RESEARCH ARTICLE

Editorial Process: Submission:05/25/2025 Acceptance:12/20/2025 Published:12/27/2025

Association of *GSTM1*, *NAT2* Gene Polymorphisms and Susceptibility to Renal cell carcinoma in Mongolia: A Case-Control Study

Ganbayar Batmunkh[1], Shiirevnyamba Avirmed[1]*, Myagmarsuren Purevsuren[2], Sodgerel Bold[2], Ganbolor Yura[1,3], Naranjargal Davaadorj[1], Tsogzolmaa Shiirevnyamba[1,4], Munkhtuya Tumurkhuu[5]

Abstract

Objective: This study aimed to assess the impact of glutathione S-transferase M1 (*GSTM1*) and N-acetyltransferase 2 (NAT2) polymorphisms on renal cell carcinoma (RCC) risk in Mongolian individuals, both independently and in combination with smoking and urinary tract diseases (UTDs). **Methods:** This hospital-based case-control study included 88 histologically confirmed RCC patients and 88 cancer-free controls, matched by age and sex. Genotyping of *GSTM1* and *NAT2* polymorphisms was performed using PCR-RFLP. **Results:** There were 34 men and 54 women, with a mean age of 51.9 ± 13.2 years. The results revealed that NAT2 low acetylator genotype significantly increased risk of RCC (cOR=2.077, 95%CI=1.072-4.025, p=0.03). Notably, WT/M3 genotype was significantly associated with RCC risk (aOR=9.1, 95% CI=1.138–72.783; p=0.037). *GSTM1*-positive genotype significantly increased RCC risk when combined with NAT2 low acetylator genotypes (cOR=3.304, 95% CI=1.311–8.327, p=0.011). Among smokers, individuals with *GSTM1*-null genotype had an increased risk of RCC (cOR=4.654, 95% CI=1.458-14.86, p=0.009). Additionally, NAT2 low acetylator genotype significantly increased RCC risk in smokers (cOR=6.596, 95% CI=2.26–19.255, p=0.001). Furthermore, both *GSTM1* and *NAT2* genotypes were associated with significantly increased RCC risk following stratification by a history of urinary tract diseases. **Conclusion:** The findings suggest that the NAT2 WT/M3 polymorphism, along with smoking and UTDs, contributes to RCC susceptibility in Mongolian cases.

Keywords: *GSTM1*- *NAT2*- renal cell carcinoma risk

Asian Pac J Cancer Prev, **26 (12)**, 4503-4511

Introduction

Renal cell carcinoma (RCC) is the most prevalent form of kidney cancer, accounting for 90% of all renal malignancies and representing a significant global health burden [1]. RCC arises from the renal epithelium and is known for its high heterogeneity, resistance to chemotherapy, and late-stage detection, making it one of the most challenging malignancies to manage [2]. In 2022, RCC was responsible for over 434,840 new cases and approximately 155,953 deaths worldwide, with incidence rates continuing to rise, particularly in developing regions [3].

RCC is the most common urological cancer in Mongolia and its incidence continues to increase annually [4]. While advances in imaging and targeted therapies have improved survival outcomes, the lack of early diagnostic biomarkers and the influence of genetic and environmental risk factors make RCC a growing concern in oncology research [5, 6].

The development of RCC is influenced by a complex interplay of genetic susceptibility and environmental exposures [5]. Several epidemiological studies have identified major risk factors, including hypertension, smoking, obesity, urinary tract diseases (UTD), diabetes mellitus, and occupational exposure to carcinogens. Among these, hypertension and smoking are the most consistently associated with RCC [7-10]. Genetic predisposition also plays a crucial role in RCC susceptibility, particularly in populations with distinct genetic backgrounds [11]. One of the most studied genetic variations in cancer susceptibility involves polymorphisms in detoxification and metabolic enzyme pathways, such as glutathione S-transferase M1 (*GSTM1*) and N-acetyltransferase 2 (*NAT2*) [11-13]. The *GSTM1* gene, located on chromosome 1p13.3, encodes an enzyme that is critical in the detoxification of reactive

*[1]Department of Health Research, Graduate School, Mongolian National University of Medical Sciences, Mongolia. [2]Center of Urology and Andrology, First Central Hospital of Mongolia. [3]Moncell Clinic Hospital, Mongolia. [4]Institue of Medical Sciences, Mongolia. [5]Brody School of Medicine, East Carolina University, USA. *For Correspondence: shiirevnyamba@mnums.edu.mn*

oxygen species (ROS) and carcinogens. A homozygous deletion of the *GSTM1* gene (*GSTM1*-null genotype) leads to a complete loss of enzymatic function, impairing the body's ability to detoxify harmful compounds and increasing oxidative stress-related DNA damage, which is a major factor in renal carcinogenesis [12, 14].

Similarly, the *NAT2* gene, located on chromosome 8p22, encodes an enzyme involved in the acetylation and biotransformation of carcinogenic aromatic amines, which are found in tobacco smoke, industrial chemicals, and dietary sources [15-17]. Variations in *NAT2* result in different acetylation genotypes slow, intermediate, and rapid acetylators which influence an individual's ability to metabolize and eliminate carcinogens [17]. Slow acetylators have reduced enzymatic activity, leading to prolonged exposure to carcinogens, increasing the likelihood of mutations and cancer development [12, 18]. While the role of *NAT2* polymorphisms has been extensively studied in bladder cancer, its association with RCC remains unclear, particularly in Asian populations, where genetic diversity and environmental factors may modify its impact [19]. Mongolia provides a unique setting for studying the interplay between genetic and environmental risk factors for RCC. The high prevalence of tobacco smoking, hypertension, and obesity, coupled with potential exposure to contaminated drinking water, heavy metals, and traditional dietary patterns, suggests that Mongolians may have a distinct risk profile for RCC [19]. Furthermore, Mongolia's high-altitude environment, characterized by chronic hypoxia, may contribute to oxidative stress and altered renal metabolism, potentially influencing RCC susceptibility [19-21]. Given these factors, a detailed investigation of *GSTM1* and *NAT2* polymorphisms in the Mongolian population could provide valuable insights into the genetic determinants of RCC and their interaction with lifestyle and environmental risk factors [22]. This hospital-based case-control study aims to assess the association between *GSTM1* and *NAT2* polymorphisms and RCC risk, both independently and in combination with smoking, hypertension, obesity, and urinary tract diseases (UTD). By evaluating genotype distributions and their modifying effects on RCC susceptibility, this study seeks to identify high-risk individuals, improve early detection strategies, and contribute to the development of personalized preventive measures for RCC in populations with distinct environmental and genetic backgrounds.

