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Genetic variations on chromosomes 5p15 and 15q25 and bladder cancer risk: findings from the Los Angeles–Shanghai bladder case–control study

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Genome-wide association studies have associated common variations at chromosomes 5p15 and 15q25 with lung cancer risk. The 5p15 locus has also been associated with increased bladder cancer risk in a recent report. The 15q25 locus has been associated with nicotine dependence and self-reported number of cigarettes smoked per day in some studies and it was proposed that its association with lung cancer may be mediated through differences in smoking behavior. Here, we investigated the roles of variations at 5p15 (rs401681, rs402710, rs2736098 and rs2736100) and 15q25 (rs1051730 and rs8034191) in bladder cancer etiology in two case–control studies conducted separately in Los Angeles County, CA, USA (498 cases and 588 controls) and in Shanghai, China (506 cases and 530 controls). We replicated the association between the 5p15 locus and bladder cancer among non-Hispanic whites (NHW) in Los Angeles [for rs2736100, per C allele odds ratio (OR) = 1.23; 95% confidence interval (CI), 1.02–1.48; $P = 0.029$] and among Chinese in Shanghai (OR = 1.22; 95% CI, 1.02–1.47; $P = 0.033$). Both rs1051730 and rs8034191 at 15q25 were rare among Chinese. Among NHW, a significant association was found between rs8034191 and bladder cancer which persisted after adjustment for cigarette smoking status, number of cigarettes smoked per day and number of years of smoking (per C allele OR = 1.26; 95% CI, 1.04–1.54; $P = 0.017$). Our results support 5p15 and 15q25 as susceptibility regions for bladder cancer risk.

Introduction

Since 2005, numerous genome-wide association studies (GWAS) have been reported and new susceptibility genes and pathways have been pointed out (1). Kiemeney *et al.* (2) reported the first GWAS of bladder cancer, which included subjects from various countries in Europe. The strongest association was observed for rs9642880 located at 8q24, which has been replicated in a Chinese study (3) and in our study in Los Angeles County, CA, USA and Shanghai, China (4). The second strongest association was observed for rs715021 located at 3q28, which we also recently replicated (5) along with a Chinese study (3). In the recently published second GWAS of bladder cancer (6), a new susceptibility variant (rs2294008) on chromosome 8q24 was identified.

Abbreviations: CI, confidence interval; GWAS, genome-wide association studies; LD, linkage disequilibrium; NHW, non-Hispanic whites; OR, odds ratio; SNPs, single nucleotide polymorphisms.

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In addition to the single nucleotide polymorphisms (SNPs) identified by GWAS of bladder cancer, two variants in 5p15 (rs401681 and rs2736098) have been found to be significantly associated with bladder cancer in a study in individuals of European ancestry, and despite being highly correlated with each other, neither variant could fully account for the association of the other (7). These variants are in linkage disequilibrium (LD) with the *CLPTM1L* gene and the telomerase reverse transcriptase (*TERT*)gene, and both variants have also been associated with basal cell carcinoma (7), lung cancer (8–11), glioma (12) and other tumors (7).

Genetic variants at rs8034191 and rs1051730 in 15q25 region have been associated with lung cancer risk (10,11,13–19), nicotine dependence (14,16,20) and smoking behavior (10,14,16–19,21–24). In prior studies, the lung cancer risk alleles at this locus have been correlated with a higher Fagerstrom Test for Nicotine Dependence scores (14) and a higher consumption of cigarettes (22). It is estimated that each copy of the A allele of rs1051730 corresponds to an increase in one cigarette smoked per day (14,22,24). These SNPs are located within an LD block containing three nicotinic receptors: CHRNA3, CHRNA5 and CHRNB4. Since smoking is a major risk factor for bladder cancer, it is plausible that variation in genes that affect smoking behavior, such as rs8034191 and rs1051730, might be associated with bladder cancer risk.

We have investigated the roles of these SNPs at 5p15 (rs401681, rs2736098 and two additional correlated SNPs rs402710 and rs2736100) and 15q25 (rs8034191 and rs1051730) in the risk of bladder cancer in non-Hispanic whites (NHW) in Los Angeles County and Chinese in Shanghai, taking into account smoking behavior and tumor characteristics. Other SNPs in the 15q25 region, such as rs2036534, rs667282, rs12910984 and rs6495309, which were also associated with bladder cancer among Chinese (18), were reported after the completion of our genotyping and therefore were not examined in the present study.

