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RESEARCH ARTICLE

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Associations between inflammasome-related gene NLRP3 Polymorphisms (rs10754558 and rs35829419) and risk of bladder cancer in a Chinese population

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Abstract

Background: NLRP3 inflammasome as a component of immune system has been found related to several cancers, but no study has assessed NLRP3 polymorphisms on risk of bladder cancer (BC). We aim to investigate whether NLRP3 polymorphisms are associated with the risk and clinical features of bladder cancer (BC) in a Chinese population.

Methods: Genotype frequency of two commonly studied NLRP3 SNPs (rs10754558 and rs35829419) was examined in 154 patients with BC and the 308 healthy controls. NLRP3 gene polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism method.

Results: The distribution frequencies of GG, AG+GG, GG, and G allele in NLRP3 (rs10754558) genotypes were significantly different between case and control group (OR = 2.296, P = .022; OR = 1.598, P = .020; OR = 1.998, P = .049; OR = 1.557, P = .006), but no statistical difference existed for rs35829419. Among smokers and alcohol drinkers, for rs10754558, individuals with AG, GG, and GG+AG genotypes had a higher BC risk compared with individuals with AA; for rs35829419, individuals with variant genotypes (AG and GG+AG) had a stronger risk of developing BC compared with individuals with AA (all P < .05). In stratified analyses of tumor size and tumor node metastasis, AG or GG genotypes of rs10754558 and rs35829419 SNPs were associated with BC risk (both P < .05).

Conclusion: NLRP3 polymorphisms (rs10754558 and rs35829419) were related to BC risk and tumor size and lymph node metastasis, especially among smokers and alcohol drinkers.

KEYWORDS
bladder cancer, Chinese population, NLRP3, polymorphism

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1 | INTRODUCTION

Bladder cancer (BC) is the second most frequent diagnosed urinary cancer, ranked ninth among all cancers by the International Agency for Research on Cancer in 2012.[1] Morbidity and mortality rates in developing regions are up to three times higher than in developed countries, and men are four times more likely to develop bladder cancer than women.[1] In 2012, a total of 165,000 deaths and 429,000 new cases were recorded globally, of which 26,820 deaths and 55,486 new cases were recorded in China, with an incidence rate of 3/100,000, and East Asia (37,491 deaths and 85,451 new cases) accounted for a large proportion.[1] Emerging evidence suggests that bladder cancer is a complex, multi-step, and multifactorial disease due to the interaction of lifestyle, environmental, and genetic factors.[2] GWAS studies have identified gene variants that contribute to bladder cancer risk,[3] and many gene variants have been demonstrated to be correlated with risk.[4-6]

It has been reported that inflammation plays a critical role in carcinogenesis and tumorigenesis, such as the initiation and promotion of cancer cells,[7,8] as well as tumor progression,[9] angiogenesis,[10] and invasion.[11] Inflammasome is a complex of proteins in cells that can be formed in response to various stimuli.[12] However, the exact role of inflammasome in heterogeneous tumorigenesis remains unclear. The Nod-like receptor protein 3 (NLRP3) is one of the most typical inflammasome components, such as pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs). Studies have shown that the dysfunction of NLRP3 inflammasome pathway is related to various inflammation-induced diseases,[13] and the genetic variation of NLRP3 inflammasome pathway gene is associated with the development of malignant tumors, such as chronic myeloid leukemia[14] and melanoma.[15] AIM2-like receptors (ALRs), leucine-rich repeat sequences containing receptors (NLR), or nucleotide-binding domains do not have inflammasome complexes. NLRP3 inflammasomes consist of NLRP3, the connector apoptosis-related speckle-like protein (ASC), and caspase-1, which are activated by intracellular process or by foreign pathogens in vivo.[16]

The NLRP3 gene is located at 1q44, with a length of ~30 kbp, including 9 exons and 8 introns).[17] NLRP3 is a member of NLR family and participates in increasing inflammatory cytokine production.[17] Genetic alterations in NLRP3 gene could alter its activity. About 60 single nucleotide polymorphisms (SNPs) have been reported within the NLRP3 gene. Two NLRP3 polymorphisms have been extensively studied: rs10754558 and rs35829419, which are located in the 3'-UTR of the NLRP3 gene and may affect the stability and expression of NLRP3 mRNA.[18] These two SNPs have been demonstrated that could be related to different multifactorial diseases such as coronary artery disease,[19] as well as cancers such as Philadelphia chromosome-negative myeloproliferative neoplasms and lung cancer,[20,21] but the role of these genetic variants in the development of BC remains unclear.

