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Association Between Single-Nucleotide Polymorphisms of *BRAF* and Papillary Thyroid Carcinoma in a Chinese Population

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Background: Papillary thyroid cancer (PTC) is a common malignancy that frequently harbors a high prevalence of somatic mutations in the oncogenic *BRAF* gene. As a novel prognostic molecular marker, this gene has drawn much attention in recent years for its potential utility in the risk prognosis and management of PTC. However, the contribution of the germline variants in this gene to PTC remains unclear. The study herein was aimed to investigate the potential association between the inherited *BRAF* variants and PTC based on a case–control study.

Methods: We selected four single-nucleotide polymorphisms (SNPs) and took a systematic step to interrogate whether these SNPs of *BRAF* are associated with PTC risk by genotyping these SNPs from 368 patients with PTC and 564 healthy controls.

Results: In comparison of cases and controls for the four SNPs, no differences were observed in the genotypic and allelic frequencies, nor was there evidence of an association between *BRAF* SNPs and overall risk of PTC. After stratification, however, we found a significantly increased risk of PTC attributed to the SNP variants rs17161747, rs1042179, and rs3748093 for those with a family history of cancer, for smokers, and for both those of age <45 years and nondrinkers, respectively. Further, in the PTC cases, those carrying the rs3748093 variant seemed to be less susceptible to developing lymph node metastases, but more likely to suffer from PTC at an earlier age (<45 years).

Conclusions: These preliminary results may provide evidence for the involvement of the common genetic variants scattered throughout the *BRAF* oncogene in the prediction of PTC onset and progression. In the future, enlarging the number of samples and performing functional studies in this gene may help to validate whether the association truly exists.

Introduction

Thyroid cancer represents the most common malignant tumor of the human endocrine system, and its incidence has increased significantly during the past decades in many areas of the world (1,2). Papillary thyroid cancer (PTC), accounting for 80%–85% of all the thyroid malignancies (1–3), is clinically described as a differentiated thyroid cancer with its tumor cells structurally and functionally resembling normal follicular cells. Although patients with PTC generally evolve favorably and present good responsiveness to surgery, this disease possesses a relatively indolent natural history and a high rate of locoregional recurrence and/or metastases. Currently, with the more advanced techniques, PTCs, especially microcarcinomas, are more frequently identified than before; molecular diagnostic markers thus seem more promising to the disease prevention/treatment planning.

Genetically, frequent alterations activating the MAP kinase/ERK pathway and the subsequent enhanced cell division and proliferation have been shown to play an important role in thyroid carcinogenesis (4), such as rearrangement of *RET* or *NTRK1* (5,6) and point mutations of the *RAS* and *BRAF* genes (7–9). Among these, *BRAF* acts as a potent activator of the MAP kinase/ERK pathway, having the T1799A

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(V600E) as its most common mutation. This mutation occurs in 29%–83% of PTC cases (10), and has been directly correlated with the classical PTC phenotype and worse patient outcomes. These are characterized by advanced age, tumor size, and tumor stage, as well as massive extrathyroidal extension and lymph node and distant metastases at presentation and at recurrence (11).

However, PTC is mostly sporadic, and it has been estimated that 5%–10% of all PTC are familial (12,13), making it unlikely that high-penetrance germline mutations in *BRAF* or other genes mediate susceptibility to PTC. On the contrary, variants conferring a moderate or low penetrance risk and their interaction with the environment seem to be more likely involved in disease etiology at the population level. Until now, only a few epidemiological studies have investigated what association occurs between the inherited polymorphisms of the *BRAF* gene and melanoma or ovarian cancers (14–16). Quaye and colleagues reported borderline evidence for the contribution of three tagging single-nucleotide polymorphisms (tSNPs) (rs10487888, rs1267622, and rs17695623) in the *BRAF* gene to mucinous ovarian cancer risk, and identified a haplotype exerting protective effect on ovarian cancer risk. Also, they found that another single tSNP and haplotype in the *BRAF* gene are important in the prediction of ovarian cancer survival (14). Melanoma case–control studies in Germans and Australians both suggested that the *BRAF* polymorphisms possessed an estimated attributable risk of ~1.6% to the burden of this disease, although the casual variant needs to be further determined (15,16). No studies have examined the role of the *BRAF* polymorphisms in the predisposition to PTC.