Materials and methods

Study population

Study subjects were participants in two population-based case–control studies: one in Los Angeles County, CA, USA and the other in Shanghai, China (25). In the Los Angeles study, case patients were Los Angeles County non-Asian residents aged 25–64 years, diagnosed with histologically confirmed bladder cancer between 1987 and 1996 identified through the Los Angeles County Cancer Surveillance Program, one of the National Cancer Institute's Surveillance, Epidemiology and End Results registries. For each case, one control subject was identified from the neighborhood of residence of the case using a previous procedure (25) and was matched to the index case on age (±5 years), gender and race/ethnicity (NHW, Hispanic or African–American). In Shanghai, case patients were Shanghai Chinese residents aged 25–74 years, diagnosed with histologically confirmed bladder cancer between 1995 and 1998 identified through the Shanghai Cancer Registry. Population-based controls were randomly selected from the city of Shanghai using an algorithm described previously (26) and were frequency matched to bladder cancer cases by gender and 5 years age groups during the recruitment period. All individuals were interviewed in person using a structured questionnaire that ascertained information on demographic, lifestyle and medical characteristics up to a reference date: 2 years before cancer diagnosis for cases and 2 years before the interview for controls (Shanghai study) or diagnosis of the index/paired case (Los Angeles County). Clinical characteristics of the tumors were obtained from pathology reports, which at the time of this study were only available for cases identified in Los Angeles County. Consistent with Kiemeney *et al.* (2), tumors with tumor-node-metastasis stage pTa and grade 1 or 2 were classified as having a low risk of progression and the rest as having a high risk of progression. Additional details from both studies can be found in the supplementary Materials and Methods, available at *Carcinogenesis* Online.

Samples of peripheral blood were requested from participants in the Shanghai study and from participants in the Los Angeles County component who were interviewed from January 1992 on. In Los Angeles, 74% of cases and 79% of controls who were asked to donate a blood sample consented, among whom, 79% of cases and 86% of controls had enough DNA materials for the

current genotyping effort. The corresponding figures in Shanghai were 89, 89, 98 and 99%. We have found no differences with respect to the distribution of demographic characteristics, including age, sex, level of education and cigarette smoking between those who were genotyped and the original study participants for both Los Angeles and Shanghai. All blood components (plasma, serum, buffy coat and red cells) were stored at −80°C until analysis. The majority of Los Angeles participants with DNA were NHW (88% cases and 89% of controls) and all participants in Shanghai were Han Chinese. Given these distributions, this report was restricted to NHW and Chinese participants with questionnaire data and DNA samples. Thus, this analysis includes 498 cases and 588 controls from Los Angeles and 506 cases and 530 controls from Shanghai.

Permissions to conduct the study were obtained from the Institutional Review Boards at the University of Southern California, the University of Minnesota and the Shanghai Cancer Institute. Informed consent forms for interview and biospecimen collection were obtained from each study participant.

Genotyping

DNA was extracted from peripheral blood lymphocytes using proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation as described previously (27). DNA samples from the Los Angeles County subjects were whole genome amplified using a REPLI-g mini kit from Qiagen (Valencia, CA). Genotyping of rs401681, rs402710, rs2736098, rs2736100, rs1051730 and rs8034191 was performed using Taqman assays from Applied Biosystems (Foster City, CA). An ABI 7900HT Sequence Detection and Scoring System was used for allele scoring. For quality control, 5% randomly selected samples were duplicated and all duplicates except one were matched for all six SNPs. The overall call rate was >96%.

Statistical analysis

For each SNP, genotypic frequencies among NHW and Chinese controls were examined separately for deviations from Hardy–Weinberg Equilibrium using exact tests. No deviations were observed among NHW controls. Among Chinese controls, statistically significant increased numbers of heterozygous genotypes of rs2736098 (270 observed versus 242 expected under Hardy–Weinberg Equilibrium; $P = 0.009$) were observed. The genotyping data of this SNP showed clear genotype separation with call rates >98% and concordance rates of 100% among replicates. Therefore, it is unlikely that we have genotyping errors. Population stratification is unlikely to explain the above deviations, considering the homogeneity of the study population in Shanghai.

