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# Frequent *BRAF*$^{V600E}$ and Absence of *TERT* Promoter Mutations Characterize Sporadic Pediatric Papillary Thyroid Carcinomas in Japan

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**Abstract** Pediatric papillary thyroid carcinoma (PTC) has unique features but requires further genetic investigation. Moreover, there has been increasing concern about the risk for pediatric PTC in Japan after the Fukushima accident. This study aims to evaluate the frequencies of *BRAF* and *TERT* promoter mutations and to examine their significance in non-radiation-associated pediatric PTCs in Japan. We enrolled 81 pediatric PTC patients aged ≤20 years. The control group included 91 adult PTCs from patients >20 years old. *BRAF* and *TERT* mutations were analyzed by allele-specific-PCR and/or Sanger sequencing. Compared with adult PTCs, pediatric PTCs exhibited larger tumor size, more frequent lymph node metastasis, and less classical histology. The prevalence of *BRAF*$^{V600E}$ in pediatric PTCs was 54% and significantly lower than that in adults of 85%. In the pediatric PTCs, *BRAF*$^{V600E}$ was positively associated with older age, classical histology, and the lymph node metastasis but independent from other clinicopathological factors. *TERT* mutations were identified in 13% of adults and in none of the pediatric PTCs. In conclusion, pediatric PTCs are characterized by more advanced clinicopathological features, lower *BRAF*$^{V600E}$ frequency, and absence of *TERT* mutation. The *BRAF*$^{V600E}$ frequency in this study is similar to the reported *BRAF*$^{V600E}$ frequency in the ultrasonographically screened pediatric PTCs in Fukushima.

**Keywords** Papillary thyroid carcinoma · Pediatric · Adult · Mutation · *BRAF* · *TERT*

## Introduction

Papillary thyroid carcinoma (PTC) is the most commonly encountered malignant endocrine tumor and accounts for up to 85% of all thyroid malignancies [1, 2]. Although PTC usually affects adults with a female predominance, small numbers of PTCs occur in children and youth [2–11]. In these pediatric PTCs, histopathologically and clinically distinct features are reported [3, 6]. For instance, children and youth have a predilection to be affected by morphologically unique variants of PTC, such as solid variant (SV), solid-follicular variant (SFV), and diffuse sclerosing variant (DSV) [8, 12–14]. Despite a higher frequency of lymph node (LN) and distant metastases, some reports indicated that the overall survival of pediatric PTCs is more favorable than adult PTCs [7, 11].

Molecular pathogenesis of adult PTCs has been clarified by previous studies [1, 15, 16], including a next-generation-sequencing analysis by the Cancer Genome Atlas [17]. Activating mutation of the *BRAF* gene is the most frequent driver alternation of PTC [1, 15, 18]. *BRAF*$^{V600E}$ is generally identified in 35 to 80% of adult PTCs [1, 15, 18]. Conversely, there have been limited studies on the *BRAF* mutation in pediatric PTCs [5, 6, 8–11, 19, 20]. In addition, the number of examined cases in those studies was relatively small, and the mutation frequency varied widely from 0 to 36.8%. Since

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mutated *BRAF* is a promising target for molecular therapy [21], it is important to discover the true incidence and significance of *BRAF* mutation in pediatric PTCs. Another important genetic alternation in thyroid cancer is *TERT* promoter mutation. Some earlier studies showed that *TERT* mutations are associated with aggressive clinicopathological features [22–24]. However, only few studies reported its incidence and significance in pediatric PTCs [20, 25].

Radiation exposure is a well-established risk factor for thyroid cancer. Indeed, after the nuclear meltdown accident at Chernobyl in 1986, the incidence of PTC had increased in the radiologically contaminated areas of Belarus, Russia, and Ukraine [26]. In Japan as well, the Fukushima Dai-ichi nuclear accident in 2011 has created great concern about the risk for pediatric thyroid cancer, especially in eastern Japan. Recently, Mitsutake et al. reported the genetic profile of thyroid carcinoma in the young population in Fukushima [27]. Their study enrolled 67 PTCs and one poorly differentiated carcinoma detected by the thyroid ultrasound screening for children in the Fukushima prefecture aged 0–18 years old at the time of the accident. Of note, quite different from post-Chernobyl PTCs, $BRAF^{V600E}$ was highly prevalent in the pediatric PTCs in Fukushima. Based on this finding, they suggested non-radiogenic etiology in the pediatric PTCs in Fukushima. The conclusion is well concordant with the lower estimated thyroid doses of radioiodine exposure in Fukushima [28]. However, their study had no comparison with sporadic non-radiation-associated pediatric PTCs in Japan. To evaluate the actual impact of the Fukushima accident, it is essential to review a large cohort of Japanese pediatric PTCs from before the disaster and to delineate their clinicopathological and genetic features.

The purpose of this study is, therefore, to evaluate the frequency of *BRAF* and *TERT* promoter mutations and their clinicopathological significance in a series of non-radiation-associated pediatric PTCs in Japan.

