BMC Cancer

RESEARCH Open Access

The role of *CDK8* gene polymorphisms in bladder cancer susceptibility and prognosis: a study in the Chinese Han population

Zhilong Li[1†], Min Su[1†], Qin Li[1], Xuelian Zheng[1], Yaping Song[1], Yanyun Wang[1], Bin Zhou[1*] and Lin Zhang[1*]

Abstract

Background *Cyclin-dependent kinase 8* (*CDK8*) has been implicated in various tumors, with its role differing across tumor types. However, the association between *CDK8* polymorphisms and bladder cancer (BC) remains unclear. This study investigated the association between *CDK8* polymorphisms and BC susceptibility and prognosis.

Methods This case-control study included 271 patients with BC and 381 healthy controls. Two-tag single-nucleotide polymorphisms in the *CDK8* gene (rs17083838 and rs7992670) were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Statistical analyses were performed using SNPstats and SPSS software to assess genetic associations.

Results The AG/AA genotypes of rs17083838 were associated with a significantly reduced risk of BC under the dominant model ($P<0.001$, odds ratio [95% confidence interval] = 0.50 [0.33–0.76]). Stratified analysis revealed that the AG genotype of rs17083838 increased the risk of postoperative recurrence in patients with stage IV BC ($P=0.007$). For rs7992670, females with the AG/AA genotype exhibited a 2.07-fold higher risk of BC than males, whereas smokers with the same genotype showed a 2.13-fold higher risk than non-smokers. The GG genotype of rs7992670 was associated with better overall survival in patients with stage III BC ($P=0.023$). Among patients with recurrent muscle-invasive BC, those with the GG/AA genotype showed significantly improved survival compared with those carrying the AG genotype ($P=0.023$).

Conclusions *CDK8* polymorphisms influence BC susceptibility and prognosis, with rs17083838 showing a protective effect and rs7992670 being associated with increased risk and survival outcomes in specific subgroups.

Impact This study highlights the potential of *CDK8* polymorphisms as biomarkers for BC susceptibility and prognosis, emphasizing the need for further research.

Keywords *CDK8*, SNP polymorphism, Bladder cancer, PCR-RFLP, Genetic susceptibility

†Zhilong Li and Min Su contributed equally to this work.

*Correspondence:
Bin Zhou
zb630@163.com
Lin Zhang
zhanglin@scu.edu.cn

[1]Laboratory of Molecular Translational Medicine, Center for Translational Medicine; Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education; Children's Medicine Key Laboratory of Sichuan Province, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, P. R. China

Introduction

Bladder cancer (BC) is the ninth most common form of cancer globally, accounting for 61,3791 new cases and 22,0349 BC-related deaths estimated in 2022 [1]. According to the GLOBOCAN 2020 data, China's annual BC mortality has reached 39,393 cases, accounting for 18.5% of global BC mortality, indicating the substantial public health challenge of this disease in China [2]. BC imposes large personal, social, and economic burdens, with the highest prevalence in developed communities globally [3]. At diagnosis, approximately 70% of patients with BC have non-muscle-invasive bladder cancer (NMIBC), 20% have muscle-invasive bladder cancer (MIBC), and 10% have metastatic disease [4]. Compared with NMIBC, patients with MIBC have worse prognoses and account for a large proportion of deaths [5, 6].

The multistep accumulation of genetic, epigenetic, and environmental factors plays an important role in the development and progression of BC [7–9]. Advanced age, male sex, and tobacco smoking are the main risk factors for the development of BC [10]. Globally, BC-related mortality rates in men are nearly three times higher than those in women. Age-standardized mortality rates (ASR) per 100,000 person-years vary by region, with rates in men generally ranging from 2.7 to 9.2 and those for women ranging from 0.8 to 1.8 [2]. Cigarette smoking is the strongest contributor to the increased incidence of BC in Western countries [11]. Additionally, individuals with a family history of BC have nearly double the risk of developing the disease [12–14], indicating a genetic predisposition to bladder carcinogenesis. For instance, the variant form of the hormone gene *3-beta-hydroxysteroid dehydrogenase type 2* (*HSD3B2)* has been associated with an increased risk of BC in the New Hampshire population [15].

Cyclin-dependent kinase 8 (CDK8), a member of *CDK* family, is encoded by the *CDK8* gene located on chromosome 13q12.13. It is a nuclear serine-threonine kinase that regulates cell cycle and proliferation at the transcriptional level [16]. Studies have reported that variants in *CDK8* cause intellectual disabilities and congenital anomalies [17]. The role of *CDK8* in tumors appears to be divergent and highly context-dependent. It functions as an oncogene that accelerates proliferation and migration in most tumors, such as colon cancer [18], leukemia [19], clear cell renal cell carcinoma [20], breast cancer [21], oral squamous cell carcinoma [22], non-small cell lung cancer [23] and gastric cancer [24]. Conversely, *CDK8* acted as a tumor suppressor in intestinal and endometrial tumors [25]. Mutations in *CDK8* were found to be associated with poor prognosis in Chinese patients with renal cell carcinoma [20]. Recently, Min et al. [26] reported that polymorphisms in *CDK8* (rs17083838 and rs7992670) are associated with an increased risk of cervical cancer in Han women from Southwest China, with the A allele of both single-nucleotide polymorphisms (SNPs) being significantly more frequent in patients with cervical cancer. Analysis of BC clinical samples revealed a notable prevalence (4.11%) of *CDK8* gene amplification [27]. However, an association between *CDK8* mutations and BC susceptibility has not been established. The present study assessed the association between polymorphic variants in *CDK8* and BC susceptibility. Two tag SNPs (rs17083838 and rs7992670) were selected, and their prevalence was determined in 271 unrelated patients with BC and 381 healthy controls from a Chinese Han population.

Materials and methods

Clinical characteristics and follow-up of subjects

Human peripheral blood samples were obtained with the approval from the Ethics Committee of West China Second University Hospital and West China Hospital, Sichuan University (Medical Research Project Approval No. 017, 2012). Informed consent was obtained from all participants. A total of 271 patients with BC and 381 healthy volunteers were enrolled at the West China Hospital between 2015 and 2020. None of the healthy participants had a personal or family history of BC or other serious diseases. Patients with a history of cancer or metastatic cancer from other origins were excluded, and none had undergone radiotherapy or chemotherapy. Information regarding intravesical Bacillus Calmette-Guérin treatment was not specifically collected because the study primarily focused on genetic associations rather than treatment outcomes. The follow-up information of the participants was obtained by telephone calls every 6 months for 5 years.

DNA extraction and genotyping

Genomic DNA was isolated from 200μL of EDTA-anticoagulated blood using a DNA extraction kit (BioTeke, Peking, China). Two tag SNPs of *CDK8* were selected according to the CHB (Han Chinese in Beijing, China) population data from the HapMap Project using SNPinfo Web Server (RRID: SCR_010589) [28]. Polymerase chain reaction (PCR) primers were designed using Primer3 (RRID: SCR_003139) version 4.1.0 (https://bioinfo.ut.ee/primer3/) [29]. Genotyping was conducted using a polymerase chain reaction-restriction fragment length polymorphism (PCR-PFLP) assay. Each 10μL reaction contained 1μL of genomic DNA together and 5μL of 2×Taq plus Master Mix (Biosharp, Hefei, China), according to the manufacturer's instruction. PCR was carried out in a thermocycler (Eppendorf, Hamburg, Germany) using the following cycling conditions: 95 °C for 3 min, followed by 34 cycles of 95 °C for 3 s, 58 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 10 min. Negative controls in which DNA was replaced

with water, were used to detect potential contamination. The PCR products of rs17083838 and rs7992670 were digested using the restriction enzymes MspI and MboII (New England BioLabs, Inc. MA, USA). Details of the primer sequences, PCR conditions, restriction enzymes, and lengths of the digested products are shown in Table 1. Finally, the digested fragments were separated on 6% polyacrylamide gels and stained with 1.0 g/L silver nitrate. In addition, approximately 10% of the samples were randomly selected to repeat the assay, yielding concordant results. The genotypes of these two SNPs were also confirmed by DNA sequencing analysis.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics (version 26; IBM Corp., Armonk, NY, USA; RRID: SCR_016479). Genotypic association tests in a case-control pattern were performed using the SNPSTATS (RRID: SCR_002142) online analysis software [30], assuming codominant, dominant, recessive, and over-dominant genetic models. Hardy–Weinberg equilibrium was calculated using the chi-squared test. We applied the Bonferroni correction method to control for the risk of false positives due to multiple tests, adjusting the significance threshold from $P<0.05$ to $P<0.005$ (0.05/10) or $P<0.0125$ (0.05/4). Differences with $P<0.005$ or $P<0.025$ were considered significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to evaluate the effects of the different genotypes and alleles. The associations between *CDK8* genotypes and patient outcomes (recurrence and death) were first assessed using Kaplan–Meier analysis and the log-rank test. Stratified analyses were performed to evaluate these associations in different subgroups according to age, sex, smoking status, tumor stage, tumor grade, clinical stage, and metastatic status. Multivariate Cox proportional hazards regression models were used to estimate the independent effects of significant factors, adjusting for age at first diagnosis, sex, smoking status, tumor grade, and clinical stage.

Results

Clinical and pathological characteristics of the study population

In total, 271 patients and 381 healthy controls were included in this study. Patient diagnoses were confirmed based on the histopathological analysis. According to the 2024 American Urological Association (AUA) [31]/European Association of Urology (EAU) guidelines and the TNM staging system [32], patients were classified as having NMIBC (stage Ta-T1) and MIBC (stage T2-T4) based on pathological staging. As shown in Table 2, the median age was 66 and 60 years in the patient and control groups, respectively. When age was analyzed as a continuous variable, no significant difference were observed between patients and controls ($P=0.093$). However, when using the median age of 63 years as a cut-off point for dichotomization, the chi-square test revealed a significant difference in age distribution between the two groups, with a higher proportion of subjects older than 63 years in the patient group than that in the control group (55.72% vs. 44.62%, $P<0.01$). Regarding sex distribution, males were predominant in the patient group (79.70%), whereas the control group had a more balanced sex ratio (48.03% males, $P<0.001$). The proportion of smokers was similar between patients and controls (52.03% vs. 49.34%, $P=0.551$).