We hypothesized that inherited *BRAF* variants may predispose to the development of PTC. In this study, we set out to test this hypothesis in a population-based case–control study, selecting three intronic tSNPs and a coding SNP in the *BRAF* gene. In addition, we attempted to detect the association between the *BRAF* SNPs and PTC phenotypes.

Materials and Methods

Subjects

All participants enrolled in this study were of the Chinese Han ethnicity. The Ethics Committee of the Tianjin Medical University Cancer Hospital approved the study protocol, and we obtained written informed consent from all patients and controls to participate in this study. A total of 368 patients with PTC and 564 healthy controls were included in the analysis. Primary PTC was histopathologically diagnosed in all patients, and patients were consecutively recruited from the Department of Head and Neck Surgery between June 2007 and December 2009. Controls were randomly selected from the general population attending health screening, genetically unrelated to the patients, and frequency-matched to the patient's sex and age (±5 years). All study participants met the following inclusion criteria: aged 18–65 years, and no previous diagnosis of cancer. Each eligible subject donated 10 mL of blood that was collected into heparinized tubes and used for biomarker assays, including DNA extraction and genotyping.

All information was collected by a structured questionnaire in a face-to-face interview, including demographic data, papillary disease history, family history of cancer, clinical data, and laboratory data. Those with a history of smoking at least one cigarette per day for more than three months, and those with history of drinking alcohol >50 mL (ardent spirits) at least once a week, were defined as smokers and drinkers, respectively. Further, we divided smoking and drinking status into the two categories of "Yes" (current smokers/drinkers) and "No" (past smokers/drinkers).

For the cases, we also collected information about tumor features and disease severity, including morphology, tumor size, lymph node metastases, distant metastases, and tumor stage. B-type ultrasonography was performed on all the patients before surgery. In accordance with the National Comprehensive Cancer Network (NCCN) guidelines for treatment of cancer by site of papillary thyroid carcinoma, 339 patients received additional neck dissection, while 29 patients only had a thyroid lobectomy. These 29 patients, with tumor sizes of <1 cm, did not have cervical lymph node metastases by B-type ultrasonography. Therefore, we classified them in the lymph node–negative group.

SNP selection and genotyping

Based on the resequencing data of Chinese Han individuals in the International HapMap Project SNP database, the tSNPs were selected using HapMap Genome Browser (Phase 1 & 2—full dataset) and Haploview version 4.2, and we selected three intronic tSNPs (rs11762469, rs17161747, and rs3748093) with an R^2 threshold of 0.80 across the whole genomic region. Additionally, from the National Center for Biotechnology Information dbSNP database, we identified another synonymous coding SNP: rs1042179, located in exon-16 near the hot spot T1799A *BRAF* mutation in exon-15. All four SNPs were identified based on the following criteria: a minor allele frequency ≥5% across the whole genomic region, and supported by literature.

Genomic DNA was extracted using the TKM method, and the amount and purity of the DNA were quantified for each sample by spectrophotometry. The four SNPs (rs11762469, rs17161747, rs3748093, and rs1042179) were genotyped using the TaqMan-MGB probe assay from Applied Biosystems, Inc. (ABI; Foster City, CA). The polymerase chain reactions (PCR) were performed in a 96-well plate with each well containing 10 ng genomic DNA, 2.5 μL TaqMan Genotyping Master Mix (ABI), and 0.125 μL 40× primer/probe Assay Mix, according to the manufacturer's manual. TaqMan assay plates were directly placed in an ABI Prism 7500HT instrument, and heated at 95°C for 10 minutes, followed by 45 cycles of 92°C for 15 seconds and 60°C for 60 seconds. Subsequently, the fluorescence intensity in each well of the plate was read after the completion of PCR amplification and analyzed by sodium dodecyl sulfate 2.2.1 automated software (ABI). Two blank (water) controls and two duplicated samples with known genotypes in each 96-well plate were used for the assay quality control. More than 5% of the samples were randomly selected for repeated assays, and the results were concordant.

