RESEARCH ARTICLE

Editorial Process: Submission:05/27/2025 Acceptance:10/02/2025 Published:10/19/2025

Evaluation of Genetic Polymorphisms of *HOTTIP* (rs5883064, rs1859168, rs2067087) in Patients with Non-Hodgkin Lymphoma and Comparison with Healthy Subjects

Masoomeh Sadraei[1,2], Seyed Mehdi Hashemi[3], Hossein Mozaffari[1,2], Mohsen Taheri[4], Reza Mahdizadeh[1], Gholamreza Bahari[1,5]*

Abstract

Background: Non-Hodgkin Lymphoma (NHL) is a group of blood cancers in which lymphoid cells develop abnormal growth due to genetic changes or other factors without Reed-Sternberg cells. Given the proven role of *HOTTIP* gene polymorphisms in the prognosis, diagnosis, and treatment of different kinds of carcinomas, this study investigated the association between rs5883064, rs1859168, and rs2067087 polymorphisms of the gene with NHL susceptibility in a population of Zahedan. **Materials and Methods:** A total of 190 NHL cases (122 males and 68 females), aged 44.45±15.29 years, and 190 healthy individuals (107 males and 83 females, 43.13±12.15 years) were collected as study subjects. The gene polymorphisms were examined by PCR RFLP and ARMS methods. **Results:** Our results showed that the rs5883064 Del>Ins polymorphism of the *HOTTIP* gene has a protective role and significantly reduces the risk of lymphoma. The 1859168 C>A variant in the co-dominant model is a risk criterion for non-Hodgkin lymphoma and significantly increases this disease's risk. The *HOTTIP* rs2067087 G>C variant had a protective role and significantly reduced the risk of lymphoma. **Conclusion:** Our results showed that the association between *HOTTIP* gene polymorphisms and NHL incidence has a significant pattern. More studies in larger populations and various ethnicities are recommended to confirm our findings.

Keywords: Non-Hodgkin lymphoma- polymorphism- *HOTTIP*

Asian Pac J Cancer Prev, **26 (10)**, 3857-3864

Introduction

Lymphomas, a heterogeneous group of cancers, arise from the clonal proliferation of B-cell, T-cell, and natural killer (NK) cell subsets at various stages of maturation [1]. Lymphomas are broadly categorized into non-Hodgkin's lymphoma (NHL), which accounts for approximately 90% of cases, and Hodgkin's lymphoma (HL), which comprises the remaining 10%. NHL is categorized based on the lineage of the malignant cells into B-cell, T-cell, and natural killer-cell subtypes, whereas Hodgkin lymphoma is subdivided into classical and nodular lymphocyte-predominant (non-classical) forms. Clinically, lymphomas are classified as either indolent (low grade) or aggressive (high grade) [2].

According to 2022 data, NHL ranks as the 11th most frequently diagnosed malignancy and the 11th leading cause of cancer-related mortality worldwide [3]. GLOBOCAN estimates from 2018 reported approximately 509,600 new cases globally, accounting for 2.8% of all cancer diagnoses. In that same year, NHL was responsible for about 248,700 deaths, or 2.6% of all deaths from cancer. Between 2010 and 2016, the five-year survival rate for NHL in the United States was 72.7%, representing a notable 158% improvement over the 46% five-year survival rate initially documented in 1975 [4]. Despite a substantial global increase in NHL incidence, its etiology remains largely unknown. According to preliminary research, genetic variations involving immune deficiency pathways may be linked to a higher or lower risk of NHL development [5].

There are two categories of risk factors for NHL: modifiable and non-modifiable. Radiation exposure, chemical exposure, obesity, alcohol use, smoking, breast implants, and vitamin deficiencies are all modifiable risk factors. On the other hand, non-modifiable factors include autoimmune diseases, immune system suppression, age, gender, race and ethnicity, and family history [4].

[1]*Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.* [2]*Student Research Committee, Zahedan University of Medical Sciences. Zahedan, Iran.* [3]*Clinical Immunology Research Center, Department of Internal Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.* [4]*Genetics of Non- Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.* [5]*Children and Adolescent Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran. *For Correspondence: r_b_1333@yahoo.com*

LncRNAs have been shown in numerous studies to be essential for both tumorigenesis and tumor progression [6]. In particular, DNA damage, immune system evasion, and cellular metabolic dysregulation in cancer cells may all be linked to aberrant lncRNA expression. The complexity of tumorigenesis is further enhanced by the diversity and functional heterogeneity of lncRNAs. Furthermore, the regulation of cell lineage and the epithelial-mesenchymal transition are closely associated with lncRNAs. All of these results point to lncRNAs as important players in tumor metastasis. As a result, lncRNA targeting may be a promising clinical approach to cancer treatment [7].

