

## 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'-GGGCAAACCTTGTTAGCAG-3'
rs1800858	Forward	5'-AAGCCTTATTCTACCATCC-3'
	Reverse	5'-AGGCTTCTCAAGGACAAAA-3'
rs74799832	Forward	5'-GTTCTGTGCCAGGAGTGC-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<sup>-1</sup>) 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  $\chi^2$  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/ alleleotype	Cases (n=135)	Controls (n=135)	X <sup>2</sup>	P value	OR (95% CI)
<b>rs1799939</b>					
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)
<b>rs1800858</b>					
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)
<b>rs74799832</b>					
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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### References

- [1] Mazzaferri EL. An overview of the management of papillary and follicular thyroid carcinoma. *Thyroid* 1999; 9: 421-427.
- [2] Faam B, Ghaffari MA, Ghadiri A and Azizi F. Epigenetic modifications in human thyroid cancer. *Biomed Rep* 2015; 3: 3-8.
- [3] Davies L and Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. *JAMA* 2006; 295: 2164-2167.
- [4] Pierotti MA, Vigneri P and Bongarzone I. Rearrangements of RET and NTRK1 tyrosine kinase receptors in papillary thyroid carcinomas. *Recent Results Cancer Res* 1998; 154: 237-247.
- [5] Ivanov VK, Tsyb AF, Nilova EV, Efendiev VF, Gorsky AI, Pitkevich VA, Leshakov SY and Shiryaev VI. Cancer risks in the Kaluga oblast of the Russian Federation 10 years after the Chernobyl accident. *Radiat Environ Biophys* 1997; 36: 161-167.
- [6] Chow SM, Law SC, Au SK, Mang O, Yau S, Yuen KT and Lau WH. Changes in clinical presentation, management and outcome in 1348 patients with differentiated thyroid carcinoma: experience in a single institute in Hong Kong, 1960-2000. *Clin Oncol (R Coll Radiol)* 2003; 15: 329-336.
- [7] Sakoda LC and Horn-Ross PL. Reproductive and menstrual history and papillary thyroid cancer risk: the San Francisco Bay Area thyroid cancer study. *Cancer Epidemiol Biomarkers Prev* 2002; 11: 51-57.
- [8] Galanti MR, Sparen P, Karlsson A, Grimelius L and Ekblom A. Is residence in areas of endemic goiter a risk factor for thyroid cancer? *Int J Cancer* 1995; 61: 615-621.
- [9] Aschebrook-Kilfoy B, Sabra MM, Brenner A, Moore SC, Ron E, Schatzkin A, Hollenbeck A and Ward MH. Diabetes and thyroid cancer risk in the National Institutes of Health-AARP Diet and Health Study. *Thyroid* 2011; 21: 957-963.
- [10] Kitahara CM, Platz EA, Beane Freeman LE, Black A, Hsing AW, Linet MS, Park Y, Schairer C and Berrington de Gonzalez A. Physical activity, diabetes, and thyroid cancer risk: a pooled analysis of five prospective studies. *Cancer Causes Control* 2012; [Epub ahead of print].
- [11] Boelaert K. The association between serum TSH concentration and thyroid cancer. *Endocr Relat Cancer* 2009; 16: 1065-1072.
- [12] Zheng T, Holford TR, Chen Y, Ma JZ, Flannery J, Liu W, Russi M and Boyle P. Time trend and age-period-cohort effect on incidence of thyroid cancer in Connecticut, 1935-1992. *Int J Cancer* 1996; 67: 504-509.
- [13] Pujol P, Daures JP, Nsakala N, Baldet L, Bringer J and Jaffiol C. Degree of thyrotropin suppression as a prognostic determinant in differentiated thyroid cancer. *J Clin Endocrinol Metab* 1996; 81: 4318-4323.
- [14] Motoi N, Sakamoto A, Yamochi T, Horiuchi H, Motoi T and Machinami R. Role of ras mutation in the progression of thyroid carcinoma of follicular epithelial origin. *Pathol Res Pract* 2000; 196: 1-7.
