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RESEARCH ARTICLE

The association of interleukin-16 gene polymorphisms with IL-16 serum levels and risk of nasopharyngeal carcinoma in a Chinese population

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Abstract Interleukin (IL)-16 plays a fundamental role in inflammatory diseases, as well as in the development and progression of tumors. Genetic variation in DNA sequence of *IL16* gene may lead to altered cytokine production and/or activity, and this variation may modulate an individual's susceptibility to nasopharyngeal carcinoma (NPC). To test this hypothesis, we investigated the association of *IL16* gene polymorphisms and serum IL-16 levels with NPC risk in a Chinese population. We analyzed *IL16* gene rs11556218 T/G, rs4778889 T/C, and rs4072111 C/T polymorphisms using PCR-RFLP and DNA sequencing, and serum IL-16 levels were measured by ELISA. The *IL16* rs11556218 T/G polymorphism was significantly associated with the susceptibility to NPC patients. The TG genotype was associated with a significantly higher risk of NPC as compared with the TT genotype (OR=2.05, 95 % CI 1.04–4.01; p=0.037). Patients carrying the G allele had a significantly higher risk for developing NPC compared with individuals carrying the T allele (OR=1.79, 95 % CI 1.07–3.01; p=0.027). The serum IL-16 levels were increased in NPC patients compared with controls (p<0.01); the genotypes carrying the *IL16* rs11556218 G variant allele were associated with increased serum IL-16 levels compared with the homozygous wild-type genotype in NPC patients (all p values <0.01). Our data suggested that *IL16* rs11556218 T/G polymorphism was associated with increased susceptibility to NPC through increasing the production of serum IL-16 levels.

Keywords Nasopharyngeal carcinoma · Interleukin-16 · PCR-RFLP · Single-nucleotide polymorphism

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy with strikingly variable racial and geographic distribution. It is rarely seen in most regions of the world but is prevalent among populations from Southeast Asia [1], especially in Guangdong and Guangxi provinces of southern China. Etiologically, carcinogenesis of NPC is a complex, multistep, and multifactor process, in which many factors are implicated. It was reported that major risk factors for the development of NPC are Epstein-Barr virus infection, long-term tobacco smoking, occupational exposure to formaldehyde, and various dietary factors [2]. However, even if a group of people are exposed to the same hazard in areas where NPC is endemic, only a few people develop the disease, suggesting that genetic differences such as single-nucleotide polymorphisms (SNP) may contribute to NPC occurrence.

Interleukin (IL)-16 is one of the potent pro-inflammatory cytokines which has a wide array of biological functions and

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was initially identified as lymphocyte chemoattractant factor in 1982 [3]. The *IL16* gene is located on chromosome 15q26.3 in humans and encodes two distinct isoforms, leukocyte IL-16 (isoform 1) and neuronal IL-16 (isoform 2), which is derived from two different transcription variants [4]. IL-16 is a precursor protein consisting of 631 amino acids that is cleaved by caspase-3 to form the active C-terminal domain containing 121 amino acids [5, 6]. IL-16 is produced by activated $CD8^+$ T cells, mast cells, and B cells [7–9] and can selectively activate $CD4^+$ T cells, monocytes, macrophages, eosinophils, and dendritic cells by binding the CD4 receptor [10, 11]. Furthermore, IL-16 can promote the secretion of tumor-associated inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-15 by monocytes [12]. All these cytokines have been demonstrated to play a critical role in the pathogenesis of human cancers [13–16]. Furthermore, the high levels of IL-16 also have been demonstrated in several malignant cancers both in vitro and in vivo [17–23]. Hence, it is biologically reasonable to hypothesize a potential relationship between the *IL16* gene polymorphisms and cancer risk.

