

# Association between functional polymorphisms in the flanking region of miR-143/145 and risk of papillary thyroid carcinoma

## A case-control study

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### Abstract

MiR-143 and miR-145 were down-regulated in papillary thyroid carcinoma (PTC) involving in cell proliferation, apoptosis, migration, invasion, and epithelial to mesenchymal transition. In this study, we aimed to investigate the association between 2 functional polymorphisms (ie, rs4705342 and rs353292) in the flanking region of miR-143/145 and risk of PTC.

A case-control study including 316 PTC patients and 347 controls was performed. The rs4705342 and rs353292 were genotyped by using the TaqMan allelic discrimination. The results were confirmed by DNA sequencing.

For the rs4705342, a reduced risk of PTC was observed in heterozygous comparison, dominant genetic model and allele comparison (CC vs TT: adjusted OR=0.37, 95% CI=0.19–0.74,  $P=.003$ ; CT/CC vs TT: adjusted OR=0.64, 95% CI=0.47–0.87,  $P=.005$ ; C vs T: adjusted OR=0.66, 95% CI=0.52–0.85,  $P=.001$ , respectively). No significant difference was found in the genotypic distributions of the rs353292 between cases and controls.

These findings indicate that the rs4705342 in the flanking region of miR-143/145 may be a protective factor against the occurrence of PTC. Further study is therefore required to investigate the correlation between the genotype and V-raf murine sarcoma viral oncogene homolog B1 V600E, rat sarcoma viral oncogene homolog mutations, rearranged in transformation/PTC1 and rearranged in transformation/PTC3.

**Abbreviations:** CIs = confidence intervals, ORs = Odds ratios, PTC = papillary thyroid carcinoma, SNPs = single nucleotide polymorphisms.

**Keywords:** miR-143, miR-145, papillary thyroid carcinoma, polymorphism

## 1. Introduction

Thyroid cancer, the most common endocrine carcinoma, is responsible for 567,233 new cases and 41,071 deaths worldwide

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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in 2018.<sup>[1]</sup> Papillary thyroid carcinoma (PTC), accounting for more than 80% of thyroid cancer, has been sharply increasing in recent years.<sup>[2,3]</sup> Although the etiology of PTC is not well clarified, some risk factors have been demonstrated to contribute to the tumorigenesis of PTC, such as ionizing radiation,<sup>[4]</sup> obesity,<sup>[5,6]</sup> smoking,<sup>[6]</sup> hormonal exposures<sup>[7]</sup> as well as environmental pollutants.<sup>[8]</sup> However, not all individuals develop PTC even though exposure to the same risk factors, indicating other factors, such as genetic variants may be involved in the pathogenesis of PTC. In our previous study, we found that rs153109 AG and AG/GG genotypes in interleukin-27, rs10877887 in the promoter of let-7, miR-34b/c rs4938723 and TP-53 Arg72Pro were associated with the susceptibility of PTC.<sup>[9–11]</sup>

MicroRNAs, a series of endogenous non-coding RNAs, can regulate gene expression at the posttranscriptional level.<sup>[12]</sup> It is evident that MicroRNAs have been implicated in various cellular processes, such as cell proliferation, apoptosis, migration, invasion and epithelial to mesenchymal transition by binding to their targeting genes or being used as sponges competing long noncoding RNAs.<sup>[13–20]</sup> Previously, miR-143 and miR-145 were down-regulated in PTC tissues and over-expression of miR-145 can inhibit cell growth by targeting dual specificity phosphatase 61,<sup>[21]</sup> suggesting that miR-143 and miR-145 may be crucial modulators in the progression of PTC.

Recently, 2 single nucleotide polymorphisms (SNPs) in the flanking region of miR-143/145 (ie, rs4705342 and rs353292) was reported to be functional, with the rs4705342 C allele carriers exhibiting higher luciferase activity by increasing the

extent of binding of NF- $\kappa$ B<sup>[22]</sup> and the rs353292 T allele carriers exhibiting a significantly lower transcriptional activity and miR-143 expression.<sup>[23]</sup> The rs4705342 C allele was associated with an increased risk of prostate cancer<sup>[24]</sup> and the rs353292 T allele was associated with an increased risk of colorectal cancer.<sup>[23]</sup> No report to date has been performed to evaluate the association between the rs4705342 and rs353292 and PTC risk. We carried out a case-control study to investigate whether the 2 polymorphisms affected the susceptibility to PTC in a Chinese population.