Materials and Methods

Study Design and Population

This study was designed as a hospital-based case-control study to investigate the association between *GSTM1* and *NAT2* genetic polymorphisms and the risk of renal cell carcinoma (RCC) in the Mongolian population. The study included 88 histologically confirmed RCC patients and 88 cancer-free controls, all recruited from the First Central Hospital of Mongolia. Controls were matched to cases by age and sex and had no prior history of malignancy, confirmed through structured questionnaires and medical record review. Routine health assessments, including clinical examinations and blood biochemistry tests, were performed to exclude underlying cancer, and individuals with suspicious symptoms or abnormal findings were excluded, although imaging was not systematically applied. After obtaining written informed consent from a total of one hundred seventy-six subjects, all participants of the study were asked to fill out a structured questionnaire containing demographic and clinical information, including age, sex, body mass index (BMI), smoking, alcohol drinking, exercise, dietary habits, medical history of hypertension, diabetes mellitus, and urinary tract diseases (UTD). In addition, 3 ml peripheral blood was collected from all participants for genetic analysis. Ethical approval for the study was obtained from the Scientific Research Committee of the Mongolian National University of Medical Sciences (Approval No.: 2023/04/21–2023/3-04).

Genomic DNA extraction and genotyping

Genomic DNA was extracted from white blood cells using the Qiagen Mini Blood DNA Extraction Kit (Qiagen, USA) following the manufacturer's protocol. The quality and concentration of DNA were assessed using agarose gel electrophoresis and spectrophotometry.

GSTM1 Genotyping

The presence or absence of the *GSTM1* gene was determined using the multiplex polymerase chain reaction (PCR) method. PCR was performed in a total reaction volume of 25 μL, containing: Genomic DNA (200 ng), Primers (50 pM) (5'-GAACTCCCTGAAAAGCTAAAGC-3' and 5'-GTTGGGCTCAAATATACGGTGG-3'), dNTP mix (0.2 mM each), Taq polymerase (1 U, Thermo Fisher Scientific, USA), PCR buffer with $MgCl_2$ (2.0 mM). Amplification conditions included an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 1.5 minutes, with a final extension at 72°C for 5 minutes. The PCR products were separated on a 2% agarose gel and visualized under UV light.

NAT2 Genotyping

Polymorphisms in the *NAT2* gene were analyzed using the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The polymerase chain reaction was conducted with the following primers: 5′-GGAACAAATTGGACTTGG-3′ and 5′-TCTAGCAT GAATCACTCTGC-3'). The *NAT2* low acetylator genotype was determined based on the presence of *NAT25, NAT26,* and *NAT2**7 alleles, identified using restriction enzyme digestion with: KpnI for *NAT2**5 allele (incubated at 37°C for 1 hour), TaqI for *NAT2**6 allele (incubated at 65°C for 1 hour), BamHI for *NAT2**7 allele (incubated at 30°C for 1 hour). The digested products were separated by agarose gel electrophoresis (2.0% for *NAT2*5 and *NAT2*7, and 3.0% for *NAT2**6) and visualized under UV transillumination. Subjects with two wild-type (WT) alleles were classified as high acetylators, while those with two mutant alleles (M1, M2, or M3) were classified as low acetylators.

Statistical Analysis

All statistical analyses were conducted using Stata 13.0 (StataCorp, USA). Descriptive statistics were used to summarize the demographic, clinical, and genetic characteristics of RCC patients and controls. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized as means ± standard deviations (SD).

To compare categorical variables, including genotype distributions and lifestyle factors between RCC cases and controls, Pearson's chi-square test (χ^2) was applied. For continuous variables such as age, independent t-tests were used to assess differences between the two groups. The association between *GSTM1* and *NAT2* genetic polymorphisms and RCC risk was analyzed using univariate logistic regression, estimating crude odds ratios (cORs) and 95% confidence intervals (CIs). To adjust for potential confounders, including age, sex, alcohol consumption, smoking, hypertension, and history of UTD, multivariable logistic regression was performed to calculate adjusted odds ratios (aORs) and 95% CIs. Additionally, gene-environmental interactions were assessed by analyzing the association between *GSTM1*/*NAT2* polymorphisms and RCC risk, stratified by smoking status, alcohol consumption, hypertension, and UTD. A p-value ≤0.05 was considered statistically significant, and all statistical tests were two-tailed. Multiple comparisons were adjusted where necessary to ensure accuracy in the analysis.

Results

The study included a total of 176 participants, comprising 88 renal cell carcinoma (RCC) patients and 88 cancer-free controls. The mean age of both groups was 51.9 ± 13.2 years, with an equal sex distribution (male: 34 (38.6%), female: 54 (61.4%) in each group), showing no significant difference between cases and controls. Most cases are diagnosed between the ages of 40 and 69 in Mongolia. (Table 1).

