

RESEARCH ARTICLE

Polymorphic Variation of Inflammation-related Genes and Risk of Non-Hodgkin Lymphoma for Uygur and Han Chinese in Xinjiang

Xia Gu^{1&*}, Yan Shen^{2&}, Ling Fu³, Hong-Yun Zuo⁴, Halida Yasen⁵, Ping He¹, Xin-Hong Guo⁵, Yu-wei Shi⁶, Muhabaiti Yusufu⁷

Abstract

Polymorphisms of inflammation-related genes have been found to be associated with non-Hodgkin lymphoma (NHL) or some of its subtypes, but only a few relevant data have been reported in China. In this study, the Snapshot method was used to assess genetic variation; a total of 14 single nucleotide polymorphisms (SNPs) for 6 inflammatory factors in 157 NHL cases (64 Uygur ethnic subjects, 93 Han Chinese) and 435 controls (231 Uygur and 204 Han Chinese) were studied from the Xinjiang province of China. Haplotype distribution was estimated using PHASE 2.3 software. Statistical differences in the genotype and haplotype frequencies between case and control groups were also considered and estimated. For the Han population, the genotype distributions for TNF- α rs1800629, TNF- α rs1800630, IL-6 rs1800795, IL-6 rs1800797, NF-KB1 rs1585215 and TLR-4 rs4986790 showed significant differences between the case and control groups ($p < 0.05$). The TNF- α gene frequencies of ACG and CCA haplotypes in the cases were higher than in the controls (OR=2.45, 95% CI: 1.55-3.89, $p=0.0002$, OR=2.53, 95% CI: 1.10-5.80, $p=0.029$, respectively), and the same findings were detected for TNF- β gene CA haplotype (OR=1.87, 95% CI: 1.21-2.90, $p=0.0054$). However, for the Uygur population, no such significant differences were detected within the gene-type distribution of the 14 SNPs. The TNF- α gene frequency of the CCA haplotype between the two groups (OR=1.98, 95% CI: 1.11-3.51, $p=0.021$) revealed a statistically significant difference. Our results showed that polymorphic variations of inflammation-related genes could be important to the NHL etiology of the Han population, and that these may only have limited influence on the Uygur population.

Keywords: Inflammation-related genes - non-Hodgkin lymphoma - SNPs - Uygur - Han - ethnicity

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Introduction

Non-Hodgkin lymphoma (NHL) is a heterogeneous malignancy closely associated with immune function. Some findings indicate that immune dysfunction may influence the risk of developing NHL, although the etiology remains enigmatic (Alexander et al., 2007). The immune response is characterized by a robust and pleiotropic network that includes cytokines, chemokines, cell adhesion molecules, interferons, and innate immune response molecules, all of which work in coordinate and remain delicately balanced in healthy individuals. Sustained perturbation of this balance, for example caused by inherited genetic mutations, can result in disease (Picard et al., 2004). Common genetic variations can also alter the expression or function of key genes and disturb the immune network, and these variations can

be associated with susceptibility and resistance to human diseases, including NHL (Forrest et al., 2006; Hollegaard et al., 2006).

Given the substantial body of evidence for the involvement of immune function in NHL, it is important to study common variations in immune-related genes in order to understand the pathogenesis mechanism behind lymphomas. Specifically, inflammation-related genes may play a role in chronic antigenic stimulation, which is a key mechanism for lymphomagenesis. Whereas a number of case-control association studies have examined the role of genetic polymorphisms of inflammation-related genes in lymphoma risk (Rothman et al., 2006; Song et al., 2012). In recent studies, enough evidence has shown that the polymorphic variations of inflammation-related genes could overall be associated with the risk of NHL or some of its subtypes. However, present studies presenting

