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Polymorphisms in DNA repair genes, smoking, and bladder cancer risk: findings from the International Consortium of Bladder Cancer

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Abstract

Tobacco smoking is the most important and well-established bladder cancer risk factor, and a rich source of chemical carcinogens and reactive oxygen species that can induce damage to DNA in urothelial cells. Therefore, common variation in DNA repair genes might modify bladder cancer risk. In this study we present results from meta- and pooled analyses conducted as part of the International Consortium of Bladder Cancer. We included data on 10 single nucleotide polymorphisms corresponding to 7 DNA repair genes from 13 studies. Pooled- and meta-analyses included 5,282 cases and 5,954 controls of non-Latino white origin. We found evidence for weak but consistent associations with *ERCC2* D312N (rs1799793) (per allele OR = 1.10; 95% CI = 1.01–1.19; p = 0.021), *NBN* E185Q (rs1805794) (per allele OR = 1.09; 95% CI = 1.01–1.18; p = 0.028), and *XPC* A499V (rs2228000) (per allele OR = 1.10; 95% CI = 1.00–1.21, p = 0.044). The association with *NBN* E185Q was limited to ever smokers (interaction p = 0.002), and was strongest for the highest levels of smoking dose and smoking duration. Overall, our study provides the strongest evidence to date for a role of common variants in DNA repair genes in bladder carcinogenesis.

INTRODUCTION

Tobacco smoking is the most important and well-established risk factor for bladder cancer, contributing up to 50% of bladder cancer occurrence in men and 20% in women (1). Another important risk factor is occupational exposures to aromatic amines (2). A common property of

these exposures is the presence of carcinogens that can induce DNA damage in the bladder epithelium. Specifically, tobacco smoke is a rich source of polycyclic aromatic hydrocarbons, aromatic amines, heterocyclic amines, and N-nitroso compounds, which can produce DNA bulky adducts that may lead to DNA damage (3). Aromatic amines have also been identified in hair dyes and use of these products has been associated with bladder cancer risk in some studies (4). Tobacco smoke is also a rich source of reactive oxygen species (ROS), which can induce damage to DNA (5) and can accumulate in the bladder as a by-product of the metabolism of chemical carcinogens present in tobacco smoke (6–9). Genetic risk factors identified so far include common polymorphisms in xenobiotic metabolism enzymes, such as NAT2 and GSTM1, both of which are involved in the detoxification of arylamines (10,11). In addition, GSTM1 participates in the detoxification of ROS.

Genotoxic compounds derived from the metabolism of chemical carcinogens can contribute to the accumulation of several forms of DNA damage such as bulky adducts, single (SSBs) and double strand breaks (DSBs), abasic sites, and modified bases. DNA repair mechanisms exist to prevent detrimental consequences of these types of DNA damage. Specifically, base damage, abasic sites, and SSBs are repaired through the base excision repair pathway (BER); whereas DSBs are repaired by either non-homologous end joining (NHEJ) or the homologous recombination repair (HRR) pathways. Bulky adducts are generally repaired by the nucleotide excision repair (NER) pathway. Individuals vary in their ability to repair DNA damage and it has been hypothesized that DNA repair gene polymorphisms may partly explain this variability. In the past decade, several groups have investigated the role of selected DNA repair single nucleotide polymorphisms (SNPs) and bladder cancer risk and the potential modifier role of these polymorphisms on the effects of smoking. Whereas some interesting common findings have emerged, larger sample sizes are required to evaluate the associations of these polymorphisms in bladder cancer risk. The role of DNA repair on bladder carcinogenesis is further supported by the finding that reduced DNA repair capacity, as measured in lymphocytes, associated with increased bladder cancer risk (12).

The International Consortium of Bladder Cancer (ICBC; http://dceg.cancer.gov/icbc/) was formed in 2005 to facilitate the pooling of comparable data on environmental and genetic risk factors across studies in order to overcome the limited statistical power of individual studies. In this report we present meta- and pooled analyses of published and unpublished data on 10 SNPs corresponding to seven DNA repair genes that play key roles in the NER, BER, HRR, and NHEJ DNA repair pathways. Specifically, we report findings on the associations between bladder cancer risk and each SNP and their interactions with cigarette smoking. These 10 SNPs were selected because each had been previously evaluated in at least three participating studies.

METHODS

We carried out a literature review in January 2008 to identify DNA repair polymorphisms that had been reported by three or more bladder cancer studies. This resulted in the identification of 12 unique polymorphisms. Ten of these SNPs had been evaluated by at least three studies participating in ICBC and are included in this report: rs1799793 *ERCC2* D312N (5 studies) and rs13181 K751Q (8 studies); rs2228000 *XPC* A499V (3 studies) and rs2228001 K939Q (4 studies); rs1799782 *XRCC1* R194W (7 studies) and rs25487 R399Q (9 studies); rs861539 *XRCC3* T241M (8 studies); rs1130409 *APEX1* D148E (6 studies); rs1805794 *NBN* E185Q (5 studies); and rs1799801 *ERCC4* S835S (4 studies).