In this study, NLRP3 SNPs rs10754558 and rs35829419 were detected and analyzed in Chinese population, aiming to explore its influence on the development and progression of BC, which may provide new insights for the prevention strategies and therapeutic target for bladder cancer.

2 | MATERIALS AND METHODS

2.1 | Recruitment of patients

The study was a case-control study conducted from March 23, 2016 to February 1, 2020. 154 patients with histologically diagnosed bladder cancer were recruited from Department of Urological Surgery, First Affiliated Hospital of Gannan Medical University, Ganzhou, China. Patients with other malignancies, chronic diseases, and prior radiation or chemotherapy were excluded from the study. All BC cases were staged according to the 2002 International Union Against Cancer TNM Staging System and graded using the World Health Organization classification: highly differentiated or poorly differentiated.

A total of 308 healthy controls with no history of cancer, who received regular health checks from the same hospital, were not genetically related individuals and were matched for sex and age (±3 years). All individuals were surveyed using a structured questionnaire, including gender, age, smoking, drinking status, and other exposure history, with the informed consent of each participant.

The study was approved by the Ethics Committee of First Affiliated Hospital of Gannan Medical University and was conducted in accordance with the Helsinki Declaration of 1964.

2.2 | Genotyping

Approximately 2.0 ml of venous blood was extracted from each person and stored at −80℃. Genomic DNA was extracted from blood samples using the QIAAMP DNA Blood Mini Kit (Qiagen, Hilden, Germany), as recommended by the manufacturer's protocol. The A/G polymorphism of NLRP3 gene was genotyped by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

The sequences of primers have been reported previously[22]: (1) rs10754558: 5′- GACAATGACAGCATCGGGTGTTGTT-3′ (forward) and 5′- TCATCACAGCGCCTCAGTTAGAGGA −3′ (reverse); (2) rs35829419: 5′- GGAAGGCCGACACCTTGATATGGTG −3′ (forward) and 5′- AGTGTGTCCTCCCAAGCTCCTCTCA −3′ (reverse). The specific primers were designed manually and synthesized by GeneScript Biotechnology (Nanjing, China). PCR conditions were as follows: Initial denaturation was carried out at 95℃ for 5 min followed by 35 cycles of 95℃ for 30 s, 35 cycles at 0℃ for 30 s, and 72℃ for 30 s and finished with a final extension cycle at 72℃ for 10 min. The PCR products were digested with EcoRI and separated in a 3% agarose gel electrophoresis and ethidium bromide staining. The sizes of the different genotypes were 261 bp for AA, 261 bp, 236 bp, and 25 bp for AG, and 236 bp, and 25 bp for GG of rs10754558; 429 bp for AA,

429 bp, 258 bp, and 171 bp for AG, and 258 bp, and 171 for GG of rs35829419, respectively.

2.3 | Statistical analysis

SPSS 20.0 software was used to analyze the data. Clinical features were tested using chi-square tests and t tests depending on variables types. SNPStats online analysis software provides genotype associations, including dominant, dominant, recessive, and dominant inheritance models, to calculate allele frequencies of NLRP3 (rs10754558, rs35829419) in case-control studies. SNPs in both bladder cancer cases and healthy controls were tested for Hardy-Weinberg equilibrium (HWE). The odds ratio (OR) and the corresponding 95% confidence interval (95% CI) were calculated by unconditional logistic regression to assess the role of different genotypes and alleles. A P value <.05 was regarded as statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

The baseline characteristics of the study population have been shown in Table 1. No remarkable differences were found between the two groups in age (P = .818) and gender (P = .677). However, statistical differences were found in the distribution of smoking or alcohol consumption between the two groups (P < .001). As for the grade of bladder cancer, 97 cases (62.99%) were highly differentiated and 227 cases (37.01%) were poorly differentiated. In terms of tumor size, 113 cases (73.38%) with a size of <3 cm; 41 cases (26.62%) were >3 cm in size. TNM stages I, II, III, and IV were 34 (22.08%), 43 (27.92%), 46 (29.87%), and 31 (20.13%), respectively. There were 142 patients (92.86%) with lymph node metastasis, and 11 patients (7.14%) without lymph node metastasis. HWE test showed that genotypes frequencies of both the polymorphisms both in cases and healthy controls were according with HWE (all P > .05).

