

Original Article

RET polymorphisms might be the risk factors for thyroid cancer

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Abstract: Aims: The purpose of the study is to investigate the relationship between rs1799939, rs1800858 and rs74799832 polymorphisms of *RET* with thyroid cancer (TC) susceptibility. Methods: Genotypes distribution of control groups were tested by Hardy-Weinberg equilibrium (HWE). Rs1799939, rs1800858 and rs74799832 polymorphisms of *RET* were researched in 135 patients with TC and 135 healthy people using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Odds ratio (OR) with 95% confidence interval (CI) were calculated to evaluate the association between *RET* polymorphisms and the risk of TC by Chi-squared test. Results: Genotypes frequencies of the control group were consistent with HWE. The frequency of genotype AA and allele A in rs1799939 were significantly higher in patients with TC than controls (OR=3.768, $P=0.046$; OR=1.695, $P=0.035$). Genotype GG and allele G of rs1800858 remarkably increased the risk of TC (OR=2.149, $P=0.039$; OR=1.45, $P=0.039$). Moreover, CC genotype and C allele in rs74799832 polymorphism was related with TC susceptibility. (OR=2.28, $P=0.049$; OR=1.566, $P=0.049$). Conclusion: In present result, *RET* rs1799939, rs1800858 and rs74799832 polymorphisms might be the risk factors for TC.

Keywords: *RET*, polymorphisms, thyroid cancer

Introduction

Thyroid cancer (TC), common in endocrine system, is one of the malignant tumors with fast growth [1], which includes papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid cancer (MTC), and anaplastic thyroid cancer (ATC). Among 80% of TC is PTC, the second is FTC (10%-15%) [2]. With rapid economic development, changes in diet and improvement of health-care, occurrence of TC, however, shows an upward tendency [3]. Previous study showed genetic and environmental factors were relative with the pathogenesis of TC [4]. The possible relevant environmental factors contain ionizing radiation [5], the anomaly uptake of iodine [6], estrogen [7], lesions in thyroid itself [8], other diseases [9, 10], TSH and its receptors [11], the popularization of neck ultrasound [12] as well as social and cultural factors [13]. Moreover, many researches have demonstrated the close correlation of thyroid cancer with some internal

oncogenes [14-17], such as *RAS*, *BRAF*, *MYC*, *CCND1*, *RET*. *RET* proto-oncogene encodes one of the receptor tyrosine kinase proteins [18], which is located on chromosome 10q11.2 including 24 exons [19]. It can transform signals for cell proliferation and differentiation as cell-surface molecules [20]. The high prevalence of *RET* cytogenetic rearrangement is a genetic lesion for the development of diseases, such as papillary thyroid carcinoma (PTC) [21], lung adenocarcinomas [22]. The mutations in *RET* are also the pathogenic factors and relevant with occurrence and development of some malignant tumors [23, 24]. In view of the functions, a mass of publications reported the influence of *RET* gene on TC. The evidence showed that *RET* mutations were related with pathogenesis of MTC [25].

In present study, 135 patients with TC and 135 TC-free controls were enrolled to evaluate the association of *RET* rs1799939, rs1800858 and rs74799832 polymorphisms with the risk of TC.

RET polymorphisms might be the risk factors for thyroid cancer

Table 1. Primers sequences in *RET* polymorphisms

Polymorphisms	Up-/down-primer	Sequences
rs1799939	Forward	5'-TGCTACCACAAGTTTGCCCA-3'
	Reverse	5'-GGGCAAACCTGTGGTAGCAG-3'
rs1800858	Forward	5'-AAGCCTTATTCTACCATCC-3'
	Reverse	5'-AGGCTTCTTCAAGGACAAAA-3'
rs74799832	Forward	5'-GTTCTGTGCCAGGAGTGTC-3'
	Reverse	5'-CTGTAGACACTCCTGGGCAC-3'

Materials and methods

Objects of the study

A case-control study was conducted, containing 135 patients with TC and 135 healthy controls. Among patients, there were 89 males and 46 females with a median age of 55. The cases were confirmed by histopathology and did not experience radiotherapy or chemotherapy before blood collection.

Controls with a median age of 51 were collected within those for health physical examination in hospitals at same period, including 93 males and 42 females. People were excluded if they had family history of tumors, suffered from genetic diseases or other diseases related to thyroid. There were no significant differences in basic information like age, gender, race and native place between two groups through statistical test. This research was approved by the Research Ethics Committee of the hospital, and they all signed informed consent before the study and all subjects were not related by blood.

DNA extraction

5 mL peripheral venous blood was collected from every subject, conducted anticoagulant using ethylene diamine tetraacetic acid (EDTA) and preserved in refrigerator at -80°C. DNA was extracted by phenol-chloroform method according to instructions, and stored at -20°C for later.

Genotyping methods

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to test genotypes distribution of *RET* rs1799939, rs1800858 and rs74799832 polymorphisms. The PCR primers were all designed by Primer 5.0 software and synthesized by Shanghai

Genecore Biotechnologies Company (**Table 1**). Total of 25 µL PCR reaction mixtures included 2.0 µL template DNA, each 1.0 µL (10 µmol·L⁻¹) of forward and reverse primers, 12.5 µL Master Mix and 8.5 µL of sterile water. PCR process consisted of the following steps: pre-denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 40 s

and extension at 74°C for 30 s, finally continuation at 72°C for 10 min after all cycles. PCR products were digested by *Bal I*, *Hal III* and *Sma I* and measured each genotype of genetic variation through agarose gel electrophoresis.

Statistical analysis

The genotypes frequencies of the control group were tested whether it was correspond with Hardy-Weinberg equilibrium (HWE). Odds ratio (OR) with 95% confidence interval (CI) were calculated by χ^2 test to examine the differences on genotype and allele distributions of each polymorphism between cases and controls using SPSS 18.0 software ($P < 0.05$ represented the statistically significant differences).

