Tumor Necrosis Factor-α Induced Protein 8 Polymorphism and Risk of Non-Hodgkin’s Lymphoma in a Chinese Population: A Case-Control Study

Yan Zhang1,2, Meng-Yun Wang2,3, Jing He2,3, Jiu-Cun Wang4,5, Ya-Jun Yang4,5, Li Jin4,5, Zhi-Yu Chen1,2, Xue-Jun Ma2,6, Meng-Hong Sun2,7, Kai-Qin Xia2,3,7, Xiao-Nan Hong1,2, Qing-Yi Wei3,8, Xiao-Yan Zhou1,3,7*

1 Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China, 2 Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China, 3 Cancer Research Laboratory, Fudan University Shanghai Cancer Center, Shanghai, China, 4 Ministry of Education Key Laboratory of Contemporary Anthropology and State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China, 5 Fudan-Taizhou Institute of Health Sciences, Taizhou, Jiangsu, China, 6 Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, China, 7 Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai, China, 8 Department of Epidemiology, the University of Texas MD Anderson Cancer Center, Houston, Texas, United States of America

Abstract

Background: Non-Hodgkin’s lymphoma (NHL) has been reported to be associated with autoimmune and pro-inflammatory response, and genetic polymorphisms of candidate genes involved in autoimmune and pro-inflammatory response may influence the susceptibility to NHL. To evaluate the role of such genetic variations in risk of NHL, we conducted a case-control study of 514 NHL patients and 557 cancer-free controls in a Chinese population.

Method: We used the Taqman assay to genotype six potentially functional single nucleotide polymorphisms (SNPs) in six previously reported inflammation and immune-related genes (TNF rs1799964T>C, LTA rs1800683G>A, IL-10 rs1800872T>G, LEP rs2167270G>A, LEPR rs1327118C>G, TNFAIP8 rs1045241C>T). Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI).

Results: We observed a significantly increased risk of NHL associated with the TNFAIP8 rs1045241C>T polymorphism (adjusted OR = 3.03; 95% CI = 1.68–5.45 for TT vs. CC and adjusted OR = 2.03; 95% CI = 1.53–2.69 for CT/TT vs. CC). The risk associated with the T allele was more evident in subgroups of 40–60 year-old, non-smokers or light-smokers (less than 25 pack-years), and subjects with normal weight or overweight. Risk for both B and T cell non-Hodgkin’s lymphoma was elevated for CT/TT genotypes (adjusted OR = 1.95, 95% CI = 1.41–2.70 for B cell NHL and adjusted OR = 2.22, 95% CI = 1.49–3.30 for T cell NHL), particularly for DLBCL (adjusted OR = 2.01, 95%CI = 1.41–2.85) and FL (adjusted OR = 2.53, 95% CI = 1.17–5.45). These risks were not observed for variant genotypes of other five SNPs compared with their common homozygous genotypes.

Conclusions: The polymorphism of TNFAIP8 rs1045241C>T may contribute to NHL susceptibility in a Chinese population. Further large-scale and well-designed studies are needed to confirm these results.

Introduction

Non-Hodgkin’s lymphoma (NHL) incidence rates have been increasing in both developed and developing countries with about 355,900 new cases in the world annually [1]. In China, the most common subtype of NHL is diffuse large B cell lymphoma (DLBCL), whereas follicular lymphoma (FL) is less common than in Western countries. Extramedullary lesions and T/NK cell NHLs (eg. Extramedullary NK/T-cell lymphoma) appear to be more common in China [2]. However, the exact causes of NHL remain largely unknown. Some evidence has shown that immune dysfunction may be one of the risk factors [3], and single nucleotide polymorphisms (SNPs) in immune and inflammatory response genes may play an important role in lymphomagenesis [4,5,6,7].