Statistical analysis

Differences in the distribution of demographic variables and known risk factors between patients with PTC and controls were tested by the Student's *t*-test for continuous variables or the χ^2 test for categorical variables, as appropriate. Two-sided χ^2 test was used to identify the Hardy–Weinberg equilibrium of each single locus in controls, and differences in

Table 1. Frequency Distributions of *BRAF* Single-Nucleotide Polymorphisms' Genotypes in Cases and Controls and the Associations with Papillary Thyroid Cancer Risk Stratified by Age

	Age <45 years			*Age ≥45 years*		
Variants	*Cases,* n *(%)*	*Controls,* n *(%)*	*OR [CI]*[a]	*Cases,* n *(%)*	*Controls,* n *(%)*	*OR [CI]*[a]
rs1042179: A>G						
AA	132 (71.4)	185 (72.5)	1.00	127 (69.8)	216 (70.4)	1.00
AG	48 (25.9)	60 (23.5)	1.24 [0.78–1.99]	50 (27.5)	86 (28.0)	0.95 [0.62–1.46]
GG	5 (2.7)	10 (3.9)	0.65 [0.21–2.05]	5 (2.7)	5 (1.6)	1.90 [0.51–7.13]
GG+AG	53 (28.6)	70 (27.5)	1.15 [0.74–1.80]	55 (30.2)	91 (29.6)	1.00 [0.66–1.51]
rs11762469: A>T						
AA	121 (65.1)	173 (67.8)	1.00	127 (69.8)	218 (70.8)	1.00
AT	61 (32.8)	77 (30.2)	1.09 [0.71–1.68]	50 (27.5)	81 (26.3)	1.04 [0.68–1.60]
TT	4 (2.2)	5 (2.0)	1.08 [0.28–4.23]	5 (2.7)	9 (2.9)	1.13 [0.35–3.68]
AT+TT	65 (34.9)	82 (32.2)	1.09 [0.72–1.66]	55 (30.2)	90 (29.2)	1.05 [0.69–1.59]
rs17161747: G>C						
GG	128 (68.8)	168 (66.1)	1.00	116 (63.7)	205 (66.8)	1.00
GC	50 (26.9)	77 (30.3)	0.80 [0.51–1.25]	61 (33.5)	88 (28.7)	1.17 [0.77–1.77]
CC	8 (4.3)	9 (3.5)	1.06 [0.38–2.95]	5 (2.7)	14 (4.6)	0.61 [0.21–1.78]
GC+CC	58 (31.2)	86 (33.9)	0.83 [0.54–1.26]	66 (36.3)	102 (32.2)	1.09 [0.73–1.63]
rs3748093: T>A						
TT	104 (55.9)	167 (66.8)	1.00	115 (63.5)	175 (58.3)	1.00
AT	76 (40.9)	70 (28.0)	**1.75 [1.14–2.69]**[b]	52 (28.7)	113 (37.7)	0.76 [0.50–1.15]
AA	6 (3.2)	13 (5.2)	1.11 [0.37–3.33]	14 (7.7)	12 (4.0)	2.33 [0.97–5.56]
AT+AA	82 (44.1)	83 (33.2)	**1.68 [1.11–2.54]**[b]	66 (36.5)	125 (41.7)	0.88 [0.60–1.31]

[a]ORs are adjusted for sex, cancer familial history, alcohol drinking, and smoking.
[b]Boldface indicates statistical significance ($p<0.05$).
CI, 95% confidence interval; OR, odds ratio.