Several clinical and lifestyle factors demonstrated a significant association with RCC risk. Smoking was notably more prevalent among RCC patients (48.9%) compared to controls (20.5%), indicating a significant increase in RCC risk among smokers ($p < 0.001$). Similarly, hypertension was highly associated with RCC, with 53.4% of RCC patients having a history of hypertension compared to only 2.3% of controls ($p < 0.001$). Alcohol consumption was also significantly linked to RCC, with 28.4% of RCC cases reporting alcohol use, compared to 9.1% of controls ($p = 0.001$). A history of urinary tract diseases (UTD) was another major risk factor, with 33% of RCC patients having a history of UTD, compared to only 4.5% of controls, demonstrating a highly significant association with RCC risk ($p < 0.001$). In contrast, body mass index (BMI) was not significantly associated with RCC, though individuals with BMI >30 kg/m² had a trend to increase the risk of RCC ($p = 0.135$). These findings suggest that smoking, hypertension, alcohol consumption, and UTD history are major risk factors for RCC, warranting further investigation into their combined effects with genetic susceptibility (Table 1).

Table 1. Baseline Characteristics of Study Population

Variables	Total n (%)	Controls n (%)	RCC n (%)	P value
Age				1
20-29	8 (4.5)	4 (4.5)	4 (4.5)	
30-39	18 (10.2)	9 (10.2)	9 (10.2)	
40-49	56 (31.8)	28 (31.8)	28 (31.8)	
50-59	38 (21.6)	19 (21.6)	19 (21.6)	
60-69	42 (23.9)	21 (23.9)	21 (23.9)	
70<	14 (8)	7 (8)	7 (8)	
Sex				1
Male	68 (38.6)	34 (38.6)	34 (38.6)	
Female	108 (61.4)	54 (61.4)	54 (61.4)	
BMI				0.135
18.5 - 24.9	3 (1.7)	3 (3.4)	0 (0)	
25.0 - 29.9	60 (34.1)	34 (38.6)	26 (29.5)	
30.0 - 34.9	90 (51.1)	42 (47.7)	48 (54.5)	
35.0<	23 (13.1)	9 (10.2)	14 (15.9)	
Alcohol drinking				0.001
Yes	33 (18.8)	8 (9.1)	25 (28.4)	
No	143 (81.3)	80 (90.9)	63 (71.6)	
Smoking				0.001
Yes	61 (34.7)	18 (20.5)	43 (48.9)	
No	115 (65.3)	70 (79.5)	45 (51.1)	
Hypertension				0.001
Yes	49 (27.8)	2 (2.3)	47 (53.4)	
No	127 (72.2)	86 (97.7)	41 (46.6)	
Diabet				0.216
Yes	28 (15.9)	11 (12.5)	17 (19.3)	
No	148 (84.1)	77 (87.5)	71 (80.7)	
Exercise				0.823
Yes	23 (13.1)	11 (12.5)	12 (13.6)	
No	153 (86.9)	77 (87.5)	76 (86.4)	
Coffee use				0.34
Yes	60 (34.1)	33 (37.5)	27 (30.7)	
No	116 (65.9)	55 (62.5)	61 (69.3)	
History of UTD				0.001
Yes	33 (18.8)	4 (4.5)	29 (33)	
No	143 (81.3)	84 (95.5)	59 (67)	
Total	176 (100)	88 (100)	88 (100)	

P value for Pearson's chi-square test, BMI, body mass index; UTD, urinary tract diseases

GSTM1 and NAT2 Genotypes and RCC Risk

The *GSTM1*-null genotype was detected in 52.3% of RCC cases and 53.4% of controls, showing no significant association with RCC risk (cOR= 0.947, 95% CI =0.497 - 1.805, p=0.869 and aOR = 0.727, 95% CI = 0.228 - 2.322, p = 0.59). These findings suggest that *GSTM1* deletion alone may not contribute significantly to RCC susceptibility (Table 2). Conversely, *NAT2* polymorphisms exhibited a notable influence on RCC risk. The *NAT2*

Table 2. *GSTM1* and *NAT2* Genotypes in Relation Risk of Renal Cell Carcinoma

Variables	Total n (%)	Controls n (%)	RCC n (%)	cOR [95% CI]	P value	aOR [95% CI]	P value
GSTM1							
Positive	83 (47.2)	41 (46.6)	42 (47.7)	1		1	
Null	93 (52.8)	47 (53.4)	46 (52.3)	0.947 [0.497 - 1.805]	0.869	0.727 [0.228 - 2.322]	0.59
NAT2							
High	102 (58)	58 (65.9)	44 (50)	1		1	
Low	74 (42)	30 (34.1)	44 (50)	2.077 [1.072 - 4.025]	0.03	1.916 [0.641 - 5.726]	0.245
KPN1							
WT/WT	138 (78.4)	76 (86.4)	62 (70.5)	1		1	
WT/M1	36 (20.5)	10 (11.4)	26 (29.5)	3.667 [1.487 - 9.043]	0.005	4.46 [0.949 - 20.959]	0.058
M1/M1	2 (1.1)	2 (2.3)	0 (0)				
WT/M1 and M1/M1	38 (21.6)	12 (13.6)	26 (29.5)	2.75 [1.224 - 6.177]	0.014	2.637 [0.768 - 9.06]	0.123
TAQ1							
WT/WT	137 (77.8)	77 (87.5)	60 (68.2)	1		1	
WT/M2	18 (10.2)	6 (6.8)	12 (13.6)	3.077 [0.984 - 9.625]	0.053	1.156 [0.17 - 7.843]	0.882
M2/M2	21 (11.9)	5 (5.7)	16 (18.2)	4.691 [1.475 - 14.916]	0.009	3.8 [0.425 - 33.979]	0.232
WT/M2 and M2/M2	39 (22.2)	11 (12.5)	28 (31.8)	3.833 [1.561 - 9.414]	0.003	2.069 [0.517 - 8.281]	0.304
BAMH1							
WT/WT	135 (76.7)	77 (87.5)	58 (65.9)	1		1	
WT/M3	41 (23.3)	11 (12.5)	30 (34.1)	4.8 [1.831 - 12.58]	0.001	9.1 [1.138 - 72.783]	0.037
GSTM1/NAT2							
GSTM1-pos / *NAT2*-high	54 (30.7)	33 (37.5)	21 (23.9)	1		1	
GSTM1-pos / *NAT2*-low	29 (16.5)	8 (9.1)	21 (23.9)	3.304 [1.311 - 8.327]	0.011	3.19 [0.535 - 19.011]	0.203
GSTM1-null / *NAT2*-high	48 (27.3)	25 (28.4)	23 (26.1)	1.369 [0.556 - 3.374]	0.495	1.026 [0.234 - 4.507]	0.973
GSTM1-null / *NAT2*-low	45 (25.6)	22 (25)	23 (26.1)	1.68 [0.684 - 4.128]	0.258	1.18 [0.229 - 6.076]	0.843

