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Incidence of *BRAF* V600E mutation in patients with papillary thyroid carcinoma: a single-institution experience

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Abstract

Objective: Papillary thyroid carcinoma (PTC) accounts for 95% of all thyroid carcinomas. PTC is an epithelial tumor characterized by the proliferation of follicular cells with distinctive nuclear features, and is heterogeneous in terms of its carcinogenesis and behavior. PTC has been associated with several genetic abnormalities, of which the *BRAF* V600E mutation is the most common. However, reported incidences of this mutation have varied depending on the patient background, population size, or methods. In this study, we investigated the incidence of *BRAF* V600E mutation and its relationships with clinicopathological characteristics in patients with PTC.

Methods: Surgical specimens were obtained from 40 patients with PTC who underwent surgery at Nippon Medical School Hospital between 2009 and 2017. DNA from exon 15 of the *BRAF* gene was extracted and amplified by polymerase chain reaction, followed by direct sequencing.

Results: The frequency of *BRAF* V600E mutation increased with age. However, there were no correlations between *BRAF* V600E mutation and other clinicopathological features including sex, Hashimoto disease, family history of thyroid disease, tumor size, pathological T stage, pathological N stage, lymphovascular invasion, extrathyroidal extension, and metastasis.

Conclusions: This study demonstrated that PTCs harboring the *BRAF* V600E mutation increased in an age-dependent manner.

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Introduction

Papillary thyroid carcinoma (PTC) is a major endocrine malignancy, with an increasing incidence in all age groups worldwide.[1] Knowledge of the genetic alterations in PTC has gradually improved in recent years, and the *BRAF* V600E mutation has been identified as the most common mutation.[2,3] *BRAF* mutation results in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway, which in turn promotes cell growth, proliferation, and differentiation.

BRAF mutations were observed in 36% to 83% of cases of PTC in all age groups,[4] and in 0% to 63% of pediatric cases.[1,5–11] However, the frequency varies and studies in pediatric patients are limited. *BRAF* mutation was previously reported to be associated with older male patients.[12–15] Many studies have suggested that *BRAF* mutation is related to aggressive features of PTCs, including large tumor size,[13,16–18] extrathyroidal extension,[15,18,19] vascular invasion,[12,20] lymph node metastasis,[5,12,20] advanced stage,[18,19,21] recurrence,[17] and higher disease-specific mortality.[5,22] However, other studies have demonstrated different results,[23,24] indicating that these clinicopathological features can vary depending on the patient background and research methodology.[22,25] Given that *BRAF* is a potential molecular target in PTC, it is important to clarify the frequency and clinical significance of *BRAF* mutation in these patients.

The aim of this study was to investigate the frequency of *BRAF* mutation in adult and pediatric patients with PTC, and to examine the relationships between the mutation rate and clinicopathological features of PTCs in our institution.

Methods

Patients

Eligible patients were pathologically diagnosed with PTC following thyroid resection at the Department of Endocrine Surgery, Nippon Medical School Hospital, Tokyo, Japan, from 2009 to 2017. Because of the small amount of tumor tissue, tumors with extensive fibrosis, calcification, hematoma, or cystic lesions were excluded from the study, and 50 cases were finally selected for analysis. Clinical and pathological data were retrieved from the patients' medical records. The tumors were staged according to the Union for International Cancer Control classification. This study was conducted according to the STROBE statement and written consent was obtained from all the patients for the use of the clinical samples for research purposes. This study was approved by the Ethics Committee of Nippon Medical School Hospital (No. 29-12-867, December 2017), and registered at the UMIN Clinical Trial Registry as UMIN00037461.

Genetic analysis

Tissues were obtained from the patients, fixed in formalin, and paraffin embedded (FFPE). Tumor tissue was then dissected

manually from the representative tissue sections and total DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. *BRAF* exon 15 was amplified by polymerase chain reaction (PCR) using the following primers: forward, 5'-CTTCATGAAGACCTCACAGT A-3'; reverse, 5'- TAGTTGAGACCTTCA ATGACTT-3'. PCR was carried out using standard buffer conditions, 100 ng DNA, and AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) with 40 cycles of denaturing at 96°C for 60 seconds, annealing at 55°C for 60 seconds, and extension at 77°C for 60 seconds in a total volume of 50 μL. Two microliters of the purified PCR product was sequenced using a BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA) for 25 cycles using 1.0 pmol primers, with denaturing at 96°C for 10 seconds, annealing at 55°C for 5 seconds, and extension at 60°C for 1 minute. Sequences were determined using a semi-automated sequencer (ABI 3130 Genetic Analyzer, Applied Biosystems) and a sequence analyzer (Data Collection, version 3.0).