the allelic and genotypic frequencies between patient and control odds ratios (ORs) and 95% confidence intervals (CIs) for PTC risk conferred by the *BRAF* polymorphisms were derived using multivariate unconditional logistic regression analyses with adjustment for age, sex, drinking alcohol, smoking, and family history of cancer. Also, potential gene–environment interaction at a multiplicative scale was assessed in the logistic regression analysis by comparing the changes in deviance between the models for main effects with or without the interaction terms. Only for patients with PTC, we also

Table 2. Frequency Distributions of *BRAF* Single-Nucleotide Polymorphisms' Genotypes in Cases and Controls and the Associations with Papillary Thyroid Cancer Risk Stratified by Alcohol Drinking Status

	No			*Yes*		
Variants	*Cases,* n *(%)*	*Controls,* n *(%)*	*OR [CI]*[a]	*Cases,* n *(%)*	*Controls,* n *(%)*	*OR [CI]*[a]
rs1042179: A>G						
AA	248 (70.7)	314 (71.9)	1.00	11 (68.8)	87 (69.6)	1.00
AG	94 (26.8)	110 (25.2)	1.09 [0.78–1.51]	4 (25.0)	36 (28.8)	0.91 [0.26–3.12]
GG	9 (2.6)	13 (3.0)	0.89 [0.37–2.16]	1 (6.3)	2 (1.6)	3.09 [0.24–39.16]
GG+AG	103 (29.3)	123 (28.1)	1.07 [0.78–1.47]	5 (31.3)	38 (30.4)	1.06 [0.34–3.34]
rs11762469: A>T						
AA	235 (66.8)	303 (69.3)	1.00	13 (81.3)	88 (69.8)	1.00
AT	108 (30.7)	124 (28.4)	1.12 [0.81–1.53]	3 (18.8)	34 (27.0)	0.62 [0.16–2.38]
TT	9 (2.6)	10 (2.3)	1.25 [0.49–3.16]	0 (0.0)	4 (3.2)	—
AT+TT	117 (33.2)	134 (30.7)	1.13 [0.83–1.53]	3 (18.8)	38 (30.2)	0.53 [0.14–2.01]
rs17161747: G>C						
GG	236 (67.0)	281 (64.6)	1.00	8 (50.0)	92 (73.0)	1.00
GC	103 (29.3)	134 (30.8)	0.88 [0.64–1.21]	8 (50.0)	31 (24.6)	2.77 [0.94.8.15]
CC	13 (3.7)	20 (4.6)	0.83 [0.40–1.72]	0 (0.0)	3 (2.4)	—
GC+CC	116 (33.0)	154 (35.4)	0.88 [0.65–1.19]	8 (50.0)	34 (27.0)	2.50 [0.85–7.31]
rs3748093: T>A						
TT	210 (59.8)	274 (64.6)	1.00	9 (56.3)	68 (54.0)	1.00
AT	122 (34.8)	137 (32.3)	1.16 [0.85–1.58]	6 (37.5)	46 (36.5)	0.97 [0.32–2.98]
AA	19 (5.4)	13 (3.1)	**2.12 [1.00–4.47]**[b]	1 (6.3)	12 (9.5)	0.67 [0.08–5.99]
AT+AA	141 (40.2)	150 (35.4)	1.24 [0.92–1.67]	7 (43.8)	58 (46.0)	0.91 [0.31–2.67]

[a]ORs are adjusted for age, sex, cancer familial history, and smoking.
[b]Boldface indicates statistical significance ($p<0.05$).

Table 3. Frequency Distributions of *BRAF* Single-Nucleotide Polymorphisms' Genotypes in Cases and Controls and the Associations with Papillary Thyroid Cancer Risk Stratified by Cancer Familial History

