

# Contribution of MRE11, RAD50, and NBS1 Genotypes to Bladder Cancer Susceptibility

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## Abstract


**Background/Aim:** Genome instability is a hallmark of cancer, often accelerated by defects in DNA damage responses. MRE11-RAD50-NBS1 (MRN) complex plays a crucial role in sensing and repairing DNA damage; however, there is limited literature on the involvement of MRN genotypes in bladder cancer (BLCA) susceptibility. This study aimed to elucidate the impact of MRN genotypes on the risk of BLCA.

**Materials and Methods:** We genotyped 14 single nucleotide polymorphisms (SNPs) in MRN genes, including rs684507, rs2155209, rs10831234, rs13447720, rs601341 in MRE11, rs17166050, rs17772583, rs6871536, rs3798134, rs2244012 in RAD50, and rs1805794, rs2735383, rs1063053, rs1063054 in NBS1, among 375 BLCA cases and 375 controls, and evaluated their contributions to BLCA susceptibility.

**Results:** Among these SNPs, only NBS1 rs2735383 was significantly associated with BLCA risk ( $p$  for trend=0.0053), with both CG and CC genotypes conferring higher risks (OR=1.48 and 1.95, respectively). Subgroup analysis showed that NBS1 rs2735383 was associated with BLCA risk in individuals older than 55 years (OR=2.29,  $p$ =0.0053), smokers (OR=2.56,  $p$ =0.0026), alcohol drinkers (OR=3.25,  $p$ =0.0005), and those with muscle-invasive disease (OR=1.97,  $p$ =0.0243), but not in younger individuals, non-smokers, non-drinkers, or non-muscle-invasive cases.

**Conclusion:** NBS1 rs2735383 genotype may serve as a genetic biomarker for BLCA susceptibility, particularly in high-risk subpopulations, including elders, smokers, drinkers, and those with muscle-invasive disease.

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## Introduction

Bladder cancer (BLCA) ranks as the fourth most common malignancy in males and the eleventh in females worldwide (1). In Taiwan, BLCA incidence is the eleventh highest among men and the sixteenth among women, with a continuous upward trend (2). The male predominance in BLCA, with an approximate male-to-female ratio of 5:2, has been primarily attributed to smoking (3). The pathogenesis of BLCA is likely driven by intricate interactions between genetic predisposition and environmental exposures. Among various risk factors, tobacco use remains the most significant contributor. Additional influences include alcohol drinking, exposure to industrial chemicals and fine particulate matter (PM 2.5), prior radiation therapy, abuse of Chinese medicine, and recurrent urinary tract infections (4-7). Furthermore, dietary patterns, particularly meat consumption, have been implicated in BLCA risk. The association may be influenced by factors such as meat type, cooking technique, and thermal processing conditions (8). Although several hereditary components in BLCA susceptibility have been identified (9-11), the precise genetic determinants and molecular mechanisms remain largely undefined.

The MRE11/RAD50/NBS1 (MRN) complex consists of two MRE11 subunits, two RAD50 units, and two NBS1 subunits (12, 13). It plays a critical role in cellular defense against genomic insults by initiating signaling pathways required for the repair of double-strand breaks (DSBs). It facilitates this process by mediating the resection of damaged DNA and participating in both homologous recombination (HR) and non-homologous end-joining (NHEJ) repair mechanisms (14, 15). Additionally, the MRN complex regulates cell cycle progression by triggering the G1/S checkpoint and promoting Chk2 phosphorylation (16). Proper telomere maintenance is essential for genome stability, and deficiencies in MRE11 or NBS1 lead to telomere attrition and dysfunction (17, 18). For a long

time, DSB repair mechanisms have been recognized as more error prone in BLCA (19, 20). Overall, the MRN complex serves as a key regulator of DNA damage sensing, repair signaling, and telomere homeostasis, highlighting its fundamental role in maintaining genomic integrity.

The *MRE11* gene, located on chromosome 11, encodes the MRE11 protein, which possesses intrinsic exonuclease activity and binds to DNA, playing a crucial role in DNA metabolism (21, 22). Mutations in *MRE11* have been implicated in the pathogenesis of ataxia telangiectasia-like disorder, highlighting its significance in neurological function (23, 24). Functionally, MRE11 is involved in multiple cellular processes, including meiotic recombination, checkpoint activation during cell cycle progression, and the repair of DSBs through HR and NHEJ pathways. Collectively, MRE11 plays a fundamental role in preserving genomic stability in eukaryotic cells (25, 26). Notably, MRE11 functions as both an exo- and endonuclease and has emerged as a potential therapeutic target in oncology (27, 28). Low MRE11 expression in BLCA tumors was associated with worse cancer-specific survival compared with high expression among cases receiving radiotherapy (29). Furthermore, genetic variants of *MRE11* have been associated with an increased susceptibility to various malignancies, such as glioblastoma (30), and breast cancer (31-34). As for BLCA, Choudhury and his colleagues found a marginal increase in the risk of BLCA for GG genotypes of *MRE11* 3' untranslated region (3'UTR) rs2155209 (35). Another group found that *MRE11* rs1805363 was associated with worse cancer-specific survival following radiotherapy among 256 muscle-invasive BLCA patients (36).

The *RAD50* gene is located on chromosome 5q31.1 (37). Within the MRN complex, RAD50 plays a dual role in tethering DNA ends and modulating MRE11's enzymatic functions (38). This protein is essential for preserving genomic stability and acts as a barrier against tumorigenesis (39). Functionally, RAD50 is a pivotal

component of the DNA DSB repair machinery and is frequently down-regulated in basal-like breast carcinomas, contributing to worse survival outcomes (40, 41). Moreover, it has been identified as a breast cancer susceptibility gene linked to genomic instability (42). Altered RAD50 expression has been reported in various malignancies, including acute myeloid leukemia (43), endometrial carcinoma (44), and Burkitt lymphoma (45). In mouse models, a homozygous mutation in the Zn-hook domain of RAD50 results in embryonic lethality, whereas heterozygous mutations predispose to hepatic tumorigenesis, emphasizing the domain's critical role in cancer development (46). Despite its recognized significance, the impact of *RAD50* genetic variants on cancer susceptibility has been scarcely reported. Several SNPs located in *RAD50* intronic regions – rs3798134, rs3798135, rs2040704, and rs2706347 – were found to be significantly associated with an increased risk of breast cancer (47). Regarding BLCA, it has been reported that a high expression of RAD50 may be correlated with a shorter overall survival for patients with muscle-invasive BLCA (48). Choudhury and his colleagues examined the contribution of *RAD50* rs1047382 to BLCA but did not find a significant association (35). In 2022, Pietzak and his colleagues reported that high-grade non-muscle-invasive BLCA harbored pathogenic and likely pathogenic variants in DNA damage response genes, including *RAD50* (1270\_1271delCT and 326\_329delCAGA) (49). However, the contribution of *RAD50* genotypes to BLCA remains largely unclear.