Study subjects

Twelve studies contributed case-control data and one study provided case-only data, to the ICBC, for a total of 6,348 cases and 6,752 controls (Table 1). Supplementary tables 1–2 contain individual data for all contributing studies. Most studies were hospital-based, with the

exception of 3 that were population-based (LABCS, NHBCS, SHBCS). We requested data from each study on gender, site of the cancer (urinary bladder, ureter, renal pelvis or other), age (continuous), race (African-descent, Caucasian, Asian or other non-Caucasian), ethnicity (non-Latino or Latino), case-control status, smoking status (never, current, former), smoking intensity (cigarettes per day), and smoking duration (years of smoking). Most studies reported data on histology (transitional cell carcinoma, in-situ carcinoma, adenocarcinoma, or other), and a few reported data on grade using the 2004 WHO grading system (carcinoma of low potential malignancy, LMP) (supplementary Table 1). For each of the 10 SNPs selected, we were able to include a range of 3–9 studies with genotypic data available for each SNPs (supplementary Table 2).

We restricted pooled and meta-analyses to non-Latino whites, who were the predominant racial/ethnic group in 11 of the 12 studies (all minus SHBCS, that included all Chinese). We present results for that study individually. We included all histological types diagnosed in the urinary bladder. Genotypic, demographic and smoking data were available from a total of non-Latino 5,282 cases and 5,954 controls that were available for the final pooled- and meta- case-control analyses, and a total of 5,434 cases that were available for pooled case-only analyses. Most studies had previously published on the SNPs included in these analyses, with the exception of five studies who also contributed non-published data (EEBCS, FBCS, HBCS, LBCS, UCLABCS, and LABCS).

Genotyping data

The genotypes contributed for these pooled and meta-analyses were obtained using a variety of techniques, which included PCR-RFLP assays (BBCS, HBCS, NCBCS, NHBCS, SSBCS, TBCS), Taqman assays (FBCS, HBCS, LABCS, LBCS, NHBCS, SBCS, SHBCS, SSBCS, TBCS), Golden Gate assays (FBCS), SNP mass-tagging systems (NHBCS, TBCS), direct sequencing (FBCS), primer extension-denaturing HPLC (NHBCS, TBCS), and SNPlex (UCLABCS). Overall, all studies included a 3–10% duplicated samples to check for concordance, with the exception of one study that did not include duplicates (BBCS). Further details on the methods used by each study can be found in the references listed in Table 1, with the exception of studies that contributed unpublished data.

Statistical analyses

Pooled analyses—We checked among controls for differences between the observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium (HWE) using chi-square tests. We found no statistically significant deviations from HWE among controls, with the exception of three SNPs from one single study (NHBCS, rs1799793 $p = 0.023$, rs1799782 $p = 0.011$, and rs25487 $p = 0.002$). Analyses excluding this study did not substantially change estimates of association.

For each individual SNP, we present genotype frequencies and estimated odds ratios (OR) and 95% confidence intervals (95%CI) obtained from logistic regression models adjusting for gender, age (at diagnosis for cases and at interview for controls) in five year categories, study, and smoking status (never, former, and current). Most studies matched controls to cases on gender and age, using 3–5-year (BBCS, FBCS, HBCS, SBCS, TBCS) or 10-year intervals (EEBCS, NCBCS, NCBCS), or other intervals (LCBS). A few studies also matched on ethnicity (SBCS, HBCS, LASBCS) and/or hospital/region/neighborhood (SBCS, LASBCS, BBCS, TBCS). Studies defined ever smoker as those that smoked ≥1 cig/day for at least one year (BBCS, FBCS, LBCS), >100 cigs during lifetime (EEBC, HBCS, LASBCS, NHBCS, SBCS, UCLABCS), ≥ 1 cig/day for at least six months (NCBCS). Most studies defined former smokers as those that quit smoking at least 12 months prior to diagnosis. We estimated per allele ORs (95%CIs) assuming a log additive mode of inheritance. We note that two studies

individually matched cases to controls (LABCS and SBCS). Analyses within these two studies comparing results from conditional logistic models using matched pairs to unconditional logistic regression adjusting for gender, age (at diagnosis for cases and at interview for controls) in five year categories, and smoking status (never, former, and current) yielded almost identical results; therefore, matched case-control pairs were broken in final analyses. Gene-gene and gene-smoking interactions were evaluated by introducing interaction terms in logistic regression models that assumed a log-additive mode of inheritance for the genotype effects. For the case-only analyses to determine the effects of genotype among cases, we used a logistic regression model with smoking as the outcome variable (never/ever status) adjusting for gender, age, and study. We evaluated the independence assumption of genotype and smoking among controls using a logistic regression model with smoking status (never/ever) as the outcome variable and the SNP as the explanatory term adjusting for study, gender, and age. We assessed heterogeneity of relative risk estimates across different studies using logistic models introducing an interaction term. There was no evidence of heterogeneity for any of the SNPs. All tests were two-sided and all analyses were done using the statistical software STATA version 9.2 (STATA Corporation). Haplotype frequencies for ERCC2 were estimated using HaploStats (version 1.2.1; http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm) using the statistical program R (http://www.r-project.org/).

