzidine Cohort, With Follow-up Through 2001

- 30 new cases identified, 62 healthy members of the cohort enrolled as controls

NAT2 Phenotype and Genotype and Bladder Cancer Risk in China Benzidine Cohort (Carreon et al., IJC 2006)

	Acetylation	Cases	Controls	OR	95% CI
Phenotype	Slow	2	15	0.3	0.1-1.6
	Rapid	26	47		
Genotype	Slow	1	15	0.2	0.0-1.4
	Rapid	29	50		

Pooled NAT2 Phenotype and Genotype and Bladder Cancer Risk Among Workers Exposed to Benzidine in China, 1993 and 2001 Studies

	Acetylation	Cases	Controls	OR	95% CI
Phenotype	**Slow**	**6**	**24**	**0.4**	**0.1-1.1**
1993, 2001	**Rapid**	**59**	**81**		
Genotype	**Slow**	**6**	**25**	**0.3**	**0.1-1.0**
1993, 2001	**Rapid**	**62**	**83**		

Comparison of *NAT2* Genotype and Bladder Cancer Risk Among Workers Exposed to Benzidine with Studies in the General Population in Asia

(Rothman et al., IJE, 2007)

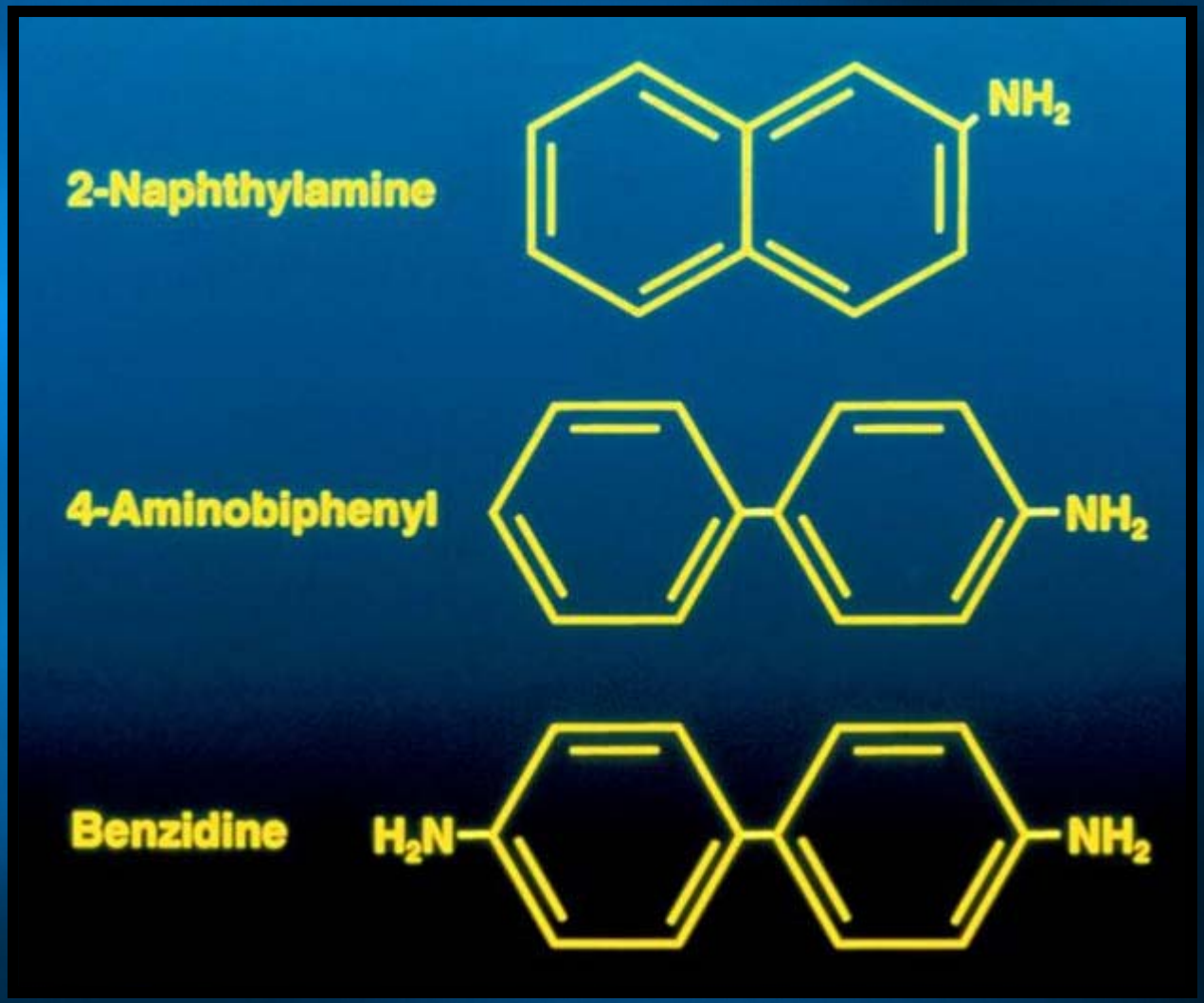
- **Benzidine-exposed workers in China:**
  OR = 0.3, 95% CI: 0.1-1.0
- **General population studies in Asia (n = 9 studies, 975 cases, 1417 controls)**
  OR = 1.4, 95% CI: 1.0-2.2