The *NBS1* gene, also known as *nibrin* or *NBN*, is located on human chromosome 8q21 (50, 51). In *NBS1* heterozygous (+/-) mice, both tumor incidence and sensitivity to ionizing radiation are markedly elevated compared to wild-type counterparts, underscoring the gene's essential function in DSB repair and tumorigenesis (52). Within the MRN complex, NBS1 is a key regulator of ATM activation, primarily through its direct interaction with ATM (53, 54). Among the genetic variants of *NBS1*, rs1805794 is the most extensively studied SNP and has been investigated for its association with multiple

malignancies, including nasopharyngeal carcinoma (55), lung cancer (56, 57), breast cancer (58), colorectal cancer (59), prostate cancer (60), and leukemia (61). Regarding BLCA, several common *NBS1* variants, including rs1805794 and two SNPs in the 3'UTR (rs2735383, rs1063054), have been investigated; however, the results are inconsistent (35, 62-67).

The primary aim of this study was to assess the impact of 14 SNPs in the MRN complex genes – *MRE11* (rs684507, rs2155209, rs10831234, rs13447720, rs601341), *RAD50* (rs17166050, rs17772583, rs6871536, rs3798134, rs2244012), and *NBS1* (rs1805794, rs2735383, rs1063053, rs1063054) – on BLCA susceptibility. Furthermore, we aimed to explore the potential role of specific *MRN* genotypes in predicting BLCA tumor grade and stage. Lastly, we conducted a comprehensive literature review on the associations between *MRN* SNPs and BLCA risk.

## Materials and Methods

*Recruitment of BLCA patients and non-cancer controls.* This hospital-based case-control study, which included both BLCA patients and cancer-free controls, was conducted with approval from the Institutional Review Board of China Medical University Hospital (CMUH111-REC1-176). All participants provided written informed consent. Clinical and pathological data were rigorously reviewed in accordance with the ethical principles outlined in the Declaration of Helsinki. A total of 375 BLCA cases were enrolled following histopathological confirmation. Each patient completed a structured questionnaire and donated 3 to 5 ml of peripheral blood. The control group consisted of an equal number (n=375) of healthy individuals, selected from the hospital's Health Examination Cohort, originally comprising 15,000 subjects. Controls were matched to cases based on age, sex, smoking status, and alcohol drinking status. Individuals with a prior history of malignancy, metastatic cancer from another site, tumors of unknown origin, or any hereditary or genetic disorders were excluded from the control group. As part of the study

Table I. Basic characteristics of the 375 bladder cancer patients and 375 non-cancer controls.

Character	Controls (n=375)			Cases (n=375)			p-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			62.9 (9.8)			61.4 (10.3)	0.7315 <sup>a</sup>
Age group (years)							0.7108 <sup>b</sup>
≤55	152	40.5%		158	42.1%		
>55	223	59.5%		217	57.9%		
Sex							0.5525 <sup>b</sup>
Male	287	76.5%		279	74.4%		
Female	88	23.5%		96	25.6%		
Personal habits							
Cigarette smoking	186	49.6%		201	53.6%		0.3063 <sup>b</sup>
Alcohol drinking	176	46.9%		189	50.4%		0.3807 <sup>b</sup>
Stage							
Non-muscle-invasive				235	62.7%		
Muscle-invasive				140	37.3%		
Grade							
Low				151	40.3%		
High				224	59.7%		

SD: Standard deviation; <sup>a</sup>Based on Student's *t*-test; <sup>b</sup>Based on Chi-square test.

design, all participants provided information on personal characteristics, lifestyle factors, and environmental exposures, with particular emphasis on smoking and alcohol consumption habits. "Ever smokers" were defined as individuals who smoked daily or nearly every day, having accumulated at least five pack-years over a minimum duration of one year. "Ever alcohol drinkers" were classified as those who had experienced intoxication at least twice or consumed more than three drinks per week for a minimum of one year. Intoxication was characterized by an impaired ability to walk in a straight line. A summary of the key demographic characteristics of the study population is presented in Table I.

**MRN genotyping methods and experimental conditions.** Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) and stored in aliquots for subsequent analysis, following previously established protocols (68-70). A comprehensive summary of the investigated SNPs, along with the corresponding forward and reverse primers, restriction enzymes, and PCR fragment sizes after enzymatic digestion or direct sequencing, is presented in

Table II. All primers used in this study were designed by the Terry Fox Cancer Research Laboratory. The specific SNP sites are illustrated in Figure 1.

**Statistical analysis.** To confirm that the control group is representative of the general population, we assessed Hardy-Weinberg equilibrium (HWE) using a goodness-of-fit test to identify any deviations in genotype frequencies of MRN SNPs. Comparisons between case and control groups, including variables such as age within subgroups, were conducted using an unpaired Student's *t*-test. Pearson's Chi-square test with Yates' correction was applied to examine genotype distribution differences across subgroups. Any *p*-value of less than 0.05 was considered statistically significant. Logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs), evaluating the association between MRN genotypes and BLCA susceptibility.

## Results

**Demographic characteristics of BLCA cases and controls.** The demographic details, including age, sex, personal habits, and

Table II. Summary of the polymorphic sites, paired primer sequences, restriction enzymes, and expected DNA fragments after digestion.

