European Review for Medical and Pharmacological Sciences 2020; 24: 4396-4403

Association between C-myc and K-ras gene polymorphisms and non-Hodgkin lymphoma

Q.-H. LIU[1], X.-D. LI[1], M.-Y. SONG[2], R.-N. RAN[3], Z.-S. WU[4], B.-X. YANG[5], H. WANG[6], R.-X. XIA[1]

[1]Department of Hematology, the First Affiliated Hospital of Anhui Medical University, Hefei, China
[2]Department of Hematology, the Chaohu Hospital Affiliated to Anhui Medical University, Chaohu, Anhui, China
[3]Department of Gastroenterology, the First Affiliated Hospital of Anhui Medical University, Hefei, China
[4]Department of Pathology, the First Affiliated Hospital of Anhui Medical University, Hefei, China
[5]Department of Laboratory, the First Affiliated Hospital of Anhui Medical University, Hefei, China
[6]Department of Radiotherapy, the First Affiliated Hospital of Anhui Medical University, Hefei, China

Abstract. – OBJECTIVE: To explore the association between c-myc and K-ras gene polymorphisms and non-Hodgkin lymphoma (NHL).

PATIENTS AND METHODS: A total of 200 NHL patients in our hospital in the past 3 years were collected as disease group, while 200 healthy people were taken as control group. The genomic deoxyribonucleic acid (DNA) in the peripheral blood was extracted in both groups, amplified *via* Polymerase Chain Reaction (PCR) and sent to the company for the detection of c-myc and K-ras gene polymorphisms. The expressions of c-myc and K-ras were detected *via* Reverse Transcription-quantitative PCR (RT-qPCR), and the levels of clinical indexes hemoglobin (Hb), platelet (PLT) and lactate dehydrogenase (LDH) were determined in the Laboratory Department.

RESULTS: The allele distribution at c-myc gene locus rs121918684 was different between control group and disease group (p=0.000), and the G allele frequency was 202 (0.505) in the control group and 263 (0.657) in the disease group. In the disease group, the GG genotype frequency at c-myc gene locus rs121918684 [97 (0.485)], the CC genotype frequency at rs775522201 [98 (0.490)], and the GA genotype frequency at K-ras gene locus rs1137188 [127 (0.635)] were all significantly higher than those in the control group (p=0.000, p=0.002, p=0.011). In the disease group, the frequency of recessive model GC+CC (p=0.003), heterozygous model GC (p=0.035), and homozygous model CC (p=0.037) at c-myc gene locus rs121918684 was significantly lower than that in the control group, and the frequency of recessive model CT+TT (p=0.046) at c-myc gene locus rs775522201 was also markedly lower than that in the control group. The haplotype frequency of c-myc CC (p=0.000), GC (p=0.000), and GT (p=0.018) in the disease group was different from that in the control group. Moreover, the CT genotype at c-myc gene locus rs775522201 was remarkably correlated with the c-myc gene expression, and the gene expression was markedly increased in the disease group. The TT genotype at K-ras gene locus rs12245 was correlated with the K-ras gene expression, and the gene expression was notably increased in the disease group. There was an association between GG genotype at c-myc gene locus rs121918684 and LDH level (p=0.000), between CT genotype at c-myc gene locus rs775522201 and PLT level (p=0.002), and between AA genotype at K-ras gene locus rs1137188 and Hb level (p=0.003).

CONCLUSIONS: The c-myc and K-ras gene polymorphisms are associated with susceptibility to NHL, gene expression and levels of Hb, PLT, and LDH.

Key Words:
Non-Hodgkin lymphoma, C-myc gene, K-ras gene, Gene polymorphism.

Introduction

Non-Hodgkin lymphoma (NHL) is a kind of frequently-occurring malignant tumor of the lymphoid hematopoietic system in the world[1,2], threatening children's health[3]. The disease mainly involves lymph nodes, spleen, and other immune organs, leads to the disorder or collapse of immune function in patients, and reduces the defense ability of patients against such harmful external stimuli as pathogens. NHL is mainly divided into B cell, T cell, and NK cell types, which have different conditions and therapeutic effects. The pathogenesis of NHL is associated with multi-level factors. Notably, the expressions of sCD27 and sCD30 in the circulatory system are associated with the risk of NHL[4], and the star molecules PD-1 and PD-L1 in immunotherapy also play roles in this disease[5,6]. Therefore, study-

Corresponding Author: Ruixiang Xia, MD; e-mail: xrx2041@163.com

ing the predisposing factors of NHL is significantly helpful in the prevention, diagnosis, and treatment of NHL.