TNF/LTA and IL-10 genes code for immunoregulatory cytokines that can mediate inflammation, apoptosis and Th1/Th2 balance [6,7], and they may be good candidate genes for studying lymphomagenesis. The cytokines TNF-α and LT-α are thought to influence lymphomagenesis through up-regulation of pro-inflammatory and anti-apoptotic signals, possibly via the NFKB pathway [5]. Some evidence also showed that polymorphisms...
suggesting that genetic variants in these regions may influence the risk of DLBCL in non-Hispanic white populations [9]. Purdue et al. reported that IL-10-3575T>A and TNF-863CA (rs1800630) were associated with an elevated risk of DLBCL in an Australian case-control study [5]. A recent genome-wide association study (GWAS) of FL has identified additional two variants in the 6p21 chromosome region [10], which is the TNF gene location, suggesting that genetic variants in these regions may influence NHL susceptibility.

The tumor necrosis factor-α induced protein 3 (TNFAIP8) family are newly identified proteins that are important for inflammation and immune homeostasis [11]. They play roles in anti-inflammation by negatively regulating T cell receptor (TCR) and Toll-like receptor (TLR) signaling [12]. But the association between TNFAIP8 polymorphisms and NHL risk has not been reported so far, particularly in Chinese populations.

The circulating levels of adipocytokines, including adiponectin, resistin and leptin may also alter immune system function and chronic inflammatory response. Leptin has pro-inflammatory properties and stimulates the growth of certain cancer cells as well as circulating pro-inflammatory cytokines, such as TNF-α and interleukin [13]. Associations between NHL and polymorphisms in the leptin (LEP) and leptin receptor (LEPR) gene have also been reported. Skibola et al. found that the LEPR Arg allele was associated with an increased risk of NHL, particularly FL [14]. A similar result was reported by a UK study, in which the LEPR 223Arg/Arg genotype was shown to be associated with an increased risk of FL among women [15].

To test the hypothesis that polymorphisms in inflammation and immune-related genes (such as TNF, LTA, IL-10, LEP, LEPR and TNFAIP8) may be associated with susceptibility to NHL, we conducted a case-control study in a Chinese population and genotyped six potentially functional SNPs in the above-mentioned candidate genes.
samples were included in each of the 384-well plates for the quality control. The assays were repeated for 5% of the samples, and the results were 100% concordant. The analyzed fluorescence results were then auto-called into the genotypes using the built-in SDS2.2 software of the system.

Statistical Analysis

BMI was calculated as weight (kg) divided by the square of the height (m). In this study, we used the BMI cutoff points as suggested by the Cooperative Meta-Analysis Group of Working Group on Obesity in China [18]. If BMI < 18.5 kg/m², the individuals were defined as lower than normal weight, 18.5 ≤ BMI < 24.0 kg/m² was defined as normal weight and BMI ≥ 24.0 kg/m² was defined as overweight. Smoking status was divided into smokers and non-smokers by whether or not they had smoked for more than one year. Those who drank alcoholic beverages at least once a week for one year or more were defined as alcohol users, while the others were non-users. Differences in the distributions of the alleles and genotypes as well as demographic characters, smoking status, alcohol use and BMI between the cases and controls were evaluated by the Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test. The Hardy–Weinberg equilibrium (HWE) of genotype distribution in the controls was tested by a goodness-of-fit Chi-square test.

Results

Characteristics of the Study Population

There were 514 NHL cases and 557 cancer-free controls included in this study, whose DNA samples were available. The frequency distributions of demographic and some selected characteristics of the participants are shown in Table 1. There was no statistical difference in the distributions of age and sex between cases and controls because of frequency matching by design. The mean age was 49.3 years for the cases (± 14.1; range, 15–85) and 49.6 years for the controls (± 15.5; range, 20–85; P = 0.895), and 64.0% of the cases and 63.4% of the controls were male (P = 0.0001) compared with the cases. Consequently, smoking status, alcohol use and BMI were adjusted for in the subsequent multivariate logistic regression analyses. Of the 514 cases, 336 (65.4%) were B cell lymphoma, and 178 (34.6%) were T cell and natural killer (NK) cell lymphoma. After stratified, 233 (45.3%), 52 (10.1%), 51 (9.9%), 146 (28.4%), 52 (6.2%) were DLBCL, FL, other B cell lymphoma, NK/T cell lymphoma, and other T cell lymphoma, respectively. Of all cases, 322 (62.6%) had an Ann Arbor Stage of I–II and 192 (37.4%) had a later Ann Arbor Stage of III–IV.