¹Department of Pathology, The First Affiliated Hospital, ²Department of Pathology, The Fourth Affiliated Hospital, Guangzhou Medical University, Guangzhou, ³Department of Hematology, People's Hospital of Xinjiang Uygur Autonomous Region, ⁴Institute of Clinical Medicine, Urumqi General Hospital of Lanzhou Command, PLA, ⁵Department of Hematology, The First Affiliated Hospital, ⁶Department of Hematology, ⁷Department of Pathology, The Second Affiliated Hospital, Xinjiang Medical University, Urumqi, China
&Equal contributors *For correspondence: guqxxia@163.com

inconsistent and conflicting results were confusing, due to certain factors, such as the population size, population stratification, and the race or ethnicity. Some relevant studies have been conducted in China, but the studies were mostly focused on the Han Chinese of south-east China (Zhao et al., 2002; Lu et al., 2008). Studies rarely identified the genetic susceptibility of NHL etiology, which were reported based on the population of western China; and the candidate genes were fewer and mostly confined to TNF- α and TNF- β . This study was conducted to evaluate the association of TNF- α , TNF- β , IL-6, IL-10, NF-KB1 and TLR4 gene polymorphisms with the risk of NHL, between the Han and Uygur Chinese population in Xinjiang province, western China.

Materials and Methods

Study Population

This study included 157 unrelated patients (64 from Uygur, 93 from Han) diagnosed with pathologically confirmed NHL. From September 1, 2008 to August 31, 2011, the patients were treated at the First and Second Affiliated Hospitals of Xinjiang Medical University and the People's Hospital of Xinjiang Uygur Autonomous Region; the age of the patients ranged from 19 to 77 years old. The patients were classified according to the World Health Organization Classification of Neoplastic Diseases of the Hematopoietic and Lymphoid Tissues. The control group included 435 unrelated healthy blood donors (231 from Uygur, 204 from Han) aged over 20 years old.

Genotyping

DNA isolation, multiplex PCR, and DNA Sequencing were done in blood samples. Genomic DNA was prepared from peripheral blood mononuclear cells using a DNA extraction kit (QIAGEN, Valencia, CA). A multiplex assay was used to detect genetic polymorphisms. The PCR primer sequences were as follows:

rs1800896F:CCGGTCCTTCCCCAGGTAGA
 rs1800896R:CCATGGAGGCTGGATAGGAGGT
 rs10871_72F:TGGTGTACCCTTGTACAGGTGATG
 rs10871_72R:TTTTACTTTCCAGAGACTGGCTTCC
 rs1800890F:ATTTTGGAGCAGGGATGGAAGA
 rs1800890R:GTGAGAAGGCAGGCACCTATGG
 rs1800797F:AGACTCAGTGGCAATGGGGAGA
 rs1800797R:TGTGTTCTGGCTCTCCCTGTGA
 rs1800795F:GCGCTAGCCTCAATGACGACCT
 rs1800795R:AGGGCAGAATGAGCCTCAGACA
 rs1585215F:TTGTGGCTGTCTGTATATCTTCAATCAA
 rs1585215R:GGAAAGGGCAGCTCTGAGTCC
 rs4986790F:GCCTGTGCAATTTGACCAATTGA
 rs4986790R:TCTGAAAAGCATTCCCACCTTTG
 rs0629_1525F:TTCTGAAGCCCCCTCCAGTCTT
 rs0629_1525R:GTTGGGGACACACAAGCATCAA
 rs1799724F:GGGGAGATGTGACCACAGCAA
 rs1799724R:ACTCCCTGGGGCCCTCTACAT
 rs1800630F:GGGGAAGCAAAGGAGAAGCTGA
 rs1800630R:GCCCTCTACATGGCCCTGTCTT
 rs2239704_rs909253F:GAGGCTCTCTGCCCACTCTC
 rs2239704_rs909253R:GAGAAACCCCAAGGTGAGCAGAG
 Polymerase chain reaction (PCR) was performed with

1 μ l of DNA in a 20 μ l total reaction mixture, containing 1x HotStarTaq buffer, 3.0mM Mg₂₊, 0.3mM dNTP, 1 U HotStarTaq polymerase (Qiagen Inc), and 2 μ l of multiplex PCR primer. Each pair of the primers in the reaction system had a concentration of 1 μ M. The PCR conditions were 95°C 2mins; 11 cycles x (94°C 20s, 65°C-0.5°C/cycle 40 s, 72°C 1min30s); 24cycles x (94°C for 20s, 59°C for 30s, 72°C for 1.5mins.); 72°C for 2mins. The reaction mixtures were collected and stored at 4°C; and the products of the PCR reactions were extracted and purified using the SNaPshot Multiplex Kit (ABI). Then, 2 μ l of purified multiplex PCR products were taken for an extended reaction by PCR consisting of 96°C for 1min, 28 cycles x (96°C for 10s, 55°C for 5s, 60°C for 30s). The extension products were purified, sequenced by using an automatic DNA sequencer (ABI 3130XL), and the data were analyzed with a GeneMapper4.0 software (Applied Biosystems Co., Ltd., USA).