P = 0.019 for difference

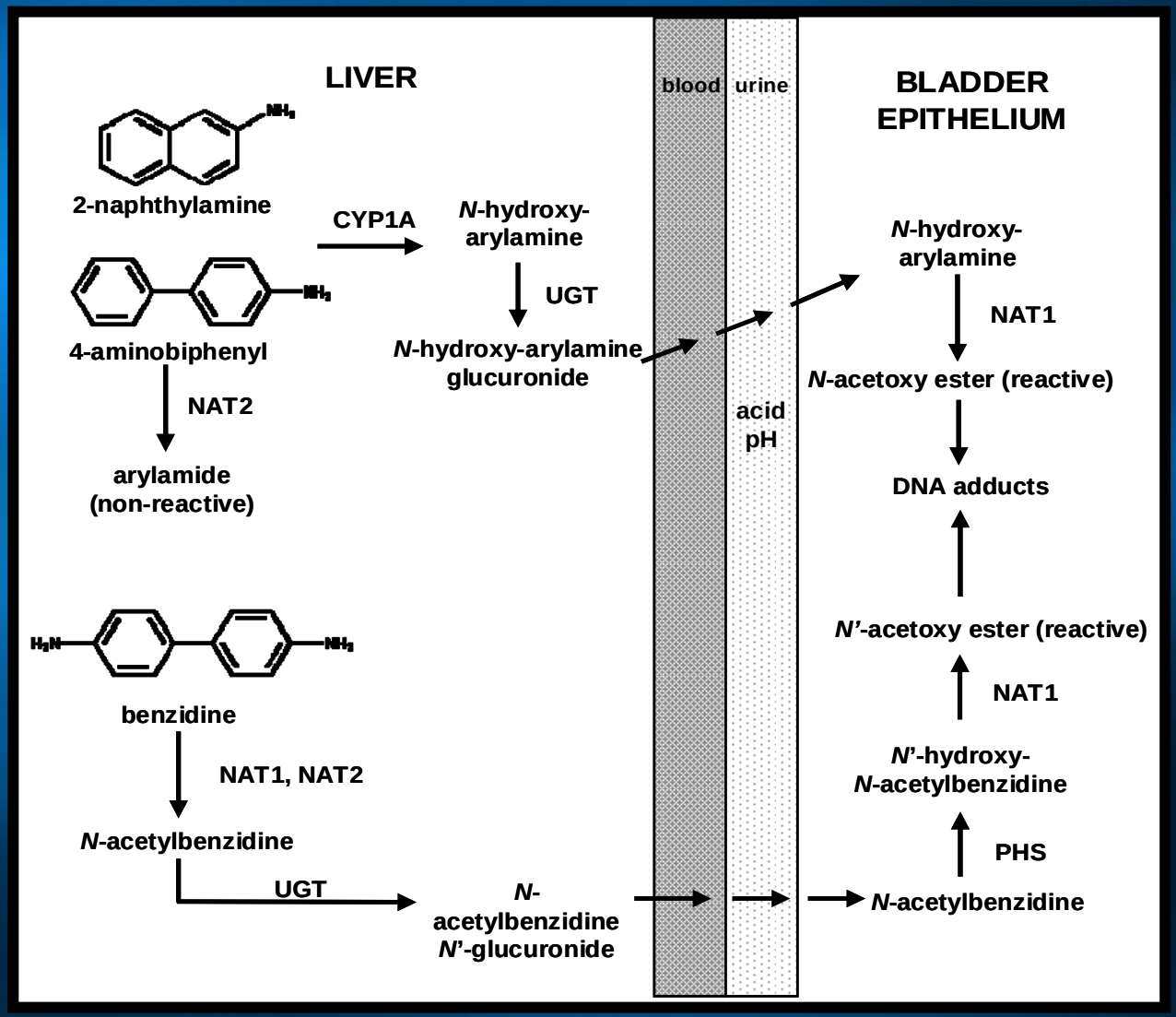
Case-Control Studies of Bladder Cancer and *NAT2* Slow Acetylation Phenotype in Workers Exposed to Aromatic Amines

68ar	Location	#Cases	O.R	(95% C.I.)	Exposure
1982	England	23	16.7	(2.2-346.3)	?Benzidine 2-Naphthylamine
1990	Poland	24	8.4	(1.6-48.9)	Benzidine 2-Naphthylamine
1993/ 2001	China	68	0.3	(0.1-1.3)	Benzidine

Carcinogenic Aromatic Amines



Metabolism of Monoarylamines and Diarylamines and Influence of Urine pH on DNA Adduct Formation

