NAT2 slow acetylation and *GSTM1* null genotypes increase bladder cancer risk: results from the Spanish Bladder Cancer Study and meta-analyses

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Abstract

Background: Many associations between common genetic polymorphisms and complex diseases have not been replicated. One of the few exceptions may be the association between *NAT2* slow acetylation, *GSTM1* null genotype and bladder cancer risk. However, current evidence is based on meta-analyses of relatively small studies (range 23-374 cases) with some evidence of publication

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Conflict of interest statement

None of the authors in this manuscript have conflicts of interest. Montserrat García-Closas had full access to all the data in the study and had final responsibility for the decision to submit for publication.

bias and study heterogeneity. Associations between polymorphisms in other *NAT* and *GST* genes and bladder cancer risk have been inconsistent.

Methods: We evaluated polymorphisms in *NAT2, GSTM1, NAT1, GSTT1, GSTM3* and *GSTP1* in 1,150 patients with transitional cell carcinoma of the urinary bladder and 1,149 control subjects of Caucasian origin in Spain. We also carried out meta-analyses of *NAT2*, *GSTM1* and bladder cancer which included more than twice the number of cases in previous reports.

Findings: In our study, the relative risks of bladder cancer for subjects with deletion of one or two (null genotype) copies of the *GSTM1* gene were 1.2 (95% CI 0.8-1.7) and 1.9 (1.4-2.7), respectively (p-trend=3×10^{-8}). Compared to *NAT2* rapid/intermediate acetylators, *NAT2* slow acetylators had an increased overall risk of bladder cancer (1.4 (95% CI 1.2-1.7)) that was stronger for cigarette smokers than for never smokers (p-interaction = 0.008). No significant associations were found with the other polymorphisms. Meta-analyses showed that the overall association for *NAT2* was robust ($p=5\times10^{-8}$) and case-only meta-analyses provided support for a *NAT2*-smoking interaction (p-interaction=0.008). The overall association for *GSTM1* also was robust ($p=9\times10^{-15}$) and was not modified by smoking status (p=0.86).

Interpretation: The *GSTM1* null genotype increases the overall risk of bladder cancer, and the *NAT2* slow acetylator genotype increases risk particularly among cigarette smokers. Although relative risks are modest, these polymorphisms could account for up to 30% of bladder cancer cases because of their high prevalence. These findings provide some of the most compelling evidence to date for the role of common polymorphisms in the etiology of cancer, and illustrate the need for large and rigorous investigations to establish effects.

Keywords

bladder cancer; genetic polymorphisms; epidemiology; glutathione S-transferases; N-acetyltransferases

INTRODUCTION

Lack of replication for many associations between common genetic polymorphisms and complex diseases has raised skepticism in this field of research (1). One of the few exceptions may be the association between bladder cancer risk with polymorphisms in two carcinogen detoxification genes, *NAT2* slow acetylation and *GSTM1* null genotype. However, evidence for an association relies on pooled and meta-analyses of relatively small studies (range 23-374 cases, average size of about 100 cases per study) and concerns have been raised about publication bias and heterogeneity of results (2-9). Tobacco smoking is an important cause of bladder cancer (10) and previous analyses have suggested that the relative risk for smoking is stronger for *NAT2* slow than rapid/intermediate acetylators (2;5;11). This interaction is biologically plausible since aromatic amines are thought to be the primary bladder carcinogen in tobacco smoke (12) and are detoxified by *NAT2* (13). However, epidemiologic evidence for this interaction is even weaker than for the overall genotype association. Also, the *GSTM1* genotype does not seem to modify the relative risk for smoking according to previous studies (8). Associations between bladder cancer risk and polymorphisms in other carcinogen detoxification genes such as *NAT1* and other glutathione S-transfereases have been less frequently explored with inconsistent results across studies (14-33).

Here, we report results on the associations of polymorphisms in *NAT* and *GST* genes with bladder cancer risk and their interaction with cigarette smoking among subjects participating in the Spanish Bladder Cancer Study. This is the first study of bladder cancer that has adequate statistical power to rigorously evaluate the proposed associations between genetic variation in *NAT2, GSTM1* and bladder cancer risk, as well as to study interactions with smoking habits.

We also conduct meta-analyses of *NAT2*, *GSTM1*, smoking and bladder cancer that include more than twice the number of cases than in previous reports.

MATERIALS AND METHODS

Study population

The Spanish Bladder Cancer Study is a hospital-based case-control study conducted in 18 hospitals from five different areas in Spain (i.e., Asturias, Barcelona metropolitan area, Vallès/Bages, Alicante, and Tenerife). Cases were patients newly diagnosed with histologically confirmed carcinoma of the urinary bladder in 1998-2001, aged 21-80 years. Diagnostic slides from each case were reviewed by a panel of expert study pathologists to confirm diagnosis and ensure uniformity of classification criteria, based on the 1998 World Health Organization/International Society of Urological Pathology system (34).

Controls were selected from patients admitted to participating hospitals for diagnoses believed to be unrelated to the exposures of interest such as tobacco use. The distribution of reasons for hospital admission was: 37% hernias, 11% other abdominal surgery, 23% fractures, 7% other orthopedic conditions, 12% hydrocele, 4% circulatory conditions, 2% dermatological conditions, 1% ophthalmologic conditions, 3% other diseases. Controls were individually matched to the cases on age at interview within 5 year categories, gender, ethnicity and region. Information on known or potential bladder cancer risk factors for cases and controls was collected using computer-assisted personal interviews during the hospital admission. Eighty-four percent of eligible cases and 88 % of eligible controls agreed to participate in the study and were interviewed. Of the 1,219 cases and 1,271 controls interviewed, 1,188 (97.5 %) cases and 1,173 (92.3%) controls provided a blood or buccal cell sample for DNA extraction. Seven cases and 11 controls were excluded because of low amounts of DNA. To reduce heterogeneity, 16 cases with neoplasias of non-transitional histology, and 6 non-white subjects (5 cases and 1 control) were excluded from the analyses. Fifteen subjects (7 cases and 8 controls) with missing smoking status information and 7 subjects (3 cases and 4 controls) with DNA quality control problems were also excluded from the analyses. Thus, the final study population available for analysis included 1,150 cases and 1,149 controls, all of whom were Caucasians.