Meta-analyses—Meta-analysis was performed to summarize our findings along with previously published studies for the association between the ERCC2 K751Q and D312N, NBN E185Q, and XPC A499V and bladder cancer risk. Counts for the genotypes for Caucasian individuals were used with the same exclusions used for the pooled analyses. In addition, peer-reviewed studies published by January 1, 2009 in English were identified using a PubMed search “bladder cancer polymorphisms” and “bladder cancer risk variants”. Data on genotype frequencies for cases and controls were obtained from published manuscripts. A random-effect model was used to estimate summary ORs and 95% CIs by weighing each study result by a factor accounting for within- and between-study variance (13)

False positive report probability (FPRP)—We used FPRP calculations to evaluate the robustness of statistically significant (P<0.05) findings as described by Wacholder *et al.* (14). As previously suggested, a cutoff value of 0.2 was used to denote a “noteworthy finding”, with values less than 0.2 indicating a robust association for a given prior probability. Statistical power and FPRP were computed by the Excel spreadsheet provided by Wacholder et al, using a range of prior probabilities from 0.25 to 0.001 and per allele ORs ranging from 1.10 to 1.30. The lower bound of prior probabilities was chosen based on Thomas and Clayton (15,16). The range of underlying ORs was based on the observed per allele OR for established associations between common polymorphisms and cancer risk[1].

RESULTS

Pooled analyses of DNA repair SNPs and bladder cancer risk

In Table 2 we summarize our findings for the pooled analyses of all ten SNPs, for non-Latino white individuals from eleven studies. We present per allele ORs assuming a log-additive model. Estimates assuming a dominant or recessive model are presented in supplementary Table 3. We observed a modest but statistically significant association between the N-allele in *ERCC2* D312N and increased bladder cancer risk (OR per risk allele = 1.10; 95% CI = 1.01–1.19; p trend= 0.021). A similar trend was observed for another SNP in *ERCC2,* K751Q, which is in linkage disequilibrium with D312N ($D' = 0.80$, $r^2 = 0.59$, according to HapMap CEU

[1]Hindorff, L. A., Junkins, H. A., Mehta, J. P., and Manolio, T. A. A Catalog of Published Genome-Wide Association Studies. www.genome.gov/26525384.

population; in our pooled study populations the D′ = 0.75, r^2 = 0.52). We explored joint effects between the two SNPs by estimating the prevalence of the four possible haplotypes and testing for genotypes interactions. We observed no consistent interactions between the two SNPs across studies, although analyses by study suggested an inverse interaction in only two studies (HBCS, NHBCS). The association of each SNP with bladder cancer risk when adjusting for each other was OR = 1.06 (95% CI = 0.95–1.19) and OR = 1.05 (95% CI = 0.94–1.17) for 312N and 751Q alleles, respectively. Assuming a recessive or dominant mode of action did not reveal significant associations that were not captured by the log-additive model (Supplementary Table 3).

We also observed a statistically significant association between bladder cancer risk and *NBN* E185Q, with OR per Q allele of 1.09 (95% CI = 1.01–1.18, p = 0.028), and XPC A499V, with an OR per V allele of 1.10 (95% CI = 1.00–1.21; p trend = 0.044). There was no evidence of a significant association for any of the other 7 SNPs, or evidence of heterogeneity across studies for any of the SNPs we studied.

Pooled analyses of DNA repair SNPs x smoking interactions and bladder cancer risk

We observed statistically significant evidence of an interaction between *NBN* E185Q and measures of cigarette smoking (Table 3). Specifically, the Q allele was associated with increased bladder cancer risk among ever smokers (OR = 1.17; 95% CI = 1.07–1.28) but did not increase among never smokers (OR = 0.89; 95% CI = 0.77–1.06)(p for interaction = 0.002). The relation between the Q allele and bladder cancer risk in current smokers was comparable to that in former smokers (Table 3). However, stratification by smoking intensity suggested that increased bladder cancer risk is strongest for the highest smoking intensity category (>20 cigarettes/day) (OR = 1.24; 95% CI = 1.04–1.48; interaction p = 0.016). When considering the effect of smoking on bladder cancer risk taking into account NBN E185Q genotypes, we found that among carriers of the EE genotype who reported smoking more than 20 cigarettes per day we estimated an OR = 2.33 (95% CI = 1.86–2.94), whereas among carriers of the QQ genotype the corresponding OR estimate was 4.90 (95% CI = 2.86–8.44).