Genes	Polymorphic sites	Primer sequences (5'→3')	Restriction enzymes	Genetic variants	DNA fragments, bp
<i>MRE11</i>	rs684507	Forward: GCACAAGGTACAGACGTCTT Reverse: AGGATTGCCTCTGTTCTCCA	Direct sequencing		
	rs2155209	Forward: TCCATAACAGGCTGAACCAA Reverse: CACTGATGGAATCCCTCTAC	<i>EcoO109I</i>	T C	316 84+232
	rs10831234	Forward: GTCACCAAGGCTTATCTCT Reverse: GGCTAAGCAACCTCATTCTAG	<i>Eae I</i>	T C	355 145+210
	rs13447720	Forward: AAGTGCCTGGCACATAGGAA Reverse: GGCGCAGATGCAAATCAGTT	<i>BsmFI</i>	T C	495 126+369
	rs601341	Forward: CCTCTCAGACTCAACCATAC Reverse: GGCTAGAAGAAGCAGCTTCA	<i>HpyCH4 III</i>	A G	312 82+230
<i>RAD50</i>	rs17166050	Forward: GGTCACTCTGAGCTCTAAGT Reverse: GTACCTGCCGAAGTGTCTTCT	<i>Drd I</i>	A G	568 199+369
	rs17772583	Forward: TGTGCCTTTGACATGAGCAA Reverse: TTCTGTGCGCCCTAATGCCAA	<i>Bcl I</i>	G A	489 161+328
	rs6871536	Forward: GGATCTCACTATGTTGCCCA Reverse: CCTATTCACAGTGGCGATAA	Direct sequencing		
	rs3798134	Forward: CTGCTGACAGTCTGTCTGAT Reverse: CGCAACAATTACTGACTG	Direct sequencing		
	rs2244012	Forward: TAGCATGTGGAAGTGTGAGC Reverse: GCAAGAAGCCACCTGGTATA	<i>Bfa I</i>	G A	170+279 129+150+170
<i>NBS1</i>	rs1805794	Forward: TGTGCTCTTCTGACCATGAG Reverse: CAGTGACCAAAGACCGACTT	<i>Hinf I</i>	G C	576 255+321
	rs2735383	Forward: GATGAAGTCTCCACATGGTC Reverse: GCATCTACTTCAGGCCAACA	<i>Sfc I</i>	G C	604 106+498
	rs1063053	Forward: GCAAGGTGTAGAACACTGCA Reverse: CTAATTCAGGCCAACAAGGT	<i>Rsa I</i>	T C	416 188+228
	rs1063054	Forward: CCACCAGCTTCACTTGGTAA Reverse: GCATCTACTTGCCAGAACCA	Direct sequencing		

the stage and grade of the 375 BLCA patients, are summarized in Table I. The mean age for the control group and patients was 62.9 and 61.4 years, respectively. The male-to-female ratio in the cases was approximately 3:1 (Table I). To ensure comparability, non-cancer controls were matched based on age, sex, smoking, and alcohol consumption habits, resulting in no significant differences between the groups in these factors ( $p$ -values for age, sex, smoking, and alcohol consumption were 0.7315, 0.5525, 0.3063, and 0.3807, respectively). Regarding the clinical characteristics of patients, 62.7% had non-muscle-invasive BLCA and 37.3% had muscle-invasive disease. The distribution of tumor grades indicated that 40.3% were classified as low-grade, while 59.7% were high-grade (Table I).

*Association between MRN genotypes and BLCA risk.* Table III presents a summary of the distribution of individual

*MRN* genotypes and their potential associations with the risk of BLCA. Genotypic distributions for all the 14 SNPs were in compliance with Hardy-Weinberg equilibrium (all  $p > 0.05$ ).

Among the five *MRE11* SNPs (rs684507, rs2155209, rs10831234, rs13447720, and rs601341), no significant associations were observed between their variant genotypes and the risk of BLCA ( $p$  for trend=0.7284, 0.8005, 0.8165, 0.6510, and 0.6310, respectively). Likewise, no significant associations were observed between the variant genotypes of the five *RAD50* SNPs and the risk of BLCA ( $p$  for trend=0.6946, 0.8135, 0.6908, 0.7221, and 0.4935 for rs17166050, rs17772583, rs6871536, rs3798134, and rs2244012, respectively).

In contrast, among the four *NBS1* SNPs (rs1805794, rs2735383, rs1063053, and rs1063054), the genotypes of rs2735383 were significantly associated with BLCA risk