Association between Selected SNPs and Risk of NHL

Genotype distributions of the selected six SNPs in cases and controls and their associations with NHL risk are presented in Table 2. All observed genotype distributions among controls were in agreement with the Hardy–Weinberg equilibrium (P = 0.285 for rs1799964, P = 0.777 for rs180683, P = 0.872 for rs1000672, P = 0.733 for rs2167270, P = 0.559 for rs1327118, P = 0.420 for rs1045241), Significant difference in the genotype frequencies was observed between the cases and controls for TNFAIP8 rs1045241 C>T (P < 0.0001). When the rs1045241 CC genotype was used as the reference, the CT heterozygous, TT homozygous and combined GT/TT genotypes were associated

| Variables | Cases No. (%) | Controls No. (%) | P-value* |
|-----------|--------------|-----------------|----------|
| All subjects | 514 (100) | 557 (100) | 0.895 |
| Age (years) | | | |
| Median (Range) | 50.5 (15–85) | 51.0 (20–85) | | |
| <40 | 129 (25.1) | 131 (23.52) | | |
| 40–60 | 269 (52.33) | 299 (53.68) | | |
| >60 | 116 (22.57) | 127 (22.80) | | |
| Sex | | | 0.830 |
| Male | 329 (64.0) | 353 (63.4) | | |
| Female | 185 (36.0) | 204 (36.6) | | |
| Smoking status | | | <0.0001 |
| Smoker | 101 (20.7) | 232 (43.4) | | |
| Non-smoker | 292 (74.3) | 303 (56.6) | | |
| Missing | 121 | 22 | | |
| Pack-years | | | <0.0001 |
| 0 | 292 (74.3) | 302 (56.4) | | |
| 0–25 | 64 (16.3) | 171 (32.0) | | |
| >25 | 37 (9.4) | 62 (11.6) | | |
| Missing | 121 | 22 | | |
| Alcohol use | | | 0.0001 |
| Yes | 58 (14.8) | 134 (25.0) | | |
| No | 335 (85.2) | 401 (75.0) | | |
| Missing | 121 | 22 | | |
| BMI (kg/m²) | | | <0.0001 |
| Median | 22.8 | 23.7 | | |
| <18.5 | 29 (6.9) | 21 (3.8) | | |
| 18.5–24.0 | 233 (59.8) | 273 (49.0) | | |
| >24.0 | 141 (33.3) | 263 (47.2) | | |
| Missing | 91 | 0 | | |
| Subtype | | | |
| B cell lymphoma | 336 (65.4) | – | – |
| DLBCL | 233 (45.3) | – | – |
| FL | 52 (10.1) | – | – |
| Other B cell NHL | 51 (9.9) | – | – |
| T cell lymphoma | 178 (34.6) | – | – |
| NK/T | 146 (28.4) | – | – |
| Other T cell NHL | 32 (6.2) | – | – |
| Ann Arbor Stage | | | – |
| I–II | 322 (62.6) | – | – |
| III–IV | 192 (37.4) | – | – |

*P value of the comparison with a two-sided Chi-square test. doi:10.1371/journal.pone.0037846.t001
with significantly increased risk of NHL (adjusted OR = 1.88; 95% CI = 1.39–2.53 for CT heterozygous genotype, adjusted OR = 3.03; 95% CI = 1.68–5.45 for TT homozygous genotype, adjusted OR = 2.03; 95% CI = 1.53–2.69 for CT/TT genotype) after adjustment for age, sex, BMI, smoking and drinking status. However, no significantly altered NHL risk was found for variant genotypes of the other five SNPs compared with their common genotypes. We also evaluated the combined effect of all the six SNPs. We had divided the subjects into seven groups according to the number of combined variant genotypes. The “0” risk genotype group was used as the reference, and unconditional logistic regression models were applied to calculate OR and 95%CI for each group. But we did not find any significant association between the combined effect of these six SNPs and risk of NHL (data not shown).