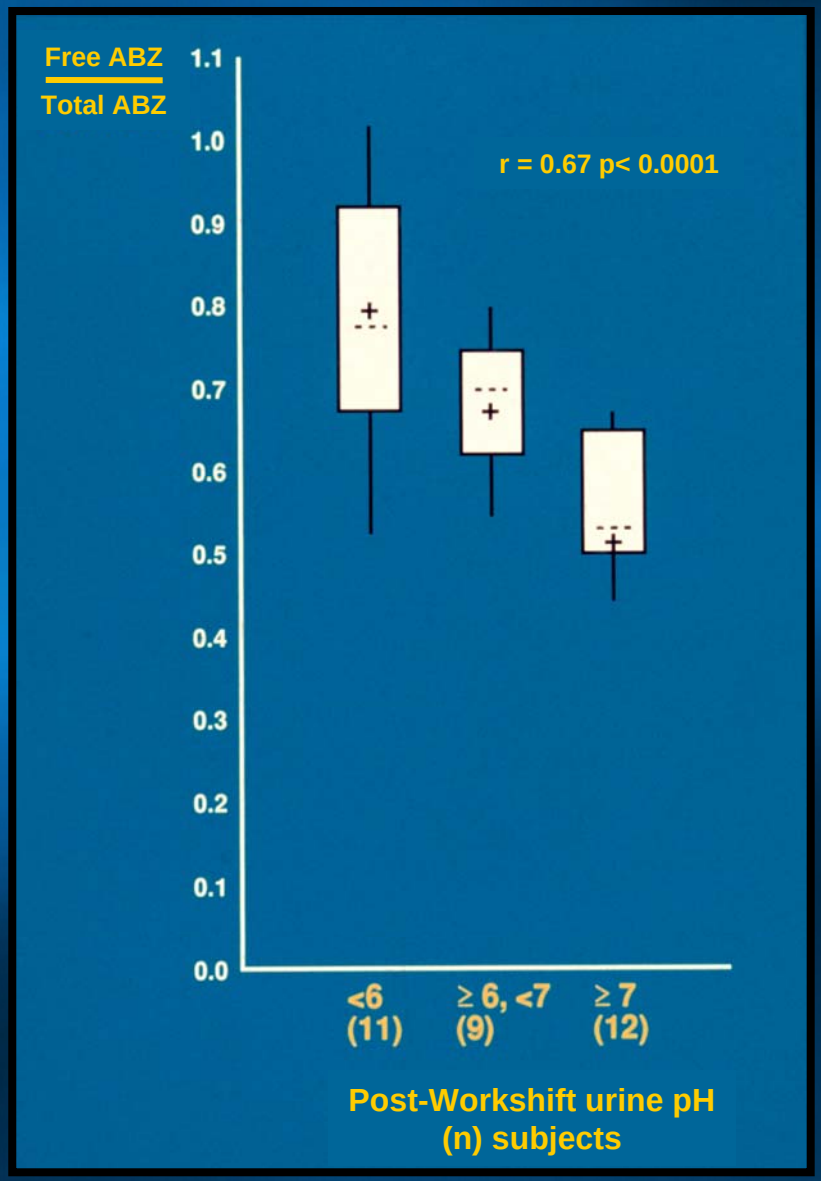


Urine pH, Hydrolysis of Glucuronidated Aromatic Amines, and Potential Contribution to Bladder Cancer Risk

Glucuronidated Compound	t1/2 at pH = 5.3 (min)	t1/2 at pH = 7.4 (min)
Benzidine	4	104
N-Acetylbenzidine	5	150
4-Aminoblphenyl	10.5	185

Cross-Sectional Study of Urothelial Cell DNA Adducts Among Benzidine-Exposed Workers in India

Influence of Urine pH on Proportion of Total *N*-Acetylbenzidine Present in Free State



Estimate of Impact of Urine pH on Urothelial DNA Adduct Levels

(Rothman et al., CEBP 1997)

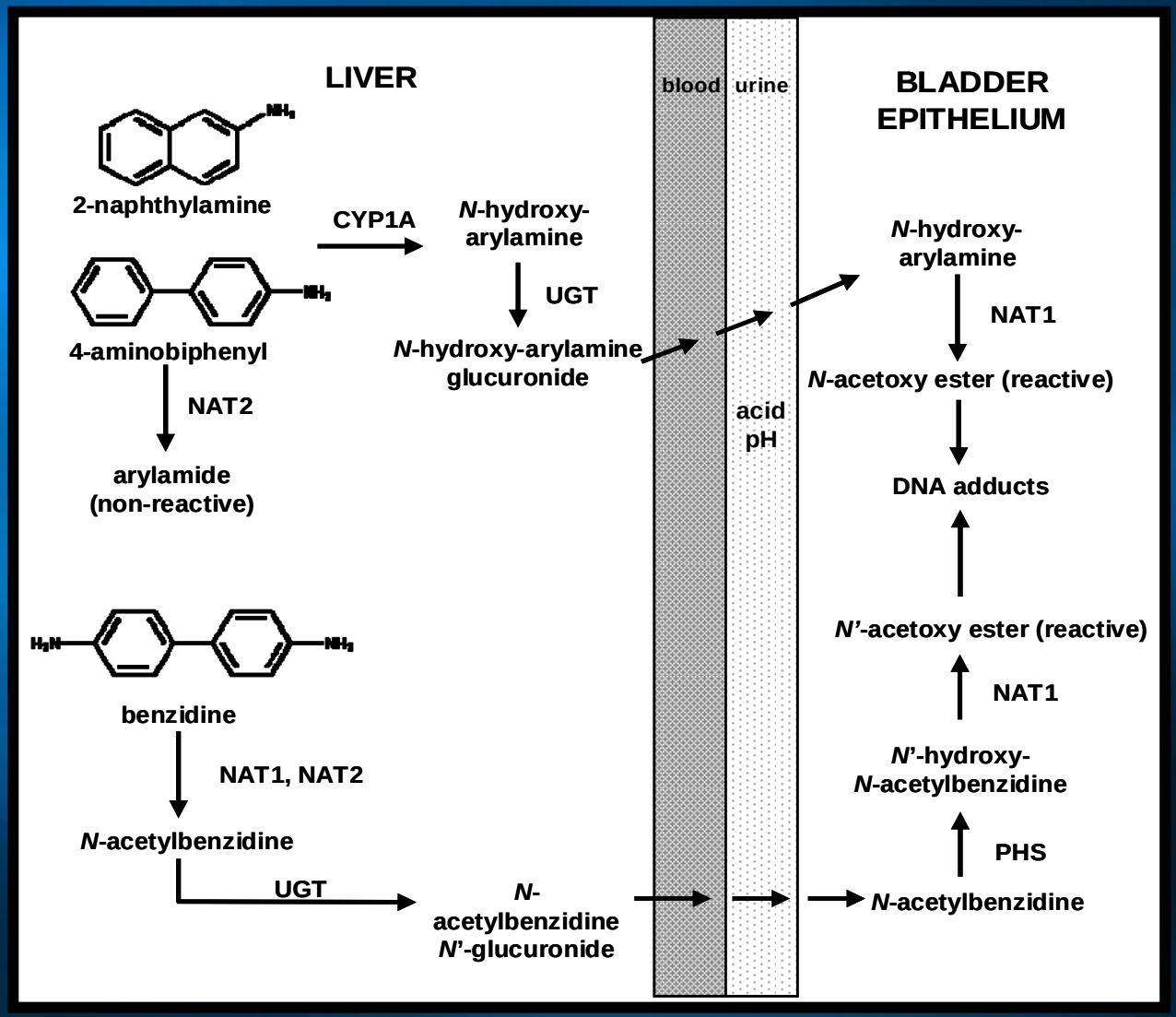
Urine pH	DNA adducts [1] RAL x 10^9
pH < 6	32.0
pH ≥ 7	3.2

