

Cigarette Smoking, *N*-Acetyltransferase 2 Acetylation Status, and Bladder Cancer Risk: A Case-Series Meta-analysis of a Gene-Environment Interaction

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Abstract

Tobacco use is an established cause of bladder cancer. The ability to detoxify aromatic amines, which are present in tobacco and are potent bladder carcinogens, is compromised in persons with the *N*-acetyltransferase 2 slow acetylation polymorphism. The relationship of cigarette smoking with bladder cancer risk therefore has been hypothesized to be stronger among slow acetylators. The few studies to formally explore such a possibility have produced inconsistent results, however. To assess this potential gene-environment interaction in as many bladder cancer studies as possible and to summarize results, we conducted a meta-analysis using data from 16 bladder cancer studies conducted in the general population ($n = 1999$ cases). Most had been conducted in European countries. Because control subjects were unavailable for a number of these studies, we used a case-series design, which can be used to assess multiplicative gene-environment interaction without inclusion of control subjects. A case-series interaction odds ratio (OR) >1.0 indicates that the relationship of cigarette smoking and bladder cancer risk is stronger among slow acetylators as compared with rapid

acetylators. We observed an interaction between smoking and *N*-acetyltransferase 2 slow acetylation (OR, 1.3; 95% confidence interval, 1.0–1.6) that was somewhat stronger when analyses were restricted to studies conducted in Europe (OR, 1.5; confidence interval, 1.1–1.9), a pooling that included nearly 80% of the collected data. Using the predominantly male European study population and assuming a 2.5-fold elevation in bladder cancer risk from smoking, we estimated that the population attributable risk percent was 35% for slow acetylators who had ever smoked and 13% for rapid acetylators who had ever smoked. These results suggest that the relationship of smoking and bladder cancer is stronger among slow acetylators than among rapid acetylators.

Introduction

Tobacco use is an established cause of bladder cancer, resulting in a 2- to 3-fold increased risk among individuals who have ever smoked. Although certain occupational exposures (*e.g.*, benzidine) confer a much greater elevation in risk, cigarette smoking is nevertheless responsible for more cases of bladder cancer than any other risk factor because of its higher prevalence (1). It is estimated that in some populations, 50% of bladder cancer in males and 25% of bladder cancer in females could be prevented with elimination of cigarette smoking (2).

Aromatic amines are suspected to be the primary causative agent for bladder cancer in tobacco smoke (3). *N*-acetylation, which occurs mainly in the liver and is chiefly regulated by the enzyme NAT2,² can detoxify monoarylamines (*e.g.*, 4-aminobiphenyl), rendering them less susceptible to metabolic activation by P-450 enzymes (3). The lack of two functional NAT2 alleles confers the slow acetylation phenotype, which is thought to compromise detoxification ability (3). For that reason, Lower *et al.* (4) hypothesized in 1979 that slow acetylators would be at an elevated bladder cancer risk.

Since then, at least 22 case-control studies have examined the relationship of NAT2 acetylation status and bladder cancer in the general population (4–24). A recent meta-analysis of those studies (25) reported a positive association between slow acetylation status and bladder cancer, although there was a suggestion that the relationship varied somewhat by geographic region; a positive association was observed for studies conducted in Europe and Asia, but not for studies conducted in the United States. Few of the 22 studies formally explored whether the relationship of cigarette smoking and bladder cancer differed by acetylation status; results were inconsistent among the studies that performed such analyses (5–7, 17, 19, 22).

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²The abbreviations used are: NAT2, *N*-acetyltransferase 2; OR, odds ratio; CI, confidence interval.

Because cigarette smoking is a relatively common habit, and the slow acetylation phenotype is a relatively common metabolic polymorphism [about 55% in populations of European descent, 35% in populations of African descent, and 15% in populations of Asian descent (26)], a potential modifying effect of acetylation status on the relationship between cigarette smoking and bladder cancer risk is of considerable interest. To explore such a possibility, as well as to summarize results of separate studies, we undertook a meta-analysis of bladder cancer studies that had been conducted in the general population and had collected data on cigarette smoking and acetylation status.

Materials and Methods

Data Collection. We conducted our first search for studies in 1991. MEDLINE was used to conduct literature searches; citations in identified articles and review articles (27) also were examined (28, 29). Any case-control study of bladder cancer that was conducted in the general population and that collected data on cigarette smoking and NAT2 acetylation status (either as a phenotype or an NAT2 genotype) was eligible for inclusion; studies conducted in or derived in part from cohorts of workers with documented exposure to carcinogenic aromatic amines were excluded. Investigators associated with eligible studies were invited to contribute data to our effort. In addition to smoking history and acetylation status, we also requested data on age, sex, history of potential occupational exposure to aromatic amines, tumor stage, tumor grade, date of diagnosis, and date of study enrollment.

After several years of data collection efforts, information had been received for only about half the identified studies, primarily because data sets were no longer available. Ultimately, we decided to employ a case-series design because this method would allow for inclusion of unavailable studies if case cross-classification of cigarette smoking (ever/never) and acetylation status (slow/rapid) had been published. Although control cross-classifications were available for some studies, it was unclear how representative the control series were of the individual base populations, especially with regard to tobacco use. Therefore, we did not use these data to address our primary research questions.