Statistical analysis

The relationship between the presence of *BRAF* V600E mutation and age was analyzed by χ^2 test and the relationship between mutation and tumor size by Mann–Whitney U test. The relationships between *BRAF* V600E mutation and all other clinicopathological features were analyzed by two-tailed Fisher's exact tests. Statistical analysis was performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). A value of $P < 0.05$ was considered significant.

Results

Clinical characteristics

DNA amplification was successful in 40 of the 50 cases, and these were therefore used for further analysis. The clinical characteristics of the 40 cases are summarized in Table 1. All patients were Asian and aged 13 to 79 years (average 31.8, median 24 years).

Disease characteristics

The disease characteristics of the 40 analyzed patients are shown in Table 2.

Table 1. Clinical characteristics in all patients with papillary thyroid carcinoma.

	Age group (years)				
	Total n = 40	13–19 n = 7	20–39 n = 24	40–59 n = 4	60–79 n = 5
Mean age [range], years	31 [13–79]	17 [13–19]	24 [23–34]	51 [43–54]	69 [61–79]
Sex (female/male)	37/3	7/0	23/1	2/2	5/0
Hashimoto disease, n (%)	12 (30)	2 (29)	7 (29)	1 (25)	2 (40)
Non-thyroid malignancy, n (%)	5 (12)	0	0	3 (75)	2 (40)
Family history of non-thyroid malignancy, n (%)	2 (5)	0	1 (4)	0	1 (20)
Family history of thyroid carcinoma, n (%)	3 (7.5)	1 (14)	1 (4)	1 (25)	0
Family history of benign thyroid disease, n (%)	4 (10)	2 (29)	1 (4)	0	1 (20)

Table 2. Disease characteristics in all patients with papillary thyroid carcinoma.

	Age group (years)				
	Total n = 40	13–19 n = 7	20–39 n = 24	40–59 n = 4	60–79 n = 5
Mean tumor size [range], cm	1.9 [0.5–7.0]	2.4 [0.7–7.0]	1.7 [0.5–4.0]	1.5 [0.6–2.2]	2.3 [1.4–3.5]
pT stage, n (%)					
pT1a	10 (25)	1 (14)	8 (33)	1 (25)	0
pT1b	10 (25)	2 (29)	5 (21)	2 (50)	1 (20)
pT2	11 (28)	3 (43)	6 (25)	1 (25)	1 (20)
pT3a	1 (2.5)	0	1 (4)	0	0
pT3b	7 (17.5)	1 (14)	4 (17)	0	2 (40)
pT4a	1 (2.5)	0	0	0	1 (20)
pN stage, n (%)					
pN0	10 (25)	0	8 (33)	1 (25)	1 (20)
pN1a	17 (42.5)	3 (7.5)	9 (23)	3 (75)	2 (40)
pN1b	7 (18)	1 (14)	5 (21)	0	1(20)
pNX	6 (15)	3 (43)	2 (8)	0	1(20)
Extrathyroidal extension, n (%)	8 (20)	1 (14)	4 (17)	0	3(60)
Lymphovascular invasion, n (%)	17 (43)	3 (43)	12 (50)	1(25)	1 (20)

pT, pathological T stage; pN, pathological N stage; pNX, no lymph node dissection.

Tumor size did not significantly differ by age, but the minimum size was seen in patients aged 20 to 39 years and the maximum size in patients aged 13 to 19 years. There was a non-significant trend towards more advanced stage in patients aged 60 to 79 years compared with younger age groups. Thirty-four patients underwent lymph node dissection and six did not. Histologically, there were 38 cases (95%) of conventional PTC, one (2.5%) of cribriform variant (Figure 1a,b), and one (2.5%) of PTC with poorly differentiated components (Figure 1c,d). The follow-up duration was 1 to 96 months (average 26, median 21). No patients died from PTC and there was no recurrence or metastasis, except for one patient who had metastasis at their initial diagnosis.

