Cancer Epidemiology 37 (2013) 930–934

Contents lists available at ScienceDirect

Cancer Epidemiology

The International Journal of Cancer Epidemiology, Detection, and Prevention

journal homepage: www.cancerepidemiology.net

Genetic variations in *CTLA-4*, *TNF-α*, and *LTA* and susceptibility to T-cell lymphoma in a Chinese population

Jie Liu [a,b,1], Jing Liu [c,1], Bao Song [a,b], Ti Wang [d], Yuhong Liu [b], Jing Hao [e], Jinming Yu [a,b,*]

[a] *Medical College, Shandong University, Jinan, China*
[b] *Department of Oncology, Shandong Cancer Hospital, Shandong Academy of Medical Sciences, Jinan, China*
[c] *Department of Epidemiology and Biostatistics, School of Public Health, Shandong University, Jinan, China*
[d] *Department of Oncology, Sishui People Hospital, Sishui, China*
[e] *Department of Oncology, Qilu Hospital, Shandong University, Jinan, China*

ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form 19 August 2013
Accepted 21 August 2013
Available online 12 September 2013

Keywords:
T-cell lymphoma
Immunogenetics
Genetic susceptibility
CTLA-4
TNF-α
LTA

ABSTRACT

Objective: T-cell lymphoma is a highly aggressive malignant lymphoma that is rare in Caucasians but relatively common in Asian populations. Factors regulating T-cell proliferation and function may play an important role in the pathogenesis of T-cell lymphoma.
Methods: A total of 8 single nucleotide polymorphisms in cytotoxic T lymphocyte antigen-4 (*CTLA-4*), tumor necrosis factor-α (*TNF-α*), and lymphotoxin-α (*LTA*) genes were detected by polymerase chain reaction–ligation detection reaction analysis in a Chinese population of 291 patients with T-cell lymphoma and 300 healthy controls. Logistic regression was used to determine the odds ratios (ORs) and 95% confidence intervals for the associations of genotypes and haplotypes with T-cell lymphoma risk.
Results: Among these polymorphisms, the *LTA* +252AA genotype was significantly associated with T-cell lymphoma risk (OR, 2.3; $P = 0.002$). Furthermore, the TNF-α/LTA haplotype C-G-G-A (*TNF-α* −857C, −308G, and −238G and *LTA* +252A) showed a significantly increased risk for T-cell lymphoma (OR, 1.6; $P = 0.001$).
Conclusion: Our study suggested that the *LTA* +252G>A polymorphism may influence susceptibility to T-cell lymphoma in the Chinese population.

With an estimated 77,000 new cases for the year 2013, non-Hodgkin lymphoma (NHL) is the fifth most common malignant neoplasm in the USA [1]. NHL is ranked the 11th most common cancer in China, with an incidence rate of approximately 3.5/100,000 [2]. NHL is generally classified as B-cell lymphoma or T-cell lymphoma. T-cell lymphoma accounts for less than 15% of all NHLs in Caucasians, whereas it accounts for approximately 30% of all NHLs in Asian populations [3]. Altered immune function and genetic factors have been suggested as risk factors for NHL [4,5]. Identification of genetic variants of immune function genes associated with T-cell lymphoma risk should improve our understanding of the pathogenesis of this malignancy.

Cytotoxic T lymphocyte antigen-4 (CTLA-4), which regulates the activation and proliferation of T-cells and NK cells, is regarded as a candidate susceptibility gene for T-cell lymphoma. The CTLA-4–ligand interaction has an inhibitory effect on T-cell proliferation, whereas antibody-mediated blockade of CTLA-4 prevents development of tolerance, augments antitumor responses, and exacerbates autoimmune disease [6]. More than 100 single nucleotide polymorphisms (SNPs) have been identified in the *CTLA-4* gene. Among the *CTLA-4* polymorphisms, −1661A>G (rs4553808) and −318C>T (rs5742909) in the promoter region, 49A>G (rs231775) in exon 1, and CT60A>G (rs3087243) in the 3′ untranslated region (3′-UTR) have been associated with susceptibility to autoimmune disorders and various cancers [7–10]. In addition, polymorphisms in the *CTLA-4* gene have been shown to have a role in the occurrence of NHL [11,12].