We also conducted case-only analyses of *NBN*-smoking interactions, which allowed us to include an additional 311 cases from the SSBCS study and thus estimate interaction ORs with slightly larger power. Under the assumption of independence between *NBN* genotype and smoking in the source population, ORs obtained from a case-only analyses are valid estimates of the interaction ORs (17). We found no evidence for an association between *NBN* E185Q and ever smoking among controls, and the case-only estimate per allele interaction OR was 1.23 (95% CI = 1.07–1.41). This interaction OR, albeit smaller, is comparable to the one estimated from case-control comparisons (interaction OR = 1.32; 95% CI = 1.11–1.56).

We did not find evidence of an interaction between smoking habits and any of the other nine SNPs (supplementary tables 4–7). Even though the association between the *ERCC2* codon 312 N and codon 751 K alleles seemed stronger among ever (supplementary Table 4), or former (supplementary table 5), or low dose or low smoking duration (supplementary Tables 6–7), neither of these interactions were statistically significant.

Meta-analyses of DNA repair SNPs and bladder cancer risk

We conducted meta-analyses across all studies which contributed data to the ICBC. To make our analyses more comprehensive, for the four SNPs for which we found a statistically significant association with bladder cancer risk, we also included two studies that also reported results for these SNPs but did not contribute data to the ICBC (18,19). Results for these four SNPs are summarized with forest plots in Figure 1. The meta-OR for *ERCC2* D312N and K751Q was 1.09 (95% CI = 1.01–1.17; p = 0.031) and 1.06 (95% CI = 0.9–1.13; p = 0.084),

respectively. The meta-OR for *NBN* E185Q was 1.09 (95% CI = 1.01–1.17; p = 0.022), and for *XPC* A499V was 1.09 (95% CI = 1.00–1.20; p = 0.045). Forest plots for all other SNPs are presented in supplementary Figure 1. Overall, there was no statistically significant heterogeneity across studies, as measured by the I^2 statistic.

We compared the overall ORs obtained for *ERCC2* and *NBN* SNPs with ORs obtained from data contributed by the SHBCS, which was the only study that contributed data on Asian individuals. The minor allele frequency (MAF) for the two *ERCC2* SNPs was significantly lower (0.088 and 0.075 for K751Q and D312N, respectively) than among Caucasians; whereas the MAF observed for the *NBN* E185Q SNP (0.449) was in the same range. Data on the *XPC* A499V SNP was unavailable from this study. Among SHBCS subjects we observed log-additive per allele ORs for *ERCC2* D312N of 0.63 (95% CI = 0.43–0.91, p = 0.013), *ERCC2* K751Q 0.76 (95% CI = 0.55–1.05, p = 0.101), and *NBN* E185Q 0.97 (95% CI = 0.82–1.15, p = 0.732) (supplementary table 8). We observed statistically significant heterogeneity between the meta-OR obtained for non-Latino whites and the OR observed for Shanghai Chinese for both SNPs in the ERCC2 gene (interaction p = 0.001 and p = 0.018 for D312N and K751Q, respectively). In contrast, no comparable heterogeneity was observed for *NBN* E185Q.

In Figure 2 we present forest plots for NBN x smoking status interaction. The meta-OR for *NBN* E185Q among never smokers was 0.89 (95% CI = 0.76–1.103) and among ever smokers was 1.17 (95% CI = 1.07–1.27). There was no statistically significant heterogeneity across studies. In contrast to what we observed for non-Latino white studies, we found no evidence of *NBN* x smoking interaction among Shanghai Chinese (data not shown). Interestingly, we found evidence of *XRCC3* T241M x smoking interaction (interaction p = 0.0108 for smoking status, p = 0.041 for smoking duration, and p = 0.004 for smoking intensity).

FPRP calculations

FPRP calculations indicated that the observed associations are noteworthy only for high prior probabilities. Specifically, the most significant association with bladder cancer risk in this report was found for *ERCC2* D312N (p = 0.021). FPRP calculations reach the threshold of < 0.2 at a prior probability level of 0.25 and statistical power to detect a true per-allele OR of 1.1 or higher. At a prior probability of 0.1, FPRP<0.2 was reached for true per-allele ORs of 1.2 or higher. The *XPC* A499V association reach the threshold of FPRP < 0.2 for priors of 0.25 and true ORs>=1.2. The *NBN* E185Q association for all subjects did not reach the FPRP threshold of < 0.2 for any of the combinations of prior probabilities and ORs, However, the observed estimates in ever smokers only were noteworthy for priors of 0.01 and ORs >=1.2.