## Materials and Methods

### Patients, Tissue Samples, and Data Collection

In the present study, we defined PTC occurring up to and including age 20 years as pediatric PTC. We explored the pathological records of University of Yamanashi Hospital and Kuma Hospital from 1991 to 2013 and collected cases of 81 pediatric PTCs. As the control group, we collected 91 cases of adult PTCs from patients aged 21 years or over. We performed additional pathological analyses on the formalin-fixed and paraffin-embedded (FFPE) tissue blocks from these cases and obtained the following clinicopathological data from the medical records under an appropriate identification: age, sex, tumor size (cm), extrathyroidal invasion, LN metastasis, and distant metastasis. All patients studied lived in iodine-sufficient areas, and there were no individuals with previous history of radiation exposure. The study protocol was approved by the ethical review board of University of Yamanashi (approval code: 1332).

### Histopathological Evaluation

Two pathologists (N.O. and R. K.) examined the histology of the most representative slide from each PTC without any prior clinicopathological or genetic information on the case. They used the histological classification of the WHO Classification Tumors of Endocrine Organs [29]. However, the WHO classification defines the SV as a PTC dominated by solid sheets of tumor cells, whereas our case studies rarely had a purely solid pattern. The solid architecture was usually intermingled with some follicular pattern. These PTCs were very similar to the SFV described by Harach and Williams [30]. Hence, in the current study, we combined these histological types in an SV/SFV group.

#### *DNA Extraction from FFPE Samples*

We extracted DNA from FFPE using RecoverAll total nucleic acid isolation kit (Applied Biosystems, Tokyo, Japan) according to the manufacturer's protocol. To validate the quality and quantity of the extracted DNA, we performed PCR for phosphoglycerate kinase 1 gene (PGK1). The PCR amplification was carried out in a reaction volume of 50 μL with 10× PCR buffer (QIAGEN, Tokyo, Japan), 200 μM of each dNTP (QIAGEN), 500 nM of each primer (listed in Table S1), and 1.25 units of HotStarTaq DNA polymerase (QIAGEN). The thermal cycling condition was as follows: 15 min at 95 °C, followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. We used 3% agarose gel electrophoresis with Midori Green DNA Stain (NIPPON Genetics Co., Ltd., Tokyo, Japan) and light-emitting diode (LED) illuminator (NIPPON Genetics) to analyze the PCR products.

#### *Allele-Specific PCR and Sanger Sequencing for BRAF Mutation*

To detect *BRAF* mutations, we carried out two-step testing with initial allele-specific PCR (AS-PCR) and subsequent Sanger sequencing. We screened for $BRAF^{V600E}$, the most common type of *BRAF* mutation, using AS-PCR, and subsequently performed Sanger sequencing for *BRAF* exon 15 in the AS-PCR-negative PTCs. AS-PCR is a concise method using the specific primer pair for $BRAF^{V600E}$ mutant allele (listed in Table S1), and we performed the AS-PCR as described previously [31, 32]. In Sanger sequencing for *BRAF* exon 15, 1 μL of DNA in a final volume of 50 μL with 10×

PCR buffer, 200 μM of each dNTP, 500 nM of each primer (listed in Table S1), and 1.25 units of the polymerase was amplified. The thermal cycling condition was as follows: 15 min at 95 °C, followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The PCR products were submitted to Sanger sequencing with the forward primer using BigDye Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, CA, USA).

*Sanger Sequencing for TERT Promoter Mutations*

We examined *TERT* promoter mutations by standard Sanger sequencing. In brief, 1 μL of DNA in a final volume of 50 μL with 5× Q-solution, 10× PCR buffer, 200 μM of each dNTP, 500 nM of each primer (listed in Table S1), and 1.25 units of the polymerase was amplified. The thermal cycling condition was the same as described in *BRAF* exon 15. After electrophoresis, the PCR products were sequenced with the forward primer.

*Sanger Sequencing for CTNNB1 and PIK3CA*

Mutations of *CTNNB1* and *PIK3CA* have been reported in cribriform-morular variant (CMV)-PTCs [33, 34]; we explored mutations at exon 3 of *CTNNB1* and exon 9 and exon 20 of *PIK3CA* in one CMV-PTC using Sanger sequencing. Used primers are listed in Table S1. The protocol for PCR and Sanger sequencing was the same as that for *BRAF* exon 15.

**Statistical Analysis**

$Chi^2$ test and the Fisher exact test were used for ordinal or nominal categorical variables and discrete numerical variables. Student's *t* test and Mann-Whitney test were used for continuous numerical data appropriately, after Shapiro-Wilk test for normality. All statistical calculations were performed with the SPSS Statistics 22 software package (IBM Japan, Tokyo, Japan), and a *p* value of <0.05 was considered statistically significant.