Statistical analysis

Each polymorphism was tested for Hardy-Weinberg equilibrium (HWE) among the controls by using a Pearson goodness-of-fit test or Fisher's exact test, with no significant departure from normality. With the genotype as a categorical variable, each SNP was separately included in a logistic regression model to estimate the odds ratios (OR) and the 95% confidence intervals (95%CI) for associations that has an overall risk of NHL or its common subtypes. Haplotype analysis was done by using the PHASE2.3 software, and the Haplotype-specific ORs were estimated. Participants with the most common genotype or haplotype, based on the frequency among controls, were the referent group. SPSS (version 11.5) software was used for statistical analysis and the results were considered statistically significant, at a *p*-value of less than 0.05. All *p*-values presented in this present study are two-sided.

Results

For the Han Chinese population, there were 15 T-NHL and 78 B-NHL in the case group. The most common NHL subtypes were DLBCL (n=34), MM (n=21) and SLL/CLL (n=10). The 14 previously mentioned gene's SNPs that had putative functional significance were evaluated with the risk of NHL and each of its common subtypes. The genotype distribution among NHL cases and controls are shown in Table 1. The results show that five SNPs were associated with the overall risk of NHL: TNF- α rs1800629 (GA versus GG: OR=2.262, 95%CI:1.017-5.032, *p*=0.041), TNF- α rs1800630 (CA+AA versus CC: OR=1.739, 95%CI:1.041-2.906, *p*=0.034), IL-6 rs1800795 (GC versus GG: OR=3.976, 95%CI:1.400-11.295, *p*=0.006), IL-6 rs1800797 (GA versus GG: OR=3.976, 95%CI:1.400-11.295, *p*=0.006) and NF-KB1 rs1585215 (TC versus TT: OR=1.753, 95%CI:1.014-3.031, *p*=0.044). Moreover, some subtype-specific associations were statistically significant (rs1800629 for B-NHL; rs1800630 for DLBCL; rs1800795 and rs1800797 for MM). From the Haplotype-based analysis shown in Table 3, we observed the A-C-G haplotype (OR=2.45, 95%CI 1.55-3.89, *p*=0.0002) and the C-C-A haplotype

Table 1. Association between Polymorphisms of Inflammation-Related Genes and Non-Hodgkin Lymphoma (NHL) in the Han Ethnic Group of Xinjiang Province