Recently, several studies have revealed that the genetic variants of the *IL16* gene were associated with cancers, including prostate cancer, colorectal, and gastric cancer [23–25]. In previous study, we also identified that the rs11556218 T/G and rs4072111 C/T polymorphisms of the *IL16* gene were significantly associated with the susceptibility to HBV-related hepatocellular carcinoma in a Chinese population [26]. However, few studies, to date, have examined the association between *IL16* polymorphisms and NPC; the relationship between *IL16* gene polymorphisms and the IL-16 serum levels remains unknown. Therefore, the aim of this study was to investigate the relationship between *IL16* gene polymorphisms and the incidence of NPC and the influence of SNPs on IL-16 serum levels in patients with NPC versus healthy controls in a Chinese population.

Materials and methods

Study population

The case–control population contained 150 unrelated adult Chinese who were selected from the same population living in China between October 2009 and April 2010 (Table 1). A total of 75 NPC patients were recruited from the Department of Otolaryngology, First Affiliated Hospital of Guangxi Medical University. The only selection criterion for patients was that their NPC diagnosis had been pathologically confirmed. The patients (55 men; 20 women) had a mean (standard deviation) age of 46.03(9.05)years. The control group comprised of 75 healthy volunteers who visited the general health checkup division at the First Affiliated Hospital of Guangxi Medical University. Selection criteria for controls were no evidence of any personal or family history of cancer or other serious illness. The mean age of the control group (50 men and 25 women) was 44.04(8.33)years. There was no significant difference between patients and control subjects in terms of sex and age distribution. Written informed consent was obtained from all of the included subjects, and the study was performed with the approval of the ethics committee of the First Affiliated Hospital of Guangxi Medical University.

Table 1 Clinical characteristics of the study participants

Variable	NPC patients $n=75$ (%)	Controls $n=75$ (%)	*P*
Age			
(mean±standard deviation)	46.03±9.05	44.04±8.33	0.121
Sex			
Male	55 (73.3)	50 (66.7)	0.373
Female	20 (26.7)	25 (33.3)	
Cigarette smoking			
Nonsmokers	23 (30.7)	27 (36.0)	0.488
Smokers	52 (69.3)	48 (64.0)	
Alcohol consumption			
Nondrinkers	17 (22.7)	21 (28.0)	0.453
Drinkers	58 (77.3)	54 (72.0)	
Clinical stages			
Stages I and II	33 (44.0)		
Stages III and IV	42 (56.0)		

NPC nasopharyngeal carcinoma

DNA extraction and genotyping

Genomic DNA was extracted from 200 μl EDTA anticoagulated peripheral blood using QIAamp DNA blood mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. *IL16* gene polymorphisms (rs11556218T/G, rs4072111C/T, and rs4778889T/C) were identified by performing polymerase chain reaction–restriction fragment length polymorphism analysis (Fig. 1). Primer sequences, reaction conditions, restriction enzymes used, and length of resulting polymerase chain reaction products are listed in Table 2. To confirm the genotyping results, polymerase chain reaction-amplified DNA samples were examined by DNA sequencing. The results were 100 % concordant.

Serum IL-16 levels

Serum samples were available for all included subjects. When blood samples were obtained, the serum was allowed to clot for 30 min at 4 °C before centrifugation at 2,000 rpm for 10 min at 4 °C. Serum was isolated and stored at −80 °C before use. We detected IL-16 concentration by using a sandwich ELISA (Quantikine EGF immunoassay kit, R&D