## Results

**Clinical and Histopathological Features**

Table 1 summarizes clinicopathological features of both pediatric and adult PTCs. In the pediatric PTC group, patients' ages were distributed from 6 to 20 years. Children 15 years or under accounted for 22% (18/81) of the cases. Females accounted for 91% (74/81) of pediatric PTCs. Although female predominance was also apparent in adult PTCs (62% (56/91)), the disparity between the sexes was more significant in the pediatric PTCs ($p < 0.001$). Figure 1 shows the representative histology of pediatric PTCs. There was a higher prevalence of non-classical PTCs including SV/SFV and DSV in the pediatric group than in the adult control group (19% (15/81) vs 4% (4/91), $p = 0.003$). Furthermore, pediatric PTCs exhibited significantly advanced feature in tumor size and LN metastasis. The mean tumor size was significantly larger in pediatric PTCs than in adult PTCs (3.0 ± 1.5 vs 1.3 ± 0.9 cm, $p < 0.001$). Microcarcinoma, defined as carcinoma with a diameter 1.0 cm or smaller, was identified in only 5% (4/75) of pediatric PTCs, while 49% (42/86) of adult PTCs were microcarcinoma ($p < 0.001$). The incidence of LN metastasis was significantly higher in pediatric PTCs than adult PTCs (90% (72/80) vs 66% (58/88), $p < 0.001$). Furthermore, extensive LN metastasis to central and lateral nodes (pN1b) was identified in 68% (54/80) of pediatric PTCs and in only 16% (14/88) of adult PTCs ($p < 0.001$). However, there was no statistically significant difference in distant metastasis between pediatric and adult PTCs (4% (3/80) vs 1% (1/88), $p = 0.27$). Table 2 indicates the clinicopathological difference of each histology in pediatric PTCs. Classical PTCs occurred in significantly older pediatric patients (17.8 ± 2.6 in classical, 15.3 ± 2.5 in SV/SFV, 16.0 ± 2.9 in DSV, $p = 0.034$). Of note, distant metastasis was exclusively observed in 29% (2/7) of SV/SFVs and 25% (1/4) of DSV ($p = 0.002$). Clinicopathological feature of the pediatric PTCs with distant metastasis is summarized in Table S2. All the three pediatric PTCs with distant metastasis exhibited multiple lung metastases. In addition, the DSV-PTC showed multiple bone metastases. While the patient with DSV-PTC died of the pulmonary fibrosis after the radioactive iodine therapy, the other two patients were alive at the last follow-up (18 and 9 years after the initial operation, respectively). Other clinicopathological factors including tumor size, extrathyroidal invasion, and LN metastasis showed no association with the histology in pediatric PTCs.

***BRAF* Mutations**

We identified $BRAF^{V600E}$ mutation in 54% (44/81) of pediatric PTCs and 85% (77/91) of adult PTCs (Table 3); the *BRAF* mutation frequency was significantly lower in pediatric PTCs than in adult PTCs ($p < 0.001$). No *BRAF* mutations other than $BRAF^{V600E}$ were identified in pediatric or adult PTCs. Table 4 and Table S3 show the association between $BRAF^{V600E}$ status and clinicopathological features in pediatric and adult PTCs, respectively. In pediatric PTCs, $BRAF^{V600E}$ was associated with an older patient's age (18.2 ± 2.1 vs 16.5 ± 3.0, $p = 0.009$). The association between $BRAF^{V600E}$ and patient's age was also significant, when the pediatric PTC patients were subdivided into two age groups: 28% (5/18) in 15-year-old or younger vs 62% in 16- to 20-year-old (39/63) ($p = 0.011$). However, the older group of pediatric PTCs still showed

**Table 1** Clinicopathological features of pediatric and adult PTCs

| | Pediatric PTCs | | Adult PTCs | | *p* value |
|---|---|---|---|---|---|
| *Age at operation* | | | | | |
| Mean ± SD | 17.4 ± 2.7 | | 58.4 ± 11.8 | | |
| Median (range) | 18 | (6–20) | 58 | (31–96) | |
| *Female/male* | 74:7 | | 56:35 | | <0.001[a] |
| *Histology* | | | | | |
| Classical | 66/81 | (81) | 87/91 | (96) | 0.003[*,a] |
| TCV | 0 | (0) | 1/91 | (1) | |
| FV | 2/81 | (2) | 3/91 | (3) | |
| SV/SFV | 8/81 | (10) | 0 | (0) | |
| DSV | 4/81 | (5) | 0 | (0) | |
| CMV | 1/81 | (1) | 0 | (0) | |
| *Tumor size* | | | | | |
| Mean (cm) ± SD | 3.0 ± 1.5 | | 1.3 ± 0.9 | | <0.001[c] |
| Microcarcinoma (≤1.0 cm) | 4/75 | (5) | 42/86 | (49) | <0.001[a] |
| *Extrathyroidal invasion* | | | | | |
| Positive | 32/80 | (40) | 59/87 | (68) | <0.001[a] |
| *LN metastasis* | | | | | |
| All | 72/80 | (90) | 58/88 | (66) | <0.001[a] |
| Central only: pN1a | 18/80 | (23) | 44/88 | (50) | |
| Central + lateral cervical: pN1b | 54/80 | (68) | 14/88 | (16) | |
| *Distant metastasis* | | | | | |
| Positive | 3/80 | (4) | 1/88 | (1) | 0.27[b] |

*PTC* papillary thyroid carcinoma, *yr* year, *TCV* tall cell variant, *FV* follicular variant, *SV* solid variant, *SFV* solid-follicular variant, *DSV* diffuse sclerosing variant, *CMV* cribriform morula variant, *SD* standard deviation, *LN* lymph node

% and *p* values are based on the total number of cases with available data

[*] Classical vs others

[a] Pearson's Chi-square test

[b] Fischer's exact test

[c] Mann-Whitney test

significantly lower frequency of $BRAF^{V600E}$ than the adult PTCs: 62% (39/63) vs 85% (77/91), $p = 0.001$.

