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Cigarette smoking and subtypes of bladder cancer

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There is little information regarding associations between suspected bladder cancer risk factors and tumor subtypes at diagnosis. Some, but not all, studies have found that bladder cancer among smokers is often more invasive than it is among nonsmokers. This population-based case-control study was conducted in Los Angeles, California, involving 1,586 bladder cancer patients and their individually matched controls. Logistic regression was used to conduct separate analyses according to tumor subtypes defined by stage and grade. Cigarette smoking increased risk of both superficial and invasive bladder cancer, but the more advanced the stage, the stronger the effect. The odds ratios associated with regular smokers were 2.2 (95% confidence intervals, 1.8–2.8), 2.7 (2.1–3.6) and 3.7 (2.5–5.5) for low-grade superficial, high-grade superficial and invasive tumors respectively. This pattern was consistently observed regardless of the smoking exposure index under examination. Women had higher risk of invasive bladder cancer than men even they smoked comparable amount of cigarettes as men. There was no gender difference in the association between smoking and risk of low-grade superficial bladder cancer. The heterogeneous effect of cigarette smoking was attenuated among heavy users of NSAIDs. Our results indicate that cigarette smoking was more strongly associated with increased risk of invasive bladder cancer than with low-grade superficial bladder cancer.

In 2009, an estimated 70,980 cases of bladder cancer were diagnosed and over 14,330 people died from the disease in the United States.[1] The majority of these cases are transitional cell carcinoma (TCC). Among them, 70–80% are diagnosed at the nonmuscle invasive (superficial) stage (Ta) and remain confined to the mucosa and submucosa through most of their natural course. While, with a substantially better prognosis and a limited potential of progression,[2] superficial tumors are prone to recur. However, risk of progression does increase for high-grade superficial tumors.[3] In contrast, most muscle-invasive tumors exhibit their invasive property at initial presentation and they are associated with a worse prognosis and a propensity to metastasis.[4] It is generally believed that different molecular pathways are involved in the carcinogenesis of muscle invasive tumors as compared with superficial tumors.[5,6] The low grade superficial TCCs are characterized by constitutive activation of the Ras-MAPK pathway, and the high-grade invasive tumors are characterized by inactivation of the p53 and pRb pathways.[2,7] Tumor invasion is promoted by factors that alter the tumor microenvironment such as overexpression of cyclooxygenase 2 (COX-2). It has been suggested that etiologic factors, such as cigarette smoking, may be differentially associated with bladder cancer severity.[8–13] Here, we examine associations between cigarette smoking and bladder cancer risk taking into account tumor subtypes.

Key words: cigarette smoking, bladder cancer, tumor subtypes, nonsteroidal anti-inflammatory drugs, Los Angeles
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Material and Methods

Subjects

The design of the Los Angeles Bladder Cancer Case-Control Study has been previously described in details.[14] Cases were non-Asian patients aged 25–64 years with histologically confirmed bladder cancer diagnosed between January 1, 1987 and April 30, 1996 and identified through the Los Angeles County Cancer Surveillance Program (CSP),[15] one of the

population-based Surveillance, Epidemiology and End Results (SEER) cancer registries. For each interviewed patient, we sought to recruit a control who was matched to the index case by sex, date of birth (within 5 years), race (non-Hispanic white, Hispanic white, African American) and neighborhood of residence at the time of cancer diagnosis. Asian cases were excluded due to the low incidence rate among the ethnic group and also to the high costs for recruiting neighborhood controls. For the 1,671 interviewed cases, 1,586 eligible controlled were interviewed. All study subjects signed informed consent forms (separate forms for interview and blood donation) approved by the Institutional Review Board at the University of Southern California.