All diagnoses were confirmed through histopathological analysis and evaluation of their clinical characteristics. Based on the pathological findings, 59.78% of the patients presented with high-grade tumors, while 40.22% had low-grade tumors. In terms of tumor invasion, 51.66% and 48.34% of patients were diagnosed with NMIBC and MIBC, respectively. Detailed clinical staging revealed that most patients were diagnosed at early stages, with 51.66% at stage I and 28.41% at stage II, whereas advanced stages were less common, with 12.18% at stage III and 7.75% at stage IV.

SNP genotyping results and consistency validation

For rs17083838, the amplification product for genotype A was a 194 bp fragment, while genotype G was digested with MspI into 126 bp and 68 bp fragments. On polyacrylamide gel electrophoresis, the GG genotype showed a single band at 126 bp (the 68 bp band was not displayed), with no band at 194 bp (Fig. 1A). The AA genotype exhibited a single band at 194 bp, whereas the AG genotype showed bands at both 194 and 126 bp (the 68 bp band was not displayed). For rs7992670, the amplification product for genotype A was a 151 bp fragment, while genotype G was digested by MboII into

Table 1 Primer sequences and reaction conditions for genotyping two Tag SNPs in *CDK8*

SNPs	Primer sequence (5′→3′)	Wild/Mutated allele	Annealing temperature (°C)	Restriction enzyme	Digest condition	Product size (bp)
rs17083838	F: TCTGTTGCACTTGCCATATCA	G/A	58	Msp I	37 °C	A: 194
	R: TCCAAAGACTTTCAAAGACACTCA				45 min	G: 126 + 68
rs7992670	F: TTTCATGCAGATACCACGTCA	G/A	58	Mbo II	37 °C	A: 151
	R: TGGATTCACGGGTTCGTTAT				30 min	G: 113 + 38

Table 2 The clinicopathological features of patients with bladder cancer and health controls

Characteristics	Patients($n=271$)	Controls($n=381$)	*P* value
Age			
Median (first quartile, third quartile)	66 (56,73)	60 (49,75)	0.093
age ≤ median(63)(n[%])	120[44.28%]	211[55.38%]	**<0.01**
age> median(63)(n[%])	151[55.72%]	170[44.62%]	
Sex (n[%])			
Male	216[79.70%]	183[48.03%]	**<0.001**
Female	55[20.30%]	198[51.97%]	
Smoking status (n[%])			
Smoker	141[52.03%]	188[49.34%]	0.551
Nonsmoker	130[47.97%]	193[50.66%]	
Tumor stage (n[%])			
MIBC	131[48.34%]		
NMIBC	140[51.66%]		
Tumor grade (n[%])			
High grade	162[59.78%]		
Low grade	109[40.22%]		
Clinical stage (n[%])			
I(Ta-T1N0m0)	140[51.66%]		
II(T2N0M0)	77[28.41%]		
III(T3N0M0, T4aN0M0)	33[12.18%]		
IV(T4bN0M0, TnNnM0,TnNnMn, $n \geq 1$)	21[7.75%]		

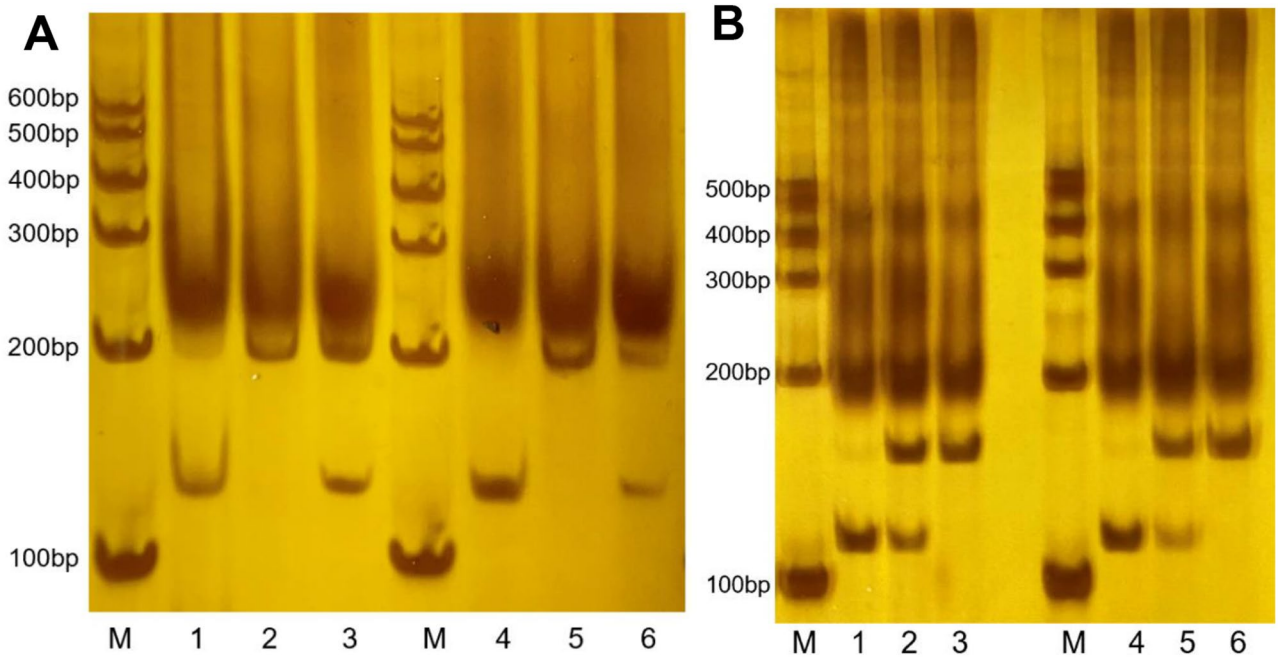

Fig. 1 Genotypes of *CDK8* rs17083838 and rs7992670 on the 6% polyacrylamide gels. (**A**) Genotypes of rs17083838. M: marker; Line 1 and 4: GG genotype. Line 2 and 5: AA genotype. Line 3 and 6: AG genotype. (**B**) Genotypes of rs7992670. M: marker; Line 1 and 4: GG genotype. Line 2 and 5: AG genotype. Line 3 and 6: AA genotype

113 bp and 38 bp fragments. On polyacrylamide gel electrophoresis, the GG genotype showed a single band at 113 bp (the 38 bp band was not displayed), with no band at 151 bp (Figure 1B). The AA genotype showed a single band at 151 bp, whereas the AG genotype showed bands at both 151 and 113 bp (the 38 bp band was not displayed). Sanger sequencing was performed on amplification products from 30 randomly selected samples, and the results were consistent with the RFLP genotyping results, indicating that the RFLP genotyping method had high accuracy.

Association between *CDK8* gene polymorphisms and BC

The genotype and allele frequencies of both tag SNPs (rs17083838 and rs7992670) conformed to Hardy–Weinberg equilibrium ($P > 0.05$) in both patients ($P = 0.061$ and $P = 0.21$, respectively) and controls ($P = 0.56$ and $P = 0.21$, respectively). For rs17083838, significant associations with BC were observed in multiple genetic models. Under the codominant model, the AG genotype showed a significantly lower frequency in patients compared to that in controls (14.8% vs. 27.3%), conferring a protective effect against BC (OR = 0.50, 95% CI: 0.33–0.76, $P < 0.001$). Similarly, in the dominant model, the combined AG/AA genotypes were associated with reduced BC risk (16.6% vs. 29.1%, OR = 0.53, 95% CI: 0.35–0.80, $P = 0.002$). The over-dominant model also revealed a protective effect of the AG genotype (14.8% vs. 27.3%, OR = 0.50, 95% CI: 0.33–0.76, $P = 0.001$). Additionally, the A allele frequency was significantly lower in BC patients than that in healthy controls (9% vs. 15%, OR = 0.61, 95% CI: 0.42–0.88, $P < 0.001$), suggesting a protective role. However, for rs7992670, no significant association was detected between genotype/allele frequencies and BC risk in any of the genetic models. The distributions of the genotypes and alleles for both SNPs are presented in Table 3. All ORs were adjusted for age, sex, and smoking status to control potential confounding variables. The crude ORs are provided in Supplementary Tables 1 to show the impact of confounders.

Linkage disequilibrium and haplotype analysis

The two SNPs exhibited low linkage disequilibrium (rs17083838 vs. rs7992670, D' = 0.2586, $r^2 = 0.0071$), suggesting independent inheritance. Haplotype analysis identified the haplotype AA (rs17083838- rs7992670), which included a low-risk A allele at rs17083838 and was associated with reduced BC susceptibility ($P = 0.0026$, OR = 0.27, 95% CI: 0.11–0.63; Table 4). All ORs were adjusted for age, sex, and smoking status to control potential confounding variables. The crude ORs are provided in Supplementary Tables 2 to show the impact of confounders.