NHW cases and controls with available data on genotypes were not all individually matched due to differential donation of blood samples within each matching pair. To maximize the sample size and match the analyses between NHW and Chinese, we created a composite variable that classified subjects according to their reference age (<45, 45–49, 50–54, 55–59 and ≥60 years for NHW and <45, 45–49, 50–54, 55–59, 60–64 and ≥65 years for Chinese), gender and study site (Los Angeles or Shanghai) and used it to stratify subjects in conditional logistic regression models. Results were not materially changed when unconditional logistic regression was used with additional adjustment for age (continuous), gender and race. We further adjusted for smoking status in reference year, number of cigarettes smoked per day (when smoking) and number of years of smoking. Relative risks were estimated by odds ratios (ORs); per allele ORs and 95% (CIs) were estimated for those carrying the high-risk allele relative to those carrying the homozygous low-risk allele at each locus assuming a log-additive mode of risk. To account for the multiple correlated tests due to LD between markers in a single region, we adjusted the final reported *P*-value using the P_{ACT} approach (28). This procedure yielded a region-level adjusted *P*-value (P_{adj}) for each SNP. P_{ACT} analyses were performed using the statistical package R 2.11.1 (29).

We tested for interaction between genotypes and other risk factors, including smoking status in reference year, number of cigarettes smoked per day, number of years of smoking, pack years of smoking, age, gender and race, on a multiplicative scale. Likelihood ratio tests were used to test the interaction assuming a log-additive mode of risk for the high-risk allele. For interaction analyses with age, age was tested as a continuous variable as well as a two-category variable dichotomized at the median age of the controls at reference date: 56 for NHW and 64 for Chinese. Among Los Angeles County participants, the SNP-bladder cancer associations were also estimated within tumors with different risks of progression (low or high) using conditional logistic regression models as described above. Heterogeneity between ORs for tumors with different risks of progression was assessed using logistic regression analyses restricted to cases (case-only analyses) with the tumor characteristic as the outcome variable. LD measures were calculated using the PROC ALLELE procedure from SAS (SAS Institute, Cary, NC).

All statistical tests (*P* values) were two sided and all analyses were conducted using SAS statistical software version 9.2 (SAS Institute).

Results

Characteristics of the two study populations have been previously reported (5). Briefly, in both Los Angeles and Shanghai, the majority (∼80%) of the study participants was male (Table I). The mean age at enrollment was ∼54 and 61 years for our Los Angeles and Shanghai participants, respectively. A much higher level of education was observed in Los Angeles participants with 67% of cases and 77% of controls having had college or higher education. The corresponding figures for Shanghai were 21 and 22%. A higher rate of cigarette smoking was reported in Los Angeles than in Shanghai (81% of cases and 61% of controls in Los Angeles versus 65 and 55% in Shanghai); however, smokers in Shanghai were more probably to be current smokers and long-term smokers.

The frequencies of alleles of each SNP in controls in our study were similar to data from previously published studies on NHW (7,14,15) and Chinese (18,30) and to the HapMap database (31). Key findings of the associations between SNPs at chromosomes 5p15 and 15q25 and bladder cancer risk are summarized in Tables II and III, respectively.

5p15 SNPs (rs401681, rs402710, rs2736098 and rs2736100) and bladder cancer risk

The correlations between the four 5p15 SNPs in our study were similar to data from the HapMap (31). Specifically, the pair-wise correlation (R^2) between rs402710 and rs401681 was 0.52 among NHW controls and 0.94 among Chinese controls, and all the other pair-wise R^2 were <0.300. All four SNPs showed association with bladder cancer risk in both populations (Table II), although among NHW, only rs2736100 reached statistical significance (per C allele OR = 1.23; 95% CI 1.02–1.48). Among Chinese, associations of similar magnitude were observed for all four SNPs, and the associations were statistically significant for all four. The OR per C allele of rs2736100 was 1.22 (95% CI, 1.02–1.47). The strongest association was found for rs2736098 among Chinese ($P = 0.019$; $P_{adj} = 0.059$). We also examined the joint effect of SNPs within the 5p15 region (data not shown; haplotype results are in supplementary Table 1). After adjustment for rs2736100, only the association of rs402710 among Chinese remained significant. There was no significant heterogeneity of risk estimates between NHW and Chinese in any of the analyses.