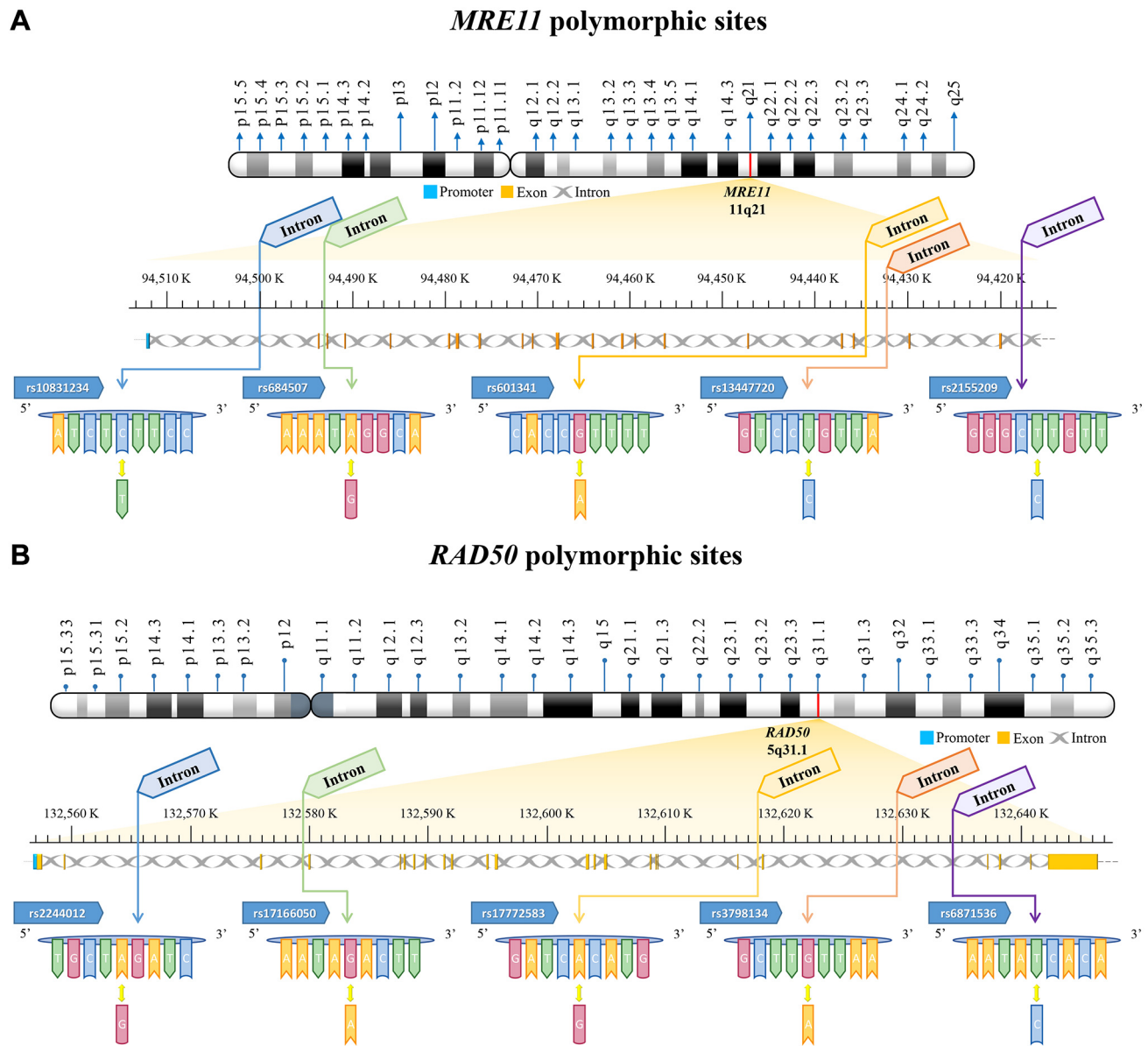


Figure 1. Continued

( $p$  for trend=0.0053). Specifically, both the heterozygous variant (CG) and the homozygous variant (CC) were linked to increased risks of developing BLCA (OR=1.48 and 1.95, 95%CI=1.06-2.06, 1.28-2.99;  $p$ =0.0246 and 0.0028, respectively). In the dominant model, the combined CG+CC genotypes were associated with a higher risk of BLCA (OR=1.59, 95%CI=1.17-2.18,  $p$ =0.0044). For the

remaining SNPs, rs1805794, rs1063053, and rs1063054, no significant associations were found.

*Association between MRN alleles and BLCA risk.* To validate the genotypic findings presented in Table III, an analysis of allelic frequency distributions was conducted. The results indicated that, with the exception of *NBS1*

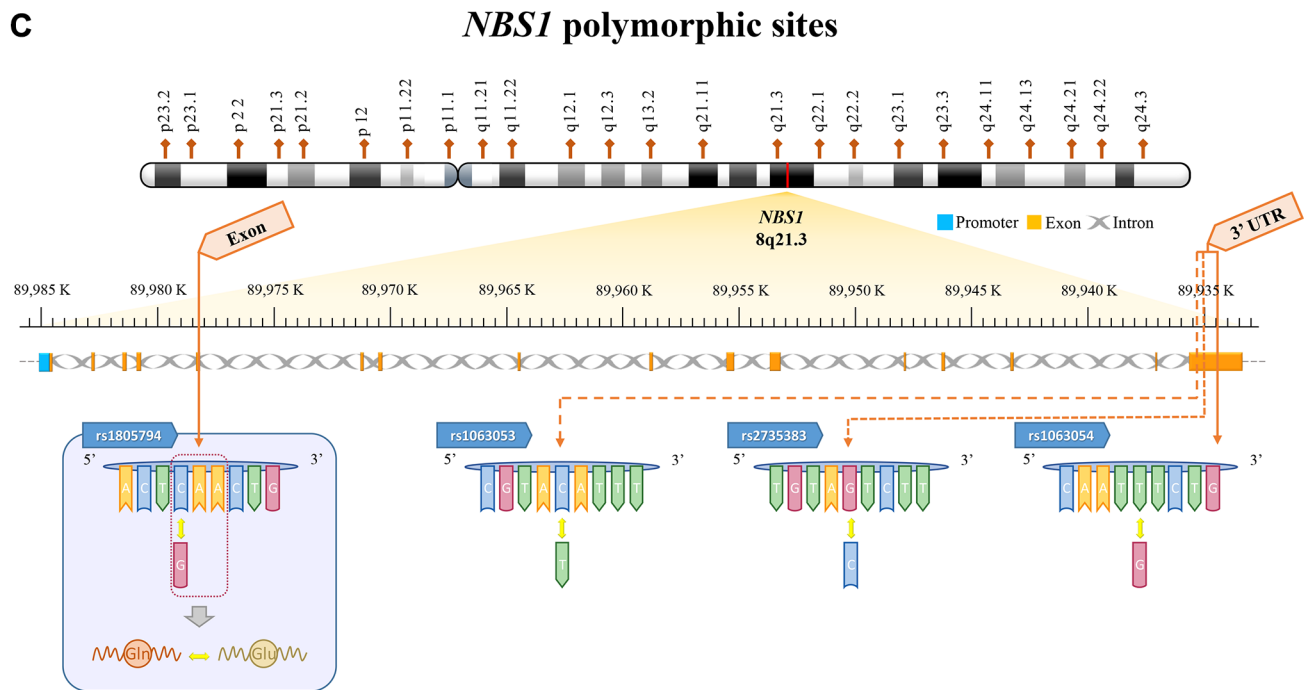


Figure 1. Physical maps of the MRN polymorphic sites in (A) *MRE11*, (B) *RAD50*, and (C) *NBS1*.

rs2735383, no significant associations were observed between the variant alleles of the *MRN* genes and BLCA risk. Only the C allele of *NBS1* rs2735383 was associated with a 1.39-fold increased BLCA risk (95%CI=1.13-1.71,  $p=0.0018$ , Table IV).