Gene name, SNP database ID (nucleotide change)	No. of controls (%)	All NHL			B-cell lymphoma			DLBCL			MM		
		No. of cases (%)	Odds ratio (95% CI)	<i>P</i>	No. of cases (%)	Odds ratio (95% CI)	<i>P</i>	No. of cases (%)	Odds ratio (95% CI)	<i>P</i>	No. of cases (%)	Odds ratio (95% CI)	<i>P</i>
TNF- α rs1799724 (-857C>T)													
CC	160	69			57			25			13		
CT+TT	42+2	24+0	1.265 (0.714-2.241)	0.42	21+0	1.340 (0.734-2.444)	0.34	9+0	1.309 (0.570-3.007)	0.525	8+0	2.238 (0.873-5.739)	0.15
TNF- α rs1800629 (-308G>C)													
GG	190	78			65			30			17		
GA+AA	14+0	13+0	2.262 (1.017-5.032)	0.041	11+0	2.297 (0.993-5.312)	0.047	3+0	1.357 (0.368-5.005)	0.923	3+0	2.395 (0.626-9.165)	0.385
TNF- α rs1800630 (-863C>A)													
CC	146	55			47			16			16		
CA+AA	55+3	34+4	1.739 (1.041-2.906)	0.034	27+4	1.660 (0.962-2.866)	0.067	14+4	2.832 (1.353-5.929)	0.005	5+0	0.787 (0.275-2.246)	0.653
TNF- α rs361525 (-863G>A)													
GG	179	83			69			31			17		
GA	24+1	8+0	0.690 (0.299-5.595)	0.383	7+0	0.726 (0.300-1.756)	0.476	2+0	0.462 (0.104-2.049)	0.457	3+0	1.264 (0.345-4.621)	1
TNF- β rs2239704 (-91C>A)													
CC	82	33			28			16			5		
CA	96	50	1.294 (0.762-2.197)	0.339	41	1.251 (0.712-2.198)	0.436	17	0.908 (0.431-1.909)	0.798	12	2.050 (0.693-6.061)	0.187
AA	25	10	0.994 (0.430-2.296)	0.989	9	1.054 (0.440-2.528)	0.906	1	0.205 (0.026-1.623)	0.185	4	2.624 (0.654-10.524)	0.316
TNF- β rs909253 (-252A>G)													
AA	69	42			37			16			10		
AG	98	39	0.654 (0.383-1.115)	0.118	31	0.590 (0.334-1.041)	0.067	14	0.616 (0.282-1.345)	0.221	9	0.634 (0.245-1.641)	0.344
GG	36	11	0.502 (0.231-1.091)	0.079	10	0.518 (0.231-1.160)	0.107	4	0.479 (0.149-1.540)	0.209	2	0.383 (0.080-1.844)	0.363
IL-6 * rs1800795 (-174G>C)													
GG	198	83			71			34			18		
GC+CC	6+0	10+0	3.976 (1.400-11.295)	0.006	7+0	3.254 (1.058-10.008)	0.065	0+0	0.853 (0.809-0.900)	0.598	3+0	5.500 (1.268-23.865)	0.041
IL-6 * rs1800797 (-598G>A)													
GG	198	83			71			34			18		
GA+AA	6+0	10+0	3.976 (1.400-11.295)	0.006	7+0	3.254 (1.058-10.008)	0.065	0+0	0.853 (0.809-0.900)	0.598	3+0	5.500 (1.268-23.865)	0.041
IL-10 rs1800871 (-819C>T)													
TT	88	40			36			19			9		
CT	94	41	0.960 (0.568-1.620)	0.877	36	0.936 (0.542-1.616)	0.813	13	0.641 (0.299-1.374)	0.25	11	1.144 (0.453-2.893)	0.776
CC	22	12	1.200 (0.541-2.661)	0.653	6	0.667 (0.250-1.781)	0.416	2	0.421 (0.091-1.945)	0.407	1	0.444 (0.053-3.696)	0.727
IL-10 rs1800872 (-592C>A)													
AA	88	40			36			19			9		
CA	95	41	0.949 (0.563-1.602)	0.846	36	0.926 (0.537-1.598)	0.783	13	0.634 (0.296-1.359)	0.239	11	1.132 (0.448-2.862)	0.793
CC	21	12	1.257 (0.564-2.803)	0.575	6	0.698 (0.260-1.873)	0.474	2	0.441 (0.095-2.043)	0.448	1	0.444 (0.053-3.696)	0.727
IL-10 rs1800890 (-3575T>A)													
TT	190	82			71			31			19		
TA+AA	14+0	11+0	1.821 (0.793-4.180)	0.153	7+0	1.338 (0.519-3.451)	0.546	3+0	1.313 (0.357-4.836)	0.959	2+0	1.429 (0.302-6.763)	0.995
IL-10 rs1800896 (-1082A>G)													
AA	172	74			66			29			19		
AG+GG	31+1	18+1	1.380 (0.735-2.590)	0.315	12+0	0.977 (0.475-2.011)	0.95	5+0	0.927 (0.334-2.573)	0.884	2+0	0.566 (0.126-2.549)	0.667
NF-KB1 rs1585215													
TT	84	28			25			9			6		
TC	89	52	1.753 (1.014-3.031)	0.044	42	1.586 (0.890-2.826)	0.117	20	2.097 (0.904-4.865)	0.08	12	1.888 (0.678-5.258)	0.218
CC	31	13	1.258 (0.579-2.734)	0.562	11	1.192 (0.525-2.707)	0.674	5	1.505 (0.468-4.842)	0.708	3	1.355 (0.319-5.752)	0.98
TLR-4 rs4986790 (+896A>G)													
AA	203	89			74			34			18		
GA+GG	1+0	4+0	9.125 (1.005-82.789)	0.06	4+0	10.973 (1.207-99.786)	0.033	0+0	0.857 (0.813-0.902)	1	3+0	33.833 (3.345-342.194)	0.003