Stratified Analysis

We further evaluated the association between the six candidate SNPs and risk of NHL by subgroups of age, sex, smoking status, alcohol use, BMI, common subtypes and Ann Arbor stage. No statistical significances were found for the SNPs except TNFAIP8 rs1045241 C>T. The stratified analysis results of TNFAIP8 rs1045241 C>T are presented in Table 3. In general, an increased risk associated with rs1045241 CT/TT genotypes was more evident in subgroups of 40–60 year-old individuals (adjusted OR = 2.99, 95% CI = 2.03–4.43), non-smokers (adjusted OR = 1.90, 95% CI = 1.35–2.68) or smoked less than 25 pack-years (adjusted OR = 2.45, 95% CI = 1.32–4.54), and normal weight (adjusted OR = 1.76, 95% CI = 1.32–2.32) or overweight groups (adjusted OR = 2.07, 95% CI = 1.32–3.35). Moreover, the patients with rs1045241 CT/TT genotypes were associated with

Table 2. Genotypes distributions of the selected functional polymorphisms among NHL cases and cancer-free controls and their associations with NHL risk.

| Genotyping | Cases | Controls | P | Crude OR (95% CI) | P | Adjusted OR* (95% CI) | P |
|------------|-------|----------|---|-----------------|---|----------------------|---|
|            | n %   | n %      |   |                 |   |                      |   |
| TNF rs1799964T>C |       |          |   |                 |   |                      |   |
| TT         | 315 61.3 | 358 64.3 | 1.00 |                 | 1.00 |                   | 1.00 |
| CT         | 183 35.6 | 182 32.7 | 1.14 (0.89–1.48) | 0.305 | 1.10 (0.82–1.47) | 0.531 |
| CC         | 16 3.1 | 17 3.0 | 1.07 (0.53–2.15) | 0.850 | 0.75 (0.33–1.70) | 0.495 |
| CT/CC      | 199 38.7 | 199 35.7 | 0.312c | 1.14 (0.89–1.46) | 0.312 | 1.06 (0.80–1.41) | 0.667 |
| LTA rs1800683G>A |       |          |   |                 |   |                      |   |
| GG         | 125 24.3 | 161 28.9 | 1.00 |                 | 1.00 |                   | 1.00 |
| AG         | 275 53.5 | 280 50.3 | 1.26 (0.95–1.68) | 0.108 | 1.30 (0.94–1.81) | 0.112 |
| AA         | 114 22.2 | 116 20.8 | 1.27 (0.89–1.79) | 0.185 | 1.30 (0.88–1.94) | 0.188 |
| AG/AA      | 389 75.7 | 396 71.1 | 0.090c | 1.26 (0.96–1.66) | 0.090 | 1.30 (0.96–1.78) | 0.094 |
| IL-10 rs1800872T>G |       |          |   |                 |   |                      |   |
| TT         | 226 44.0 | 269 48.3 | 1.00 |                 | 1.00 |                   | 1.00 |
| CT         | 228 44.3 | 235 42.2 | 1.15 (0.90–1.49) | 0.267 | 1.23 (0.92–1.65) | 0.158 |
| GG         | 60 11.7 | 53 9.5 | 1.35 (0.89–2.03) | 0.154 | 1.46 (0.92–2.31) | 0.106 |
| GT/GG      | 288 56.0 | 288 51.7 | 0.156c | 1.19 (0.94–1.51) | 0.156 | 1.28 (0.97–1.68) | 0.083 |
| LEP rs2167270G>A |       |          |   |                 |   |                      |   |
| GG         | 322 62.6 | 338 60.7 | 1.00 |                 | 1.00 |                   | 1.00 |
| AG         | 166 32.3 | 190 34.1 | 0.92 (0.71–1.19) | 0.512 | 0.93 (0.69–1.25) | 0.631 |
| AA         | 26 5.1 | 29 5.2 | 0.94 (0.54–1.63) | 0.829 | 0.92 (0.48–1.76) | 0.792 |
| AG/AA      | 192 37.4 | 219 39.3 | 0.509c | 0.92 (0.72–1.18) | 0.509 | 0.93 (0.70–1.23) | 0.607 |
| LEPR rs1327118C>G |       |          |   |                 |   |                      |   |
| CC         | 390 75.9 | 412 74.0 | 1.00 |                 | 1.00 |                   | 1.00 |
| CG         | 116 22.6 | 136 24.4 | 0.90 (0.68–1.20) | 0.472 | 0.93 (0.67–1.29) | 0.645 |
| GG         | 8 1.5 | 9 1.6 | 0.94 (0.36–2.46) | 0.898 | 1.19 (0.39–3.68) | 0.760 |
| CG/GG      | 124 24.1 | 145 26.0 | 0.472c | 0.90 (0.69–1.19) | 0.473 | 0.94 (0.68–1.29) | 0.705 |
| TNFAIP8 rs1045241C>T |       |          |   |                 |   |                      |   |
| CC         | 293 57.0 | 381 68.4 | 1.00 |                 | 1.00 |                   | 1.00 |
| CT         | 180 35.0 | 156 28.0 | 1.50 (1.15–1.95) | 0.003 | 1.88 (1.39–2.53) | <0.0001 |
| TT         | 41 8.0 | 20 3.6 | 2.67 (1.53–4.55) | 0.0005 | 3.03 (1.68–5.45) | 0.0002 |
| CT/TT      | 221 43.0 | 176 31.6 | 0.0001c | 1.63 (1.27–2.10) | 0.0001 | 2.03 (1.53–2.69) | <0.0001 |