HOTTIP (HOXA transcript at the distal tip) is a long non-coding RNA (lncRNA) 3,764 nucleotides long. At the 5' end of the HOXA gene cluster, the gene encoding *HOTTIP* coordinates the activation of several genes in the 5' HOXA cluster [8]. *HOTTIP* exerts its function by directly interacting with the WD repeat-containing protein 5 (WDR5), thereby facilitating the recruitment of WDR5/MLL complexes along the HOXA locus. This recruitment promotes histone H3 lysine 4 (H3K4) methylation, ultimately leading to transcriptional activation of HOXA genes. Emerging evidence suggests that *HOTTIP* is critically involved in the pathogenesis of a broad spectrum of human cancers [9].

Clinical studies have demonstrated that *HOTTIP* is frequently upregulated in cancerous tissues compared to corresponding non-malignant counterparts [10]. Additionally, its expression levels and the severity of the disease are correlated, either rising or falling in afflicted individuals. Taken together, these findings support the view of *HOTTIP* as a widely recognized oncogenic lncRNA implicated in most human cancer types and has been known as both a potential therapeutic target and a biomarker for cancer diagnosis and prognosis [11].

Extensive evidence suggests that genetic variations in the form of single nucleotide polymorphisms (SNPs) can arise within lncRNAs, potentially altering their expression and regulatory functions [12, 13]. In addition, the association between *HOTTIP* SNPs and various complications, including cancer, has been reported previously [14, 15]. The rs5883064 is a variant in a regulatory region, and it was reported that the *HOTTIP* rs5883064 had an association with raised risk of lung cancer [11]. The rs2067087 is localized within the second exon of the *HOTTIP* gene and has been implicated in gastric carcinogenesis through the upregulation of mature *HOTTIP* expression [16]. Additionally, the rs1859168 variant resides within an intronic region of the *HOTTIP* gene. Although introns are not present in the mature form of long non-coding RNAs, they can influence transcriptional regulation and alternative splicing, potentially affecting gene expression. The rs1859168 reportedly decreased the occurrence of tumorigenesis by *HOTTIP* downregulation [17].

Accordingly, this study investigated the potential association between polymorphisms within the *HOTTIP* gene and susceptibility to non-Hodgkin's lymphoma. To this end, we compared the *HOTTIP* gene rs5883064, rs1859168, and rs2067087 SNPs in patients with non-Hodgkin's lymphoma to those in an Iranian population who are healthy.

Materials and Methods

subjects

This investigation was planned as a case-control study. All patients with NHL referred to Ali Ibn Abi-Talib Hospital had their diagnosis verified by a pathologist, and who had no underlying medical conditions were included in the case group. These patients had blood samples taken. Blood samples from people with no known systemic diseases who were routinely checked out at Ali Ibn Abi-Talib Hospital and private clinics in Zahedan were included in the control group. The study included 190 NHL patients (122 men and 68 women) with an average age of 45.44 ± 15.29 years and 190 healthy people (107 men and 83 women) with an average age of 43.13 ± 12.15 years.

Genotyping

Five milliliters of peripheral blood were collected from patients and healthy control individuals and kept at -20°C. Genomic DNA was extracted via the salting-out technique. Using the PCR-RFLP and ARMS-PCR methods, the samples were genotyped. Specific primers were designed to identify genetic polymorphisms (Table 1). Following the optimization of PCR conditions, the samples underwent PCR under ideal circumstances. The RFLP method allowed for the determination of the samples' genotypic profile by digesting the amplified products with particular restriction enzymes, as presented in Table 1, and then electrophoresing them on an agarose gel.

PCR amplification for genotyping was performed using 20 μL reaction volumes. Each reaction tube for the PCR-RFLP method contained 1 μL of genomic DNA (~100 ng/μL), 1 μL of each primer, 10 μL of master mix, and 7 μL of double-distilled water (ddH$_2$O). The thermal cycling conditions for rs5883064 were as follows: initial denaturation at 95°C for 7 minutes; 30 cycles of 95°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds; followed by a final extension at 72°C for 10 minutes. For rs1859168, the PCR conditions were: initial denaturation at 95°C for 6 minutes; 30 cycles of 95°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 5 minutes. PCR products (10 μL) were subsequently digested using specific restriction enzymes for genotyping.

For analysis of the *HOTTIP* rs2067087 variant, the amplification-refractory mutation system PCR (ARMS-PCR) technique was employed. Each 20 μL reaction contained 1 μL of genomic DNA (~100 ng/μL), 1 μL of each primer, 10 μL of master mix, and 7 μL of ddH$_2$O. The cycling conditions were: initial denaturation at 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. To ensure genotyping accuracy, approximately 20% of the samples were randomly selected for repeat genotyping, and the results showed 100% concordance.