The associations between rs2736100 and bladder cancer were slightly more pronounced among individuals younger than the median age than among older individuals (supplementary Table 2 is available at *Carcinogenesis* Online). The per allele OR of rs2736100 was 1.30 (95% CI, 0.99–1.69) among NHW <56 years and 1.38 (95% CI, 1.06–1.81) among Chinese <64 years, whereas the corresponding figures among older subjects were 1.15 (95% CI, 0.89–1.49) and 1.11 (95% CI, 0.85–1.44), respectively. However, the differences by age were only marginally significant in NHW (*P* for interaction with age = 0.069, 0.17 and 0.064 for NHW, Chinese and both races combined, respectively). When stratified by smoking variables, among NHW, the association between rs2736100 and bladder cancer risk was stronger in current than in never or former smokers (Table II), and this difference was more pronounced among those in the higher category of smoking (data not shown). However, similar differences were not observed among Chinese. No consistent pattern was observed in the rs2736100–bladder cancer association by number of cigarettes smoked per day, number of years of smoking or pack years of smoking. Among NHW, we also investigated potential heterogeneity of the rs2736100–bladder cancer association across tumors with different risk of progression (Table IV). The per allele ORs of rs2736100 were 1.30 (1.04–1.62) and 1.15 (0.90–1.47) for tumors with low and high risk of progression, respectively (*P* for heterogeneity = 0.47).

15q25 SNPs (rs1051730 and rs8034191) and bladder cancer risk

The A allele of rs1051730 and the C allele of rs8034191 were rare (<5%) among Chinese, as previously noted (18). Among NHW, rs1051730 and rs8034191 were highly correlated ($R^2 = 0.84$). The

Table I. Characteristics of NHW in Los Angeles County and Chinese in Shanghai, China

	Los Angeles County		Shanghai	
	Cases (n = 498)	Controls (n = 588)	Cases (n = 506)	Controls (n = 530)
Age at enrollment				
≤45 years	52 (10%)	73 (12%)	50 (10%)	44 (8%)
45–49 years	57 (11%)	69 (12%)	29 (6%)	17 (3%)
50–54 years	92 (18%)	108 (18%)	36 (7%)	27 (5%)
55–59 years	149 (30%)	165 (28%)	40 (8%)	64 (12%)
60–64 years	148 (30%)	133 (23%)	135 (27%)	120 (23%)
≥65 years	—	40 (7%)	216 (43%)	258 (49%)
Mean (years)	54.5	54.4	60.7	61.7
Male	396 (80%)	475 (81%)	397 (78%)	407 (77%)
Level of education				
High school or below	166 (33%)	137 (23%)	—	—
1–3 years of college	164 (33%)	180 (31%)	—	—
College graduate	168 (34%)	271 (46%)	—	—
Elementary or below	—	—	167 (33%)	174 (33%)
Middle–high school	—	—	235 (46%)	242 (46%)
>High school	—	—	104 (21%)	114 (22%)
Cigarette smoking status				
Never smokers	93 (19%)	228 (39%)	175 (35%)	237 (45%)
Former smokers	189 (38%)	256 (44%)	64 (13%)	84 (16%)
Current smokers	216 (43%)	104 (18%)	267 (53%)	209 (39%)
Among all smokers				
<20 Cigarettes/day	82 (16%)	106 (18%)	152 (30%)	156 (29%)
≥20 Cigarettes/day	323 (65%)	254 (43%)	179 (35%)	137 (26%)
<30 Years of smoking	181 (36%)	235 (40%)	105 (21%)	112 (21%)
≥30 Years of smoking	224 (45%)	125 (21%)	226 (45%)	181 (34%)
<22.5 Pack years of smoking	117 (23%)	173 (29%)	139 (27%)	135 (25%)
≥22.5 Pack years of smoking	288 (58%)	187 (32%)	192 (38%)	158 (30%)
Probability of progression[a]				
Low: Ta/G1–2	281 (56%)	—	—	—
High: all other stages/grades	214 (43%)	—	—	—
Unknown	3 (1%)	—	—	—

[a]As defined by Kiemeney *et al.* 2008 (2).

association between rs1051730 and bladder cancer risk was of borderline statistical significance (Table III; per A allele OR = 1.19; 95% CI, 0.98–1.45). A statistically significant association between rs8034191 and risk of bladder cancer was observed (per C allele OR = 1.26; 95% CI, 1.04–1.54; P = 0.017; P_{adj} = 0.025). Adjustment for cigarette smoking status, number of cigarettes smoked per day and number of years of smoking did not seem to affect the above associations. When stratified by smoking variables, the rs8034191–bladder cancer association appeared to be stronger among former smokers (Table III; per C allele OR = 1.44; 95% CI, 1.06–1.95), particularly among those in the highest smoking categories (data not shown). The rs8034191–bladder cancer association was similar for tumors with low and high risk of progression (Table IV).