	No			*Yes*		
Variants	*Cases*, n *(%)*	*Controls*, n *(%)*	*OR [CI]*[a]	*Cases*, n *(%)*	*Controls*, n *(%)*	*OR [CI]*[a]
rs1042179: A > G						
AA	231 (69.6)	358 (71.5)	1.00	28 (80.0)	43 (70.5)	1.00
AG	91 (27.4)	130 (25.9)	1.11 [0.80–1.55]	7 (20.0)	16 (26.2)	0.72 [0.25–2.08]
GG	10 (2.8)	13 (2.6)	1.19 [0.49–2.90]	0 (0)	2 (3.3)	—
GG + AG	101 (30.4)	143 (28.5)	1.12 [0.81–1.54]	7 (20.0)	18 (29.5)	0.59 [0.21–1.67]
rs11762469: A > T						
AA	226 (67.9)	351 (69.9)	1.00	22 (62.9)	40 (65.6)	1.00
AT	100 (30.0)	139 (27.7)	1.10 [0.80–1.52]	11 (31.4)	19 (31.3)	0.80 [0.31–2.07]
TT	7 (2.1)	12 (2.4)	0.97 [0.36–2.62]	2 (5.7)	2 (3.3)	2.41 [0.26–22.60]
AT + TT	107 (32.2)	151 (30.1)	1.09 [0.80–1.50]	13 (37.1)	21 (34.4)	0.91 [0.37–2.25]
rs17161747: G > C						
GG	229 (68.8)	332 (66.3)	1.00	15 (42.9)	41 (68.3)	1.00
GC	93 (27.9)	152 (30.3)	0.83 [0.60–1.14]	18 (51.4)	13 (21.7)	**4.74 [1.71–13.18]**[b]
CC	11 (3.3)	17 (3.4)	0.87 [0.39–1.94]	2 (5.7)	6 (10.0)	0.84 [0.15–4.84]
GC + CC	104 (3.2)	169 (33.7)	0.83 [0.61–1.14]	20 (57.1)	19 (31.7)	**3.24 [1.29–8.12]**[b]
rs3748093: T > A						
TT	196 (59.0)	303 (61.6)	1.00	23 (65.7)	39 (67.2)	1.00
AT	116 (34.9)	166 (33.7)	1.13 [0.83–1.55]	12 (34.3)	17 (29.3)	1.32 [0.51–3.40]
AA	20 (6.0)	23 (4.7)	1.98 [0.99–3.99]	0 (0.0)	2 (3.4)	—
AT + AA	136 (41.0)	189 (38.4)	1.21 [0.90–1.64]	12 (34.3)	19 (32.8)	1.19 [0.47–3.04]

[a]ORs are adjusted for age, sex, drinking alcohol, and smoking.
[b]Boldface indicates statistical significance ($p < 0.05$).

performed stratified case–series analysis of the genotype data by clinical phenotypes.

All statistical analyses were conducted by SPSS software version 16.0 (SPSS Ltd., Surrey, United Kingdom), and a two-sided test with $p < 0.05$ was considered statistically significant.

Results

Characteristics of the study population

Detailed information regarding selected baseline characteristics of the cases and controls is presented in Supplementary Table S1 (Supplementary Data are available online at

Table 4. Frequency Distributions of *BRAF* Single-Nucleotide Polymorphisms' Genotypes in Cases and Controls and the Associations with Papillary Thyroid Cancer Risk Stratified by Smoking Status