Statistical Analysis

All statistical analyses were conducted using SPSS 22

Table 1. The Primers Used for Detection of HOTTIP rs5883064, rs1859168, rs2067087 Polymorphisms

HOTTIP SNPs	PCR primers (5'→3')	Restriction Enzyme	Fragment, bp
rs5883064 Del/Ins	F: CTGGTCAGACACGGCTGGAG	Hinf1	Del=227
	R: AGTCGGTAAACACCGCCCCAA		Ins=162+65
rs1859168 C/A	F: GGAGCCACAAGCAGGTTTGTCTG	HaeIII	A=263
	R: AGCAGGGTGCCTTCCTCTAAAT		C=148+115
rs2067087 G/C	F1(C Allele): GCCTAGAACCCTACTCTGTCTG	-	Product Size= 51
	F2(G Allele): GCCTAGAACCCTACTCTGTCTC		
	R:AGTGGCATTGTAAAACTCACAGTGACAAA		
B2M	F: TGTAAACACTTGGTGCCTGATATAGCTTGA	-	Product Size= 574
	R: CATCAGTATCTCAGCAGGTGCCACTAATCT		

software. Continuous variables were compared using the independent samples t-test, while categorical variables were analyzed using the chi-square (χ^2) test. Logistic regression analysis was applied to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs). A p-value of less than 0.05 was considered statistically significant.

Results

The study enrolled 190 pathologically confirmed NHL patients. No statistically noticeable differences were observed between the groups for sex and age (p> 0.05).

All three investigated SNPs of the *HOTTIP* gene rs5883064, rs1859168, and rs2067087 were found to be in Hardy-Weinberg equilibrium in the control group, with p-values of 0.996, 0.909, and 0.894, respectively. These results confirm that the observed genotype distributions are consistent with expected Mendelian proportions and support the validity of the genotyping process, minimizing the likelihood of genotyping errors or population stratification Figure 1.

Genotypic and allelic frequencies of *HOTTIP* rs5883064, rs1859168, and rs2067087 variants in Non-Hodgkin Lymphoma patients and controls are shown in Table 2. The results showed that rs5883064 Del>Ins variant of the *HOTTIP* gene significantly decreased the risk of lymphoma; codominant (OR = 0.494, 95% CI = 0.316–0.772, P=0.002, Del/Ins vs Del/Del; OR = 0.414, 95% CI = 0.226–0.760, p = 0. 004, Ins/Ins vs Del/Del), dominant (OR = 0.471, 95% CI = 0.310–0.716, P<0.001, Del/Ins + Ins/Ins vs Del/Del) and allelic (OR = 0.589, 95% CI = 0.438–0.792, p <0.001, Ins vs Del) inheritance models were utilized. The 1859168 C>A variant, under the codominant model, significantly increased the risk of lymphoma (OR = 1.597, 95% CI = 1.012–2.518, P=0.044, C/A vs C/C). But, recessive (OR = 0.448, 95% CI = 0.244–0.821, P=0.008, A/A vs C/A+C/C) decreased the risk of lymphoma. The *HOTTIP* rs2067087 G>C variant significantly decreased the risk of lymphoma; codominant (OR=0.368, 95% CI= 0.193-0.701, P=0.002, C/C vs G/G), recessive (OR = 0.404, 95% CI = 0.225- 0.726, P=0.002, C/C vs G/G+G/C) and allelic (OR = 0.667, 95% CI = 0.499- 0.892, p = 0.006, C vs G) inheritance models Figure 2.

The haplotype analysis results between NHL patients and healthy controls are summarized in Table 3. The haplotype analysis result revealed that Ins/A/G and Ins/C/C haplotype compare with Del/C/G, were significantly associated with the risk of NHL (OR=0.35, 95%CI= 0.16-0.77, P= 0.009; OR= 0.29, 95% CI= 0.13- 0.65, P= 0.0028 respectively). These haplotypes were significantly lower in NHL patients Figure 3.

Table 4 summarizes the association between the three

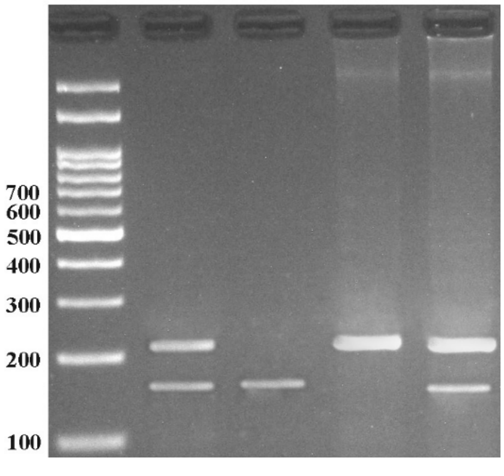

Figure 1. Electrophoresis Pattern of HOTTIP rs5883064 polymorphism. M: DNA marker, lanes 1,4: Del/Ins; lane2: Ins and lane 3: Del