Subjects were categorized as never smokers if they smoked less than 100 cigarettes in their lifetime and ever smokers otherwise. Ever smokers were further classified as regular smokers if they smoked one cigarette per day for 6 months or longer and occasional smokers otherwise. Regular smokers were classified as current smokers if they smoked within a year of the reference date and former smokers otherwise. Smokers of black tobacco alone, black and blond tobacco, and unknown tobacco type had similarly elevated bladder cancer risks compared to never smokers (data not shown), and were grouped as known or likely black tobacco smokers. We obtained informed consent from potential participants in accordance with the National Cancer Institute and local Institutional Review Boards.

Laboratory techniques

DNA for genotype assays was extracted from leukocytes using the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN) for most cases (N=1,107) and controls (N=1,032) included in the analysis. DNA from additional 43 cases and 117 controls was extracted from mouthwash samples using phenol-chloroform. Genotype assays were performed at the core genotyping facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute using Applied Biosystems TaqMan® (Foster City, CA), Epoch Biosciences MGB Eclipse® (Bothel, WA), or Sequenom MASSArray® (San Diego, CA) assays. Description and methods for each specific assay can be found at http://snp500cancer.nci.nih.gov. Genotype assays were performed for *NAT1* (Ex1-88A>T rs1057126, Ex1-81A>C rs15561, V149I

rs4987076, R187Q rs4986782, R187* rs5030839, R33*, D251V, R64W), *NAT2* (K268R rs1208, G286E rs1799931, R64Q rs1801279, Y94Y rs1041983, I114T rs1801280, L161L rs1799929, R197Q rs1799930), *GSTM1* deletion (SNP500Cancer ID - GSTM1-02), *GSTT1* deletion (SNP500Cancer ID - GSTT1-02), *GSTP1* (I105V rs947894, A114V) and *GSTM3* (V224I rs7483, IVS7 -30G>T rs1537234). All genotypes under study were in Hardy-Weinberg equilibrium among the control population. Duplicate quality control samples showed 100% agreement for all assays, except for four assays (range 98.2% to 99.6%).

Information from the *NAT1* and *NAT2* SNPs analyzed in this study was used to assign the most likely *NAT1* and *NAT2* alleles previously identified in human populations (35) (updated at www.louisville.edu/medschool/pharmacology/NAT.html). Individuals homozygous for rapid *NAT2* acetylator alleles (*NAT2*4, NAT2*11A, NAT2*12A, NAT2*12B, NAT2*12C, NAT2*13*) were classified as rapid acetylator phenotype; individuals homozygous for slow acetylator alleles were classified as slow acetylator phenotype and heterozygous individuals (one rapid and one slow *NAT2* allele) were classified as intermediate acetylator phenotype. Subjects with missing information for four rare *NAT1* SNPs (R187*, R33*, D251V and R64W with > 99% homozygous wild-type subjects) were assumed to be *4/*4. Based on previous studies, the *NAT1*10* allele was considered the "at risk" allele. The two *GSTP1* (I105V and A114V) and *GSTM3* (V224I and IVS7 -30G>T) genotypes evaluated were in strong linkage disequilibrium (D'=1.0, R^2=0.10 and D'=1.0, R^2=0.68, respectively). Subjects were classified according to the presence of three *GSTP1* variants that have been found to encode functionally different *GSTP1* proteins: *GSTP1*A* (105 Ile; 114Ala), *GSTP1*B* (105 Val; 114 Ala) and *GSTP1*C* (105 Val; 114 Val) (36).

Statistical analysis

Odds ratios (OR), as measure of relative risk, and 95% confidence intervals (95%CI) were estimated using logistic regression models, adjusting for gender, age at interview, region, and smoking status defined as never, occasional, former and current smoker categories. These unconditional models provided estimates similar to conditional logistic regression models for individually matched pairs. Interactions between genotypes and smoking habits were also evaluated using semi-parametric maximum likelihood estimator (SPMLE) (37) to allow estimation of parameters under the assumption of genotype-smoking and genotype-gender independence in the source population. This assumption is supported by strong evidence from previous studies for independence of *NAT2* and *GSTM1* genotypes from cigarette smoking status (8;11;38) and gender (38) in the general population. Tests for multiplicative interaction were used to evaluate if the genotype ORs within categories of smoking habits were significantly different from each other, or if smoking ORs within genotype categories were significantly different from each other. We also tested for additive interactions since departures from the additive model may have biological implications under certain biological models (39). The synergy index was used as a measure of additive interaction and its confidence interval was calculated using previously published formulae (40).

We updated previous meta-analyses on *NAT2*, *GSTM1* and bladder cancer following similar study selection criteria, i.e. case-control studies conducted in the general population (4;8;11). Relevant studies published through February 2005 were identified in a Medline search. Random-effects summary measures were calculated by weighting each study result by a factor of within- and between-study variance (41). Homogeneity of study results in different groups was assessed by the *Q* statistic and publication bias by Begg (42) and Egger's tests (43). A case-only design (44) was used in meta-analyses performed to assess the presence of a multiplicative interaction between *NAT2* and *GSTM1* genotypes and smoking status (ever/never) because it allowed us to include some studies without information on the cross-classification of genotype and smoking status among controls, it removed possible biases due

to the inclusion of hospital controls with diseases related to tobacco use, and it is a powerful design to test for multiplicative interactions under the assumption of independence of *NAT2* and *GSTM1* from smoking status in the population. Statistical analyses were done with STATA (Version 8.2, Special Edition).