From a morphological aspect, the *BRAF* mutation was positively associated with classical histology. Indeed, all of 44 *BRAF*-mutated pediatric PTCs were classical PTC; conversely, 67% (44/66) of pediatric classical PTCs were positive for *BRAF* mutation. On the other hand, non-classical pediatric PTCs exhibited no *BRAF* mutation ($p < 0.001$). We also confirmed this correlation between *BRAF* mutation and histological appearance in adult PTCs ($p = 0.011$) (Table S3). However, regarding PTCs with classical histology, the frequency of *BRAF* mutation was still lower in pediatric *classical* PTCs than in adult *classical* PTCs (67% (44/66) vs 87% (76/87), $p = 0.003$).

*BRAF*-mutated pediatric PTCs showed significant association with the presence of LN metastasis (98% (43/44) vs 81% (29/36), $p = 0.014$). However, when focusing on the pediatric PTCs with LN metastasis, extensive LN metastasis (pN1b) was paradoxically less frequent in *BRAF*-mutant PTCs than *BRAF*-wild-type PTCs: 65% (28/43) vs 90% (26/29), $p = 0.016$. Furthermore, all the three pediatric PTCs with distant metastasis were *BRAF*-wild-type (Table S2). Other three factors of sex, tumor size, and extrathyroidal invasion were independent from *BRAF* mutation status in both pediatric and adult PTCs.

### *TERT* Promoter Mutations

Table 3 indicates *TERT* promoter statuses in both pediatric and adult PTCs. All of the pediatric PTCs and all but six adult PTCs were informative for *TERT* promoter mutation status. None of the 81 pediatric PTCs exhibited *TERT* promoter mutations, while 13% (11/85) of adult PTCs harbored the mutations ($p = 0.001$). Among the 11 *TERT*-mutated adult PTCs, C250T and C228T accounted for 27% (3/11) and 73% (8/11), respectively. All the *TERT* promoter mutations in adult PTCs were

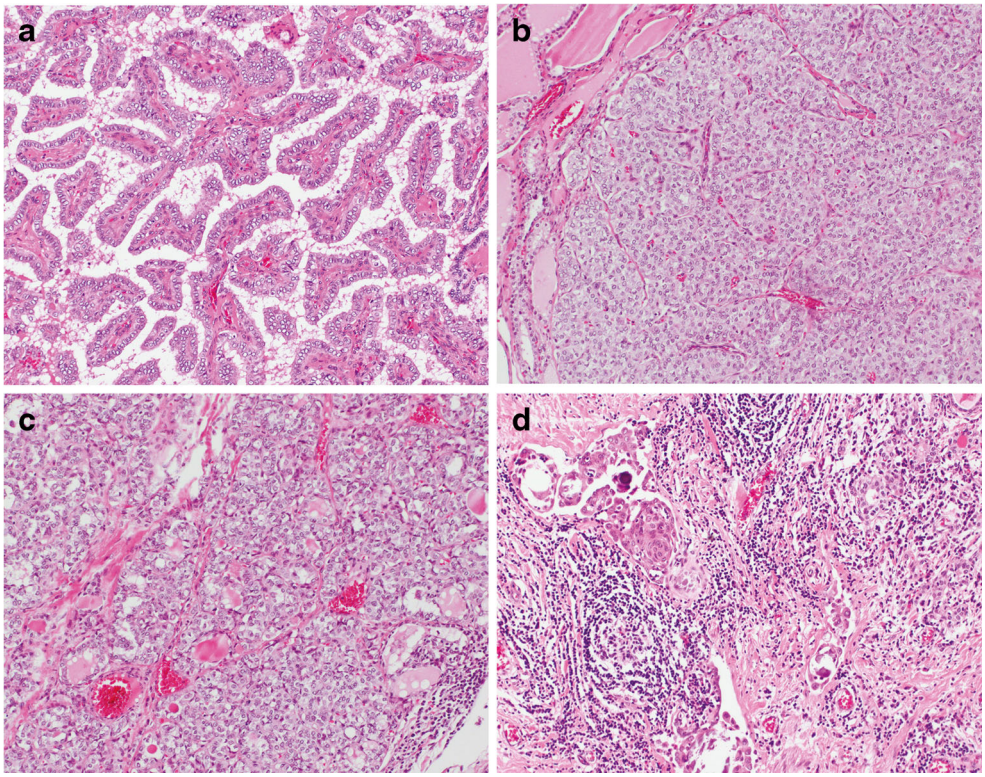


**Fig. 1** Representative histology in pediatric papillary thyroid carcinoma (PTC). **a** Classical PTC. The predominant papillary configuration is present. **b** Solid variant (SV). Tumor cells exhibit predominantly solid sheet-like architecture. **c** Solid-follicular variant (SFV). Intermingled follicular pattern is identified in the solid component. **d** Diffuse sclerosing variant (DSV). Neoplastic cells accompanied by psammoma bodies and squamous metaplasia present in a lymphatic sinus. Note the marked infiltration of lymphocytes