Association between *CDK8* gene polymorphisms and clinical characteristics of patients with BC

The stratified analysis investigated the associations between two SNP genotypes (rs17083838 and rs7992670) and clinical characteristics in patients with BC. The analysis was stratified by age (≤63 vs. >63 years), sex (male vs. female), smoking status (smoker vs. non-smoker), tumor stage (MIBC vs. NMIBC), tumor grade (high

Table 3 Distribution of SNPs in *CDK8* between patients and controls and their polymorphisms with genetic susceptibility to BC

Model	Genotype	Patients	Controls	Logistic regression	
		n[%]	*n*[%]	OR(95% CI)	Global *P* value
rs17083838					
Codominant	GG	226[83.4%]	270[70.9%]	1	**< 0.001**
	AG	40[14.8%]	104[27.3%]	**0.50(0.33–0.76)**	
	AA	5[1.8%]	7[1.8%]	1.06(0.30–3.70)	
Dominant	GG	226[83.4%]	270[70.9%]	1	**0.002**
	AG/AA	45[16.6%]	111[29.1%]	**0.53(0.35–0.80)**	
Recessive	GG/AG	266[98.2%]	374[98.2%]	1	0.74
	AA	5[1.8%]	7[1.8%]	1.23(0.35–4.35)	
Over-dominant	GG/AA	231[85.2%]	277[72.7%]	1	**0.001**
	AG	40[14.8%]	104[27.3%]	**0.50(0.33–0.76)**	
Allele	G	492[91%]	644[85%]	1	**< 0.001**
	A	50[9%]	118[15%]	**0.61(0.42–0.88)**	
rs7992670					
Codominant	GG	94[34.7%]	118[31.0%]	1	0.34
	AG	140[51.7%]	196[51.4%]	0.88(0.61–1.28)	
	AA	37[13.7%]	67[17.6%]	0.68(0.41–1.14)	
Dominant	GG	94[34.7%]	118[31.0%]	1	0.32
	AG/AA	177[65.3%]	263[69.0%]	0.83(0.59–1.19)	
Recessive	GG/AG	234[86.3%]	314[82.4%]	1	0.18
	AA	37[13.7%]	67[17.6%]	0.74(0.46–1.16)	
Over-dominant	GG/AA	131[48.3%]	185[48.6%]	1	0.97
	AG	140[51.7%]	196[51.4%]	1.01(0.72–1.41)	
Allele	G	328[61%]	432[57.0%]	1	0.16
	A	214[39%]	330[43.0%]	0.84(0.65–1.08)	

All ORs and their 95% CIs are adjusted for age, sex, and smoking status; the values in **boldfaced** indicate significant difference ($P < 0.005$). OR: odds ratio; CI: confidence interval

Table 4 Haplotype frequencies of *CDK8* in BC patients and controls

Haplotype	rs17083838	rs7992670	Controls (%)	Patients (%)	OR (95% CI)	*P* value
1	G	G	47.04%	53.04%	1	-
2	G	A	37.47%	37.74%	0.87(0.66–1.14)	0.31
3	A	G	9.65%	7.48%	0.73(0.45–1.19)	0.21
4	A	A	5.84%	1.74%	**0.31(0.13–0.74)**	**0.0081**

All ORs and their 95% CIs are adjusted for age, sex, and smoking status; **bold values** denote statistically significant results ($P < 0.0125$). OR: odds ratio; CI: confidence interval

Table 5 The association between two SNPs genotypes and clinical characteristics of patients with BC in dominant model

Characteristics	rs17083838 Genotype		*P* value[a]	rs7992670 Genotype		*P* value[a]
	GG	AG/AA		GG	AG/AA	
Age						
≤63 years old	101(84.2%)	19(15.8%)	0.81	46(38.3%)	74(61.7%)	0.18
>63 years old	125(82.8%)	26(17.2%)		48(31.8%)	103(68.2%)	
Sex						
Male	179(82.9%)	37(17.1%)	0.23	78(36.1%)	138(63.9%)	**0.034**
Female	47(85.5%)	8(14.6%)		16(29.1%)	39(70.9%)	
Smoke						
Smoker	120(85.1%)	21(14.9%)	0.26	42(29.8%)	99(70.2%)	**0.0098**
Nonsmoker	106(81.5%)	24(18.5%)		52(40%)	78(60%)	
Tumor stage						
MIBC	110(84%)	21(16%)	0.69	44(33.6%)	87(66.4%)	0.62
NMIBC	116(82.9%)	24(17.1%)		50(35.7%)	90(64.3%)	
Tumor grade						
High grade	136(84%)	26(16.1%)	0.68	55(34%)	107(66%)	0.99
Low grade	90(82.6%)	19(17.4%)		39(35.8%)	70(64.2%)	
Recurrence						
Recurrent	69(87.3%)	10(12.7%)	0.12	28(35.4%)	51(64.6%)	0.83
Nonrecurrent	157(81.8%)	35(18.2%)		66(34.4%)	126(65.6%)	
Metastasis						
Metastatic	38(79.2%)	10(20.8%)	0.19	19(39.6%)	29(60.4%)	0.38
Nonmetastatic	188(84.3%)	35(15.7%)		75(33.6%)	148(66.4%)	

The P values in **boldfaced** indicates significant difference. [a] The P value was adjusted by age, sex, smoking status, tumor stage, tumor grade, recurrence status and metastasis status

vs. low), recurrence status, and metastasis status. For rs7992670, significant associations were observed in sex and smoking status subgroups (Table 5). After adjusting for other clinical factors, the AG/AA genotypes were significantly more prevalent in female patients than that in male patients (70.9% vs. 63.9%, $P = 0.034$, OR = 2.07, 95% CI: 1.04–4.66). Additionally, the A allele carriers showed a higher frequency in smokers than that in non-smokers (70.2% vs. 60%, $P = 0.0098$, OR = 2.13, 95% CI: 1.19–3.70). Based on these observations, we further analyzed the interaction between rs7992670 and smoking status on BC risk among females using the dominant genetic model adjusted for age. Interestingly, a different pattern was observed when examining the case-control analysis, specifically within the female population. Among female smokers, carriers of the A allele were associated with significantly decreased disease risk (OR = 0.12, 95% CI: 0.04–0.39), whereas no such effect was observed in female non-smokers (OR = 1.04, 95% CI: 0.50–2.16). This apparent contradiction with the overall population findings suggests complex gene-environment interactions that may be sex-specific. The p-value for the interaction was 0.08, which approached statistical significance. Notably, the study cohort included only 55 female patients with BC, with only four patients being smokers (three with the AG genotype and one with the AA genotype), which may limit the robustness of these findings and warrant validation in larger studies. For rs17083838, no significant associations were found with any clinical characteristics, with the GG genotype being predominant (approximately 80–85%) across all groups. Furthermore, neither SNP was significantly associated with age, tumor stage, tumor grade, recurrence, or metastatic status. All analyses used a dominant genetic model with p-values adjusted for potential confounding factors.

Impact of SNP genotypes on BC prognosis: univariate and multivariate analyses

Overall survival and recurrence analysis in the entire cohort

Among the 271 enrolled patients, 47 (17.34%) died and 79 (29.2%) experienced disease recurrence during the follow-up period. We performed Kaplan–Meier and Cox regression analyses focusing on recurrence-free survival (RFS) and overall survival (OS) to explore potential prognostic factors. Kaplan–Meier analysis revealed no significant association between rs17083838 or rs7992670 and either OS or RFS across all genetic models. In the multivariate Cox regression analysis for RFS, after adjusting for demographic and clinicopathological characteristics (including age, sex, smoking status, clinical stage, tumor stage, tumor grade, and metastasis status) and SNP genotypes, metastasis status emerged as the only independent risk factor for disease recurrence (HR ≈ 3.5, 95% CI: 2.0–6.3, $P < 0.001$). For OS, the multivariate model identified metastasis (HR ≈ 35.728, 95% CI: 14.5–88.1, $P < 0.001$) and recurrence status (HR ≈ 2.09, 95% CI: 1.06–4.12, $P < 0.05$) as independent prognostic factors.

Importantly, no significant associations were detected between the two tag SNPs and RFS or OS in any genetic model (Supplementary Table 3). These consistent findings across all genetic models suggest that while metastasis and recurrence status are robust prognostic indicators, the genetic polymorphisms rs17083838 and rs7992670 are unlikely to affect patient survival or the risk of disease recurrence directly.

Stratification analysis by tumor stage in all patients with BC

Further stratified analysis using the Kaplan–Meier codominant genetic model revealed a significant association between the rs17083838 polymorphism and postoperative recurrence risk in stage IV patients ($P = 0.007$). Patients with the AG genotype exhibited the highest risk of recurrence, with a rapidly increasing trend observed during the early follow-up period. Patients with the GG genotype showed a moderate risk of recurrence, with a cumulative recurrence rate of approximately 60% at 60 months. In contrast, no recurrence was observed throughout the follow-up period in patients with AA genotype (Fig. 2A). These findings suggested that the AG heterozygous genotype of rs17083838 may be an important genetic marker for predicting postoperative recurrence in patients with stage IV disease. Similar results were observed in the over-dominant genetic model ($P = 0.002$; Fig. 2B).

Additionally, the study found that rs7992670 polymorphism was significantly associated with OS in patients with stage III disease (Fig. 3). Specifically, under the recessive model analysis ($P = 0.023$), patients with the GG/AG genotypes had significantly better prognoses than those with the AA genotype. The 5-year survival rate of the GG/AG genotype carriers was approximately 65%, whereas it decreased significantly to approximately 35% for the AA genotype carriers (Fig. 3A). In the dominant model analysis, although statistical significance was not reached ($P = 0.050$), the data suggested a trend toward better survival for GG genotype carriers compared with the AA/AG genotype carriers. The 5-year survival rate of patients with the GG genotype was approximately 80%, whereas that of patients with the AA/AG genotype was approximately 40% (Fig. 3B). In the codominant model ($P = 0.040$), patients with the GG genotype exhibited the best survival outcomes, with a 5-year survival rate of approximately 80%. In contrast, patients with the AA genotype had the worst prognosis, with a 5-year survival rate of approximately 35%. In contrast, those with the AG genotype showed intermediate survival outcomes, with a 5-year survival rate of approximately 45% (Fig. 3C). These findings suggest that the GG genotype at the rs7992670 locus may be a protective factor associated with favorable prognosis. In contrast, the AA genotype may represent a risk factor for poor prognosis.