Table 2. Association between Polymorphisms of Inflammation-Related Genes and Non-Hodgkin Lymphoma (NHL) in the Uygur Ethnic Group of Xinjiang Province

Gene name, SNP database ID (nucleotide change)	No. of controls (%)	All NHL			B-cell lymphoma			DLBCL		
		No. of cases (%)	Odds ratio (95% CI)	P	No. of cases (%)	Odds ratio (95% CI)	P	No. of cases (%)	Odds ratio (95% CI)	P
TNF- α rs1799724 (-857C>T)										
CC	164	47			38			12		
CT+TT	57+8	15+2	0.913 (0.489-1.704)	0.774	13+2	0.996 (0.513-1.933)	0.99	5+1	1.262 (0.454-3.503)	0.655
TNF- α rs1800629 (-308G>C)										
GG	186	49			41			11		
GA+AA	40+5	12+3	1.265 (0.652-2.457)	0.486	9+3	1.210 (0.588-2.488)	0.604	6+1	2.630 (0.966-7.164)	0.099
TNF- α rs1800630 (-863C>A)										
CC	158	40			34			12		
CA+AA	66+7	21+3	1.299 (0.729-2.313)	0.374	16+3	1.210 (0.647-2.262)	0.551	5+1	1.082 (0.391-2.997)	0.879
TNF- α rs361525 (-863G>A)										
GG	205	59			48			15		
GA+AA	24+2	5+0	0.668 (0.246-1.816)	0.427	5+0	0.821 (0.300-2.249)	0.701	3+0	1.577 (0.428-5.815)	0.758
TNF- β rs2239704 (-91C>A)										
CC	82	30			24			9		
CA	100	21	0.574 (0.306-1.077)	0.082	18	0.615 (0.312-1.211)	0.157	7	0.638 (0.228-1.786)	0.389
AA	47	13	0.756 (0.360-1.589)	0.46	11	0.800 (0.360-1.777)	0.583	2	0.388 (0.080-1.870)	0.374
TNF- β rs909253 (-252A>G)										
AA	104	27			25			7		
AG	97	29	1.152 (0.637-2.083)	0.641	21	0.901 (0.474-1.713)	0.75	9	1.378 (0.494-3.845)	0.538
GG	28	8	1.101 (0.451-2.687)	0.833	7	1.040 (0.408-2.652)	0.935	2	1.061 (0.209-5.394)	1
IL-6 rs1800795 (-174G>C)										
GG	165	51			43			14		
GC+CC	57+9	13+0	0.637 (0.325-1.249)	0.187	10+0	0.581 (0.276-1.225)	0.15	4+0	0.714 (0.227-2.250)	0.564
IL-6 rs1800797 (-598G>A)										
GG	167	49			41			14		
GA+AA	56+8	15+0	0.799 (0.419-1.524)	0.495	12+0	0.764 (0.377-1.545)	0.453	4+0	0.746 (0.237-2.350)	0.819
IL-10 rs1800871 (-819C>T)										
CC	70	23			19			9		
CT	122	30	0.748 (0.404-1.388)	0.357	25	0.755 (0.388-1.468)	0.407	6	0.383 (0.131-1.120)	0.071
TT	39	11	0.858 (0.379-1.946)	0.714	9	0.850 (0.351-2.059)	0.719	3	0.598 (0.153-2.341)	0.671
IL-10 rs1800872 (-592C>A)										
CC	69	23			19			9		
CA	122	30	0.738 (0.398-1.369)	0.334	25	0.744 (0.382-1.448)	0.383	6	0.377 (0.129-1.104)	0.066
AA	40	11	0.825 (0.364-1.868)	0.644	9	0.817 (0.338-1.977)	0.654	3	0.575 (0.147-2.248)	0.627
IL-10 rs1800890 (-3575T>A)										
TT	143	48			37			14		
TA+AA	78+10	16+0	0.542 (0.290-1.012)		16+0	0.703 (0.369-1.338)	0.281	4+0	0.464 (0.148-1.455)	0.179
IL-10 rs1800896 (-1082A>G)										
AA	125	43			33			12		
AG+GG	94+12	21+1	0.603 (0.339-1.072)	0.083	19+1	0.715 (0.387-1.319)	0.281	6+0	0.590 (0.214-1.625)	0.302
NF- κ B1 rs1585215										
TT	94	22			18			5		
TC	108	33	1.306 (0.712-2.394)	0.388	27	1.306 (0.677-2.519)	0.426	11	1.915 (0.642-5.710)	0.237
CC	29	9	1.326 (0.550-3.198)	0.529	8	1.441 (0.568-3.655)	0.441	2	1.297 (0.239-7.040)	1
TLR-4 rs4986790 (+896A>G)										
AA	210	60			49			15		
GA+GG	20+1	3+1	0.667 (0.220-2.017)	0.47	3+1	0.816 (0.268-2.486)	0.929	2+1	2.000 (0.535-7.474)	0.526