## 2. Materials and methods

### 2.1. Study population

The study design was described in our previous work.<sup>[10]</sup> Briefly, a total of 316 PTC patients and 347 controls were selected for genotyping from West China Hospital of Sichuan University between January 2010 and October 2014. PTC diagnosis was confirmed by pathological result of an ultrasonography-guided fine needle aspiration biopsy or resected specimens. We excluded those patients combined with other cancer types. The demographic characteristics and clinical information were obtained from medical records, such as age, gender, tumor node metastasis status, and multiplicity of tumor. During the same period, 347 healthy subjects visiting the hospital for physical examination were selected as controls. We excluded those controls with thyroid disease or a personal or family history of cancer. All cases and controls were unrelated ethnic Han Chinese, living in Southwest China. The study was approved by the ethics committee of the West China Hospital of Sichuan University, and written informed consent was obtained from all participants enrolled in this study.

### 2.2. DNA isolation and genotyping

Genomic DNA was isolated from ethylene diamine tetraacetic acid-anticoagulated peripheral blood using an extraction kit (Bioteke, Beijing, China). The rs4705342 and rs353292 were genotyped by using the TaqMan allelic discrimination, as described previously.<sup>[23,25,26]</sup> Details of quality control for genotyping have been described previously,<sup>[26]</sup> including performing double-blindness procedure, negative control and DNA sequencing to assure the genotyping reliability.

### 2.3. Statistical analysis

All statistical analyses were performed using SPSS software version 19.0 (SPSS, Chicago, IL). Departure of the frequencies of rs4705342 and rs353292 under Hardy-Weinberg equilibrium was assessed using  $\chi^2$  test. The association between the 2 SNPs and PTC risk was evaluated using unconditional logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated after adjustment based on age and gender. *P* values were corrected to be .017 (.05/3) in multiple comparisons.

## 3. Results

### 3.1. Population characteristics

The baseline characteristics of PTC cases and controls are presented in Table 1. Mean age of the cases were  $43.8 \pm 13.3$  years and male gender was 21.8%. Mean age of the controls were  $44.0 \pm 8.7$  years and male gender was 24.2%. There was no

**Table 1**

**Demographics of study population.**

	Controls (n = 347)	PTC (n = 316)	<i>P</i> value
Age (yr, mean $\pm$ SD)	44.0 $\pm$ 8.7	43.8 $\pm$ 13.3	.84
Gender			
Male	84 (24.2)	69 (21.8)	.47
Female	263 (75.8)	247 (78.2)	
T classification			
Tx		20 (6.3)	
T1 and T2		89 (28.2)	
T3 and T4		207 (65.5)	
N classification			
N0		118 (37.3)	
N1a and N1b		198 (62.7)	
M classification			
Mx		2 (0.6)	
M0		312 (98.7)	
M1		2 (0.6)	
Multiplicity of tumor			
Yes		87 (27.5)	
No		229 (72.5)	

PTC=papillary thyroid carcinoma, SD=standard deviation.

significant difference in age and gender distribution between the 2 groups.

### 3.2. Genotypic distributions of the rs4705342 and rs353292 in the flanking region of miR-143/145 in the cases and controls

The genotypic frequencies of the rs4705342 and rs353292 among cases and controls did not differ from the values expected by the Hardy-Weinberg model. For the rs4705342, a reduced risk of PTC was observed in heterozygous comparison, dominant genetic model and allele comparison (CC vs TT: adjusted OR = 0.37, 95% CI = 0.19–0.74, *P* = .003; CT/CC vs TT: adjusted OR = 0.64, 95% CI = 0.47–0.87, *P* = .005; C vs T: adjusted OR = 0.66, 95% CI = 0.52–0.85, *P* = .001, respectively). No significant difference was found in the genotypic distributions of the rs353292 between cases and controls (Table 2). No significant association was also found in stratified analyses of the rs4705342 and rs353292 with clinical features of PTC (Tables 3 and 4).