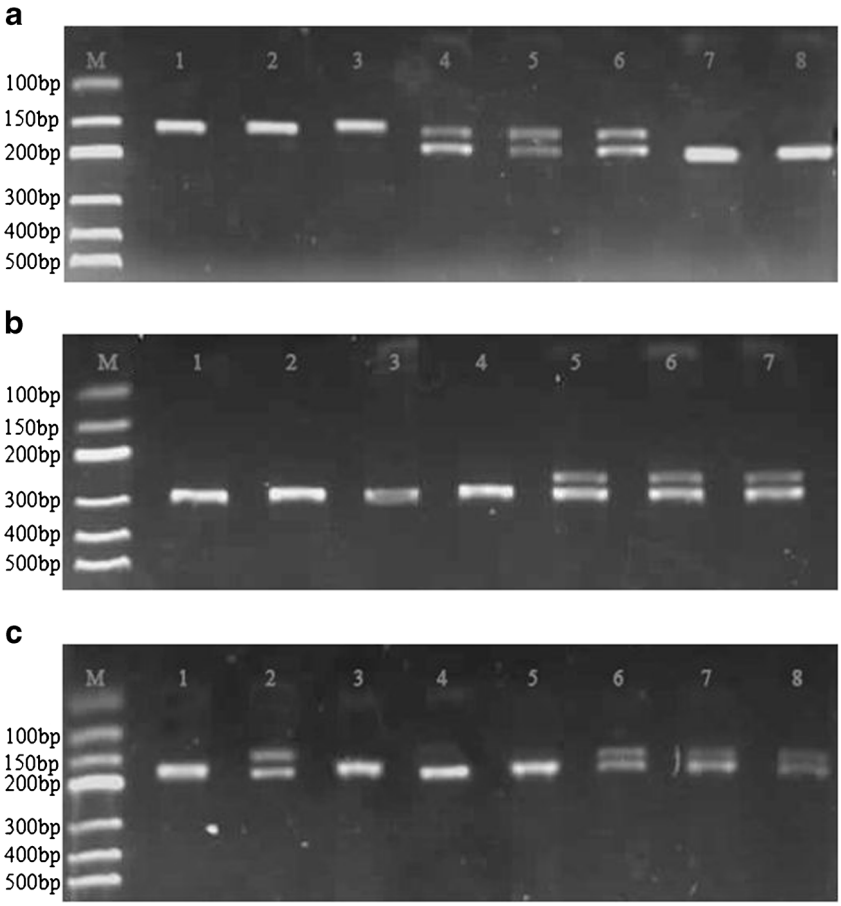

Fig. 1 PCR-RFLP assay for analyzing the rs11556218 T/G, rs4778889 T/C, and rs4072111 C/T polymorphisms of the *IL16* gene. PCR product was digested by restriction enzyme and separated on 8 % polyacrylamide gels electrophoresis. **a** *Lanes 1*, *2*, and *3* show rs11556218 TT genotypes; *lanes 4*, *5*, and *6* show rs11556218 TG genotypes; *lanes 7* and *8* show rs11556218 GG genotypes. **b** *Lanes 1*, *2*, *3*, and *4* show rs4778889 TT genotypes; *lanes 5*, *6*, and *7* show rs4778889 TC genotypes. **c** *Lanes 1*, *3*, *4*, and *5* show rs4072111 CC genotypes; *lanes 2*, *6*, *7*, and *8* show rs4072111 TC genotypes

Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The minimum level of detection for IL-16 was 5 pg/mL. No cross-detection of other cytokines was observed. Each sample was assayed in duplicate, and the intra-assay coefficient of variation was 10 %.

Statistical analysis

Genotype and allele frequencies of *IL16* were compared between NPC patients and controls using the χ^2 test and Fisher's exact test when appropriate. Odds ratio (OR) and 95 % confidence intervals (CIs) were calculated by binary logistic regression and adjusted for age, gender, smoking, and drinking status to assess the relative risk conferred by a particular allele and genotype. Demographic and clinical data among groups were compared using the χ^2 test and the Student's t test. Hardy–Weinberg equilibrium (HWE) was tested with a goodness of fit χ^2 test with 1° of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. Differences in IL-16 serum levels among patients with NPC versus control subjects were examined using the one-way analysis of variance, and if significant, followed by multiplied paired analyses. To elucidate the interaction of the factors studied (genotype and/or severity) on IL-16 serum levels, the data were analyzed by two-way analysis of variance for independent

Table 2 Primer sequence and the reaction condition for genotyping *IL16* polymorphisms

