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Original Article

The correlation analysis of RET gene polymorphism with papillary thyroid carcinoma

Dong-Guo Wang[1*], Jia-Yu Chen[2*], Hua-Yuan Zhang[3], Fang-Fang Zhang[3], Lin-Jun Yang[4], Yong-Hua Mu[5]

[1]Department of Clinical Laboratory Medicine, Taizhou Municipal Hospital Affiliated with Taizhou University, Taizhou, China; [2]Department of Laboratory Medicine, Taizhou University, Taizhou, China; [3]Department of Pathology, Taizhou Municipal Hospital Affiliated with Taizhou University, Taizhou, China; [4]Pepartment of Thyroid and Breast Surgery, Taizhou Municipal Hospital Affiliated with Taizhou University, Taizhou, China; [5]Department of Hepatobiliary Surgery, Taizhou Municipal Hospital Affiliated with Taizhou University, Taizhou, China. [*]Equal contributors.

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Abstract: Objective: Thyroid cancer is a common malignant tumor occurred in head and neck. Studies also shown that the occurrence and development of thyroid cancer is related with some genes and gene polymorphisms. In this study, we used case control to test the polymorphisms of rs1799939 in RET gene exon 11. Methods: A total of 350 cases of papillary thyroid carcinoma (PTC) were collected, 320 healthy control were recruited from subject received routine medical examination. Their PCR product from blood sample and gene sequencing were performed to confirm their polymorphism distribution of rs1799939 in RET gene. Results: There is no difference in age and gender distribution between the 2 groups. correlation analysis showed the three genotypes frequency distribution (G/G, G/A and A/A) have no significant difference between PTC group and control group. There is statistical difference between PTC patients with different gender in gene frequency of rs1799939 site distributions (G/G, G/A and A/A) but not healthy control. In addition, the allele frequency distribution of G/A also have statistical difference (in gender frequency). "A" allele in rs1799939 did not increase the risk of PTC occurrence in female group. Conclusions: The polymorphism of rsl799939 locus is associated with the occurrence and development of PTC in the Han population in southern China.

Keywords: Papillary thyroid carcinoma (PTC), single nucleotide polymorphism (SNP), rsl799939, Chinese

Introduction

Thyroid cancer is a common malignant tumor occurred in head and neck [1]. Thyroid cancer accounted for 1% in all of the body malignant tumor, and mainly includes 4 kinds of types: papillary thyroid carcinoma (PTC), medullary thyroid carcinoma (MTC), follicular carcinoma and undifferentiated carcinoma, of them, PTC is the most common type [2, 3]. The key point of treating thyroid cancer relying on timely early diagnosis. Neck ultrasonography, thyroid scintigraphy, fine needle aspiration cytology and CT examination are commonly usedclinical diagnosis methods for thyroid carcinoma [4-7]. However, the clinical diagnostic accuracy of thyroid cancer needs to be improved,even with the help of imaging, preoperative diagnosis rate of thyroid cancer is only about 50% [8]. An addition, the clinical manifestations of thyroid carcinoma in early stage is similar to benign thyroid tumor, this easily lead to misdiagnosis and missed diagnosis of thyroid cancer [9, 10].

Tumor marker detection has the advantages of easy operation, high sensitivity and high specificity, and it has gradually become one of the important methods for the early diagnosis of thyroid cancer. The commonly used hematological markers in clinical ismainly the functional marker of thyroglobulin, such as Tg, T3, T4, FT3, FT4, TSH and TPO, etc., while the specific markers are rare for the diagnosis of benign and malignant thyroid cancer. The pathogenesis of thyroid cancer has not yet entirely clear, a study showed that women, Asians, highly educated, a history of thyroid goiter, neck received radiation and a family history of thyroid disease are risk

Table 1. Tested result of Hardy-Weinberg genetic equilibrium of rsl799939 sites

Group	HO	HE	χ^2	*P*
Control	0.168	0.157	1.121	0.277
PTC	0.191	0.174	1.419	0.251

Note: HO means "observed heterozygosity", HE means expected heterozygosity, df=1.

factors for thyroid cancer [11]. Studies also shown that the occurrence and development of thyroid cancer is related with some genes and gene polymorphisms, single nucleotide polymorphism (SNP) refers to the difference in the same sequence of single nucleotide (single base conversion, insertion and deletion) between different individuals, and SNP the generally-means the two and other single nucleotide substitution. Researches have reported that RET proto oncogene, TSHR gene, BRAF gene and Ras gene were related with the occurrence of thyroid cancer.