Table 3. The Case- Control Analysis of Haplotypes of SNPs in TNF- α and TNF- β Gene in the Han and Uygur Ethnic Group

Haplotype	Han ethnic group			Uygur ethnic group		
	Freq	OR (95% CI)	P-value	Freq	OR (95% CI)	P-value
TNF- α (rs1800630/rs1799724/ rs1800629)						
C-C-G	0.633	1	-	0.486	1	-
A-C-G	0.204	2.45(1.55-3.89)	0.0002	0.184	1.43 (0.77 - 2.68)	0.26
C-T-G	0.112	1.29(0.67-2.50)	0.45	0.201	0.98 (0.52 - 1.84)	0.96
C-C-A	0.05	2.53(1.10-5.80)	0.029	0.128	1.98(1.11-3.51)	0.021
TNF- β (rs2239704/ rs909253)						
C-G	0.406	1	-	0.318	1.11 (0.68 - 1.81)	0.69
A-A	0.328	1.31(0.82-2.09)	0.27	0.433	1	-
C-A	0.266	1.87(1.21-2.90)	0.0054	0.246	1.12 (0.64 - 1.97)	0.69

(OR=2.53, 95%CI: 1.10-5.80 $p=0.029$) associations in TNF- α -rs1800630/rs1799724/rs1800629; with an overall risk of NHL. In addition, we also found an association between the C-A haplotype in TNF- β -rs909253/rs2239704 and the NHL risk.

For the Uygur population, 11 cases of T-NHL and 53 cases of B-NHL were included in the case group; and the most common NHL subtypes were also DLBCL (n=18), MM (n=13) and SLL/CLL (n=13). The genotype distribution among the NHL cases and controls are shown in Table 2. None of the SNPs were individually associated with the overall risk of NHL or its common subtypes. An association was observed from the haplotype analysis between the TNF- α C-C-A haplotype (OR=1.98, 95%CI:1.11- 3.51, $p=0.021$) and the NHL risk which was also observed from the Han Chinese population. However, there was no significant association between TNF- α A-C-G and TNF- β C-A haplotypes with all NHL risks.

Discussion

Cytokines participate in regulating cell differentiation, proliferation, death, as well as the inflammatory and immune responses. These also have an important influence in the development and differentiation of the lymphoid tissue and its roles in the inflammatory and immune responses can be divided into two major categories: proinflammatory and anti-inflammatory cytokines. Among these set of gene candidates, TNF- α , TNF- β and IL-6 are important proinflammatory cytokines, due to its characteristic of promoting or inducing inflammatory and immune responses (Chen et al., 2002; Pfeffer, 2003; Kishimoto, 2006) and IL-10 is a significant anti-inflammatory cytokine that has a main role of limiting the inflammatory response in vivo (Akdis et al., 2001). Innate cytokine amounts are closely and genetically regulated at the level of transcription, which greatly varies in different individuals due to genetic variations.