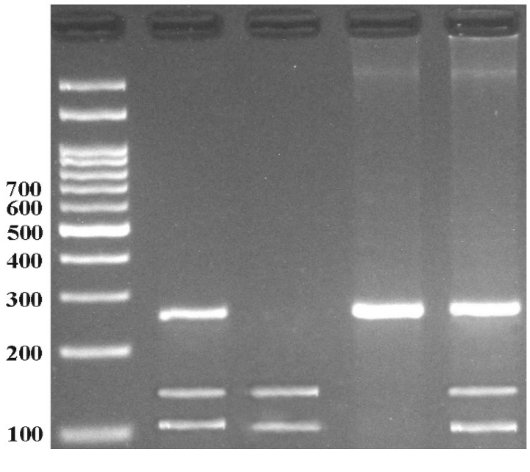

Figure 2. Electrophoresis Pattern of HOTTIP rs1859168 polymorphism. M: DNA marker, lanes 1,4: CA; lane2: CC and lane 3: AA

Table 2. Association of *HOTTIP* (rs5883064, rs1859168, rs2067087), Polymorphisms and the Risk of NHL

Polymorphism	Case n(%)	Control n(%)	OR(95% CI)	P- Value
rs5883064				
Codominant				
Del/Del	94 (49.5)	60 (31.6)	Ref	-
Del/Ins	72 (37.9)	93 (48.9)	0.494 (0.316-0.772)	0.002
Ins/Ins	24 (12.6)	37(19.5)	0.414 (0.226-0.760)	0.004
Dominant				
Del/Del	94 (49.5)	60 (31.6)	Ref	-
Del/Ins+Ins/Ins	96 (50.5)	130 (68.4)	0.471 (0.310- 0.716)	< 0.001
Recessive				
Del/Del+Del/Ins	166 (87.4)	153 (80.5)	Ref	-
Ins/Ins	24 (12.6)	37 (19.5)	0.598 (0.342- 1.045)	0.069
Allele				
Del	260 (68.4)	213(56.1)	Ref	-
Ins	120 (31.6)	167(43.9)	0.589 (0.438- 0.792)	< 0.001
rs1859168				
Codominant				
C/C	53 (27.9)	64 (33.7)	Ref	-
C/A	119 (62.6)	90 (47.4)	1.597 (1.012-2.518)	0.044
A/A	18 (9.5)	36 (18.9)	0.604 (0.308-1.183)	0.142
Dominant				
C/C	53 (27.9)	64 (33.7)	Ref	-
C/A+A/A	137 (72.1)	126 (66.3)	1.313 (0.848- 2.033)	0.222
Recessive				
C/C+C/A	172 (90.5)	154 (81.1)	Ref	-
A/A	18 (9.5)	36 (18.9)	0.448 (0.244- 0.821)	0.008
Allele				
C	225 (59.2)	218 (57.4)	Ref	-
A	155 (40.8)	162 (42.6)	0.927 (0.695- 1.237)	0.606
rs2067087				
Codominant				
G/G	73 (38.4)	58 (30.5)	Ref	-
G/C	98 (51.6)	91 (47.9)	0.856 (0.547-1.339)	0.495
C/C	19 (10.0)	41 (21.6)	0.368 (0.193-0.701)	0.002
Dominant				
G/G	73 (38.4)	58 (30.5)	Ref	-
G/C+C/C	117 (61.6)	132 (69.5)	0.704 (0.460- 1.077)	0.105
Recessive				
G/G+G/C	171 (90.0)	149 (78.4)	Ref	-
C/C	19 (10.0)	41 (21.6)	0.404 (0.225- 0.726)	0.002
Alleles				
G	244 (64.2)	207 (54.5)	Ref	-
C	136 (35.8)	173 (45.5)	0.667 (0.499-0.892)	0.006

HOTTIP polymorphisms (rs5883064, rs1859168, and rs2067087) and various clinicopathological characteristics of NHL patients, including age, histological subtype, disease stage, treatment regimen, radiotherapy status, and recurrence type. The distribution of genotypes across these clinical subgroups did not reveal any statistically significant associations (all p-values > 0.05). The genotype frequencies of rs5883064 did not differ significantly between patients aged ≤50 and >50 years (p = 0.335), and no significant differences were observed between diffuse large B-cell lymphoma (DLBCL) and other subtypes for any of the SNPs. Similarly, treatment type (R-CHOP vs. others), disease stage, and relapse status were not associated with variant distribution, suggesting these

Table 3. Association of *HOTTIP* Haplotypes and Risk of NHL

rs5883064	rs1859168	rs2067087	Case (frequency)	control (frequency)	OR (95%CI)	P-value
Del	C	G	0.2932	0.1867	1	-
Del	A	G	0.1693	0.1231	0.84(0.39- 1.79)	0.65
Del	C	C	0.1265	0.152	0.56 (0.25 - 1.23)	0.15
Ins	C	G	0.1027	0.1112	0.69 (0.31 - 1.55)	0.37
Ins	A	G	0.0768	0.1238	0.35 (0.16 - 0.77)	0.009
Ins	C	C	0.0696	0.1238	0.29 (0.13 - 0.65)	0.0028
Del	A	C	0.0951	0.0988	0.56 (0.28 - 1.13)	0.11
Ins	A	C	0.0666	0.0807	0.63 (0.26 - 1.52)	0.31

