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Original Article

Role of gene polymorphisms and plasma levels of interleukin-16 in susceptibility to papillary thyroid carcinoma

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Abstract: Objective: The aim of this study was to examine the association of Interleukin-16 (IL-16) gene polymorphisms (rs11556218, rs4072111 and rs4778889) and IL-16 plasma levels with papillary thyroid carcinoma (PTC) in Chinese population. Methods: Our study included 538 patients with PTC and 625 healthy controls. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay to analyze genotype IL-16 in rs11556218, rs4072111 and rs4778889. Results: There were no significant differences in the genotype and allele frequency of rs4072111 and rs4778889 between PTC and healthy controls. Interesting, the frequencies of rs11556218 genotypes had statistical differences in PTC patients compared with healthy controls, and the frequencies of rs11556218 allele were increased in PTC patients compared with healthy controls ($P<0.05$). Increased plasma IL-16 levels were observed in PTC patients compared to healthy controls (9.7±7.42 vs. 3.8±4.86 ng/ml, $P<0.001$). Plasma IL-16 concentration in PTC patients with TG/GG genotypes were higher than those with the TT genotype (11.5±6.52 vs. 7.6±7.88 ng/ml, $P = 0.015$). Conclusions: Our data suggested that IL-16 (rs11556218) genotype is associated with increased risk of PTC in the Chinese population, and IL-16 polymorphism may be used as a genetic susceptibility marker in patients with PTC.

Keywords: Interleukin-16, papillary thyroid carcinoma, genetic susceptibility

Introduction

Papillary thyroid carcinoma (PTC) is the most common cancer in endocrine malignancy, and accounts for approximately 70% in all thyroid cancers [1]. Although the potential pathogenesis of PTC has not been fully elucidated. However, some investigations suggested that PTC might be induced by the interaction between genetic and environmental factor, and the molecular mechanism might be involved in the etiology of PTC [2]. Multiple genetic variants interact with each other and with the environment and modulate individual susceptibility in patients with PTC [3]. In fact, inflammation has been demonstrated to be one of the major hallmarks of multiple cancers [4], and the genetic polymorphism of inflammatory cytokines plays a pivotal role in the development of cancers in the human genome [5].

Interleukin-16 (IL-16) locates on chromosome 15q26.3 in the human genome, and encodes IL-16 cytokine [6]. IL-16 is originally found as a lymphocyte chemoattractant factor in human body [7]. Further, IL-16 is secreted by activated CD8 + T cells, and it is able to promote the secretion of other inflammatory cytokines including tumour necrosis factor (TNF), interleukin-1β (IL-1β) and interleukin-6 (IL-6) [8]. It has been reported that IL-16 as a modulator is a pleiotropic cytokine in inflammatory processes and tumorigenesis [9]. It is well known that IL-16 is associated with various cancer, and participates aberrant regulation in human malignancies [10]. Obviously, these finds highlight the important role that IL-16 is linked with the pathogenesis of cancer. Indeed, genetic polymorphism of IL-16 has been found to be associated with nasopharyngeal carcinoma, gastric cancer and hepatocellular carcinoma [11-13]. However, the gene polymorphisms in IL-16 and serum levels of IL-16 have not been reported in patients with PTC. Thus, the aim of this study was to examine the association of IL-16 gene polymorphisms (rs11556218,

Table 1. The primer sequence for genotyping IL-16 polymorphisms

Polymorphism	Primer sequence
rs11556218T/G	F: 5'-GCTCAGGTTCACAGAGTGTTTCCATA-3'
	R: 5'-TGTGACAATCACAGCTTGCCTG-3'
rs4072111C/T	F: 5'-CACTGTGATCCCGGTCCAGTC-3'
	R: 5'-TTCAGGTACAAACCCAGCCAGC-3'
rs4778889T/C	F: 5'-CTCCACACTCAAAGCCTTTTGTTCCTATGA-3'
	R: 5'-CCATGTCAAAACGGTAGCCTCAAGC-3'

Table 2. The demographic and clinical data of the study participants

	PTC patients	Controls	*P*-values
	N = 538, %	N = 625, %	
Age			
≤45	242 (44.9)	298 (47.7)	0.358
>45	296 (55.1)	327 (52.3)	
Gender			
Male	113 (21.0)	125 (20.0)	0.672
Female	425 (79.0)	500 (80.0)	
T status			
T1 + T2	420 (78.1)	-	-
T3 + T4	118 (21.9)	-	-
N status			
N0	278 (51.7)	-	-
N1	260 (48.3)	-	-
M status			
M0	500 (92.9)	-	-
M1	38 (7.1)	-	-
Clinical stage			-
I + II	306 (56.9)	-	-
III + IV	232 (43.1)	-	-

rs4072111 and rs4778889) and IL-16 plasma levels with PTC in Chinese population.