Tumor necrosis factor (TNF), a major cytokine involved in the promotion of inflammatory responses, plays a crucial role in the pathogenesis of various inflammatory, autoimmune, and malignant diseases. Three classes of TNF have been identified: TNF-α, lymphotoxin-α (LTA), and LT-β [13]. Both TNF-α and LTA influence lymphomagenesis by upregulating proinflammatory and anti-apoptotic signals *via* the NK-kβ pathway [14]. The *TNF/LTA* gene is located on chromosome 6q21.3, and several studies have suggested that genetic variations in *TNF-α* (−857C>T [rs1799724], −308G>A [rs1800629], and −238G>A [rs361525])

* Corresponding author at: Shandong Cancer Hospital, Shandong Academy of Medical Sciences, 440 Jiyan Road, Jinan 250117, China. Tel.: +86 531 67626332; fax: +86 531 8798 4079.
E-mail address: yu_jinming@126.com (J. Yu).
[1] These authors contributed equally to this work.

1877-7821/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canep.2013.08.011

and *LTA* (+252G>A [rs909253]) may contribute to susceptibility to several cancers, including NHL [15–19].

Although large-scale studies of the genetic susceptibility to B-cell lymphoma have been published in Caucasian populations [17–20], little has been done to explore the genetic risk factors for T-cell lymphoma. In the present study, we investigated the association between *CTLA-4* (−1661A>G, −318C>T, +49A>G, and CT60A>G), *TNF-α* (−857C>T, −308G>A, and −238G>A), and *LTA* (+252G>A) polymorphisms and susceptibility to T-cell lymphoma in a Chinese population.

1. Patients and methods

1.1. Patients and control subjects

This study included 291 patients who were newly diagnosed with T-cell lymphoma at Shandong Cancer Hospital, Shandong University Qilu Hospital, Shandong Provincial Hospital, Jinan Fourth People's Hospital or Affiliated Hospital of Taishan Medical University between January 2007 and December 2010. All patients were diagnosed according to the World Health Organization Classification of Tumors of the Hematopoietic and Lymphoid Tissues (2001) criteria. The pathologic classification was based on hematoxylin and eosin staining of tissue sections, immunohistochemistry, and clinical characteristics. Other immunostains, polymerase chain reaction analysis, and fluorescence *in situ* hybridization were performed as needed. The most common histopathologic subtypes were extranodal NK/T-cell lymphoma, nasal type (29.8%; 87/291); PTL, unspecified (27.1%; 79/291); and precursor T lymphoblastic leukemia/lymphoma (14.4%; 42/291).

Between January 2007 and December 2010, 300 control subjects were accrued from healthy volunteers who visited the general health check-up division of Shandong Cancer Hospital, Shandong University Qilu Hospital, or Shandong Provincial Hospital and patients with non-cancer diagnoses who attended any of the 3 hospitals. None of the controls had malignancy or any autoimmune or immune-mediated disease. Randomly selected controls were matched to the cases by age (±5 years) and gender. All study subjects were Han Chinese. The demographic characteristics of the patients and controls are listed in Table 1.

Informed consent was obtained from each subject at the time of recruitment. The study was approved by the institutional review board of Shandong Cancer Hospital and Institute. All participants provided peripheral blood samples.

Table 1
Characteristics of study participants.