RET protein is a transmembrane tyrosine kinase receptor protein, its coding gene is located in chromosome 10q11.2. The shearing of the 3' end of RET gene in different ways form three different forms of protein: RET9, RET43, and RET51. RET protein is activated through binding to ligands protein of glial cell line-derived neurotrophic factor [12, 13]. Now, it has been found that RET associated tumor pathogenesis mainly is the abnormal expression of RET gene and the mutations of wild-type RET gene. Lonn [14] and Ho [15] *et al* and have reported RET gene polymorphism is related with the occurrence of thyroid cancer. The mutationsof RET proto oncogene can cause the occurrence of thyroid papillary carcinoma, multiple endocrine adenoma type 2 (men2) and sporadic medullary thyroid carcinoma (MTC) [16, 17]. RET proto oncogeneis activated in thyroid papillary carcinoma through gene rearrangement, and is in activated in type 2 multiple endocrine adenoma and sporadic medullary thyroid carcinoma via point mutations [18]. The mutation in men2 wereoccurredin species level, and it can be passed on to the offspring. In this study, we used case control to test the polymorphisms of rs1799939 in RET gene exon 11, and investigatedits association with PTC to provide a genetic basis for the pathogenesis study of thyroid cancer.

Patients and methods

Patients

A total of 350 cases of papillary thyroid carcinoma (PTC) were collected, the patients were all diagnosed as PTC by pathology after surgery in Taizhou Municipal Hospital during 2010 to 2015, PTC was diagnosed according to diagnosis treatment guidelines of 2009 American Thyroid Association of thyroid nodules and differentiated thyroid carcinoma [19]. 320 healthy-control were recruited from subject received routine medical examination in Taizhou Municipal hospital from same periods, the age, gender and ethnic distribution and geographic location in health control were matched with selected PTC patients. Our research subjects were all Han population of south China.

The study has been approved and registered in Ethics Committeeof Taizhou Municipal Hospital Affiliated with Taizhou University in January 2015, the Ethics committee approved relating screening, and data collection of these patients, all subjects signed written informed consent form. All works were undertaken following the provisions of the Declaration of Helsinki.

Sample collection and DNA extraction

5 ml of peripheral venous blood was drawn to vacuum blood tube containing ethylene diamine tetraacetic acid (EDTA) from each subject. The samples were then stored at -20°C to prepare for the extraction of genomic DNA. The DNA was extracted bya Promega trace DNA Extraction Kit (Madison, WI, USA); the DNA contentwas used to detected using UV spectrophotometer (Beckman DU640, Brea, CA, USA). The absorbance value of A260/A280 ratio should be 1.6~2.0; extracted DNA was preserved in 4°C refrigerator.

Primer design and gene sequencing for genotype confirmation

The primers were designed by Premier Primer 5 software according to the template sequence of rsl799939 site of RET gene in NCBI SNP database. Upstream primer: 5'-CACAAGCCACCCATCTCC-3', downstream primer: 5'-GAACGGCACCTCATCATAGTC-3', the length of PCR product fragment is 342 BP. The PCR product was send for direct gene sequencing to confirm their polymorphism distribution, the sequenc-

Table 2. Distribution of genotypes frequency and the allele frequency of rs1799939 sites (N(%)] in PTC patients and healthy control

		Control N (%)	PTC N (%)	*P*
Genotypes frequency	GG	239 (74.7%)	252 (72.0%)	
	GA	62 (19.4%)	74 (21.1%)	
	AA	19 (5.94%)	24 (6.86%)	0.725
Allelic frequency	G	540 (84.4%)	578 (82.6)	
	A	100 (15.6%)	122 (17.4%)	0.379

Table 3. Distribution of genotypes frequency and the allele frequency of rs1799939 sites N (/%) in PTC patients with different gender