Statistically significant results (P<0.05) are highlighted in bold. ORs were obtained from logistic regression models with adjustment for age, sex, smoking status, alcohol use and BMI. Two-sided Chi-square test for distribution of three genotypes.

*Two-sided Chi-square test for distribution of combined genotypes.

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risk of both B and T cell lymphoma (adjusted OR = 1.95, 95% CI = 1.41–2.70 and adjusted OR = 2.22, 95% CI = 1.49–3.30, respectively). After stratifying by histological subtypes, we observed increased risk for DLBCL (adjusted OR = 2.01, 95% CI = 1.41–2.85) and FL (adjusted OR = 2.53, 95% CI = 1.17–5.45), but no significant association was found for NK/T cell lymphoma (adjusted OR = 0.79, 95% CI = 0.27–2.33). However, after we verified homogeneity assumption by using a Chi square-based Q-test. The results indicated that an increased NHL risk associated with CT/TT genotypes was particularly more pronounced only in subgroups of 40–60 year-old (P = 0.03), and patients with a later Ann Arbor stage of III–IV (P = 0.04).

### Discussion

Genetic polymorphisms in immune-related genes that regulate the immune and inflammation response may play an important role in the incidence of NHL [19,20]. In this case-control study, we reported that TNFAIP8 rs1045241C>T was significantly associated with an increased risk of NHL in a Chinese population. Stratified analyses revealed that subgroups of 40–60 years, non-smokers or light-smokers (≤25 pack-years), subjects with normal weight or overweight were more likely to have been diagnosed with NHL, especially the subtypes of DLBCL and FL. According to epidemiologic data about NHL in China, the main subtypes of lymphoma are DLBCL, FL and NK/T lymphoma, and other subtypes have a small sample size, and therefore we only analyzed the dominant subtypes of the cases. These results support the