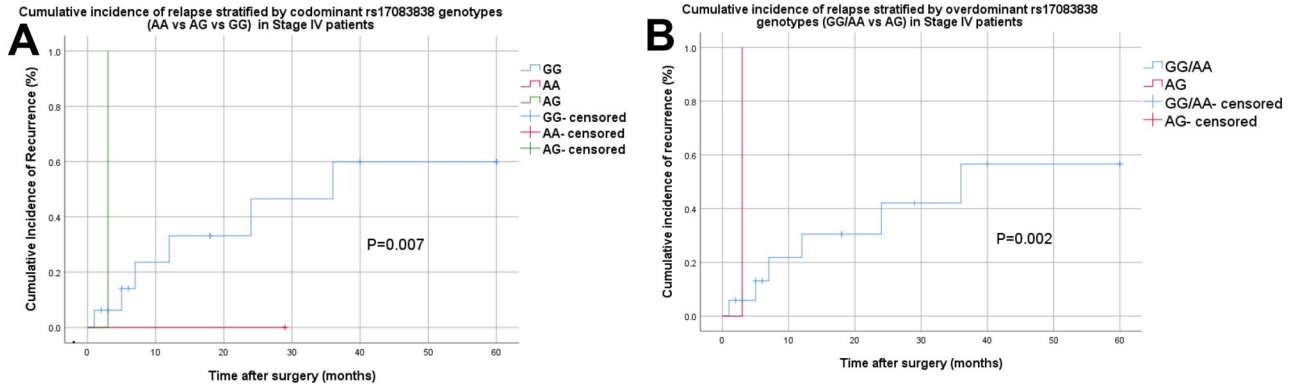

Fig. 2 The association between rs17083838 polymorphism and postoperative recurrence risk in stage IV patients. **A**, codominant model; **B**, over-dominant model

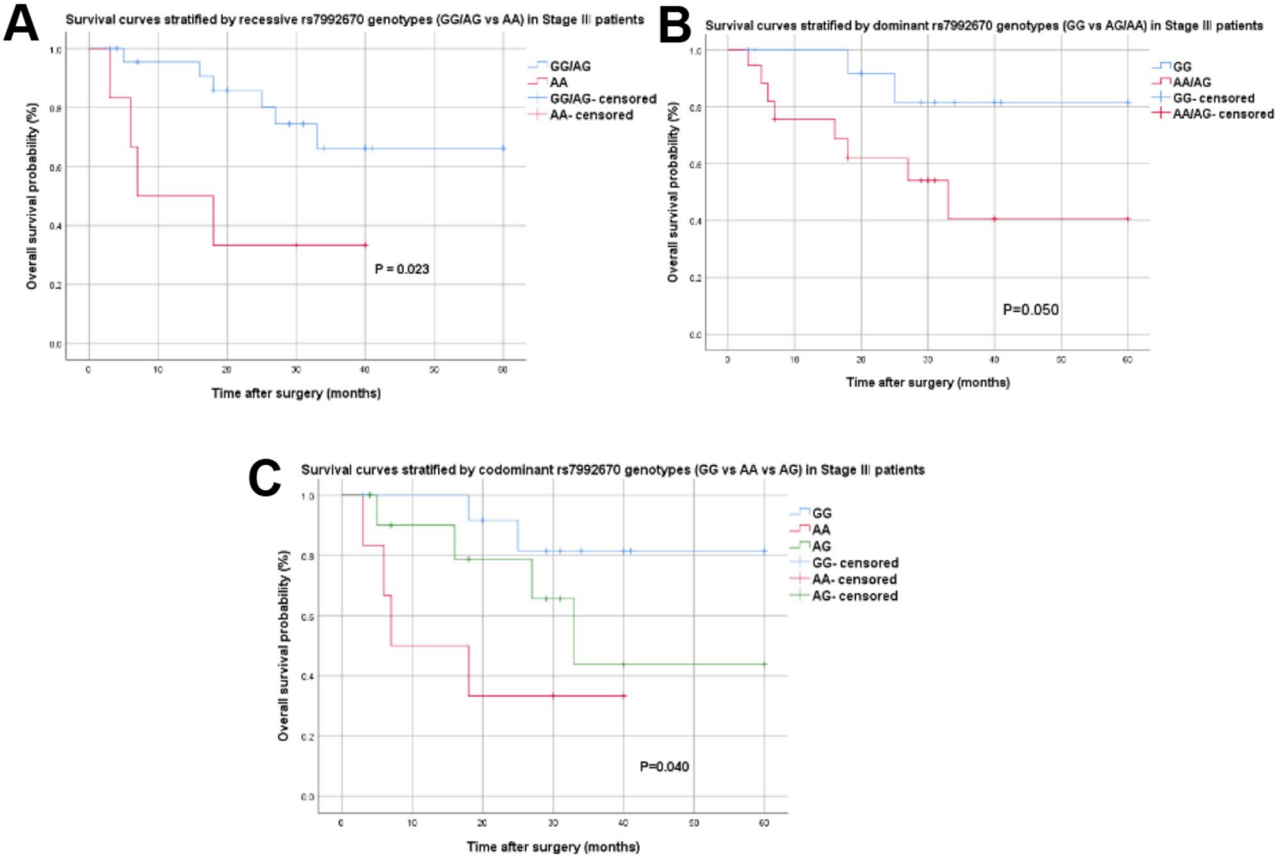

Fig. 3 The association between rs7992670 polymorphism and overall survival in stage III patients. **A**, recessive model; **B**, dominant model; **C**, codominant model

Subgroup analyses and prognostic factors in patients with MIBC and NMIBC

According to the 2024 AUA/EAU guidelines [31] and the TNM staging system [32], patients were categorized into NMIBC (Ta-T1) and MIBC (T2-T4) based on pathological staging. Stratified analyses were performed for NMIBC and MIBC groups. No significant associations were observed between SNPs and either OS or RFS in these subgroups (Supplementary Tables 4 and 5).

NMIBC The rs17083838 polymorphism showed significant prognostic relevance in female patients with NMIBC. Significant associations were observed in the codominant (GG vs. AA vs. AG; $P = 0.011$), dominant (GG vs. AA/AG; $P = 0.025$), and over-dominant models (GG/AA vs. AG; $P = 0.003$) (Supplementary Fig. 1A–C). Survival curve trends indicated that patients with the GG genotype generally exhibited the best survival outcomes, whereas those with the AG genotype had poorer prognoses. This suggests that the rs17083838 polymorphism may be an important prognostic marker in female patients. In non-smoking patients, the over-dominant model (GG/AA vs. AG) analysis of rs17083838 revealed significant differences between genotypes ($P = 0.044$), with GG/AA genotype carriers showing significantly better OS compared with AG genotype carriers (Supplementary Fig. 1D).

Survival analysis of patients with NMIBC also revealed significant prognostic correlations with the rs7992670 polymorphism in different populations. Among patients with low-grade tumors, the over-dominant model (AA/GG vs. AG) survival curves showed statistically significant differences ($P = 0.036$), with patients with the AA/GG genotype exhibiting significantly better OS than those with the AG genotype (Supplementary Fig. 2A). Additionally, in older patients aged > 63 years, the dominant model (GG vs. AA/AG) analysis of rs7992670 demonstrated statistical significance ($P = 0.049$), with GG genotype carriers exhibiting a better survival advantage (Supplementary Fig. 2B). In the smoking group (Supplementary Fig. 2C), cumulative recurrence incidence analysis under the recessive model (GG/AA vs. AG) showed significant differences ($P = 0.046$), with GG/AG genotype carriers exhibiting a higher recurrence risk than those with the AA genotype. Conversely, in the non-smoking group, the recessive model also showed significant differences ($P = 0.046$) but with an opposite trend, where GG/AG genotype carriers exhibited a lower recurrence risk (Supplementary Fig. 2D). These findings suggest that

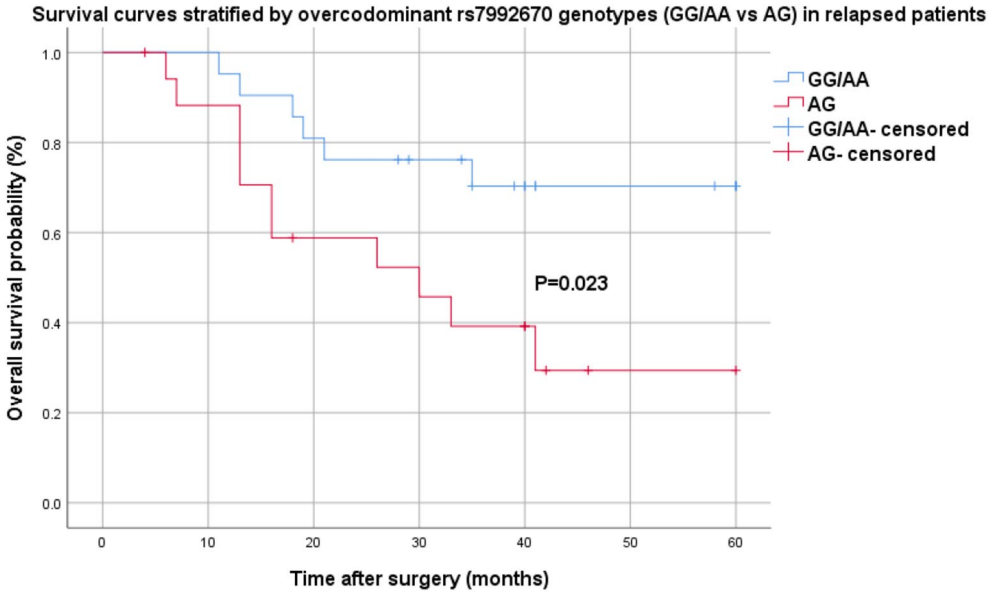

Fig. 4 Association of rs7992670 polymorphism with overall survival in relapsed MIBC patients

these two genetic polymorphisms may serve as important prognostic markers for specific populations, including females, non-smokers, smokers, patients with low-grade tumors, and older patients.