TABLE 1 Clinical characteristics of bladder cancer cases and controls included in the data analysis

Variables	Case (n = 154)	Control (n = 308)	t/X^2	P
Age, years	63.87 ± 11.24	64.12 ± 10.87	0.230	.818
Sex, n (%)				
Male	126 (81.82)	247 (80.19)	0.174	.677
Female	28 (18.18)	61 (19.81)		
Smokers, n (%)				
Yes	108 (70.13)	121 (39.29)	39.072	<.001
No	46 (29.87)	187 (60.71)		
Alcohol drinkers, n (%)				
Yes	101 (65.58)	110 (35.71)	36.917	<.001
No	53 (34.42)	198 (64.29)		
Tumor grade, n (%)[a]				
High	97 (62.99)	-		
Low	57 (37.01)	-		
Tumor Size (cm), n (%)				
<3	113 (73.38)	-		
≥3	41 (26.62)	-		
TNM Stage, n (%)				
I	34 (22.08)	-		
II	43 (27.92)	-		
III	46 (29.87)	-		
IV	31 (20.13)	-		
Lymph node metastasis, n (%)				
Yes	143 (92.86)	-		
No	11 (7.14)	-		

[a]Classified by WHO classification: Highly differentiated tumors are defined as showing only mild degrees of cytological atypia and infrequent mitotic figures; poorly differentiated tumors are defined as showing marked nuclear pleomorphism, loss of maturation from the base to the surface, and mitotic activity.

3.2 | Association of NLRP3 gene A/G polymorphism with the risk of bladder cancer

First, we examined the distribution of single nucleotide polymorphisms in NLRP3 in the case group and the control group (Table 2). For rs10754558, the distribution frequencies of GG, AG + GG, GG, and G allele were statistically different between the case and control groups, and their ORs and 95% CIs were 2.296 (1.125–4.685, P = .022), 1.598 (1.076–2.375, P = .020), 1.998 (1.004–3.975, P = .049), and 1.557 (1.138–2.131, P = .006), respectively. But A allele showed no statistically significant differences in distribution between the case and the control group (P > .05). For rs35829419, no statistical differences were found in the distribution of various genotypes between the case and the control group (all P > .05).

Stratified analysis was conducted by smoking and drinking status (Table 3). When stratified by smoking and alcohol consumption, the risk of BC was significantly increased for rs10754558: smoker AG or/and GG genotypes (AG vs. AA, OR = 1.829, 95%CI 1.042–3.211, P = .035; GG vs. AA, OR = 11.82, 95%CI 1.424–98.180, P = .022; GG+AG vs. AA, OR = 2.115, 95%CI 1.224–3.654, P = .007) and alcohol drinkers (AG vs. AA, OR = 2.724, 95%CI 1.426–5.201, P = .002; GG vs. AA, OR = 4.284, 95%CI 1.314–13.970, P = .016; GG+AG vs. AA, OR = 3.019, 95%CI 1.657–5.500, P < .001). For rs35829419, smokers with AG or/and GG genotypes had a significantly increased risk of BC (AG vs. AA, OR = 1.784, 95%CI 1.031– 3.089, P = .039; GG+AG vs. AA, OR = 1.815, 95%CI 1.055–3.123, P = .031) and non-alcohol drinkers (GG vs. AA, OR = 10.278, 95%CI 4.035–26.178, P < .01; GG+AG vs. AA, OR = 2.570, 95%CI 1.381–4.784, P = .003).

TABLE 2 Distribution of the NLRP3 polymorphisms and association analysis in patients with bladder cancer and healthy controls

SNP	Genotype	Case (n = 154)	Control (n = 308)	OR (95%CI)	P
rs10754558	AA	81 (52.60)	197 (63.96)	1.000	
	AG	56 (36.36)	93 (30.19)	1.464 (0.960, 2.231)	.076
	GG	17 (11.04)	18 (5.84)	2.296 (1.125, 4.685)	.022
	AG+GG	73 (47.40)	111 (29.21)	1.598 (1.076, 2.375)	.020
	AA+AG	137 (88.96)	290 (94.15)	1.000	
	GG	17 (11.04)	18 (5.84)	1.998 (1.004, 3.975)	.049
	A allele	218 (70.78)	487 (79.06)	1.000	
	G allele	90 (29.22)	129 (20.94)	1.557 (1.138, 2.131)	.006
rs35829419	AA	88 (57.14)	197 (63.96)	1.000	
	AG	51 (33.12)	90 (29.22)	1.254 (0.819, 1.918)	.298
	GG	15 (9.74)	21 (6.82)	1.679 (0.821, 3.433)	.156
	AG+GG	66 (42.86)	111 (36.04)	1.328 (0.887, 1.989)	.168
	AA+AG	139 (90.26)	287 (93.18)	1.000	
	GG	15 (9.74)	21 (6.82)	1.553 (0.771, 3.126)	.218
	A allele	227 (73.70)	484 (78.57)	1.000	
	G allele	81 (26.30)	132 (21.43)	1.320 (0.959, 1.818)	.088