Gene polymorphism refers to the individual differences in alleles or genotypes at the same locus in the biological community, and such differences may be related to the susceptibility to some diseases. Single nucleotide polymorphism, the most common type of gene polymorphism, is involved in the occurrence of lymphoid hematopoietic system diseases[7,8]. Both c-myc and K-ras genes are well-known oncogenes, and their high expressions can promote the occurrence of a variety of cancers, such as bladder cancer[9,10] and cervical cancer[11]. It can be predicted that the c-myc and K-ras genes may exert crucial effects in the development of malignant tumors. However, the association between c-myc and K-ras gene polymorphisms and NHL has not been reported yet.

In the present work, therefore, the gene polymorphisms at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 were explored in NHL patients and healthy people. Besides, the haplotype of the two genes was analyzed, and blood routine, coagulation index, and lactate dehydrogenase (LDH) were detected, so as to explore the association between susceptibility to NHL and c-myc and K-ras gene polymorphisms.

Patients and Methods

Patients

A total of 200 NHL patients in our hospital in the past 3 years (disease group) and 200 healthy people (control group) were collected as the objects of study. The clinical data in both groups were collected, including the patient's name, ID number, age, gender, body mass index (BMI), disease history, and family history. The mean age was (45.21±4.25) years old in the control group and (43.13±4.31) years old in the disease group. There were no statistically significant differences in such general data as age and gender between the two groups (p>0.05). In the disease group, NHL patients were diagnosed by the pathologist with intermediate title or above. This investigation was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University, and signed written informed consents were obtained from all participants before the study.

Sample Collection and Processing

A total of 8 mL of peripheral blood was collected from both groups in the Laboratory Department, and centrifuged using a centrifuge at 3500 rpm for 8 min within 2 h. Then, the upper-layer serum and mid-layer nucleated cells were transferred into new centrifuge tubes. The upper-layer serum was stored in liquid nitrogen for later detection, and the genomic deoxyribonucleic acid (DNA) was extracted from the mid-layer nucleated cells.

Genomic DNA Extraction

The genomic DNA was extracted from the peripheral blood in both groups using the blood genome extraction kit (Tiangen, Beijing, China) in strict accordance with the instructions of the kit. Specifically, according to the volume of sample, 250 μL of protease K solution was added into the centrifuge tube, and peripheral blood samples and 2 mL of buffer (GE) were also added. The mixture was mixed evenly using a vortex oscillator for 1 min, and placed at 65°C for 8 min. Then, 2 mL of absolute alcohol was added into the mixture, mixed evenly, and transferred into an absorption column. After that, 2 mL of buffer was added into the absorption column, followed by centrifugation at 4000 rpm for 1 min. Subsequently, 200 μL of elution buffer was added into the absorption column, and the resulting solution was the genomic DNA. Finally, the purity of DNA was detected using a spectrophotometer, and the samples with the optical density $(OD)_{260}/OD_{280}$ of 1.8-2.0 were qualified for subsequent studies.

Polymerase Chain Reaction (PCR) Amplification and Analysis of C-myc and K-ras Gene Polymorphism

The polymorphic regions at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 were amplified using the PCR instrument. The total PCR system was 25 μL, including 1 μL of forward primers, 1 μL of reverse primers, 0.5 μL of DNA template, 12.5 μL of Taq DNA polymerase, and 10 μL of dH_2O. PCR conditions: 95°C for 5 min (95°C for 30 s, 54°C for 45 s and 72°C for 35 s) × 40 cycles, and 72°C for 5 min. The primers of polymorphic loci are as follows: c-myc gene locus rs121918684: forward (5'→3'): GTCAAGAGGCGAACACACAACAAC [temperature of melting (Tm)=62.6], reverse (5'→3'): TTGGACGGACAGGATGTATGC (Tm=62.8), c-myc gene locus rs775522201: for-

ward (5'→3'): ATGCCCCTCAACGTGAACTTC (Tm=61.1), reverse (5'→3'): GTCGCAGATGAAATAGGGCTG (Tm=60.8). K-ras gene locus rs12245: forward (5'→3'): CCCTATTTCATCTGCGACGAG (Tm=61.5), reverse (5'→3'): GAGAAGGACGTAGCGACCG (Tm=60.7), K-ras gene locus rs1137188: forward (5'→3'): TTCATCTGCGATCCTGACGAC (Tm=60.2), reverse (5'→3'): CACTGAGGGGTCAATGCACTC (Tm=60.2). The PCR products were sent to the Jiangsu Biotechnology Co., Ltd. (Nanjing, China) for sequencing, and the polymorphisms at c-myc and K-ras gene loci were analyzed in both groups.