	No			*Yes*		
Variants	*Cases*, n *(%)*	*Controls*, n *(%)*	*OR [CI]*[a]	*Cases*, n *(%)*	*Controls*, n *(%)*	*OR [CI]*[a]
rs1042179:A > G						
AA	243 (72.3)	302 (70.7)	1.00	16 (51.6)	99 (73.3)	1.00
AG	86 (25.6)	114 (26.7)	0.97 [0.69–1.37]	12 (38.7)	32 (23.7)	2.26 [0.94–5.42]
GG	7 (2.1)	11 (2.6)	0.69 [0.26–1.83]	3 (9.7)	4 (3.0)	4.55 [0.88–23.67]
GG + AG	93 (27.7)	125 (29.3)	0.95 [0.68–1.31]	15 (48.4)	36 (26.7)	**2.51 [1.10–5.74]**[b]
rs11762469:A > T						
AA	223 (66.2)	296 (69.2)	1.00	25 (80.6)	95 (70.4)	1.00
AT	105 (31.2)	122 (28.5)	1.15 [0.83–1.59]	6 (19.4)	36 (26.7)	0.58 [0.21–1.59]
TT	9 (2.7)	10 (2.3)	1.31 [0.51–3.36]	0 (0)	4 (3.0)	—
AT + TT	114 (33.8)	132 (30.8)	1.16 [0.85–1.59]	6 (19.4)	40 (29.6)	0.54 [0.20–1.45]
rs17161747:G > C						
GG	224 (66.5)	280 (65.7)	1.00	20 (64.5)	93 (68.9)	1.00
GC	103 (30.6)	128 (30.0)	0.97 [0.70–1.34]	8 (25.8)	37 (27.4)	0.97 [0.38–2.46]
CC	10 (3.0)	18 (4.2)	0.68 [0.30–1.53]	3 (9.7)	5 (3.7)	2.22 [0.47–10.61]
GC + CC	113 (33.5)	146 (34.3)	0.93 [0.68–1.27]	11 (35.5)	42 (31.1)	1.14 [0.49–2.66]
rs3748093: T > A						
TT	199 (59.2)	270 (65.1)	1.00	20 (64.5)	72 (53.3)	1.00
AT	120 (35.7)	132 (31.8)	1.23 [0.90–1.69]	8 (25.8)	51 (37.8)	0.60 [0.24–1.51]
AA	17 (5.1)	13 (3.1)	1.90 [0.88–4.10]	3 (9.7)	12 (8.9)	1.20 [0.29–4.90]
AT + AA	137 (40.8)	145 (34.9)	1.29 [0.95–1.75]	11 (35.5)	63 (46.7)	0.70 [0.30–1.61]

[a]ORs are adjusted for age, sex, cancer familial history, and drinking alcohol.
[b]Boldface indicates statistical significance ($p < 0.05$).

Table 5. Frequency Distributions of *BRAF* Genotypes by Lymph Node Metastases in Patients with Papillary Thyroid Cancer

Variants	*No,* n *(%)*	*Yes,* n *(%)*	*OR [CI]*[a]
rs1042179: A > G			
AA	115 (74.2)	144 (67.9)	1.00
AG	36 (23.2)	62 (29.2)	1.32 [0.81–2.17]
GG	4 (2.6)	6 (2.8)	1.21 [0.31–4.64]
GG + AG	40 (25.8)	68 (32.1)	1.31 [0.81–2.12]
rs11762469: A > T			
AA	109 (70.3)	139 (65.3)	1.00
AT	44 (28.4)	67 (31.5)	1.21 [0.75–1.93]
TT	2 (1.3)	7 (3.3)	3.24 [0.65–16.24]
AT + TT	46 (29.7)	74 (34.7)	1.29 [0.81–2.04]
rs17161747: G > C			
GG	104 (67.1)	140 (65.7)	1.00
GC	47 (30.3)	64 (30.0)	1.02 [0.63–1.64]
CC	4 (2.6)	9 (4.2)	1.45 [0.42–5.08]
GC + CC	51 (32.9)	73 (34.4)	1.05 [0.67–1.67]
rs3748093: T > A			
TT	77 (50.0)	142 (66.7)	1.00
AT	68 (44.2)	60 (28.2)	**0.40 [0.25–0.64]**[b]
AA	9 (5.8)	11 (5.2)	0.77 [0.29–2.01]
AT + AA	77 (50)	71 (33.3)	**0.44 [0.28–0.69]**[b]

[a]ORs are adjusted for age, sex, cancer familial history, drinking alcohol, and smoking.
[b]Boldface indicates statistical significance ($p < 0.05$).