- [15] Fukushima T, Suzuki S, Mashiko M, Ohtake T, Endo Y, Takebayashi Y, Sekikawa K, Hagiwara K and Takenoshita S. BRAF mutations in papillary carcinomas of the thyroid. *Oncogene* 2003; 22: 6455-6457.
- [16] Bieche I, Franc B, Vidaud D, Vidaud M and Lidereau R. Analyses of MYC, ERBB2, and CCND1 genes in benign and malignant thyroid follicular cell tumors by real-time polymerase chain reaction. *Thyroid* 2001; 11: 147-152.
- [17] Rusinek D, Szpak-Ulczo S and Jarzab B. Gene expression profile of human thyroid cancer in relation to its mutational status. *J Mol Endocrinol* 2011; 47: R91-103.
- [18] Goodfellow PJ and Wells SA Jr. RET gene and its implications for cancer. *J Natl Cancer Inst* 1995; 87: 1515-1523.
- [19] Sovrea AS, Dronca E, Galatar M, Radian S, Vornicescu C and Georgescu C. Diagnostic correlation between RET proto-oncogene mutation, imaging techniques, biochemical markers and morphological examination in MEN2A syndrome: case report and literature review. *Rom J Morphol Embryol* 2014; 55: 389-400.
- [20] Schuchardt A, D'Agati V, Larsson-Blomberg L, Costantini F and Pachnis V. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor RET. *Nature* 1994; 367: 380-383.

## RET polymorphisms might be the risk factors for thyroid cancer

- [21] Liu RT, Chou FF, Wang CH, Lin CL, Chao FP, Chung JC, Huang CC, Wang PW and Cheng JT. Low prevalence of *RET* rearrangements (*RET/PTC1*, *RET/PTC2*, *RET/PTC3*, and *ELKS-RET*) in sporadic papillary thyroid carcinomas in Taiwan Chinese. *Thyroid* 2005; 15: 326-335.
- [22] Mukhopadhyay S, Pennell NA, Ali SM, Ross JS, Ma PC and Velcheti V. *RET*-rearranged lung adenocarcinomas with lymphangitic spread, psammoma bodies, and clinical responses to cabozantinib. *J Thorac Oncol* 2014; 9: 1714-1719.
- [23] Valdes N, Navarro E, Mesa J, Casteras A, Alcazar V, Lamas C, Tebar J, CastaNo L, Gaztambide S and Forga Llenas L. *RET* Cys-634Arg mutation confers a more aggressive multiple endocrine neoplasia type 2A phenotype than Cys634Tyr mutation. *Eur J Endocrinol* 2015 ; 172: 301-7.
- [24] Sosonkina N, Hong SK, Starenki D and Park JI. Kinome sequencing reveals *RET* G691S polymorphism in human neuroendocrine lung cancer cell lines. *Genes Genomics* 2014; 36: 829-841.
- [25] Romei C, Tacito A, Molinaro E, Agate L, Bottici V, Viola D, Matrone A, Biagini A, Casella F, Ciampi R, Materazzi G, Miccoli P, Torregrossa L, Ugolini C, Basolo F, Vitti P and Elisei R. Twenty years of lesson learning: how does the *RET* genetic screening test impact the clinical management of medullary thyroid cancer? *Clin Endocrinol (Oxf)* 2015; 82: 892-9.
- [26] Akslen LA, Haldorsen T, Thoresen SO and Glattre E. Incidence of thyroid cancer in Norway 1970-1985. Population review on time trend, sex, age, histological type and tumour stage in 2625 cases. *APMIS* 1990; 98: 549-558.
- [27] Albores-Saavedra J, Henson DE, Glazer E and Schwartz AM. Changing patterns in the incidence and survival of thyroid cancer with follicular phenotype—papillary, follicular, and anaplastic: a morphological and epidemiological study. *Endocr Pathol* 2007; 18: 1-7.
