



NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population

[Original Article]

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Abstract

The *NAT2* gene is involved in phase II detoxification of aromatic monoamines, a class of known bladder carcinogens. Certain allelic combinations result in the slow acetylation phenotype, which is thought to increase bladder cancer risk. We conducted a meta-analysis of all identifiable published case-control studies conducted in the general population that had examined the relationship of acetylation status and bladder cancer risk (22 studies, 2496 cases,

3340 controls). Using meta-analysis techniques that employed weighting based on individual-study variation, slow acetylators had an approximately 40% increase in risk compared with rapid acetylators [odds ratio (OR) 1.4, 95% confidence interval (CI) 1.2–1.6]. Statistical tests indicated, however, that pooling of all studies, or of studies conducted in Caucasian populations, hid potentially important heterogeneity in the individual study results, and suggested that the relationship of NAT2 slow acetylation and bladder cancer risk might differ by geographical region. Studies conducted in Asia generated a summary OR of 2.1 (CI 1.2–3.8), in Europe, a summary OR of 1.4 (CI 1.2–1.6), and in the USA, a summary OR of 0.9 (CI 0.7–1.3). Among European studies, the relationship between NAT2 slow acetylation and bladder cancer risk did not differ by method used to assess acetylation status (older drug-based phenotyping methods: 10 studies, OR 1.5, CI 1.2–1.8; more recent *NAT2* genotyping methods: four studies, OR 1.4, CI 1.1–1.7). Our results suggest that in most populations studied to date, NAT2 slow acetylation status is associated with a modest increase in bladder cancer risk.

Introduction

Lower et al. (1979b) hypothesized that individuals expressing the slow NAT2 acetylation phenotype would be at elevated risk of bladder cancer due to their decreased ability to detoxify aromatic amines. Several studies of *N*-acetylation phenotype and bladder cancer risk among populations with documented occupational exposure to aromatic monoamines or mixed amines showed that the prevalence of NAT2 slow acetylation in case series was much greater than that in control series or the general population (Cartwright et al., 1982; Hanke & Krajewska, 1990). It is generally accepted that NAT2 slow acetylators with past occupational exposure to aromatic amines, with the exception of benzidine, which is a diamine (Hayes et al., 1993), are at elevated bladder cancer risk (Silverman et al., 1996).

Case-control studies conducted outside of occupational cohorts (Lower & Bryan, 1979a; Lower et al., 1979b; Woodhouse et al., 1982; Evans et al., 1983; Miller & Cosgriff, 1983; Cartwright, 1984; Hanssen et al., 1985; Ladero et al., 1985; Karakaya et al., 1986; Mommsen et al., 1985; Kaisary et al., 1987; Horai et al., 1989; Roots et al., 1989; Dewan et al., 1995; Ishizu et al., 1995; Risch et al., 1995; Su et al., 1998; Brockmüller et al., 1996; Okkels et al., 1997; Peluso et al., 1998; Taylor et al., 1998) have observed elevations in risk, but inconsistently so. The magnitude of the observed positive associations has tended to be modest, and some of the more recently published studies (Brockmüller et al., 1996; Okkels et al., 1997; Taylor et al., 1998), which are among those with both the largest sample size and that have analysed *NAT2* genotype, have suggested that there is no, or a very minimal, elevation in risk. Because the NAT2 slow acetylation phenotype is relatively common [approximately 55% in populations of European descent, 35% in populations of African descent and 15% in populations of Asian descent (Yu et al., 1994)], even modest elevations in bladder cancer risk may be of potential public health importance.

To summarize the results of published epidemiologic literature, we conducted a meta-analysis of studies that explored the relationship of NAT2 acetylation status with bladder cancer risk in the general population. We were interested in examining whether specific study characteristics, in particular the method used to assess acetylation status and ethnicity/country of study participants, were discriminating factors for the presence of a positive association.