Table 6. Frequency Distributions of *BRAF* Genotypes by Age in Patients with Papillary Thyroid Cancer

Variants	*< 45 years,* n *(%)*	*≥ 45 years,* n *(%)*	*OR [CI]*[a]
rs1042179: A > G			
AA	132 (71.4)	127 (69.8)	1.00
AG	48 (25.9)	50 (27.5)	1.49 [0.68–1.75]
GG	5 (2.7)	5 (2.7)	0.87 [0.24–3.20]
rs11762469: A > T			
AA	121 (65.1)	127 (69.8)	1.00
AT	61 (32.8)	50 (27.5)	0.84 [0.51–1.27]
TT	4 (2.2)	5 (3.6)	1.21 [0.31–4.64]
rs17161747: G > C			
GG	128 (68.8)	116 (63.7)	1.00
GC	50 (26.9)	61 (33.5)	1.34 [0.84–2.12]
CC	8 (4.3)	5 (2.7)	0.65 [0.20–2.10]
rs3748093: T > A			
TT	104 (55.9)	115 (63.5)	1.00
AT	76 (40.9)	52 (28.7)	**0.64 [0.41–0.99]**[b]
AA	6 (3.2)	14 (7.7)	2.05 [0.75–5.59]

[a]ORs are adjusted for sex, cancer familial history, drinking alcohol, and smoking.
[b]Boldface indicates statistical significance ($p < 0.05$).

www.liebertpub.com/thy). We observed that significantly smaller proportions of patients than control subjects had a history of tobacco and alcohol use (both $p < 0.01$). No differences were found in the distribution of age, sex, family history of cancer, and body mass index.

Association of the selected BRAF SNPs with PTC risk

No significant deviations from the Hardy–Weinberg equilibrium existed for genotypic frequencies of all these polymorphisms in the controls ($p = 0.970$ for rs1042179; $p = 0.975$ for rs11762469; $p = 0.840$ for rs17161747; $p = 0.996$ for rs3748093). Analysis of the four *BRAF* SNPs did not yield any significant differences, either for genotypic frequencies between patients with PTC and controls or their associations with PTC risk (data not shown).

When the results were further stratified by the traditional risk factors for PTC, we found that the rs3748093 AT genotype and the combined AT + AA genotypes were associated with an increased risk of PTC in those of age < 45 years (OR 1.75 [CI 1.14–2.69], $p = 0.011$ for AT genotype; OR 1.68 [CI 1.11–2.54], $p = 0.015$ for AT + AA genotype; see Table 1). Furthermore, the AA genotype was an independent risk factor of PTC for nondrinkers (OR 2.12 [CI 1.00–4.47], $p = 0.049$; see Table 2).

For those with a family history of cancer, genotypes carrying the rs17161747 variant (GC and GC + CC) conferred a higher risk of PTC (GC genotype: OR 4.74 [CI 1.73–13.18], $p = 0.003$; GC + CC genotypes: OR 3.24 [CI 1.29–8.12], $p = 0.012$; see Table 3). Meanwhile, for another *BRAF* gene SNP, rs1042179, the combined genotype GG + AG indicated an increased risk of PTC among the smokers (OR 2.51 [CI 1.10–5.74], $p = 0.029$; see Table 4), as compared with the AA genotype.

Distribution of BRAF genotypes by clinical phenotypes in the patient-only analysis

Having demonstrated that the aforementioned *BRAF* SNPs were associated with PTC risk, we next sought to determine whether these SNPs also affected PTC prognosis in the patient-only analysis. We compared differences in genotypic distribution of *BRAF* SNPs stratified by each individual clinical phenotype and calculated ORs by modeling the probability of the worse prognostic phenotype for breast cancer. Specifically, we divided each phenotype into two groups, one with good prognosis and the other with poor prognosis. We observed that when compared with the SNP rs3748093 TT genotype carriers, patients carrying the AT and combined AT + AA genotypes were less susceptible to developing lymph node metastases (AT genotype: OR 0.40 [CI 0.25–0.64], $p < 0.001$; AT + AA genotypes: OR 0.44 [CI 0.28–0.69], $p < 0.001$; see Table 5), and the AT genotype carriers were more likely to have PTC at an earlier age (< 45 years; OR 0.64 [CI 0.41–0.99], $p = 0.047$; see Table 6). Also, patients with the rs17161747 CC genotype demonstrated an extraordinarily increased risk of extrathyroidal extension than those with the GG genotype (OR 5.72 [CI 1.22–26.77], $p = 0.027$; see Supplementary Table S2), a finding by chance possibly due to the few number of patients with the CC genotype.