		Male N (%)	Female N (%)	*P*
Genotypes frequency	GG	59	193	
	GA	13	61	
	AA	1	23	0.029
Allelic frequency	G	131	447	
	A	14	107	0.006

Table 4. Distribution of genotypes frequency and the allele frequency of rs1799939 sites N (/%) in healthy control with different gender

		Male N (%)	Female N (%)	*P*
		86	234	
Genotypes frequency	GG	61	178	
	GA	16	46	
	AA	6	13	0.845
Allelic frequency	G	138	402	
	A	28	72	0.620

ing company is Life Technology Ltd (Shang Hai, China).

Statistical analysis

Data analysis was performed using SPSS 19.0 statistical software, chi square test of goodness - of - fit testswhether the genotype distribution of the 2 groups were consistent with the Hardy-Weinberg's law of equilibrium, and whether the genotype of the locus, allele frequency distribution of allele was associated with PTC.

Results

Demographic data

A total of 350 cases of patient with PTC and 320 health control were recruited successfully. The average age of PTC patients was 45.32±9.24 year; 73 of them were male and 277 of them were female. The average of healthy control were 43.86±10.28 year; 86 of them were male and 234 of them were female. Comparison results showed there is no difference in age and gender distribution between the 2 groups (*P*=0.466 and 0.714, respectively).

PCR amplification and enzyme cutting

The rsl799939 sites of RET gene is a dimorphic SNPs containing 2 allelic genes: G and A. The PCR amplified length for gene fragments is 342 bp. We send the PCR product for gene sequencing, and detected three genotypesrespectively: homozygous incised G/G genotype, heterozygous incised G/A genotype and thehomozygous unincisedgenotype of A/A.

Hardy-Weinberg genetic equilibrium test

Goodness - of - fit test showed the genotype frequency distribution in the PTC group and control group are in line with the Weinberg - Hardy genetic equilibrium law (*P*>0.05, **Table 1**).

Correlation analysis of rs1799939 sites with PTC

Correlation analysis showed the threegenotypes frequency distribution (G/G, G/A and A/A) have no significant difference between PTC group and control group (**Table 2**); the allele frequency distribution of G/A also did not have significant difference between PTC group and control group (**Table 2**).

Rs1799939 sites distribution in patients with different gender

We divided PTC patients into two groups according to their gender: male and female group. The gene frequency and allele frequency distribution results showed that there is statistical difference between PTC patients with different gender in gene frequency of rs1799939 site distributions (G/G, G/A and A/A). In addition, the allele frequency distribution of G/A also have statistical difference (**Table 3**). Although the genotype frequency on rs1799939 site is significant different between male and female in PTC patients, we did not find same result in healthy control (**Table 4**), there is no significant difference between genders in healthy control.

Table 5. Relationship between rs1799939 polymorphisms of RET with PTC risk in female group

		Female control N (%)	Female PTC N (%)	*P*	OR (95% CI)
Genotypes frequency	GG	178	193		
	GA	46	61	0.381	1.223 (0.793-1.887)
	AA	13	23	0.221	1.632 (0.802-3.319)
Allelic frequency	G	402	447		
	A	72	107	0.084	1.337 (0.963-1.855)

"A" allele in rs1799939 did not increase the risk of PTC occurrence in female group

As shown in **Table 5**, among PTC female patients, although there was higher ratio of AA genotype and A allele compared to male group, there is no significant difference of genotype distribution between PTC female patients and female healthy control. Although there is possibility of A allele could increase the risk of PTC in female population, but the *p* value is 0.084, above 0.05.

Discussions

The international agency for research for cancer of World Health Organization: the thyroid cancer incidence rate of male increased from of 1.2/million to 1.5/10 million from year 2000 to 2008; the female incidence rate increased from 3.0/10 million to 4.7/million from year 2000s to 2008 word widely (http://www.iarc.fr/). Theincidence rate of thyroid cancer shows an increasing trend in recent years, and the incidence rate of female were higher than male all over the world [20].