heterozygous. The age of the youngest and oldest patient with *TERT*-mutated PTC was 61 and 96 years, respectively. Furthermore, all these *TERT*-mutated PTCs (11/11) harbored *BRAF* mutation (Fig. 2). As indicated in Table S4, the adult PTCs with *TERT* promoter mutation were associated with an older age at operation (73.3 ± 9.8 vs 55.7 ± 10.3, $p < 0.001$) and UICC stage IV (46% (5/11) vs 14% (10/74), $p = 0.019$).

**Table 2** Histology and clinicopathological features of pediatric PTCs

| | Classical | | SV/SFV | | DSV | | *p* value |
|---|---|---|---|---|---|---|---|
| *Age at operation* | | | | | | | |
| Mean ± SD | 17.8 ± 2.6 | | 15.3 ± 2.5 | | 16.0 ± 2.9 | | 0.034[a] |
| Median (range) | 19 | (6–20) | 15 | (12–19) | 15.5 | (13–20) | |
| *Female/male* | 62:4 | | 7:1 | | 2:2 | | 0.023[b] |
| *Tumor size* | | | | | | | |
| Mean (cm) ± SD | 2.9 ± 1.4 | | 3.2 ± 1.5 | | 5.7 ± 3.3 | | 0.27[a] |
| Microcarcinoma (≤1.0 cm) | 4/64 | (6) | 0/7 | (0) | 0/2 | (0) | 1.00[b] |
| *Extrathyroidal invasion* | | | | | | | |
| Positive | 26/66 | (39) | 3/7 | (43) | 2/4 | (50) | 1.00[b] |
| *LN metastasis* | | | | | | | |
| All | 59/66 | (89) | 7/7 | (100) | 4/4 | (100) | 1.00[b] |
| Central only: pN1a | 17/66 | (26) | 1/7 | (14) | 0 | (0) | |
| Central + lateral cervical: pN1b | 42/66 | (64) | 6/7 | (86) | 4/4 | (100) | |
| *Distant metastasis* | | | | | | | |
| Positive | 0/66 | (0) | 2/7 | (29) | 1/4 | (25) | 0.002[b] |

*SV* solid variant, *SFV* solid-follicular variant, *DSV* diffuse sclerosing variant, *SD* standard deviation, *NA* not available, *LN* lymph node

% and *p* values are based on the total number of cases with available data

[a] Kraskal-Wallis test

[b] Fischer's exact test

**Table 3** *BRAF* and *TERT* mutation status in pediatric and adult PTCs

| | Pediatric PTCs | | Adult PTCs | | *p* value |
|---|---|---|---|---|---|
| *BRAF* | | | | | |
| V600E | 44/81 | (54) | 77/91 | (85) | <0.001[a] |
| *TERT* promoter mutation | | | | | |
| All | 0/81 | (0) | 11/85 | (13) | 0.001[a] |
| C250T | 0 | (0) | 3/85 | (4) | |
| C228T | 0 | (0) | 8/85 | (9) | |

% and *p* values are based on the total number of cases with available data

[*] Mutant vs wild-type

[a] Pearson's Chi-square test

### *Mutation Analysis in a CMV-PTC*

In the pediatric PTC group, there was one CMV-PTC. The patient was an 18-year-old female without history of familial adenomatous polyposis. In genotyping, no *BRAF*, *CTNNB1*, *PIK3CA*, or *TERT* promoter mutation was evident in the CMV-PTC.

## Discussion

We reviewed and investigated 81 PTCs from patients aged 20 years or less and compared them to 91 adult PTCs. We found that $BRAF^{V600E}$-mutated tumors accounted for 54% of overall pediatric PTCs. Although reported proportions of $BRAF^{V600E}$ in pediatric PTCs have varied widely from 0 to 36.8% [11], our current study identified relatively higher frequency of *BRAF* mutation in Japanese pediatric PTCs. There may be several potential causes for this observation. First, the inclusion criteria for pediatric PTC can affect the *BRAF* mutation frequency. Our definition of pediatric PTC was PTC in a patient of 20-year-old or younger, while some other studies set the cutoff age of 15 or 16 years. Supporting this hypothesis, as shown in the present study, an older patient's age was significantly associated with higher *BRAF* mutation frequency even in pediatric PTCs. Second, racial and/or geographical factors may be another cause. Comparing with the reported data in USA and Europa, the frequency of *BRAF* mutation in adult PTCs is quite higher in East Asia, particularly in South Korea

