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Original Article

IL-13 polymorphisms are associated with the risk of Non-Hodgkin's lymphoma

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Abstract: Interleukin-13 (IL13) is an immunoregulatory cytokine which plays an important role in carcinogenesis through affecting tumor immunosurveillance. In this study, we investigated the association of two functional polymorphisms of IL-13 (rs1800925 and rs20541) with risk of Non-Hodgkin's lymphoma (NHL). Overall, significant differences of genotype distribution were observed between 130 NHL cases and 30 controls at the IL-13 rs1800925 T/G genotypes. Compared with the IL-13 rs1800925 T/G homozygote TT, the heterozygous TG genotype was associated with significantly increased risk for NHL (OR = 2.32, 95% CI = 1.21-3.92, P = 0.025). Moreover, the genotype GG of IL-13 rs1800925 T/G carried a higher risk of NHLmetastasis and later stages, compared with the TT genotype. However, the genotype and allele frequencies of IL-13 rs20541 C/T polymorphisms in NHL patients were not significantly different from controls. Conclusion: IL-13 rs1800925 T/G genotype was associated with increased risk for development and metastasisof NHL in Chinese Han population.

Keywords: IL-13, Non-Hodgkin's lymphomas, single-nucleotide polymorphism

Introduction

Non-Hodgkin's lymphomas (NHL) are heterogeneousgroup of lymphoproliferative disorders, the most commonhematologic malignancy worldwide and has shown apronounced increase in incidence in the past 10 years [1]. Although the survival rate of NHL has improved substantially, the incidence of the disease has still been increasing steadily worldwide over thelast two decades [2]. NHL is generally classified into two major groups: B cell lymphoma and T celllymphoma. Of B cell lymphoma, diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL) are the two most common subtypes, constituting 30-40 and 20-30% of lymphomas in western countries, respectively. Biologic mechanisms leading to the development of NHL are not clearly understood. It has been suggested that the immune system deregulation may be linked to the rising incidence of lymphoma [3]. Molecular epidemiology studies suggested that single nucleotide polymorphisms (SNPs) in specific genes and pathways may play an important role in the pathogenesis of NHL.

The human IL-13 gene is together with the IL-4 gene located within 15 kb on chromosome 5q315. IL-4 and IL-13 share many (but not all) functions. To date, variation screening has been extensively performed for these genes and several single-nucleotide polymor-phisms (SNPs) have been found to show functional significance and to be strongly associated with immune-related disease. IL-4 and IL-13 share immunoregulatory functions and the common IL-4R chain on their receptor, inducing immunoglobulin E (IgE) production and inhibiting inflammatory cytokines [4, 5]. However, the effect of IL-13 still remains not clear in the NHL. The aim of our study was to investigate the possible association between the polymorphisms oftwo SNPs of IL-13 (rs1800925 and rs20541) and risk of NHL in Chinese Han population.

Material and methods

Study population

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the ethics review committee of each participating center. All patients gave writ-

Table 1. Distribution of selected variables between cases and controls

Characteristics	Cases (%) N = 130	Controls (%) N = 130	*P* value*
Mean Age (years)	55.3 (± 11.3)	56.8 (± 14.4)	0.241
≤60	98 (75.4)	87 (66.9)	
>60	32 (24.6)	43 (33.1)	
Gender			0.139
Male	76 (58.5)	82 (63.1)	
Female	54 (41.5)	48 (36.9)	
Ann arbor stages	23.5 (± 4.3)		
Localized (I + II)	48 (36.9)		
Advanced (III + IV)	82 (63.1)		
Bone marrow involvement			
No	101 (77.7)		
Yes	29 (22.3)		
B symptoms			
With	58 (44.6)		
Without	72 (55.4)		

*, *P*-value is < 0.05.

ten informed consent. This study included 130 patients with NHL and 130 healthy controls from Han ethnic group from Jining NO. 1 People's Hospital between March 2011 and March 2014. All of them were histologically/pathologically confirmed by two experienced pathologists. Patients were eligible if they had previously untreated, biopsy-confirmed aggressive NHL according to the Revised European-American Lymphoma Classification (translated into the WHO classification). The control group comprised 130 healthy volunteers for the general health checkup in our hospital during the same period. All the healthy controls had been under the health screening, and their clinical characteristics were matched to the sex and age distribution with the NHL, as outlined in **Table 1**.