TABLE 3 Subgroup analysis of distribution of the NLRP3 polymorphisms and association analysis in patients with bladder cancer and healthy controls by smoking and alcohol drinking status

			Case/Control			AG vs. AA	
SNP	Factors		AA	AG	GG	OR (95%CI)	P
rs10754558	Smokers[a]	Yes	58/86	42/34	8/1	1.829 (1.042, 3.211)	.035
		No	30/111	9/56	7/20	0.595 (0.264, 1.341)	.210
	Alcohol drinkers[b]	Yes	60/87	36/19	12/4	2.724 (1.426, 5.201)	.002
		No	28/110	15/71	3/17	0.810 (0.403, 1.627)	.553
rs35829419	Smokers[a]	Yes	60/84	46/36	2/1	1.784 (1.031, 3.089)	.039
		No	21/113	10/57	15/17	0.990 (0.483, 2.029)	.978
	Alcohol drinkers[b]	Yes	62/50	38/33	8/12	1.207 (0.673, 2.164)	.528
		No	19/147	18/60	9/6	1.429 (0.692, 2.951)	.335

[a]Smokers refer to daily smokers or smoking at least 1 time per day for at least 6 months.

[b]Alcohol drinkers refer to those reporting ≥1, standard drink per week over the past 30 days.

3.3 | Association of NLRP3 gene A/G polymorphism with the characteristics of bladder cancer

We also investigated whether NLRP3 A/G polymorphisms influence the clinical characteristics of patients with bladder cancer (Table 4). The results showed that for rs10754558, GG or AG + GG genotype polymorphisms were related to BC risk in groups with tumor size ≥3 cm (GG vs. AA, 0.236, 95%CI 0.079–0.704, P = .010; AG + GG vs. AA, OR = 0.480, 95%CI 0.234–0.985, P = .046), with null association observed for AG (P > .05). This association was also shown in analysis of tumor node metastasis (GG vs. AA, OR = 4.284, 95%CI 1.455–12.616, P = .008), with null interaction observed for tumor grade and TNM Stage (P > .05). For rs35829419, GG or AG + GG genotype polymorphism was associated with bladder cancer risk (GG vs. AA, 0.225, 95%CI 0.072–0.704, P = .010; AG + GG vs. AA, OR = 0.439, 95%CI 0.213–0.905, P = .026). This association was also shown in analysis of tumor node metastasis (GG vs. AA, OR = 3.999, 95%CI 1.286–12.439, P = .017), with null interaction found for tumor grade and TNM Stage (P > .05). This result suggests that NLRP3 A/G polymorphism is related to tumor size and tumor node metastasis in patients with bladder cancer. No statistical association was found when stratified by tumor histology and TNM stage (both P > .05).

4 | DISCUSSION

In this study, we found that NLRP3 (rs10754558 and rs35829419) A/G polymorphisms were related to a higher risk of BC. In subgroup analysis, these associations were only significant among smokers and drinkers. In addition, both of these two SNPs were related to tumor size and lymph node metastasis in patients with bladder cancer.

Bladder cancer is a complex disease and genetic factor involving interactions between various environments.[23] Studies have suggested that immune cells play a critical role in bladder cancer development.[24] NLRP3 inflammasome works in innate immune response and affects many diseases, involving metabolic, cardiovascular, renal, and neurodegenerative diseases.[25] NLR can be activated by cellular stress and tissue injury. NLRP3 inflammasome can be activated by ROS, when thioredoxin-interacting protein (TXNIP) dissociates from thioredoxin and binds to NLRP3. Lack of TXNIP weakens NLRP3 inflammasome activation and subsequent IL-1β secretion, which could be correlated to the development of diabetes.[26] However, the association between NLRP3 and bladder cancer requires further study. NLRP3 inflammasomes play a role in the immune response, but also in susceptibility to inflammation-induced abnormalities.[27]

NLRP3 gene is located on the long arm of chromosome 1q44 and contains 9 exons in its 32.9 KB sequence.[17] Studies have shown the relationship between NLRP3 SNP and susceptibility to inflammation-induced abnormalities.[28] About 60 SNPs of NLRP3 gene have been reported, but few have tested the relationship between NLRP3 genotype and cancer.[29] NLRP3 (rs35829419) genotype is correlated to poorer outcomes in patients with colorectal cancer.[30] Nevertheless, this genotype is neither related to myeloid leukemia 14 nor pancreatic cancer.[31] In this study, rs10754558 G and rs35829419 G NLRP3 alleles tended to be related to the risk of BC.