[1] for average benzidine internal dose (22.1 ng/umol benzidine and metabolites)

Determinants Of Urine pH In Healthy Individuals

Factor	Impact on Urine pH
DIET	
Hard cheeses	↓↓↓
Animal protein	↓↓
Grains	↓
Fruits/vegetables	↑
BODY WEIGHT	
Obesity	↓↓

Metabolism of Monoarylamines and Diarylamines and Influence of Urine pH on DNA Adduct Formation



Susceptibility, Tobacco, and Bladder Cancer in the General Population

Spanish Bladder Cancer Study

Hospital-based case-control study in 5 areas of Spain (1998-2001)

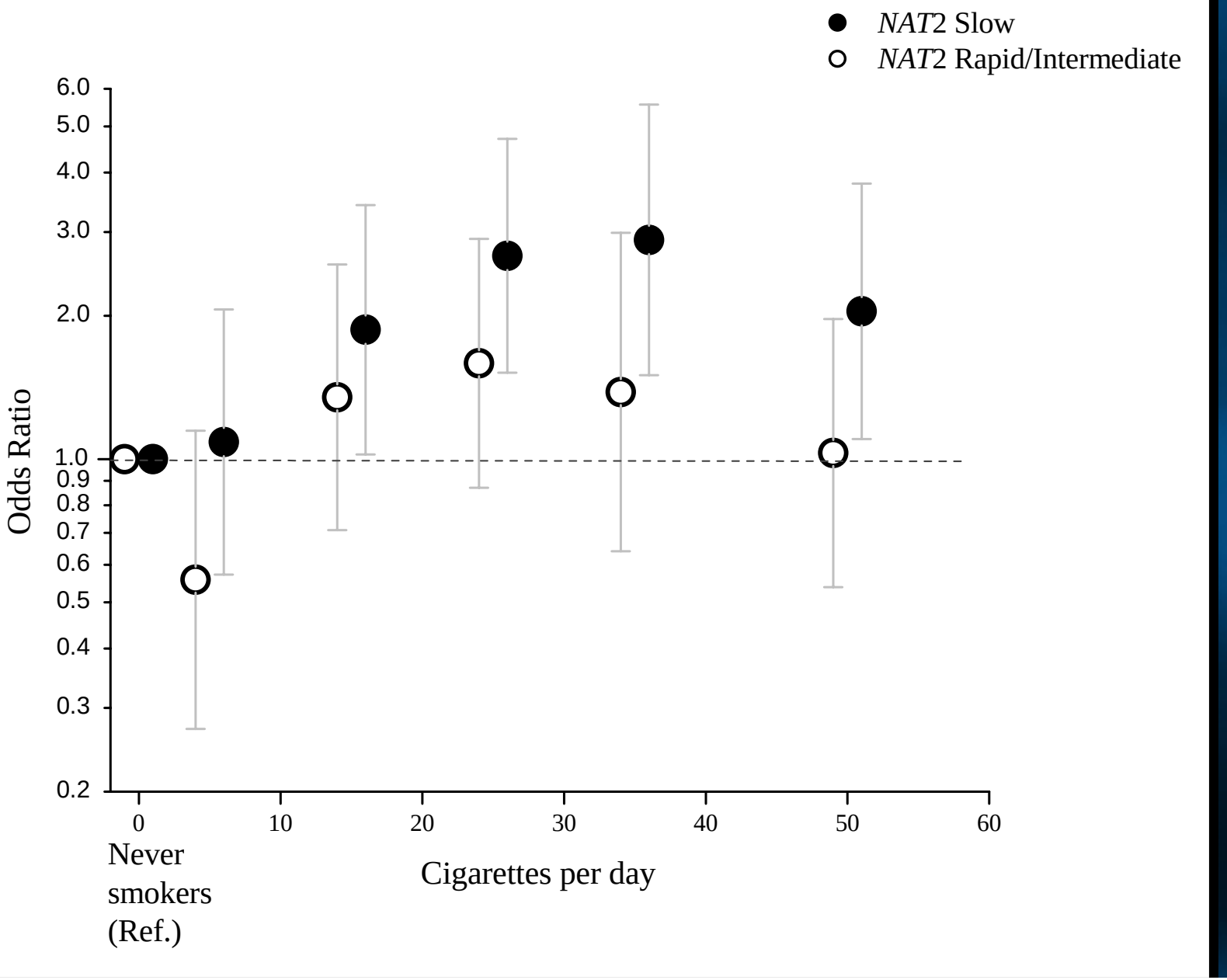
1219 cases, 1271 controls

NAT slow acetylation genotypes and bladder cancer risk : Spanish Bladder Cancer Study