BRAF V600E analysis

The *BRAF* V600E mutation status (Figure 2) and clinicopathological features of the included cases are listed in Table 3 and summarized in Table 4. In the pediatric cases (13–19 years), *BRAF* V600E was detected in one 17-year-old and one 18-year-old patient. The frequency of *BRAF* V600E mutation increased significantly with increasing age ($P = 0.022$).

There were no significant associations between mutational status and other clinicopathological features including sex, Hashimoto disease, family history of thyroid disease, tumor size, pathological T stage, pathological N stage, lymphovascular invasion, extrathyroidal extension, and metastasis

Discussion

The aim of this study was to investigate the frequencies of *BRAF* mutation in adult and pediatric patients with PTC, and to clarify the relationships between mutation status and clinicopathological features in our institution. *BRAF* V600E

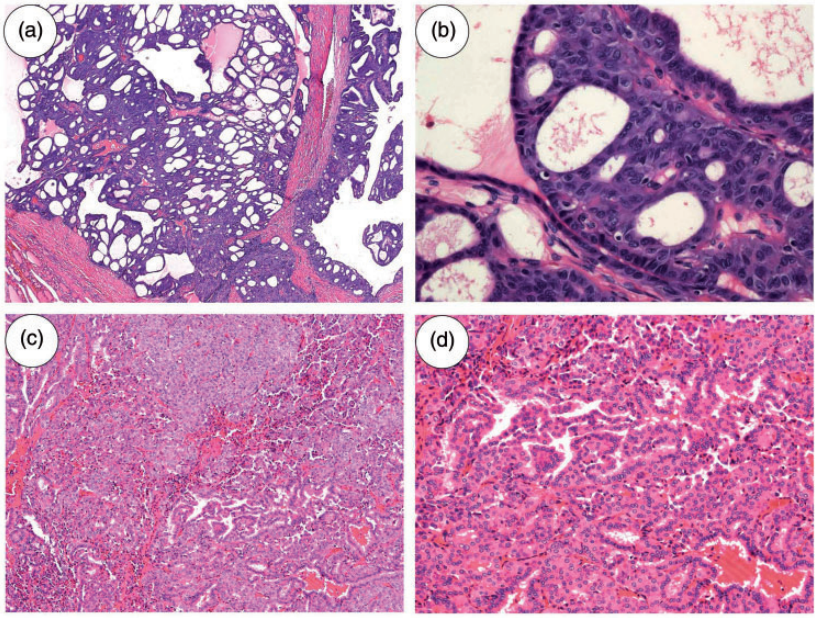

Figure 1. Representative histology of papillary thyroid carcinoma (PTC) subtypes. (a) Low-magnification image of cribriform variant of PTC. Follicular epithelial cells proliferated in cribriform patterns. Original magnification ×20. (b) High-magnification image of cribriform variant of PTC. Tumor cells showed distinct nuclear features, such as grooves and ground-glass appearance. Original magnification ×400. (c) Low-magnification image of poorly differentiated PTC with poorly differentiated components. The tumor contained papillary structures and poorly differentiated components, including solid and insular proliferations. The solid and insular components comprised about 20% of the tumor. Original magnification ×20. (d) High-magnification image of PTC with poorly differentiated components. The tumor cells of the poorly differentiated components showed no distinct nuclear features of PTC. Original magnification ×200. Hematoxylin and eosin staining.

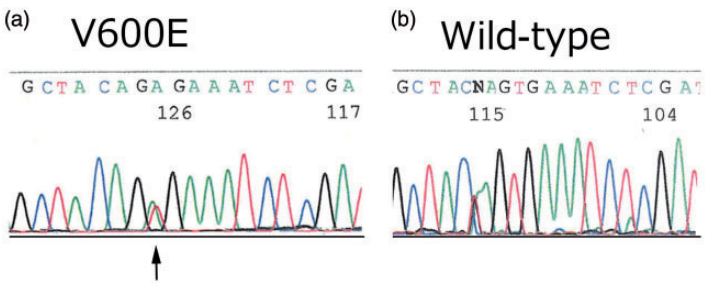

Figure 2. Representative sequence of exon 15 of the *BRAF* gene. Arrow shows heterozygous T to A mutation (a) and wild-type (b).

mutation occurred in 45% of patients overall and in 29% of pediatric cases (two patients aged 17 and 18 years, respectively). The frequency of *BRAF* V600E mutation increased significantly with age, but no other clinicopathological characteristics were associated with *BRAF* V600E mutation status.