Role of the funding source

The study sponsors had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

RESULTS

Genotype main effects and gene-gene interactions

The study population was of Caucasian origin, predominantly male and with a high prevalence of smoking, mostly black tobacco (Table 1). In this population, *NAT2* slow acetylator and *GSTM1* null (−/−) genotypes significantly increased bladder cancer risk (Table 2). *NAT2* slow acetylators had a 40% increase in bladder cancer risk compared to *NAT2* rapid/intermediate acetylators (OR (95% CI) = 1.4 (1.2-1.7)); the risk for *NAT2* rapid and intermediate acetylators was similar (Table 2). The relative risks of bladder cancer for subjects with deletion of one or two (null genotype) copies of the *GSTM1* gene were 1.2 (95% CI 0.8-1.7) and 1.9 (1.4-2.7), respectively (p for trend test=3×10^{-8}). Individuals with the null genotype had a 70% increased risk of bladder cancer, compared to subjects with one or two copies of the *GSTM1* gene (95% CI = 1.4-2.0) (Table 2). The associations for *NAT2* and *GSTM1* genotypes were similar regardless of tumor grade or stage (footnote to Table 2), and there was no evidence that these associations differed by age or gender (data not shown).

The joint association for the combined *NAT2* slow acetylator and *GSTM1* null genotype, present in 28% of the control population, compared to *NAT2* rapid/intermediate and *GSTM1* present genotype (OR (95%CI) = 2.2 (1.7-2.9)) was consistent with a weak multiplicative interaction between these two genetic variants; however, the test for multiplicative interaction was not significant (p=0.15). None of the other genetic polymorphisms evaluated was significantly associated with an increased risk of bladder cancer (Table 2), and there was no evidence of multiplicative interactions between them (data not shown).

Interaction of NAT2 and GSTM1 genotypes with cigarette smoking

Conventional logistic regression analyses showed a significant multiplicative interaction between *NAT2* slow acetylation and cigarette smoking status (ever/never) with an interaction OR (95%CI) of 1.8 (1.2-2.8), p=0.008 (Table 3). The evidence for a multiplicative interaction became somewhat weaker (interaction OR (95% CI) = 1.4 (1.0-1.9), p =0.08) when using SPMLE logistic regression which assumes genotype-smoking and genotype-gender independence conditional on age, in the source population. Estimates for the *NAT2* slow acetylation association with bladder cancer were similar for occasional, current and former smokers (Table 3, SPMLE OR (95%CI) were 1.5 (0.8-2.8), 1.5 (1.2-1.9) and 1.4 (1.1-1.8), respectively). The data suggested that the association of *NAT2* slow acetylation genotype with bladder cancer was stronger for known or likely black tobacco smokers than for blond tobacco smokers (Table 3, SPMLE OR (95%CI) = 1.5 (1.3-1.8) and 1.0 (0.6-1.7), respectively). However, this difference was not statistically significant (Table 3, SPMLE p interaction = 0.08). *NAT2* slow acetylators were at a higher risk than rapid/intermediate acetylators compared to never smokers for all levels of smoking intensity (average cigarettes per day) (Figure 1). At the same time, the magnitude of the association between *NAT2* slow acetylation and bladder cancer risk among regular smokers was similar across different levels of smoking intensity (Table 3), duration and pack-years (data not shown).

Neither conventional nor SPMLE logistic regression showed a significant multiplicative interaction (OR (95%CI) was 0.7 (0.4-1.1), p=0.09, and 0.8 (0.5-1.1), p=0.15, respectively) for the association of *GSTM1* null and smoking status (ever/never) on bladder cancer risk. This indicated that the relative risk of bladder cancer for *GSTM1* null compared to present genotypes does not vary by smoking status. Multiplicative interactions were also not found for other smoking characteristics such as smoking cessation (current vs. former smokers), smoking intensity or duration. Given that an additive interaction can exist in the absence of a multiplicative interaction, and that departures from the additive model might have biological implications under certain assumptions, we then tested for an additive interaction. Both conventional and SPMLE logistic regressions showed significant departures from the additive model (i.e. additive interactions) with a synergy index (95% CI) of 1.3 (1.0-1.6), p=0.04 and 1.4 (1.1-1.7), p=0.001, respectively.

Meta-analyses

We updated a previously published meta-analysis of 22 studies of *NAT2* and bladder cancer (4) to include data from our study and 8 additional studies (17-19;27;28;45-47) including a total of 5,096 cases and 6,519 controls (Figure 2A). The summary relative risk for *NAT2* slow acetylators compared to rapid/intermediate acetylators was 1.4 (1.2-1.6), $p=5\times10^{-8}$, with no evidence for publication bias according to Begg's (p=0.94) and Egger's tests (p=0.91). There was some evidence for study heterogeneity (*Q* statistic p=0.04) which was not present when small studies (14 studies with less than 100 cases each) were excluded (summary OR (95%CI) = 1.4 (1.2-1.5), *Q* statistic p=0.31). Summary estimates for Caucasians (56% prevalence of *NAT2* slow acetylators in controls) and Asians (11% prevalence of *NAT2* slow acetylators in controls) were similar (p=0.87) (Figure 2A). The summary relative risk for studies of Caucasians conducted in the US was lower than for studies conducted in Europe, which accounted for most (82%) Caucasian cases; however this difference was not statistically significant (p=0.17) (Figure 2A).