Another MEDLINE search was conducted to identify studies that had been published after our initial search and before the end of 1998. The results of this search brought the number of eligible published studies to 20 (4–13, 15–23), 15 (4–6, 8–13, 15–17, 19, 20, 22) of which (1908 of 2179 cases; 88%) could be included in the meta-analysis. For the newly identified studies, acetylation and smoking were abstracted directly from published manuscripts, although in one instance it was necessary to contact authors for clarification (17). We also included data from one study that had been supplied to us as unpublished data during the early stages of our project but is presently submitted (91 cases).³ The final data set for the meta-analysis included 16 studies and 1999 cases. Of those, response rates were available for only one study (17).

Data on acetylation status (phenotype or NAT2 genotype) and cigarette smoking (never/ever smoked) were available for all cases. Some investigator-supplied data sets also provided

information on age (9, 12, 13, 16, 20) and potential occupational exposure to aromatic amines (13).³ Participants' sex was known for all studies except two (4),³ although sex-specific cross-tabulations of acetylation status and cigarette smoking were available for only seven (6, 9, 11–13, 16, 20). Race was not available for most studies, but it is reasonable to assume that studies conducted in Europe were comprised primarily of Caucasians. Of the two studies conducted in the United States, one was known to be comprised solely of Caucasians (15); in the other, 93% of cases were known to be Caucasian (22).

In two studies, individuals who used tobacco products other than cigarettes could be identified (16);³ they were excluded to minimize tobacco exposure among never-smokers. Two studies were known to have included prevalent cases (5, 17), although it is likely that many other studies included such cases also. Ten studies had data on smoking and acetylation status for control subjects (5, 6, 10–12, 16, 19, 20, 22).³ Most of these series (5, 6, 11, 16, 19, 20, 22)³ consisted of either clinic attendees or hospital in-patients.

Statistical Analyses. In a case-series study of gene-environment interaction, an OR (referred to as a "case-series interaction OR" in the remainder of this paper) is calculated from cross-classification of exposure and genetic information among cases only (30–33). A case-series interaction OR >1 in the present study indicates that the relationship of cigarette smoking and bladder cancer is stronger among slow acetylators than among rapid acetylators. Independence of exposure and the genetic factor in the base population is necessary for valid interpretation of a case-series interaction OR. In the present study, the validity of that assumption was assessed by calculating χ^2 statistics for the available controls series as well as for a pooled analysis of those series (34, 35). Logistic regression was used to estimate ORs and 95% CIs in the individual case series (36, 37). Meta-analysis techniques that weighted the estimated β coefficient for each individual study by a function of its variance were used to calculate a summary estimate (38, 39). Because results for fixed and random effects models were nearly identical and because the hypothesis of homogeneity was not rejected in any instance [using the Q-statistic (38) at a significance level of 0.05], results from only fixed effect models are presented.

Our analyses addressed the association between ever having smoked cigarettes (*versus* never smoking) and slow acetylation status (*versus* rapid acetylation status) among bladder cancer cases. Because a number of studies have shown excellent correlation between NAT2 phenotype determined pharmacologically and that predicted by NAT2 genotyping (40–44) and because the relationship of NAT2 acetylation status and bladder cancer risk did not vary by method used to assess NAT2 in a recent meta-analysis (25), only in one instance do we present separate results from studies using genotyping. The result of that analysis further supports pooling of studies.

Data on age were available for 32% of the pooled data set, and data on potential occupational exposure to aromatic amines were available for 19%. By limiting analyses to subsets where these variables were available, we assessed potential confounding effects. Age was categorized as <55 years, 55–64 years, 65–74 years, and ≥ 75 years. Potential occupational exposure to aromatic amines was coded as history or no history of exposure. If the OR of interest changed by >10% with inclusion of the variable, confounding was said to exist. Potential effect modification by sex could not be assessed owing to the small cell

³ M. Romkes, N. Paulsen, C. M. Fleming, R. A. Persad, P. J. B. Smith, C. Collins, A. Schwartz, and R. A. Branch. The *N*-acetyltransferase slow acetylator phenotype as a major risk factor for aggressive bladder cancer following industrial occupational exposure, submitted for publication.

Table 1 Data sources used in the meta-analysis

First author, Date	No. of cases	Data source	Country (city or state)	Phenotyping or genotyping (drug used for phenotyping or mutant allele) ^a
Brockmoller, 1996 (5)	374	Publication	Germany (Berlin)	Genotyping (NAT2*5, 6, 7)
Okkels, 1997 ^a (17)	253	Publication	Denmark (Aarhus)	Genotyping (NAT2*5, 6, 7)
Taylor, 1998 (22)	230	Publication	United States (North Carolina)	Genotyping (NAT2*5, 6, 7, 14)
Risch, 1995 (19)	178	Publication	England (Birmingham)	Genotyping (NAT2*5, 6, 7)
Mommsen, 1986 (16)	149	Investigator	Denmark (Aarhus)	Phenotyping (sulfamethazine)
Ladero, 1985 (13)	130	Investigator	Spain (Madrid)	Phenotyping (sulfamethazine)
Hanssen, 1985 (8)	105	Publication	Germany (Hamburg)	Phenotyping (sulfamethazine)
Roots, 1989 (20)	101	Investigator	Germany (Berlin)	Phenotyping (caffeine)
Kaisary, 1987 (11)	98	Investigator	England (Bristol)	Phenotyping (dapsone)
Romkes, 2000 ^b	91	Investigator	England (Bristol)	Phenotyping (dapsone)
Dewan, 1995 (6)	77	Publication	India (Ahmedabad)	Phenotyping (isoniazid)
Lower, 1979 (4)	67	Publication	Denmark (Copenhagen)	Phenotyping (sulfamethazine)
Horai, 1989 (9)	50	Investigator	Japan (Tokyo)	Phenotyping (dapsone)
Ishizu, 1995 (10)	47	Publication	Japan (Tokyo)	Phenotyping (isoniazid)
Miller, 1983 (15)	26	Publication	United States (Western NY state)	Phenotyping (sulfamethazine)
Karakaya, 1986 (12)	23	Investigator	Turkey (Ankara)	Phenotyping (sulfamethazine)

^a Authors were contacted to verify joint-frequencies derived from published data.