Inflammation works in the occurrence and development of cancer. Over-activation of Nuclear factor-kappa B and STAT signaling, these inflammatory pathways, leading to abnormal increase of inflammatory cytokines and over-response of immune cells, thereby promoting the progression of cancer.[32] Accumulated evidence indicates that NLRP3 is closely associated with susceptibility, development, and outcome of cancer.[33] NLRP3 derived from cancer-associated fibroblasts accelerates the progression of breast carcinoma.[34] NLRP3 complex activation results in the production of IL-1β and IL-18, and the generation of pyrolytic cell death. Inhibition of NLRP3 by inhibiting S100A9 or inhibitors of NLRP3 can effectively attenuate the development of cancer.[35] In addition, NLRP3 is indispensable for adaptive immunity to tumors. Deficiency of functional NLRP3 leads to failure of CD8+T cell initiation, thereby inhibiting the antitumor

GG vs. AA		GG vs. AA+AG		GG+AG vs. AA	
OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
11.82 (1.424, 98.18)	.022	0.103 (0.013, 0.837)	.033	2.115 (1.224, 3.654)	.007
1.292 (0.497, 3.355)	.599	1.496 (0.590, 3.796)	.396	0.776 (0.394, 1.528)	.463
4.284 (1.314, 13.97)	.016	3.304 (1.019, 10.71)	.046	3.019 (1.657, 5.500)	<.001
0.664 (0.181, 2.429)	.536	2.638 (1.114, 6.249)	.027	1.632 (0.884, 3.015)	.117
2.795 (0.247, 31.58)	.406	2.259 (0.202, 25.27)	.508	1.815 (1.055, 3.123)	.031
0.326 (0.108, 0.982)	.046	0.325 (0.110, 0.964)	.043	0.980 (0.504, 1.950)	.953
0.698 (0.267, 1.825)	.464	0.651 (0.254, 1.667)	.370	1.001 (0.580, 1.726)	.997
10.28 (4.035, 26.178)	<.001	9.070 (3.725, 22.08)	<.001	2.570 (1.381, 4.784)	.003

TABLE 4 Subgroup analysis of distribution of the NLRP3 polymorphisms and association analysis in patients with bladder cancer and healthy controls by tumor clinical characteristics

	Characteristics	AA	AG	GG	AG+GG
rs10754558	Tumor Grade (high/low)	55/26	34/22	8/9	42/31
	OR (95%CI) P	1.000	0.731 (0.359, 1.488) 0.387	0.420 (0.145, 1.214) 0.109	0.640 (0.331, 1.237) 0.184
	Tumor Size (cm) (<3/≥3)	64/17	39/17	8/9	47/26
	OR (95%CI) P	1.000	0.609 (0.279, 1.331) 0.214	0.236 (0.079, 0.704) 0.010	0.480 (0.234, 0.985) 0.046
	TNM Stage (I+II/III+IV)	46/42	22/34	7/10	29/44
	OR (95%CI) P	1.000	0.590 (0.298, 1.168) 0.130	0.639 (0.222, 1.838) 0.406	0.602 (0.321, 1.129) 0.114
	Lymph node metastasis (Y/N)	22/66	18/38	10/7	28/45
	OR (95%CI) P	1.000	1.420 (0.677, 2.980) 0.353	4.284 (1.455, 12.616) 0.008	1.866 (0.949, 3.670) 0.070
rs35829419	Tumor Grade (high/low)	59/29	31/20	8/7	39/27
	OR (95%CI) P	1.000	0.762 (0.372, 1.561) 0.457	0.562 (0.185, 1.703) 0.308	0.710 (0.365, 1.370) 0.312
	Tumor Size (cm) (<3/≥3)	70/18	35/16	7/8	41/24
	OR (95%CI) P	1.000	0.563 (0.256, 1.237) 0.563	0.225 (0.072, 0.704) 0.010	0.439 (0.213, 0.905) 0.026
	TNM Stage (I+II/III+IV)	47/41	22/29	7/8	29/37
	OR (95%CI) P	1.000	0.662 (0.330, 1.327) 0.245	0.763 (0.254, 2.286) 0.628	0.683 (0.358, 1.302) 0.247
	Lymph node metastasis (Y/N)	24/64	17/34	9/6	26/40
	OR (95%CI) P	1.000	1.332 (0.630, 2.817) 0.452	3.999 (1.286, 12.439) 0.017	1.733 (0.876, 3.428) 0.114