Data collection

In-person structured interviews were conducted in each subject's home. A reference date was defined as 2 years before the diagnosis of bladder cancer for case patients and this same reference date was used for each case patient's matched control subject. Information was requested on demographic characteristics, use of tobacco products and alcohol, usual adult dietary habits, occupational history, prior medical conditions, and prior use of medications at or before the reference date. Lifetime exposure was defined as all past exposures that occurred prior to the reference date. All interviews were conducted by the same team of interviewers throughout the entire data collection; and most case patient and control subjects in a given case-control pair were interviewed by the same interviewer.

For eligible cases, their pathological reports were retrieved through CSP and abstracted by trained CSP personnel for histology, stage and grade. The tumor node metastasis (TNM) system was used for stage classification.[16] On the basis of the T-stage, bladder cancer cases were classified as Tis (carcinoma *in situ*), Ta (noninvasive papillary carcinoma), T1 (tumors that have grown into the connective tissue beneath the bladder lining) and T2-4 (tumors that have grown through the connective tissue into the muscle). Grade was recorded as well (Grade 1), moderately (Grade 2) or poorly (Grades 3 or 4) differentiated. We excluded cases with unknown stage from all analyses. Consistent with Guey *et al.*,[17] tumors were classified into three categories according to stage and grade: low-grade superficial tumors (Ta and grade < 3, $N = 725$), high-grade superficial tumors (Ta and grade ≥ 3, T1 and grade ≥ 2, $N = 456$) and muscle-invasive tumors (T2, T3, T4, $N = 258$). A total of 147 cases were excluded from our analyses: 14 non-TCC cases (11 cases of adenocarcinoma, one pheochromocytoma, one spindle cell carcinoma and one lymphoma), nine TCC cases with unknown stage, 74 Tis cases, five Ta cases with unknown grade and 45 T1 cases with unknown grade or Grade 1.

Statistical analysis

The associations of bladder cancer with medical histories were measured by odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) and *p*-values. Unconditional polytomous logistic regression was used in the analyses of cigarette smoking, with additional adjustment for strata defined by age and sex (age groups of <45, 45–49, 50–54, 55–59 and ≥60 years for each sex), racial/ethnic groups (non-Hispanic white, Hispanic white or African American) and level of education (high school or less, some college, college or above). Analyses of nonsmoking factors were also adjusted for number of cigarettes smoked per day, number of years of smoking, and smoking status in reference year (smoker or nonsmoker). Similar results were obtained when we limited our analyses to non-Hispanic whites only. Therefore, we presented results based on all study subjects with adjustment for race to have a larger sample size and more stable risk estimates.

We tested for heterogeneity of the association between cigarette smoking and bladder cancer risk by subtypes using likelihood ratio tests of case–case comparisons with the tumor characteristic as the outcome variable. For stratified analyses by age, age was dichotomized at 56 years, the median age of the controls at reference date.

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). ORs with two-sided *p*-values <0.05 were considered statistically significant. All *p*-values quoted are two sided.

Results

A total of 725 low-grade superficial, 456 high-grade superficial and 258 muscle invasive tumors of the bladder was included in the current analysis. Compared with cases with low-grade superficial tumors, cases with high-grade superficial tumors or muscle-invasive tumors were diagnosed at slightly older ages (55.0 *vs.* 56.7 *vs.* 56.9 years), more likely to be men, and less educated (Supporting Information Table 1). No difference in racial/ethnic distribution was observed.

Table 1 shows the heterogeneity of cigarette smoking's effects on various subtypes of bladder cancer. Risks of all subtypes of bladder cancer were significantly higher among smokers and increased with increasing number of cigarettes smoked per day and number of years of regular smoking. Quitting smoking reduced the risk of all subtypes of bladder cancer, and the reduction in risk was proportional to the duration of smoking cessation. However, risks associated with cigarette smoking were higher for more advanced tumors, and in particular muscle-invasive tumors. For example, individuals who smoked ≥40 cigarettes per day had an OR of 7.2 (95% CI, 4.5–11.6) of muscle-invasive tumors, more than twice that of low-grade superficial tumors (OR = 3.3, 95% CI = 2.4–4.4). This difference in the risk of subtypes of bladder cancer was more pronounced among current smokers, especially those who smoked ≥20 cigarettes/day or ≥20 years. The elevated ORs for all three subtypes of bladder cancer were similar among relatively lighter smokers (*i.e.*, <20 cigarettes/day, <20 years of smoking or quitting smoking for ≥20 years).