Detection of C-myc and K-ras Gene Expressions

The c-myc and K-ras gene expressions were detected *via* Reverse Transcription-quantitative PCR (RT-qPCR), and the gene primers were designed using Primer Premier 5.0 and synthesized by Sangon (Shanghai, China): C-myc: forward (5'→3'): GGCTCCTGGCAAAAGGTCA, reverse (5'→3'): CTGCGTAGTTGTGCTGATGT, K-ras: forward (5'→3'): TCCCTCCACTCGGAAGGAC, reverse (5'→3'): CTGGTGCATTTTCGGTTGTTG. The total PCR system was 25 μL, including 1 μL of forward primers, 1 μL of reverse primers, 0.5 μL of cDNA template, 12.5 μL of SYBR premix Taq, and 10 μL of dH_2O. PCR conditions: 94°C for 2 min (95°C for 30 s, 58°C for 40 s and 72°C for 35 s) × 40 cycles, and 72°C for 5 min.

Detection of Clinical Indexes

The levels of clinical indexes hemoglobin (Hb), platelet (PLT), and LDH in both groups were detected in the Laboratory Department using the hematology analyzer, full-automatic coagulometer, and full-automatic biochemical analyzer within 2 h.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 23.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Enumeration data were compared using χ^2-test, and the Hardy-Weinberg equilibrium test was performed. Haplotype analysis was conducted at the SHEsis website. $p<0.05$ suggested the statistically significant difference.

Results

Allele Distribution at C-myc Gene Loci rs121918684 and rs775522201, and K-ras Gene Loci rs12245 and rs1137188

As shown in Table I, the allele distribution at c-myc gene locus rs775522201 and K-ras gene loci rs12245 and rs1137188 had no differences between the control group and disease group. In addition, the allele distribution at c-myc gene locus rs121918684 was different between the two groups (p=0.000), and the G allele frequency was 202 (0.505) in the control group and 263 (0.657) in the disease group.

Genotype Distribution at C-myc Gene Loci rs121918684 and rs775522201, and K-ras Gene Loci rs12245 and rs1137188

As shown in Table II, the GG genotype frequency at c-myc gene locus rs121918684 [97 (0.485)], the CC genotype frequency at rs775522201 [98 (0.490)], and the GA genotype frequency at K-ras gene locus rs1137188 [127 (0.635)] were all significantly higher in the disease group than those in the control group (p=0.000, p=0.002, p=0.011).

Table I. Allele distribution at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188.

Gene	Locus	Allele	Control group	Disease group	OR	95% CI	χ^2	p
C-myc	rs121918684	G	202 (0.505)	263 (0.657)	0.53	0.39-0.70	19.11	0.000
		C	198 (0.495)	137 (0.343)				
	rs775522201	C	221 (0.552)	263 (0.657)	1.55	1.16-2.06	3.22	0.141
		T	179 (0.448)	137 (0.343)				
K-ras	rs12245	A	212 (0.530)	209 (0.522)	0.97	0.73-1.28	0.04	0.830
		T	188 (0.470)	19 1 (0.477)				
	rs1137188	G	183 (0.458)	185 (0.463)	0.98	0.74-1.29	0.02	0.886
		A	217 (0.542)	215 (0.537)				

Table II. Genotype distribution at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188.

Gene	Locus	Genotype	Control group	Disease group	OR	95% CI	χ^2	*p*
C-myc	rs121918684	GG	50 (0.250)	97 (0.485)	1.41	1.21-1.74	23.78	0.000
		GC	102 (0.510)	69 (0.345)				
		CC	48 (0.240)	34 (0.170)				
	rs775522201	CC	64 (0.320)	98 (0.490)	1.24	1.11-1.58	12.18	0.002
		CT	93 (0.465)	67 (0.335)				
		TT	43 (0.215)	35 (0.175)				
K-ras	rs12245	AA	54 (0.270)	39 (0.195)	0.87	0.58-0.97	2.74	0.582
		AT	104 (0.520)	131 (0.655)				
		TT	42 (0.210)	30 (0.150)				
	rs1137188	GG	43 (0.215)	29 (0.145)	1.34	1.15-1.54	9.21	0.011
		GA	97 (0.485)	127 (0.635)				
		AA	60 (0.300)	44 (0.220)				