DNA extraction and genotyping

Genomic DNA was extracted from 5 mL frozen whole blood using the DNA Extraction Kit (Fastagen, China) according to the manufacturer's protocol. The IL-13 rs1800925 and rs20541 of genotypes were determined using a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay and DNA sequencing analysis. For quality control, genotyping was repeated randomly in at least 5% of the samples, and two of the authors independently reviewed all results. PCR reactions were carried out in a total volume 10 μl containing 20 ng of genomic DNA, 0.25 mM of each Dntp (Ecogen, Biologia Molecular S.L.), 0.2 units of Taq polymerase (Biotools, Inc.) and 2.5 pmol of each primer in a 1× PCR buffer (Sigma-Aldrich Co.).

Statistical analysis

Genotype and allele frequencies of IL-16 polymorphisms were compared between NHL cases and controls using the Chi-square test and odds ratios (OR), and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a particular allele and genotype. Demographic and clinical data between groups were compared by Chi-squaretest and Student's t-test. Similarly, the Hardy-Weinberg equilibrium (HWE) of each subject was examined by implying a two-sided chi-square (χ^2) test which was performed by comparison of observed and expected genotype frequencies. We managed all the statistical analysis with the SPSS software version 19.0. A two-sided *P* value less than 0.05 was considered to be statistically significant for all the analyses.

Results

Patients and control individuals

The clinical characteristics of NHL cases and controls were summarized in **Table 1**.

This study included 130 NHL patients and 130 healthy controls including the information on their age, gender, Ann arbor stages, Bone marrow involvement and B symptoms. The mean age (± SD) for case and control groups was 55.5 (± 13.3) and 56.8 (± 14.4) years, respectively. No significant difference was detected in age, gender, Ann arbor stages, Bone marrow involvement and B symptoms between two groups (P > 0.05). Regarding the clinical stage, 36.9% of patients were instage I and II, whereas 63.1% of patients presented III and IV stage. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for the polymorphisms in IL-13 rs1800925 and rs20541.

Distributions of IL-13 (rs1800925 and rs20541) genotypes and risk of NHL

The genotype and allele frequencies of the IL-13 (rs1800925 and rs20541) polymorphi-

Table 2. Association between two SNPs (rs1800925 and rs20541) of IL-13 gene and patients susceptibility

Polymorphisms	Cases (N = 130) (%)	Controls (N = 130) (%)	OR (95% CI)	*P*-value*
rs1800925				
TT	64 (49.2)	95 (73.1)	1	
TG	42 (32.3)	25 (19.2)	2.32 (1.21-3.92)	***0.025****
GG	24 (18.5)	10 (7.7)	1.81 (1.42-3.99)	***0.013****
TG + GG	66 (48.7)	35 (29.5)	1.93 (1.36-3.94)	***0.021****
T	170 (65.9)	215 (81.3)	1	
G	90 (34.1)	45 (18.7)	1.86 (1.35-4.21)	***0.026****
rs20541				
CC	59 (45.4)	63 (48.5)	1	
CT	50 (48.5)	48 (36.9)	1.47 (0.81-4.63)	0.251
TT	21 (16.1)	19 (14.6)	1.76 (0.89-5.36)	0.272
CT + TT	71 (54.7)	67 (52.6)	1.69 (0.84-4.81)	0.149
C	168 (68.9)	174 (70.5)	1	
T	92 (31.1)	86 (29.5)	1.48 (0.89-6.41)	0.162

OR, odds ratio; CI, confidence interval. *Bold numbers indicate that the *P*-value is < 0.05.

sms for all the studied variations are analyzed. All genotype frequencies of the control group conformed to the Hardy-Weinberg equilibrium.

There were significant differences in the genotype and allele frequencies of IL-13 rs1800925 T/G genotypes between NHL cases and controls. Compared with the IL-13 rs1800925 homozygote TT, the heterozygous TG genotype was associated with significantly increased risk for NHL (OR = 2.32, 95% CI = (1.21-3.92), P = 0.025); the GG genotype was associated with increased risk for NHL (OR = 1.81, 95% CI = 1.42-3.99, P = 0.013). TG and GG combined variants were associated with increased risk for NHL compared with the TT genotype (OR = 1.93, 95% CI = 1.36-3.94, P = 0.021). However, the genotype and allele frequencies of IL-13 rs20541 C/T polymorphisms in NHL patients were not significantly different from controls (P > 0.05) as shown in **Table 2**.