Table 3. Clinicopathological features and *BRAF* V600E status in all patients with papillary thyroid carcinoma.

Case	Age (years)	Sex	Hashimoto disease	Non-thyroid malignancy	FHx of benign thyroid disease	FHx of non-thyroid malignancy	FHx of thyroid cancer	Tumor size (cm)	Histology	pT	pN	Ex	Ly/V	M	Follow-up (months)	*BRAF* V600E
1	13	F	−	−	−	−	−	7	Conv	3b	1b	1	+	0	12	WT
2	14	F	−	−	−	−	−	2	Conv with poorly differentiated component	2	X	0	−	0	20	WT
3	16	F	−	−	−	−	−	2.3	Cribriform variant	2	X	0	−	0	93	WT
4	17	F	−	−	+	−	−	1.8	Conv	1b	1a	0	+	0	19	MT
5	18	F	−	−	+	−	+	0.7	Conv	1a	X	0	−	0	24	MT
6	19	F	+	−	−	−	−	2.2	Conv	2	1a	0	+	0	24	WT
7	19	F	+	−	−	−	−	1	Conv	1b	1a	0	−	0	18	WT
8	20	F	−	−	−	−	−	0.6	Conv	1a	X	0	−	0	21	WT
9	20	F	−	−	−	−	−	1.1	Conv	1b	1a	0	−	0	47	WT
10	20	F	−	−	−	−	−	3.5	Conv	2	1b	0	+	0	29	WT
11	21	F	−	−	−	−	−	1.8	Conv	1b	1a	0	+	0	24	WT
12	21	F	−	−	−	−	−	4	Conv	3a	1b	0	+	1	19	WT
13	21	F	−	−	−	−	−	0.8	Conv	1a	0	0	−	0	32	WT
14	21	F	−	−	−	−	−	1.8	Conv	1b	1a	0	+	0	96	WT
15	22	F	+	−	+	−	−	0.3	Conv	1a	0	0	+	0	29	WT
16	22	F	+	−	−	−	+	0.8	Conv	1a	0	0	−	0	16	WT
17	23	F	+	−	−	−	−	3	Conv	2	X	0	−	0	39	MT
18	23	F	−	−	−	−	−	2.8	Conv	2	0	0	−	0	32	WT
19	23	F	+	−	−	−	−	1.5	Conv	3b	0	1	−	0	80	WT
20	24	F	−	−	−	−	−	1.1	Conv	1b	1a	0	+	0	26	MT
21	24	F	−	−	−	−	−	3	Conv	2	1b	0	+	0	3	MT
22	24	M	−	−	−	+	−	2.5	Conv	2	1b	0	+	0	68	MT
23	24	F	+	−	−	−	−	0.6	Conv	1a	1a	0	−	0	30	MT
24	24	F	−	−	−	−	−	2.3	Conv	3b	1a	1	−	0	25	MT

(continued)

Table 3. Continued.

Case	Age (years)	Sex	Hashimoto disease	Non-thyroid malignancy	FHx of benign thyroid disease	FHx of non-thyroid malignancy	FHx of thyroid cancer	Tumor size (cm)	Histology	pT	pN	Ex	Ly/V	M	Follow-up (months)	*BRAF* V600E
25	24	F	+	−	−	−	−	2.1	Conv	2	1a	0	+	0	34	WT
26	25	F	+	−	−	−	−	1.4	Conv	3b	1a	1	+	0	25	WT
27	26	F	−	−	−	−	−	0.5	Conv	1a	1a	0	−	0	59	WT
28	28	F	−	−	−	−	−	0.5	Conv	1a	0	0	−	0	13	MT
29	28	F	−	−	−	−	−	0.7	Conv	1a	0	0	−	0	4	WT
30	34	F	−	−	−	−	−	1.7	Conv	1b	0	0	+	0	15	MT
31	34	F	−	−	−	−	−	2	Conv	3b	1b	1	+	0	3	WT
32	43	F	+	−	−	−	−	2.2	Conv	2	1a	0	−	0	8	MT
33	49	M	−	+	−	−	−	0.6	Conv	1a	0	0	−	0	1	MT
34	54	M	−	+	−	−	−	1.5	Conv	1b	1a	0	+	0	12	WT
35	58	F	−	+	−	−	+	1.5	Conv	1b	1a	0	−	0	8	MT
36	61	F	−	+	−	+	−	1.4	Conv	1b	0	0	−	0	10	MT
37	67	F	+	−	−	−	−	1.4	Conv	4a	X	1	−	0	7	MT
38	68	F	−	−	−	−	−	2.2	Conv	3b	1a	1	−	0	8	MT
39	72	F	+	−	−	−	−	3.5	Conv	3b	1a	1	+	0	14	MT
40	79	F	−	+	+	−	−	3	Conv	2	1b	0	−	0	11	MT