Table 2 shows the ORs for three subtypes of bladder cancer with the joint exposure levels of smoking intensity and duration. Smoking with higher number of cigarettes/day and

Table 1. Cigarette smoking and risk of subtypes of bladder cancer

		Low-grade superficial		High-grade superficial		Muscle-invasive	
Cigarette smoking	Controls	Cases	OR (95% CI)[1]	Cases	OR (95% CI)[1]	Cases	OR (95% CI)[1]
Nonsmokers	574	148	1.0 (ref)	71	1.0 (ref)	30	1.0 (ref)
Regular smokers	1012	577	2.2 (1.8–2.8)	385	2.7 (2.1–3.6)	228	3.7 (2.5–5.5)
			$P_{heterogeneity}$[3] = 0.082				
No. of cigarettes/day							
<10	127	45	1.3 (0.9–1.9)	23	1.4 (0.8–2.3)	7	0.9 (0.4–2.2)
10-<20	186	71	1.5 (1.1–2.0)	52	2.2 (1.4–3.2)	20	1.9 (1.1–3.5)
20-<30	384	216	2.3 (1.8–2.9)	144	2.7 (2.0–3.7)	85	3.7 (2.4–5.8)
30-<40	130	101	3.4 (2.4–4.7)	63	3.5 (2.4–5.2)	37	5.2 (3.0–8.8)
≥40	185	144	3.3 (2.4–4.4)	103	3.9 (2.7–5.6)	79	7.2 (4.5–11.6)
			$P_{heterogeneity}$[3] = 0.002				
No. of years of smoking							
<10	128	40	1.2 (0.8–1.8)	29	1.9 (1.2–3.0)	6	0.9 (0.4–2.2)
10-<20	216	101	1.8 (1.3–2.4)	37	1.4 (0.9–2.1)	20	1.7 (1.0–3.1)
20-<30	245	132	2.1 (1.6–2.8)	89	2.8 (2.0–3.9)	43	3.1 (1. 9–5.1)
30-<40	251	175	2.9 (2.2–3.8)	127	3.6 (2.6–5.1)	93	6.4 (4.1–10.2)
≥40	172	129	3.4 (2.5–4.7)	103	4.2 (2.9–6.1)	66	6.4 (3.9–10.7)
			$P_{heterogeneity}$[3] = 0.002				
Smoking cessation							
Current smokers	374	310	3.2 (2.5–4.1)	217	4.2 (3.1–5.7)	142	6.1 (4.0–9.3)
Ex-smokers	638	266	1.7 (1.3–2.1)	168	1.9 (1.4–2.6)	86	2.3 (1.5–3.5)
			$P_{heterogeneity}$[3] = 0.054				
Years since quitting[2]							
<10	200	102	2.0 (1.5–2.7)	65	2.3 (1.6–3.4)	37	3.0 (1.8–5.0)
10-<20	190	93	1.9 (1.4–2.7)	55	2.1 (1.4–3.1)	29	2.6 (1.5–4.6)
≥20	248	71	1.2 (0.8–1.6)	48	1.4 (0.9–2.1)	20	1.4 (0.8–2.5)
			$P_{heterogeneity}$[3] = 0.41				

[1]Results were estimated from unconditional polytomous logistics regression, adjusted for age, gender, race, and level of education. [2]One patient with low-grade superficial bladder cancer was excluded from this analysis due to missing number of years since quitting smoking. [3]Case-case comparison of the effect of smoking status and the trends of smoking intensity, duration and number of years since quitting.

larger number of years of smoking experienced a greater risk of bladder cancer for each subtype examined. Heavy smokers (*i.e.*, ≥40 cigarettes/day and ≥40 years of smoking) had statistically significantly higher risk for invasive bladder cancer (OR = 9.0, 95% CI = 4.8–16.8) than for low-grade superficial bladder cancer (OR = 3.6, 95% CI = 2.3–5.8) as compared to nonsmokers, respectively ($p = 0.006$ for the difference between the two ORs).