| Variables | CT+TT (cases/controls) | CC (cases/controls) | p* | OR (95%CI) | Adjustedb | p*c |
|-----------|------------------------|---------------------|----|------------|-----------|-----|
| All subjects | 221/176 | 43.0/31.6 | 293/381 | 57.0/68.4 | 0.0001 | 1.63 (1.27–2.10) | 2.03 (1.53–2.69) |
| Age (year) | | | | | | |
| <40 | 56/42 | 43.3/32.1 | 73/89 | 56.6/67.9 | 0.259 | 1.43 (0.77–2.67) |
| 40–60 | 119/81 | 44.2/27.1 | 150/218 | 55.8/72.9 | <0.0001 | 2.13 (1.50–3.03) | 2.99 (2.03–4.43) |
| >60 | 46/53 | 39.7/41.7 | 70/74 | 60.3/58.3 | 0.840 | 0.94 (0.51–1.72) |
| Sex | | | | | | |
| Male | 143/106 | 43.5/30.0 | 186/247 | 56.5/70.0 | 0.003 | 1.79 (1.31–2.45) | 2.09 (1.46–3.01) |
| Female | 78/70 | 42.2/34.3 | 107/134 | 57.8/65.7 | 0.006 | 1.91 (1.21–3.04) |
| Smoking status | | | | | | |
| Never | 137/101 | 46.9/33.7 | 155/201 | 53.1/66.3 | 0.008 | 1.76 (1.26–2.45) | 1.90 (1.35–2.68) |
| ≤25 pack-year | 31/47 | 48.4/27.5 | 33/124 | 51.6/66.1 | 0.0024 | 2.48 (1.37–4.49) | 2.45 (1.32–4.54) |
| >25 pack-year | 18/21 | 48.6/33.9 | 19/41 | 51.4/66.1 | 0.145 | 2.06 (0.85–5.02) |
| Missing | 35/7 | 28.9/31.8 | 86/15 | 71.1/68.2 | 0.0024 | 2.45 (1.32–4.54) | 2.45 (1.32–4.54) |
| Drinking status | | | | | | |
| Yes | 29/38 | 50/28.4 | 29/96 | 50/71.6 | 0.004 | 2.53 (1.34–4.78) | 2.66 (1.36–5.19) |
| No | 157/131 | 46.9/32.7 | 178/270 | 53.1/67.3 | 0.0001 | 1.82 (1.35–2.45) | 1.88 (1.37–2.58) |
| Missing | 35/7 | 28.9/31.8 | 86/15 | 71.1/68.2 | 0.145 | 2.06 (0.85–5.02) |
| BMI(kg/m²) | | | | | | |
| <18.5 | 9/4 | 31.0/19.1 | 20/17 | 69.0/80.9 | 0.340 | 1.91 (0.50–7.33) | 1.62 (0.37–7.05) |
| 18.5–24.0 | 117/93 | 46.2/34.1 | 136/180 | 53.8/65.9 | 0.0044 | 1.67 (1.17–2.37) | 1.76 (1.23–2.52) |
| >24.0 | 70/79 | 49.6/30.0 | 71/184 | 50.4/70.0 | <0.0001 | 2.07 (1.32–3.25) |
| Missing | 25/0 | 27.5/0 | 66/0 | 72.5/0 | 0.0001 | 1.82 (1.35–2.45) | 1.88 (1.37–2.58) |
| Ann Arbor Stage | | | | | | |
| I– II | 137/176 | 42.5/31.6 | 185/381 | 57.5/68.4 | 0.001 | 1.60 (1.21–2.13) | 1.89 (1.38–2.59) |
| III–IV | 84/176 | 43.7/31.6 | 108/381 | 56.3/68.4 | 0.002 | 1.68 (1.20–2.36) | 2.28 (1.51–3.46) |
| Subtype | | | | | | |
| B cell NHL | 140/176 | 41.7/31.6 | 196/381 | 58.3/68.4 | 0.002 | 1.55 (1.17–2.05) | 1.95 (1.41–2.70) |
| DLBCL | 100/176 | 42.9/31.6 | 133/381 | 57.1/68.4 | 0.002 | 1.63 (1.19–2.23) | 2.01 (1.41–2.85) |
| FL | 29/176 | 55.8/31.6 | 23/381 | 44.2/68.4 | 0.0004 | 2.73 (1.53–4.85) | 2.53 (1.17–5.45) |
| T cell NHL | 81/176 | 45.3/31.6 | 97/381 | 54.5/68.4 | 0.007 | 1.81 (1.28–2.55) | 2.22 (1.49–3.30) |
| NK/T | 11/176 | 21.6/31.6 | 40/381 | 78.4/68.4 | 0.137 | 0.79 (0.27–2.33) | 0.79 (0.27–2.33) |