Discussion

Somatic alterations that activate oncogenes and drive cells to an unregulated proliferation are a well-defined feature for most cancers. The RAS/RAF/MAPK signal transduction pathway is involved in the regulation of cell proliferation or differentiation. *BRAF*, encoding a serine–threonine kinase, is a downstream effector of RAS and critical for the cellular response to growth signals. A somatic mis-sense mutation (V600E, formerly termed V599E) in the kinase domain of BRAF has been reported to be present in 29%–83% of PTCs (17), benign melanocytic nevi,

and cutaneous melanomas, as well as in other types of cancers, including ovarian cancer and colon cancer (14,18). Germline mutations of *BRAF* have not yet been detected in sporadic or familial PTC, but several studies have found that *BRAF* may be indeed a low-risk susceptibility gene for melanoma and ovarian cancer.

Here, we genotyped 368 classical PTC cancers and 564 healthy controls for the presence of four SNPs in the *BRAF* gene, these being representative of the majority of variants, including a previously reported coding variant. To our knowledge, this is the first report concerning the risk of PTC and common tSNPs of *BRAF* in a Chinese population. We found no evidence of an association between the risk of PTC and polymorphisms of the *BRAF* gene, after calculating the ORs with adjustment for the known risk factors of PTC. There may be several reasons for this. One is that our sample size may have been too small to detect a modest change in the risk of PTC that might actually be associated with rare *BRAF* alleles. We also cannot exclude the possibility that there is an association between PTC and other *BRAF* variants that are less common or even as common as the variants we tested for.

Carcinogenesis of the thyroid and other organs is a multifactorial process, usually involving an interaction between multiple genetic and environmental events. Thereafter, in the present study, it is not surprising to find minor effects of polymorphisms on the prevalence of different behaviors of PTC. Similar to previous studies (19), we also found that smoking and drinking alcohol were significantly and independently associated with a reduced risk for developing PTC (data not shown). Among smokers, the *BRAF* gene SNP rs1042179 with the combined genotype GG + AG conferred an increased risk for PTC. Meanwhile, another variant SNP, the rs3748093 AA genotype, was positively correlated with the risk of PTC in nondrinkers.

A major hereditary component is apparent for the predisposition to PTC (12,13,20), as individuals with a family history of cancer are at a higher risk of developing thyroid cancer (19). In the present study, most of the patients were sporadic cases. We found the *BRAF* SNP rs17161747 carrying the C variant (GC and GC + CC genotypes) increased the risk of PTC in those with a family history of tumors.

Age is an important factor as far as the prognosis of PTC is concerned, especially with age 45 years as the cut point. There is an excellent overall survival rate of >90% in patients <45 years old (21). In the present study, there was a moderate impact of *BRAF* variants on the risk of PTC among individuals under 45 years, with a positive correlation between the SNP rs3748093, an allele genotype (genotype AT and AT + AA), and the risk of PTC. In addition, carriers with the AT genotype of the *BRAF* gene SNP rs3748093 also tended to be younger (<45 years) at their onset of PTC. It is interesting, but unclear, why *BRAF* SNP rs3748093 with the AT genotype was associated with less lymph node metastases in patients with PTC.

In conclusion, in this report, we describe an association between three tSNPs and one coding SNP in *BRAF* and the risk of PTC in a Chinese population. Although we did not observe a linear correlation, there appears to be an interaction between the presence of *BRAF* variants and environmental factors with predisposition and behavior of PTC. Further studies of the *BRAF* polymorphisms and PTC, with greatly increased power, should be conducted in Chinese and other ethnic populations.

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Disclosure Statement

The authors declare that no competing financial interests exist.

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