Analysis of Polymorphisms at C-myc gene loci rs121918684 and rs775522201, and K-ras Gene loci rs12245 and rs1137188

As shown in Table III, the frequency of the recessive model GC+CC (p=0.003), heterozygous model GC (p=0.035), and homozygous model CC (p=0.037) at c-myc gene locus rs121918684 was markedly lower in the disease group than that in the control group, and the frequency of recessive model CT+TT (p=0.046) at c-myc gene locus rs775522201 was also significantly lower in the disease group than that in the control group.

Haplotype Analysis of C-myc gene Loci rs121918684 and rs775522201, and K-ras Gene Loci rs12245 and rs1137188

According to the haplotype analysis of c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 (Table IV), the haplotype frequency of c-myc CC (p=0.000), GC (p=0.000), and GT (p=0.018) in the disease group was different from that in the control group.

Associations of Genotypes at C-myc Gene Loci rs121918684 and rs775522201, and K-ras Gene Loci rs12245 and rs1137188 With Gene Expressions

The associations of genotypes at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 with gene expressions were detected, and the results revealed that the CT genotype at c-myc gene locus rs775522201 was remarkably correlated with the c-myc gene expression, and the gene expression was remarkably increased in the disease group. The TT genotype at K-ras gene locus rs12245 was correlated with the K-ras gene expression, and the gene expression was remarkably increased in disease group (Figures 1-4).

Associations of Genotypes at C-myc Gene Loci rs121918684 and rs775522201, and K-ras Gene Loci rs12245 and rs1137188 With Clinical Indexes

There was an association between GG genotype at c-myc gene locus rs121918684 and LDH level (p=0.000), between CT genotype at c-myc gene locus rs775522201 and PLT level (p=0.002), and between AA genotype at K-ras gene locus rs1137188 and Hb level (p=0.003) (Table V).

Discussion

NHL is a highly malignant lymphoid hematopoietic tumor, whose pathogenesis has not been fully clarified[12]. As one of the frequently-occurring malignant tumors in the world, NHL has similar characteristics to other cancers, such as uncontrolled proliferation of cancer cells, proneness to recurrence, and difficulty in cure[13,14]. Therefore, it is speculated that the oncogenes c-myc and K-ras in lung cancer and liver cancer may also be related to the occurrence and development of NHL. Both c-myc and K-ras genes can cause changes in the condition of NHL patients or affect the therapeutic effect[15,18]. Therefore, the polymorphisms of c-myc and K-ras genes may be associated with susceptibility to NHL.

In this work, it was found that the allele distribution at c-myc gene locus rs775522201 and K-ras

Table III. Analysis of polymorphisms at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188.

	Gene	Locus	Genotype	Control group	Disease group	χ^2	*p*
Dominant model	C-myc	rs121918684	GG+GC	152 (0.760)	166 (0.830)	3.71	0.132
			CC	48 (0.240)	34 (0.170)		
		rs775522201	CC+CT	157 (0.785)	165 (0.825)	2.12	0.384
			TT	43 (0.215)	35 (0.175)		
	K-ras	rs12245	AA+AT	158 (0.790)	170 (0.850)	3.24	0.261
			TT	42 (0.210)	30 (0.150)		
		rs1137188	GG+GA	140 (0.700)	156 (0.780)	2.71	0.251
			AA	60 (0.300)	44 (0.220)		
Recessive model	C-myc	rs121918684	GG	50 (0.250)	97 (0.485)	9.11	0.003
			GC+CC	150 (0.750)	103 (0.515)		
		rs775522201	CC	64 (0.320)	98 (0.490)	6.43	0.046
			CT+TT	136 (0.680)	102 (0.510)		
	K-ras	rs12245	AA	54 (0.270)	39 (0.195)	2.42	0.231
			AT+TT	146 (0.730)	161 (0.805)		
		rs1137188	GG	43 (0.215)	29 (0.145)	1.35	0.513
			GA+AA	157 (0.785)	171 (0.855)		
Heterozygous model	C-myc	rs121918684	GG	50 (0.250)	97 (0.485)	7.41	0.035
			GC	102 (0.510)	69 (0.345)		
		rs775522201	CC	64 (0.320)	98 (0.490)	2.21	0.245
			CT	93 (0.465)	67 (0.335)		
	K-ras	rs12245	AA	54 (0.270)	39 (0.195)	1.24	0.572
			AT	104 (0.520)	131 (0.655)		
		rs1137188	GG	43 (0.215)	29 (0.145)	2.74	0.201
			GA	97 (0.485)	127 (0.635)		
Homozygous model	C-myc	rs121918684	GG	50 (0.250)	97 (0.485)	7.23	0.037
			CC	48 (0.240)	34 (0.170)		
		rs775522201	CC	64 (0.320)	98 (0.490)	2.14	0.264
			TT	43 (0.215)	35 (0.175)		
	K-ras	rs12245	AA	54 (0.270)	39 (0.195)	1.27	0.619
			TT	42 (0.210)	30 (0.150)		
		rs1137188	GG	43 (0.215)	29 (0.145)	1.64	0.582
			AA	60 (0.300)	44 (0.220)		