We further assessed whether the heterogeneity in the association between cigarette smoking and risk of subtype bladder cancer was dependent on age, gender and level of education. In all these subgroup analyses, cigarette smoking was associated with consistently higher ORs for invasive bladder cancer than for low-grade superficial bladder cancer. In particular, even though a heterogeneous effect of smoking was observed in both men and women, we noticed the magnitude of the heterogeneity appeared to be larger among women (Table 3). As consequence, the gender-smoking interaction we observed previously,[14] with a higher risk of bladder cancer observed in women who smoked than that in men who smoked comparable amount of cigarettes, seemed to be more pronounced for high-grade superficial and muscle-invasive tumors than for low-grade superficial tumors. The p values for interaction between gender and smoking duration on risk of low-grade and high-grade superficial bladder cancer and invasive bladder cancer were 0.41, 0.007 and 0.068, respectively ($p = 0.002$ for the later two combined). Among lifelong nonsmokers, there was a higher proportion of high-grade superficial and muscle-invasive bladder cancer in men (30% and 14%) than in women (24% and 7%) ($P_{Mantel\text{-}Haenszel\ \chi 2} = 0.048$). We also examined the modifying effect of NSAIDs, known inhibitors of COX2. The heterogeneous effect of cigarette smoking was attenuated among regular users of NSAIDs. Our sample size was too small for further tests by different amounts of exposure to NSAIDs.

Table 2. Joint effects of intensity and duration of cigarette smoking on risk of subtypes of bladder cancer

		Low-grade superficial		High-grade superficial		Muscle-invasive	
	Controls	Cases	OR (95% CI)[1]	Cases	OR (95% CI)[1]	Cases	OR (95% CI)[1]
Nonsmokers	574	148	1.0 (ref)	71	1.0 (ref)	30	1.0 (ref)
<20 years of smoking							
<20 cigs/day	149	46	1.2 (0.8–1.7)	26	1.4 (0.9–2.4)	8	1.0 (0.5–2.3)
20-39 cigs/day	141	72	1.9 (1.3–2.6)	30	1.6 (1.0–2.5)	14	1.8 (0.9–3.5)
≥40 cigs/day	23	23	2.3 (1.3–3.9)	10	1.9 (0.9–3.9)	4	1.8 (0.6–5.5)
20-39 years of smoking							
<20 cigs/day	154	54	1.5 (1.0–2.1)	39	2.1 (1.4–3.3)	15	1.8 (1.0–3.5)
20-39 cigs/day	263	172	2.7 (2.1–3.6)	116	3.3 (2.3–4.6)	74	4.9 (3.1–7.8)
≥40 cigs/day	97	81	3.7 (2.6–5.3)	61	4.8 (3.2–7.3)	47	9.1 (5.4–15.4)
≥40 years of smoking							
<20 cigs/day	41	16	2.9 (1.5–5.6)	10	3.2 (1.4–7.1)	4	2.8 (0.9–8.9)
20-39 cigs/day	92	73	3.4 (2.3–5.0)	61	4.4 (1.4–7.1)	34	5.7 (3.2–10.1)
≥40 cigs/day	52	40	3.6 (2.3–5.8)	32	4.3 (2.5–7.2)	28	9.0 (4.8–16.8)
				$P_{heterogeneity}$[2] = 0.0002			

[1]Results were estimated from unconditional polytomous logistics regression, adjusted for age, gender, race, and level of education. Nonsmokers were the reference group for the OR estimates. [2]Case-case comparison of the effect of lifetime smoking (the total number of cigarettes smoked over a lifetime, calculated from the average number of cigarette smoked per day ×365.25 days/year × the number of years of smoking cigarettes).