effect of chemotherapy in the mouse model.[36] Although the effect of inflammasomes on the progression of BC is unclear, the effect of inflammasomes in the development of cancer has been investigated in a series of other cancer studies using colitis-induced colon cancer as an animal model. One study found that inflammasome components prevented colitis-associated colon cancer.[37] Other studies have suggested that inflammation associated with cancer may promote tumor growth and metastasis. For example, one study reported that NLRP3 promotes inflammation to induce skin cancer and is essential for asbestos-induced mesothelioma.[38] Other studies have shown that the activation of NLRP3 inflammasome promotes the metastasis of breast cancer to liver and lung tissue,[39] and NLRP3 inflammasome can activate the secretion of IL-18 and IL-1β in lung adenocarcinoma A549 cell line, and IL-18 and IL-1β are the main components of the inflammatory response.[40] These studies suggest that NLRP3 inflammasomes are involved in carcinogenesis in various organs.

On one hand, smoking is a significant risk factor for BC. In 11 case-control analyses, men's risk of BC increased with the consumptions of cigarette.[41] A linear increased risk of up to 15–20 cigarettes per day was reported. After treatment was discontinued, the risk of bladder cancer dropped sharply by about 30 percent after 1 to 4 years. But even after 25 years, operating rooms still have not reached the number of nonsmokers. These results were next validated in a meta-analysis involving 43 comprehensive studies.[42] Subsequent to adjusting for age and sex, the risk for all smokers was significantly increased compared with nonsmokers, which revealed a confirmed association of consumption and exposure package year.[43] On the other hand, until now, the relationship between BC and alcohol has been ambiguous. Early studies were unable to establish a relation between BC risk and alcohol consumption.[44] This is in contrast to a recent study that suggested that high alcohol consumption, especially the frequent consumption of high-strength liquor, could cause BC. The average OR of male drinkers was 2.1 and that of female drinkers was 3.4. These results suggest that regular alcohol consumption is independently correlated with an increased risk of BC.[45] In the contrast, other studies have shown that alcohol consumption is related to a relatively lower BC risk.[46,47] Systematic meta-analyses of 30 epidemiological trials were unable to establish an association between alcohol consumption and bladder cancer.[43] In the subgroup analysis, we observed important findings in the population of smokers and drinkers, suggesting that exposure to smoking and alcohol may be more likely to develop bladder cancer.

In addition, as previously reported, NLRP3 was associated with the tumor metastasis.[48,49] We also found that the A/G polymorphism of NLRP3 gene (rs10754558, rs35829419) is associated with tumor size and tumor node metastasis in patients with BC, suggesting that the SNP may interact with tumor size and tumor node metastasis. NLRP3 may be associated with tumor size, invasion, lymph node metastasis, and TNM staging by activating the epithelial-mesenchymal transition process through inflammation response.[50]

We should acknowledge the potential limitations of this study. First, selection bias cannot be addressed in such case-control studies. Second, only two loci in NLRP3 gene were explored, and other genetic variations should be further studied. Third, the potential mechanism of A/G polymorphism in NLRP3 gene (rs10754558, rs35829419) and increased risk of BC should also be investigated. Fourth, further cross-analysis is needed to verify the combined impact of environmental and genetic factors on bladder cancer susceptibility.

In conclusion, our study firstly indicated that NLRP3 (rs10754558 and rs35829419) A/G polymorphism was associated with an increased risk of BC in the Chinese population in this study. Larger studies with larger sample sizes from other ethnic groups are needed to confirm these findings.