*Stratified analysis of NBS1 rs2735383 genotypes based on demographic and clinical characteristics.* We further conducted subgroup analyses to explore the relationship between *NBS1* rs2735383 genotypes and BLCA risk, stratified by age, sex, smoking, alcohol consumption, and cancer stage and grade. However, a notable difference in risk estimate was found between individuals older than 55 years and those 55 years or younger. Specifically, in the older age group, both the heterozygous CG and homozygous CC variants were significantly associated with higher BLCA risks (OR=1.72 and 2.29, 95%CI=1.12-2.65 and 1.31-4.01,  $p=0.0176$  and 0.0053, respectively, Table V). However, no significant associations were found in the younger age group. When stratified by sex, the variant genotypes were

associated with increased risks of BLCA in both males and females. However, statistical significance was reached only in males (Table V), while females showed a borderline association (Table V). This is likely due to the smaller sample size of females and the resulting lack of statistical power. When stratified by smoking status, significant differences in genotype distribution were found among smokers, but not non-smokers (Table V). The homozygous CC variant was significantly associated with an increased BLCA risk in the smoker subgroup (OR=2.56, 95%CI=1.42-4.63,  $p=0.0026$ ) but not in the non-smokers (OR=1.44, 95%CI=0.77-2.70,  $p=0.3228$ , Table V). For alcohol consumption, genotype distribution differed significantly among alcohol drinkers, while no such association was observed in non-drinkers (Table V). Both the heterozygous CG and homozygous CC variants were associated with significantly increased BLCA risks in drinkers (OR=1.73 and 3.25, 95%CI=1.08-2.78 and 1.70-6.23,  $p=0.0298$  and 0.0005, respectively, Table V), but not in non-drinkers. Interestingly, significant differences in genotype frequencies were

Table III. Distributions of MRE11, RAD50, and NBS1 genotypes among the bladder cancer patients and control subjects.

Genotype	Controls		Patients		OR (95%CI)	p-Value <sup>a</sup>
	n	%	n	%		
<i>MRE11</i>						
rs684507						
AA	121	32.3%	124	33.1%	1.00 (reference)	
AG	178	47.5%	168	44.8%	0.92 (0.66-1.28)	0.6823
GG	76	20.2%	83	22.1%	1.07 (0.71-1.59)	0.8334
p-Value for trend						0.7284
AG+GG	254	67.7%	251	66.9%	0.96 (0.71-1.31)	0.8763
rs2155209						
TT	190	50.7%	181	48.3%	1.00 (reference)	
CT	160	42.6%	167	44.5%	1.10 (0.81-1.48)	0.5988
CC	25	6.7%	27	7.2%	1.13 (0.63-2.03)	0.7829
p-Value for trend						0.8005
CT+CC	185	49.3%	194	51.7%	1.10 (0.83-1.47)	0.5590
rs10831234						
CC	338	90.1%	341	90.9%	1.00 (reference)	
CT	35	9.3%	33	8.8%	0.93 (0.57-1.54)	0.8896
TT	2	0.6%	1	0.3%	0.50 (0.04-5.49)	0.6233
p-Value for trend						0.8165
CT+TT	37	9.9%	34	9.1%	0.91 (0.56-1.49)	0.8030
rs13447720						
TT	242	64.5%	232	61.9%	1.00 (reference)	
CT	119	31.8%	125	33.3%	1.10 (0.80-1.49)	0.6163
CC	14	3.7%	18	4.8%	1.34 (0.65-2.76)	0.5370
p-Value for trend						0.6510
CT+CC	133	35.5%	143	38.1%	1.12 (0.83-1.51)	0.4956
rs601341						
GG	178	47.5%	191	50.9%	1.00 (reference)	
AG	153	40.8%	144	38.4%	0.88 (0.65-1.19)	0.4456
AA	44	11.7%	40	10.7%	0.85 (0.53-1.36)	0.5724
p-Value for trend						0.6310
AG+AA	197	52.5%	184	49.1%	0.87 (0.65-1.16)	0.3808
<i>RAD50</i>						
rs17166050						
GG	255	68.0%	265	70.7%	1.00 (reference)	
AG	103	27.5%	96	25.6%	0.90 (0.65-1.24)	0.5691
AA	17	4.5%	14	3.7%	0.79 (0.38-1.64)	0.6580
p-Value for trend						0.6946
AG+AA	120	32.0%	110	29.3%	0.88 (0.65-1.20)	0.4760
rs17772583						
AA	157	41.9%	149	39.7%	1.00 (reference)	
AG	169	45.1%	173	46.2%	1.08 (0.79-1.47)	0.6875
GG	49	13.0%	53	14.1%	1.14 (0.73-1.78)	0.6474
p-Value for trend						0.8135
AG+GG	218	58.1%	226	60.3%	1.09 (0.82-1.46)	0.6030
rs6871536						
TT	240	64.0%	229	61.1%	1.00 (reference)	
CT	124	33.1%	133	35.4%	1.12 (0.83-1.52)	0.4992
CC	11	2.9%	13	3.5%	1.24 (0.54-2.82)	0.7634
p-Value for trend						0.6908
CT+CC	135	36.0%	146	38.9%	1.13 (0.84-1.52)	0.4506

Table III. Continued

Table III. *Continued*

Genotype	Controls		Patients		OR (95%CI)	<i>p</i> -Value <sup>a</sup>
	n	%	n	%		
rs3798134						
GG	244	65.1%	232	61.9%	1.00 (reference)	
AG	122	32.5%	132	35.2%	1.14 (0.84-1.54)	0.4512
AA	9	2.4%	11	2.9%	1.29 (0.52-3.16)	0.7487
<i>p</i> -Value for trend						0.7221
AG+AA	131	34.9%	143	38.1%	1.15 (0.85-1.55)	0.4042
rs2244012						
AA	246	65.6%	231	61.6%	1.00 (reference)	
AG	118	31.5%	130	34.7%	1.17 (0.86-1.60)	0.3465
GG	11	2.9%	14	3.7%	1.36 (0.60-3.05)	0.5940
<i>p</i> -Value for trend						0.4935
AG+GG	129	34.4%	144	38.4%	1.19 (0.88-1.60)	0.2880
<i>NBS1</i>						
rs1805794						
CC	177	47.2%	170	45.4%	1.00 (reference)	
CG	162	43.2%	164	43.7%	1.05 (0.78-1.43)	0.7918
GG	36	9.6%	41	10.9%	1.19 (0.72-1.94)	0.5826
<i>p</i> -Value for trend						0.7874
CG+GG	198	52.8%	205	54.6%	1.08 (0.81-1.44)	0.6604
rs2735383						
GG	134	35.7%	97	25.9%	1.00 (reference)	
CG	183	48.8%	196	52.2%	1.48 (1.06-2.06)	<b>0.024</b>
CC	58	15.5%	82	21.9%	1.95 (1.28-2.99)	<b>0.0028</b>
<i>p</i> -Value for trend						<b>0.0053</b>
CG+CC	241	64.3%	278	74.1%	1.59 (1.17-2.18)	<b>0.0044</b>
rs1063053						
CC	142	37.9%	128	34.1%	1.00 (reference)	
CT	175	46.6%	181	48.3%	1.15 (0.84-1.57)	0.4409
TT	58	15.5%	66	17.6%	1.26 (0.82-1.93)	0.3349
<i>p</i> -Value for trend						0.5109
CT+TT	233	62.1%	247	65.9%	1.18 (0.87-1.59)	0.3227
rs1063054						
TT	128	34.1%	137	36.5%	1.00 (reference)	
GT	188	50.1%	185	49.3%	0.92 (0.67-1.26)	0.6581
GG	59	15.8%	53	14.2%	0.84 (0.54-1.31)	0.5067
<i>p</i> -Value for trend						0.7221
GT+GG	247	65.9%	238	63.5%	0.90 (0.67-1.21)	0.5411