Materials and methods

We used review articles (Weber, 1987; Hein, 1988; Vineis & Pirastu, 1997), a previously published

meta-analysis (D'Errico et al., 1996), and MEDLINE searches (using the terms 'bladder neoplasms', 'arylamine *n*-acetyltransferase' and 'NAT2') to identify articles with information on NAT2 acetylation status and bladder cancer. Eligible studies were those published before December 1998 that had utilized a case-control design, had a cross-tabulation of acetylation status and case status presented in a published manuscript, and that had been conducted in the general population. Studies conducted in or derived in part from groups of workers with documented exposure to carcinogenic aromatic amines were excluded (Cartwright et al., 1982; Hanke & Krajewska, 1990; Hayes et al., 1993; Golka et al., 1996), as were certain participants of one study who were known to have extensive environmental arsenic exposure (Su et al., 1998). A total of 22 eligible studies were identified and included; 14 were conducted in Europe (Lower et al., 1979b; Woodhouse et al., 1982; Evans et al., 1983; Cartwright, 1984; Hanssen et al., 1985; Ladero et al., 1985; Mommsen et al., 1985; Kaisary et al., 1987; Roots et al., 1989; Risch et al., 1995; Brockmüller et al., 1996; Okkels et al., 1997; Peluso et al., 1998), three in Asia (Horai et al., 1989; Ishizu et al., 1995; Su et al., 1995), three in the USA (Lower & Bryan, 1979a; Miller & Cosgriff, 1983; Taylor et al., 1998), one in India (Dewan et al., 1995), and one in the Middle East (i.e. Turkey) (Karakaya et al., 1986).

We estimated odds ratios (OR) and 95% confidence intervals (CI) for individual studies by fitting logistic regression models, using PROC GENMOD of the statistical software package SAS (Breslow & Day, 1980; SAS Institute Inc., 1996). Random effects models (DerSimonian & Laird, 1986; Laird & Mosteller, 1990; Whitehead & Whitehead, 1991), which estimate summary measures by weighting each individual-study result by a factor of individual- and between-study variance, were fit to determine groups of studies with homogenous results. *Q*-statistics associated with $P < 0.05$ were considered to indicate meaningful lack of homogeneity. Fixed effects models (DerSimonian & Laird, 1986; Laird & Mosteller, 1990; Whitehead & Whitehead, 1991), which weight only by a factor of individual-study variance, also were fit. We report results from fixed effects models only: random effects models are inappropriate for heterogeneous groupings, and produced very similar results for homogeneous groupings.

A summary odds ratio was calculated for a pooling of all studies, as well as for subpoolings defined by geographical region and method used to assess acetylation status. Geographical poolings were conducted for three of the five regions [Europe, Asia and USA]. The two omitted regions (India and the Middle East) each had only one study. The Asian subpooling included studies conducted in Japan and Taiwan; the European subpooling included studies conducted in Denmark, England, Germany, Italy, Portugal, Spain and Sweden. We also pooled studies whose participants were known or presumed to be Caucasian: this pooling consisted of the European studies, the Middle Eastern and Indian studies, and the three studies conducted in the USA. Of the three US studies, one had been conducted among Caucasian individuals only (Miller & Cosgriff, 1983), one had been conducted in Wisconsin (Lower, 1979b) [where the population is estimated to be 92% Caucasian (Census of Population & Housing, 1990, 1992)], and one provided separate data for Caucasians and African-Americans (Taylor et al., 1998), allowing us to include only the Caucasian data in the pooling.

Results

Selected characteristics of the 22 individual studies are reported in Table 1. Results were published as early as 1979 and as late as 1998, and most studies were conducted in European countries. Six employed genotyping methods, while the remaining 16 employed drug-based phenotyping methods. Individual datasets ranged in size from 23 to 374 cases. Data were available for a total of 2496 cases and 3340 controls.