Results

HWE test

The HWE test in controls showed that the goodness of fit to the law in each site was fine ($P > 0.05$), indicating that controls were in equilibrium state and had good representativeness.

Analysis on the correlation of *RET* alleles and genotypes with TC patients

Genotypes distributions of rs1799939, rs1800858 and rs74799832 polymorphisms in *RET* showed that (**Table 2**) AA genotype and A allele of rs1799939 polymorphism significantly increased the risk of thyroid cancer (OR=3.768, 95% CI=1.006-14.111; OR=1.695, 95% CI=1.061-2.708), the genotype and allele frequencies of GG and G in rs1800858 were higher in cases than controls (OR=2.149, 95% CI=1.078-4.285; OR=1.450, 95% CI=1.033-2.036). CC genotype and C allele in rs74799832 were the risk factors for thyroid cancer (OR=2.280, 95% CI=1.018-5.109; OR=1.566, 95% CI=1.078-2.275).

RET polymorphisms might be the risk factors for thyroid cancer

Table 2. Relationship between three polymorphisms of *RET* with TC risk

Genotype/ allelotype	Cases (n=135)	Controls (n=135)	X ²	P value	OR (95% CI)
rs1799939					
GG	92	104	-	-	1
GA	33	28	0.96	0.38	1.33 (0.75-2.37)
AA	10	3	4.39	0.046	3.77 (1.01-14.11)
G	217 (80.4%)	236 (87.4%)	-	-	1
A	53 (19.6%)	34 (12.6%)	4.95	0.035	1.70 (1.06-2.71)
rs1800858					
AA	24	39	-	-	1
AG	70	65	3.26	0.093	1.75 (0.95-3.22)
GG	41	31	4.78	0.038	2.15 (1.08-4.29)
A	118 (43.7%)	143 (53.0%)	-	-	1
G	152 (56.3%)	127 (47.0%)	4.64	0.039	1.45 (1.03-2.04)
rs74799832					
TT	63	79	-	-	1
TC	52	45	1.97	0.19	1.45 (0.86-2.43)
CC	20	11	4.14	0.049	2.28 (1.02-5.11)
T	178 (65.9%)	203 (75.2%)	-	-	1
C	92 (34.1%)	67 (24.8%)	5.57	0.023	1.57 (1.08-2.28)

Discussion

For thyroid cancer, it has been demonstrated that incidence of the disease is higher in females than in males [26] and increases with aging [27]. So far, many therapies are applied for treatment of TC, such as surgery, chemotherapy, endocrine and radioactive therapies, but the results were unsatisfactory [28]. Genes associated with TC are inevitable objects in study. The study of Lee et al. demonstrated that *IL17RA* polymorphisms are associated with both development and bilaterality of PTC in Korean population [29]. Wei et al. has uncovered the expression level of mir-149-5p has an influence on local progression of PTC and susceptibility in Chinese populations [30]. In research of Chen et al., *ECRG4* could regulate cell cycle in PTC cells which transit from the G1 to G2 phase and promote tumors growth [31].

RET is a proto-oncogene encoding receptor thyroid kinase protein. Researches show that *RET* rearrangement is one of the most common types of gene modification in PTC [32], and is related to the incidence of radioactive TC [33]. *RET* mutants are common in MTC [34, 35]. In recent years, many studies have discussed the relationship of *RET* polymorphisms with thyroid

cancer. According to the study of Santos et al., minor alleles of *RET* G691S and S904S polymorphisms significantly increase tumors size in patients with TC at diagnosis, and S836S polymorphism is a risk factor for PTC, but not FTC [36]. A result that *RET* polymorphisms have an additive influence on evaluating the risk of MTC metastasis was found by Lucieli et al [37]. Ho et al. has reported that *RET* exon 7 (and possibly 14) polymorphism is a risk factor increased the morbidity of differentiated TC (DTC) [38].

In our case-control study, three polymorphisms of *RET* (rs1799939, rs1800858 and rs74799832) were selected to explore the correlation with pathogenesis of TC. The mutant homozygous genotype AA and A allele of *RET* rs1799939 polymorphisms significantly increased the risk of TC. The results reported from many publications were consistent with ours. Cardot et al. research demonstrated *RET* G691S (rs1799939) variant was an independent predictor with a high basal calcitonin synthesis rate for sporadic MTC (sMTC) [39]. A meta-analysis of Lantieri et al. have uncovered G691S mutant allele can increase the risk of MTC, especially in females [40]. The same as rs1799939 polymorphism, the frequencies of genotype GG and allele G in *RET* rs1800858 polymorphism were observably higher in cases than controls, so is the role of rs74799832. This two polymorphisms were hardly reported in past years.

We have clarified *RET* rs1799939, rs1800858 and rs74799832 polymorphisms significantly increase TC susceptibility. In other words, *RET* polymorphisms maybe the risk predictors for developing aggressive of TC. This result has a positive influence on diagnosis and treatment of TC. But it may present some limitations due to neglecting environmental factors. Further study with well-design and enough large sam-

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RET polymorphisms might be the risk factors for thyroid cancer

ple size is needed to assess the relationship of RET polymorphisms with TC susceptibility.

At the moment, the rapid increasing incidence of thyroid cancer in China has seriously harmed people's health. We should promote the programs of general examination, early diagnosis and timely treatment as well as advocating healthy lifestyle and diet custom among high-risk population in high-prevalence areas. What is more, more efforts are made to explore the pathogenic gene of TC.

Disclosure of conflict of interest

None.

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RET polymorphisms might be the risk factors for thyroid cancer

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