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interests exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87-108.
2. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat Rev Cancer*. 2015;15(1):25-41.
3. Dudek AM, Grotenhuis AJ, Vermeulen SH, et al. Urinary bladder cancer susceptibility markers. What do we know about functional mechanisms? *Int J Mol Sci*. 2013;14(6):12346-12366.
4. Zeng FF, Liu SY, Wei W, et al. Genetic polymorphisms of glutathione S-transferase T1 and bladder cancer risk: a meta-analysis. *Clin Exp Med*. 2010;10(1):59-68.
5. Wang M, Zhu H, Fu G, et al. Polymorphisms of methylenetetrahydrofolate reductase and methionine synthase genes and bladder cancer risk: a case-control study with meta-analysis. *Clin Exp Med*. 2009;9(1):9-19.
6. Grando JPS, Kuasne H, Losi-Guembarovski R, et al. Association between polymorphisms in the biometabolism genes CYP1A1, GSTM1, GSTT1 and GSTP1 in bladder cancer. *Clin Exp Med*. 2009;9(1):21-28.
7. Le Magnen C, Virk RK, Dutta A, et al. Cooperation of loss of and inflammation in prostate cancer initiation. *Dis Model Mech*. 2018;11(11):35139.
8. Engels EA. Inflammation in the development of lung cancer: epidemiological evidence. *Expert Rev Anticancer Ther*. 2008;8(4):605-615.
9. Bremnes RM, Al-Shibli K, Donnem T, et al. The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer. *J Thorac Oncol*. 2011;6(4):824-833.
10. De la Garza MM, Cumpian AM, et al. COPD-Type lung inflammation promotes K-ras mutant lung cancer through epithelial HIF-1α mediated tumor angiogenesis and proliferation. *Oncotarget*. 2018;9(68):32972-32983.