- [28] Lkhoyaali S, Benhmida S, Ait Elhaj M, Layachi M, Bensouda Y and Errihani H. [Targeted therapy in thyroid cancer: Towards a treatment card]. *Pathol Biol (Paris)* 2015 ; 63: 1-6.
- [29] Lee YC, Chung JH, Kim SK, Rhee SY, Chon S, Oh SJ, Hong IK and Eun YG. Association between interleukin 17/interleukin 17 receptor gene polymorphisms and papillary thyroid cancer in Korean population. *Cytokine* 2015; 71: 283-288.
- [30] Wei WJ, Lu ZW, Li DS, Wang Y, Zhu YX, Wang ZY, Wu Y, Wang YL and Ji QH. Association of the miR-149 Rs2292832 polymorphism with papillary thyroid cancer risk and clinicopathologic characteristics in a Chinese population. *Int J Mol Sci* 2014; 15: 20968-20981.
- [31] Chen J, Liu C, Yin L and Zhang W. The tumor-promoting function of *ECRG4* in papillary thyroid carcinoma and its related mechanism. *Tumour Biol* 2015 ; 36: 1081-9.
- [32] Klugbauer S, Pfeiffer P, Gassenhuber H, Beimfohr C and Rabes HM. *RET* rearrangements in radiation-induced papillary thyroid carcinomas: high prevalence of topoisomerase I sites at breakpoints and microhomology-mediated end joining in *ELE1* and *RET* chimeric genes. *Genomics* 2001; 73: 149-160.
- [33] Maenhaut C, Detours V, Dom G, Handkiewicz-Junak D, Oczko-Wojciechowska M and Jarzab B. Gene expression profiles for radiation-induced thyroid cancer. *Clin Oncol (R Coll Radiol)* 2011; 23: 282-288.
- [34] Wiench M, Kwasniewski M, Gubala E, Wygoda Z, Pawlaczek A, Oczko M and Jarzab B. [Proto-oncogene *RET* somatic mutations in medullary thyroid carcinoma]. *Wiad Lek* 2001; 54 Suppl 1: 415-421.
- [35] Elisei R, Cosci B, Romei C, Agate L, Piampiani P, Miccoli P, Berti P, Basolo F, Ugolini C, Ciampi R, Nikiforov Y and Pinchera A. Identification of a novel point mutation in the *RET* gene (Ala883Thr), which is associated with medullary thyroid carcinoma phenotype only in homozygous condition. *J Clin Endocrinol Metab* 2004; 89: 5823-5827.
- [36] Santos M, Azevedo T, Martins T, Rodrigues FJ and Lemos MC. Association of *RET* genetic polymorphisms and haplotypes with papillary thyroid carcinoma in the Portuguese population: a case-control study. *PLoS One* 2014; 9: e109822.
- [37] Ceolin L, Siqueira DR, Ferreira CV, Romitti M, Maia SC, Leiria L, Crispim D, Ashton-Prolla P and Maia AL. Additive effect of *RET* polymorphisms on sporadic medullary thyroid carcinoma susceptibility and tumor aggressiveness. *Eur J Endocrinol* 2012; 166: 847-854.
- [38] Ho T, Li G, Zhao C, Wei Q and Sturgis EM. *RET* polymorphisms and haplotypes and risk of differentiated thyroid cancer. *Laryngoscope* 2005; 115: 1035-1041.
- [39] Cardot-Bauters C, Leteurtre E, Leclerc L, Vantghem MC, Do Cao C, Wemeau JL, d'Herbomez M, Carnaille B, Barbu V, Pinson S and Pigny P. Does the *RET* variant G691S influence the features of sporadic medullary thyroid carcinoma? *Clin Endocrinol (Oxf)* 2008; 69: 506-510.
- [40] Lantieri F, Caroli F, Ceccherini I and Griseri P. The involvement of the *RET* variant G691S in medullary thyroid carcinoma enlightened by a meta-analysis study. *Int J Cancer* 2013; 132: 2808-2819.