Materials and methods

Study population

Our study included 538 patients with PTC and 625 healthy controls. The controls for age and gender were matched to cases (p = 0.358; p = 0.672). None of the patients received surgical treatment, chemotherapy and radiotherapy before admission. Healthy subjects who had no personal or family history of thyroid disease were included on examination, and they had normal thyroid functions and negative thyroid autoantibodies, and they had no cardiovascular diseases, hypertension, diabetes mellitus, infectious diseases, smoking, history of any cancer and family history of any cancer in this study. This study was performed according to the relevant guidelines. The study was approved by the Affliated ZhongDa Hospital, School of Medicine, Southeast University, and informed consent was obtained from all subjects.

Fasting blood samples were collected in all individuals for genotype analysis. Clinical data of all patients were extracted from medical records such as age, gender, clinical stage and metastasis. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay to analyze genotype IL-16 in rs11556218, rs4072111 and rs4778889. The preparation for PCR primer sequences, annealing temperature, restriction enzymes used and length of PCR products were performed in accordance with described previously [14]. The Sanger sequencing was used to confirm the genotyping results. The sequencing data was shown in **Table 1**.

Blood samples from patients with PTC and controls were centrifuged at 1000 g for 10 min, and the plasma was stored at -80°C until analysis. We used enzyme-linked immunosorbent assay (ELISA) to measure plasma IL-16 levels in accordance with the manufacturer's instructions. For the ELISA kit, the minimum detectable dose was 5 pg/mL for the human IL-16. All samples were assayed in duplicate with the intra-assay coefficient of variation was less than 10%.

Statistical analysis

SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL, USA) was used to complete all statistical analyses. The differences for age and gender were evaluated by using Student's *t*-test and chi-square test between the study groups. The goodness-of-ft χ^2 test was used to test Hardy-Weinberg equilibrium (HWE). We used the chi-square test to compare genotype and allele frequencies between cases and controls. The multiple logistic regression analysis was used to adjust the factor potential associated with The genotype and allele frequency of IL-16. The Mann-Whitney U test was used to assess the differences of

Table 3. The genotype and allele frequencies of IL-16 polymorphism inPCT patients and healthy controls

	PTC patients	Controls	Crude OR	Beta	*Adjusted OR (95% CI)	*Adjusted *P*-values
rs11556218	538	625				
TT	242	350	1.00 (Reference)	-	1.00 (ref)	
TG	252	247	1.577 (1.238-2.009)	0.484	1.580 (1.241-2.015)	0.001
GG	44	28	1.951 (1.241-3.069)	0.336	1.953 (1.244-3.072)	0.008
Allele						
T	736	947	1.00 (Reference)	-	1.00 (ref)	
G	340	303	1.455 (1.215-1.743)	0.177	1.458 (1.217-1.746)	0.012
rs4072111						
CC	329	357	1.00 (Reference)	-	1.00 (ref)	
CT	186	241	0.837 (0.657-1.068)	-0.045	0.839 (0.658-1.071)	0.159
TT	23	27	0.924 (0.520-1.644)	-1.134	0.925 (0.522-1.647)	0.799
Allele						
C	844	955	1.00 (Reference)	-	1.00 (ref)	
T	232	295	0.890 (0.732-1.082)	-0.053	0.889 (0.733-1.084)	0.240
rs4778889						
TT	323	373	1.00 (Reference)	-	1.00 (ref)	
TC	190	217	1.011 (0.791-1.292)	0.133	1.012 (0.792-1.293)	0.932
CC	25	35	0.825 (0.483-1.408)	-0.090	0.825 (0.482-1.406)	0.479
Allele						
T	836	963	1.00 (Reference)	-	1.00 (ref)	
C	240	287	0.963 (0.793-1.171)	-0.075	0.963 (0.793-1.171)	0.707

*Adjusted for sex and age by logistic regression model.

plasma IL-16 concentrations among cases and controls. A $P<0.05$ was considered as statistical significance.