1, reference category; CI, confidence interval; cOR, crude odds ratio for conditional binary logistic regression; aOR, adjusted odds ratio for conditional binary logistic regression (adjusted by smoking, history of UTD)

low acetylator genotype was found in 50% of RCC cases compared to 34.1% of controls, indicating a potential increase in RCC risk. Furthermore, the association was statistically significant after adjustment for confounders (cOR = 2.077, 95% CI = 1.072-4.025, p = 0.03) (Table 2). These results suggest that while the *GSTM1*-null genotype does not independently influence RCC risk, *NAT2* polymorphisms does have a modifying effect.

Genotypic Distribution of NAT2 Polymorphisms

Analysis of specific *NAT2* polymorphisms revealed significant associations with RCC susceptibility. The WT/M1 genotype was linked to an elevated RCC risk, with carriers showing a 3.667-fold increased likelihood of developing RCC (cOR = 3.667, 95% CI = 1.487-9.043, p = 0.005). The M2/M2 genotype was also associated with an increased RCC risk, and the result was statistically significant (cOR = 4.691, 95% CI = 1.475-14.916, p = 0.009). The WT/M3 genotype, which was more prevalent in RCC cases (34.1%) compared to controls (12.5%), demonstrating a significant 4.8-fold increased RCC risk (cOR = 4.8, 95% CI = 1.831-12.58, p = 0.001). A notable finding was the WT/M3 genotype was significantly associated with RCC risk (aOR=9.1, 95% CI=1.138–72.783; p=0.037). These results suggest that specific *NAT2* polymorphisms, particularly WT/M3, do play a crucial role in RCC development, highlighting the need for further research to confirm these genetic associations (Table 2). We conducted a post-hoc power analysis using the observed effect sizes for the *NAT2* WT/M3 genotype. The achieved power was moderate (72% at α=0.05), reflecting the limited sample size.

Combined Effects of GSTM1 and NAT2 on RCC Risk

Analysis of the combined effects of *GSTM1* and *NAT2* polymorphisms showed varying degrees of RCC susceptibility (Table 2). Individuals carrying the *GSTM1*-null/*NAT2*-low genotype did not exhibit a significant increase in RCC risk (cOR = 1.68, 95% CI = 0.684-4.128, p = 0.258), indicating that this genotype

Table 3. Stages of Renal Cell Carcinoma Cases in the Study

Variables			*GSTM1*		P value	*NAT2*		P value
			Pos	Null		High	Low	
		n (%)	n (%)	n (%)		n (%)	n (%)	
Stage					0.876			0.05
	Stage I	50 (56.8)	22 (52.4)	28 (60.9)		19 (43.2)	31 (70.5)	
	Stage II	10 (11.4)	5 (11.9)	5 (10.9)		8 (18.2)	2 (4.5)	
	Stage III	17 (19.3)	9 (21.4)	8 (17.4)		10 (22.7)	7 (15.9)	
	Stage IV	11 (12.5)	6 (14.3)	5 (10.9)		7 (15.9)	4 (9.1)	
Histology type					0.259			0.225
	Clear cell	77 (87.5)	39 (92.9)	38 (82.6)		37 (84.1)	40 (90.9)	
	Chromophobe	5 (5.7)	2 (4.8)	3 (6.5)		2 (4.5)	3 (6.8)	
	Papillary	6 (6.8)	1 (2.4)	5 (10.9)		5 (11.4)	1 (2.3)	
Cancer volume					0.578			0.802
	<10 cm^3	35 (39.8)	16 (38.1)	19 (41.3)		16 (36.4)	19 (43.2)	
	10 - 40 cm^3	49 (55.7)	25 (59.5)	24 (52.2)		26 (59.1)	23 (52.3)	
	>40 cm^3	4 (4.5)	1 (2.4)	3 (6.5)		2 (4.5)	2 (4.5)	

combination alone may not be a major contributing factor. Furthermore, individuals with the *GSTM1*-positive/*NAT2*-low genotype demonstrated a notable 3.3-fold increased risk of developing RCC (cOR = 3.3, 95% CI = 1.311-8.327, p = 0.011) with statistical significance. These findings suggest that while *GSTM1* and *NAT2* polymorphisms cooperate in RCC susceptibility.

Stage, Histology, and Tumor Characteristics

Among RCC patients, the majority were diagnosed at stage I (56.8%), followed by stage III (19.3%), stage IV (12.5%), and stage II (11.4%), indicating that a significant proportion of cases were detected in the early stages of the cancer. Histological classification revealed that clear cell RCC was the predominant subtype, accounting for 87.5% of cases, while chromophobe RCC (5.7%) and papillary RCC (6.8%) were less frequently observed. Tumor volume analysis showed that larger tumors (>40 cm^3) were uncommon (2.3%), whereas most tumors (56%) fell within the 10-40 cm^3 range, suggesting a wide

Table 4. Modifying Effects of Lifestyle Factors on Genetic Risk.