gene loci rs12245 and rs1137188 had no differences between control group and disease group. The allele distribution at c-myc gene locus rs121918684 was different between the two groups (p=0.000), and the G allele frequency was 202 (0.505) in the control group and 263 (0.657) in the disease group,

Table IV. Haplotype analysis of c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188.

Gene	Haplotype	Control group	Disease group	OR	95% CI	χ^2	*p*
C-myc	CC	113.55 (0.284)	67.55 (0.169)	0.513	0.365-0.720	15.1	0.000
	CT	84.45 (0.211)	69.45 (0.174)	0.785	0.552-1.117	1.811	0.178
	GC	107.45 (0.269)	195.45 (0.489)	2.601	1.936-3.496	41.14	0.000
	GT	94.55 (0.236)	67.55 (0.169)	0.656	0.463-0.930	5.638	0.018
K-ras	AA	129.23 (0.323)	147.08 (0.368)	1.218	0.910-1.632	1.762	0.184
	AG	82.77 (0.207)	61.92 (0.155)	0.702	0.488-1.009	3.669	0.056
	TA	87.77 (0.219)	67.92 (0.170)	0.728	0.511-1.035	3.143	0.076
	TG	100.23 (0.251)	123.08 (0.308)	1.329	0.975-1.813	3.244	0.072

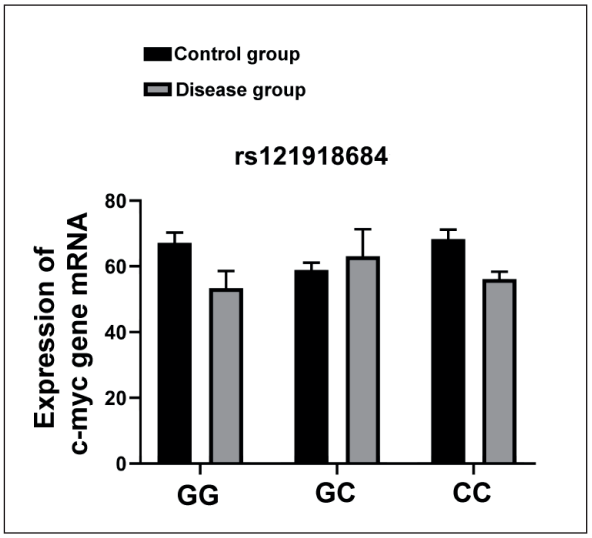

Figure 1. Association between genotype at c-myc gene locus rs121918684 and c-myc gene expression.

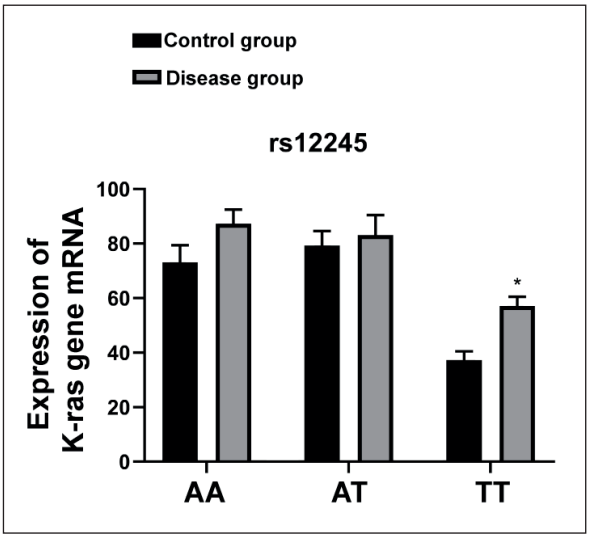

Figure 3. Association between genotype at K-ras gene locus rs12245 and K-ras gene expression (*p<0.05).