OR: Odds ratio; CI: confidence interval; <sup>a</sup>Based on chi-square test with Yates' correction ( $n \geq 5$ ) or Fisher's exact test ( $n < 5$ ); *p*-Values for trend were calculated by 2×3 chi-square tests. Significant *p*-Values are shown in bold.

observed in muscle-invasive BLCA cases, but not in non-muscle-invasive cases (Table V). The homozygous CC variant was associated with a significantly increased risk of muscle-invasive BLCA (OR=1.97, 95%CI=1.13-3.45,  $p=0.0243$ , Table V). Finally, no significant differences in genotype distributions were found between BLCA cases with low or high-grade tumors (Table V).

## Discussion

BLCA is one of the most prevalent malignancies worldwide, and its incidence continues to rise, especially in Taiwan. Genetic and environmental factors, such as smoking and alcohol consumption, contribute significantly to the pathogenesis of BLCA. While much

Table IV. Allelic frequencies of *MRE11*, *RAD50*, and *NBS1* genotypes among the bladder cancer patients and control subjects.

Gene	Polymorphism	Allele	Control (%)	Case (%)	OR (95%CI)	p-Value <sup>a</sup>
<i>MRE11</i>	rs684507	A	420 (56.0)	416 (55.5)	1.00 (Reference)	0.8761
		G	330 (44.0)	334 (44.5)	1.02 (0.83-1.25)	
	rs2155209	T	540 (72.0)	529 (70.5)	1.00 (Reference)	
		C	210 (28.0)	221 (29.5)	1.07 (0.86-1.34)	
	rs10831234	C	711 (94.8)	715 (95.3)	1.00 (Reference)	
		T	39 (5.2)	35 (4.6)	0.89 (0.56-1.42)	
	rs13447720	T	603 (80.4)	589 (78.5)	1.00 (Reference)	
		C	147 (19.6)	161 (21.5)	1.12 (0.87-1.44)	
	rs601341	G	509 (67.9)	526 (70.1)	1.00 (Reference)	
		A	241 (32.1)	224 (29.9)	0.90 (0.75-1.12)	
<i>RAD50</i>	rs17166050	G	613 (81.7)	626 (83.5)	1.00 (Reference)	0.4138
		A	137 (18.3)	124 (16.5)	0.89 (0.68-1.16)	
	rs17772583	A	483 (64.4)	471 (62.8)	1.00 (Reference)	
		G	267 (35.6)	279 (37.2)	1.07 (0.87-1.32)	
	rs6871536	T	604 (80.5)	591 (78.8)	1.00 (Reference)	
		C	146 (19.5)	159 (21.2)	1.11 (0.87-1.43)	
	rs3798134	G	610 (81.3)	596 (79.5)	1.00 (Reference)	
		A	140 (18.7)	154 (20.5)	1.13 (0.87-1.45)	
	rs2244012	A	610 (81.3)	592 (78.9)	1.00 (Reference)	
		G	140 (18.7)	158 (21.1)	1.16 (0.90-1.50)	
<i>NBS1</i>	rs1805794	C	516 (68.8)	504 (67.2)	1.00 (Reference)	0.5426
		G	234 (31.2)	246 (32.8)	1.08 (0.87-1.34)	
	rs2735383	G	451 (60.1)	390 (52.0)	1.00 (Reference)	
		C	299 (39.9)	360 (48.0)	1.39 (1.13-1.71)	
	rs1063053	C	459 (61.2)	437 (58.3)	1.00 (Reference)	
		T	291 (38.8)	313 (41.7)	1.13 (0.92-1.39)	
	rs1063054	T	444 (59.2)	459 (61.2)	1.00 (Reference)	
		G	306 (40.8)	291 (38.8)	0.92 (0.75-1.13)	

<sup>a</sup>Based on Chi-square test with Yates' correction. OR: Odds ratio; CI: confidence interval; Significant p-Values are shown in bold.

attention has been given to environmental exposures, the role of genetic factors, particularly those involved in DNA damage response pathways, has garnered increasing interest. Efficient DSB repair is essential for preserving genomic stability in BLCA, as the deficiencies not only promote tumorigenesis through the accumulation of genetic alterations but also influence therapeutic responses by modulating sensitivity to DNA-damaging agents such as platinum-based chemotherapy and PARP inhibitors. However, there is limited literature investigating the involvement of the DSB repair pathway and its associated genes in BLCA. This study aimed to investigate the associations between SNPs in three crucial

DNA DSB repair genes – *MRE11*, *RAD50*, and *NBS1* – and BLCA susceptibility, focusing on the Taiwanese population.

We explored 14 SNPs in the *MRE11*, *RAD50*, and *NBS1* genes (Figure 1) among 375 BLCA cases and 375 cancer-free controls (Table I). Our findings showed no significant association between SNPs in *MRE11* or *RAD50* and the risk of BLCA, suggesting that these genetic variants may not play a significant role in predisposition to BLCA in this Taiwanese cohort. This aligns with previous studies, which also failed to establish a link between *MRE11* and *RAD50* genetic variants and various cancers (41, 71).