MIBC In patients with recurrent MIBC, dominant model analysis (GG/AA vs. AG) revealed that GG/AA genotype carriers had significantly higher overall survival rates than those with AG genotype carriers ($P = 0.023$). Over a 60-month follow-up period, the survival rate for patients with the GG/AA genotype remained around 70%, dropping to approximately 30% for patients with the AG genotype (Fig. 4). These findings suggest that the GG/AA genotype may be a favorable prognostic marker for OS in patients with recurrent MIBC.

Discussion

Multiple large-scale genome-wide association studies (GWAS) have explored the genetic susceptibility to BC [33–35], with subsequent research analyzing the mechanisms of specific SNPs from various perspectives. These include the Hippo signaling pathway, where *WWC1* rs755813 shows protective effects in Chinese populations [36]; carcinogen metabolism processes, where *AKR1C2* rs7087341 influences tobacco carcinogen metabolism [37]; epigenetic regulation, where *SOD2* rs5746136 affects BC risk through m6A modification [38]; and SNP–SNP interactions showing significant differences across ethnic groups [39]. Kourie et al.'s review [40] identified 343 SNPs in 197 genes associated with BC, including several cell cycle-related genes. Although *CDK* family members, such as *CDK1*, *CDK2*, *CDK4*, and *CDK6*, have been studied, *CDK8*, an important member of this family in BC genetic susceptibility, has not been sufficiently explored. Multiple studies have revealed that *CDK8* is associated with various cancers, with divergent roles across tumor types [18–23]. Among various cancer types, BC demonstrates a considerable *CDK8* amplification frequency of 4.11% in clinical specimens [27]. However, the relationship between *CDK8* polymorphisms and BC susceptibility has not yet been reported. We collected peripheral blood samples from 271 patients with BC and 381 healthy controls. The genotypes of the two tag SNPs in *CDK8* were determined using PCR-RFLP. Based on linkage disequilibrium (LD) analysis of the HapMap CHB population, four major tag SNPs exist within the *CDK8* gene region: rs17083838, rs543474, rs17538850, and rs7992670. We prioritized the two tagged SNPs located at opposite ends of the gene region because the LD analysis indicated that these SNPs represented different LD blocks with minimal correlations. This selection strategy aimed to capture more diverse genetic information through the most distantly located tag SNPs rather than closely linked variants.

Our study identified a significant association between *CDK8* polymorphisms and the risk of BC. For rs17083838, we found that the AG/AA genotype was associated with decreased BC risk (OR = 0.50), contradicting the findings of Min et al. [26]. Their research on Southwest Chinese Han women with cervical cancer reported that the A allele of rs17083838 was significantly more frequent in patients with cervical cancer compared to that in controls (25% vs. 12%, $P < 0.0001$). This suggests that rs17083838 may play a tissue-specific role in different cancer types. Regarding rs7992670, our results showed that females and smokers carrying the AG/AA

genotype had a 2.07-fold and 2.13-fold increased risk of BC, respectively ($P = 0.034$ and $P = 0.0098$). Interestingly, Min et al.'s study [26] revealed similar trends, finding that the A allele of rs7992670 was more prevalent in the cervical cancer group compared to that in controls (52% vs. 45%, $P = 0.026$). This consistency across different cancer types suggests that rs7992670 may play a conserved role in cancer susceptibility, particularly in specific subgroups. The contrasting effects of rs17083838 and similar trends for rs7992670 across these two studies highlight the complex nature of *CDK8* polymorphisms in cancer development. These findings suggest that *CDK8* genetic variations may contribute to cancer risk through diverse mechanisms influenced by tissue type and patient characteristics.

The JAK/STAT pathway is implicated in cell proliferation, cell cycle progression, and apoptosis in BC [41]. As a critical regulator of this pathway, CDK8 specifically phosphorylates STAT1 at S727, which selectively modulates over 40% of the interferon-responsive genes. This phosphorylation event can positively or negatively regulate downstream gene expression, potentially influencing BC cell proliferation, survival, and immune evasion by altering cytokine responses and inflammatory signaling [42]. Building on this understanding, Steinparzer et al. further elucidated the specificity of this mechanism, demonstrating that CDK8, but not its paralog CDK19, phosphorylates the STAT1 transcription factor during IFN-γ stimulation. Importantly, CDK8 inhibition directly blocked the activation of JAK-STAT pathway transcription factors. This molecular mechanism exists in BC, where CDK8 regulates the pathway that influences interferon signaling and tumor immune surveillance, thereby contributing to disease progression [43]. However, a direct association between *CDK8* and BC has not been fully elucidated. In this study, we focused on tag SNPs that can be regarded as haplotype representatives of specific regions. Our findings revealed that rs17083838 in *CDK8* was associated with a decreased risk of BC, consistent with previous observations that modulation of the JAK/STAT pathway activity affects BC cell proliferation [41].

Furthermore, according to data from The Cancer Genome Atlas, molecular alterations in the RTK/RAS/PI3K/AKT/mTOR pathway are common in BC. Multiple independent studies have demonstrated the frequent overactivation of the PI3K signaling pathway in muscle-invasive or metastatic BC. The high frequency of PI3K signaling deregulation in BC, combined with the availability of small-molecule inhibitors, makes it a promising therapeutic target. Notably, activation of the PI3K/AKT/mTOR pathway in BC is associated with PIK3CA mutations (21–25%) and loss of PTEN expression (39–94%) [44]. Although direct evidence in BC is limited, compelling studies in breast cancer have demonstrated significant functional interactions between CDK8/19 and the PI3K/AKT/mTOR pathway. In HER2-positive breast cancer, the synergistic effects of CDK8/19 inhibitors and HER2-targeting drugs are partly mediated through the PI3K/AKT/mTOR pathway [45]. Given the critical role of the PI3K/AKT/mTOR pathway in BC progression and the established regulatory functions of CDK8 in transcriptional reprogramming, investigating the relationship between CDK8 expression and the activation of the PI3K/AKT/mTOR pathway in BC is an important research direction. Studies have demonstrated that the mTOR signaling pathway is significantly dysregulated in CDK8-deficient cells [19], a correlation that is particularly important in BC, as the mTOR pathway regulates critical processes such as cell growth, survival, and metastasis. As a transcriptional regulator, CDK8 likely influences BC progression by modulating the expression of mTOR pathway components. CDK8 regulates the activity of STAT transcription factors, particularly by enhancing transcriptional activity through STAT1S727 phosphorylation [19], while the STAT signaling pathway plays a crucial role in BC cell proliferation and invasion. Large cohort studies have revealed a significant positive correlation between CDK8 and mTOR pathway members in patients with acute myeloid leukemia and acute lymphoblastic leukemia, with CDK8-deficient cells showing deregulation of the mTOR signaling pathway and increased sensitivity to mTOR inhibitors [19], suggesting that similar regulatory relationships may exist in BC. Moreover, considering CDK8 has been shown to exert kinase-independent functions in leukemia [19], future studies should explore whether similar mechanisms exist in BC. This could provide new insights into developing CDK8-targeted therapeutic strategies for BC. These findings suggest that CDK8 genetic variations and expression levels may influence BC susceptibility, progression, and treatment response by regulating multiple critical signaling pathways, particularly the PI3K/AKT/mTOR and JAK/STAT pathways. The potential synergistic effects of CDK8/19 and PI3K/AKT/mTOR pathway inhibitors, as demonstrated in breast cancer models, warrant investigation as a promising therapeutic strategy for BC.

Although the initial analysis revealed no significant differences between the two tag SNPs and patient outcomes in the entire cohort, subsequent stratified analyses revealed several important associations. For rs17083838, the AG genotype was associated with a higher recurrence risk in stage IV patients ($P = 0.007$) while showing a significant prognostic value in NMIBC females ($P = 0.011$) and non-smokers ($P = 0.044$). In patients with recurrent MIBC, GG/AA genotypes demonstrated higher survival (approximately 70% at 60 months) than AG (approximately 30%, $P = 0.023$). For rs7992670, stage III patients

with the GG genotype showed better survival (≈ approximately 80% at 5 years) than those with AA genotype (approximately 35%, $P = 0.040$). In patients with NMIBC, AA/GG genotypes were associated with better survival in low-grade tumors ($P = 0.036$), and the GG genotype was associated with better prognosis in older patients aged > 63 years ($P = 0.049$). In contrast, opposite recurrence trends were observed between smokers and non-smokers. These findings highlight the importance of personalized risk assessments based on genetic variations in specific patient populations. In multivariate Cox regression analysis, metastasis status remained an independent factor influencing both OS (HR = 35.728, 95% CI: 14.5–88.1, $P < 0.001$) and RFS (HR = 3.5, 95% CI: 2.0–6.3, $P < 0.001$). Together, these results underscore the critical role of metastasis in determining patient outcomes and suggest that genetic variations may serve as additional prognostic markers in specific patient subgroups.