Statistically significant results (P<0.05) are highlighted in bold.

*P value of the comparison with a two-sided Chi-square test.

*ORs were obtained from logistic regression models with adjustment for age, sex, smoking status, alcohol use and BMI.

*P value of the heterogeneity assumption with a Chi-square-based Q-test.

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hypothesis that some polymorphism in inflammation and immune-related genes may be associated with risk of NHL and its common subtypes.

TNFAIP8, also known as GG2-1, MDC-3.13, SCC-S2, is located on chromosome 5 (5q23.1). It was first identified in a human head and neck squamous cell carcinoma (HNSCC) cell line [21]. Recently, it has been reported to be associated with oncogenesis, immunity, and inflammation in several studies [11,12,22]. TNFAIP8 mRNA over-expression has been found in various malignant cell lines, such as breast cancer [23], non-small cell lung cancer [24], and esophageal squamous cell carcinoma [25]. Evidence showed that TNFAIP8 expression was upregulated by TNF-α-induced NF-κB pathway activation in cancer cell lines, which can inhibit caspase-8 and reduce apoptosis [26,27]. Tumor necrosis factor-α induced protein 8-like 2 (TIPE2), a member of the TNFAIP8 family, was originally identified as a gene abnormally expressed in the inflamed spinal cord of mice with experimental autoimmune encephalomyelitis [12]. TIPE2-deficient mice were more likely to suffer from chronic inflammation diseases and multiple organ inflammation [12]. In humans, the abnormal expression of TIPE2 was associated with systemic autoimmunity [28], diabetic nephropathy [29], and hepatitis B [30]. These studies supported that TIPE2 plays an important role in maintaining immune homeostasis.

However, fewer studies have focused on the association between polymorphisms of TNFAIP8 and NHL risk. The SNP TNFAIP8 rs1045241C>T included in the present study is located on the 3′UTR of TNFAIP8, and the SNP function prediction shows that it may affect the microRNA-binding sites activity (http://snpinfo.niehs.nih.gov/snpfunc.htm). Some evidence has indicated that a genetic polymorphism in a microRNA target site influence transcriptional and post-transcriptional gene expression in cancers [31]. Though we found that rs1045241C>T was associated with the risk of NHL, the exact mechanisms of this relationship remained unknown. Further functional studies have been conducting to confirm our results.

In the present study, we did not find any significant association between the investigated polymorphisms of TNF rs1799964T>C, LTA rs1800636G>A, IL-10 rs1800872T>G, LEP rs2167270G>A, LEPR rs1327116C>G and NHL risk in a Chinese population. However, many pieces of evidence have shown that other SNPs in these genes like TNF-308 G>A (rs1800629), LTA 252A>G (rs909253), IL10-308G>A (rs1800890), LEPR 233Q>R (rs1137101) increased the risk of NHL, particularly DLBCL in non-Hispanic white populations [9,14,15,32,33]. Our studies did not cover these SNPs because their MAF was <5% in CHB population according to Hapmap database (http://hapmap.ncbi.nlm.nih.gov/). But Xiao et al. reported that no association between TNF-308, LTA 252 polymorphisms and histological subtypes, disease stages in Chinese NHL patients [34]. We believe that this inconsistency may be due to ethnic and demographic differences between Chinese populations and white populations as well as the smaller sample size that may also have led to insufficient statistical power. So our results need to be replicated in additional studies in different populations with large sample sizes.