DISCUSSION

We report findings from pooled- and meta-analyses of 10 DNA repair polymorphisms and bladder cancer risk among studies from the US and Europe participating in the ICBC, along with a comparison to results from a large study from China. These analyses provide evidence for an association between bladder cancer risk and polymorphisms in three DNA repair genes: *ERCC2*(D312N and K751Q), *NBN (*E185Q) and *XPC* (A499V). Furthermore, the *NBN* E185Q polymorphism might modify the association between cigarette smoking and bladder cancer risk.

To our knowledge, there is no available data on the functional consequences of the *XPC* A499V polymorphism for which we found a positive association with bladder cancer risk. This SNP is in linkage disequilibrium with two other polymorphisms in the 3′UTR region of the gene; therefore, any of these, or other linked variants, might be responsible for the observed association (20). The XPC protein plays a key role in global NER by recognizing the distortion of damaged DNA (21,22). This pathway repairs bulky adducts, such as those induced by

tobacco chemical carcinogens. Interestingly, emerging evidence suggests an additional role for XPC in the removal of oxidative damage (23–25). The ERCC2 protein also plays a key role in NER as an ATP dependent DNA helicase. In our data two SNPs in the *ERCC2* were associated with bladder cancer risk. The *ERCC2* D312N, but not K751Q, has been reported to associate with a 2.5 fold increase in UV induced apoptosis in a lymphoblastoid cell line (26). The literature for genotype-phenotype association studies for *ERCC2* SNPs have been inconsistent, hindering final conclusions regarding the mechanistic bases for the observed associations between these SNPs and disease, but an etiologic role for D312N seems plausible (27). Finally, we also report an association with the *NBN* E185Q polymorphism, which supports the conclusions of a recent overview of previous studies done on DNA repair and bladder cancer risk (28). The NBN protein is a member of the MRN complex, which participates in DSB detection and signaling (21,29). The functional impact of the E185Q variant has not been characterized, although recent studies support an association between this polymorphism and differences in DNA repair outcomes (30,31).

Overall, our findings support a role in bladder carcinogenesis for SNPs that play key roles in the repair of bulky adducts (*XPC*, *ERCC2*) and oxidative damage (XPC, NBN). Many environmental exposures can contribute to these types of damages. We considered cigarette smoke, which is the strongest risk factor for bladder cancer and contains chemical carcinogens that are known to induce bulky adducts, base damage, and DNA strand breaks in the bladder epithelium. In addition, the metabolism of tobacco carcinogens generates free radicals that can contribute to further oxidative damage. A role of oxidative damage in bladder carcinogenesis is consistent with our finding of an interaction between the NBN E185Q polymorphism and cigarette smoking. Our results indicate that the association between cigarette smoking and bladder cancer risk is stronger among carriers of the NBN codon 185 Q allele. Even though we observed a stronger association between NBN E185Q and bladder cancer risk among individuals that reported the highest smoking doses or duration, we saw little evidence of a dose-response trend with increasing dose or duration of smoking. We did not find evidence of differences in associations with bladder cancer risk for *ERCC2* or *XPC* when considering smoking habits. The presence of an association between these SNPs and bladder cancer risk among non-smokers suggest that there are endogenous or environmental exposures other than smoking that might be important sources of DNA damage in the bladder. Potential candidates include passive smoking, use of hair dyes, occupational exposures, and dietary sources of oxidative damage.

A strength of these analyses is the inclusion of both published and unpublished data from ICBC participating studies, thus minimizing the possibility of publication bias. To minimize confounding by population admixture we restricted our analyses to non-Latino whites which represented the majority of subjects included in participating studies. Given that studies were conducted in different populations in the US and Europe using a variety of study designs, it seems unlikely that any remaining biases would consistently occur in the same direction across studies. Therefore, the consistency of findings across studies indicates that results are unlikely to be driven by biases specific to particular studies.

A comparison of our pooled and meta-ORs to those obtained using data contributed by one large study in Shanghai highlighted interesting differences in estimates for both SNPs in the *ERCC2* gene and suggested a role for the *XRCC3* M241T SNP as an effect modifier of smoking. Future studies within the ICBC including more Asian populations will allow us to follow-up on these findings.

Although the statistical power of the pooled analyses was adequate (>0.80) to detect per allele ORs of 1.2 or higher for most SNPs evaluated, the power to detect per allele ORs of about 1.1 was more limited. FPRP calculations that take into account power and prior probability of an

association to evaluate the robustness of significant findings indicated that the observed associations for *ERCC2*, *XPC* and *NBN* are noteworthy only for relatively high prior probabilities (>0.01). Given that the knowledge on functional consequences of the identified SNPs is still limited (see discussion above), such high priors are difficult to justify (15,16). To our knowledge this report includes most bladder cancer studies with DNA available to date, therefore, it will take sometime before pooled analyses can include significantly larger number of bladder cancer cases.