The above results were similar when different classifications of histology were used (data not shown). For example, significant heterogeneity in the effect of duration of cigarette smoking was also found between tumors with different stages (P for heterogeneity = 0.008 for Ta *vs.* T1 *vs.* T2-4), grades (P = 0.006 for G1 *vs.* G2 *vs.* G3/4) or risks of progression (P = 0.039 for low *vs.* high risk) with the most pronounced difference in risk found between Ta and T2-T4 tumors. Consistent with our findings above, these heterogeneities were limited to long-term smokers (≥20 years of smoking).

Discussion

This study examined associations between cigarette smoking and bladder cancer subtypes at diagnosis. Regardless of the exposure index under examination (smoking status, number of cigarettes per day or number of years of smoking), risks associated with cigarette smoking were higher for invasive tumors.

Some,[11–13,18,19] but not all,[20–23] studies have found that bladder cancer among smokers is often more invasive than it is among nonsmokers. In some studies,[12] age appeared to modify this association with the association limited to or more pronounced among younger individuals (<60 years old). Among the studies that did not observe an impact of smoking on tumor invasiveness, two studies found that smokers had poorer prognosis.[20,23] Most of these studies were based on simple comparisons of a small number of subjects and none of these studies formally tested for the heterogeneous effect of smoking. By far, the largest study was that of Sturgeon *et al.*,[18] which found that risks of each stage of bladder cancer (invasive, noninvasive) increased with number of cigarettes smoked per day, but the more advanced the stage the higher the relative risk. Similarly, in this study we found that smokers were proportionally more likely than nonsmokers to be diagnosed with invasive disease and this difference was statistically significant.

The exact mechanism of bladder carcinogenesis in smokers remains unknown. Allelic loss on chromosome 9 has been reported as an early lesion in the development of bladder cancer and does not distinguish between different subtypes of tumors.[24] Other events may occur during independent evolution of different tumor subclones. Experimental studies have observed distinctive genetic defects in subtypes of bladder cancer: the low-grade noninvasive papillary tumors are characterized by activating mutations in the FGFR3 gene, while the high-grade invasive tumors are characterized by structural and functional defects in the p53 and Rb protein tumor-suppressor pathways.[2] Tumor invasion is promoted by over-expression of COX-2, a downstream target of p53 that regulates cell growth, angiogenesis, immune surveillance and apoptosis. Zhang *et al.*[25] found that chromosome 9 alterations were more common in smokers compared to those in nonsmokers. Chromosome 9p21 has been found to be a molecular target for damages induced by benzopyrene diolepoxide (BPDE), the metabolic product of benzopyrene, a constituent of tobacco smoke.[26] Smoking seems to affect the pattern of TP53 mutations, but have no effect on the incidence and pattern of FGFR3 mutation.[27] Consistently, occupational exposure to polycyclic aromatic hydrocarbons, which are also commonly found in cigarette smoke, did not influence the frequency or spectrum of FGFR3 mutations.[28] These

Table 3. Cigarette smoking and risk of subtype of bladder cancer stratified by gender and use of NSAIDs