Results

Characteristics of participants

The demographics of the patients with PTC and controls are shown in **Table 2**. A total of 113 males and 425 females were included in patients with PTC, and 125 males and 500 females were considered as controls. There were no statistical differences between PTC patients and healthy controls with respect to the age and gender distributions.

The genotype and allele frequency of PTC patients versus healthy controls

The genotype and allele frequency of IL-16 (rs11556218, rs4072111 and rs4778889) polymorphism are summarized in PTC patients and controls (**Table 3**). Genotype distributions of all the three SNPs among the healthy controls were in Hardy-Weinberg equilibrium (P>0.05). There were no significant differences in the genotype and allele frequency of rs4072111 and rs4778889 between PTC and controls. Interesting, the frequencies of rs11556218 genotypes had statistical differences in PTC patients compared with controls (TG vs. TT: adjusted OR = 1.580, 95% CI: 1.241-2.015, P = 0.001; GG vs. TT: adjusted OR = 1.953, 95% CI: 1.244-3.072, *P* = 0.008), and the frequencies of rs11556218 allele were increased in PTC patients compared with healthy controls (G vs. T: adjusted OR = 1.458, 95% CI: 1.217-1.746, P = 0.012). There were no linkage disequilibrium in the three SNPs (for rs11556218 and rs4778889, D' = 0.18; for rs11556218 and rs4072111, D' = 0.24; and for rs4778889 and rs4072111, D' = 0.27). Further, the stratifcation analysis was performed in accordance with clinical stage there were no statistical differences for IL-16 (rs11556218) genotype and allele frequencies among any subgroup of PTC patients, as shown in **Table 4**.

Table 4. Genotype and allele frequencies of rs11556218T/G polymorphism in accordance with clinical stage

Variables	Genotypes (%)				Alleles (%)		
	TT	TG	GG	*P*	T	G	*P*
T status							
T1 + T2	189	194	37	0.570	572	268	0.684
T3 + T4	53	58	7		164	72	
N status							
N0	125	130	23	0.996	380	176	0.967
N1	117	122	21		356	164	
M status							
M0	226	234	40	0.998	686	314	0.974
M1	17	18	3		52	24	
Clinical stage							
I + II	138	144	24	0.948	420	192	0.855
III + IV	104	108	20		316	148	

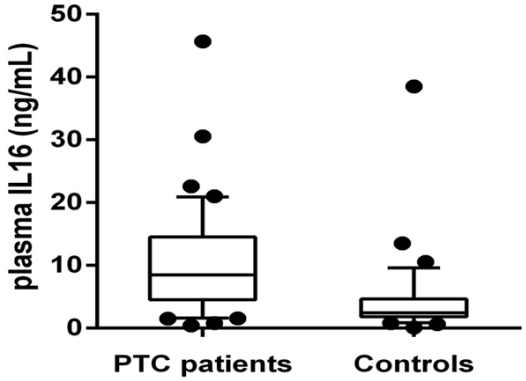

Figure 1. Increased interleukin-16 concentrations are observed in patients with papillary thyroid carcinoma (n = 84) compared with controls (n = 68).

The association between SNPs and IL-16

Plasma IL-16 concentrations were estimated in PTC patients and controls, and the gender and age were matched in 84 PTC patients and 64 controls. Increased plasma IL-16 levels were observed in PTC patients compared to healthy controls (9.7±7.42 vs. 3.8±4.86 ng/ml, P<0.001), as shown in **Figure 1**. The relations between plasma IL-16 levels and IL-16 polymorphisms were analyzed in patients with PTC. Plasma IL-16 concentration in PTC patients with TG/GG genotypes were higher than those with the TT genotype (11.5±6.52 vs. 7.6±7.88 ng/ml, P = 0.015) (**Figure 2**). Nevertheless, no statistical differences were found between the TG genotype and the GG genotype for plasma IL-16 concentrations in patients with PTC (P>0.005), and there were no statistical differences in plasma IL-16 between the metastatic patients and non-metastatic patients (P>0.005).