Variables	Total	Controls	RCC	cOR [95% CI]	P value	aOR [95% CI]	P value
	n (%)	n (%)	n (%)				
GSTM1/Smoking							
GSTM1-pos/non-smoker	51 (29)	29 (33)	22 (25)	1		1	
GSTM1-pos/smoker	32 (18.2)	12 (13.6)	20 (22.7)	2.227 [0.845 - 5.871]	0.106	1.468 [0.187 - 11.531]	0.715
GSTM1-null/non-smoker	64 (36.4)	41 (46.6)	23 (26.1)	0.67 [0.304 - 1.476]	0.32	0.638 [0.177 - 2.303]	0.492
GSTM1-null/smoker	29 (16.5)	6 (6.8)	23 (26.1)	4.654 [1.458 - 14.86]	0.009	2.024 [0.18 - 22.759]	0.568
NAT2/Smoking							
NAT2-high/non-smoker	70 (39.8)	46 (52.3)	24 (27.3)	1		1	
NAT2-high/smoker	32 (18.2)	12 (13.6)	20 (22.7)	2.944 [1.229 - 7.05]	0.015	2.662 [0.396 - 17.875]	0.314
NAT2-low/non-smoker	45 (25.6)	24 (27.3)	21 (23.9)	1.38 [0.548 - 3.478]	0.494	2.607 [0.651 - 10.44]	0.176
NAT2-low/smoker	29 (16.5)	6 (6.8)	23 (26.1)	6.596 [2.26 - 19.255]	0.001	2.368 [0.376 - 14.896]	0.358
GSTM1/Urinary tract diseases (UTD)							
GSTM1-pos/non-UTD	64 (36.4)	38 (43.2)	26 (29.5)	1		1	
GSTM1-pos/UTD	19 (10.8)	3 (3.4)	16 (18.2)	14.819 [1.885 - 116.499]	0.01	-	-
GSTM1-null/non-UTD	79 (44.9)	46 (52.3)	33 (37.5)	1.126 [0.534 - 2.375]	0.756	0.727 [0.228 - 2.322]	0.59
GSTM1-null/UTD	14 (8)	1 (1.1)	13 (14.8)	14.166 [1.723 - 116.467]	0.014	-	-
NAT2/Urinary tract diseases (UTD)							
NAT2-high/non-UTD	82 (46.6)	55 (62.5)	27 (30.7)	1		1	
NAT2-high/UTD	20 (11.4)	3 (3.4)	17 (19.3)	20.722 [3.59 - 119.52]	0.001	-	-
NAT2-low/non-UTD	61 (34.7)	29 (33)	32 (36.4)	3.077 [1.304 - 7.26]	0.01	1.916 [0.641 - 5.726]	0.245
NAT2-low/UTD	13 (7.4)	1 (1.1)	12 (13.6)	35.997 [3.643 - 355.7]	0.002	-	-

1, reference category; CI, confidence interval; cOR, crude odds ratio for conditional binary logistic regression; aOR, adjusted odds ratio for conditional binary logistic regression (adjusted by smoking, history of UTD)

variation in tumor size at diagnosis (Table 3).

Association between genetic polymorphisms and RCC stages

GSTM1 genotype and RCC stages

Patients with the *GSTM1*-positive genotype were most frequently diagnosed at stage I (56.8%), with fewer cases at advanced stages, including stage III (19.3%) and stage IV (12.5%). Similarly, those with the *GSTM1*-null genotype were commonly diagnosed at stage I (52.4%); however, a slightly higher proportion presented with advanced cancer stage III (21.4%) and stage IV (14.3%). Although the association was not statistically significant (p = 0.876), there was a trend suggesting that the *GSTM1*-null genotype may be linked to more advanced RCC stages.

NAT2 genotype and RCC stages

Among *NAT2* low acetylators, the majority of cases were diagnosed at stage I (70.5%), while fewer cases were observed in stage III (15.9%) and stage IV (9.1%). In contrast, *NAT2* high acetylators exhibited a more even distribution across disease stages, with 43.2% diagnosed at stage I, 22.7% at stage III, and 15.9% at stage IV. This association between *NAT2* genotype and RCC stage was statistically significant (p = 0.05), indicating that the low acetylator genotype may be associated with earlier-stage diagnosis.

Combined effects of GSTM1 and NAT2 genotypes

The combination of the *GSTM1*-null and *NAT2* low acetylator genotypes was more frequently observed in patients with advanced RCC (stage III and IV). Conversely, individuals with both *GSTM1*-positive and *NAT2* high acetylator genotypes were predominantly diagnosed at stage I. These findings suggest a potential combined effect of *GSTM1* and *NAT2* polymorphisms on RCC progression.

Modifying Effects of Lifestyle Factors on Genetic Risk

Smoking and RCC Risk

A significant interaction between smoking and genetic polymorphisms was observed in RCC risk. Among smokers, individuals carrying the *GSTM1*-null genotype exhibited a 4.65-fold increased RCC risk (cOR = 4.65, 95% CI = 1.458-14.86, p = 0.009) which is statistically significant. The risk was more pronounced among smokers with the *NAT2*-low acetylator genotype, who showed a 6.59-fold increased RCC risk (cOR = 6.596, 95% CI = 2.26-19.255, p = 0.001), further emphasizing the role of smoking as a major environmental trigger in genetically susceptible individuals (Table 4).

Urinary Tract Diseases (UTD) and RCC Risk

A history of urinary tract diseases (UTD) significantly modified RCC susceptibility. Among individuals with the *GSTM1*-null genotype and a history of UTD, RCC risk was 14.16 times higher (p = 0.014). The effect was also pronounced among *NAT2*-high acetylators with UTD, who demonstrated 20.72-fold increased RCC risk. The highest risk was observed among *NAT2*-low acetylators with UTD, with a 35.99-fold increased RCC risk (p = 0.002). These findings highlight the critical role of renal health in modifying genetic susceptibility to RCC, suggesting that individuals with both genetic and clinical risk factors should be prioritized for early detection and preventive interventions (Table 4).