^b M. Romkes, N. Paulsen, C. M. Fleming, R. A. Persad, P. J. B. Smith, C. Collins, A. Schwartz, and R. A. Branch. The *N*-acetyltransferase slow acetylator phenotype as a major risk factor for aggressive bladder cancer following industrial occupational exposure, submitted for publication.

Table 2 Independence of NAT2 acetylation status and cigarette smoking among available control subjects

First author	Never-smokers <i>n</i> (%)			Ever-smokers <i>n</i> (%)		<i>P</i>
	Total controls	Slow acetylators	Rapid acetylators	Slow acetylators	Rapid acetylators	
Brockmoller (5)	373	51 (61)	33 (39)	165 (57)	124 (43)	0.55
Taylor (22)	203	44 (60)	30 (40)	65 (50)	64 (50)	0.21
Risch (19)	39	6 (55)	5 (45)	11 (40)	17 (60)	0.39
Mommsen (16)	69	11 (50)	11 (50)	25 (53)	22 (47)	0.81
Roots (20)	101	6 (33)	12 (67)	39 (47)	44 (53)	0.29
Kaisary (11)	116	29 (40)	44 (60)	20 (47)	23 (53)	0.48
Romkes ^b	70	10 (48)	11 (52)	24 (49)	25 (51)	0.92
Dewan (6)	80	11 (35)	20 (63)	17 (35)	32 (65)	0.94
Ishizu (10)	91	7 (16)	38 (84)	6 (13)	40 (87)	0.73
Karakaya (12)	109	39 (65)	21 (35)	28 (57)	21 (43)	0.42
Combined	1251	214 (49)	225 (51)	400 (49)	412 (51)	0.40 ^a

^a Weighted by study site; *P* for *Q*-statistic (test for homogeneity): 0.88.

^b M. Romkes, N. Paulsen, C. M. Fleming, R. A. Persad, P. J. B. Smith, C. Collins, A. Schwartz, and R. A. Branch. The *N*-acetyltransferase slow acetylator phenotype as a major risk factor for aggressive bladder cancer following industrial occupational exposure, submitted for publication.

sizes produced by cross-tabulations of acetylation status and cigarette smoking, even in analyses restricted to males.

To provide a more accustomed interpretation, we converted our case-series interaction OR to the corresponding measures that would be generated using data from a case-control study (that is, the ORs for non-smoking slow acetylators, smoking rapid acetylators, and smoking slow acetylators, all relative to nonsmoking rapid acetylators). Four additional parameters were necessary: prevalence of smoking and NAT2 slow acetylation in the base population, and the bladder cancer ORs for smoking and NAT2 slow acetylation. Details of this method are presented in the "Appendix." Calculations were restricted to European studies because several of the necessary parameters (*e.g.*, prevalence of NAT2 slow acetylation and the association of NAT2 slow acetylation with bladder cancer) vary by geographic region (25) and substantial amounts of data were available for the European region only (77% of the total data set). Population attributable risk percents (45) were calculated using the European smoking and slow acetylation prevalences and the derived case-control ORs.

Results

Selected characteristics of the 16 data sets are shown in Table 1. Most were obtained from publications and provided NAT2 phenotype rather than genotype information. The majority of cases were European (77%) and male (73%, based on the 14 studies with that information). Individual data sets ranged in size from 23 to 374 cases. The pooled data set contained 1999 cases.

Smoking history and acetylation status were independent among available control subjects (Table 2). Overall, 49% of non-smokers were slow acetylators, as were 49% of ever-smokers (*P* = 0.40). In many of the control series, the percentages of slow acetylators among smokers and non-smokers were quite similar.

A case-series interaction OR of 1.3 (95% CI, 1.0–1.6) was observed when data were pooled (Table 3), suggesting that the relationship of cigarette smoking and bladder cancer is stronger among slow acetylators compared with rapid acetylators. Neither age nor potential occupational exposure to aromatic amines confounded that relationship (data not shown). Restriction to studies that used genotyping produced results similar to the overall finding. Individual study results varied (Table 3; Fig. 1),

Table 3 Case-series interaction ORs and 95% CIs for NAT2 slow acetylation, cigarette smoking, and bladder cancer