		Low-grade superficial		High-grade superficial		Muscle-invasive	
	Controls	Cases	OR (95% CI)[1]	Cases	OR (95% CI)[1]	Cases	OR (95% CI)[1]
Among men							
Nonsmokers	414	101	1.0	55	1.0	25	1.0
<20 years of smoking	273	104	1.5 (1.1–2.1)	59	1.6 (1.1–2.4)	20	1.2 (0.6–2.2)
≥20 years of smoking	550	335	2.6 (2.0–3.4)	257	3.1 (2.2–4.3)	160	4.1 (2.6–6.4)
		$P_{heterogeneity}$[2] = 0.055					
Among women							
Nonsmokers	160	47	1.0	16	1.0	5	1.0
<20 years of smoking	71	37	1.7 (1.0–3.0)	7	1.0 (0.4–2.6)	6	3.0 (0.9–10.1)
≥20 years of smoking	118	101	3.0 (2.0–4.6)	62	4.9 (2.7–9.1)	42	10.2 (3.9–26.8)
		$P_{heterogeneity}$[2] = 0.019					
Among NSAID nonusers							
Nonsmokers	392	113	1.0	50	1.0	23	1.0
<20 years of smoking	219	100	1.7 (1.3–2.3)	44	1.6 (1.1–2.4)	17	1.5 (0.8–2.8)
≥20 years of smoking	413	269	2.7 (2.1–3.4)	226	3.8 (2.8–5.2)	138	5.4 (3.5–8.4)
		$P_{heterogeneity}$[2] = 0.0004					
Among NSAID users							
Nonsmokers	175	34	1.0	21	1.0	7	1.0
<20 years of smoking	123	40	1.3 (0.9–1.9)	19	1.2 (0.7–2.1)	9	1.4 (0.6–3.0)
≥20 years of smoking	251	163	2.6 (2.0–3.5)	91	2.5 (1.8–3.6)	62	4.1 (2.5–6.5)
		$P_{heterogeneity}$[2] = 0.15					

[1]Results were estimated from unconditional polytomous logistic regression, adjusted for age, sex, race and level of education, with both strata estimated in one model. [2]Case-case comparison of the trend effect of smoking duration.

observations support our findings that even though cigarette smoking was associated with an increased risk of all subtypes of bladder cancer, possibly by inducing alterations in chromosome 9, a stronger effect was observed for invasive tumors which are characterized by TP53 mutations.

The above hypothesis is strengthened by the observation that the heterogeneity of smoking's effect was attenuated among regular users of NSAIDs, which function to inhibit COX-2. The presence of a wild-type p53 is known to suppress COX-2 transcription, and the loss or mutation of p53 up-regulates the expression of COX-2.[29] If the heterogeneous effect of smoking functions by deactivating the p53 pathway therefore leading to over-expression of COX-2, use of NSAIDs may counteract, at least partially, this heterogeneous effect by blocking COX-2 activity. Consistent with this notion, we found that the gender-smoking interaction was limited to invasive diseases. In rabbit studies, estrogen deficiency induces a significant decrease in prostaglandin E_2, one of COX-2's enzymatic products, in the urinary bladder mucosa and estrogen treatment restored the level of prostaglandin E_2.[30] It has been reported that estrogen stimulates prostaglandin synthesis by activating COX-2.[31,32] Therefore, it is possible that through COX-2, estrogen may promote the progression of bladder tumor.

Alternatively, the heterogeneous effect of smoking can be due to different surveillance for smokers. Compared with nonsmokers, cigarette smokers might be less likely to seek medical care, thus more likely to be diagnosed with higher grade and/or more invasive bladder cancer. One Japanese study[19] found that, at the time of diagnosis, current smoker were more likely to be diagnosed at a higher stage and have larger tumor size than nonsmokers; however, there were no differences between smokers and nonsmokers in initial symptoms, indicating that the difference in diagnosis was not likely due to delayed presentation of smokers to medical attentions.

While this study has several strengths including population-based design, relatively large sample size, and well characterized risk/protective factors, a potential weakness is the fact that the bladder cancer histology was recorded by trained personnel from pathology reports and was not determined by a single reference laboratory. The various grading schemes used by pathologists who produced the original pathology reports can be a source for misclassification. Since it is unlikely the pathologist would know the status of the exposures we measured, the variations in grading schemes is most likely to bias results to the null and lead to false negative findings. This misclassification is unlikely to result in differential effects of exposures such as cigarettes by stage at diagnosis as we observed here. In addition, by reviewing individual pathology reports, we were able to separate Ta from Tis, which is not possible if relying on SEER registry data alone. Another potential limitation of our study is

the confinement in case selection to relatively younger cases of bladder cancer, making our findings not directly applicable or generalizable to patients diagnosed at older ages. Finally, some of our subgroup analyses, especially those conducted among women and regular users of NSAIDs, were based on very small numbers; so interpretation must be cautious.