Overall, our study indicates that alterations in the NER and DSB repair pathways could be relevant contributors to bladder carcinogenesis. Further studies comprehensively characterizing these pathways and accounting for relevant environmental exposures, should offer further insight into the role of DNA repair in bladder cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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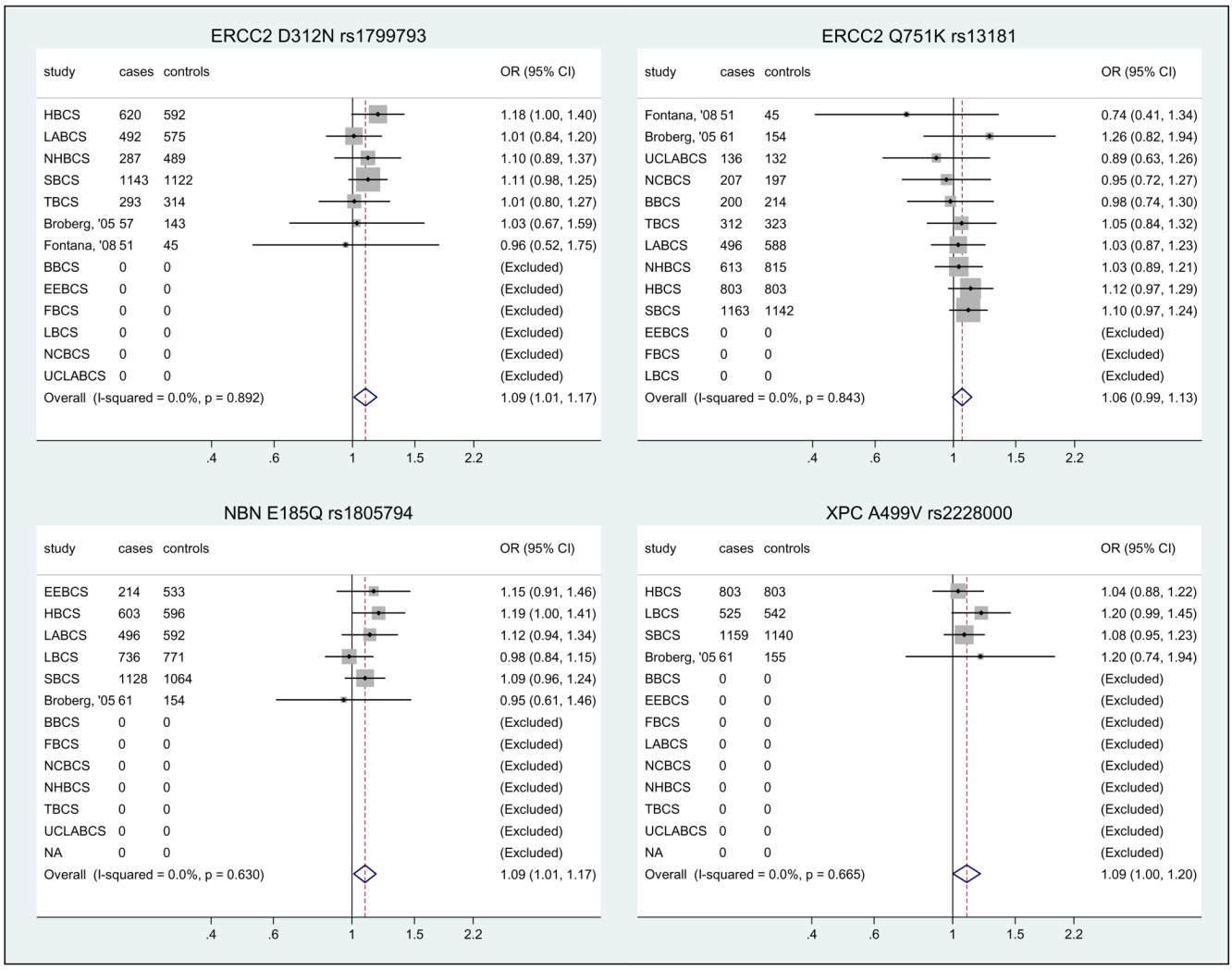

Figure 1.

Meta-plots for DNA repair SNPs that showed a statistically significant association with bladder cancer risk in pooled analyses. The I^2 statistics provides an estimate of the heterogeneity across studies.