Polymorphism	Primer sequence	Annealing temperature	Restriction enzyme	Product size (bp)
rs11556218T/G	F: GCTCAGGTTCACAGAGTGTTTCCATA	61.0 °C	Nde I	T: 171 bp
	R: TGTGACAATCACAGCTTGCCTG			G: 147+24 bp
rs4778889T/C	F: CTCCACACTCAAAGCCTTTTGTTCCTATGA	63.0 °C	Ahd I	T: 280 bp
	R: CCATGTCAAAACGGTAGCCTCAAGC			C: 246+34 bp
rs4072111C/T	F: CACTGTGATCCCGGTCCAGTC	67.0 °C	BsmA I	C: 164 bp
	R: TTCAGGTACAAACCCAGCCAGC			T: 140+24 bp

samples. Statistical significance was assumed at the $p<0.05$ level. The SPSS statistical software package version 13.0 was used for all of the statistical analyses.

Results

Characteristics of the study subjects

Table 1 summarizes the characteristics of the 75 cancer patients and 75 control subjects included in this study. No significant differences in age, gender distribution, or smoking and alcohol consumption status were identified between cancer patients and control subjects, suggesting that subject matching based on these variables were adequate.

Genotype and allele distribution of *IL16* polymorphisms

The genotype and allele frequencies of *IL16* gene polymorphisms among NPC patients and controls are summarized in Table 3. The genotype distributions of these three polymorphisms in control subjects were presented consistent with HWE. For the rs11556218 T/G, there was a significant difference in the genotype and allele frequencies of this polymorphism between NPC patients and control subjects. The frequencies of the TT, TG, and GG genotypes of rs11556218 T/G were 61.3, 34.7, and 4.0 % in controls, and 42.7, 49.3, and 8.0 % in NPC patients, respectively. The TG genotype was associated with a significantly increased risk of NPC as compared with the TT genotype (OR=2.05, 95 % CI 1.04–4.01; $p=0.037$). The G allele was associated with a significantly increased risk of NPC as compared with the T allele (OR=1.79, 95 % CI 1.07–3.01; $p=0.027$). Under the dominant model, genotype TG+GG appeared to be associated with an increased risk of NPC when compared with TT genotype (OR=2.13, 95 % CI 1.11–4.09; $p=0.022$). However, genotype and allele frequencies of the *IL16* gene rs4778889 T/C and rs4072111 C/T polymorphisms in NPC patients were not significantly different from those in healthy controls ($p>0.05$).

The genotype and allele frequencies of *IL16* polymorphisms in relation to pathological indices of NPC severity

No significant association was found between *IL16* gene rs11556218 T/G, rs4778889 T/C, and rs4072111 C/T polymorphisms and different clinical stage as shown in Table 4. Genotype and allele frequencies of the *IL16* gene rs11556218 T/G, rs4778889 T/C, and rs4072111 C/T polymorphisms in stages I and II were not significantly different from that in stages III and IV of PNC patients (all p values are larger than 0.05).

Table 3 Genotype and allele frequencies of three SNPs in the *IL16* gene between NPC patients and controls

Polymorphisms	NPC patients $n=75$ (%)	Controls $n=75$ (%)	OR (95%CI) [a]	P [a]
rs11556218T/G				
Genotypes				
TT	32 (42.7)	46 (61.3)	1.00[ref]	
TG	37 (49.3)	26 (34.7)	2.05 (1.04–4.01)	0.037
GG	6 (8.0)	3 (4.0)	2.88 (0.67–12.35)	0.142
TG+GG	43 (57.3)	29 (38.7)	2.13 (1.11–4.09)	0.022
T Allele	101 (67.3)	118 (78.7)	1.00 [ref]	
G allele	49 (32.7)	32 (21.3)	1.79 (1.07–3.01)	0.027
rs4778889T/C				
Genotypes				
TT	39 (52.0)	49 (65.3)	1.00[ref]	
TC	36 (48.0)	26 (34.7)	1.74 (0.90–3.35)	0.097
CC	0 (0.0)	0 (0.0)	NC	NC
T allele	114 (76.0)	124 (83.0)	1.00[ref]	
C allele	36 (24.0)	26 (17.0)	1.50 (0.90–3.35)	0.154
rs4072111C/T				
Genotypes				
CC	41 (54.7)	44 (56.0)	1.00[ref]	
TC	34 (45.3)	31 (44.0)	1.17 (0.62–2.25)	0.621
TT	0 (0.0)	0 (0.0)	NC	NC
C allele	116 (77.3)	119 (79.3)	1.00[ref]	
T allele	34 (22.7)	31 (20.7)	1.12 (0.64–1.96)	0.674