First author (date)	Number of cases	Number of controls	Country	Phenotyping or genotyping (drug used for phenotyping or mutant allele ^a)
Brockmüller (1996)	374	373	Germany (Berlin)	Genotyping (NAT2*5A,B,C; 6A; 7B)
Okkels (1997)	254	242	Denmark (Aarhus)	Genotyping (NAT2*5A,B,C; 6; 7)*
Taylor (1998)	230	203	USA (North Carolina)	Genotyping (NAT2*5; 6; 7; 14)*
Mommsen (1985)	228	100	Denmark (Aarhus)	Phenotyping (Sulphamethazine)
Risch (1995)	178	59	England (Birmingham)	Genotyping (NAT2*5A,B,C; 6A; 7B)
Ladero (1985)	130	157	Spain (Madrid)	Phenotyping (Dapsone)
Lower (1979)	115	118	Sweden (Lund)	Phenotyping (Sulphamethazine)
Peluso (1998)	114	46	Italy (Torino)	Genotyping (NAT2*5A; 6A; 7A)
Hanssen (1985)	105	42	Germany (Hamburg)	Phenotyping (Sulphamethazine)
Roots (1989)	102	292	Germany (Berlin)	Phenotyping (Caffeine)
Evans (1983)	100	852	England (Liverpool)	Phenotyping (Sulphamethazine)
Kaisary (1987)	98	110	England (Bristol)	Phenotyping (Dapsone)
Dewan (1995)	77	80	India (Ahmedabad)	Phenotyping (Isoniazid)
Lower (1979)	71	74	Denmark (Copenhagen)	Phenotyping (Sulphamethazine)
Ishizu (1995)	71	91	Japan (Tokyo)	Phenotyping (Isoniazid)
Horai (1989)	51	203	Japan (Tokyo)	Phenotyping (Dapsone)
Cartwright (1984)	47	35	Portugal (unknown)	Phenotyping (not stated)
Lower (1979)	34	41	USA (Wisconsin)	Phenotyping (Sulphamethazine)
Woodhouse (1982)	30	27	England (North-east)	Phenotyping (Isoniazid)
Su (1998)	27	60	Taiwan (Tianan City)	Genotyping (NAT2*5; 6; 7)*
Miller (1983)	26	26	USA (Western NY State)	Phenotyping (Sulphamethazine)
Karakaya (1986)	23	109	Turkey (Ankara)	Phenotyping (Sulphamethazine)

^aFurther specification of allele subtypes was not provided in manuscripts.

Table 1. Source and description of data used in the pooled analysis of acetylation status and bladder cancer risk ^aFurther specification of allele subtypes was not provided in manuscripts.

Table 2 presents results of the individual studies. Although odds ratios for the association of NAT2 slow acetylation and bladder cancer risk varied substantially (range 0.4–3.3), only four were 1.0 or less; more than half were 1.5 or greater. The magnitude of the association varied somewhat by study sample size (Fig. 1). As expected, studies with the smallest sample size produced many of the extreme results.

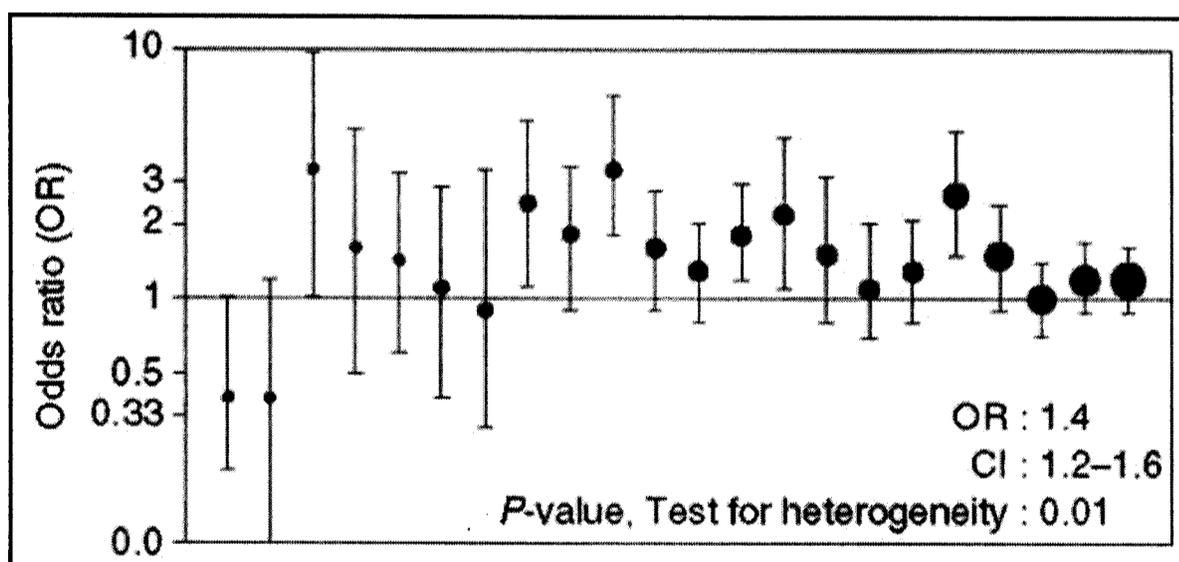


Fig. 1. Odds ratios and 95% confidence intervals for NAT2 slow acetylation and bladder cancer risk. Circles are proportional to study sample size. The smallest study has a sample size of 23, the largest study has a sample size of 374.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for the association of NAT2 acetylation status and bladder cancer - individual studies