Given the important role of smoking in bladder cancer and the previous findings of rs8034191's association with nicotine addiction and smoking intensity (14,16,20,21), we also investigated the association between rs8034191 and cigarette smoking behavior among NHW in our study (supplementary Table 3 is available at *Carcinogenesis* Online). The overall evidence did not appear to support an association between SNP rs8034191 and cigarette smoking. The frequency of the C allele varied only slightly by smoking status. Among case patients, high-risk genotypes (CT/CC) were associated with a greater number of cigarettes smoked per day (P trend = 0.093). Similar trends were not observed among controls.

Discussion

The present study confirmed that rs2736100 at 5p15 region is a potential susceptibility marker of bladder cancer in both NHW and Chinese, providing the first evidence of an association in an Asian population. In addition, to our knowledge, we are the first to report an association between the 15q25 region, recently identified through GWAS as a susceptibility locus for both lung cancer and smoking behavior and bladder cancer risk among NHW.

5p15 SNPs and bladder cancer

The SNPs rs401681, rs402710, rs2736098 and rs2736100 are located on chromosome 5p15 with the first two SNPs residing within the *CLPTM1L* gene and the latter two SNPs within the *TERT* gene. Various mechanisms have been proposed to explain the observed associations between this locus at 5p15 and cancer risk. One candidate is the *TERT* gene, which is essential for telomere maintenance and cell immortalization. Shorter telomeres have been associated with an increased risk of bladder cancer (32,33). Rafnar *et al.* (7) reported that the risk alleles of rs401681 and rs2736098 were significantly associated with shorter telomeres in older women but not in younger women, leading the authors to hypothesize that the effect of these variants may only become apparent after a certain age. If these 5p15 SNPs indeed increase bladder cancer risk through their associations with shorter telomeres, one would expect the associations between these SNPs and bladder cancer to be more pronounced among older individuals. However, in our study, the 5p15 SNPs–bladder cancer associations were slightly more pronounced among younger individuals, although the differences were not statistically significant.

Another potential candidate causal gene for the 5p15 region is the *CLPTM1L* gene, which has been reported to be involved in cellular response to genotoxic stress and cisplatin resistance (34). In a recent study, the risk allele of rs402710, which localizes within the *CLPTM1L* gene, was associated with statistically significantly higher levels of bulky aromatic/hydrophobic DNA adducts (formed by polycyclic aromatic hydrocarbons and aldehydes found in cigarette smoke) in adjacent normal lung tissues of lung cancer patients (35).

Table II. SNPs at 5p15 region and bladder cancer risk among NHW in Los Angeles County and Chinese in Shanghai

	NHW			Chinese		
	Cases/controls	OR (95% CI)[a]	P^a	Cases/controls	OR (95% CI)[a]	P^a
rs401681						
TT	73/106	1.00 (ref)		45/66	1.00 (ref)	
CT	235/278	1.23 (0.85–1.76)	0.27	207/226	1.28 (0.83–1.97)	0.27
CC	164/170	1.36 (0.93–2.01)	0.12	248/237	1.53 (1.00–2.35)	0.052
Per C allele		1.16 (0.96–1.40)	0.13		1.22 (1.01–1.48)	0.036
rs402710						
TT	42/51	1.00 (ref)		46/65	1.00 (ref)	
CT	169/201	1.04 (0.64–1.69)	0.87	197/222	1.21 (0.79–1.87)	0.38
CC	260/276	1.21 (0.76–1.93)	0.43	260/240	1.53 (1.00–2.35)	0.049
Per C allele		1.12 (0.92–1.37)	0.26		1.25 (1.03–1.51)	0.021
rs2736098						
CC	217/278	1.00 (ref)		178/203	1.00 (ref)	
CT	189/210	1.17 (0.89–1.55)	0.27	236/270	1.00 (0.76–1.32)	0.99
TT	43/43	1.33 (0.82–2.17)	0.25	85/54	1.84 (1.23–2.77)	0.003
Per T allele		1.16 (0.94–1.43)	0.16		1.25 (1.04–1.51)	0.019
rs2736100						
AA	86/127	1.00 (ref)		141/174	1.00 (ref)	
AC	239/262	1.41 (1.00–1.98)	0.053	260/274	1.12 (0.84–1.49)	0.44
CC	146/158	1.55 (1.06–2.26)	0.022	98/77	1.54 (1.06–2.26)	0.025
Per C allele		1.23 (1.02–1.48)	0.029		1.22 (1.02–1.47)	0.033
Per C allele of rs2736100 stratified by smoking status						
Never-smokers	73/156[b]	1.29 (0.91–1.83)	0.15	118/159[b]	1.22 (0.91–1.63)	0.18
Former smokers	147/197[b]	0.94 (0.71–1.25)	0.67	46/51[b]	1.24 (0.75–2.03)	0.40
Current smokers	165/67[b]	1.71 (1.19–2.45)	0.004	194/141[b]	1.17 (0.88–1.55)	0.29
P for interaction between rs2736100 and smoking status[c]			0.038			0.89