F, female; M, male; FHx, family history; Conv, conventional type; pT, pathological T stage; Ex, extrathyroidal extension; pN, pathological N stage; Ly/V, lymphovascular invasion; M, clinical M stage; WT, wild-type; MT, mutant.

Table 4. Summary of the *BRAF* V600E status and clinicopathological features in all patients with papillary thyroid carcinoma.

				Age group (years)							
		Total n = 40		13–19 n = 7		20–39 n = 24		40–59 n = 4		60–79 n = 5	
BRAF		−	+	−	+	−	+	−	+	−	+
n (%)		22 (55)	18 (45)	5 (71)	2 (29)	16 (67)	8 (33)	1 (25)	3 (75)	0	5 (100)
Mean age [range], years		23 [13–54]	41 [17–79]	16 [13–19]	18 [17–18]	26 [20–34]	24 [23–24]	54 [54]	50 [43–58]	–	69 [61–79]
Sex	M, n (%)	1 (3)	2 (5)	0	0	0	1 (4)	1 (25)	1 (25)	0	0
	F, n (%)	21 (53)	16 (40)	5 (71)	2 (29)	16 (67)	7 (29)	0	2 (50)	0	5 (100)
Hashimoto disease, n (%)	−	15 (38)	13 (33)	3 (43)	2 (29)	11 (46)	6 (25)	1 (25)	2 (50)	0	3 (60)
	+	7 (18)	5 (13)	2 (29)	0	5 (20)	2 (8)	0	1 (25)	0	2 (40)
Non-thyroid malignancy, n (%)	−	21 (53)	14 (35)	5 (71)	2 (29)	16 (67)	8 (33)	0	1 (25)	0	3 (60)
	+	1 (3)	4 (10)	0	0	0	0	1 (25)	2 (50)	0	2 (40)
Family history of non-thyroid malignancy, n (%)	−	22 (55)	16 (40)	5 (71)	2 (29)	16 (67)	7 (29)	1 (25)	3 (75)	0	4 (80)
	+	0	2 (5)	0	0	0	1 (4)	0	0	0	1 (20)
Family history of thyroid carcinoma, n (%)	−	21 (53)	16 (40)	5 (71)	1 (14)	15 (63)	8 (33)	1 (25)	2 (50)	0	5 (100)
	+	1 (3)	2 (5)	0	1 (14)	1 (14)	0	0	1 (25)	0	0
Family history of benign thyroid disease, n (%)	−	21 (53)	15 (38)	5 (71)	0	15 (63)	8 (33)	1 (25)	3 (75)	0	4 (80)
	+	1 (3)	3 (8)	0	2 (29)	1 (4)	0	0	0	0	1 (20)
Mean tumor size [range], cm		2 [0.5–7.0]	1.8 [0.5–3.5]	2.9 [1.0–7.0]	1.3 [0.7–1.8]	1.7 [0.5–3.5]	1.8 [0.6–3.0]	1.5	1.4 [0.6–2.2]	–	2.3 [1.4–3.5]
pT stage, n (%)	pT1a	6 (15)	4 (10)	0	1 (14)	6 (25)	2 (3)	0	1 (25)	0	0
	pT1b	5 (13)	5 (13)	1 (14)	1 (14)	3 (13)	2 (3)	1 (25)	1 (25)	0	1 (20)
	pT2	6 (15)	5 (13)	3 (43)	0	3 (13)	3 (13)	0	1 (25)	0	1 (20)
	pT3a	1 (3)	0	0	0	1 (4)	0	0	0	0	0
	pT3b	4 (10)	3 (8)	1 (14)	0	3 (13)	1 (4)	0	0	0	2 (40)
	pT4a	0	1 (3)	0	0	0	0	0	0	0	1 (20)
pN stage, n (%)	pN0	6 (15)	4 (10)	0	0	6 (25)	2 (3)	0	1 (25)	0	1 (20)
	pN1a	9 (23)	8 (20)	2 (29)	1 (14)	6 (25)	3 (13)	1 (25)	2 (50)	0	2 (40)
	pN1b	4 (10)	3 (8)	1 (14)	0	3 (13)	2 (3)	0	0	0	1 (20)
	pNX	3 (8)	3 (8)	2 (29)	1 (14)	1 (4)	1 (4)	0	0	0	1 (20)
Extrathyroidal extension, n (%)	−	18 (45)	14 (35)	4 (57)	2 (29)	13 (54)	7 (29)	1 (25)	3 (75)	0	2 (40)
	+	4 (10)	4 (10)	1 (14)	0	3 (13)	1 (4)	0	0	0	3 (60)
Lymphovascular invasion, n (%)	−	11 (28)	12 (30)	3 (43)	1 (14)	8 (33)	4 (17)	0	3 (75)	0	4
	+	11 (28)	6 (15)	2 (29)	1 (14)	8 (33)	4 (17)	1 (25)	0	0	1 (20)