First author (date)	Total number of cases	Cases n (%)		Controls n (%)		OR (CI)
		Slow acetylators	Rapid acetylators	Slow acetylators	Rapid acetylators	
Brockmüller (1996)	374	233 (62)	141 (38)	215 (43)	158 (57)	1.2 (0.9-1.6)
Okkels (1997)	254	154 (61)	100 (39)	135 (56)	107 (44)	1.2 (0.9-1.7)
Taylor (1998)	230	121 (53)	109 (47)	109 (38)	94 (62)	1.0 (0.7-1.4)
Mommsen (1985)	228	145 (64)	83 (36)	54 (54)	46 (46)	1.5 (0.9-2.4)
Risch (1995)	189	127 (67)	62 (33)	26 (44)	33 (56)	2.6 (1.5-4.7)
Ladero (1985)	130	83 (64)	47 (36)	90 (57)	67 (43)	1.3 (0.8-2.1)
Lower (1979) (Sweden)	115	80 (70)	35 (30)	79 (67)	39 (33)	1.1 (0.7-2.0)
Peluso (1998)	114	76 (67)	38 (33)	26 (57)	20 (43)	1.5 (0.8-3.1)
Hanssen (1985)	105	65 (62)	40 (38)	18 (43)	24 (57)	2.2 (1.1-4.5)
Roots (1989)	102	67 (66)	35 (34)	149 (51)	143 (49)	1.8 (1.2-2.9)
Evans (1983)	100	66 (66)	34 (34)	510 (60)	342 (40)	1.3 (0.8-2.0)
Kaisary (1987)	98	59 (60)	39 (40)	54 (49)	56 (51)	1.6 (0.9-2.7)
Dewan (1995)	77	50 (65)	27 (35)	28 (35)	52 (65)	3.3 (1.8-6.6)
Lower (1979) (Denmark)	71	46 (65)	25 (35)	38 (51)	36 (49)	1.8 (0.9-3.4)
Ishizu (1995)	71	20 (28)	51 (72)	13 (14)	78 (86)	2.4 (1.1-5.2)
Horai (1989)	51	3 (6)	48 (94)	13 (6)	190 (94)	0.9 (0.3-3.3)
Cartwright (1984)	47	14 (30)	33 (70)	10 (29)	25 (71)	1.1 (0.4-2.8)
Lower (1979) (Wisconsin)	34	20 (59)	14 (41)	20 (49)	21 (51)	1.5 (0.6-3.8)
Woodhouse (1982)	30	21 (70)	9 (30)	16 (59)	11 (41)	1.6 (0.5-4.8)
Su (1998)	27	8 (30)	19 (70)	7 (12)	53 (88)	3.3 (1.0-9.9)
Miller (1983)	26	12 (46)	14 (54)	18 (69)	8 (31)	0.4 (0.1-1.2)
Karakaya (1986)	23	9 (39)	14 (61)	67 (61)	42 (39)	0.4 (0.2-0.9)

OR, Odds ratio; CI, confidence interval.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for the association of NAT2 acetylation status and bladder cancer—individual studies OR, Odds ratio; CI, confidence interval.

A pooling of all studies generated an odds ratio of 1.4 (CI 1.2-1.6) (Table 3). Statistical tests indicated, however, that pooling of all studies (P -value, Q -statistic 0.01) as well as restriction to studies conducted in Caucasian populations (P -value, Q -statistic 0.02) hid potentially important heterogeneity in the individual study results. Exclusion of the Indian and Middle Eastern studies, the two studies that were geographically distinct from all other studies and that produced the most extreme results, resulted in a Q -statistic that was no longer statistically significant ($P = 0.2$). Nevertheless, additional geographical subdivisions of the remaining studies suggested other regional differences. Studies conducted in Asia produced an OR of 2.1 (CI 1.2-3.8, $n = 3$); in Europe, an OR of 1.4 (CI 1.2-1.6, $n = 14$), and in the USA, an OR of 0.9 (CI 0.7-1.3, $n = 3$) (Table 3, Fig. 2). A very similar US result was obtained even when the pooling was restricted to Caucasians (Table 3).