11. Zhang L, Chen C, Duanmu J, et al. Cryptotanshinone inhibits the growth and invasion of colon cancer by suppressing inflammation and tumor angiogenesis through modulating MMP/TIMP system, PI3K/Akt/mTOR signaling and HIF-1α nuclear translocation. *Int Immunopharmacol*. 2018;65:429-437.
12. Masumoto J. The inflammasomes. *Nihon Rinsho Meneki Gakkai Kaishi*. 2011;34(5):346-354.
13. Song Z, Lin Z, He F, et al. NLRP3 is expressed in human dental pulp cells and tissues. *J Endod*. 2012;38(12):1592-1597.
14. Zhang A, Yu J, Yan S, et al. The genetic polymorphism and expression profiles of NLRP3 inflammasome in patients with chronic myeloid leukemia. *Hum Immunol*. 2018;79(1):57-62.
15. Zhao X, Hua M, Yan S, et al. The genetic polymorphisms of NLRP3 inflammasome associated with T helper cells in patients with multiple myeloma. *J Immunol Res*. 2018;2018:7569809.
16. Martinon F, Burns K. Tschopp J The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*. 2002;10(2):417-426.
17. Zaki MH, Lamkanfi M, Kanneganti TD. The Nlrp3 inflammasome: contributions to intestinal homeostasis. *Trends Immunol*. 2011;32(4):171-179.
18. Zhang AQ, Zeng L, Gu W, et al. Clinical relevance of single nucleotide polymorphisms within the entire NLRP3 gene in patients with major blunt trauma. *Crit Care*. 2011;15(6):R280.
19. Zhou D, Wang X, Chen T, et al. The NLRP3 rs10754558 polymorphism is associated with the occurrence and prognosis of coronary artery disease in the chinese han population. *Biomed Res Int*. 2016;2016:3185397.
20. Zhou Y, Yan S, Liu N, et al. Genetic polymorphisms and expression of NLRP3 inflammasome-related genes are associated with Philadelphia chromosome-negative myeloproliferative neoplasms. *Hum Immunol*. 2020;81(10–11):606-613.
21. Tang D, Liu H, Zhao Y, et al. Genetic variants of and in the NLRP3 inflammasome pathway are associated with non-small cell lung cancer survival. *Am J Cancer Res*. 2020;10(8):2582-2595.
22. Imani D, Azimi A, Salehi Z, et al. Association of nod-like receptor protein-3 single nucleotide gene polymorphisms and expression with the susceptibility to relapsing-remitting multiple sclerosis. *Int J Immunogenet*. 2018;45(6):329-336.
23. Tran L, Xiao JF, Agarwal N, Duex JE. Theodorescu D Advances in bladder cancer biology and therapy. *Nat Rev Cancer*. 2021;21(2):104-121.
24. Wu J, Abraham SN. The Roles of T cells in Bladder Pathologies. *Trends Immunol*. 2021;42(3):248-260.
25. Swanson KV, Deng M, Ting JPY. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol*. 2019;19(8):477-489.
26. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat Immunol*. 2010;11(2):136-140.
27. Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol*. 2006;7(12):1250-1257.
28. Paramel GV, Sirsjö A. Fransén K Role of genetic alterations in the NLRP3 and CARD8 genes in health and disease. *Mediators Inflamm*. 2015;2015:846782.
29. International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426(6968):789-796.
30. Ungerbäck J, Belenki D, Jawad ul-Hassan A, et al. Genetic variation and alterations of genes involved in NFκB/TNFAIP3- and NLRP3-inflammasome signaling affect susceptibility and outcome of colorectal cancer. *Carcinogenesis*. 2012;33(11):2126-2134.
31. Miskiewicz A, Szparecki G, Durlik M, et al. The Q705K and F359L single-nucleotide polymorphisms of NOD-like receptor signaling pathway: association with chronic pancreatitis, pancreatic cancer, and periodontitis. *Arch Immunol Ther Exp (Warsz)*. 2015;63(6):485-494.
32. Mantovani A, Garlanda C, Allavena P. Molecular pathways and targets in cancer-related inflammation. *Ann Med*. 2010;42(3):161-170.
33. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature*. 2012;481(7381):278-286.
34. Ershaid N, Sharon Y, Doron H, et al. NLRP3 inflammasome in fibroblasts links tissue damage with inflammation in breast cancer progression and metastasis. *Nat Commun*. 2019;10(1):4375.
35. Basiorka AA, McGraw KL, Eksioglu EA, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. *Blood*. 2016;128(25):2960-2975.
36. Ghiringhelli F, Apetoh L, Tesniere A, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nat Med*. 2009;15(10):1170-1178.
37. Chapkin RS, Kamen BA, Callaway ES, et al. Use of a novel genetic mouse model to investigate the role of folate in colitis-associated colon cancer. *J Nutr Biochem*. 2009;20(8):649-655.
38. Chow MT, Tschopp J, Möller A, Smyth MJ. NLRP3 promotes inflammation-induced skin cancer but is dispensable for asbestos-induced mesothelioma. *Immunol Cell Biol*. 2012;90(10):983-986.
39. Hu Q, Zhao F, Guo F, Wang C, Fu Z. Polymeric nanoparticles induce NLRP3 inflammasome activation and promote breast cancer metastasis. *Macromol Biosci*. 2017;17(12).
40. Schmidt RL, Lenz LL. Distinct licensing of IL-18 and IL-1β secretion in response to NLRP3 inflammasome activation. *PLoS One*. 2012;7(9):e45186.
41. Brennan P, Bogillot O, Cordier S, et al. Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies. *Int J Cancer*. 2000;86(2):289-294.
42. Zeegers MPA, Goldbohm RA, van den Brandt PA. A prospective study on active and environmental tobacco smoking and bladder cancer risk (The Netherlands). *Cancer Causes Control*. 2002;13(1):83-90.
43. Zeegers MPA, Kellen E, Buntinx F, van den Brandt PA. The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review. *World J Urol*. 2004;21(6):392-401.
44. Saginala K, Barsouk A, Aluru JS, et al. Epidemiology of bladder cancer. *Med Sci (Basel)*. 2020;8(1):15.
45. Donato F, Boffetta P, Fazioli R, et al. Bladder cancer, tobacco smoking, coffee and alcohol drinking in Brescia, northern Italy. *Eur J Epidemiol*. 1997;13(7):795-800.
46. Geoffroy-Perez B, Cordier S. Fluid consumption and the risk of bladder cancer: results of a multicenter case-control study. *Int J Cancer*. 2001;93(6):880-887.
47. Pelucchi C, Negri E, Franceschi S, et al. Alcohol drinking and bladder cancer. *J Clin Epidemiol*. 2002;55(7):637-641.
48. Cong J, Gong J, Yang C, Xia Z, Zhang H. miR-22 suppresses tumor invasion and metastasis in colorectal cancer by targeting NLRP3. *Cancer Manag Res*. 2020;12:5419-5429.
49. Yin H, Liu YG, Li F, et al. Resibufogenin suppresses growth and metastasis through inducing caspase-1-dependent pyroptosis via ROS-mediated NF-κB suppression in non-small cell lung cancer. *Anat Rec (Hoboken)*. 2021;304(2):302-312.
50. Shao X, Lei Z, Zhou C. NLRP3 promotes colorectal cancer cell proliferation and metastasis via regulating epithelial mesenchymal transformation. *Anticancer Agents Med Chem*. 2020;20(7):820-827.

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