pT, pathological T stage; pN, pathological N stage; M, male; F, female; pNX, no lymph node dissection.

The reported incidence of *BRAF* V600E mutation has ranged from 36% to 83%,[4] with a higher prevalence in Asian countries, including Japan and Korea, compared with western countries[26] associated with differences in iodine intake.[27] The overall frequency of *BRAF* V600E in the present study was within the reported range, but relatively low for an Asian population. There are several possible explanations for this apparent discrepancy. First, 60% of the cases analyzed in the current study were in their second and third decades, and this age distribution might have affected findings. Second, we detected the mutation by Sanger sequencing using FFPE tissues. Sanger sequencing with FFPE[28,29] or frozen tissues and real-time PCR are currently the main methods used, though droplet digital PCR (ddPCR) and next-generation sequencing have also been used to assess genetic profiles.[30,31] The sensitivity for detecting the mutation is increased by manual tissue dissection because this is more effective at excluding normal tissues in which the occurrence of the single nucleotide polymorphism is negligible. Sanger sequencing has been reported to be more sensitive for detecting *BRAF* V600E than real-time PCR, but less sensitive than ddPCR.[30,32] We therefore used manually dissected FFPE samples and Sanger sequencing as a cost-effective way of detecting the *BRAF* V600E mutation in surgical specimens. Nasirden et al.[29] reported *BRAF* V600E in 38% of Japanese PTC patients using FFPE samples and Sanger sequencing, with a similar population, methodology, and mutation frequency to our current study. The geographic background of the patients and the methodology may thus be key factors affecting the frequency of the *BRAF* V600E mutation. Kowalska et al.[33] demonstrated time-dependent trends in the prevalence of this mutation in PTC, with significant increases in its prevalence during 2000 to 2004, 2005 to 2009, and 2010 to 2013. Further investigations of the relationships between *BRAF* V600E frequency and the clinicopathological features of PTC should thus take account of different time periods and regions.

The current results indicated that the frequency of the *BRAF* V600E mutation increased with increasing age in patients with PTC, in line with previous reports.[15] However, few reports have investigated the influence of age on mutation incidence. A review of previous publications regarding the incidence of *BRAF* V600E mutation in relation to median age showed that it occurred in 49% of 45-year olds[34]63% of 47-year olds,[35] and 85% of 58-year olds.[5] One recent study also demonstrated a positive correlation between *BRAF* V600E and age. Patients were divided into age groups and the *BRAF* V600E mutation was found in approximately 55%, 80%, 85%, 88%, and 90% of patients aged <25 years, 25 to 35, 35 to 45, 45 to 55, and >55 years old, respectively.[36] This trend was also seen in patients with colorectal cancer.[37] In a study investigating the relationship between age and driver mutations, Yokoyama et al.[38] demonstrated that remodeling of the esophageal epithelium by numerous driver-mutated clones was an inevitable consequence of normal aging. It is therefore plausible that the frequency of the *BRAF* V600E mutation might be higher in older PTC patients.