(Garcia-Closas M et al. Lancet, 2005)

	Phenotypes*	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

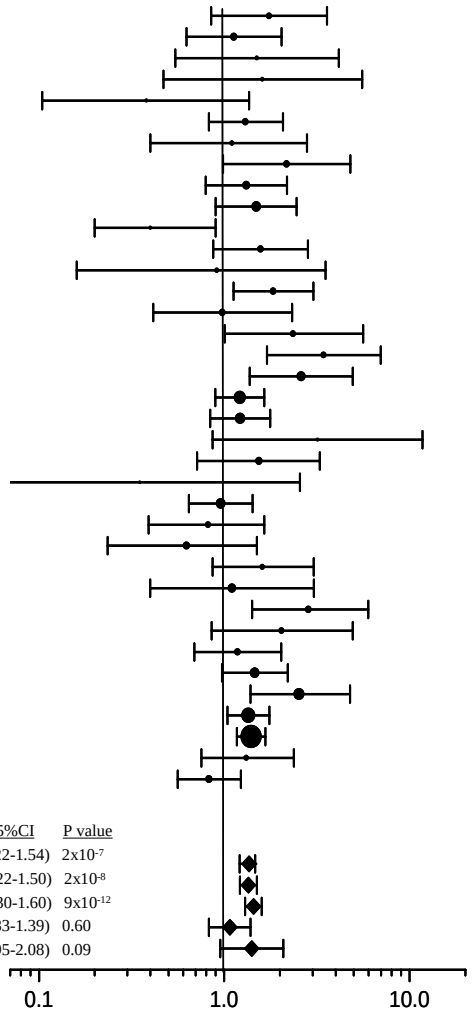
* Inferred from genotype data


*NAT*2 Slow
*NAT*2 Rapid/Intermediate
Odds Ratio
6.0
5.0
4.0
3.0
2.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
10
20
30
40
50
60
Never smokers (Ref.)
Cigarettes per day

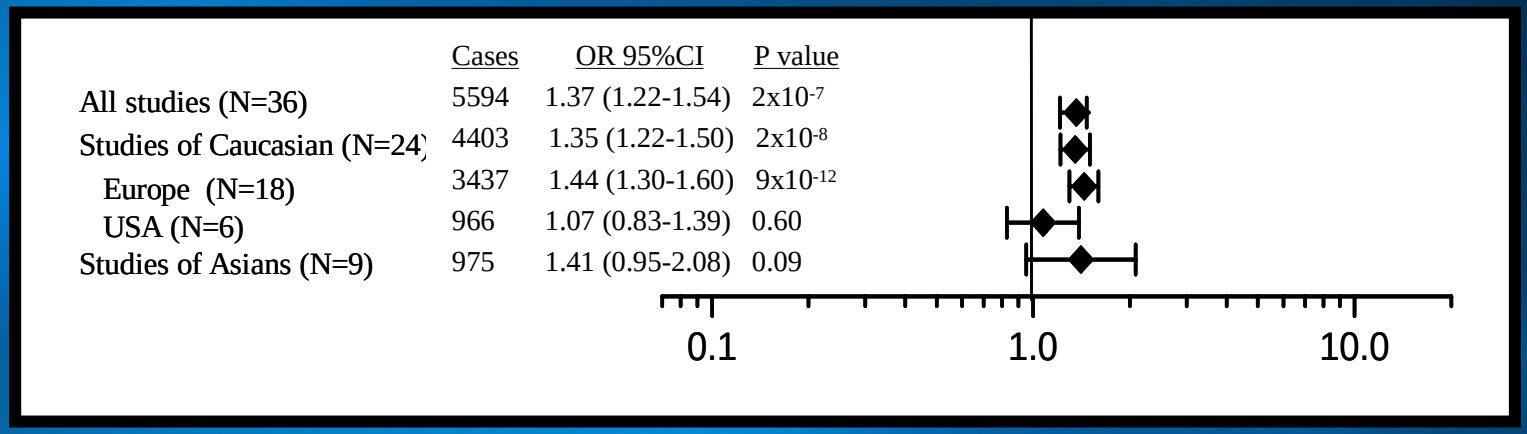
Meta-Analysis of Case-Control Studies of *NAT2* Slow Acetylation and Bladder Cancer (Rothman et al., IJE, 2007)