Smoking is one of the main risk factors for BC development [3, 10, 11, 46], accounting for slightly less than 50% of cases [47]. An analysis of 10 population-based Japanese cohorts revealed a strong association between smoking and BC risk, with a risk reduction observed after long-term smoking cessation [48]. In our study, patients who smoked developed BC at a younger average age. The percentage of patients with MIBC, high tumor grade, recurrence, metastasis, and late clinical stage was higher among smokers than that in non-smokers (Supplementary Table 6). However, no significant association was observed between smoking status and BC progression. China consumes more cigarettes than any other country globally [49]. The prevalence of smoking is 30%, with 57% of men and 3% of women representing approximately one-third of the world's smoking population [50]. However, the incidence rate of BC in China (7.3 per 100,000 in men) is below the global average (9.6 per 100,000 in men) [51] which suggests that genetic background also contributes to BC development. Our study found significant associations between rs7992670 and sex as well as smoking status subgroups. Notably, we observed a complex gene-environment interaction pattern, and in the overall population, the AG/AA genotypes were associated with a higher BC risk in females and smokers; however, carriers of the A allele demonstrated significantly reduced disease risk among female smokers. The A allele-carrying subgroup of rs7992670 had a higher frequency of patients who were smokers (70.2% vs. 60%, $P = 0.0098$, OR = 2.13, 95% CI: 1.19–3.70), suggesting a greater impact of smoking on these individuals. Additionally, our study revealed the AG/AA genotype of rs7992670 had a significantly higher percentage in female patients than that in male patients (70.9% vs. 63.9%, $P = 0.034$, OR = 2.07, 95% CI: 1.04–4.66). This is particularly noteworthy, as BC is generally more common in men than that in women, with global incidence and mortality rates in men being approximately four times those in women [46, 51, 52]. Sex hormones may play a crucial role in this process, with estrogen levels in females potentially interacting with the function of rs7992670, thereby altering its impact on the risk of BC. Previous studies have shown that estrogen receptors can regulate the expression of various cancer-related genes [53–55], and the region containing rs7992670 may include estrogen response elements or influence related signaling pathways. Additionally, smoking-related carcinogens may have different metabolic pathways across the sexes, which could explain the sex-specific effects we observed. Aromatic amines produced by smoking require activation through specific enzyme systems to exert their carcinogenic effects [56, 57], and the activity of these enzyme systems may be co-regulated by genetic variations and sex hormone levels. Notably, our study included a limited sample of female patients with BC (only 55), with only four female smokers, which may have affected the stability of our results. Therefore, these findings should be validated in larger cohorts. Future studies should expand the sample size, particularly the proportion of female smokers, and combine functional studies to reveal the molecular mechanisms underlying rs7992670's role in BC development and its interaction with sex and smoking factors. Furthermore, our findings emphasize the importance of stratified analysis in genetic association studies, especially when exploring the risk factors for complex diseases such as BC. The consideration of sex and environmental factors (such as smoking) may reveal important associations that are masked in the overall population analyses. This has potential clinical significance for developing personalized prevention and treatment strategies, particularly for patients of different sexes and lifestyles.

Several prognostic scoring models and risk stratification systems exist for NMIBC [31, 58–61]. In these models, clinicopathological features serve as primary assessment indicators, whereas demographic characteristics are less frequently employed. Currently, only the CUETO scoring model incorporates age and sex as prognostic factors, identifying poorer prognoses in female patients with T1 G3 tumors [58]. Based on the SEER-Medicare cohort findings, older age was significantly associated with poorer oncological outcomes among patients with NMIBC [62]. Russell et al. [63] found that younger patients with BC (< 50 years) demonstrated significantly better survival outcomes than older patients (50–70 years), particularly in NMIBC (HR = 0.43, 95% CI: 0.28–0.64), highlighting the importance of age stratification in prognostic assessment of BC. Radkiewicz et al. [64] demonstrated that female patients with BC, particularly those with muscle-invasive tumors, have a significantly worse prognosis than males (adjusted HR 1.24,

95% CI: 1.14–1.34), with survival disadvantages mainly occurring within 2 years after diagnosis, emphasizing the necessity of sex-stratified analysis in BC management. Although smoking is the most significant risk factor for BC, it has not been included as an assessment parameter in the existing prognostic scoring models. The COBLAnCE study emphasized the importance of establishing a large-scale prospective cohort to investigate gene-environment interactions in BC [65].

Our study attempted to reveal significant interactions between *CDK8* genetic polymorphisms and these traditionally considered limited-value predictive factors, providing new insights into improving existing prognostic assessment systems. This analysis revealed significant associations between *CDK8* polymorphisms and clinical factors. The rs17083838 polymorphism showed prognostic significance in female patients with NMIBC under multiple genetic models (codominant $P = 0.011$, dominant $P = 0.025$, and over-dominant $P = 0.003$), with the GG genotype showing the best survival outcome. These findings not only support the molecular rationale for including sex as a prognostic factor in the CUETO model but also provide potential molecular mechanisms explaining the poorer prognosis in female patients. The age-related analysis demonstrated rs7992670's prognostic value in older patients with NMIBC aged > 63 years ($P = 0.049$), with carriers of the GG genotype showing better survival rates. These results suggest that age may influence disease prognosis by regulating gene expression, providing new theoretical support for incorporating age into prognostic assessments. Regarding smoking status, rs17083838 was significantly associated with non-smoking ($P = 0.044$), whereas rs7992670 displayed opposite recurrence trends between smokers and non-smokers ($P = 0.046$). These interactions revealed, at the molecular level, how smoking modulates disease progression through gene expression.

These findings have significant clinical implications, supporting the integration of genetic polymorphism testing with traditional clinical factors to construct a comprehensive prognostic assessment system. This integration may not only improve predictive accuracy, particularly for specific populations (such as females, older individuals, and smokers), but also provide new evidence for developing individualized treatment strategies. From a clinical perspective, these SNPs may serve as valuable biomarkers for BC risk assessment and treatment planning. For instance, rs17083838 genotyping may help identify individuals with lower BC susceptibility who may benefit from modified screening protocols, whereas rs7992670 could be particularly useful for risk stratification in female patients and smokers. Furthermore, the stage-specific prognostic associations we observed suggest their potential utility in tailoring follow-up strategies and treatment intensity based on patients' genetic profiles. Our research indicates that certain traditionally considered limited-value predictive factors may gain new clinical significance when combined with molecular markers, thereby offering important implications for improving NMIBC prognostic assessment systems.

Conclusion

This study provides novel insights into the role of *CDK8* polymorphisms in BC. Our findings demonstrate that the AG genotype of rs17083838 confers a protective effect against BC susceptibility. In contrast, the AG/AA genotypes of rs7992670 are significantly associated with increased BC risk in specific populations, particularly in females and smokers. Stratified analyses revealed distinct prognostic implications of these polymorphisms across different patient subgroups, with rs17083838 showing significant prognostic value in stage IV patients, female patients with NMIBC, and non-smokers, whereas rs7992670 demonstrated prognostic relevance in stage III patients and older patients with NMIBC. When integrated with traditional clinical factors, these genetic variations may enhance risk stratification and prognostic assessment of patients with BC. Future investigations should explore the integration of these genetic markers into clinical prediction models and examine whether targeted interventions based on CDK8 pathway alterations can improve patient outcomes. However, our study has several limitations. First, analyzing only two tag SNPs does not comprehensively cover all genetic variations within the *CDK8* gene region. Second, the study population was geographically restricted to southwestern China. We prioritized these representative SNPs for the initial investigation due to resource constraints. Future research should include additional tag SNPs identified through LD plot, recruit larger and more diverse cohorts, and examine CDK8 expression differences between tumors and normal adjacent tissues. Despite these limitations, our findings provide valuable molecular evidence supporting the integration of genetic markers into personalized BC management and prognostic models.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14132-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements

None.

Author contributions
Z.L. and M.S. conceived and designed the study. Q.L. and M.S. performed the experiments and collected the data. Z.L. and Q.L. analyzed the data and prepared the statistical models. X.Z., and Y.S. verified the data accuracy and consistency. Z.L. wrote the main manuscript text. Q.L. and M.S. contributed to the literature review and provided critical revisions. Y.W. and B.Z. managed laboratory resources and ensured the availability of experimental materials. B.Z. and L.Z. secured funding and supervised the overall progress of the project. All authors reviewed and approved the final manuscript.

Funding
This study was supported by grants from the National Natural Science Foundation of China (Nos. 32171264 and 81974365).

Data availability
The datasets generated and analysed during the current study are available in two forms: All statistical analysis results and summary data are included in this published article and its supplementary information files. The raw genotyping and clinical data used in this study have been deposited in the Figshare repository (https://doi.org/10.6084/m9.figshare.28218404). Additional raw data are available from the corresponding author upon reasonable request.

Declarations

Ethical approval
This study was approved by the Ethics Committee of West China Second University Hospital and West China Hospital, Sichuan University (Institutional Review Board Approval Number: Medical Research Project Approval No. 017, 2012). This study adhered to the ethical principles of the Helsinki Declaration of the World Medical Association.

Consent to participate
Written informed consent was obtained from all sample donors.

Competing interests
The authors declare no competing interests.

Received: 4 January 2025 / Accepted: 10 April 2025
Published online: 16 April 2025