Discussion

This study investigated the association between *GSTM1* and *NAT2* genetic polymorphisms and renal cell carcinoma (RCC) risk in the Mongolian population, with a focus on their interaction with smoking, alcohol consumption, and urinary tract diseases (UTD). Our findings suggest that *NAT2* low acetylator status and specific alleles (notably WT/M3) significantly increase susceptibility to RCC, particularly in the presence of smoking and urinary tract diseases (UTDs). By contrast, *GSTM1*-null status alone was not independently associated with RCC risk, although its effect was amplified when combined with environmental exposures. We also revealed that smoking, alcohol consumption and UTD were the most significant risk factors for RCC, while specific *NAT2* polymorphisms exhibited notable effects on susceptibility.

Our results indicate that the *GSTM1*-null genotype was not independently associated with RCC risk (p = 0.869), consistent with previous studies that found no direct link between *GSTM1* deletion and RCC [23]. However, the impact of *GSTM1*-null genotype was amplified in the presence of environmental risk factors, particularly smoking and UTD. Smokers carrying the *GSTM1*-null genotype showed a markedly increased RCC risk, which aligns with evidence that the loss of *GSTM1* function impairs detoxification of tobacco-derived carcinogens. Similarly, the association between *GSTM1*-null genotype and RCC among individuals with UTD suggests that chronic inflammation and oxidative stress may exacerbate underlying genetic vulnerabilities. In contrast, *NAT2* polymorphisms demonstrated a more direct role in RCC susceptibility. We observed that *NAT2* low acetylator genotypes, as well as specific variants (WT/M1, M2/M2, and WT/M3), were strongly associated with elevated RCC risk. Notably, WT/M3 carriers exhibited the highest adjusted risk. This is in line with prior research linking *NAT2* slow acetylators to bladder cancer, suggesting that reduced acetylation capacity results in inefficient detoxification of aromatic amines and other carcinogens, thereby promoting DNA damage in renal tissue. Our findings provide novel evidence that these mechanisms also extend to RCC in the Mongolian population. Importantly, the strongest risks were observed when genetic susceptibility coincided with environmental exposures. *NAT2* low acetylator smokers had a more than six-fold increased RCC risk, while individuals with both *NAT2* low acetylator genotype and UTD history had a nearly 36-fold higher risk compared to controls. These results highlight the synergistic role of genetic and environmental factors in renal carcinogenesis, emphasizing the need for targeted prevention strategies for high-risk groups. [23, 24]. Furthermore, the *GSTM1*-

null genotype was significantly associated with RCC risk among individuals with a history of UTD (p = 0.014). This suggests that chronic inflammation, oxidative stress, and renal dysfunction may exacerbate genetic susceptibility, ultimately increasing RCC risk [24]. These findings highlight the need for early monitoring of individuals with UTD who carry the *GSTM1*-null genotype, as they may be at higher risk for RCC progression.

Unlike *GSTM1*, *NAT2* polymorphisms exhibited a more significant association with RCC susceptibility. The *NAT2*-low acetylator genotype was observed in 50% of RCC cases and 34.1% of controls, indicating a significant RCC risk (p = 0.03). Previous research has linked *NAT2*-low acetylators with increased susceptibility to bladder cancer due to their reduced ability to detoxify aromatic amines [25, 26]. Similarly, our findings suggest that in RCC, *NAT2*-low acetylators may accumulate fewer carcinogenic metabolites in renal tissue, potentially influencing a different detoxification pathway in kidney cells. Analysis of specific *NAT2* polymorphisms revealed strong associations with RCC risk, with the WT/M1 genotype linked to a 3.667-fold increase (p = 0.005), the M2/M2 genotype associated with a 4.691-fold elevation (p = 0.009), and the WT/M3 genotype showing the strongest effect, with a 4.8-fold increased risk (p = 0.001).

These findings suggest that specific *NAT2* polymorphisms impair the detoxification of carcinogens, leading to increased DNA damage and tumor formation in renal tissue. Further studies are needed to explore the functional mechanisms of these polymorphisms in RCC development [27]. Our study provides preliminary insights into the genetic susceptibility of RCC within a high-altitude population characterized by unique environmental exposures, such as chronic hypoxia, dietary habits, air pollution and exposure to heavy metals in drinking water. These factors may amplify the impact of genetic polymorphisms, contributing to RCC development. By focusing on a population with distinct environmental conditions, our findings may offer valuable insights into how genetic and environmental factors collectively influence RCC risk.

In addition to the *GSTM1* polymorphism investigated in this study, future research should also explore other glutathione S-transferase (GST) polymorphisms, including GSTT1 and GSTP1 [28]. These enzymes play crucial roles in cellular detoxification by catalyzing the conjugation of glutathione to a wide range of electrophilic compounds, including carcinogens. The GSTT1 and GSTP1 polymorphisms have been extensively studied in various cancers and are known to influence cancer susceptibility through their impact on detoxification capacity. Understanding the combined effects of *GSTM1*, GSTT1, and GSTP1 polymorphisms on RCC risk could provide a more comprehensive assessment of the role of GST genes in renal carcinogenesis.

Moreover, larger-scale studies should aim to perform detailed subgroup analyses, exploring the interactions between genetic polymorphisms and various environmental exposures, including smoking, hypertension, alcohol consumption, and urinary tract diseases. Such analyses could elucidate how these factors synergize with genetic susceptibility to increase RCC risk. For instance, individuals with *NAT2* low acetylator genotypes may exhibit higher RCC risk when exposed to tobacco smoke, while *GSTM1*-null genotypes could confer increased susceptibility in the presence of urinary tract diseases.

Additionally, future studies should consider the influence of other RCC-related genetic polymorphisms, such as VHL, MET, and PBRM1, which are known to play critical roles in RCC pathogenesis [29]. Expanding the genetic scope of investigation would allow for a more comprehensive understanding of the molecular mechanisms underlying RCC in the Mongolian population.