OR, Odds Ratio; CI, Confidence Interval; Significant p-values are shown in bold (p < 0.05)

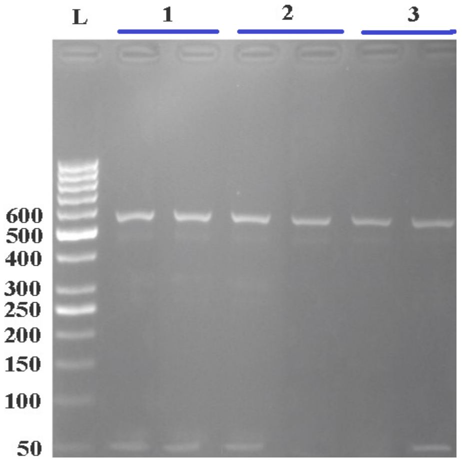

Figure 3. Electrophoresis Pattern of HOTTIP rs2067087 polymorphism. M: DNA marker, lanes 1: GC; lane2: GG and lane 3: CC. Product size for rs2067087= 51 bp and for B2M=574 bp

polymorphisms may not influence disease progression or treatment response.

Discussion

The current case-control study investigated the association between *HOTTIP* gene polymorphisms and susceptibility to NHL. Our findings revealed that the rs5883064 polymorphism in the *HOTTIP* gene significantly reduced the risk of lymphoma. Conversely, the rs1859168 variant was associated with a statistically significant increase in lymphoma risk under the codominant model. Furthermore, it was discovered that the rs2067087 polymorphism in *HOTTIP* considerably reduced the risk of developing lymphoma.

Genetic variations can be very important for determining the prognosis, treating cancer, and preventing

Table 4. Association of *HOTTIP* (rs5883064, rs1859168 and rs2067087) Polymorphisms with Clinicopathological Characteristics of NHL Patients

Characteristic of Patients	rs5883064			P- value	rs1859168			P-value	rs2067087			P-Value
	Del/Del	Del/Ins	Ins/Ins	0.335	C/C	C/A	A/A	0.648	G/G	G/C	C/C	0.542
Age												
≤50	56	50	17		32	80	11		44	67	12	
>50	38	22	7		21	39	7		29	31	7	
Histology				0.347				0.151				0.527
DLBCL	43	34	15		22	64	6		35	50	7	
Others	50	38	15		30	55	12		37	48	12	
Stage				0.355				0.215				0.49
I	51	43	10		33	65	6		40	51	13	
II	13	8	5		8	15	3		12	11	3	
III	8	3	4		2	12	1		5	8	2	
IV	22	18	4		10	26	8		16	27	1	
Treatment				0.434				0.139				0.623
R-CHOP	53	38	10		32	63	6		39	50	12	
Others	41	34	14		21	56	12		34	48	7	
Radio Therapy				261				0.309				0.909
No	85	59	20		49	100	15		63	84	17	
Yes	9	13	4		4	19	3		10	14	2	
Kind				0.246				0.344				0.306
Primary	84	66	24		49	107	18		65	90	19	
Recurrent	10	6	0		4	12	0		8	8	0	

it. According to our findings and related research, using genetic methods to target *HOTTIP* and control its expression may aid in preventing the growth and metastasis of cancer cells, thereby improving patients' capacity to fight the illness more successfully [18, 19]. Certain lncRNAs exhibit dysregulated expression across various cancer types and demonstrate pronounced tissue specificity [20]. They are potential biomarkers for cancer diagnosis because they can be non-invasively extracted from circulating blood.

Recent studies elucidated the role of *HOTTIP* in normal hematopoiesis and lymphoid development. For instance, a 2023 comprehensive review on lncRNA signatures in lymphopoiesis and lymphoid malignancies reported that *HOTTIP* is actively expressed during early stages of B-cell and T-cell lineage commitment, suggesting its involvement in lymphoid differentiation and cell fate decisions [21].

In at least some cancer types, *HOTTIP* has been linked to immune evasion. Targeting tumor cells and modifying the tumor microenvironment are just two of the obvious ways that *HOTTIP* can affect carcinogenesis. Additionally, a growing body of research indicates that *HOTTIP* plays an oncogenic role in almost every type of cancer, indicating that a broad spectrum of cancer patients may benefit from *HOTTIP*-targeted treatments [22]. The significance of this lncRNA in tumorigenesis is further highlighted by the discovery of a correlation between *HOTTIP* SNPs and cancer susceptibility and their effect on patient prognosis. Although *HOTTIP* may be a common molecular biomarker, the basic mechanisms underlying its role in human cancer are still mostly unknown. To date, no studies have been published assessing the association between *HOTTIP* gene polymorphisms and the risk of NHL.