We also updated a case-only meta-analysis of *NAT2* and smoking interaction on bladder cancer risk (11) to include results from our study and 5 additional studies published after the meta-analysis (17;19;46;47) (Figure 2B). This analysis included a total of 4,305 cases and showed evidence for an interaction with a summary estimate of 1.2 (95%CI 1.1-1.5, p=0.008) for all populations combined. The point estimate for interaction was higher in Caucasian than Asian populations (1.3 versus 0.9, respectively), as well as in European compared to US Caucasian populations (1.4 versus 1.0, respectively); however, these differences were not statistically significant (p=0.32 and 0.08, respectively) (Figure 2B).

A meta-analysis of 17 studies of *GSTM1* (8) was also updated to include our study, 10 additional studies (17;21;22;24;26;29;30;48-50) and an update from a previously published study (45) yielding a total of 5,108 cases and 6,483 controls (Figure 3A). The summary odds ratio for *GSTM1* null versus present genotype for all populations combined was 1.5 (95% CI 1.3-1.6), $p=9\times10^{-15}$, with no evidence for publication bias according to Begg's (p=0.25) and Egger's tests (p=0.56). Summary estimates were similar and statistically significant in Caucasians (51% of *GSTM1* null genotype in controls) and Asians (53% of *GSTM1* null genotype in controls), as well as in US and European Caucasians (Figure 3A).

An updated case-only meta-analysis of studies that evaluated the *GSTM1*-smoking interaction (8) to include our study and 7 additional studies (17;21;22;29;30;48;50) (17 studies of 4,059 cases), confirmed the absence of a multiplicative interaction with a summary OR (95%CI) of 1.0 (0.9-1.2), p=0.86 (Figure 3B). *Q* statistics showed no evidence for study heterogeneity and Begg's and Egger's tests did not show evidence for publication bias among any of the population subgroups evaluated. Summary estimates for the interaction were very similar for all population subgroups (Figure 3B).

DISCUSSION

This report provides compelling evidence for an increased bladder cancer risk associated with the *GSTM1* null and *NAT2* slow acetylation genotypes. The association of the latter was particularly important among cigarette smokers. Although the relative risks for polymorphisms in *NAT2* and *GSTM1* genes are modest, they could account for a large percentage of bladder cancer cases because of their high prevalence in the population. Based on our data, we estimated that these polymorphisms are responsible for 31% (95% CI 20%-46%) of bladder cancer cases. In addition, we provide strong evidence against a substantial overall association for polymorphisms in other *NAT* and *GST* genes, with the possible exception of small to moderate associations for *NAT1 *10/*10* and *GSTP1 114Val/Val* genotypes.

A new meta-analysis of studies of *NAT2* slow acetylation and bladder cancer risk shows that this association is robust ($p=5\times10^{-8}$) and similar for Caucasian and Asian populations. The fact that the association for Asian populations was not stistically significant might be explained by a substantially lower statistical power to detect associations in Asian studies due to a lower prevalence of *NAT2* slow acetylators (11% for Asians versus 56% for Caucasians) along with a smaller number of cases available for the meta-analysis. We also show that *NAT2* slow acetylators are especially susceptible to the adverse effects of cigarette smoking on bladder cancer risk. This gene-environment interaction has strong biological plausibility since *NAT2* slow acetylators have a decreased capacity to detoxify aromatic monoamines by N-acetylation (13), tobacco smoking is a primary source of exposure to aromatic amines in the general population, and aromatic amines are suspected of being the primary bladder carcinogen in tobacco smoke (12). Although our data suggest that *NAT2* slow acetylation might not increase bladder cancer risk among never smokers, although it does not rule out a small increase in risk in this group of subjects.

Because the content of aromatic amines is higher in black than in blond tobacco (51), it is conceivable the effect of *NAT2* slow acetylation may be stronger for smokers of black tobacco. Out data are consistent with this hypothesis, although differences were not statistically significant. The magnitude of the association between *NAT2* slow acetylation and bladder cancer risk is similar for different levels of smoking intensity in our study population. Our meta-analysis of the interaction between smoking status and *NAT2* slow acetylation genotype suggested a stronger interaction with ever/never smoking in European than in US studies. This could be due to a smaller number of studies conducted in the US than in Europe, or the lower aromatic amine content in blond tobacco generally smoked in the US compared to black tobacco commonly smoked in parts of Europe. Interestingly, a report from a population in the US recently reported an interaction between *NAT2* slow acetylation genotype and smoking only for heavy smokers (47).

Distinction of subjects with one and two copies of the *GSTM1* gene, an issue that has not been adequately addressed in prior studies of bladder cancer, suggests the presence of a gene-dosage effect with relative risks of 1.2 (95% CI 0.8-1.7) and 1.9 (1.4-2.7) for subjects with one or no copies of *GSTM1*, respectively, compared to subjects with two copies ($ptrend=3\times10^{-8}$). Meta-analyses of the association between the deletion of two copies of the *GSTM1* gene (null genotype) compared to subjects with one or two copies (present genotype), as presented in previous studies that could not distinguish between these two groups of subjects, indicated that this association is robust ($p=9\times10^{-15}$), and similar in magnitude and significant across different population subgroups.

The relative risk for *GSTM1* null genotype and bladder cancer is similar for smokers and never smokers in our study population and meta-analysis within population subgroups, suggesting that the *GSTM1* activity protects equally against tobacco-related and non-tobacco related

bladder cancers. This finding indicates that *GSTM1* may reduce the risk of bladder cancer through mechanisms that are not specific to the detoxification of polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke. Other mechanisms of action for *GSTM1* could be protection from oxidative damage through metabolism of reactive oxygen species (52). Our data did not confirm previously suggested differences in risk for *NAT2* slow acetylation and *GSTM1* null genotypes by tumor grade or stage at presentation (26;53-56). Our findings are consistent with a potential interaction between *NAT2* slow acetylation and *GSTM1* null genotypes; however, additional evidence is needed to confirm this interaction (17;28).