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

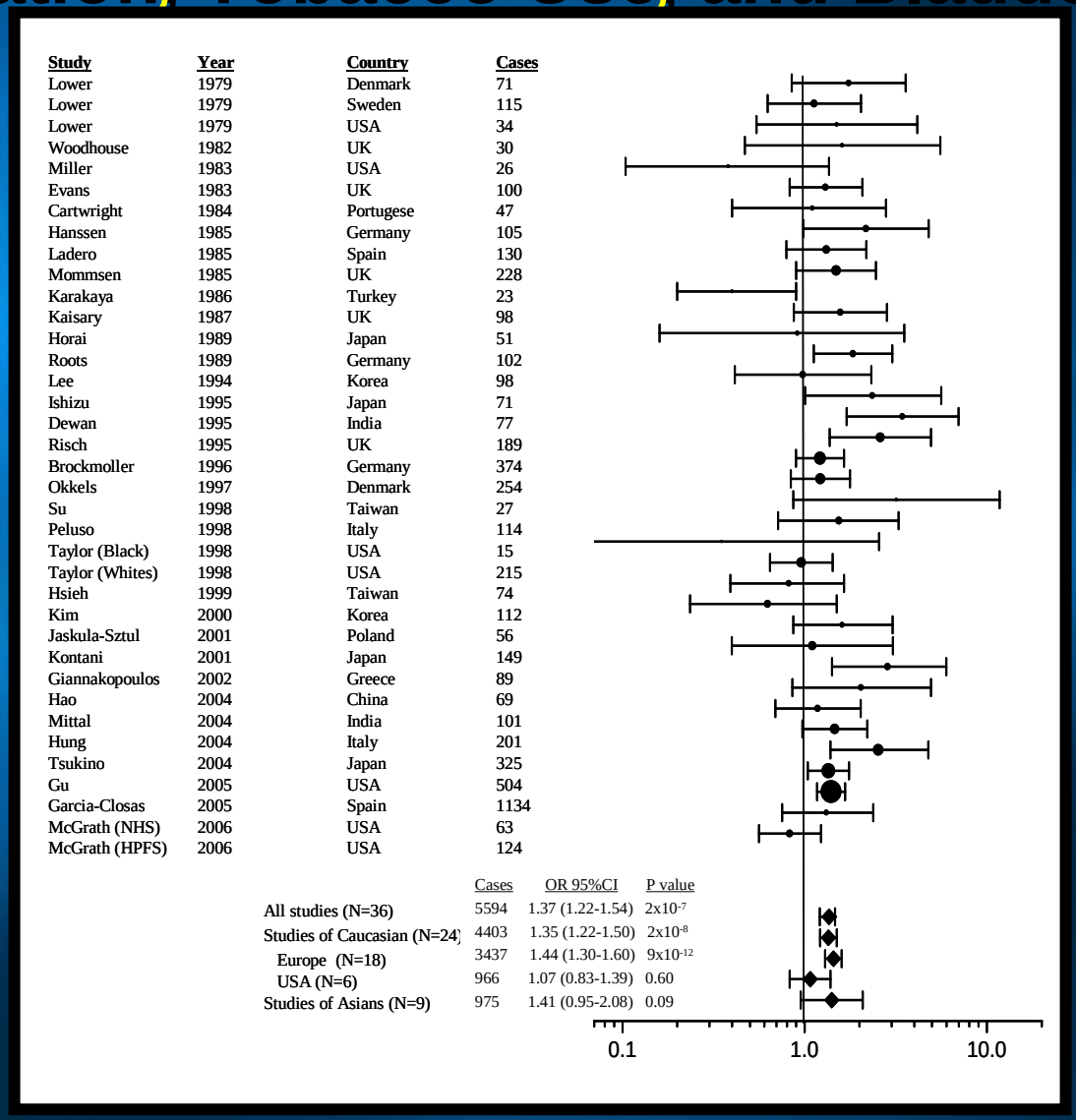
	Cases	OR 95%CI	P value
All studies (N=36)	5594	1.37 (1.22-1.54)	$2x10^{-7}$
Studies of Caucasian (N=24)	4403	1.35 (1.22-1.50)	$2x10^{-8}$
Europe (N=18)	3437	1.44 (1.30-1.60)	$9x10^{-12}$
USA (N=6)	966	1.07 (0.83-1.39)	0.60
Studies of Asians (N=9)	975	1.41 (0.95-2.08)	0.09