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63.
2. Weber A, Vignat J, Shah R, Morgan E, Laversanne M, Nagy P, Kenessey I, Znaor A. Global burden of bladder cancer mortality in 2020 and 2040 according to GLOBOCAN estimates. World J Urol. 2024;42(1):237.
3. Richters A, Aben KKH, Kiemeney L. The global burden of urinary bladder cancer: an update. World J Urol. 2020;38(8):1895–904.
4. Spiess PE, Agarwal N, Bangs R, Boorjian SA, Buyyounouski MK, Clark PE, Downs TM, Efstathiou JA, Flaig TW, Friedlander T, Greenberg RE, Guru KA, Hahn N, Herr HW, Hoimes C, Inman BA, Jimbo M, Kader AK, Lele SM, Meeks JJ, Michalski J, Montgomery JS, Pagliaro LC, Pal SK, Patterson A, Plimack ER, Pohar KS, Porter MP, Preston MA, Sexton WJ, Siefker-Radtke AO, Sonpavde G, Tward J, Wile G, Dwyer MA, Gurski LA. Bladder cancer, version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2017;15(10):1240–67.
5. Zhang W, Zhang J, Zhang Z, Guo Y, Wu Y, Wang R, Wang L, Mao S, Yao X. Overexpression of indoleamine 2,3-Dioxygenase 1 promotes Epithelial-Mesenchymal transition by activation of the IL-6/STAT3/PD-L1 pathway in bladder Cancer. Transl Oncol. 2019;12(3):485–92.
6. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BWG, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. EAU guidelines on Non-Muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol. 2017;71(3):447–61.
7. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015;15(1):25–41.
8. Huang M, Dong W, Xie R, Wu J, Su Q, Li W, Yao K, Chen Y, Zhou Q, Zhang Q, Li W, Cheng L, Peng S, Chen S, Huang J, Chen X, Lin T. HSF1 facilitates the multistep process of lymphatic metastasis in bladder cancer via a novel PRMT5-WDR5-dependent transcriptional program. Cancer Commun (Lond). 2022;42(5):447–70.
9. Jubber I, Ong S, Bukavina L, Black PC, Comperat E, Kamat AM, Kiemeney L, Lawrentschuk N, Lerner SP, Meeks JJ, Moch H, Necchi A, Panebianco V, Sridhar SS, Znaor A, Catto JWF, Cumberbatch MG. Epidemiology of bladder Cancer in 2023: A systematic review of risk factors. Eur Urol. 2023;84(2):176–90.
10. Lenis AT, Lec PM, Chamie K, Mshs MD. Bladder Cancer: Rev JAMA. 2020;324(19):1980–91.
11. Patel VG, Oh WK, Galsky MD. Treatment of muscle-invasive and advanced bladder cancer in 2020. CA Cancer J Clin. 2020;70(5):404–23.
12. Murta-Nascimento C, Silverman DT, Kogevinas M, Garcia-Closas M, Rothman N, Tardon A, Garcia-Closas R, Serra C, Carrato A, Villanueva C, Dosemeci M, Real FX, Malats N. Risk of bladder cancer associated with family history of cancer: do low-penetrance polymorphisms account for the increase in risk? Cancer Epidemiol Biomarkers Prev. 2007;16(8):1595–600.
13. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol. 2013;63(2):234–41.
14. Koutros S, Decker KL, Baris D, Pardo LA, Johnson A, Hosain GMM, Rothman N, Karagas MR, Schwenn MR, Silverman DT. Bladder cancer risk associated with family history of cancer. Int J Cancer. 2021;148(12):2915–23.
15. Andrew AS, Hu T, Gu J, Gui J, Ye Y, Marsit CJ, Kelsey KT, Schned AR, Tanyos SA, Pendleton EM, Mason RA, Morlock EV, Zens MS, Li Z, Moore JH, Wu X, Karagas MR. HSD3B and gene-gene interactions in a pathway-based analysis of genetic susceptibility to bladder cancer. PLoS ONE. 2012;7(12):e51301.
16. Wu D, Jia H, Zhang Z, Li S. Capsaicin suppresses breast cancer cell viability by regulating the CDK8/PI3K/Akt/Wnt/betacatenin signaling pathway. Mol Med Rep. 2020;22(6):4868–76.
17. Uehara T, Abe K, Oginuma M, Ishitani S, Yoshihashi H, Okamoto N, Takenouchi T, Kosaki K, Ishitani T. Pathogenesis of CDK8-associated disorder: two patients with novel CDK8 variants and in vitro and in vivo functional analyses of the variants. Sci Rep. 2020;10(1):17575.
18. Ron Firestein KS, Nosho K, Irahara N, Baba Y, Bojarski E, Giovannucci EL, Hahn WC. Fuchs, and Shuji Ogino, CDK8 expression in 470 colorectal cancers in relation to BCatenin activation, other molecular alterations and patient survival. Int J Cancer. 2010;126(12):2863–73.
19. Ingeborg Menzl TZ, Berger-Becvar A, Grausenburger R, Heller G, Prchal-Murphy M, Edlinger L, Vanessa M, Knab, Iris Z, Uras M, Roth A, Skucha Y, Liu JM, Hatcher Y, Liang NP, Kwiatkowski S. Gray & Veronika Sexl, A kinase-independent role for CDK8 in BCR-ABL1 + leukemia, Nature communication 10 (2019) 4741.
20. Bi H, Yin J, Zhou L, Wu Y, Ge L, Lu M, Liu L, Zhang H, Zhao Y, Liu C, Ma L. Clinicopathological and prognostic impact of somatic mutations in Chinese patients with clear cell renal cell carcinoma. Transl Androl Urol. 2020;9(6):2751–63.
21. Spear JM, Lu Z, Russu WA. Pharmacological Inhibition of CDK8 in Triple-Negative breast Cancer cell line MDA-MB-468 increases E2F1 protein, induces phosphorylation of STAT3 and apoptosis. Molecules 25(23) (2020).
22. Chen R, Wang X, Zhou S, Zeng Z. LncRNA HOXA-AS2 promotes tumor progression by suppressing miR-567 expression in oral squamous cell carcinoma. Cancer Manag Res. 2021;13:5443–55.
23. Elkady MA, Doghish AS, Elshafei A, Elshafey MM. MicroRNA-567 inhibits cell proliferation and induces cell apoptosis in A549 NSCLC cells by regulating cyclin-dependent kinase 8. Saudi J Biol Sci. 2021;28(4):2581–90.
24. Sun H, Yan J, Tian G, Chen X, Song W. LINC01224 accelerates malignant transformation via MiR-193a-5p/CDK8 axis in gastric cancer. Cancer Med. 2021;10(4):1377–93.
25. Gu W, Wang C, Li W, Hsu FN, Tian L, Zhou J, Yuan C, Xie XJ, Jiang T, Addya S, Tai Y, Kong B, Ji JY. Tumor-suppressive effects of CDK8 in endometrial cancer cells. Cell Cycle. 2013;12(6):987–99.
26. Min S, Li Q, Li Z, Huang H, Zheng X, Song Y, Ye Z, Tang Z, Zhou B, Li T, Wang Y. Association of CDK8 gene polymorphisms with cervical Cancer in Han women in Southwest China. Genet Test Mol Biomarkers. 2025;29(2):39–47.
27. Roninson IB, Gyorffy B, Mack ZT, Shtil AA, Shtutman MS, Chen M, Broude EV. Identifying Cancers Impacted by CDK8/19, Cells 8(8) (2019).
28. Xu Z, Taylor JA. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res 37(Web Server issue) (2009) W600–5.