Associations between bladder cancer risk and polymorphisms in genes coding for the NAT1 enzyme involved in the activation of aromatic amines by O-acetylation (13), and other GST enzymes that play an important role in the detoxification of PAHs and other carcinogens (57), have been less explored. Previous studies have provided inconsistent evidence for an association between bladder cancer risk and *NAT1**10 alone or in combination with *NAT2* slow acetylation (14-19;47), *GSTT1* null alone or in combination with *GSTM1* null genotype (17; 20-31;50), and *GSTP1 105 Val/Val* genotype (17;21;32;33). Data from our study does not support a substantial association between *GSTT1* and *GSTM3* genotypes and bladder cancer risk. We find no significant increases in bladder cancer risk associated with polymorphisms in *NAT1* or *GSTP1* genes; however, our estimates do not exclude a small to moderate association for the *NAT1*10/*10* compared to the *NAT1*4/*4* genotype, or for genotypes with the *GSTP1 114Val* allele compared to the *114Ala/Ala* genotype.

Analyses using conventional logistic regression suggested a modification of the association between bladder cancer with *NAT2, GSTM1* and *NAT1* genotypes by gender. However, the modifications by gender are explained by unexpected differences in the genotype distribution for male and female controls.

Our study has several strengths of note: high participation rates, large sample size, high quality exposure and genotype information and use of state-of-the art statistical methods. Specifically, we made an effort to improve the precision in genotype estimation by genotyping the seven SNPs in *NAT2* that likely account for virtually all genetic variation in Caucasian populations, (58) and developed assays that sucesfully distinguished individuals with one or two copies of the *GSTM1* and *GSTT1* genes. We also used the SPMLE method (37) to increase power and reduce bias in the estimation of interactions, because of the strong evidence from previous studies for independence of *NAT2* and *GSTM1* genotypes from cigarette smoking status (8; 11;38) and gender (38) in the general population. In order to minimize selection bias, we carefully selected controls from patients admitted for a variety of diagnoses that were thought to be unrelated to exposures of interest including tobacco use. Genotype frequencies among the control population were similar to those previously published. We found no significant overall differences in genotype frequencies across control diagnoses that could have biased our results.

Although this is the largest study on the role of genetic polymorphisms and bladder cancer risk published to date and had adequate statistical power to detect small genotype associations, the power to detect small to moderate interactions was limited. Meta-analyses including previous studies improved our ability to make inferences on interactions, when there were an adequate number of previous studies with homogeneous results. A consortium of bladder cancer studies is currently being formed to facilitate the pooling of comparable data on environmental and genetic risk factors across studies that will help overcome the limited power of individual studies to evaluate complex interrelationships.

Overall, these findings are among the most consistent for common genetic polymorphisms and risk of any tumor site in the literature, and provide compelling evidence for the role of common

polymorphisms in cancer risk. This report also illustrates that large and rigorous investigations are required to establish or dismiss effects of common polymorphisms on complex diseases.

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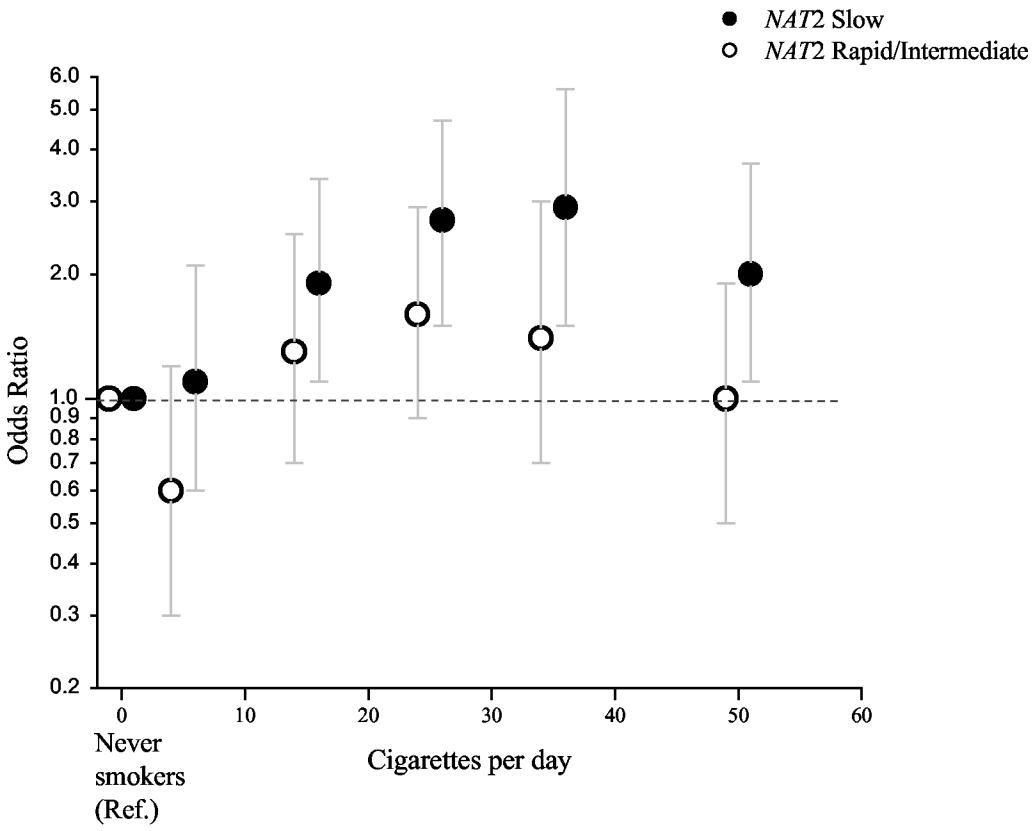

Figure 1.