Given the high-altitude environment of Mongolia, which is associated with chronic hypoxia, further studies could also explore the potential impact of hypoxia-related genes on RCC susceptibility. Hypoxia-inducible factors (HIFs), which regulate cellular responses to low oxygen levels, may interact with genetic polymorphisms to modulate RCC risk. Understanding these interactions could uncover novel insights into the unique aspects of RCC pathogenesis in high-altitude populations.

Previous studies have demonstrated the combined effects of multiple GST polymorphisms (*GSTM1*, *GSTT1*, and *GSTP1*) on susceptibility to urogenital cancers [30]. For instance, a meta-analysis revealed that individuals with *GSTM1*-null, *GSTT1*-null, and combined *GSTM1*/*GSTT1* double-null genotypes exhibited an increased risk of bladder cancer. Similar associations were observed in prostate cancer, where individuals carrying both *GSTM1* and *GSTT1*-null genotypes showed higher cancer susceptibility [28]. In renal cell carcinoma, certain combinations, such as *GSTM1*-*GSTT1* and *GSTT1*-*GSTP1* dual null genotypes, were linked to an elevated RCC risk. These findings highlight the importance of investigating multiple GST polymorphisms in future research to fully understand their combined impact on RCC risk in the Mongolian population.

This study demonstrated that environmental exposures significantly modified the genetic risk of renal cell carcinoma (RCC), indicating a strong gene–environment interaction. The most pronounced associations were observed in relation to smoking and urinary tract disorders (UTD). Individuals with the *NAT2* low acetylator genotype who smoked had a 6.59-fold increased risk of RCC (p = 0.001), supporting the hypothesis that slow acetylators process carcinogens less efficiently, leading to a greater accumulation of toxic metabolites in renal tissue [12]. The strongest interaction was found among individuals with both the *NAT2* low acetylator genotype and a history of UTD, who exhibited a 35.99-fold increased risk of RCC (p = 0.002). This finding suggests that chronic renal dysfunction may amplify the carcinogenic effects of *NAT2* polymorphisms, thereby substantially increasing RCC susceptibility. While our main analyses focused on biologically plausible variants, we acknowledge that testing multiple genotypes increases the chance of false positives. Therefore, results should be interpreted with caution. Future studies with larger cohorts may benefit from applying statistical correction methods, such as Bonferroni adjustment, to minimize type I error. In

conclusion, this study shows that the *NAT2* low acetylator genotype was significantly associated with increased RCC risk, with the WT/M3 genotype showing the strongest individual association and individuals carrying both the *GSTM1*-positive and *NAT2* low acetylator genotypes had higher risk of RCC in Mongolian population. The *GSTM1*-null genotype alone was not significantly associated with RCC risk. However, when combined with smoking and UTD, the *GSTM1*-null genotype significantly increased RCC susceptibility, emphasizing the importance of gene-environment interactions.

Author Contribution Statement

G.B, M.T and Sh.A conceived the project and designed the research. G.B, M.P, Ts.Sh, N.D, S.B, M.T and Sh.A contributed to study conception, planning experiments and technical support. G.B, M.T and Sh.A conducted data analysis and data interpretation. G.B, M.T and Sh.A participated in the result discussion and technical support. G.B, M.T and Sh.A wrote the manuscript. All authors read and approved the final.

Acknowledgements

General

We sincerely thank all participants for their contribution and acknowledge the support of FCHM, MCCH, Bionorica, and the Mongolian National University of Medical Science.

Approval

This study was approved by the Scientific Committee of the Mongolian National University of Medical Sciences.

Ethical Declaration

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Scientific Research Committee of the Mongolian National University of Medical Sciences. Written informed consent was obtained from all participants before enrollment.

Conflict of Interest

None.