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



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



Candidate Pathways for Bladder Cancer

NAT2

Carcinogen metabolism

GSTM1

Activation

Detoxification

Oxidative damage

DNA damage

Cell-cycle control DNA repair

Normal cell

VEGF

ERCC2/XPD

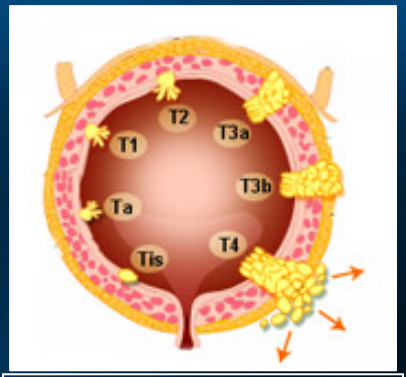
Apoptosis

Malignant cell

Malignant tumor

Cell death

Tumor growth



Bladder: tumor stages

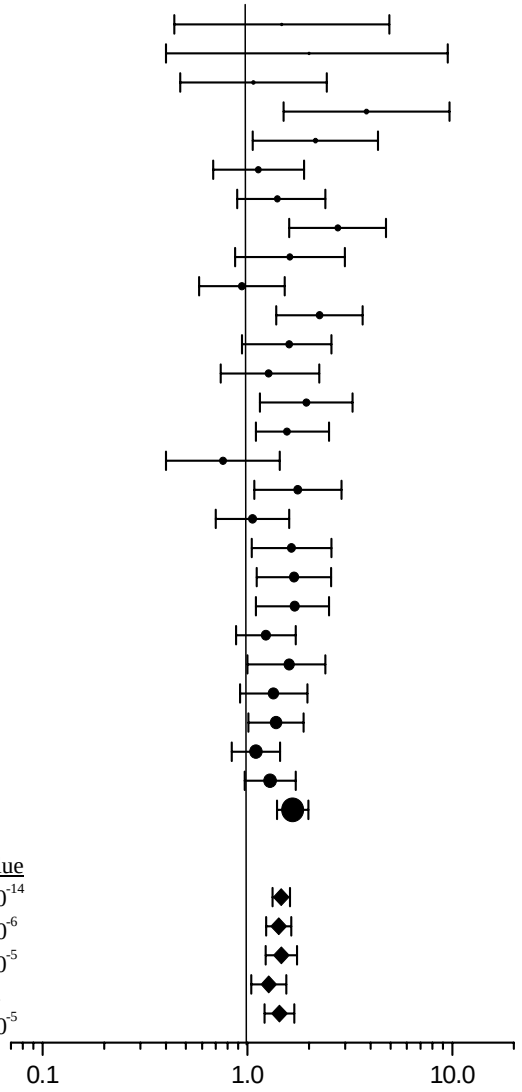
GSTM1 Null Genotype Increases Bladder Cancer Risk (Garcia-Closas M et al. Lancet, 2005)

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
				1.7	(1.4-2.0)	10^{-8}

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

	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)	$1x10^{-6}$
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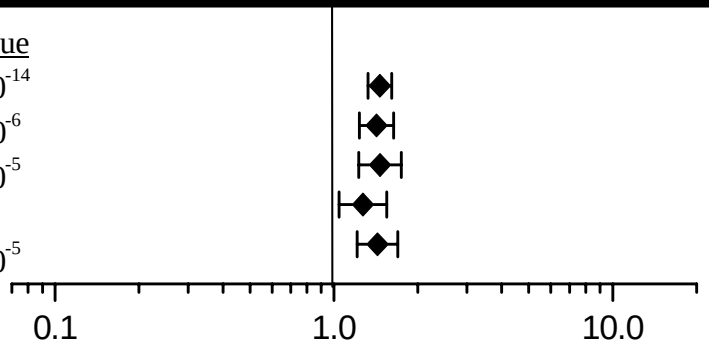
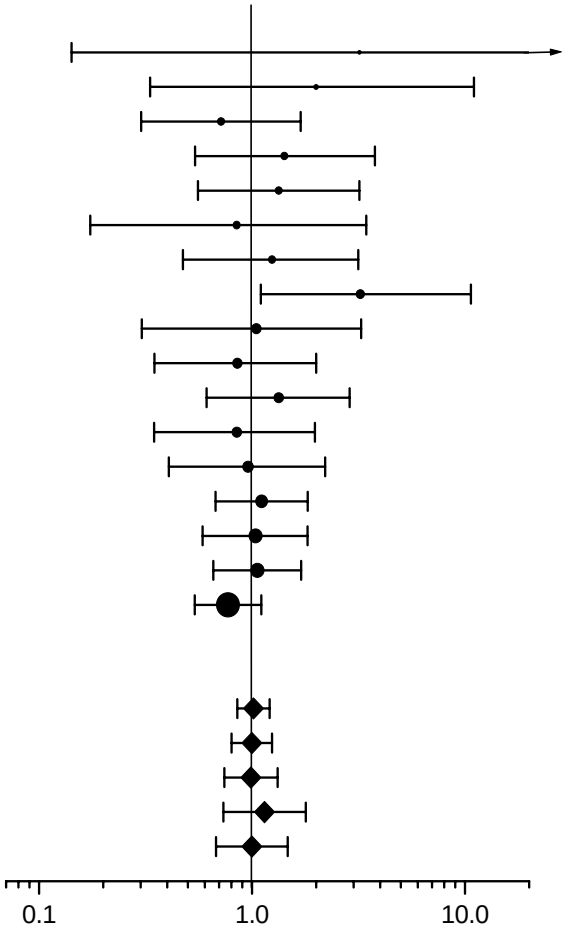


Case-Only Meta-Analysis of *GSTM1* Null Genotype, Smoking, and Bladder Cancer Risk