Distributions of IL-13 (rs1800925 and rs20541) genotypes and clinicopathological characteristics

The associations between the IL-13 (rs1800925 and rs20541) genotypes polymorphisms and clinicopathological parameters were calculated. The resultsare given in **Table 3**. For IL-13 rs1800925 T/G, the genotype GG frequency in tumor later stages (III + IV) patients was greater compared to patients with early stages (I + II), and the difference in frequency distribution between genotypes reached significance (P = 0.018). No significant difference was observed with respect to their age, gender, Bone marrow involvement and B symptomsand the IL-13 rs1800925 T/G genotypes. For IL-13 rs20541 C/T, there are no any obvious differences in the relations between their age, gender, Ann arbor stages, Bone marrow involvement and B symptoms respectively, and IL-13 rs20541 C/T genotypes.

Discussion

IL-13 acts as a tumor inhibitor to downregulate the tumor immunosurveillance through the IL-4R signaling pathway [6, 7]. Besides, IL-13 was identified to induce myeloid cells to make transforming growth factor-b to mediate the tumor immunosurveillance in several mouse tumor models [8-10] and the increased production of IL-13 was found in patients with bladder cancerso thatit is possible from the immune response against inflammation caused by bladder tumors [11, 12]. Therefore, the variants of the IL-4R and IL-13 genes could be expected to have an effect on tumor immune responses, and thus, carcinogenesis.

In present study, we assessed the association between the polymorphisms of two SNPs of IL-13 (rs1800925 and rs20541) and risk of NHL in Chinese Han population and found the significant association between IL-13 rs1800925 T/G polymorphisms and risk of NHL. The genotype and allele distribution of IL-13 rs1800925 T/G genotypes were significantly different between case and control groups, indicating that IL-13 rs1800925 T/GT/G might be related to NHL development. In addition, our study showed the genotype GG frequency of IL-13 rs1800925 T/G in tumor metastasis patients was greater compared to patients without tumor metastasis. These results indicated that the genotype GG frequency of IL-13 rs1800925 T/G carried a higher risk

Table 3. Correlations between genotypes of two SNP (rs1800925 and rs20541) of IL-13 gene and clinicopathological features of patients

Genotypes		rs1800925 T/G				rs20541 C/T			
Variable	n	TT	TG	GG	*P* value	CC	CT	TT	*P* value
Mean Age (years)		64	42	24		59	50	21	
≤60	98	48	31	19	0.421	42	40	16	0. 281
>60	32	16	11	5		17	10	5	
Gender									
Male	76	39	24	13	0.215	38	44	9	0. 408
Female	54	25	18	11		48	46	5	
Ann arbor stages									
Localized (I + II)	48	81	47	14	0.018*	74	66	2	0.136
Advanced (III + IV)	82	37	20	31		32	29	27	
Bone marrow involvement									
No	101	94	46	24	0.238	86	68	10	0.241
Yes	29	24	21	21		20	27	19	
B symptoms									
With	58	20	23	25	0.219	25	32	11	0.321
Without	72	79	40	3		61	58	33	

*Student's t-test and the chi-square (χ^2) test.

of NHL metastasis, compared with the TT genotype. To the best of our knowledge, our study is the first report to describe the possible role of two polymorphisms of IL-13 (rs1800925 and rs20541) as a risk factor for NHL and found that IL-13 rs1800925 T/G genotype variations do influence susceptibility to NHL development and metastasis in the Chinese Han population.

The effects of human IL-13 on clonal growth of breast cancer cells were investigated that revealed IL-13 can reduce clonal growth of 3 cell type [13]. The 1055 C/T was located in promoter region of IL-13 gene. There were several polymorphisms in IL-13 gene. These studies concluded that IL-13 have important role in regulation of antitumor activities. The change of C to T nucleotide in this point may alter attachment and transcription factor type and then alter the expression of IL-13 gene. Several studies revealed that -1055 C/T polymorphism was associated with allergic asthma, altered regulation of IL-13 production and increased binding of nuclear protein [14].

In spite of interesting findings on the association of IL-13 polymorphisms with NHL risk, there were several limitations that need to be addressed regarding the present study. We did not collect lifestyle data for individual participants, e.g. on local environmental factors, diet, or level of physical activity, which potentially could interact with genetic variations in influencing overall risk of developing NHL. Studies need to be performed in larger study groups to confirm our preliminary results.

In conclusion, our study provided the evidence of association between the polymorphisms of IL-13 (rs1800925 and rs20541) and the risk of NHL and found the IL-13 rs1800925 T/G genotype was associated with increased risk for development and metastasis of NHL in Chinese Han population.

Disclosure of conflict of interest

None.

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