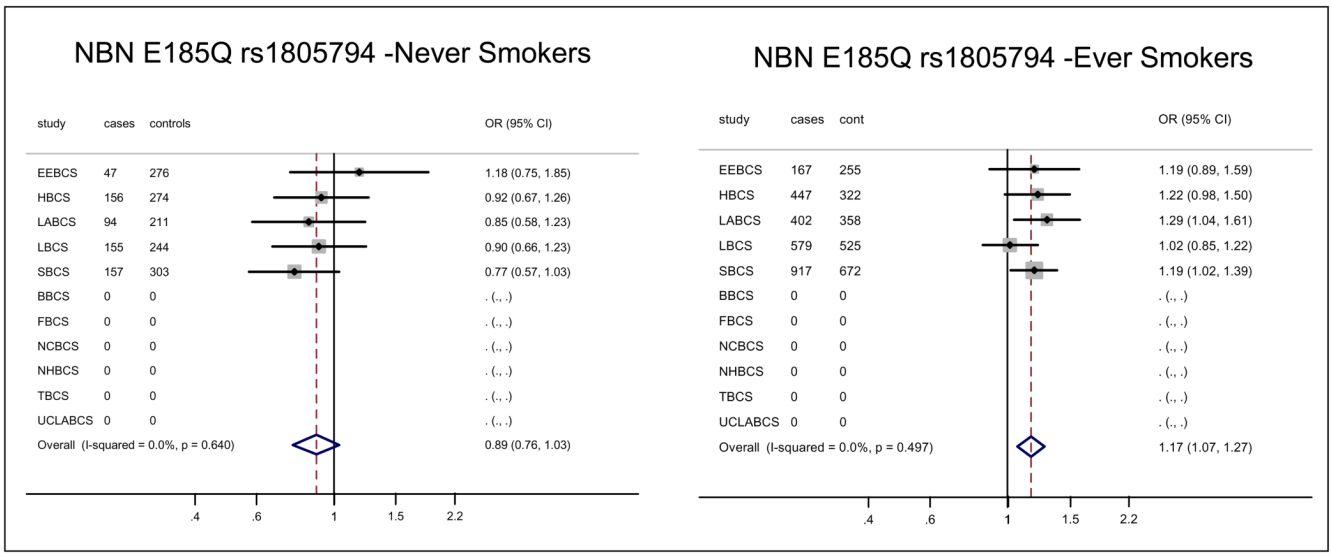

Figure 2.
Meta-plots for *NBN* E185Q stratified by smoking status (ever versus never). The I^2 statistics provides an estimate of the heterogeneity across studies.

Table 1

Characteristics of the studies included in pooled analyses

Study abbreviation	Study name	Controls	Cases	% Males[1]	Source	References[2]
BBCS	Brescia Bladder Cancer Study	214	200	100	Italy: hospital-based	(32,33)
EEBCS	Eastern European Bladder Cancer Study[3]	540	214	83	Hungary, Romania, Slovakia: hospital-based	(34)
FBCS	French Bladder Cancer Study	316	190	84	France: hospital-based	NA
HBCS	Houston Bladder Cancer Study	849	901	79	USA: hospital-based	(35,36)
LABCS	Los Angeles Bladder Cancer Study	676	581	78	USA: population-based	(37)
LBCS	Leeds Bladder Cancer Study	800	771	71	UK: hospital-based	(20,38–40)
NCBCS	North Carolina Bladder Cancer Study	215	245	77	USA: hospital-based	(41–44)
NHBCS	New Hampshire Bladder Cancer Study	899	675	76	USA: population-based	(45,46)
SBCS	Spanish Bladder Cancer Study	1149	1170	87	Spain: hospital-based	(10)
SHBCS	Shanghai Bladder Cancer Study	535	541	78	China: population-based	NA
SSBCS	Stockholm Bladder Cancer Study	N/A	311	65	Sweden: hospital-based	(47)
TBCS	Torino Bladder Cancer Study	401	379	100	Italy: hospital-based	(48)
UCLABCS	UCLA Bladder Cancer Study	158	170	80	USA: hospital-based	(49)
TOTAL		6,752	6,348			

[1] Percentage refers to cases;

[2] References that describe the original study and references that report data on the SNPs analyzed in pooled analyses. The EEBCS, FBCS, HBCS, LACBS, and SHBCS contributed original unpublished genotype data for these analyses;

[3] The EEBCS refers to the bladder cancer component of the ASHRAM case control study of bladder, skin and kidney cancers.