indicating that the people with G allele at c-myc gene locus rs121918684 are prone to NHL. At the same time, the GG genotype frequency at c-myc gene locus rs121918684 [97 (0.485)], the CC genotype frequency at rs775522201 [98 (0.490)], and the GA genotype frequency at K-ras gene locus rs1137188 [127 (0.635)] were all significantly higher in the disease group than those in the control group (p=0.000, p=0.002, p=0.011). The above results prove that G allele and GG genotype at c-myc gene locus rs121918684 can indeed increase the susceptibility to NHL. Therefore, such patients should be highly suspected of NHL, and the corresponding treatment should be well prepared. Moreover, the people with CC genotype at c-myc gene locus rs775522201 and GA genotype at K-ras gene locus rs1137188 are prone to NHL, and those with CC (p=0.000), GC (p=0.000), and GT (p=0.018) haplotypes at c-myc gene loci rs121918684 and rs775522201 are also highly susceptible to NHL. It can be seen that the c-myc gene may play a key role in the occurrence of NHL.

According to the analysis of polymorphisms of c-myc and K-ras genes, the frequency of re-

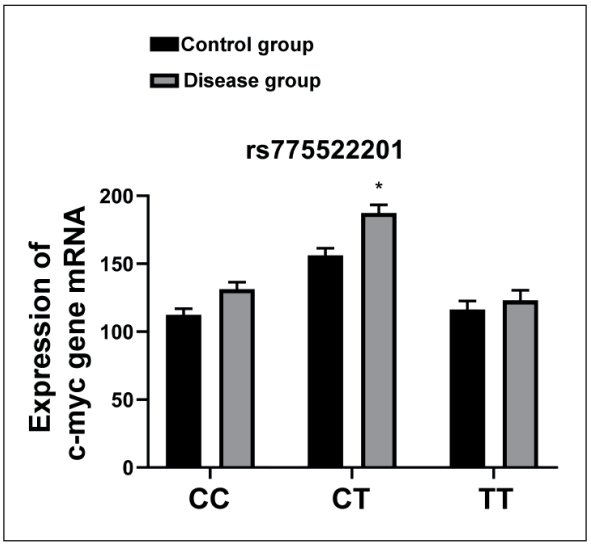

Figure 2. Association between genotype at c-myc gene locus rs775522201 and c-myc gene expression (*p<0.05).

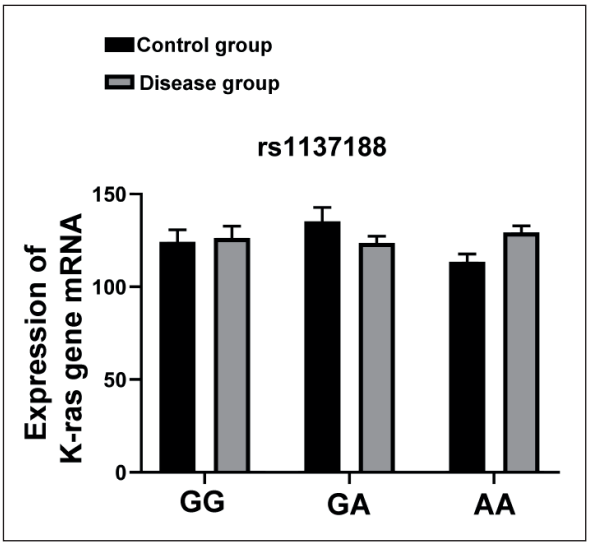

Figure 4. Association between genotype at K-ras gene locus rs1137188 and K-ras gene expression.

Table V. Associations of genotypes at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 with Hb, PLT and LDH.