Fewer studies reported an association between LEPR 19 Δ>G, LEPR 233Q>R and risk of NHL in Chinese populations, though some previous published studies revealed that obesity was a risk factor of NHL in developed countries [35]. Obesity may play the role of inducing lymphomagenesis by the co-effects of LEPR 19 Δ>G, LEPR 233Q>R polymorphisms and immune dysfunction [14,15,36]. Inconsistently, we found the NHL cases were more likely to have lower BMI compared with the cancer-free controls (P<0.0001). One possible reason may due to ethnic difference in BMI and its contribution to lymphoma. It is likely that populations of developing and developed countries have different body size, and Asians have lower BMI than Westerners. Thus the Chinese standard of obesity was applied to the subjects in this study, but obesity did not appear closely related to lymphomagenesis. However, another possibility was that obesity may not a direct risk factor for NHL in Chinese populations. There may be some intrinsic interaction among obesity, diet, hormone, genetics, and susceptibility to NHL but the present study did not have enough power to detect such interactions. Further larger, transcultural studies are needed to fully explore and elaborate the extent of such interactions.

In the stratified analyses, the risk effect of the TNFAIP8 rs1045241 CT/TT genotype was more evident in subgroups of 40–60 year-old, non-smoker or light-smoker, and normal BMI or overweight. Stratifying by subtypes and stage, the risk effect of rs1045241 CT/TT genotype was evident for both B and T cell lymphoma, especially for DLBCL and FL as well as later Ann Arbor Stage of ?–?. But we noticed that after verification of homogeneity assumption, only age and stage showed marginally statistical significance. So, there was no strong evidence of an effect between the selected covariates and NHL risk. The exact association between selected covariates and NHL risk needs to be further investigated by large studies. Up to now, there is still inconsistency about the effect of smoking, alcohol use, and BMI on NHL risk, and the mechanisms are multiple. Wang et al. once reported that autoimmune conditions, obesity, and later birth order could contribute to lymphomagenesis through an alteration of the proinflammatory pathway, specifically involving common genetic variants in TNF and IL-10 [8]. Given the results of our study, NHL development may be of complex process that includes a great number of events. A single factor would have a limited effect on the susceptibility. Once the exposure of environmental factors was accumulated to a certain level, alteration took place in immune and proinflammatory pathways, and genetic variants or other unknown events may also participate in the process. On the other hand, our study was not large enough, and possible selection bias may exist due to a higher-than-expected non-response rate in case group. Well-designed large studies are needed to test the hypothesis that TNFAIP8 polymorphisms may influence NHL susceptibility, particularly by the possible interactions with environmental risk factors. Several limitations exist in the present study. First, the sample size was not large enough, so selection bias may be inevitable, and the study did not provide enough statistical power to detect gene-gene and gene-environment interaction. Second, we selected only one functional SNP for each candidate gene, which restricted further analysis to identify other potentially important associations. Moreover, different histological subtypes of NHL, such as DLBCL, FL, and NK/T cell lymphoma may have different risk factors and etiologies. We did not have enough information about environmental exposure from NHL patients, such as use of hair dye, family history of NHL, occupation and EB-virus infection to be included in stratified analyses.

In summary, we identified TNFAIP8 rs1045241C>T to be associated with risk of NHL in a Chinese population, particularly DLBCL and FL. To the best of our knowledge, this is the first study to report a TNFAIP8 polymorphism associated with NHL susceptibility in a Chinese population, which provides additional evidence that inflammation and immune-related genetic polymorphisms play an important role in lymphomagenesis. Further large-scale well-designed population-based studies are needed to validate
our findings, define the high-risk population and deepen our understanding of NHL pathogenesis.

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