29. Koressaar T, Lepamets M, Kaplinski L, Raime K, Andreson R, Remm M. Primer3_masker: integrating masking of template sequence with primer design software. Bioinformatics. 2018;34(11):1937–8.
30. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22(15):1928–9.
31. Holzbeierlein JM, Bixler BR, Buckley DI, Chang SS, Holmes R, James AC, Kirkby E, McKiernan JM. Schuckman, diagnosis and treatment of Non-Muscle invasive bladder cancer: AUA/SUO guideline: 2024 amendment. J Urol. 2024;211(4):533–8.
32. Babjuk M, Burger M, Capoun O, Cohen D, Comperat EM, Dominguez Escrig JL, Gontero P, Liedberg F, Masson-Lecomte A, Mostafid AH, Palou J, van Rhijn BWG, Roupret M, Shariat SF, Seisen T, Soukup V, Sylvester RJ. European association of urology guidelines on Non-muscle-invasive bladder Cancer (Ta, T1, and carcinoma in Situ). Eur Urol. 2022;81(1):75–94.
33. Ma Z, Hu Q, Chen Z, Tao S, Macnamara L, Kim ST, Tian L, Xu K, Ding Q, Zheng SL, Sun J, Xia G, Xu J. Systematic evaluation of bladder cancer risk-associated single-nucleotide polymorphisms in a Chinese population. Mol Carcinog. 2013;52(11):916–21.
34. Figueroa JD, Ye Y, Siddiq A, Garcia-Closas M, Chatterjee N, Prokunina-Olsson L, Cortessis VK, Kooperberg C, Cussenot O, Benhamou S, Prescott J, Porru S, Dinney CP, Malats N, Baris D, Purdue M, Jacobs EJ, Albanes D, Wang Z, Deng X, Chung CC, Tang W, Bas Bueno-de-Mesquita H, Trichopoulos D, Ljungberg B, Clavel-Chapelon F, Weiderpass E, Krogh V, Dorronsoro M, Travis R, Tjonneland A, Brenan P, Chang-Claude J, Riboli E, Conti D, Gago-Dominguez M, Stern MC, Pike MC, Van Den Berg D, Yuan JM, Hohensee C, Rodabough R, Cancel-Tassin G, Roupret M, Comperat E, Chen C, De Vivo I, Giovannucci E, Hunter DJ, Kraft P, Lindstrom S, Carta A, Pavanello S, Arici C, Mastrangelo G, Kamat AM, Lerner SP, Barton Grossman H, Lin J, Gu J, Pu X, Hutchinson A, Burdette L, Wheeler W, Kogevinas M, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Schwenn M, Karagas MR, Johnson A, Schned A, Armenti KR, Hosain GM, Andriole G Jr., Grubb R, Black A, Ryan Diver W, Gapstur SM, Weinstein SJ, Virtamo J, Haiman CA, Landi MT, Caporaso N, Fraumeni JF Jr., Vineis P, Wu X, Silverman DT. Hum Mol Genet. 2014;23(5):1387–98. S. Chanock, N. Rothman, Genome-wide association study identifies multiple loci associated with bladder cancer risk.
35. Koutros S, Kiemeney LA, Pal Choudhury P, Milne RL, Lopez de Maturana E, Ye Y, Joseph V, Florez-Vargas O, Dyrskjot L, Figueroa J, Dutta D, Giles GG, Hildebrandt MAT, Offit K, Kogevinas M, Weiderpass E, McCullough ML, Freedman ND, Albanes D, Kooperberg C, Cortessis VK, Karagas MR, Johnson A, Schwenn MR, Baris D, Furberg H, Bajorin DF, Cussenot O, Cancel-Tassin G, Benhamou S, Kraft P, Porru S, Carta A, Bishop T, Southey MC, Matullo G, Fletcher T, Kumar R, Taylor JA, Lamy P, Prip F, Kalisz M, Weinstein SJ, Hengstler JG, Selinski S, Harland M, Teo M, Kiltie AE, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Schned A, Lenz P, Riboli E, Brennan P, Tjonneland A, Otto T, Ovsiannikov D, Volkert F, Vermeulen SH, Aben KK, Galesloot TE, Turman C, De Vivo I, Giovannucci E, Hunter DJ, Hohensee C, Hunt R, Patel AV, Huang WY, Thorleifsson G, Gago-Dominguez M, Amiano P, Golka K, Stern MC, Consortium U, Yan W, Liu J, Li SA, Katta S, Hutchinson A, Hicks B, Wheeler WA, Purdue MP, McGlynn KA, Kitahara CM, Haiman CA, Greene MH, Rafnar T, Chatterjee N, Chanock SJ, Wu X, Real FX, Silverman DT, Garcia-Closas M. Eur Urol. 2023;84(1):127–37. K. Stefansson, L. Prokunina-Olsson, N. Malats, N. Rothman, Genome-wide Association Study of Bladder Cancer Reveals New Biological and Translational Insights.
36. Huang Z, Wang X, Ma L, Guo Z, Liu H, Du M, Chu H, Wang M, Wang Z, Zhang Z. Genetic variations in Hippo pathway genes influence bladder cancer risk in a Chinese population. Arch Toxicol. 2020;94(3):785–94.
37. Xiao Y, Shen Y, Song H, Gao F, Mao Z, Lv Q, Qin C, Yuan L, Wu D, Chu H, Wang M, Du M, Zheng R, Zhang Z. AKR1C2 genetic variants mediate tobacco carcinogens metabolism involving bladder cancer susceptibility. Arch Toxicol. 2024;98(7):2269–79.
38. Liu H, Gu J, Jin Y, Yuan Q, Ma G, Du M, Ge Y, Qin C, Lv Q, Fu G, Wang M, Chu H, Yuan L, Zhang Z. Genetic variants in N6-methyladenosine are associated with bladder cancer risk in the Chinese population. Arch Toxicol. 2021;95(1):299–309.
39. Yu EY, Tang QY, Chen YT, Zhang YX, Dai YN, Wu YX, Li WC, Mehrkanoon S, Wang SZ, Zeegers MP, Wesselius A. Genome-wide exploration of genetic interactions for bladder cancer risk. Int J Cancer. 2024;154(1):81–93.
40. Kourie HR, Zouein J, Succar B, Mardirossian A, Ahmadieh N, Chouery E, Mehawej C, Jalkh N, Kattan J, Nemr E. Genetic polymorphisms involved in bladder cancer: A global review. Oncol Rev. 2023;17:10603.
41. Huang W, Li Y, Zhang C, Zha H, Zhou X, Fu B, Guo J, Wang G. IGF2BP3 facilitates cell proliferation and tumorigenesis via modulation of JAK/STAT signalling pathway in human bladder cancer. J Cell Mol Med. 2020;24(23):13949–60.
42. Bancerek J, Poss ZC, Steinparzer I, Sedlyarov V, Pfaffenwimmer T, Mikulic I, Dolken L, Strobl B, Muller M, Taatjes DJ, Kovarik P. CDK8 kinase phosphorylates transcription factor STAT1 to selectively regulate the interferon response. Immunity. 2013;38(2):250–62.
43. Steinparzer I, Sedlyarov V, Rubin JD, Eislmayr K, Galbraith MD, Levandowski CB, Vcelkova T, Sneezum L, Wascher F, Amman F, Kleinova R, Bender H, Andrysik Z, Espinosa JM, Superti-Furga G, Dowell RD, Taatjes DJ, Kovarik P. Transcriptional responses to IFN-gamma require mediator Kinase-Dependent pause release and mechanistically distinct CDK8 and CDK19 functions. Mol Cell. 2019;76(3):485–e4998.
44. Anuja RN, Sathe. Targeting the PI3K/AKT/mTOR pathway in bladder Cancer. Urothelial Carcinoma (2018) 335–50.
45. Ding X, Sharko AC, McDermott MSJ, Schools GP, Chumanevich A, Ji H, Li J, Zhang L, Mack ZT, Sikirzhytski V, Shtutman M, Ivers L, O'Donovan N, Crown J, Gyorffy B, Chen M, Roninson IB, Broude EV. Inhibition of CDK8/19 mediator kinase potentiates HER2-targeting drugs and bypasses resistance to these agents in vitro and in vivo. Proc Natl Acad Sci U S A. 2022;119(32):e2201073119.
46. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder Cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96–108.
47. van Osch FH, Jochems SH, van Schooten FJ, Bryan RT, Zeegers MP. Quantified relations between exposure to tobacco smoking and bladder cancer risk: a meta-analysis of 89 observational studies. Int J Epidemiol. 2016;45(3):857–70.
48. Masaoka H, Matsuo K, Oze I, Kimura T, Tamakoshi A, Sugawara Y, Tsuji I, Sawada N, Tsugane S, Ito H, Wada K, Nagata C, Kitamura T, Zha L, Sakata R, Ozasa K, Lin Y, Mizoue T, Tanaka K, Abe SK, Inoue M. Cigarette smoking, smoking cessation, and bladder Cancer risk: A pooled analysis of 10 cohort studies in Japan. J Epidemiol. 2023;33(11):582–8.
49. Yang G, Ma J, Liu N, Zhou L. Smoking and passive smoking in Chinese. Chin J Epidemiol. 2005;26(2):77–83.
50. Lu S. Development of treatment options for Chinese patients with advanced squamous cell lung cancer: focus on Afatinib. Onco Targets Ther. 2019;12:1521–38.
51. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
52. DeGEORGE MKATHARINEC, HARRY MS, HOLT R. HODGES, bladder cancer: diagnosis and treatment. Am Family Phys. 2017;96(8):507–15.
53. Dessources K, Miller KM, Kertowidjojo E, Da Cruz Paula A, Zou Y, Selenica P, da Silva EM, Benayed R, Ashley CW, Abu-Rustum NR, Dogan S, Soslow RA, Hensley ML, Weigelt B, Chiang S. ESR1 hotspot mutations in endometrial stromal sarcoma with high-grade transformation and endocrine treatment. Mod Pathol. 2022;35(7):972–8.
54. Skrzypczak M, Wolinska E, Adaszek L, Ortmann O, Treeck O. Epigenetic modulation of Estrogen receptor signaling in ovarian Cancer. Int J Mol Sci 26(1) (2024).
55. Tabe-Bordbar S, Song YJ, Lunt BJ, Alavi Z, Prasanth KV, Sinha S. Mechanistic analysis of enhancer sequences in the Estrogen receptor transcriptional program. Commun Biol. 2024;7(1):719.
56. Chevereau M, Glatt H, Zalko D, Cravedi JP, Audebert M. Role of human sulfotransferase 1A1 and N-acetyltransferase 2 in the metabolic activation of 16 heterocyclic amines and related heterocyclics to genotoxicants in Recombinant V79 cells. Arch Toxicol. 2017;91(9):3175–84.
57. Habil MR, Salazar-Gonzalez RA, Doll MA, Hein DW. Differences in beta-naphthylamine metabolism and toxicity in Chinese hamster ovary cell lines transfected with human CYP1A2 and NAT2*4, NAT2*5B or NAT2*7B N-acetyltransferase 2 haplotypes. Arch Toxicol. 2022;96(11):2999–3012.
58. Fernandez-Gomez J, Madero R, Solsona E, Unda M, Martinez-Pineiro L, Gonzalez M, Portillo J, Ojea A, Pertusa C, Rodriguez-Molina J, Camacho JE, Rabadan M, Astobieta A, Montesinos M, Isorna S, Muntanola P, Gimeno A, Blas M. Martinez-Pineiro, predicting nonmuscle invasive bladder cancer recurrence and progression in patients treated with bacillus Calmette-Guerin: the CUETO scoring model. J Urol. 2009;182(5):2195–203.
59. Lammers RJ, Hendriks JC, Rodriguez Faba OR, Witjes WP, Palou J, Witjes JA. Prediction model for recurrence probabilities after intravesical chemotherapy in patients with intermediate-risk non-muscle-invasive bladder cancer, including external validation. World J Urol. 2016;34(2):173–80.
60. Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, Newling DW, Kurth K. Predicting recurrence and progression in individual

patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol. 2006;49(3):466–5. discussion 475-7.
61. Chappidi MR, Welty C, Choi W, Meng MV, Porten SP. Evaluation of the Cancer of bladder risk assessment (COBRA) score in the Cancer genome atlas (TCGA) bladder Cancer cohort. Urology. 2021;156:104–9.
62. Lobo N, Duan Z, Sood A, Tan WS, Grajales V, Contieri R, Lindskrog SV, Dyrskjot L, Zhao H, Giordano SH, Williams SB, Bree KK, Kamat AM. Association of age with Non-muscle-invasive bladder cancer: unearthing a biological basis for epidemiological disparities?? Eur Urol Oncol. 2024;7(5):1069–79.
63. Russell B, Liedberg F, Hagberg O, Ullén A, Söderkvist K, Ströck V, Aljabery F, Gårdmark T, Jerlström T, Sherif A, Holmberg L, Bryan RT, Enting D, Hemelrijck MV. Risk of bladder cancer death in patients younger than 50 with non-muscle-invasive and muscle-invasive bladder cancer. Scand J Urol. 2022;56(1):27–33.
64. Radkiewicz C, Edgren G, Johansson ALV, Jahnson S, Haggstrom C, Akre O, Lambe M, Dickman PW. Sex differences in urothelial bladder Cancer survival. Clin Genitourin Cancer. 2020;18(1):26–e346.
65. Lebret T, Bonastre J, Fraslin A, Neuzillet Y, Droupy S, Rebillard X, Vordos D, Guy L, Villers A, Schneider M, Coloby P, Lacoste J, Mejean A, Lacoste J, Descotes JL, Eschwege P, Loison G, Blanche H, Mariani O, Ghaleh B, Mangin A, Sirab N, Groussard K, Radvanyi F, Allory Y, Benhamou S. Cohort profile: coblance: a French prospective cohort to study prognostic and predictive factors in bladder cancer and to generate real-world data on treatment patterns, resource use and quality of life. BMJ Open. 2023;13(12):e075942.

Publisher's note