Gene	Locus	Genotype	Hb (g/L)			PLT (10^9/L)			LDH (U/L)		
			Control group	Disease group	*p*	Control group	Disease group	*p*	Control group	Disease group	*p*
C-myc	rs121918684	GG	145	103	0.091	234	121	0.235	163.32	265.35	0.000
		GC	135	123		212	102		142.45	203.51	
		CC	138	93		271	110		146.42	217.72	
	rs775522201	CC	135	113	0.311	265	135	0.002	153.36	201.81	0.136
		CT	154	105		216	65		136.84	211.35	
		TT	136	113		263	113		174.17	235.75	
K-ras	rs12245	AA	133	114	0.256	214	132	0.135	137.37	212.64	0.065
		AT	153	94		274	101		163.59	275.23	
		TT	123	123		251	98		153.83	223.15	
	rs1137188	GG	132	131	0.003	275	106	0.158	166.35	231.35	0.231
		GA	127	124		265	126		159.23	226.35	
		AA	125	83		231	93		152.46	216.46	

cessive model GC+CC (p=0.003), heterozygous model GC (p=0.035), and homozygous model CC (p=0.037) at c-myc gene locus rs121918684 in the disease group was significantly lower than that in the control group, and the frequency of recessive model CT+TT (p=0.046) at c-myc gene locus rs775522201 was also significantly lower than that in the control group. The above findings demonstrate that the effects of c-myc and K-ras gene polymorphisms on NHL are not simply triggered by a single allele or genotype, but may be the result of multiple genotypes, which also provide deeper insights into the occurrence of the disease.

The effects of c-myc and K-ras genes on NHL may be realized by regulating their mRNA and protein expressions[19,20], so the associations of genotypes at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 with c-myc and K-ras gene expressions were analyzed in this paper. The results revealed that the CT genotype at c-myc gene locus rs775522201 was remarkably correlated with the c-myc gene expression, and the gene expression was notably increased in the disease group. The TT genotype at K-ras gene locus rs12245 was correlated with the K-ras gene expression, and the gene expression was remarkably increased in the disease group. Therefore, the effects of c-myc and K-ras genes on susceptibility to NHL may be realized by regulating gene expressions.

Finally, the associations of genotypes at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 with blood routine index, coagulation index, and LDH were also explored. The results showed that there was an association between GG genotype at c-myc gene locus rs121918684 and LDH level (p=0.000), between CT genotype at c-myc gene locus rs775522201 and PLT level (p=0.002), and between AA genotype at K-ras gene locus rs1137188, and Hb level (p=0.003). However, a limitation also existed in the present study. Given that we can only enroll Chinese patients in our present study, we did not exclude the effects of ethnicity on this research. In our further research, we should take this factor into consideration.

Conclusions

This investigation verifies that the polymorphisms at c-myc gene loci rs121918684 and rs775522201, and K-ras gene loci rs12245 and rs1137188 can indeed influence the clinical indexes of NHL, thereby affecting the development of the disease.

Conflict of Interests

The Authors declared that they have no conflict of interests.

References

1) Sorigue M, Gual-Capllonch F, Garcia O, Sarrate E, Franch-Sarto M, Ibarra G, Grau J, Orna E, Ribera JM, Sancho JM. Incidence, predictive factors, management, and survival impact of atrial fibril-

lation in non-Hodgkin lymphoma. Ann Hematol 2018; 97: 1633-1640.

2) Armitage JO, Gascoyne RD, Lunning MA, Cavalli F. Non-Hodgkin lymphoma. Lancet 2017; 390: 298-310.

3) Choeyprasert W, Anurathapan U, Pakakasama S, Sirachainan N, Songdej D, Lertthammakiat S, Hongeng S. Pediatric non-Hodgkin lymphoma: characteristics, stratification, and treatment at a single institute in Thailand. Pediatr Int 2019; 61: 49-57.

4) Purdue MP, Lan Q, Hoffman-Bolton J, Hildesheim A, Callahan CL, Strickland P, Visvanathan K, Rothman N. Circulating sCD27 and sCD30 in pre-diagnostic samples collected fifteen years apart and future non-Hodgkin lymphoma risk. Int J Cancer 2019; 144: 1780-1785.

5) Gravelle P, Burroni B, Pericart S, Rossi C, Bezombes C, Tosolini M, Damotte D, Brousset P, Fournie JJ, Laurent C. Mechanisms of PD-1/PD-L1 expression and prognostic relevance in non-Hodgkin lymphoma: a summary of immunohistochemical studies. Oncotarget 2017; 8: 44960-44975.

6) Hu B, Jacobs R, Ghosh N. Checkpoint inhibitors Hodgkin lymphoma and non-Hodgkin lymphoma. Curr Hematol Malig Rep 2018; 13: 543-554.

7) Yang L, Wu H, Gelder TV, Matic M, Ruan JS, Han Y, Xie RX. SLCO1B1 rs4149056 genetic polymorphism predicting methotrexate toxicity in Chinese patients with non-Hodgkin lymphoma. Pharmacogenomics 2017; 18: 1557-1562.