Early detection and early diagnosis of thyroid cancer are very important for patients with thyroid cancer, because the early treatment of thyroid cancer could improve the cure rate significantly [21, 22]. There are mainly 3 kind of methods for the diagnosis of thyroid cancer currently: physics, histocytology and chemical methods [23-25]. Carotid ultrasonography, CT, fine needle aspiration cytology, thyroid radionuclide scanning inspection methods are commonly used inclinical, they all belong to thephysics and histocytology method. However, theyare mostly used for advanced thyroid cancer diagnosis, the operationsare complex and the cost are high, and there is a certain degree of misdiagnosis. Tumor marker detection has the advantages of easy operation, high sensitivity and high specificity, and it has gradually become one of the important methods for the early diagnosis of thyroid cancer.

Papillary thyroid carcinoma (PTC) is the most common type of malignant thyroid tumors originated from thyroid follicular epithelial cells, and PTC accounted for 80% of the thyroid cancer [26]. RET proto oncogene mainly involved in regulating the normal physiological function of cells. It plays an important role in regulating the proliferation, differentiation and migration of neural crest cells and the development of enteric nervous system [18]. The activation mechanism of RET proto oncogene in papillary thyroid carcinoma is gene rearrangement. There are mainly 3 kinds of gene rearrangement: the ret gene rearranged with H4 and RFG gene at the same chromosome produces oncogene ret/ptc1 and ret/ptc3; and ret gene rearranged with RIa gene at chromosome 17 producesoncogene ret/ptc2 gene. Rearrangement of ret gene causecontinuous activation of tyrosine kinase function area of its encoding protein, thus caused malignant transformation of cells through the downstream signaling pathways [27, 28].

Researches have demonstrated that missense germline mutations in the RET proto-oncogene is the susceptibility gene for familial medullary thyroid cancer (FMTC) and multiple endocrine neoplasia type 2 (MEN2) [29]. Elisei et al [30] detected rs1799939 locus gene type in 106 healthy people and 106 MTC patients, they found the occurrence of MTC was correlated with rs1799939; A meta-analysis performed by Figlioli et al [31] also confirmed that polymorphisms of rsl799939 locus increase the risk of thyroid cancer. However, in our study, we did not find genotypes frequency distribution (G/G, G/A and A/A) have statistical difference between PTC group and control group, this result is different from some other studies. Although we did not prove the role rs1799939 polymorphism in PTC, we did demonstrate that

there is significantly difference between female and male population in PTC group, and female has higher ratio of A allele. It has possibility that A allele can increase the risk of PTC in female population, of course, this hypothesis need further study to be confirmed.

Interestingly, research performed by Ho et al showed the genotype distributions were similar between differentiated thyroid carcinoma (DTC) cases and benign thyroid disease (BTD) cases; Polymorphic allele frequencies were similar between the cases and controls in exons 11 of the RET proto-oncogene. His research is inconsistent with our results, this may cause by the following reason: firstly, the sample size is relative small, which limited the quantity of provided information, and could not draw the conclusion that the results were statistically significant, Ho's research include 163 patients participant, of whom 101 had DTC and 62 had BTD, the relatively small sample size may have a certain impact on the conclusion. Secondly, the pathogenesis of thyroid cancer is not very clear, in addition to the known signal transduction pathway of RAF/MEK/ERK and PI3K/Akt, there may still exist other unknown pathway plays an important role. Thirdly, except for genetic factors, environmental factors also play important role in the occurrence and development of thyroid cancer, the differences between research object with the control in the geographical location, genetic background, race and living environment may cause the negative results. In addition, the recruited subject was PTC patients, this also has difference with DTC patient and could cause different conclusions.

To sum up, our study finds that the polymorphism of rsI799939 locus is associated with the occurrence and development of PTC in the Han population in southern China. Further study is needed to expand the sample size and increase studied polymorphic loci site and simultaneous detecting thyroid carcinoma related genes and analyzing gene-gene interactions and gene-environment interaction, to further explore the pathogenesis of thyroid cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dong-Guo Wang, Department of Clinical Laboratory Medicine, Taizhou Municipal Hospital Affiliated with Taizhou University, 381 East Road of Zhongshan, Taizhou 318000, Zhejiang, China. E-mail: dongguowang65@163.com

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