In summary, this study reported a higher risk associated with cigarette smoking for invasive bladder tumors than for superficial tumors. Studies with bigger sample sizes are needed to confirm these findings and to understand the molecular pathways that might explain the observed heterogeneity by cancer subtypes.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225–49.
2. Wu X-R. Urothelial tumorigenesis: a tale of divergent pathways. *Nat Rev Cancer* 2005; 5:713–25.
3. Dalbagni G. The management of superficial bladder cancer. *Nat Clin Pract Urol* 2007;4: 254–60.
4. Raghavan D, Shipley WU, Garnick MB, Russell PJ, Richie JP. Biology and management of bladder cancer. *N Engl J Med* 1990;322:1129–38.
5. Cutler SJ, Heney NM, Friedel GH. Longitudinal study of patients with bladder cancer: factors associated with disease recurrence and progression. In: Bonney WW, Prout GRJ, eds. Bladder cancer, vol. 1. Baltimore: Williams & Wilkins, 1982. 35–47.
6. Kaye KW, Lange PH. Mode of presentation of invasive bladder cancer: reassessment of the problem. *J Urol* 1982;128:31–3.
7. Wolff EM, Liang G, Jones PA. Mechanisms of Disease: genetic and epigenetic alterations that drive bladder cancer. *Nat Clin Pract Urol* 2005;2:502–10.
8. Cartwright RA, Adib R, Appleyard I, Glashan RW, Gray B, Hamilton-Stewart PA, Robinson M, Barham-Hall D. Cigarette smoking and bladder cancer: an epidemiological inquiry in West Yorkshire. *J Epidemiol Commun Health* 1983;37:256–63.
9. Cartwright RA, Glashan RW, Gray B. Survival of transitional cell carcinoma cases in 2 Yorkshire centres. *Br J Urol* 1980;52: 497–9.
10. Glashan RW, Wijesinghe DP, Riley A. The early changes in the development of bladder cancer in patients exposed to known industrial carcinogens. *Br J Urol* 1981;53:571–7.
11. Brooks DR, Geller AC, Chang J, Miller DR. Occupation, smoking, and the risk of high-grade invasive bladder cancer in Missouri. *Am J Ind Med* 1992;21:699–713.
12. Hayes RB, Friedell GH, Zahm SH, Cole P. Are the known bladder cancer risk-factors associated with more advanced bladder cancer? *Cancer Causes Control* 1993;4:157–62.
13. Thompson IM, Peek M, Rodriguez FR. The impact of cigarette smoking on stage, grade and number of recurrences of transitional cell carcinoma of the bladder. *J Urol* 1987; 137:401–3.
14. Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, Ross RK, Yu MC. Gender- and smoking-related bladder cancer risk. *J Natl Cancer Inst* 2001;93:538–45.
15. Bernstein L, Ross RK. Cancer in Los Angeles Countyed. Los Angeles, CA: University of Southern California, 1991.
16. Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/ International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. *Am J Surg Pathol* 1998;22:1435–48.
17. Guey LT, Garcia-Closas M, Murta-Nascimento C, Lloreta J, Palencia L, Kogevinas M, Rothman N, Vellalta G, Calle ML, Marenne G, Tardon A, Carrato A, *et al.* Genetic susceptibility to distinct bladder cancer subphenotypes. *Eur Urol* 2010;57:283–92.
18. Sturgeon SR, Hartge P, Silverman DT, Kantor AF, Linehan WM, Lynch C, Hoover RN. Associations between bladder cancer risk factors and tumor stage and grade at diagnosis. *Epidemiology* 1994;5: 218–25.