In addition to its genetic factors, cytokine levels are extremely influenced by immune regulation, wherein, the nuclear factor - κ B (NF- κ B) and the Toll-like receptors (TLRs) play a very important role. It is known that NF- κ B is a pleiotropic transcription factor, consisting of 5 family members: RelA (p65), RelB, c-Rel, NF- κ B 1/p50 and NF- κ B2/p52; which functions as various homodimers and heterodimers the heterodimer of NF κ B1 p50/RelA is the major form among these family members. NF- κ B is involved in many physiological and pathological processes, which are known to crucially regulate immune and inflammatory responses, due to its capability of regulating the transcription of the genes encoding pro-inflammatory cytokines, chemokines and adhesion molecules (Silverman et al., 2001; Hayden et al., 2006; Ghosh et al., 2008). The immediate targets of NF- κ B-dependent pro-inflammatory cytokines such as TNF α tend to be receptors that, in turn, activate NF- κ B. Furthermore, activated NF- κ B could inhibit cell apoptosis induced by TNF α (Beg et al., 1996). TLRs are a family of transmembrane receptors that play a key role in mounting an immune response against microbial pathogens (Aderem et al., 2000; Park et al., 2009). Up to the present time, 10

functional human TLRs have been identified; and among these TLRs, TLR4 is well known for its response to lipopolysaccharide (LPS) an outer cell wall component of gram-negative bacteria. As a whole, recognizing LPS and initiating TLR4 signals are both complex processes. Two pathways initiating downstream TLR4 signals are known, namely, the MyD88 and the TRIF-dependent pathways. Initiating the MyD88-dependent pathway activation leads to the activation of the NF- κ B and the transcription of proinflammatory genes.

As mentioned, cytokines interact in the body to promote or inhibit each other and the signaling pathways of NF- κ B and TLRs form a complex regulatory network in the immune and inflammatory responses. Related genes and molecules involved in this network becomes a hot spot for a variety of diseases, such as infectious diseases, autoimmune diseases and cancer-which also includes lymphomas (now that various lines of evidence suggest that infections and immune dysregulation play a role in the risk of disease).

Up to now, many case-control studies have been done to examine the association of the polymorphic variations of genes with the risk of lymphoma these studies partly showed positive results. A pooled analysis of 3, 586 cases of NHL and 4, 018 controls from the International Lymphoma Epidemiology (InterLymph) consortium (Rothman et al., 2006) showed that the TNF- α -308 G \rightarrow A polymorphism was associated with the increased risk of NHL, particularly, for DLBCL. Additionally, the IL-10 -3575T \rightarrow A and the TNF- β -252 A \rightarrow G polymorphisms were found to be also associated with the increased risk level, particularly for DLBCL. But no association was found between the IL6 promoter polymorphism (-174G $>$ C) and the risk of NHL. Subsequently, Purdue et al (2007) was able to establish similar results, wherein, the polymorphic variations in IL-10 and TNF genes could increase the risk of NHL. Fernberg et al (2010) also described that variant alleles in IL-10- 3575 (rs1800890) can increase the risk of diffuse large B-cell lymphomas, but found no associations between the polymorphisms of TNF- α -G308A (rs1800629) and the pathogenesis of DLBCL. However, this study highlighted notable associations between TNF- α -308, T-cell lymphoma and mantle cell lymphoma. Wang et al (2006) detected a total of 57 single nucleotide polymorphisms (SNPs) in 36 candidate immune genes of 1, 172 NHL cases and 982 controls, and the results show that a haplotype C-G-C-A-G comprising of SNPs, in TNF- α and LT- α (rs1800629- rs361525- rs1799724- rs909253- rs2239704), can generally increase the risk of NHL; and this influence is clearly seen in diffuse large B-cell lymphoma. Chang et al (2009) compared a total of 20 SNPs of seven genes in 473 Hodgkin Lymphoma (HL) cases and 373 controls; and found that the NF-KB 1 polymorphism (rs1585215) was associated with the risk of HL. The risk of AG and GG genotype carriers suffering from HL was 2.1 and 3.5 times, compared with the AA genotype, respectively. In addition, the TLR-4 Asp299Gly (rs4986790) and Lymphomagenesis research results were extremely inconsistent. Hellmig (2005) and Forrest et al (2006) were able to establish that loci genetic variations could reduce the risk of lymphoma of mucosa-associated