Notably, our analysis identified a significant association between the *NBS1* rs2735383 SNP and BLCA

Table V. Stratified analysis of NBS1 rs2735383 genotypes and bladder cancer risk by demographic and clinical characteristics.

	Controls (%)	Cases (%)	OR (95%CI)	p-Value <sup>a</sup>
<b>Age</b>				
≤55 (years)				
GG	49 (32.2)	43 (27.2)	1.00 (reference)	
CG	78 (51.3)	81 (51.3)	1.18 (0.71-1.98)	0.6094
CC	25 (16.5)	34 (21.5)	1.55 (0.80-3.00)	0.2547
>55 (years)				
GG	85 (38.1)	54 (24.9)	1.00 (reference)	
CG	105 (47.1)	115 (53.0)	1.72 (1.12-2.65)	<b>0.0176</b>
CC	33 (14.8)	48 (22.1)	2.29 (1.31-4.01)	<b>0.0053</b>
<b>Sex</b>				
<b>Male</b>				
GG	99 (34.5)	73 (26.2)	1.00 (reference)	
CG	142 (49.5)	143 (51.3)	1.37 (0.93-2.00)	0.1317
CC	46 (16.0)	63 (22.5)	1.85 (1.14-3.02)	<b>0.0170</b>
<b>Female</b>				
GG	35 (39.8)	24 (25.0)	1.00 (reference)	
CG	41 (46.6)	53 (55.2)	1.89 (0.97-3.65)	0.0845
CC	12 (13.6)	19 (19.8)	2.31 (0.95-5.62)	0.1014
<b>Smoking behaviors</b>				
<b>Non-smokers</b>				
GG	62 (32.8)	43 (24.7)	1.00 (reference)	
CG	95 (50.3)	99 (56.9)	1.50 (0.93-2.43)	0.1225
CC	32 (16.9)	32 (18.4)	1.44 (0.77-2.70)	0.3228
<b>Smokers</b>				
GG	72 (38.7)	54 (26.9)	1.00 (reference)	
CG	88 (47.3)	97 (48.2)	1.47 (0.93-2.32)	0.1228
CC	26 (14.0)	50 (24.9)	2.56 (1.42-4.63)	<b>0.0026</b>
<b>Alcohol drinking behaviors</b>				
<b>Non-drinkers</b>				
GG	66 (33.2)	52 (28.0)	1.00 (reference)	
CG	95 (47.7)	95 (51.1)	1.27 (0.80-2.01)	0.3703
CC	38 (19.1)	39 (20.9)	1.30 (0.73-2.32)	0.4510
<b>Drinkers</b>				
GG	68 (38.6)	45 (23.8)	1.00 (reference)	
CG	88 (50.0)	101 (53.4)	1.73 (1.08-2.78)	<b>0.0298</b>
CC	20 (11.4)	43 (22.8)	3.25 (1.70-6.23)	<b>0.0005</b>
<b>Cancer stages</b>				
<b>Non-muscle-invasive</b>				
GG	97 (25.9)	70 (29.8)	1.00 (reference)	
CG	196 (52.3)	128 (54.5)	0.91 (0.62-1.32)	0.6755
CC	82 (21.8)	37 (15.7)	0.63 (0.38-1.03)	0.0818
<b>Muscle-invasive</b>				
GG	97 (25.9)	27 (19.3)	1.00 (reference)	
CG	196 (52.3)	68 (48.6)	1.24 (0.75-2.07)	0.4689
CC	82 (21.8)	45 (32.1)	1.97 (1.13-3.45)	<b>0.0243</b>
<b>Cancer grade</b>				
<b>Low grade</b>				
GG	97 (25.9)	55 (29.6)	1.00 (reference)	
CG	196 (52.3)	95 (51.1)	0.85 (0.57-1.29)	0.5213
CC	82 (21.8)	36 (19.3)	0.77 (0.46-1.29)	0.3960
<b>High grade</b>				
GG	97 (25.9)	42 (22.2)	1.00 (reference)	
CG	196 (52.3)	101 (53.4)	1.19 (0.77-1.84)	0.4989
CC	82 (21.8)	46 (24.4)	1.30 (0.78-2.16)	0.3880

OR: Odds ratio; CI: confidence interval. <sup>a</sup>Based on Chi-square test with Yate's correction; Statistically significant p-values are shown in bold.

susceptibility. Individuals carrying the heterozygous (CG) or homozygous (CC) genotypes of *NBS1* rs2735383 exhibited a significantly elevated risk of developing BLCA compared to those with the wild-type (GG) genotype (Table III and Table IV). In contrast, other SNPs located in the 3'UTR of *NBS1*, including rs1063053 and rs1063054, as well as rs1805794 within the exon, showed no significant association with BLCA risk (Table III and Table IV). These findings suggest that genetic variations in *NBS1* may differentially influence cellular DNA repair mechanisms, thereby contributing to BLCA susceptibility. Moreover, the increased BLCA risk associated with the rs2735383 variant genotypes underscores the potential of *NBS1* as a predictive biomarker for BLCA susceptibility. This finding supports the hypothesis that genetic variations in *NBS1* could modulate an individual's DNA repair capacity, thereby contributing to BLCA development. The identification of such variants provides valuable insights into the molecular mechanisms underlying BLCA and paves the way for future investigations, particularly in elucidating how *NBS1* may interact with other genetic and environmental risk factors.

To the best of our knowledge, relatively few studies have investigated the association between *MRN* genotypes and BLCA susceptibility (35, 36, 49, 62-67). Below, we summarize the key findings of these studies, discuss their contributions, and compare them with our own results.