OR odds ratio, *CI* confidence interval, and *NC* not calculated

[a] Adjusted by age, sex, smoking, and drinking status by logistic regression model

Table 4 Genotype and allele frequencies of *IL16* polymorphisms in relation to pathological indices of NPC severity

Polymorphisms	NPC patients		OR (95%CI)[a]	*P* [a]
	Stages III and IV *n* = 42 (%)	Stages I and II *n* = 33 (%)		
rs11556218T/G				
Genotypes				
TT	18 (42.9)	14 (42.4)	1.00[ref]	
TG	21 (50.0)	16 (48.5)	1.03 (0.41–2.67)	0.949
GG	3 (7.1)	3 (9.1)	0.78 (0.17–4.47)	0.780
T allele	57 (67.9)	44 (66.7)	1.00[ref]	
G allele	27 (32.1)	22 (33.3)	0.95 (0.48–1.89)	0.879
rs4778889T/C				
Genotypes				
TT	22 (52.4)	17 (51.5)	1.00[ref]	
TC	20 (47.6)	16 (48.5)	0.98 (0.40–2.42)	0.940
CC	0 (0.0)	0 (0.0)	NC	NC
T allele	64 (76.2)	50 (75.8)	1.00[ref]	
C allele	20 (23.8)	16 (24.2)	0.98 (0.47–2.09)	0.953
rs4072111C/T				
Genotypes				
CC	25 (59.5)	16 (48.5)	1.00[ref]	
TC	17 (40.5)	17 (51.5)	0.64 (0.27–1.61)	0.342
TT	0 (0.0)	0 (0.0)	NC	NC
C allele	67 (79.8)	49 (74.2)	1.00[ref]	
T allele	17 (20.2)	17 (25.8)	0.73 (0.34–1.58)	0.423

OR odds ratio, *CI* confidence interval, and *NC* not calculated

[a] Adjusted by age, sex, smoking, and drinking status by logistic regression model

Association between *IL16* gene polymorphisms and serum IL-16 levels

The serum IL-16 concentration was significantly higher in the NPC patients ($n = 75$, 40.22±9.09 pg/mL) than that in the controls ($n = 75$, 28.31±5.28 pg/mL; $p < 0.01$). As shown in Table 5, we found no association between the serum IL-16 concentration and the *IL16* gene polymorphisms in the controls. However, the genotypes of the rs11556218 T/G polymorphism were significantly associated with serum IL-16 levels in NPC patients. The serum IL-16 levels were significantly higher in individuals with homozygous GG genotypes (45.91±8.31 pg/mL, $n = 6$) or heterozygous TG genotypes (43.12±9.98 pg/mL, $n = 37$) than homozygous TT genotypes (35.80±5.82 pg/mL, $n = 32$; $p < 0.05$, respectively); however, there were no significant differences in the serum

Table 5 The association between *IL16* gene polymorphisms and serum concentration in NPC patients

Polymorphisms	NPC patients *n* = 75 (%)	IL-16 concentration ($X(-)$±S, pg/mL)	*t* value	*P*
rs11556218T/G				
Genotypes				
TT	32 (42.7)	35.80±5.82		
TG	37 (49.3)	43.12±9.98	3.641	0.001
GG	6 (8.0)	45.91±8.31	3.647	0.001
TG+GG	43 (57.3)	43.51±9.72	4.267	0.000
rs4778889T/C				
Genotypes				
TT	39 (52.0)	38.46±9.52		
TC	36 (48.0)	42.13±8.32	1.775	0.080
rs4072111C/T				
Genotypes				
CC	41 (54.7)	40.47±8.20		
TC	34 (45.3)	39.92±10.17	0.260	0.796

IL-16 levels between GG and TG genotypes (Fig. 2). In addition, there were no significant associations of the *IL16* gene rs4778889 T/C and rs4072111 C/T polymorphisms with serum levels of IL-16 in patients with NPC.