The rs2067087 polymorphism was shown to alter mature *HOTTIP* levels in gastric cancer, with eQTL analysis directly linking this SNP to differential transcript abundance [16]. At the molecular level, *HOTTIP* binds WDR5/MLL complexes to mediate H3K4 methylation and maintain an active chromatin state at 5′ HOXA loci [11]. In hematopoietic stem cells, *HOTTIP* orchestrates higher order chromatin structures and promotes transcription of HOXA genes crucial for lymphoid lineage commitment. SNP-induced changes in *HOTTIP* expression or folding could disrupt this scaffolding function, attenuating chromatin binding or WDR5 recruitment [23]. Specific alleles like rs5883064 and rs2067087 may weaken oncogenic HOXA activation providing protective effects in lymphoma whereas rs1859168 may enhance *HOTTIP* function, thereby heightening NHL risk.

The association between *HOTTIP* expression and cancer prognosis was examined in a 2017 meta-analysis by Fan et al., which comprised 652 cancer patients from seven relevant studies. The findings suggested that a poorer prognosis for cancer patients may be linked to elevated *HOTTIP* expression [24]. Additionally, there was a significant linkage between distant and lymph node metastasis and elevated *HOTTIP* expression.

Geng et al. (2016) discovered that individuals with the rs1859168 or rs5883064 had an increased risk of lung cancer. However, in the current study, it was revealed that the rs5883064 polymorphism in the *HOTTIP* gene significantly decreased the risk of lymphoma, while the rs1859168 polymorphism in the codominant model significantly increased the risk of lymphoma [16].

Furthermore, *HOTTIP* SNPs rs2067087 and rs3807598 were linked to an elevated risk of gastric cancer in 2020, according to Wang et al. On the other hand, our research found that the *HOTTIP* rs2067087 variant significantly protected against lymphoma [15]. Three SNPs (rs3807598, rs2067087, and rs17427960) were linked to an increased risk of colorectal cancer, according to research published in 2019 by Lv et al. Similarly, our results also showed that the *HOTTIP* rs2067087 variant and the rs5883064 polymorphism significantly reduced the risk of lymphoma [15].

According to Abdi et al.'s 2020 study, the risk of gastric cancer was considerably increased by the HOTAIR SNPs rs17720428, rs7958904, and rs1899663, as well as their interaction with the *HOTTIP* rs1859168 polymorphism. Consistent with these results, our investigation verified that the codominant model's 1859168 variant markedly elevated the risk of NHL [25].

While genetic factors, particularly SNPs within lncRNA genes, have been recognized as contributors to an elevated risk of cancers, our study provides the initial finding illustrating the correlation between *HOTTIP* genetic variants and the risk of NHL.

In addition, in the current study although significant associations were observed between *HOTTIP* polymorphisms and overall NHL risk, no correlations were found with clinicopathological features such as histology subtype, disease stage, or treatment response. This finding is consistent with other lncRNA-focused studies: for instance, polymorphisms in the H19 lncRNA have been shown to influence cancer susceptibility in hematologic malignancies, but did not correlate with clinical or pathological variables within those patient groups [26]. Such patterns suggest that while certain lncRNA SNPs may contribute to the initial development of malignancy, they might not affect disease progression, subtype, or therapeutic outcomes.

Our haplotype analysis revealed two protective combinations Ins/A/G and Ins/C/C whose frequencies were significantly lower in NHL patients than in controls. Such findings mirror similar observations in colorectal cancer, where specific *HOTTIP* haplotypes were associated with altered cancer risk. For example, in a Chinese cohort, haplotypes containing rs2067087 were linked to CRC susceptibility, suggesting that combinations of *HOTTIP* variants work synergistically to affect gene function and disease risk. One possible mechanism is that these protective haplotypes modify *HOTTIP*'s secondary structure or its interaction with chromatin modifier complexes, tempering transcription of oncogenic HOXA genes. This implies that SNPs may act jointly not additively to fine-tune lncRNA activity. Functional assays evaluating *HOTTIP* expression, RNA folding, or chromatin binding in the presence of these haplotypes would help elucidate the underlying biology [15].

There are some restrictions on this study. There were

190 cases and 190 controls in all, and more data seems required to draw more accurate conclusions. Furthermore, selection bias might have existed because sampling was limited to the Zahedan population and done in a single center. Furthermore, non-functional SNPs were excluded from this study, which only looked at three potentially functional polymorphisms. Another limitation of our study is the potential for selection bias, as the control group was recruited from individuals attending hospital clinics. Although these participants were free of systemic disease, their health-seeking behavior or underlying conditions not screened for in this study could have influenced the observed genotype distributions. Future studies should aim to include population-based controls to minimize this source of bias.