[a]Results were estimated from conditional logistic regression with adjustment for smoking status in reference year, number of cigarettes smoked per day and number of years of smoking.
[b]Number of cases and controls carrying at least one C allele at rs2736100.
[c]Likelihood ratio test of the interaction term between rs2736100 and three-category smoking status variable assuming a log-additive mode of risk for the C allele.

Table III. SNPs at 15q25 region and bladder cancer risk among NHW

	Cases/controls	Crude		Adjusted for smoking status in reference year, number of cigarettes smoked and number of years of smoking	
		OR (95% CI)[a]	P^a	OR (95% CI)[a]	P^a
rs1051730					
GG	182/244	1.00 (ref)		1.00 (ref)	
GA	231/262	1.17 (0.90–1.52)	0.23	1.17 (0.88–1.54)	0.28
AA	65/63	1.40 (0.94–2.09)	0.093	1.45 (0.96–2.20)	0.081
Per A allele		1.18 (0.99–1.42)	0.072	1.19 (0.98–1.45)	0.072
rs8034191					
TT	176/248	1.00 (ref)		1.00 (ref)	
CT	234/259	1.25 (0.96–1.63)	0.090	1.27 (0.96–1.68)	0.088
CC	66/61	1.54 (1.04–2.30)	0.033	1.59 (1.04–2.42)	0.031
Per C allele		1.25 (1.04–1.50)	0.019	1.26 (1.04–1.54)	0.017
Per C allele of rs8034191 stratified by smoking status					
Never-smokers	56/116[b]	1.22 (0.85–1.75)	0.29	1.22 (0.85–1.75)	0.29
Former smokers	120/148[b]	1.44 (1.06–1.95)	0.018	1.44 (1.06–1.95)	0.020
Current smokers	124/56[b]	1.15 (0.81–1.63)	0.45	1.08 (0.76–1.55)	0.66
P for interaction between rs8034191 and smoking status[c]			0.58		0.56

[a]Results were estimated from conditional logistic regression.
[b]Number of cases and controls carrying at least one C allele at rs8034191.
[c]Likelihood ratio test of the interaction term between rs8034191 and three-category smoking status variable assuming a log-additive mode of risk for the C allele.

Similarly, in our study, among bladder cancer patients who were smokers, rs402710 was associated with a marginally significantly higher level of hemoglobin adducts of 4-aminobyphenyl (P = 0.075, data not shown), a known human bladder carcinogen present in cigarette smoke, although a similar trend was not observed among controls (data not shown). In addition, if genetic variants in this region affect the level of accumulated carcinogen adducts, we would expect to observe stronger associations for SNPs in this region among subjects with higher exposure to carcinogens, such as smokers. Consistent with this notion, in our study, the rs2736100–bladder cancer association among NHW appeared to be stronger in current smokers, particularly among those in the higher category of smoking. However, no differences by smoking status were found among Chinese, which is consistent with findings from Rafnar *et al.* (7), who reported similar associations between 5p15 SNPs and bladder cancer risk among both smokers and nonsmokers. Thus, it is possible that the heterogeneity

Table IV. rs2736100, rs8034191 and bladder cancer risk by tumor risk of progression among NHW