## 4. Discussion

To the best of our knowledge, this is the first study investigating the association of the rs4705342 and rs353292 in the flanking region of miR-143/145 with the risk of PTC. We here showed a significant association between the rs4705342 the PTC risk. These findings implied that the rs4705342 in the flanking region of miR-143/145 may be a protective factor against the occurrence of PTC.

Several studies have surveyed the rs4705342 as a potential candidate SNP for prostate cancer,<sup>[24]</sup> intracranial aneurysm,<sup>[25]</sup> ischemic stroke,<sup>[26]</sup> and essential hypertension.<sup>[27]</sup> Kotarac et al<sup>[24]</sup> reported that the rs4705342 C allele was associated with an increased risk of prostate cancer in codominant and recessive model. Sima et al<sup>[25]</sup> reported that individuals carrying the rs4705342 C allele had a 0.82-fold reduced risk of intracranial aneurysm. Both the rs4705342 CC/CT genotypes and the rs4705342 C-rs4705343 T haplotype were associated with higher levels of miR-143. Fu et al<sup>[27]</sup> reported that individuals carrying the

**Table 2****Association of rs4705342 and rs353292 in The Flanking Region of miR-143/145 with PTC Risk.**

Polymorphisms	Controls n=347 (%)	PTC n=316 (%)	Adjusted OR (95% CI)*	P value
rs4705342				
TT	168 (48.4)	188 (59.5)	1.00	
CT	148 (42.7)	115 (36.4)	0.70 (0.51–0.96)	.03
CC	31 (8.9)	13 (4.1)	0.37 (0.19–0.74)	.003
CT/CC	179 (51.6)	128 (40.5)	0.64 (0.47–0.87)	.005
T allele	484 (69.7)	491 (77.7)	1.00	
C allele	210 (30.3)	141 (22.3)	0.66 (0.52–0.85)	.001
rs353292				
CC	246 (70.9)	214 (67.7)	1.00	
CT	97 (28.0)	97 (30.7)	1.15 (0.82–1.61)	.42
CT/TT	101 (29.1)	102 (32.3)	1.16 (0.83–1.61)	.38
C allele	589 (84.9)	525 (83.1)	1.00	
T allele	105 (15.1)	107 (16.9)	1.14 (0.85–1.53)	.38

CI=confidence interval, OR=odds ratio, PTC=papillary thyroid carcinoma.

\* Adjusted by age and gender.

rs4705342 CC or CT genotype had a significantly reduced risk of essential hypertension, particularly for females, nonsmokers and nondrinkers. Wei et al<sup>[26]</sup> reported that the rs4705342 TC/CC genotypes were associated with a decreased risk of ischemic stroke and lower levels of miR-145 in a southern Chinese Han population. However, Zhu et al<sup>[28]</sup> reported that the rs4705342 was not associated with the risk of ischemic stroke under genotypic and allelic comparisons in a northern Chinese Han population. Li et al<sup>[29]</sup> reported that the rs4705342 was not a risk factor for colorectal cancer. In agreement with the positive results, we found that the rs4705342 was associated with a decreased risk of PTC in heterozygous comparison, dominant genetic model and allele comparison, indicating that the rs4705342 may be used as a biomarker for the susceptibility of PTC.

Previously, another SNP rs353292 was associated with an increased risk for developing colorectal cancer in heterozygous comparison, dominant genetic model and allele comparison.<sup>[23,29]</sup> The rs353292 CT/TT carriers exhibited a lower expression of miR-143 and the rs353292 T allele displayed a significantly lower promoter activity than the rs353292 C allele.<sup>[23]</sup> Failure to replicate genetic association of the rs353292 with PTC risk in single site analysis.