**Table 4** $BRAF^{V600E}$ mutation status and clinicopathological features in pediatric PTCs

| | *BRAF* mutated | | *BRAF* wild-type | | *p* value |
|---|---|---|---|---|---|
| *Age at operation* | | | | | |
| Mean ± SD | 18.2 ± 2.1 | | 16.5 ± 3.0 | | 0.009[c] |
| Median (range) | 19 | (13–20) | 17 | (6–20) | |
| *Female/male* | 42:2 | | 32:5 | | 0.15[b] |
| *Histology* | | | | | |
| Classical | 44/44 | (100) | 22/37 | (59) | <0.001[*,a] |
| FV | 0 | (0) | 2/37 | (5) | |
| SV/SFV | 0 | (0) | 8/37 | (22) | |
| DSV | 0 | (0) | 4/37 | (11) | |
| CMV | 0 | (0) | 1/37 | (3) | |
| *Tumor size* | | | | | |
| Mean (cm) ± SD | 3.2 ± 1.8 | | 2.8 ± 1.3 | | 0.54[c] |
| Microcarcinoma (≤1.0 cm) | 3/44 | (7) | 1/31 | (3) | 0.45[b] |
| *Extrathyroidal invasion* | | | | | |
| Positive | 16/44 | (36) | 16/36 | (44) | 0.46[a] |
| *LN metastasis* | | | | | |
| All | 43/44 | (98) | 29/36 | (81) | 0.014[b] |
| Central only: pN1a | 15/44 | (34) | 3/36 | (8) | |
| Central + lateral cervical: pN1b | 28/44 | (64) | 26/36 | (72) | |
| *Distant metastasis* | | | | | |
| Positive | 0/44 | (0) | 3/36 | (8) | 0.087[b] |

*PTC* papillary thyroid carcinoma, *yr* year, *TCV* tall cell variant, *FV* follicular variant, *SV* solid variant, *SFV* solid-follicular variant, *DSV* diffuse sclerosing variant, *CMV* cribriform morula variant, *LN* lymph nodes

% and *p* values are based on the total number of cases with available data

[*] Classical vs others

[a] Pearson's Chi-square test

[b] Fischer's exact test

[c] Mann-Whitney test

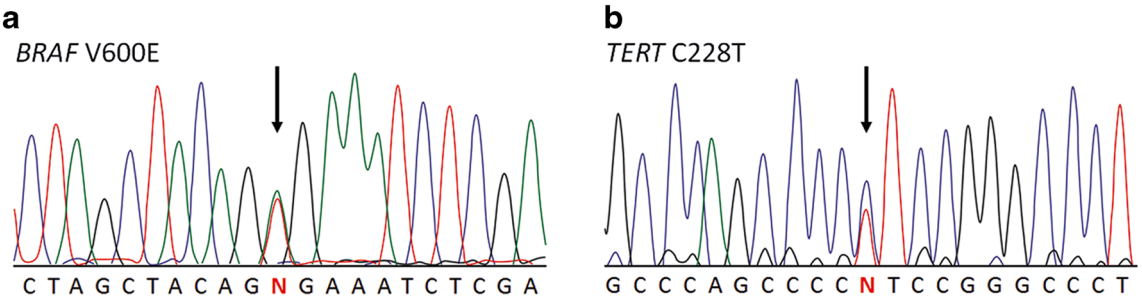


**Fig. 2** A representative adult PTC with *BRAF* and *TERT* promoter mutations is shown. **a** Sanger sequencing shows heterozygous T1799A (V600E) mutation in *BRAF* (*arrow*). **b** Heterozygous cytidine-to-thymidine transition at chr5, 1,295,228 (*TERT* promoter mutation C228T) is identified (*arrow*)

[35]. In fact, the frequent *BRAF* mutation of 85% in our adult PTCs is well concordant with previous data from East Asia. Hence, as well as adult PTC, the relatively higher *BRAF* mutation in Japanese pediatric PTCs may stem the racial and/or geographical factors.

To evaluate the impact of internal exposure after the Fukushima accident, it is important to compare our findings with the recently published study of Japanese pediatric PTCs detected by ultrasound screening program in Fukushima [27]. The Fukushima study reported slightly higher $BRAF^{V600E}$ prevalence in pediatric PTCs: 64% (43/67) in Fukushima vs 54% (44/81) in our study, although the difference is not statistically significant. This minor discordance in $BRAF^{V600E}$ frequency can be largely attributed to the difference of how to detect pediatric PTCs. The Fukushima study investigated ultrasonographically screened pediatric PTCs, while our study enrolled clinically symptomatic pediatric PTCs. This may bias the proportion of histological type in pediatric PTCs. Indeed, the Fukushima study had no SV/SFV- and DSV-PTCs that are clinically aggressive and negative for *BRAF* mutation, while our study enrolled eight and four cases of SV/SFV and DSV, respectively. When comparing with pediatric *classical* PTCs of Fukushima and our study, the difference of the $BRAF^{V600E}$ frequencies becomes much smaller: 70% (43/61) in Fukushima vs 67% (44/66) in our study. Collectively, the *BRAF* mutation status is quite similar between our non-radiation-associated Japanese pediatric PTCs and pediatric PTCs in Fukushima. This finding suggests no alternations in driver point mutation after the Fukushima accident and implies non-radiogenic etiology of PTCs in Fukushima.