Discussion

In the present study, we investigated whether *IL16* gene polymorphisms are related to the occurrence of NPC and whether *IL16* gene polymorphisms correlate with serum levels of IL-16 in a Chinese population. Our results showed that the rs11556218 T/G polymorphism of *IL16* gene and the serum levels of IL-16 were significantly associated with the presence of NPC. The rs11556218 T/G polymorphism may affect the serum levels of IL-16. People carrying the *IL16* gene rs11556218 G variant allele had an increased ability to produce IL-16, which may contribute to NPC susceptibility. Our results suggest that *IL16* gene rs11556218 T/G may play an important role in the development of NPC. Thus, *IL16* gene rs11556218 T/G polymorphism may serve as the novel genetic marker of susceptibility to NPC in Chinese population.

In most parts of the world, NPC is rare, but it occurs at a high frequency in Southeast Asia and China and at a somewhat lower but still elevated rate in North and East Africa. Microscopically, one striking feature of NPC is a heavy infiltration of nonmalignant lymphocytes, and most of these lymphocytes have been shown to be T cells. Another important feature of NPC is its association with the EBV infection. Studies have shown that the expression of EBV gene products is involved in the latent and lytic cycles in NPC specimens, and some of these viral gene products might have the capacity to induce or influence inflammatory cytokine production, such as IL-10, IL-6, and TGF-β [27, 28]. The contribution

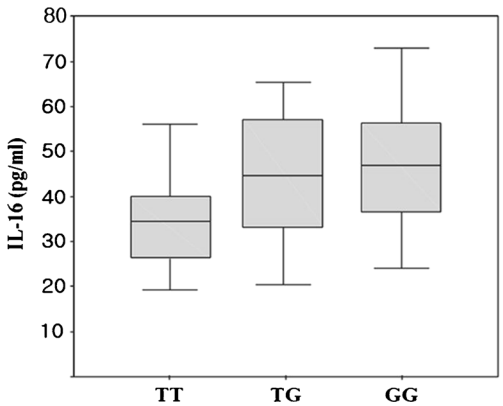

Fig. 2 Association between serum IL-16 levels and *IL16* rs11556218T/G polymorphism in NPC patients. Serum IL-16 level with TT homozygous was significantly lower than that of the GG homozygous or TG heterozygotes, respectively. However, there were no significant differences in the serum IL-16 levels between GG and TG genotypes

of inflammation and inflammatory cells to the process of tumor development and progression has been increasingly recognized [29, 30]. Elevated expression of cytokines is a common phenomenon of tumor cell lines derived from many cancers, such as melanomas, leukemias, and gastric and ovarian carcinoma [31–34]. Evidence has shown that a substantial proportion of malignant tumors worldwide arise from infection and chronic inflammation [35]. Both inflammatory and tumor cells produce an assorted array of cytokines and chemokines, which mediate all aspects of inflammation and profoundly affect the development and progression of cancer [36, 37]. Previous studies have shown that NPC is associated with overexpression of numerous cytokines in NPC biopsies. EBV infection is ubiquitous in the world, but NPC incidence differs according to geographic region, and the reasons for this selective susceptibility are not fully understood. However, it is clear that environmental, viral, and host factors play a role.