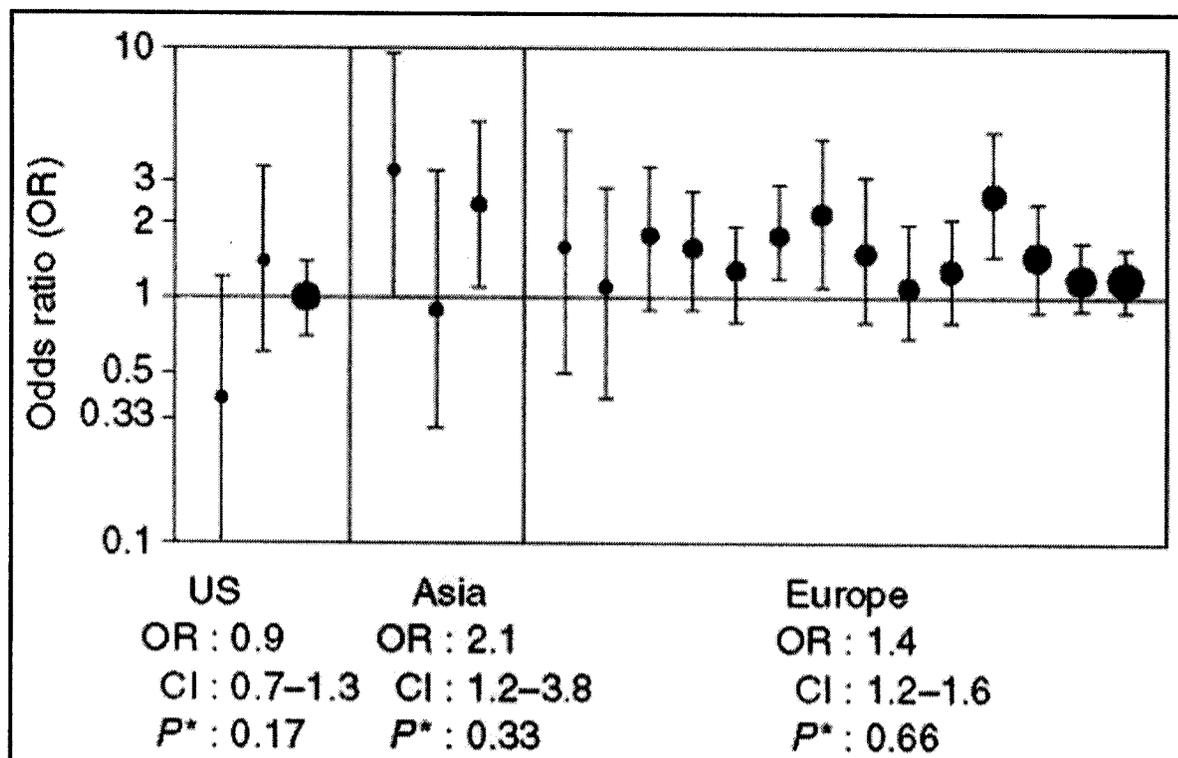


Fig. 2. Odds ratios and 95% confidence intervals for NAT2 slow acetylation and bladder cancer risk by geographical location. Turkey and India are excluded. Circles are proportional to study sample size. The smallest study has a sample size of 23, the largest study has a sample size of 374. * P-value, test for heterogeneity.

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for the association of NAT2 acetylation status and bladder cancer - pooled analyses

	Cases/controls	Cases n (%)		Controls n (%)		OR (CI)
		Slow acetylators	Rapid acetylators	Slow acetylators	Rapid acetylators	
All studies	2496/3340	1479 (59)	1017 (41)	1695 (51)	1645 (49)	1.4 (1.2-1.5)*
All studies except India and Turkey	2416/3171	1420 (59)	996 (41)	1600 (50)	1571 (50)	1.4 (1.2-1.5)
Asia ^b	149/354	31 (21)	118 (79)	33 (9)	321 (91)	2.1 (1.2-3.8)
Europe ^c	1957/2527	1236 (63)	721 (37)	1420 (56)	1107 (44)	1.4 (1.2-1.6)
US only	290/270	153 (53)	137 (47)	147 (54)	123 (46)	0.9 (0.7-1.3)
Europe and Asia	2106/2881	1267 (60)	839 (40)	1453 (50)	1428 (50)	1.5 (1.3-1.7)
Caucasians	2255/2894	1395 (62)	860 (38)	1629 (56)	1265 (44)	1.4 (1.2-1.5)*
Caucasians - US only	275/258	150 (55)	125 (45)	142 (55)	116 (45)	1.0 (0.7-1.4)
Genotyping - Europe only	931/720	590 (63)	341 (37)	402 (56)	318 (44)	1.4 (1.1-1.7)
Phenotyping - Europe only	1026/1807	646 (63)	380 (37)	1018 (56)	789 (44)	1.5 (1.2-1.8)
Sulphamethazine - Europe only	619/1186	402 (65)	217 (35)	699 (59)	487 (41)	1.4 (1.1-1.8)
Dapsone - Europe only	228/267	142 (62)	86 (38)	144 (54)	123 (46)	1.4 (1.0-2.0)