	Total cases	Never-smokers n (%)		Ever-smokers n (%)		OR (CI)
		Slow acetylators	Rapid acetylators	Slow acetylators	Rapid acetylators	
Pooled data						
All sites	1999	233 (52)	215 (48)	950 (61)	601 (39)	1.3 (1.0–1.6)
Genotyping only ^a	1035	121 (55)	98 (45)	511 (63)	305 (37)	1.4 (1.0–1.9)
About ≥100 cases ^b	1618	207 (57)	159 (43)	791 (63)	461 (37)	1.4 (1.1–1.7)
About ≥150 cases ^c	1184	144 (55)	118 (45)	583 (63)	339 (37)	1.5 (1.1–2.0)
European countries only ^d	1546	190 (56)	148 (44)	786 (65)	422 (35)	1.5 (1.1–1.9)
European countries, about ≥150 cases ^e	954	122 (55)	101 (45)	484 (66)	247 (34)	1.7 (1.2–2.3)
Individual studies (ordered by no. of cases)						
Brockmoller (5)	374	63 (56)	50 (44)	171 (66)	90 (34)	1.5 (1.0–2.4)
Okkels (17)	253	14 (47)	16 (53)	140 (62)	83 (38)	1.9 (0.9–4.2)
Taylor (22)	230	22 (56)	17 (44)	99 (52)	92 (48)	0.8 (0.4–1.6)
Risch (19)	178	22 (59)	15 (41)	101 (72)	40 (28)	1.7 (0.8–3.6)
Mommsen (16)	149	23 (53)	20 (47)	72 (68)	34 (32)	1.8 (0.9–3.8)
Ladero (13)	130	13 (62)	8 (38)	70 (64)	39 (36)	1.1 (0.4–2.9)
Hanssen (8)	105	14 (61)	9 (39)	51 (62)	31 (38)	1.1 (0.4–2.7)
Roots (20)	101	11 (69)	5 (31)	55 (65)	30 (35)	0.8 (0.2–2.5)
Kaisary (11)	98	25 (53)	19 (43)	32 (59)	22 (41)	1.1 (0.5–2.5)
Romkes (30)	91	3 (38)	5 (63)	52 (63)	31 (38)	2.8 (0.6–10.7)
Dewan (6)	77	4 (40)	6 (60)	42 (63)	25 (37)	2.5 (0.6–9.7)
Lower (4)	67	2 (67)	1 (33)	42 (66)	22 (34)	1.0 (0.1–11.0)
Horai (9)	50	2 (7)	28 (93)	1 (5)	19 (95)	0.7 (0.0–8.2)
Ishizu (10)	47	7 (39)	11 (61)	9 (31)	20 (69)	0.7 (0.2–2.4)
Miller (15)	26	3 (50)	3 (50)	9 (45)	11 (55)	0.8 (0.1–5.4)
Karakaya (12)	23	5 (71)	2 (29)	4 (25)	12 (75)	0.1 (0.0–0.9)

^a Includes references (5,17,19,22).

^b Includes references (5,8,11,13,16,17,19,20,22).

^c Includes references (5,16,17,19,22).

^d Includes references (4,5,8,11,13,16,17,19,20) and M. Romkes, N. Paulsen, C. M. Fleming, R. A. Persad, P. J. B. Smith, C. Collins, A. Schwartz, and R. A. Branch. The N-acetyltransferase slow acetylator phenotype as a major risk factor for aggressive bladder cancer following industrial occupational exposure, submitted for publication.

^e Includes references (5,16,17,19).

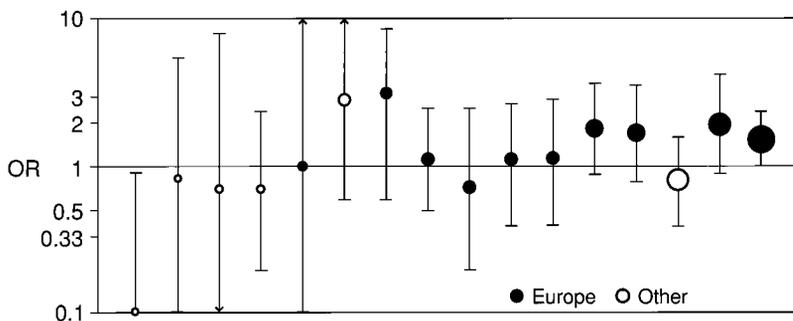


Fig. 1. Case-series interaction ORs and 95% CIs for NAT2 slow acetylation, cigarette smoking, and bladder cancer: individual studies. Circles are proportional to the study sample size. The smallest study has a sample size of 23; the largest study has a sample size of 374.

although the summary ORs generated by the five studies consisting of about 150 subjects (OR, 1.5; CI, 1.1–2.0) and by the 10 studies conducted in European countries (OR, 1.5; CI, 1.1–1.9) suggested stronger interaction. An OR of 1.7 (CI, 1.2–2.3) was generated from the four European studies that had about ≥150 subjects (48% of all data).

We converted our European OR of 1.5 to a series of point estimates that would be expected using data from a comparable case-control study (see “Appendix”). Calculations were made using the following values: 54% and 59% prevalence for NAT2 slow acetylation and ever smoking, respectively; bladder cancer ORs of 1.5 and 2.5 for NAT2 slow acetylation and ever smoking, respectively. All parameters were derived from the European studies included in this meta-analysis, with exception of the bladder cancer OR for smoking, which was obtained from

a review article (see “Appendix”). Based on these assumptions, nonsmoking slow acetylators are predicted to have no elevation in bladder cancer risk, relative to nonsmoking rapid acetylators (Table 4). Relative to that same group, rapid acetylators who smoke are predicted to have about a 2-fold elevation in risk, and slow acetylators who smoke, about a 3-fold elevation in risk. Using these findings, the estimated population attributable risk percent for smoking was 48%, which partitioned to 13% for rapid acetylators and 35% for slow acetylators.

Discussion

This meta-analysis suggests that the association of cigarette smoking and bladder cancer risk is stronger (30–50%) among NAT2 slow acetylators as compared with rapid acetylators.