Association between increasing smoking intensity (average number of cigarettes per day in categories of 10 cigarettes) and bladder cancer risk compared to never smokers, stratified by *NAT2* acetylation genotype. Odds ratios are from conventional logistic regression models adjusted for age, gender, region, smoking duration (< 20 years, 20-<30 years, 30-<40 years, 40-<50 years, ≥ 50 years) and smoking cessation (current/former smokers). Error bars represent 95% confidence intervals.

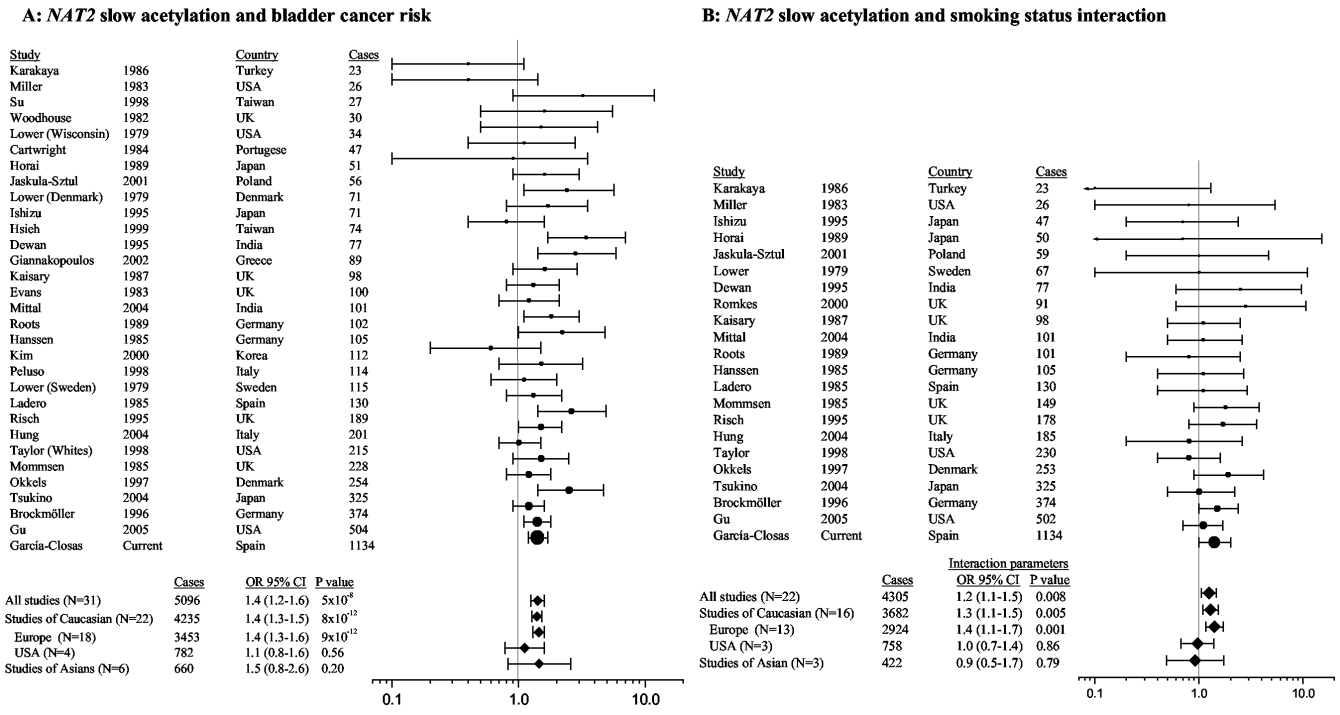

Figure 2.

Meta-analysis of studies of *NAT2* slow acetylation genotype and bladder cancer risk (A) and case-only meta-analysis of studies of *NAT2* slow acetylation gentype, cigarette smoking and bladder cancer. The horizontal axis plots odds ratios and 95% CI on a logarithmic scale. The size of black circles are proportional to the study size for studies with 50 or more cases, Disagreements between the number of cases for the same study in panels A and B are due to cases with missing smoking information.