8) Mashhadi MA, Miri-Moghaddam E, Arbabi N, Bazi A, Heidari Z, Sepehri Z, Karimkoshte A, Rezvan A, Hashemi SM. C677T and A1298C polymorphisms of methylene tetrahydrofolate reductase in non-Hodgkin lymphoma: southeast Iran. Tumori 2018; 104: 280-284.

9) Sun J, Zhang H, Tao D, Xie F, Liu F, Gu C, Wang M, Wang L, Jiang G, Wang Z, Xiao X. CircCDYL inhibits the expression of C-MYC to suppress cell growth and migration in bladder cancer. Artif Cells Nanomed Biotechnol 2019; 47: 1349-1356.

10) Nanda MS, Sameer AS, Syeed N, Shah ZA, Murtaza I, Siddiqi MA, Ali A. Genetic aberrations of the K-ras proto-oncogene in bladder cancer in Kashmiri population. Urol J 2010; 7: 168-173.

11) Dun S, Gao L. Tanshinone I attenuates proliferation and chemoresistance of cervical cancer in a KRAS-dependent manner. J Biochem Mol Toxicol 2019; 33: e22267.

12) Etter JL, Cannioto R, Soh KT, Alquassim E, Almohanna H, Dunbar Z, Joseph JM, Balderman S, Hernandez-Ilizaliturri F, Moysich KB. Lifetime physical inactivity is associated with increased risk for Hodgkin and non-Hodgkin lymphoma: a case-control study. Leuk Res 2018; 69: 7-11.

13) Thacker N, Bakhshi S, Chinnaswamy G, Vora T, Prasad M, Bansal D, Agarwala S, Kapoor G, Radhakrishnan V, Laskar S, Kaur T, Rath GK, Dhaliwal RS, Arora B. Management of non-Hodgkin lymphoma: ICMR Consensus Document. Indian J Pediatr 2017; 84: 382-392.

14) Pratap S, Scordino TS. Molecular and cellular genetics of non-Hodgkin lymphoma: diagnostic and prognostic implications. Exp Mol Pathol 2019; 106: 44-51.

15) Landsburg DJ, Petrich AM, Abramson JS, Sohani AR, Press O, Cassaday R, Chavez JC, Song K, Zelenetz AD, Gandhi M, Shah N, Fenske TS, Jaso J, Medeiros LJ, Yang DT, Nabhan C. Impact of oncogene rearrangement patterns on outcomes in patients with double-hit non-Hodgkin lymphoma. Cancer 2016; 122: 559-564.

16) Zappasodi R, Ruggiero G, Guarnotta C, Tortoreto M, Tringali C, Cavane A, Cabras AD, Castagnoli L, Venerando B, Zaffaroni N, Gianni AM, De Braud F, Tripodo C, Pupa SM, Di Nicola M. HSPH1 inhibition downregulates Bcl-6 and c-Myc and hampers the growth of human aggressive B-cell non-Hodgkin lymphoma. Blood 2015; 125: 1768-1771.

17) Ohtake A, Aoki Y, Saito Y, Niihori T, Shibuya A, Kure S, Matsubara Y. Non-Hodgkin lymphoma in a patient with cardiofaciocutaneous syndrome. J Pediatr Hematol Oncol 2011; 33: e342-e346.

18) Rigter LS, Snaebjornsson P, Rosenberg EH, Atmodimedjo PN, Aleman BM, Ten HJ, Geurts-Giele WR, van Ravesteyn TW, Hoeksel J, Meijer GA, Te RH, van Leeuwen FE, Dinjens WN, van Leerdam ME. Double somatic mutations in mismatch repair genes are frequent in colorectal cancer after Hodgkin's lymphoma treatment. Gut 2018; 67: 447-455.

19) Bretz CL, Langohr IM, Lee S, Kim J. Epigenetic instability at imprinting control regions in a Kras(G12D)-induced T-cell neoplasm. Epigenetics 2015; 10: 1111-1120.

20) Provencio M, Rodriguez M, Cantos B, Sabin P, Quero C, Garcia-Arroyo FR, Rueda A, Maximiano C, Rodriguez-Abreu D, Sanchez A, Silva J, Garcia V. mRNA in exosomas as a liquid biopsy in non-Hodgkin Lymphoma: a multicentric study by the Spanish Lymphoma Oncology Group. Oncotarget 2017; 8: 50949-50957.