Table 4 The interaction of NAT2 acetylation status and cigarette smoking with bladder cancer risk among European countries: calculation of case-control ORs from the case-series interaction OR of 1.5^a

	Rapid acetylation	Slow acetylation
Never-smokers	1.00 ^b	1.10
Ever-smokers	1.95	3.21

^a Calculated assuming: prevalence of ever having smoked cigarettes, 59%; prevalence of NAT2 slow acetylation, 54%; OR of NAT2 slow acetylation and bladder cancer, 1.5; OR of bladder cancer among ever smokers, 2.5 (see "Appendix" for methods and data sources).

^b Reference category.

These results, coupled with a number of assumptions about smoking and NAT2 slow acetylation prevalence and their relationships with bladder cancer risk, indicate that smokers who are slow acetylators have a higher risk of bladder cancer than smokers who are rapid acetylators and that slow acetylators who do not smoke are at a similar risk compared to nonsmoking rapid acetylators. Using the data of our predominantly male European study population and assuming a 2.5-fold elevation in bladder cancer risk from smoking, we calculated the attributable risk percent for smoking to be 48%. That figure partitioned into 13% for rapid acetylators and 35% for slow acetylators.

Although the pooled analysis suggested a modest interaction between NAT2 status and smoking, the magnitude of the individual study case-series interaction ORs varied somewhat. Use of the Q-statistic indicated that pooling was not inappropriate, but that test has low power to detect heterogeneity (38). To investigate variability in results, we conducted analyses on subsets of our data and observed stronger, more consistent associations among the larger studies. The larger studies may have produced consistent results because their point estimates were more precise. We also observed stronger and more consistent associations among the 10 studies conducted in Europe. Patterns of tobacco use, including intensity and type of tobacco smoked, impact the extent of aromatic amine exposure (46) and vary across geographic region; they therefore could be responsible for some variation in results. Variation in results could not be explained by the method used to assess NAT2 acetylation status, which is not surprising given how well results from NAT2 phenotyping and genotyping correlate (40–44).

Five eligible studies, representing only 16% of eligible cases, could not be included in this meta-analysis (4, 7, 18, 21, 23), yet there is no obvious reason to believe that their omission makes the group of included studies unrepresentative in some manner. The omitted studies are similar to the included studies in terms of the magnitude of the crude NAT2 main effect; using the same meta-analysis techniques, we obtained a bladder cancer OR of 1.4 (CI, 1.0–1.8) using the omitted studies, and an OR of 1.5 (CI, 1.2–1.8) using the included studies. Because good correlation exists between the crude NAT2 main effect and the NAT2-smoking interaction (Spearman correlation coefficient of 0.46 for the 16 studies included in this meta-analysis), it is unlikely that inclusion of the 5 omitted studies would have changed our findings substantially.

Publication bias, which occurs when studies with null or unexpected results are not published and therefore cannot, in most instances, be included in meta-analyses, could affect this meta-analysis. Our ability to assess the degree of publication bias is limited, but some evidence exists suggesting the absence of substantial publication bias. Fig. 1 indicates a wide range of results for studies with small sample size and a narrower range of results for studies with larger sample

size, as would be expected given the usual effects of random variation (47) both within and across studies. Although many of the studies producing null or inverse associations were published at a time when the slow acetylator hypothesis was not firmly established (thus minimizing the chance that results would not be published), two studies published in the second half of the 1990s (10, 22), a time when the slow acetylator hypothesis was better known and more widely accepted, produced null associations also. The validity of our results may be affected by confounding, misclassification, or other limitations of our data. Adjustment for age and possible occupational exposure to aromatic amines, the most plausible confounding variables, did not meaningfully change the ORs of interest, but these analyses could only be carried out on a small subset of the data and therefore may not be generalizable to the rest. Limited information on use of tobacco products other than cigarettes, as well as exposure to environmental tobacco smoke, prevented us from excluding from our unexposed category all individuals who were exposed to aromatic amines from other tobacco sources. Such misclassification would tend to attenuate our results. Our findings also may be affected by error in assigned acetylation status, as well as misreport of cigarette smoking history. Certain unusual misclassification scenarios could bias results away from the null, but the most probable situation, the one in which smoking and acetylation status misclassification are independent of one another and sensitivity and specificity of the two exposures are not severely compromised (that is, the sum of sensitivity and specificity is ≥ 1), would result in bias toward the null (48). Given these limitations, it is likely these findings, if anything, are underestimates of the true relationship.

With regard to the assumptions required for valid interpretation of case-series findings, we are confident that in these studies, smoking and acetylation status were independent, but we are less certain about the representativeness of the bladder cancer cases. At least two studies included prevalent cases (5, 17), and it is likely that some of the older studies did as well. It has been suggested that the NAT2 slow polymorphism is more influential in aggressive bladder cancer (49) and as such, it would have been best to analyze data separately for certain tumor characteristics. A large study published in 1996, however, observed similar proportions of NAT2 slow acetylators among incident and prevalent cases, as well as for different tumor grades and histological subtypes (5).

We were unable to examine whether the presence of a gene-environment interaction differs by level of smoking intensity (amount smoked per day) or duration (years smoked and pack-years smoked). Although such data were available for a small subset of the studies (around 20% of all subjects), we were concerned that such limited information would not produce generalizable results. Furthermore, a dearth of light smokers made point estimates for such categories very imprecise. The findings of a cross-sectional study that addressed the influence of NAT2 acetylation on the development of 4-aminobiphenyl hemoglobin adducts support the notion that the magnitude of the gene-environment interaction differs by smoking level, but suggests that the interaction may be most pronounced at lower levels of use (50).