*Fails test for heterogeneity. ^bJapan, Taiwan. ^cDenmark, England, Germany, Italy, Portugal, Spain, Sweden. OR, odds ratio; CI, confidence interval.

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for the association of NAT2 acetylation status and bladder cancer-pooled analyses *Fails test for heterogeneity. ^bJapan, Taiwan. ^cDenmark, England, Germany, Italy, Portugal, Spain, Sweden. OR, odds ratio; CI, confidence interval.

Among studies conducted in European countries, the relationship between NAT2 slow acetylation and bladder cancer risk did not differ by method used to assess acetylation status (older drug-based phenotyping methods: 10 studies, OR 1.5, CI 1.2–1.8; more recent *NAT2* genotyping methods: four studies, OR 1.4, CI 1.1–1.7). Results from European studies that had used sulphamethazine to assess NAT2 phenotype were similar to those from studies that had used dapsone (sulphamethazine– five studies, OR 1.4, CI 1.1–1.8; dapsone– two studies, OR 1.4, CI 1.0–2.0).

Discussion

The results of this meta-analysis suggest that in some but perhaps not all populations, individuals with the NAT2 slow-acetylation phenotype are at greater risk of bladder cancer. This result reflects all identifiable studies of acetylation status and bladder cancer, and it is unlikely that published reports covering studies with substantial numbers of participants were overlooked. The large number of studies conducted in European countries allowed us to address whether the magnitude of the NAT2 slow acetylation/bladder cancer relationship varied systematically by method used to assess acetylation status, which is an important issue as epidemiologic studies switch from drug-based phenotyping to genotyping technology. Our findings indicate that phenotyping and genotyping studies produce comparable results, which is not surprising given that a number of studies have shown excellent correlation between NAT2 phenotype determined pharmacologically and that predicted by *NAT2* genotyping (Blum et al., 1991; Hickman & Sim, 1991; Graf et al., 1992; Mrozikiewicz et al., 1994; Cascorbi et al., 1995).

Our results suggest that the relationship of NAT2 acetylation status and bladder cancer risk may vary by geographical region. We observed the greatest odds ratio for studies conducted in Asia, a modest odds ratio for studies conducted in Europe, and an odds ratio suggesting no association for studies conducted in the US. Of particular interest is the finding of no association in the US, driven primarily by one large population-based study conducted in North Carolina, coupled with the observation of a modest, statistically significant association in the pooling of European studies. Why the results of that study should differ from those conducted in Europe is unclear. A likely explanation is chance. Only three studies have been conducted in the USA, perhaps too few to reliably assess the relationship of NAT2 slow acetylation and bladder cancer. It is possible that the prevalence of other genetic factors interacting with NAT2 to impact bladder cancer risk could be different [for example, NAT1 (Taylor et al., 1998)], but such a situation is unlikely to explain why the North Carolina results differed from those of Europe: most Caucasians in North Carolina trace their origins back to the European countries represented in our meta-analysis (Census of Population & Housing, 1990, 1992).

If the slow acetylation phenotype only impacts risk in the presence of aromatic amine exposure, then differences in the prevalence and intensity of such exposure might explain the region-specific differences. However, smoking habits are unlikely to be substantially different in North Carolina and Europe, with perhaps the exception of Southern Europe (which had very limited representation in this meta-analysis), where black as well as blond tobacco is smoked. As for occupational exposure to aromatic amines, a similar series of job titles were reported by participants in the North Carolina study (Taylor et al., 1998) and those in the one European study that provided such information (Brockmüller et al., 1996).