$BRAF^{V600E}$ showed significant predilection to occur in classical PTCs. More recently, some studies have reported that classical morphology of PTC such as papillary configuration, typical nuclear features, and infiltrative growth is morphological hallmarks of *BRAF* mutation in adults [36–38]. Our findings confirmed the genotype-phenotype correlation even in pediatric PTCs. However, it should be noted that the frequency of *BRAF* mutation in pediatric *classical* PTCs was still lower than adult classical PTCs. This finding suggests that it is difficult to attribute the lower *BRAF* mutation frequency of pediatric PTCs to the lower fraction of *classical* PTCs in pediatric PTCs and implies that pediatric *classical* PTC is a distinct entity from *classical* PTC of the adult.

$BRAF^{V600E}$ mutation status was associated with the presence of LN metastasis in pediatric PTCs. However, paradoxically, the extensive LN metastasis was observed more frequently in pediatric PTCs without *BRAF* mutation. Furthermore, although not statistically significant, all the pediatric PTCs with distant metastasis were negative for *BRAF* mutation. In adult PTCs, there was no association between *BRAF* mutational status and aggressive risk factors. Collectively, in our case series, *BRAF* mutation may have less predictive value for clinical aggressiveness in both pediatric and adult PTCs. The clinicopathological significance of *BRAF* mutation and its prognostic impact have been widely discussed. A number of studies found *BRAF* mutation to be associated with worse prognostic factors including larger tumor size, extrathyroidal invasion, LN and distant metastases, and recurrence-free survival [39–41]. However, some reports failed to confirm its clinicopathological significance [42–44]. For instance, Cheng et al. reported that *BRAF* mutational status did not provide independent risk estimates within morphologically divided subgroups such as *classical* PTC and FV-PTC [45]. Li et al. also demonstrated that *BRAF* mutation is associated with aggressive clinicopathological factors not in *classical* PTCs but in FV, TCV, and in PTCs overall [44]. Based on this information, we can attribute the lack of clinicopathological significance of *BRAF* mutation in our adult PTCs to the abundance of classical PTC in this group. Because classical PTCs accounted for more than 90% of our adult subset of PTCs, the prognostic impact of *BRAF* mutation could be diminished by this histological bias. Focusing on pediatric PTCs, the absence of clinicopathological significance of *BRAF* mutation was also reported in several smaller studies in the United States, Europe, and Japan [8–11, 19, 20]. We confirmed these observations in our study with the largest patient series so far and demonstrated that the *BRAF* mutation

is not a predictor of aggressiveness and adverse prognosis of pediatric PTCs.

The lack of *TERT* promoter mutations in pediatric PTCs is one of the pivotal findings of the current study: none of our pediatric PTCs exhibited *TERT* promoter mutations, whereas 13% of adult PTCs showed the mutations. Furthermore, the *TERT*-mutated adult PTCs were identified in significantly elder patients. Notably, Liu et al. demonstrated the close association between *TERT* promoter mutation and shorter telomere length of PTC cells, a biomarker of cell aging [23]. Taken together, our study with pediatric PTCs enhanced the evidence of the age dependence of *TERT* promoter mutations and suggested that *TERT* promoter mutation is a result of the aging tumor cells. The absence of *TERT* promoter mutations can have some implications on the prognosis of pediatric PTCs. As described above, our study showed that pediatric PTC is usually detected in advanced presentation. However, the prognosis of pediatric PTC has been reported to be generally favorable, and a critical case is quite rare [3, 7]. Since *TERT* promoter mutations are an independent indicator of a worse prognosis, the absence of *TERT* mutation rather than the presence or absence of the *BRAF* mutation status in pediatric PTCs may explain the paradox between clinical aggressiveness and overall survival in pediatric PTCs. Nonetheless, there were three pediatric PTCs with distant metastasis (one DSV- and two SFV-PTCs), and all they were negative for *BRAF* or *TERT* promoter mutation. This observation indicates that genetic biomarkers for clinical aggressiveness may be different between pediatric and adult PTCs. In pediatric PTCs, therefore, further exploration for a genetic biomarker should be done using advanced methods such as whole-exome-sequencing.

In conclusion, we examined a large series of Japanese pediatric PTCs and defined their clinicopathological, histopathological, and genetic features. In comparison to adults, pediatric PTC is characterized by more advanced clinicopathological features, a lower frequency of *BRAF* mutation, and complete absence of the *TERT* promoter mutations. $BRAF^{V600E}$ was significantly associated with classical PTC histology but did not affect the clinical aggressiveness. The lack of *TERT* promoter mutation may explain the favorable prognosis of pediatric PTCs. These findings outline the nature of radiation-independent pediatric PTCs in Japan.

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**Compliance with Ethical Standards**

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**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** All procedures in this study were in accordance with the ethical standards of the institutional research committee. For this type of study, formal consent is not required.