The study of gene-environment interactions may help enhance our understanding of how some exposures impact bladder cancer risk. Our meta-analysis of cigarette smoking, NAT2 acetylation status, and bladder cancer risk has addressed a number of pertinent issues, but our summary result, which was based in part on a number of small, older studies, must be

replicated in larger studies. Future studies should address the impact of varied smoking habits, as well as the joint impact of NAT2 and other genetic factors, including the *GSTM1* null genotype, which consistently has been associated with bladder cancer (51), and polymorphisms in NAT1, thought to be involved in *o*-acetylation of aromatic amines (52). Interestingly, Taylor *et al.* (22) reported a three-way multiplicative interaction of NAT1 genotype, NAT2 genotype, and cigarette smoking: a synergistic interaction of NAT2 and cigarette smoking was observed only among individuals who carried the *NAT1*10* allele. Examination of urinary pH (53), which influences the hydrolysis of aromatic amines in the bladder, as well as water intake (54) and voiding frequency, also may shed light on other susceptibility factors for tobacco-induced bladder cancer.

Acknowledgments

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Appendix

Calculations used to determine case-control ORs from case-series interaction ORs and other information. The ORs for the effect of smoking among NAT2 rapid acetylators ($OR_{E=1|G=0}$), and the effect of NAT2 slow acetylation among nonsmokers ($OR_{G=1|E=0}$) were calculated using these five estimates:

- Interaction effect (OR_{int}) of 1.5. Obtained using the 10 European studies included in this meta-analysis.
- Prevalence of NAT2 slow acetylation ($P(G = 1)$) of 54%. Obtained using the 10 European studies included in this meta-analysis.
- Crude OR for the main effect of NAT2 slow acetylation and bladder cancer risk ($OR_{G=1}$) of 1.5. Obtained using the 10 European studies included in this meta-analysis.
- Crude OR for the main effect of smoking and bladder cancer risk ($OR_{E=1}$) of 2.5. Midpoint of the 2-to-3-fold increase in risk reported by Silverman *et al.* (1). The crude OR for the main effect of ever smoking was not calculated from European studies included in this meta-analysis because of concerns that smokers were overrepresented in the hospital/clinic based control series.
- Prevalence of smoking ($P(E = 1)$) of 59%. Derived using the OR for smoking (2.5) and the prevalence of ever smoking among cases (78%) in the 10 European studies included in this meta-analysis.

The following equations* were used to solve for $OR_{E=1|G=0}$ and $OR_{G=1|E=0}$.

$$OR_{E=1} = \frac{P(G=0) \times OR_{E=1|G=0} + P(G=1) \times OR_{E=1|G=0} \times OR_{G=1|E=0} \times OR_{int}}{P(G=0) + P(G=1) \times OR_{G=1|E=0}}$$

$$OR_{G=1} = \frac{P(E=0) \times OR_{G=1|E=0} + P(E=1) \times OR_{E=1|G=0} \times OR_{G=1|E=0} \times OR_{int}}{P(E=0) + P(E=1) \times OR_{E=1|G=0}}$$

The joint effect for NAT2 slow acetylation and smoking ($OR_{E=1,G=1}$) was then calculated by multiplying $OR_{G=1|E=0} \times OR_{E=1|G=0} \times OR_{int}$.

*These equations are derived as follows: The crude OR for the main effect of exposure on disease risk ($OR_{E=1}$) can be expressed in terms of the cell counts of two 2×2 tables— E by D for $G = 1$ and E by D for $G = 0$. The crude OR for the main effect of the genetic factor on disease risk ($OR_{G=1}$) can be expressed in terms of the cells counts of two 2×2 tables— G by D for $E = 1$ and G by D for $E = 0$. Algebraic manipulation of $OR_{E=1}$ and $OR_{G=1}$ expressed in terms of the cell counts results in the formulas we present in the "Appendix" because all of the terms on the right-hand side of the equation can also be expressed in terms of the cell counts in the two 2×2 tables. These formulas assume that E and G are independent among the controls. In the instance of no interaction ($OR_{int} = 1$), the formulas for the crude ORs will be the same as the Mantel-Haenszel OR. These formulas were presented in Ref. 55.

References

1. Silverman, D. T., Morrison, A. S., and Devesa, S. S. Bladder cancer. *In: D. Schottenfeld, and J. F. Fraumeni, Jr., Cancer Epidemiology and Prevention*, New York: Oxford University Press, 1996, pp. 1156–1179.
2. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Smoking. Lyon, France: WHO, 1986.
3. Ross, R. K., Jones, P. A., and Yu, M. C. Bladder cancer epidemiology and pathogenesis. *Semin. Oncol.*, 23: 536–545, 1996.