Table 2

DNA repair SNPs and urinary bladder cancer OR among non-Latino whites

Gene	Genotype		Studies N	Controls N	Controls %	Cases N	Cases %	OR[1]	95% CI	p
APEX1	D148E	*DD*	6	1043	28%	909	29%	1[ref]		
	rs1130409	*DE*		1822	49%	1494	48%	0.94	0.84–1.06	0.312
		EE		837	23%	700	23%	0.94	0.82–1.09	0.425
					per allele OR[2]			0.97	0.90–1.04	0.394
ERCC2	D312N	*DD*	5	1324	44%	1166	42%	1[ref]		
	rs1799793	*DN*		1303	44%	1248	45%	1.12	0.99–1.25	0.061
		NN		351	12%	363	13%	1.18	1.00–1.41	0.057
					per allele OR[2]			1.10	1.01–1.19	0.023
ERCC2	K751Q	*KK*	8	1669	41%	1542	40%	1[ref]		
	rs13181	*KQ*		1899	46%	1770	46%	1.03	0.93–1.13	0.605
		QQ		527	13%	560	14%	1.17	1.01–1.35	0.034
					per allele OR[2]			1.07	1.00–1.14	0.060
ERCC4	S835S	*TT*	4	746	48%	742	47%	1[ref]		
	rs1799801	*TC*		665	42%	691	44%	1.07	0.92–1.25	0.391
		CC		157	10%	146	9%	0.99	0.76–1.29	0.942
					per allele OR[2]			1.02	0.91–1.15	0.691
NBN	E185Q	*EE*	5	1642	48%	1404	45%	1[ref]		
	rs1805794	*EQ*		1451	42%	1376	44%	1.10	0.98–1.22	0.093
		QQ		347	10%	341	11%	1.18	0.99–1.40	0.063
					per allele OR[2]			1.09	1.01–1.18	0.028
XPC	A499V	*AA*	3	1343	56%	1316	54%	1[ref]		
	rs2228000	*AV*		906	38%	929	38%	1.05	0.93–1.19	0.429
		VV		143	6%	184	8%	1.32	1.04–1.68	0.021
					per allele OR[2]			1.10	1.00–1.21	0.044
XPC	K939Q	*KK*	4	1031	35%	925	35%	1[ref]		
	rs2228001	*KQ*		1408	48%	1277	48%	1.01	0.90–1.14	0.835
		QQ		497	17%	443	17%	0.98	0.83–1.15	0.776
					per allele OR[2]			0.99	0.92–1.07	0.854
XRCC1	R194W	*RR*	7	3244	87%	3180	88%	1[ref]		
	rs1799782	*RW*		458	12%	430	12%	0.93	0.80–1.07	0.296
		WW		16	0%	19	1%	1.45	0.73–2.90	0.287
					per allele OR[2]			0.96	0.84–1.10	0.570
XRCC1	R399Q	*RR*	9	1939	42%	1708	42%	1[ref]		
	rs25487	*RQ*		2043	45%	1888	46%	1.03	0.94–1.13	0.548
		QQ		593	13%	470	12%	0.89	0.77–1.04	0.126
					per allele OR[2]			0.97	0.91–1.03	0.358
XRCC3	T241M	*TT*	8	1625	39%	1427	38%	1[ref]		
	rs861539	*TM*		1980	47%	1724	46%	1.00	0.90–1.11	0.997
		MM		606	14%	574	15%	1.09	0.95–1.26	0.210
					per allele OR[2]			1.04	0.97–1.11	0.308

[1] ORs were adjusted for gender, age at diagnosis, study, and smoking;

[2] per allele OR assuming a log-additive model

Table 3

NBN E185Q x smoking interactions and bladder cancer risk among non-Latino whites

	Controls/Cases by genotype			Q allele risk[1]		
Smoking variables	**EE**	**EQ**	**QQ**	**OR[2]**	**95% CI**	**p-value**
Smoking status						
Never	616/311	542/241	150/57	0.89	0.77–1.03	0.110
Ever	1026/1093	909/1135	197/284	1.17	1.07–1.28	0.0005
Test of interaction p-value[3]	*0.002*					
Smoking status						
Never	616/311	542/241	150/57	0.89	0.77–1.03	0.128
Former	707/600	626/659	141/157	1.19	1.06–1.33	0.003
Current	319/493	283/476	56/127	1.15	1.00–1.34	0.058
Test of interaction p-value[3]	*0.007*					
Cigarettes per day						
Never	616/311	542/241	150/57	0.89	0.76–1.03	0.105
1–10	219/203	195/182	40/55	1.13	0.93–1.38	0.212
11–20	390/442	373/500	85/116	1.14	0.99–1.31	0.070
21+	261/339	213/346	40/80	1.24	1.04–1.48	0.014
Test of interaction p-value[3]	*0.016*					
Years of smoking						
Never	616/311	542/241	150/57	0.89	0.76–1.03	0.107
0–19	234/114	205/152	50/31	1.26	1.01–1.57	0.040
20–39	362/415	339/403	64/99	1.11	0.96–1.29	0.158
40+	286/472	246/482	51/124	1.20	1.02–1.39	0.024
Test of interaction p-value[3]	*0.014*					

[1] Assuming an log-additive mode of action;

[2] ORs adjusted for gender, age at diagnosis, and study

[3] Test of interaction on a multiplicative scale