As a multifunctional cytokine, IL-16 is an important mediator in inflammatory diseases as well as tumor growth and progression. Passam et al. [21] reported that non-Hodgkin's lymphoma patients who responded to standard treatment had lower levels of IL-16 compared with pretreatment levels. In human astrocytic brain tumors and rat C6 gliomas, Liebrich et al. [18] reported higher levels of IL-16 present. Similarly, Blaschke et al. [22] reported that IL-16 messenger RNA expression increased with the stage of cutaneous T cell lymphoma diagnosed. A few studies have shown that higher serum levels of IL-16 can be associated with advanced stages of cancer [17] and a worse patient outcome depending on the type of tumor [20]. Recently, IL-16 has been reported to be a candidate susceptibility gene to colorectal and gastric cancer [23]. Our previous study also has shown that the rs11556218 T/G and rs4072111C/T polymorphisms of the *IL16* gene were significantly associated with the susceptibility to hepatocellular carcinoma in a Chinese population [26]. The mechanisms by which *IL16* gene polymorphisms affect cancer risk remain unknown, but evidence shows that chronic inflammation pathogenesis is the underlying pathological event in these malignancies. NPC is an epithelial cancer that is causally associated with EBV infection; studies have shown that cytokines synthesis might contribute to lymphocyte infiltration and/or tumor growth during NPC development. Therefore, it is plausible that the various phenotypes of IL-16 may result in individuals with various inflammatory response and NPC risk.

Our study shows that the *IL16* gene polymorphisms probably play a major role in susceptibility to NPC. The rs11556218 TG genotype was associated with a significantly increased risk of NPC when compared with the TT genotypes (OR=2.05, 95 % CI 1.04–4.01; p=0.037). Patients carrying the rs11556218 G allele had a significantly higher risk for developing NPC compared to individuals carrying the T allele (OR=1.79, 95 % CI 1.07–3.01; p=0.027). A possible mechanism for *IL16* rs11556218 T/G polymorphism in modulating

NPC susceptibility and tumor development is that the rs11556218 T/G polymorphism regulate the expression of serum IL-16 levels; the overexpressed IL-16 activates $CD4^+$ T lymphocytes, monocyte macrophages, eosinophils, and dendrite cells by binding to CD4 (the main receptor of IL-16). The binding of IL-16 stimulates the monocytes to secrete multiple cytokines, including TNF-α, IL-1β, IL-6, and IL-15 [14, 16, 38], which have been shown to play an important role in tumorigenesis of NPC.

To our knowledge, the influence of *IL16* polymorphisms on severity to NPC has not been previously studied. Moreover, no studies have been performed to investigate the association between serum IL-16 levels and NPC. In this study, our results showed that there were no significant differences when patients with the stages I and II were compared with patients with stages III and IV. Based on our results, we suggest that the *IL16* polymorphisms may not have an influence on the severity of NPC. However, we observed that the serum IL-16 levels were significantly higher in individuals with homozygous rs11556218 GG genotypes or heterozygous rs11556218 TG genotypes than homozygous rs11556218 TT genotypes in NPC patients. Our results suggested that the *IL16* gene rs11556218 T/G polymorphism may regulate expression of the serum IL-16 levels and associate with an increased risk of NPC. Thus, genotypes carrying the *IL16* rs11556218 G variant allele may contribute to NPC susceptibility through increasing the ability to produce IL-16, which validated our hypothesis above.

There are several limitations in this study. First, detection of serum IL-16 concentration with the method of ELISA cannot completely and directly reflect the *IL16* gene expression, and mRNA expression evaluated in peripheral blood mononuclear cells by RT-PCR may be helpful. Second, we do not identify whether *IL16* gene polymorphism plays an important role in protein posttranslational modification. Further study will be required to explore the exact mechanism on how the *IL16* gene polymorphisms were involve in the pathogenesis of NPC.

In summary, we found that the *IL16* rs8034928 T/C polymorphism and the levels of serum IL-16 were significantly associated with increased risk of NPC. These results suggest that the *IL16* rs8034928 T/C polymorphism may contribute to an inherited predisposition to NPC through increased expression of IL-16 levels. Additional studies with larger sample sizes will be necessary to confirm our findings. Because genetic polymorphisms often vary among different ethnic groups, further studies are needed to clarify the association of the *IL16* polymorphism with the risk of NPC in diverse ethnic populations.

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Conflicts of interest None.

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