19. Hinotsu S, Akaza H, Miki T, Fujimoto H, Shinohara N, Kikuchi E, Mizutani Y, Koga H, Okajima E, Okuyama A. Bladder cancer develops 6 years earlier in current smokers: analysis of bladder cancer registry data collected by the cancer registration committee of the Japanese Urological Association. *Int J Urol* 2009;16:64–9.
20. Carpenter AA. Clinical experience with transitional cell carcinoma of the bladder with special reference to smoking. *J Urol* 1989;141:527–8.
21. Jensen OM, Wahrendorf J, Blettner M, Knudsen JB, Sorensen BL. The Copenhagen case-control study of bladder cancer: role of smoking in invasive and non-invasive bladder tumours. *J Epidemiol Commun Health* 1987;41:30–6.
22. Morrison AS, Proppe KH, Verhoek WG, Aoki K, Leck I, Ohno Y, Obata K. Histologic features of bladder cancer in Boston. USA, Manchester, UK and Nagoya Japan. *Int J Cancer* 1982;30:701–5.
23. Raitanen MP, Nieminen P, Tammela TL. Impact of tumour grade, stage, number and size, and smoking and sex, on survival in patients with transitional cell carcinoma of the bladder. *Br J Urol* 1995;76:470–4.
24. Knowles MA. Molecular subtypes of bladder cancer: Jekyll and Hyde or chalk and cheese? *Carcinogenesis* 2006;27:361–73.
25. Zhang D, Okada S, Yu Y, Zheng P, Yamaguchi R, Kasai H. Vitamin E inhibits apoptosis. DNA modification, and cancer incidence induced by iron-mediated peroxidation in. Wistar rat kidney. *Cancer Res* 1997;57:2410–4.
26. Hazra A, Grossman HB, Zhu Y, Luo S, Spitz MR, Wu X. Benzo[a]pyrene diol epoxide-induced 9p21 aberrations associated with genetic predisposition to bladder cancer. *Genes Chromosomes Cancer* 2004;41:330–8.
27. Wallerand H, Bakkar AA, de Medina SGD, Pairon J-C, Yang Y-C, Vordos D, Bittard H, Fauconnet S, Kouyoumdjian J-C, Jaurand M-C, Zhang Z-F, Radvanyi F, *et al.* Mutations in TP53, but not FGFR3, in urothelial cell carcinoma of the bladder are influenced by smoking: contribution of exogenous versus endogenous carcinogens. *Carcinogenesis* 2005;26:177–84.
28. Bakkar AA, Allory Y, Iwatsubo Y, de Medina SG, Maille P, Khreich N, Riou A, Leroy K, Vordos D, Abbou CC, Andujar P, Billebaud T, *et al.* Occupational exposure to polycyclic aromatic hydrocarbons influenced neither the frequency nor the spectrum of FGFR3 mutations in bladder urothelial carcinoma. *Mol Carcinog* 2010;49:25–31.
29. Pruthi RS, Derksen E, Gaston K, Wallen EM. Rationale for use of cyclooxygenase-2 inhibitors in prevention and treatment of bladder cancer. *Urology* 2004;64:637–42.
30. Hass MA, Nichol P, Lee L, Levin RM. Estrogen modulates permeability and prostaglandin levels in the rabbit urinary bladder. *Prostaglandins Leukotrienes Essential Fatty Acids* 2009;80:125–9.
31. Egan KM, Lawson JA, Fries S, Koller B, Rader DJ, Smyth EM, Fitzgerald GA. COX-2-derived prostacyclin confers atheroprotection on female mice. *Science* 2004;306:1954–7.
32. Wu WX, Ma XH, Zhang Q, Buchwalder L, Nathanielsz PW. Regulation of prostaglandin endoperoxide H synthase 1 and 2 by estradiol and progesterone in nonpregnant ovine myometrium and endometrium in vivo. *Endocrinology* 1997; 138:4005–12.