In conclusion, our results showed that the *HOTTIP* gene's rs5883064 polymorphism has a protective function by considerably lowering the risk of lymphoma. In the codominant model, the 1859168 variant was found to be a risk factor for non-Hodgkin's lymphoma, considerably raising the likelihood of developing the illness. Furthermore, the *HOTTIP* rs2067087 variant demonstrated a protective effect, considerably lowering the risk of lymphoma.

To confirm our findings, more research on larger populations with more ethnic diversity is advised. Future studies should also look into other pertinent SNPs in the *HOTTIP* gene and how they affect changes in gene expression, in addition to the polymorphisms examined in this study. Moreover, if these associations are validated, *HOTTIP* polymorphisms may serve as useful biomarkers in the development of genetic risk prediction models or non-invasive diagnostic panels for NHL. Incorporating such variants into personalized risk stratification tools could help in the early identification of high-risk individuals and contribute to precision oncology strategies.

Author Contribution Statement

SMH and MT recruited the subjects, collected clinical data. HM and RM collected clinical data. MS prepared materials, performed experiments, and drafted the manuscript. GB designed the study, analyzed the data, interpreted data, and approved the final version and proofread the manuscript. All authors approved the final manuscript.

Acknowledgements

The authors would like to thank all participants in this study and the research council of Zahedan University of Medical Sciences for their assistance. This article was extracted from an approved MSc student thesis at Zahedan University of Medical Sciences. The protocol of the current study was verified by the Ethics Committee of Zahedan Medical Sciences University (Zahedan, Iran). All supporting data have been shown in the manuscript.

Ethical Approval

Zahedan University of Medical Sciences' local ethics committee approved this project, and each participant gave informed consent (IR.ZAUMS.REC.1402.409).

Funding

This study was supported by a research grant (No. 409) from Zahedan University of Medical Sciences.

Data availability

The datasets generated during and/or analyzed dur¬ing the current study are available from the corresponding author on reasonable request.

Conflicting Interest

The authors declare that they have no conflicting interests.

References

1. de Leval L, Gaulard P, Dogan A. A practical approach to the modern diagnosis and classification of t- and nk-cell lymphomas. Blood. 2024;144(18):1855-72. https://doi.org/10.1182/blood.2023021786.
2. Jamil A, Mukkamalla SKR. Lymphoma. Statpearls. Treasure Island (FL): StatPearls PublishingCopyright © 2025, StatPearls Publishing LLC.; 2025.
3. Luo J, Craver A, Bahl K, Stepniak L, Moore K, King J, et al. Etiology of non-hodgkin lymphoma: A review from epidemiologic studies. J Natl Cancer Cent. 2022;2(4):226-34. https://doi.org/10.1016/j.jncc.2022.08.003.
4. Thandra KC, Barsouk A, Saginala K, Padala SA, Barsouk A, Rawla P. Epidemiology of non-hodgkin's lymphoma. Med Sci (Basel). 2021;9(1):5. https://doi.org/10.3390/medsci9010005.
5. Skibola CF, Curry JD, Nieters A. Genetic susceptibility to lymphoma. Haematologica. 2007;92(7):960-9. https://doi.org/10.3324/haematol.11011.
6. Ahadi A. Functional roles of lncrnas in the pathogenesis and progression of cancer. Genes Dis. 2021;8(4):424-37. https://doi.org/10.1016/j.gendis.2020.04.009.
7. Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W. Emerging roles of lncrna in cancer and therapeutic opportunities. Am J Cancer Res. 2019;9(7):1354-66.
8. Chen Z, He A, Wang D, Liu Y, Huang W. -long noncoding rna hottip as a novel predictor of lymph node metastasis and survival in human cancer: A systematic review and meta-analysis. Oncotarget. 2017;8(8):14126-32. https://doi.org/10.18632/oncotarget.12981.
9. Feng H, Zhao F, Luo J, Xu S, Liang Z, Xu W, et al. Long non-coding rna hottip exerts an oncogenic function by regulating hoxa13 in nasopharyngeal carcinoma. Mol Biol Rep. 2023;50(8):6807-18. https://doi.org/10.1007/s11033-023-08598-9.
10. Hakiminia F, Jannat Alipoor F, Keshavarz M, Asadi MH. Lncrna pnky is upregulated in breast cancer and promotes cell proliferation and emt in breast cancer cells. Noncoding RNA. 2023;9(2):25. https://doi.org/10.3390/ncrna9020025.
11. Ghafouri-Fard S, Dashti S, Taheri M. The hottip (hoxa transcript at the distal tip) lncrna: Review of oncogenic roles in human. Biomed Pharmacother. 2020;127:110158. https://doi.org/10.1016/j.biopha.2020.110158.
12. Harati-Sadegh M, Kohan L, Teimoori B, Mehrabani M, Salimi S. The effects of placental long noncoding rna h19 polymorphisms and promoter methylation on h19 expression in association with preeclampsia susceptibility. IUBMB Life. 2020;72(3):413-25. https://doi.org/10.1002/iub.2199.