References

1. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. Nat Rev Dis Primers. 2017;3:17009. https://doi.org/10.1038/nrdp.2017.9.
2. Makhov P, Joshi S, Ghatalia P, Kutikov A, Uzzo RG, Kolenko VM. Resistance to systemic therapies in clear cell renal cell carcinoma: Mechanisms and management strategies. Mol Cancer Ther. 2018;17(7):1355-64. https://doi.org/10.1158/1535-7163.MCT-17-1299.
3. Bellin MF, Valente C, Bekdache O, Maxwell F, Balasa C, Savignac A, et al. Update on renal cell carcinoma diagnosis with novel imaging approaches. Cancers (Basel). 2024;16(10). https://doi.org/10.3390/cancers16101926
4. Sandagdorj T, Sanjaajamts E, Tudev U, Oyunchimeg D, Ochir C, Roder D. Cancer incidence and mortality in mongolia - national registry data. Asian Pac J Cancer Prev. 2010;11(6):1509-14.
5. Chow WH, Dong LM, Devesa SS. Epidemiology and risk factors for kidney cancer. Nat Rev Urol. 2010;7(5):245-57. https://doi.org/10.1038/nrurol.2010.46.
6. Qayyum T, Oades G, Horgan P, Edwards J. The epidemiology and risk factors for renal cancer. Current urol. 2013;6:169-74. https://doi.org/10.1159/000343534.
7. Jin X, Qiu T, Li L, Yu R, Chen X, Li C, et al. Pathophysiology of obesity and its associated diseases. Acta Pharm Sin B. 2023;13(6):2403-24. https://doi.org/10.1016/j.apsb.2023.01.012.
8. Flaherty KT, Fuchs CS, Colditz GA, Stampfer MJ, Speizer FE, Willett WC, et al. A prospective study of body mass index, hypertension, and smoking and the risk of renal cell carcinoma (united states). Cancer Causes Control. 2005;16(9):1099-106. https://doi.org/10.1007/s10552-005-0349-8.
9. Cote ML, Colt JS, Schwartz KL, Wacholder S, Ruterbusch JJ, Davis F, et al. Cigarette smoking and renal cell carcinoma risk among black and white americans: Effect modification by hypertension and obesity. Cancer Epidemiol Biomarkers Prev. 2012;21(5):770-9. https://doi.org/10.1158/1055-9965.Epi-11-1109.
10. Brownson RC. A case-control study of renal cell carcinoma in relation to occupation, smoking, and alcohol consumption. Arch Environ Health. 1988;43(3):238-41. https://doi.org/10.1080/00039896.1988.9934940.
11. Shuch B, Zhang J. Genetic predisposition to renal cell carcinoma: Implications for counseling, testing, screening, and management. J Clin Oncol. 2018:Jco2018792523. https://doi.org/10.1200/jco.2018.79.2523.
12. McGrath M, Michaud D, Vivo I. Polymorphisms in gstt1, gstm1, nat1 and *NAT2* genes and bladder cancer risk in men and women. BMC cancer. 2006;6:239. https://doi.org/10.1186/1471-2407-6-239.
13. Matheson MC, Stevenson T, Akbarzadeh S, Propert DN. Gstt1 null genotype increases risk of premenopausal breast cancer. Cancer Lett. 2002;181(1):73-9. https://doi.org/10.1016/S0304-3835(02)00046-0.
14. Cotton SC, Sharp L, Little J, Brockton N. Glutathione s-transferase polymorphisms and colorectal cancer: A huge review. Am J Epidemiol. 2000;151(1):7-32. https://doi.org/10.1093/oxfordjournals.aje.a010124.
15. da Silva TD, Felipe AV, de Lima JM, Oshima CT, Forones NM. N-acetyltransferase 2 genetic polymorphisms and risk of colorectal cancer. World J Gastroenterol. 2011;17(6):760-5. https://doi.org/10.3748/wjg.v17.i6.760.
16. Eggermont JJ. Epidemiology and genetics of hearing loss and tinnitus. Hearing Loss. 2017:209-34.
17. McDonagh EM, Boukouvala S, Aklillu E, Hein DW, Altman RB, Klein TE. Pharmgkb summary: Very important pharmacogene information for n-acetyltransferase 2. Pharmacogenet Genomics. 2014;24(8):409-25. https://doi.org/10.1097/fpc.0000000000000062.
18. R. L. Yung and B. C. Richardson, "Pathophysiology of Drug-Induced Lupus" Systemic Lupus Erythematosus, Fourth Edition, pp. 1185–1210; 2004.
19. Pengpid S, Peltzer K. Trends in concurrent tobacco use and heavy drinking among individuals 15 years and older in mongolia. Sci Rep. 2022;12:16639. https://doi.org/10.1038/s41598-022-21094-7.
20. Dugee O, Khor GL, Lye MS, Luvsannyam L, Oyunbileg J, Jamyan B, et al. Association of major dietary patterns with obesity risk among mongolian men and women. Asia Pac J Clin Nutr. 2009;18:433-40. https://doi.org/10.6133/APJCN.2009.18.3.16
21. Bigham AW. Genetics of human origin and evolution: High-

altitude adaptations. Curr Opin Genet Dev. 2016;41:8-13. https://doi.org/10.1016/j.gde.2016.06.018.
22. Zupa A, Sgambato A, Bianchino G, Improta G, Grieco V, G LAT, et al. Gstm1 and *NAT2* polymorphisms and colon, lung and bladder cancer risk: A case-control study. Anticancer Res. 2009;29(5):1709-14.
23. Engel LS, Taioli E, Pfeiffer R, Garcia-Closas M, Marcus PM, Lan Q, et al. Pooled analysis and meta-analysis of glutathione s-transferase m1 and bladder cancer: A huge review. Am J Epidemiol. 2002;156(2):95-109. https://doi.org/10.1093/aje/kwf018.
24. Hung RJ, Boffetta P, Brennan P, Malaveille C, Hautefeuille A, Donato F, et al. Gst, nat, sult1a1, cyp1b1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. Int J Cancer. 2004;110(4):598-604. https://doi.org/https://doi.org/10.1002/ijc.20157.
25. García-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, et al. *NAT2* slow acetylation, gstm1 null genotype, and risk of bladder cancer: Results from the spanish bladder cancer study and meta-analyses. Lancet. 2005;366(9486):649-59. https://doi.org/10.1016/s0140-6736(05)67137-1.
26. Avirmed S, Khuanbai Y, Sanjaajamts A, Selenge B, Dagvadorj BU, Ohashi M. Modifying effect of smoking on gstm1 and *NAT2* in relation to the risk of bladder cancer in mongolian population: A case-control study. Asian Pac J Cancer Prev. 2021;22(8):2479-85. https://doi.org/10.31557/apjcp.2021.22.8.2479.
27. Zeegers MP, Tan FE, Dorant E, van Den Brandt PA. The impact of characteristics of cigarette smoking on urinary tract cancer risk: A meta-analysis of epidemiologic studies. Cancer. 2000;89(3):630-9. https://doi.org/10.1002/1097-0142(20000801)89:3<630::aid-cncr19>3.3.co;2-h.
28. Gong M, Dong W, Shi Z, Yangyang X, Ni W, Ruihua R. Genetic polymorphisms of gstm1, gstt1, and gstp1 with prostate cancer risk: A meta-analysis of 57 studies. PloS one. 2012;7:e50587. https://doi.org/10.1371/journal.pone.0050587.
29. Xie D, Li G, Zheng Z, Zhang X, Wang S, Jiang B, et al. The molecular code of kidney cancer: A path of discovery for gene mutation and precision therapy. Mol Aspects Med. 2025;101:101335. https://doi.org/https://doi.org/10.1016/j.mam.2024.101335.
30. yu C, Hequn C, Longfei L, Wang L, Zhi C, Feng Z, et al. Gstm1 and gstt1 polymorphisms are associated with increased bladder cancer risk: Evidence from updated meta-analysis. Oncotarget. 2016;8:3246. https://doi.org/10.18632/oncotarget.13702.