In 2008, Choudhury and his colleagues examined the associations of six SNPs within *MRE11* in a UK BLCA cohort. Their findings indicated a marginal increase in BLCA risk for individuals carrying the GG genotype of the *MRE11* 3'UTR rs2155209 polymorphism (35). They also investigated potential gene-environment interactions between *MRE11* 3'UTR rs2155209 genotypes and smoking habits or occupational dye exposure; however, no significant interactions were observed (35). Notably, their study included a relatively large sample size of 771 cases and 800 controls. In our study, we also assessed the association between *MRE11* rs2155209 and BLCA but did not observe significant association (Table III and Table IV). In 2013, another UK-based study reported that *MRE11*

rs1805363 was associated with poorer cancer-specific survival following radiotherapy in a cohort of 256 patients with muscle-invasive BLCA (36). More importantly, they found that carriers of the *MRE11* rs1805363 A allele exhibited higher *MRE11A* mRNA expression compared to G allele carriers in primary tumor samples (36). However, in our Taiwanese cohort, *MRE11* rs1805363 is not polymorphic (data not shown). In 2019, the same research group further investigated the functional impact of *MRE11* rs1805363 on radiotherapy outcomes in muscle-invasive BLCA. They reported that variations in *MRE11A* isoform expression did not significantly influence cell survival or DNA DSB repair capacity following ionizing radiation (72). Overall, despite considerable efforts to elucidate the genotypic contributions to BLCA, particularly muscle-invasive BLCA, further investigations are warranted to fully understand the role of *MRE11* genetic variants in BLCA susceptibility and treatment response.

Genotypic studies investigating the association between *RAD50* and BLCA remain relatively scarce. As previously mentioned, Choudhury and his colleagues examined only a single *RAD50* SNP, rs1047382, and found no significant association with BLCA risk (35). In our Taiwanese cohort, this SNP is not polymorphic (data not shown). More recently, in 2022, Pietzak *et al.* reported that high-grade non-muscle-invasive BLCA harbored pathogenic and likely pathogenic variants in DNA damage response genes, including *RAD50* and *NBS1* (49). In the present study, we selected and analyzed five SNPs within the intronic regions of *RAD50*; however, none of them demonstrated a significant association with BLCA susceptibility.

Among the MRN complex genes, *NBS1* has been the most extensively studied in relation to BLCA; however, the majority of these investigations have been conducted in Western populations, including cohorts from the UK, USA, and Sweden. Regarding *NBS1* rs1805794, Sanyal and his colleagues reported a marginal but statistically non-significant association with BLCA risk in a Swedish cohort (64). Wu's and Figueroa's groups further reinforced the lack of association in U.S. populations (63, 67). Similarly, our team was the first to provide evidence from an East

Asian population, demonstrating no significant association between *NBS1* rs1805794 genotypes and BLCA susceptibility (73). For *NBS1* rs2735383, Teo and his colleagues previously reported no significant association in a UK population, based on a relatively large cohort of 711 BLCA cases and 680 controls (65). Interestingly, in the present study, we identified a significant association between *NBS1* rs2735383 and BLCA susceptibility in the Taiwanese population (Table III and Table IV). The observed discrepancies may stem from ethnic differences that influence genetic backgrounds, emphasizing the need for further validation studies. Regarding *NBS1* rs1063054, Broberg and his colleagues found no significant association with BLCA risk in a Swedish cohort (62). Subsequent studies by Choudhury's and Park's groups provided additional evidence against the hypothesis that *NBS1* rs1063054 modulates BLCA susceptibility, based on analyses of UK and U.S. populations (35, 66). Beyond individual *NBS1* SNP associations, Pietzak and his colleagues identified pathogenic and likely pathogenic variants in *NBS1* (2140C>T and 657\_661delACAAA) among patients with high-grade non-muscle-invasive BLCA, further highlighting the potential role of *NBS1* in BLCA pathogenesis (49). Overall, our findings suggest that *NBS1* genetic variants may contribute to BLCA susceptibility, reinforcing the need for further studies to elucidate their gene-environment interactions and underlying molecular mechanisms in BLCA development.

In contrast to previous studies that primarily focused on a limited number of SNPs in DNA repair genes, our study comprehensively assessed a panel of variants in *MRE11*, *RAD50*, and *NBS1*. While no significant associations were observed for *MRE11* and *RAD50*, the positive association of *NBS1* rs2735383 highlights the importance of further investigating additional SNPs in these genes and other components of the DNA damage response pathway(s).

Furthermore, the potential role of gene-environment interactions in modulating BLCA risk should not be overlooked. Environmental exposures, such as smoking,

alcohol consumption, chemical exposure, use of traditional Chinese medicine, and dietary factors, may influence the impact of genetic variants on BLCA susceptibility. Notably, Choudhury *et al.* examined the interaction between *MRE11* 3'UTR rs2155209 genotypes and environmental factors, including smoking habits and dye exposure, in a UK cohort; however, no significant interactions were found (35). Overall, the observed association between *NBS1* genotypes and BLCA risk, along with our preliminary findings presented in Table V, underscores the need for further investigations. Future studies should not only explore gene-environment interactions but also examine the clinical relevance of these genetic variants in relation to BLCA tumor grade and stage.

*Study limitations.* First, the sample size of BLCA patients, although substantial, may still be underpowered to detect smaller effects of genetic variants. Second, the study only included individuals from Taiwan, and results may not be generalizable to other populations. Future studies with larger, more diverse cohorts would help strengthen these findings and elucidate the broader applicability of *NBS1* rs2735383 in BLCA susceptibility. Third, we were unable to assess the prognostic roles of the SNPs due to insufficient and/or incomplete follow-up data on the survival status of BLCA patients.

In conclusion, our study contributes to the growing body of evidence suggesting that genetic factors involved in DNA repair mechanisms, particularly in the MRN complex, may play a significant role in BLCA susceptibility. Although further studies with larger sample sizes and diverse populations are needed to validate these findings, our research provides valuable insights into the potential genetic determinants of BLCA and highlights the importance of investigating the molecular basis of cancer susceptibility in different ethnic groups.

### Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

## Authors' Contributions

Research design: Liao CH, Tsai CW, Chang WS, Bau DT; patient and questionnaire summaries: Liao CH, Chang SY, Chang CH, Chen WC; experimental work: Chang WS, Wang YC, Shih HY, Tsai CW; statistical analysis: Hsu CL, Tsai CW, Chang WS; manuscript writing: Tsai CW, Chang WS, Bau DT; manuscript checking and discussing: Liao CH, Tsai CW, Chang WS, Wang YC, Shih HY, Hsu CL, Chang SY, Chang CH, Chen WC, Bau DT.

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## Artificial Intelligence (AI) Disclosure

The Authors announce that no artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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