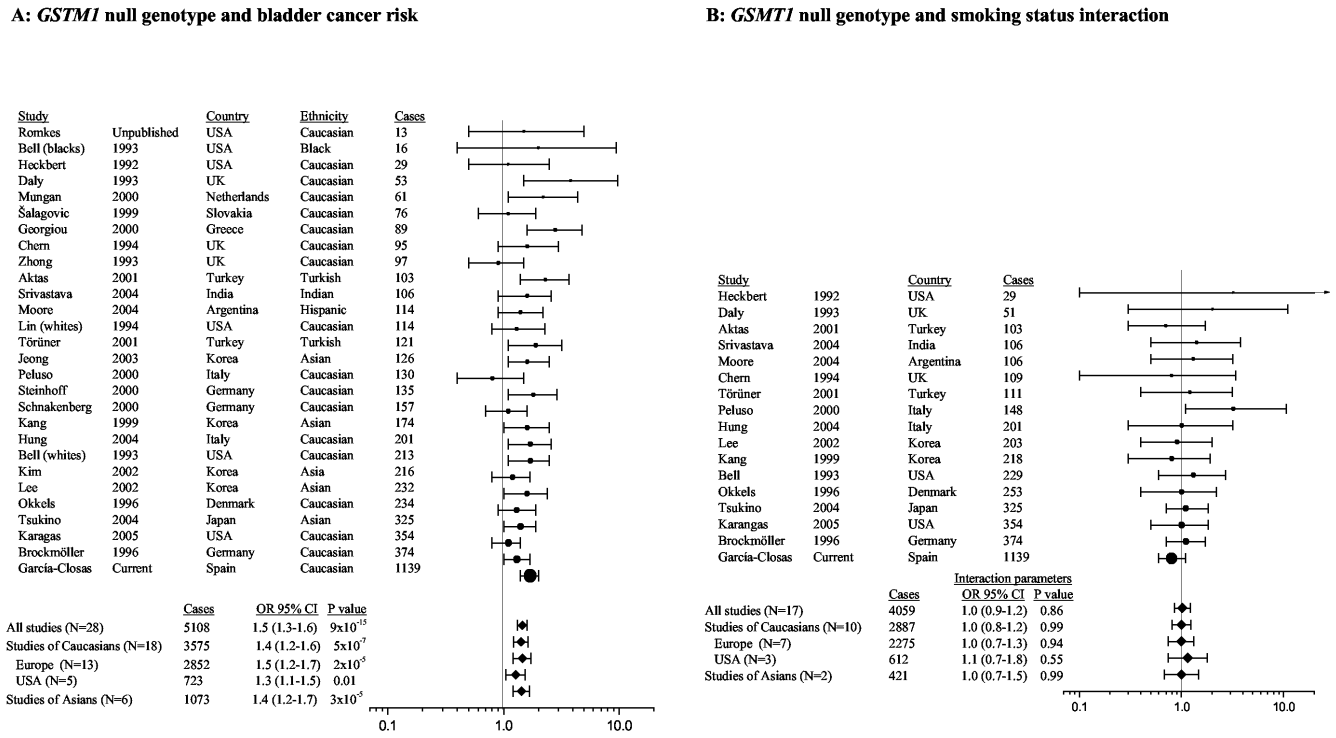

Figure 3.

Meta-analysis of studies of *GSTM1* null genotype and bladder cancer risk (A) and case-only meta-analysis of studies of *GSTM1* null genotype, cigarette smoking and bladder cancer. The horizontal axis plots odds ratios and 95% CI on a logarithmic scale. The size of black circles are proportional to the study size for studies with 50 or more cases, Disagreements between the number of cases for the same study in panels A and B are due to cases with missing smoking information.

Table 1

Characteristics of study population (1,150 cases and 1,149 controls)

Characteristic	Cases		Controls	
Age, mean±SD	66±10		65±10	
Gender, N (%)				
Female	146	13%	147	13%
Male	1004	87%	1002	87%
Educational level*, N (%)				
Less than primary	525	46%	539	47%
Primary and less than high school	452	39%	437	38%
At least high school	156	14%	154	13%
Other	14	1%	14	1%
Smoking status, N (%)				
Never	159	14%	338	29%
Occasional smokers	50	4%	88	8%
Regular smokers Former	474	41%	458	40%
Current	467	41%	265	23%
Type of tobacco smoked†, N (%)				
Blond tobacco only	92	10%	114	16%
Black tobacco only	383	41%	281	39%
Both types of tobacco	284	30%	194	27%
Unknown tobacco type	182	19%	132	18%

*Information on education is missing for 3 cases and 5 controls.

†Defined only for regular smokers. Information on type of tobacco is missing for 2 controls. Subjects who smoked black tobacco alone, black and blond tobacco, or unknown tobacco type (84% of regular smokers) were categorized as “known or likely black tobacco smokers”.

Table 2

Odds ratios (OR), 95% confidence intervals (95%CI) and p values for the associations of polymorphisms in NAT and GST genes on bladder cancer risk (1,150 cases and 1,149 controls).

Genotypes		Cases[†]	Controls[†]	OR[*]	(95% CI)	p
NAT2 [‡]	Rapid	55	66	1.0		
	Intermediate	351	427	1.0	(0.7-1.5)	0.97
	Slow	728	637	1.4	(0.9-2.1)	0.10
	Slow vs. rapid/ intermediate			1.4	(1.2-1.7)	2×10^{-4}
GSTM1 [§]	+/+	70	107	1.0		
	+/−	352	454	1.2	(0.8-1.7)	0.38
	−/−	716	571	1.9	(1.4-2.7)	2×10^{-4}
	Null vs. present			1.7	(1.4-2.0)	1×10^{-8}
NAT1	NAT1*4/NAT1*4	585	574	1.0		
	NAT1*10/NAT1*4	327	326	1.0	(0.8-1.2)	0.62
	NAT1*10/NAT1*10	53	42	1.2	(0.8-1.8)	0.48
GSTT1 [‖]	+/+	327	340	1.0		
	+/−	572	533	1.2	(1.0-1.5)	0.05
	−/−	230	248	1.0	(0.8-1.3)	0.90
GSTP1 I105V	Ile/Ile	486	488	1.0		
	Ile/Val	525	531	1.0	(0.8-1.2)	0.93
	Val/Val	130	119	1.2	(0.9-1.5)	0.35
GSTP1 A114V [¶]	Ala/Ala	966	917	1.0		
	Ala/Val	113	85	1.3	(1.0-1.8)	0.07
	Val/Val	4	5	0.9	(0.2-3.4)	0.85
GSTP1 I105V/ A114V Combination[**]	GSTP1*A/GSTP1*A	456	441	1.0		
	GSTP1*A/GSTP1*B	409	402	1.0	(0.8-1.2)	0.92
	GSTP1*B/GSTP1*B	95	69	1.4	(1.0-1.9)	0.09
	GSTP1*C	84	56	1.3	(0.9-1.8)	0.12
GSTM3 V224I	Val/Val	565	588	1.0		
	Val/Ile	472	451	1.1	(0.9-1.3)	0.30
	Ile/Ile	92	88	1.0	(0.7-1.4)	0.98
GSTM3 IVS7 -30G>T	GG	439	464	1.0		
	GT	529	504	1.1	(0.9-1.4)	0.19
	TT	160	154	1.1	(0.8-1.4)	0.64

[*] Odds ratios from conventional logistic regression models adjusted for gender, age, region and smoking status.