Regarding the pediatric population, previous studies[1,5–11] have reported the *BRAF* V600E mutation in 0% to 63% of pediatric PTC patients (Table 5). Although the results varied, a higher rate of *BRAF* mutation reflected an older age distribution of the analyzed cases, particularly showing a higher frequency in patients above 15 years of age. BRAF mutation was detected in two of the pediatric cases in the present study, both of whom were over 15 years old.

Genetic alterations detected in PTCs include *BRAF* V600E, RAS, *RET*/*PTC*

Table 5. Previous studies on *BRAF* V600E mutation frequency in pediatric patients with papillary thyroid carcinoma.

Reference	Age (years)	Population	*BRAF* mutation
Penko et al.[6]	10–21 (mean 17.5 ± 3.5)	USA	0
Rosenbaum et al.[7]	10–17 (mean 15)	USA	5%
Kumagai et al.[8]	Mean 11	Japan	3.2%
	<15 (mean 18.6)	Ukraine	24%
Givens et al.[9]	2–18 (mean 13.6)	USA	36.8% (Conventional type PTC 63%)
Mostoufi-Moab et al.[10]	<18	USA	44%
Oishi et al.[5]	6–20 (17.4 ± 2.7)	Japan	54%
Henke et al.[11]	5.8–21.2 (mean 18.6)	USA	63%

PTC, papillary thyroidcarcinoma.

rearrangement, and *ETV6-NTRK*, of which *BRAF* V600E is the most common. *BRAF* mutation stimulates the MAPK pathway, resulting in cell growth, proliferation, and differentiation.[39] Accumulated studies have demonstrated associations between *BRAF* V600E and some clinicopathological characteristics of PTC, including aggressive features. PTC with *BRAF* mutation was shown to occur in patients in their 50s[15] and predominantly in males.[12–14] *BRAF*-mutated tumors also tend to be large,[13,16–18] with conventional tumor histology,[34] and extrathyroidal extension,[15,18,19] vascular invasion,[12,20] and lymph node metastasis[5,12,20] were also frequently observed. The pathological stage is usually advanced,[18,19,21] and recurrence was noted in 25% of patients.[17] The prognosis in patients with PTC and *BRAF* mutation is generally unfavorable, and disease-specific mortality is higher than in *BRAF*-mutation-negative cases.[5,22] However, some reports have shown the opposite results or no association, and these apparent differences may be attributable to differences between the studies in terms of patient background, including factors such as race, iodine intake, radiation, family history, methodology, or sampling.[22] Mitsutake et al.[23] found that *BRAF* V600E-mutated PTC was not associated with aggressive features in an Asian population, which was in accord with the present study also conducted in Asian patients. These results suggest that *BRAF* V600E is not a marker of tumor aggressiveness in Asian patients with PTC, but may have a positive predictive value for diagnosing thyroid tumors.

This study was conducted in a single institution and had several limitations. First, our study involved a limited number of PTC cases and could therefore not thoroughly reflect the general population. Second, the analyzed patients were predominantly female. Our institution employs video-assisted neck surgery for the resection of thyroid tumors,[40] which is a safe and practical method with cosmetic benefits leading to a predominance of female patients, with a male:female ratio of approximately 1:3. The number of male patients in this study may therefore have been too small to allow the evaluation of

any association between *BRAF* mutation and sex. Third, few included cases had tumors <1 cm. Clinically, our institution prefers to carry out active surveillance as an alternative to immediate surgery in patients with very low-risk PTC, in accord with the strategy officially approved by the 2015 American Thyroid Association guidelines.[41] The number of surgical specimens of microcarcinomas, representing very low-risk PTC, was therefore limited during the study period. However, although our sample had a bias in term of tumor size, the clinical features were nonetheless of practical value given that active surveillance is standard in clinical practice.

In conclusion, our results showed that 45% of PTCs had *BRAF* mutations, which was within the previously reported range, and that the frequency of these mutations increased significantly with increasing age. Genetic alterations can inform the prognosis, therapy, follow-up, and molecular targeted therapy of PTC, highlighting the need to clarify the genetic profile of these patients, including in terms of *BRAF* mutation status. However, the genetic profiles should take account of population data and time periods. Despite the limited number of cases, the results of the current study may contribute to the genetic profile of PTC in Asian populations.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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