13. Harati-Sadegh M, Sargazi S, Sheervalilou R, Hosseini Teshnizi S, Saravani R, Mirinejad S. Association of il-1ra ser133ser variant with susceptibility to immune-mediated and inflammatory diseases: A meta-analysis of 2622 cases and 3854 controls. Iran J Public Health. 2020;49(12):2320-9. https://doi.org/10.18502/ijph.v49i12.4814.
14. Majidpour M, Saravani R, Sargazi S, Sargazi S, Harati-Sadegh M, Khorrami S, et al. A study on associations of long noncoding rna hotair polymorphisms with genetic susceptibility to chronic kidney disease. J Clin Lab Anal. 2024;38(11-12):e25086. https://doi.org/10.1002/jcla.25086.
15. Lv Z, Xu Q, Sun L, Wen J, Fang X, Xing C, et al. Four novel polymorphisms in long non-coding rna hottip are associated with the risk and prognosis of colorectal cancer. Biosci Rep. 2019;39(5):BSR20180573. https://doi.org/10.1042/bsr20180573.
16. Wang BG, Li YZ, Ding HX, Lv Z, Xu Q, Yuan Y. Hottip polymorphism may affect gastric cancer susceptibility by altering hottip expression. Biosci Rep. 2020;40(8):BSR20200837. https://doi.org/10.1042/bsr20191687.
17. Zhou W, Chen L, Chao L, Huang R, Guo M, Ning S, et al. The multifaceted roles of long noncoding rnas in pancreatic cancer: An update on what we know. Cancer cell int. 2020;20:41. https://doi.org/10.1186/s12935-020-1126-1.
18. Zhang J, Zhang L, Fan R, Guo N, Xiong C, Wang L, et al. The polymorphism in the let-7 targeted region of the lin28 gene is associated with increased risk of type 2 diabetes mellitus. Mol Cell Endocrinol. 2013;375(1-2):53-7. https://doi.org/10.1016/j.mce.2013.04.022.
19. Lu M-Y, Li X-H, Niu J-L, Liu B. Lin28b polymorphisms confer a higher postoperative recurrence risk in reproductive-age women with endometrial polyps. Disease Markers. 2022;2022(1):4824357. https://doi.org/10.1155/2022/4824357.
20. Mirinejad S, Salimi S, Sargazi S, Heidari Nia M, Sheervalilou R, Majidpour M, et al. Association of genetic polymorphisms in long noncoding rna hottip with risk of idiopathic recurrent spontaneous abortion. Biochem Genet. 2024;62(4):2884-906. https://doi.org/10.1007/s10528-023-10571-x.
21. Baghdadi H, Heidari R, Zavvar M, Ahmadi N, Shakouri Khomartash M, Vahidi M, et al. Long non-coding rna signatures in lymphopoiesis and lymphoid malignancies. Noncoding RNA. 2023;9(4):44. https://doi.org/10.3390/ncrna9040044.
22. Liu J, Hu HB, Liu YM, Li FX, Zhang LP, Liao ZM. Lncrna hottip promotes the proliferation and invasion of ovarian cancer cells by activating the mek/erk pathway. Mol Med Rep. 2020;22(5):3667-76. https://doi.org/10.3892/mmr.2020.11452.
23. Luo H, Zhu G, Xu J, Lai Q, Yan B, Guo Y, et al. Hottip lncrna promotes hematopoietic stem cell self-renewal leading to aml-like disease in mice. Cancer Cell. 2019;36(6):645-59.e8. https://doi.org/10.1016/j.ccell.2019.10.011.
24. Fan Y, Yan T, Chai Y, Jiang Y, Zhu X. Long noncoding rna hottip as an independent prognostic marker in cancer. Clin Chim Acta. 2018;482:224-30. https://doi.org/10.1016/j.cca.2017.07.031.
25. Abdi E, Latifi-Navid S, Zahri S, Kholghi-Oskooei V, Mostafaiy B, Yazdanbod A, et al. Snp-snp interactions of oncogenic long non-coding rnas hotair and hottip on gastric cancer susceptibility. Sci Rep. 2020;10(1):16763. https://doi.org/10.1038/s41598-020-73682-0.
26. Kashani S, Sasan H, Mollashahi B, Bahari G, Hashemi SM, Taheri M. A preliminary association study of h19 non-coding gene variants with risk of non-hodgkin lymphoma: A case-control study and computational analysis. J Clin Lab Anal. 2025;39(11):e70024. https://doi.org/10.1002/jcla.70024.