lymphoid tissue (MALT), and diffuse the large B-cell lymphoma; on the other hand, Nieters et al (2006) found that it could increase the risk of MALT and HL; while, Purdue et al (2009) did not find any significant association between all NHL types or any of its subtypes.

In our study, the results showed no significant correlation between the 14 SNPs of the 6 gene candidates from the Uyghur population and risks of NHL or its common histological types; however, there were 6 SNPs that are closely related to the occurrence of NHL or certain subtypes from the Han population. TNF- α -G308A (rs1800629) was found to be associated with NHL and B-NHL, overall, for the Han population; and carriers with the rs1800629 GA genotype (AA genotype was not found) had more two-fold risks of NHL and B-NHL than those with the rs1800629 GG genotype; and TNF- α rs1800630 variant genotypes (CA/AA) were found to be associated with the overall increased risks of NHL and DLBCL, on the Han population. In addition, the results showed that two SNPs of IL-6 (rs1800795 and rs1800797) and one SNP of TLR-4 (rs4986790) were strongly associated with MM which was not previously reported. Meanwhile, we found that the polymorphic site of NF-KB1 rs1585215 had an overall association with NHL, but no associations were found from any of its common subtypes. Haplotype analysis results reflected single SNP findings in magnitude and direction. From the Han population, individuals carrying TNF- α (rs1800630/rs1799724/rs1800629) ACG/CCA haplotype or TNF- β (rs909253/rs2239704) CA haplotype had greater risk of NHL than those carrying the TNF- α CCG haplotype or TNF- β CG haplotype, respectively. From the Uyghur population, carriers with TNF- α CCA haplotype had a higher risk of NHL, compared to those with TNF- α CCG.

SNP in rs1800629 (TNF- α -G308A) influenced the overall risks of NHL and B-NHL, while SNP in rs909253 (TNF- β -A252G) did not affect the risk of NHL for the Han population. These findings were consistent with the reports previously mentioned for the Caucasian population (Rothman et al., 2006), however, these were also inconsistent with some findings that were reported by domestic scholars for the Han Chinese population. For instance, Zhao et al (2002) investigated the possible association of TNF α -308 and lymphotoxin- α (TNF- β)+252 gene polymorphisms with the pathogenesis of NHL in the Han population; these results showed that TNF α -308 gene polymorphism had no effect on NHL, but TNF- β +252 allelic type may be related to the susceptibility of NHL for the Chinese Han population to some extent. Lu et al (2008) examined the SNPs in TNF α -308 of the control group, and the aggressive and high-aggressive group of NHL from the Han population. Between the control group and the aggressive groups, no significant differences were found from the TNF- α -308 genetic polymorphism, but these inferred that the TNF- α -308 G \rightarrow A variant might be an important risk factor for the high-aggressive NHL of this study.

It is worth mentioning, that our study reported a relationship between inflammation-related gene polymorphisms and the NHL of the Uyghur ethnic group, for the first time. Several SNPs -as potential NHL

susceptibility loci for the Han ethnic group-- showed no associations with the NHL for Uyghur ethnic group in our study, which had same results as the haplotypes of TNF- α ACG and TNF- β CA. We also found that the TNF- α CCA haplotype was closely related with the NHL susceptibility of the Uyghur ethnic group; though SNPs did not show the association. These findings indicates that the correlation vary with different ethnic groups, and that the haplotype analysis was somewhat better than single SNP marker; for associating it with the study of human diseases to provide more abundant information; which also helps in using low frequency variation information.

In conclusion, our case-control study provides further support for the genetic component of NHL in both Han and Uyghur ethnic groups. However, replications and a multi-center pooled analysis are still necessary to eliminate the false positive and further clarify the relationship between inflammation-related gene polymorphisms and NHL.

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