4. Lower, G. M., Nilsson, T., Nelson, C. E., Wolf, H., Gamsky, T. E., and Bryan, G. T. *N*-Acetyltransferase phenotype and risk in urinary bladder cancer: approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. *Environ. Health Perspect.*, 29: 71–79, 1979.
5. Brockmüller, J., Cascorbi, I., Kerb, R., and Roots, I. Combined analysis of inherited polymorphisms in arylamine *N*-acetyltransferase 2, glutathione *S*-transferase M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Res.*, 56: 3915–3925, 1996.
6. Dewan, A., Chattopadhyay, P., and Kulkarni, P. K. *N*-Acetyltransferase activity—a susceptibility factor in human bladder carcinogenesis. *Indian J. Cancer*, 32: 15–19, 1995.
7. Evans, D. A. P., Eze, L. C., and Whibley, E. J. The association of the slow acetylator phenotype with bladder cancer. *J. Med. Genet.*, 20: 330–333, 1983.
8. Hanssen, H. P., Agarwal, D. P., Goedde, H. W., Bucher, H., Huland, H., Brachmann, W., and Ovenbeck, R. Association of *N*-acetyltransferase polymorphism and environmental factors with bladder carcinogenesis. *Eur. Urol.*, 11: 263–266, 1985.
9. Horai, Y., Fujita, K., and Ishizaki, T. Genetically determined *N*-acetylation and oxidation capacities in Japanese patients with non-occupational urinary bladder cancer. *Eur. J. Clin. Pharmacol.*, 37: 581–587, 1989.
10. Ishizu, S., Hashida, C., Hanaoka, T., Maeda, K., and Ohishi, Y. *N*-Acetyltransferase activity in the urine in Japanese subjects: comparison in healthy persons and bladder cancer patients. *Jpn. J. Cancer Res.*, 86: 1179–1181, 1995.
11. Kaisary, A., Smith, P., Jaczq, E., McAllister, B., Wilkinson, G. R., Ray, W. A., and Branch, R. A. Genetic predisposition to bladder cancer: ability to hydroxylate debrisoquine and mephenytoin as risk factors. *Cancer Res.*, 47: 5488–5493, 1987.
12. Karakaya, A. E., Cok, I., Sardas, S., Gogus, O., and Sardas, O. S. *N*-Acetyltransferase phenotype of patients with bladder cancer. *Hum. Toxicol.*, 5: 333–335, 1986.
13. Ladero, J. M., Kwok, C. K., Jara, C., Fernández, L., Silmi, A. M., Tapia, D., and Usón, A. C. Hepatic acetylator phenotype in bladder cancer patients. *Ann. Clin. Res.*, 17: 96–99, 1985.
14. Lower, G. M., and Bryan, G. T. Etiology and carcinogenesis: natural systems approaches to causality and control. *In: N. Javadpour (ed.), Principles and Management of Urologic Cancer*, pp. 29–53. Baltimore: Williams and Wilkins, 1979.
15. Miller, M. E., and Cosgriff, J. M. Acetylator phenotype in human bladder cancer. *J. Urol.*, 130: 65–66, 1983.
16. Mommsen, S., and Aagaard, J. Susceptibility in urinary bladder cancer: acetyltransferase phenotypes and related risk factors. *Cancer Lett.*, 32: 199–205, 1986.
17. Okkels, H., Sigsgaard, T., Wolf, H., and Autrup, H. Arylamine *N*-Acetyltransferase 1 (NAT1), and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. *Cancer Epidemiol. Biomark. Prev.*, 6: 225–231, 1997.
18. Peluso, M., Airoldi, L., Armelle, M., Martone, T., Coda, R., Malaveille, C., Giacomelli, G., Terrone, C., Casetta, G., and Vineis, P. White blood cell DNA adducts, smoking, and NAT2 and GSTM1 genotypes in bladder cancer: a case-control study. *Cancer Epidemiol. Biomark. Prev.*, 7: 341–346, 1998.
19. Risch, A., Wallace, D. M. A., Bathers, S., and Sim, E. Slow *N*-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. *Hum. Mol. Genet.*, 4: 231–236, 1995.
20. Roots, I., Drakoulis, N., Brockmüller, J., Janicke, I., Cuprunov, M., and Ritter, J. Hydroxylation and acetylation phenotypes as genetic risk factors in certain malignancies. *In: R. Kato, R. W. Estabrook, and M. N. Cayen (eds.), Xenobiotic Metabolism and Disposition*, pp. 499–506. London: Taylor and Francis, 1989.
21. Su, H. J., Guo, Y. L., Lai, M. D., Huang, J. D., Cheng, Y., and Christiani, D. C. The NAT2* slow acetylator genotype is associated with bladder cancer in Taiwanese, but not in the Black Foot Disease endemic area population. *Pharmacogenetics*, 8: 187–190, 1998.
22. Taylor, J. A., Umbach, D. M., Stephens, E., Castranio, T., Paulson, D., Robertson, C., Mohler, J. L., and Bell, D. A. The role of *N*-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res.*, 58: 3603–3610, 1998.
23. Woodhouse, K. W., Adams, P. C., Clothier, A., Mucklow, J. D., and Rawlins, M. D. *N*-acetylation phenotype in bladder cancer. *Hum. Toxicol.*, 1: 443–445, 1982.
24. Cartwright, R. Epidemiologic studies on *N*-acetylation and C-center ring oxidation in neoplasia. *In: G. S. Omenn and H. V. Gelboin (eds.), Banbury Report 16: Genetic Variability in Responses to Chemical Exposure*, pp. 359–368. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1984.