Discussion

Human genetic variation of SNPs is the most common cause that may result in an individual susceptibility to cancer. Some early studies have demonstrated that genetic variation may contribute to susceptibility of PTC, which is helpful for early diagnosis, prognosis and metastasis in patients with PTC. In the present study, the relationships between single nucleotide polymorphism (SNPs) (rs11556218, rs4072111 and rs4778889) in IL-16 and risk of PTC were investigated in a Chinese population. The rs11556218 in IL-16 was found to be associated with an increased risk of PTC in genotype and allele comparison. In addition, an increased IL-16 concentrations in PTC patients were observed compared with healthy controls, and rs11556218 TG/GG genotypes was related with higher IL-16 levels in patients with PTC. These significant findings suggested that rs11556218 in IL-16 may be a useful genetic susceptibility marker in patients with PTC.

Meta-analysis provided an strong evidence that IL-16 rs11556218 is associated with increased cancer risk [15]. Interesting, rs11556218 TG/GG genotypes were found to increase risk in patients with PTC in our study. The role of IL-16 as a crucial mediator in inflammatory diseases has been regarded [16]. Some studies examined the relationship between IL-16 and development of cancer. The association of IL-16 messenger RNA expression with cutaneous T cell lymphoma has been reported by Blaschke et al [17], and serum IL-16 concentrations have been considered as a marker to differentiate tumor stage in different tumor types [18, 19]. IL-16 tends to stimulate the production of some inflammatory cytokines such as TNF, IL-1β and IL-6 [8]. Moreover, several lines of evidence have suggested that dysregulation of these inflammatory cytokines might promote the development and progression in patients with gastric and colon carcinoma [20, 21]. Accumulated data have indicated these inflammatory cytokines may also be associated with PTC, and are key elements in the pa-

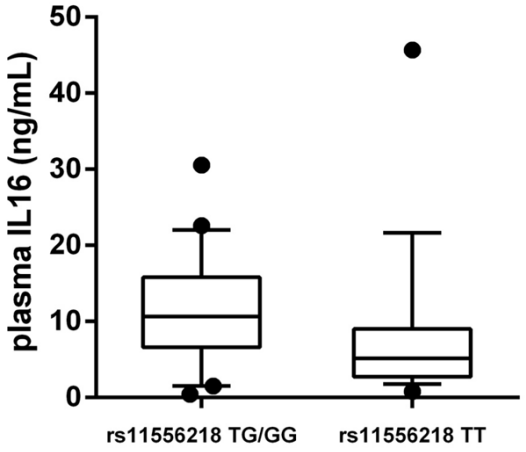

Figure 2. Increased interleukin-16 concentrations are observed in papillary thyroid carcinoma patients with rs11556218 TG/GG genotypes (n = 46) compared with papillary thyroid carcinoma patients with rs11556218 TT genotypes (n = 38).

thological physiology of PTC [22-24]. Thus, the positive results for the association between IL-16 (rs11556218) and PTC are biologically reasonable.

The SNPs (rs4072111) is located in an exon region, and single-nucleotide changes can lead to an amino acid substitution. There is increasing evidence that the IL-16 SNPs (rs4072111) are linked with colorectal and gastric cancer [12, 25]. With regard to the relationship between IL-16 rs4778889 and cancer risk, the association of IL-16 rs4778889 CC genotype with increased risk of non-cardia gastric cancer and renal cell carcinoma have been reported [26-27]. Nevertheless, IL-16 rs4778889 CC genotype has been found to be associated with a decreased risk of colorectal cancer and renal cell carcinoma [25, 28]. On the other hand, recent evidences have indicated that IL-16 rs4778889 was not related to the increased or decreased risk of cancer in patients with gastric cancer, colorectal cancer and nasopharyngeal carcinoma [11, 12, 29]. In our study, the relationships between the SNPs (rs4072111 and rs4778889) and PTC were not observed. The conflicting results may be explained by the following possibilities. First, the roles of SNP have differences in various cancer types, especially in different ethnicities. Second, limited sample size should be considered as a potential reason for the contradiction. Finally, a selection bias may be noted in different studies.

There were some limitations in this study. First, gene-environment interactions were not analyzed with available data in our study, since PTC is a disease induced by complex interactions between genetic and environmental factors. Second, all subjects were enrolled from a single hospital, although HWE test was performed in each SNP. Finally, a limited sample size should be considered in the current study. Our data suggested that IL-16 (rs11556218) genotype is associated with increased risk of PTC in the Chinese population, and IL-16 polymorphism may be used as a genetic susceptibility marker in patients with PTC. However, further studies are needed to evaluate the molecular mechanisms between IL-16 and PTC in various ethnic populations and larger sample size.

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Disclosure of conflict of interest

None.

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