[†] Missing information on *NAT2* for 16/19 cases/controls; on *NAT1* for 123/124 including subjects with uncommon or undeterminable alleles (62/83 with other *NAT1* genotypes are not shown); on *GSTM1* for 11/17; on *GSTT1* for 4/12; on *GSTP1 I105V* for 9/11; on *GSTP1 A114V* for 24/25 on *GSTM3 V224I* for 21/22; and on *GSTM3 IVS7* -30G>T for 22/27.

[‡] The percentage of *NAT2* slow acetylators among cases with superficial tumors (Ta) grades 1, 2 and 3, tumors involving the submucosa (T1) grades 2/3, tumors infiltrating muscle (T2) grades 2/3 or metastatic tumors (T3/T4) were 64%, 65% , 65%, 67%, 61% and 64% (p of 0.72, 0.80, 0.55, 0.80, 0.94, respectively, compared to Ta/grade 1 and adjusting for age, region and smoking status).

[§] GSTM1 +/+ and +/− could not be distinguished for 1 case. This subject contributed to the estimation of OR for *GSTM1* present vs. null genotypes. The percentages of *GSTM1* null genotype among cases with superficial tumors (Ta) grades 1, 2 and 3, tumors involving the submucosa (T1) grades 2/3, tumors infiltrating muscle (T2) grades 2/2 or metastatic tumors (T3/T4) were 61%, 62%, 61%, 67%, 61%, 66% (p of 0.79, 0.93, 0.14, 0.80 and 0.35, respectively, compared to Ta/grade 1 and adjusting for age, region and smoking status)

[‖] GSTT1 +/+ and +/− could not be distinguished for 17/16 cases/controls.

[¶] Assay performed only among cases and controls with blood DNA (96% of cases and 90% of controls).

[**] Categorized according to Ali-Osman et al. (36) to reflect three functionally different *GSTP1* variants: *GSTP1*A* (105 Ile; 114Ala), *GSTP1*B* (105 Val; 114 Ala) and *GSTP1*C* (105 Val; 114 Val)

Table 3

Association for *NAT2* slow acetylation genotype with bladder cancer risk stratified by smoking characteristics, and joint association for cigarette smoking characteristics and *NAT2* acetylation genotype with bladder cancer risk compared to never smokers with NAT2 rapid/intermediate acetylation genotype

	Frequencies				NAT2 slow genotype association by smoking		Joint NAT2 slow genotype and smoking association			
	NAT2 rapid/inter.		NAT2 slow				NAT2 rapid/inter.		NAT2 slow	
Smoking characteristics	**cases**	**controls**	**cases**	**controls**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Smoking status †										
Never	66	131	91	199	0.9	(0.6-1.3)	1.0		0.9	(0.6-1.3)
Ever	340	362	637	438	1.6	(1.3-1.9)	2.9	(2.0-4.2)	4.6	(3.2-6.6)
Occasional	16	37	32	48	1.2	(0.6-2.4)	1.2	(0.6-2.4)	1.6	(0.9-2.9)
Former	161	212	310	240	1.7	(1.3-2.2)	2.4	(1.6-3.7)	4.1	(2.8-6.1)
Current	163	113	295	150	1.4	(1.1-2.0)	5.2	(3.4-8.0)	7.5	(5.0- 11.3)
Type of tobacco ‡										
Never	66	131	91	199	0.9	(0.6-1.3)	1.0		0.9	(0.6-1.3)
Black	284	272	553	328	1.6	(1.3-2.0)	3.6	(2.4-5.4)	5.9	(4.0-8.7)
Blond	40	52	52	61	1.2	(0.7-2.1)	2.5	(1.4-4.3)	2.9	(1.7-4.9)
Smoking intensity §										
Never	66	131	91	199	0.9	(0.6-1.3)	1.0		0.9	(0.6-1.3)
< 10 cig/day	26	55	43	61	1.7	(0.9-3.2)	0.6	(0.3-1.1)	0.9	(0.5-1.8)
10-<20 cig/day	67	57	106	77	1.2	(0.7-1.9)	1.3	(0.7-2.6)	1.6	(0.9-3.0)
20-<30 cig/day	143	108	263	133	1.4	(1.0-2.0)	1.6	(0.9-2.9)	2.3	(1.3-4.1)
30-<40 cig/day	31	27	88	42	1.8	(0.9-3.5)	1.4	(0.6-3.0)	2.5	(1.3-4.8)
≥ 40 cig/day	54	73	102	71	1.7	(1.1-2.8)	1.0	(0.5-2.0)	1.8	(0.9-3.3)

ORs for *NAT2* slow acetylation for different categories of smoking intensity are not significantly different from the highest intensity category (p for interaction for categories <10, 10-<20, 20-<30, 30-<40 cig/day compared to ≥40 cig/day are 0.96, 0.30. 0.58 and 0.90 respectively for total intensity).

* p value for differences between the OR for *NAT2* slow acetylation genotype within strata defined by smoking characteristics compared never smokers. This test is equivalent to testing if the observed joint OR for *NAT2* slow genotype and smoking characteristics differs from the product of the OR for each factor alone.

† Odds ratios are from conventional logistic regression models adjusted for gender, age and region.

‡ Black tobacco are “known or likely black tobacco smokers” defined as subjects who smoked black tobacco alone, black and blond tobacco, or unknown tobacco type. Odds ratios are from conventional logistic regression models adjusted for gender, age, region and smoking cessation (former/current). The p for interaction for former versus current smokers is 0.44 and for blond versus black tobacco is 0.33.

§ Odds ratios are from conventional logistic regression models adjusted for age, gender and region, smoking duration (< 20 years, 20-<30 years, 30-<40 years, 40-<50 years, ≥ 50 years) and smoking cessation (current/former).