25. Marcus, P. M., Vineis, P., and Rothman, N. NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. *Pharmacogenetics*, *10*: 115–122, 2000.
26. Yu, M. C., Skipper, P. L., Taghizadeh, K., Tannenbaum, S. R., Chan, K. K., Henderson, B. E., and Ross, R. K. Acetylator phenotype, aminobiphenyl-hemoglobin adduct levels, and bladder cancer risk in white, black, and Asian men in Los Angeles, California. *J. Natl. Cancer Inst.*, *86*: 712–716, 1994.
27. Hein, D. W. Acetylator genotype and arylamine-induced carcinogenesis. *Biochim. Biophys. Acta*, *948*: 37–66, 1988.
28. Vineis, P., and Pirastu, R. Aromatic amines and cancer. *Cancer Causes Control*, *8*: 346–355, 1997.
29. Weber, W. W. *The Acetylator Genes and Drug Response*. New York: Oxford University Press, 1987.
30. Begg, C. B., and Zhang, Z. Statistical analysis of molecular epidemiology studies employing case-series. *Cancer Epidemiol. Biomark. Prev.*, *3*: 173–175, 1994.
31. Khoury, M. J., and Flanders, W. D. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! *Am. J. Epidemiol.*, *144*: 207–213, 1996.
32. Piegorsch, W. W., Weinberg, C. R., and Taylor, J. A. Non-hierarchical logistic models and case-only designs for assessing susceptibility in population-based case-controls studies. *Stat. Med.*, *13*: 153–162, 1994.
33. Umbach, D. M., and Weinberg, C. R. Designing and analysing case-control studies to exploit independence of genotype and exposure. *Stat. Med.*, *16*: 1731–1743, 1997.
34. Snedecor, G. W., and Cochran, W. G. *Statistical Methods*. Ames: Iowa State University Press, 1989.
35. SAS Institute Inc. *SAS/STAT User's Guide*. Cary, NC: SAS Institute, 1994.
36. SAS Institute Inc. *SAS/STAT Software: Changes and Enhancements Through Release 6.11*. Cary, NC: SAS Institute, Inc., 1996.
37. Breslow, N. E., and Day, N. E. *Statistical Methods in Cancer Research. Volume 1—The Analysis of Case-Control Studies*. Lyon, France: International Agency for Research on Cancer, 1980.
38. Laird, N. M., and Mosteller, F. Some statistical methods for combining experimental results. *Int. J. Technol. Assessment Health Care*, *6*: 5–30, 1990.
39. Whitehead, A., and Whitehead, J. A general parametric approach to the meta-analysis of randomized clinical trials. *Stat. Med.*, *10*: 1665–1677, 1991.
40. Blum, M., Demierre, A., Grant, D. M., Heim, M., and Meyer, U. A. Molecular mechanisms of slow acetylation of drugs and carcinogens in humans. *Proc. Natl. Acad. Sci. USA*, *88*: 5237–5241, 1991.
41. Cascorbi, I., Drakoulis, N., Brockmüller, J., Maurer, A., Sperling, K., and Roots, I. Arylamine *N*-acetyltransferase (NAT2) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. *Am. J. Hum. Genet.*, *57*: 581–592, 1995.
42. Graf, T., Broly, F., Hoffmann, F., Probst, M., Meyer, U. A., and Howald, H. Prediction of phenotype for acetylation and for debrisoquine hydroxylation by DNA-tests in healthy human volunteers. *Eur. J. Clin. Pharmacol.*, *43*: 399–403, 1992.
43. Hickman, D., and Sim, E. *N*-acetyltransferase polymorphism: comparison of phenotype and genotype in humans. *Biochem. Pharmacol.*, *42*: 1007–1014, 1991.
44. Mrozikiewicz, P. M., Drakoulis, N., and Roots, I. Polymorphic arylamine *N*-acetyltransferase (NAT2) genes in children with insulin-dependent diabetes mellitus. *Clin. Pharmacol. Ther.*, *56*: 626–634, 1994.
45. Kleinbaum, D., Kupper, L., and Morgenstern, H. *Epidemiologic Research: Principles and Quantitative Methods*. New York: Van Nostrand Reinhold, 1982.
46. Vineis, P., and Ronco, G. Interindividual variation in carcinogen metabolism and bladder cancer risk. *Environ. Health Perspect.*, *98*: 95–99, 1992.
47. Begg, C. B. Publication bias. *In*: H. Cooper and L. V. Hedges (eds.), *The Handbook of Research Synthesis*, pp. 399–409. New York: Russell Sage Foundation, 1994.
48. Garcia-Closas, M., Thompson, W. D., and Robins, J. Differential misclassification and the assessment of gene-environment interactions in case-control studies. *Am. J. Epidemiol.*, *147*: 426–433, 1998.
49. Cartwright, R. A., Rogers, H. J., Barham-Hall, D., Slashan, R. W., Ahmod, R. A., Higgins, E., and Kahn, M. A. Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, *2*: 842–845, 1982.
50. Vineis, P., Bartsch, H., Caparaso, N., Harrington, A. M., Kadlubar, F. F., Landi, M. T., Malaveille, C., Shields, P. G., Skipper, P., Talaska, G., and Tannenbaum, S. R. Genetically based *N*-acetyltransferase metabolic polymorphism and low-level environmental exposure to carcinogens. *Nature (Lond.)*, *369*: 154–156, 1994.
51. D'Errico, A., Taoli, E., Chen, X., and Vineis, P. Genetic metabolism polymorphisms and the risk of cancer: a review of the literature. *Biomarkers*, *1*: 149–173, 1996.
52. Kadlubar, F. F., and Badawi, A. F. Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis. *Toxicol. Lett.*, *82*: 627–632, 1995.
53. Bois, F. Y., Krowech, G., and Zeise, L. Modeling human interindividual variability in metabolism and risk: the example of 4-aminobiphenyl. *Risk Anal.*, *15*: 205–213, 1995.
54. Michaud, D., Spiegelman, D., Clinton, S., Rimm, E., Curhan, G., Willett, W., and Giovannucci, E. Fluid intake and the risk of bladder cancer in men. *N. Engl. J. Med.*, *340*: 1390–1397, 1999.
55. Yang, Q., Khoury, M. J., and Flanders, W. D. Sample size requirements in case-only designs to detect gene-environment interaction. *Am. J. Epidemiol.*, *146*: 713–720, 1997.