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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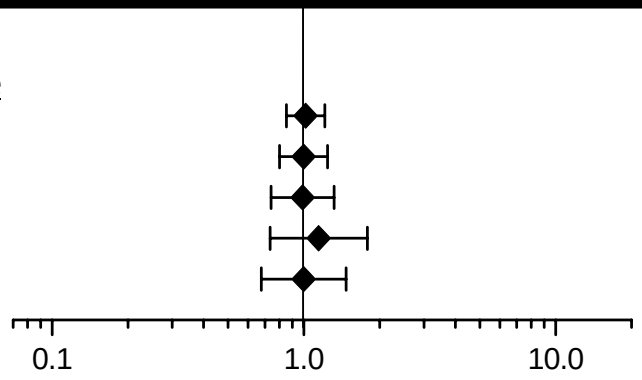
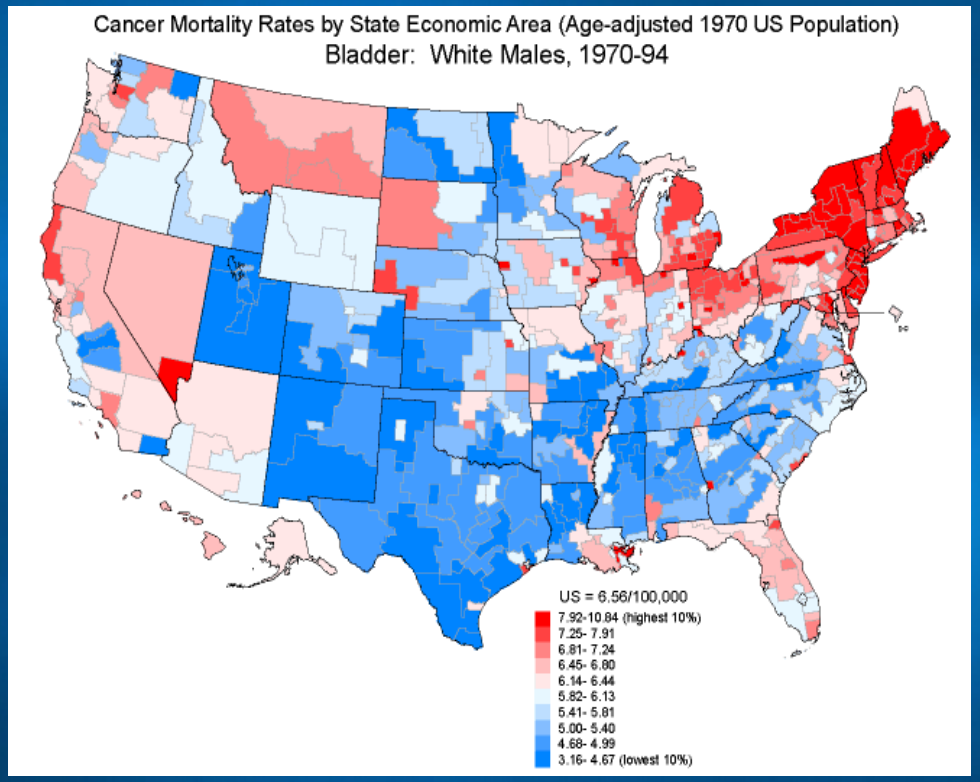


Bladder Cancer

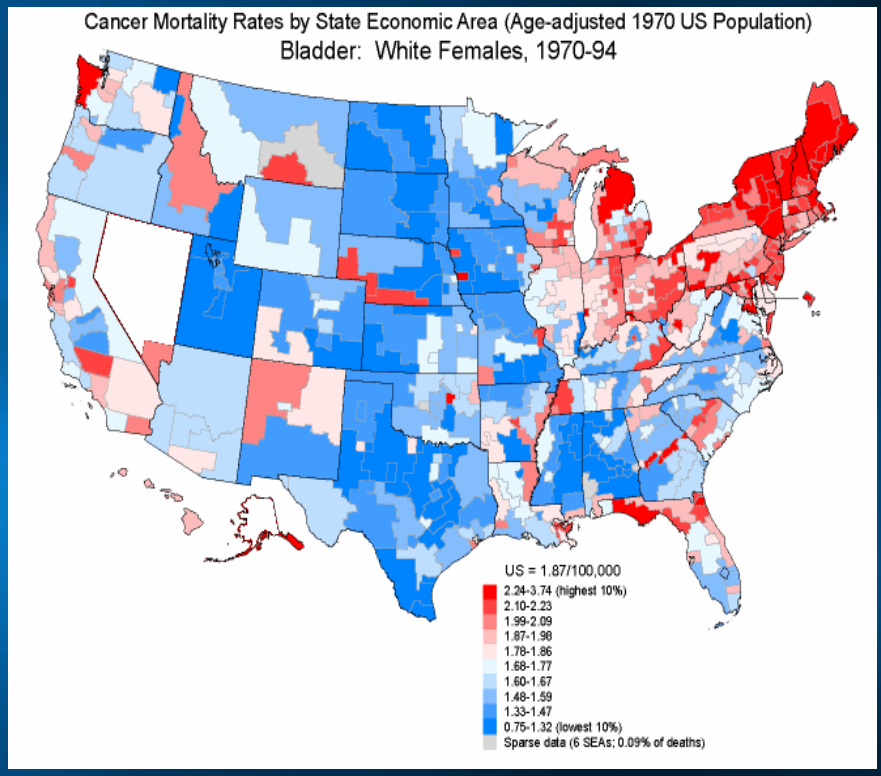
Excellent model to evaluate susceptibility and gene-environment interactions

- Relatively homogeneous histology
- Major causes well described
  - Tobacco smoking
  - Occupational exposures
  - 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, and genome-wide association studies being planned

Excess bladder cancer mortality in males and females in New England



Males, 1970-94



Females, 1970-94

Source: Atlas of Cancer Mortality in the United States, 1950-1994, NCI

Bladder Cancer Consortium

Investigator Web Portal

International Consortium of Case-Control Studies of Bladder Cancer

Opportunities for replication

> 20 studies currently participating
> 8,000 cases, 9,000 controls

Acknowledgments –China, India Studies

- China Study

  Wenfang Bi (CAPM, China)

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  Fred Kadlubar (U. Arkansas, USA)

  Douglas Bell (NIEHS, USA)

  Richard Hayes (NCI, USA)

- India Study

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Acknowledgments – Spanish Bladder Study

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Núria Malats
Francisco X Real
Manolis Kogevinas
Gemma Castaño Vinyals
Francisco Fernandez

Core Genotyping Facility

Stephen Chanock
Robert Welch
Meredith Yeager

Other Study Centers in Spain

Alfredo Carrato (Alicante)
Reina García-Closas (Tenerife)
Consol Serra (Vallès/Bages)
Adonina Tardón (Asturias)