Another possible explanation is population stratification, which has been suggested by some as a major source of bias in admixed populations (Lander & Schork, 1994). Population stratification is a special form of confounding that occurs when individuals from ethnic groups that differ with

respect to allele frequencies and cancer rates are combined without accounting for ethnic background. Wacholder *et al.* (unpublished work) show how to estimate the bias due to population stratification in studies of admixed Caucasians of European origin residing in the USA by calculating a confounding relative risk (Breslow & Day, 1980). This approach uses the distribution of ethnic origin in the population, the rates of cancer in the countries of origin, and the allele frequency in the countries of origin. Using 1990 US census data, we obtained the distribution of primary country of origin for Caucasian North Carolinians (England, Ireland, Scotland, Germany, Holland, France and Italy) (Census of Population & Housing, 1990, 1992), and using data from *Cancer Incidence in Five Continents* (World Health Organization, 1997), we obtained rates of bladder cancer for the same countries. *NAT2* allele frequency was determined using control subjects from studies of *NAT2* phenotype or genotype (Iwainsky *et al.*, 1961; Fantoli *et al.*, 1963; Ellard *et al.*, 1975; Kergueris *et al.*, 1986; Holland *et al.*, 1991; Hubbard *et al.*, 1997). Our calculations suggest that the odds ratio produced in the study of Taylor *et al.* (1998) could potentially be biased upwards by 3% due to population stratification; that is, the estimate of 1.0 might actually be a slight overestimate of the true relationship of *NAT2* slow acetylation and bladder cancer among North Carolinians. It is therefore highly unlikely that population stratification explains the discrepancy between that study and the genotyping studies conducted in Europe.

Because of limited data, we were unable to examine whether the relationship of *NAT2* slow acetylation and bladder cancer risk differs by tumour aggressiveness (histological grade). We also were unable to limit analyses to incident cases. If *NAT2* is more influential in the development of aggressive disease, then inclusion of prevalent cases would attenuate the magnitude of the association. We also had limited information for most of the control series and, as such, could not assess the influence of different control sources on the results.

The six studies included in this meta-analysis that employed genotyping methods (Risch *et al.*, 1995; Su *et al.*, 1995; Brockmüller *et al.*, 1996; Okkels *et al.*, 1997; Peluso *et al.*, 1998; Taylor *et al.*, 1998) identified the most common *NAT2* alleles, which have been shown to predict *N*-acetylation phenotype with excellent sensitivity and specificity. Although exclusion of certain alleles (such as the rare *NAT2**12 and *13 alleles) may result in misclassification of acetylation status, we calculate (Rothman *et al.*, 1993) that under realistic misclassification scenarios the effect would be minimal. If sensitivity and specificity of genotyping (with regard to the slow acetylation phenotype) were both 90% and the true prevalence of *NAT2* slow acetylation was 56% (as was observed in our European data), then a true odds ratio of 1.5 would be attenuated only to 1.4. A sensitivity of 90% coupled with a specificity of 100% would affect the odds ratio similarly.

We have included, to the best of our knowledge, all accounts of the *NAT2* acetylation/bladder cancer relationship published prior to 1999. We are aware of two studies whose results were presented at the 1999 Annual Meeting of the American Association for Cancer Research (Katoh *et al.*, 1999; Tiemersma *et al.*, 1999). Each study showed an elevation in bladder cancer risk for *NAT2* slow acetylation genotype that was comparable to, or perhaps stronger than, our region-specific findings (study conducted in the Netherlands—OR 1.9, CI 0.9–5.2; study conducted in Japan—OR 4.8, CI 1.6–15.9). Because unpublished (and unrepresented) analyses are more likely than published analyses to have produced unexpected results (in this setting, null or inverse associations), it is possible that our summary measures are overestimates. Our meta-analysis does include a few published studies that produced inverse and null associations, though, suggesting that publication bias, should it exist, is probably not extreme.

We observed a positive association of *NAT2* slow acetylation and bladder cancer risk in most of the 22 studies included in our meta-analysis. Future studies may help to clarify if geographic differences exist. Incorporation of data on other bladder cancer risk and susceptibility factors

with the goal of assessing potential interactions with NAT2, as well as inclusion of information on tumour characteristics, may also help to enhance our understanding of the NAT2/bladder cancer relationship.

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