	Controls	Low risk of progression			High risk of progression		
		Cases[a]	OR (95% CI)[b]	P^b	Cases[a]	OR (95% CI)[b]	P^b
rs2736100							
AA	127	46	1.00 (ref)		40	1.00 (ref)	
AC	262	134	1.53 (1.01–2.34)	0.047	103	1.25 (0.80–1.95)	0.33
CC	158	87	1.75 (1.11–2.75)	0.016	58	1.34 (0.82–2.20)	0.24
Per C allele			1.30 (1.04–1.62)	0.021		1.15 (0.90–1.47)	0.25
		Heterogeneity P value for low-risk versus high-risk tumors[c] = 0.47					
rs8034191							
TT	248	100	1.00 (ref)		74	1.00 (ref)	
CT	259	126	1.22 (0.87–1.70)	0.25	107	1.38 (0.96–1.98)	0.083
CC	61	43	1.72 (1.06–2.78)	0.027	23	1.36 (0.76–2.42)	0.30
Per C allele			1.29 (1.03–1.61)	0.029		1.23 (0.95–1.59)	0.12
		Heterogeneity P value for low-risk versus high-risk tumors[c] = 0.49					

[a]Risk of progression was unknown for three cases.
[b]Results were estimated from conditional logistic regression with adjustment for smoking status in reference year, number of cigarettes smoked per day, and number of years of smoking.
[c]Likelihood ratio test from case–case analysis assuming a log-additive mode of risk for the high risk allele.

by cigarette smoking that we have observed in this study may be due to chance alone.

15q25 SNPs and bladder cancer

Two studies have assessed the associations between 15q25 SNPs and bladder cancer risk. Kiemeney *et al.* (2) did not find a significant association between rs1051730 and bladder cancer risk (combined OR = 1.03, P = 0.26). In a case–control analysis by Spitz *et al.* (16) that included bladder and renal cancers combined, no significant association was found with rs1051730 (for GA genotype, OR = 1.15, 95% CI = 0.91–1.45; for AA genotype, OR = 0.87, 95% CI = 0.69–1.26). In our study, rs8034191, which is highly correlated with rs1051730, was associated with bladder cancer among NHW (per C allele, OR = 1.26; 95% CI, 1.04–1.54). We did not find comparable associations among Chinese, but this could not be properly evaluated given the low prevalence of the minor allele in this population. However, other SNPs in this same region, such as rs2036534, rs667282, rs12910984 and rs6495309, have been previously reported to be associated with bladder cancer among Chinese by Wu *et al.* (18), suggesting that a causal variant in this region might also contribute to susceptibility to bladder cancer among this ethnic group. SNP rs16969968, another GWAS-identified variant at 15q25, was not examined in our study because of its high LD with rs8034191 and rs1051730, which exhibited stronger disease associations in the earlier GWAS of lung cancer (13–15).

SNPs rs1051730 and rs8034191 map to a region of strong LD on chromosome 15q25, containing *CHRNB4*, *CHRNA3*, *CHRNA5*, *PSMA4*, *IREB2* and *LOC123688* (10,13). The most promising candidates for a casual gene/s in this region are *CHRNB4*, *CHRNA3* and *CHRNA5*. These three genes encode the nicotinic acetylcholine receptor subunits, which play important roles in the development of nicotine dependence. Genetic variants on 15q25 have also been associated with nicotine dependence (14,16) and smoking behavior (10,14,16,18,19), and adjustment for smoking reduced the 15q25 SNP-associated lung risk in some studies (14,19). However, it is still under debate whether this locus affects lung cancer directly or the association is mediated primarily by affecting smoking behavior (10,13–19,36,37). In a recent study, smokers who carry the high bladder cancer risk allele (allele A) in *CHRNA3* (rs1051730) were found to have higher levels of nicotine per cigarette dose and of urinary metabolites of 4-(methylnitrosamino)-I-(3-pyridyl)-1-butanone, a tobacco-specific nitrosamine carcinogenic to humans (36). We found no evidence or very limited evidence of an association between rs8034191 and a greater number of cigarettes smoked per day among bladder cancer patients; furthermore, such association was not consistently observed among control subjects. Additionally, adjustment for cigarette smoking did not materially change the rs8034191–bladder cancer association in our study and there was also little evidence of a gene × smoking interaction, suggesting that this locus may affect bladder cancer also through other mechanisms, in addition to its potential impact on smoking behavior.

In conclusion, we report that SNPs in the 5p15 and 15q25 regions appear to play important roles in bladder cancer development. The power of our study is limited due to the modest sample size, particularly in subgroup analyses stratified by cigarette smoking and age, increasing thus the likelihood of spurious associations and precluding firm conclusions. Further investigations to validate our findings and fine map these regions to identify the functional variants are needed.

Supplementary material

Supplementary Materials and Methods and Tables 1–3 can be found at http://carcin.oxfordjournals.org/

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