Characteristic	T-cell lymphoma (n = 291)	Controls (n = 300)	P value
Age, years			
Mean ± SD	42.5 ± 15.9	42.4 ± 15.7	0.986
Sex			
Male	187 (64.3)	198 (66.0)	
Female	104 (35.7)	102 (34.0)	0.667
Histopathological subtype			
NK/T	87 (29.9)		
PTL	79 (27.1)		
T-LBL	42 (14.4)		
ALCL	23 (7.9)		
AILT	14 (4.8)		
MF	7 (2.4)		
SPTCL	3 (1.0)		
Other and unknown type T-cell NHL	36 (12.4)		

NK/T: extranodal NK/T-cell lymphoma, nasal type; PTL: peripheral T cell lymphoma, unspecified; T-LBL: precursor T lymphoblastic leukemia/lymphoma; ALCL: anaplastic large cell lymphoma; AILT: angioimmunoblastic T-cell lymphoma; MF: mycosis fungoides; SPTCL: subcutaneous panniculitis-like T-cell lymphoma.

1.2. Genotyping

Genomic DNA was extracted from 2 ml of frozen whole blood using the Genomic DNA Extraction Kit (Fastagen, Shanghai, China) according to the manufacturer's protocol. All genotyping experiments were performed by the Shanghai BioWing Applied Biotechnology Company (http://www.biowing.com.cn/) using the polymerase chain reaction–ligation detection reaction (PCR–LDR) method.

Target DNA sequences were amplified using a multiplex PCR method. The ligation reaction was conducted in a final volume of 10 μl containing 1× buffer, 100 ng of Multi-PCR product, 1 pmol of each discriminating oligonucleotide, 1 pmol of each common probe, and 2 U Taq DNA ligase (New England Biolabs, Boston, MA). The LDR cycling parameters were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s and 50 °C for 2 min. Following the LDR reaction, 1 μl of the LDR reaction product was mixed with 1 μl ROX and 1 μl loading buffer. The mixtures were analyzed using the ABI Prism 373 DNA Sequencer (Applied Biosystems, Foster City, CA). In addition, representative PCR products were subjected to direct DNA sequencing on the ABI Prism 310 Sequencer to confirm the accuracy of the PCR–LDR method.

1.3. Statistical analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Demographic data between the study groups were compared using chi-square and Student's t-tests. Genotype frequencies were compared between groups using the chi-square test, and odds ratios (ORs) and 95% confidence intervals were calculated using unconditional logistic regression. Gene-dose effects were estimated by Cochran–Armitage trend test based on the number of variant alleles present. Bonferroni correction was used to adjust for false positive association discovery in multiple testing. The linkage disequilibrium of the polymorphic loci and haplotypes of CTLA-4 and TNF-α/LTA was analyzed using SHEsis software (Bio-X Inc., Shanghai, China). All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

2. Results

Results of the polymorphism analyses for all T-cell lymphoma cases and the 2 main histological subtypes (PTL and NK/T-cell lymphoma) are shown in Table 2. *CTLA-4* −1661 and −318 polymorphisms were associated with a 0.5-fold decreased risk for PTL (per-G allele OR, 0.5; P trend = 0.016 for −1661 site; per-T allele OR, 0.5; P trend = 0.021 for −318 site). After accounting for multiple comparisons, these associations were not significant. The *LTA* +252 polymorphism was associated with increased risk for overall T-cell lymphoma (GA genotype, OR, 2.0 and P = 0.009; AA genotype, OR, 2.3 and P = 0.002) and NK/T-cell lymphoma (GA genotype, OR, 3.8 and P = 0.007; AA genotype, OR, 3.7 and P = 0.011).

Haplotype analysis and details of the linkage disequilibrium tests (D' and r^2) are shown in Tables 3 and 4, respectively. Strong linkage disequilibrium existed between *CTLA-4* SNPs. The common haplotype A-C-G-G (−1661A, −318C, +49G, and CT60G) accounted for 68.3% of all CTLA-4 haplotypes in the control group. Haplotype G-T-A-G, which contains −1661G and −318T, was associated with a 0.5-fold decreased risk of PTL (OR, 0.5; P = 0.026), but this association was non-significant after accounting for multiple comparisons. The TNF-α/LTA haplotype C-G-G-A (*TNF-α* −857C, −308G, and −238G and *LTA* +252A) showed a significantly increased risk for T-cell lymphoma (OR, 1.6; P = 0.001).