It has been demonstrated that miR-143 and miR-145 are tumor suppressors in thyroid cancer and repress multiple target genes of signaling pathways involving in cell proliferation,

apoptosis, migration, invasion, and epithelial to mesenchymal transition.<sup>[13–16,30–33]</sup> Down-regulation of miR-143 and miR-145 was observed in thyroid cancer and the down-regulation was related to the aggressive behavior in cases of classic PTC.<sup>[13,21,30]</sup> Over-expression of miR-143 attenuated glycolysis in poorly differentiated thyroid cancer both in vitro and in vivo,<sup>[33]</sup> suggesting the therapeutic potentiality of miR-143 playing in thyroid cancer treatment. Based on this background, we speculated that the reason for the rs4705342 C decreasing PTC risk may be CC carriers exhibiting higher levels of miR-143, which has been reported in hepatitis B virus-positive hepatocellular carcinoma<sup>[22]</sup> and intracranial aneurysm.<sup>[25]</sup> Further studies are warranted to verify this hypothesis.

We have to admit some limitations in this study. Firstly, all subjects were ethnic Han Chinese, and thus the results in this study cannot be directly applicable to other ethnic groups. Further confirmation studies are of great importance in different ethnicities. Secondly, environmental factors were not taken into consideration during the study design. Gene-environment interaction therefore cannot be analyzed. Thirdly, the molecular mechanism of the rs4705342 influencing PTC risk was not investigated in this study. Further functional analysis is helpful for understanding how the rs4705342 affecting the tumorigenesis of PTC. Finally, we were unable to investigate the correlation between the genotype and V-raf murine sarcoma viral oncogene

**Table 3****Stratified analyses of rs4705342 in the flanking region of miR-143/145 with clinical features of PTC.**

Variants	TT (%)	CT/CC (%)	Adjusted OR (95% CI)*	P value
T classification				
T1 and T2	49 (27.5)	40 (33.9)	1.00	
T3 and T4	129 (72.5)	78 (66.1)	0.74 (0.45–1.23)	.25
N classification				
N0	73 (38.8)	45 (35.2)	1.00	
N1a and N1b	115 (61.2)	83 (64.8)	1.17 (0.72–1.91)	.52
Multiplicity of tumor				
Yes	51 (27.1)	36 (28.1)	1.00	
No	137 (72.9)	92 (71.9)	1.05 (0.63–1.74)	.85

CI=confidence interval, OR=odds ratio, PTC=papillary thyroid carcinoma.

\* Adjusted by age and gender.

**Table 4**  
**Stratified analyses of rs353292 in the flanking region of miR-143/145 with clinical features of PTC.**

Variants	CC (%)	CT/TT (%)	Adjusted OR (95% CI) <sup>a</sup>	P value
T classification				
T1 and T2	55 (27.5)	34 (35.4)	1.00	
T3 and T4	145 (72.5)	62 (64.6)	0.69 (0.41–1.16)	.16
N classification				
NO	75 (35.0)	43 (42.2)	1.00	
N1a and N1b	139 (65.0)	59 (57.8)	0.75 (0.45–1.23)	.25
Multiplicity of tumor				
Yes	63 (29.4)	24 (23.5)	1.00	
No	151 (70.6)	78 (76.5)	0.73 (0.42–1.26)	.25

CI=confidence interval, OR=odds ratio, PTC=papillary thyroid carcinoma.

<sup>a</sup> Adjusted by age and gender.

homolog B1 V600E, rat sarcoma viral oncogene homolog mutations, rearranged in transformation/PTC1 and rearranged in transformation/PTC3, due to the financial constraints. In the future, further study is therefore required to clarify this question.

In conclusion, this study showed that the rs4705342 C allele in the flanking region of miR-143/145 was a protective factor against the development of PTC in the Chinese population, suggesting that genetic basis for the crucial roles of miR-143 and miR-145 in PTC pathogenesis may partially originate from SNPs. Extension of the findings of the current study to other ethnicities will be of use after confirmation in larger cohorts of diverse populations.

### Author contributions

**Conceptualization:** Zhihui Li, Jingqiang Zhu, Tao Wei, Haolan Song.

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