## References

1. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol 21 Suppl 2:S37-S43, 2008.
2. LiVolsi VA. Papillary thyroid carcinoma: an update. Mod Pathol 24 Suppl 2(S2):S1–S9, 2011.
3. Giuffrida D, Scollo C, Pellegriti G, Lavenia G, Iurato MP, Pezzin V, et al. Differentiated thyroid cancer in children and adolescents. J Endocrinol Invest 25:18–24, 2011.
4. Nikiforova MN, Ciampi R, Salvatore G, Santoro M, Gandhi M, Knauf JA, et al. Low prevalence of BRAF mutations in radiation-induced thyroid tumors in contrast to sporadic papillary carcinomas. Cancer Lett 209:1–6, 2004.
5. Penko K, Livezey J, Fenton C, Patel A, Nicholson D, Flora M, et al. BRAF mutations are uncommon in papillary thyroid cancer of young patients. Thyroid 15:320–325, 2005.
6. Rosenbaum E, Hosler G, Zahurak M, Cohen Y, Sidransky D, Westra WH. Mutational activation of BRAF is not a major event in sporadic childhood papillary thyroid carcinoma. Mod Pathol 18: 898–902, 2005.
7. Ito Y, Kihara M, Takamura Y, Kobayashi K, Miya A, Hirokawa M, et al. Prognosis and prognostic factors of papillary thyroid carcinoma in patients under 20 years. Endocr J 59:539–545, 2012.
8. Sassolas G, Hafdi-Nejjari Z, Ferraro A, Decaussin-Petrucci M, Rousset B, Borson-Chazot F, et al. Oncogenic alterations in papillary thyroid cancers of young patients. Thyroid 22:17–26, 2012.
9. Givens DJ, Buchmann LO, Agarwal AM, Grimmer JF, Hunt JP. BRAF V600E does not predict aggressive features of pediatric papillary thyroid carcinoma. Laryngoscope 124:E389-E393, 2014.
10. Henke LE, Perkins SM, Pfeifer JD, Ma C, Chen Y, DeWees T, et al. BRAF V600E mutational status in pediatric thyroid cancer. Pediatr Blood Cancer 61:1168–1172, 2014
11. Cordioli MICV, Moraes L, Cury AN, Cerutti JM. Are we really at the dawn of understanding sporadic pediatric thyroid carcinoma? Endocr Relat Cancer 22:R311–324, 2015.
12. Nikiforov YE, Erickson LA, Nikiforova MN, Caudill CM, Lloyd R V. Solid variant of papillary thyroid carcinoma: incidence, clinicalpathologic characteristics, molecular analysis, and biologic behavior. Am J Surg Pathol 25:1478–1484, 2001.
13. Sheu S-Y, Schwertheim S, Worm K, Grabellus F, Schmid KW. Diffuse sclerosing variant of papillary thyroid carcinoma: lack of BRAF mutation but occurrence of RET/PTC rearrangements. Mod Pathol 20:779–787, 2007.
14. Pillai S, Gopalan V, Smith R a, Lam AK-Y. Diffuse sclerosing variant of papillary thyroid carcinoma-an update of its clinicopathological features and molecular biology. Crit Rev Oncol Hematol 94:64–73, 2014
15. Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Nat Rev Cancer 6:292–306, 2006.
16. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 13:184–199, 2013.
17. The Cancer Genome Atral Research Network. Integrated Genomic Characterization of Papillary Thyroid Carcinoma. Cell 159:676–690, 2014.
18. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol. 7:569–580, 2011.

19. Kumagai A, Namba H, Saenko V a., Ashizawa K, Ohtsuru A, Ito M, et al. Low frequency of BRAFT1796A mutations in childhood thyroid carcinomas. J Clin Endocrinol Metab 89:4280–4284, 2004.
20. Onder S, Ozturk Sari S, Yegen G, Sormaz IC, Yilmaz I, Poyrazoglu S, et al. Classic Architecture with Multicentricity and Local Recurrence, and Absence of TERT Promoter Mutations are Correlates of BRAF V600E Harboring Pediatric Papillary Thyroid Carcinomas. Endocr Pathol 27:153–161, 2016
21. Cantwell-Dorris ER, O'Leary JJ, Sheils OM. BRAFV600E: implications for carcinogenesis and molecular therapy. Mol Cancer Ther 10:385–394, 2011.
22. Landa I, Ganly I, Chan T a., Mitsutake N, Matsuse M, Ibrahimpasic T, et al. Frequent Somatic TERT Promoter Mutations in Thyroid Cancer: Higher Prevalence in Advanced Forms of the Disease. J Clin Endocrinol Metab 98:E1562–1566, 2013.
23. Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, et al. The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene. 33:4978–4984, 2014.
24. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer.20:603–610, 2013.
25. Alzahrani AS, Qasem E, Murugan AK, Al-Hindi HN, AlKhafaji D, Almohanna M, et al. Uncommon TERT Promoter Mutations in Pediatric Thyroid Cancer. Thyroid. 26:235–241, 2016.
26. Cardis E, Howe G, Ron E, Bebeshko V, Bogdanova T, Bouville A, et al. Cancer consequences of the Chernobyl accident: 20 years on. J Radiol Prot. 26(2):127–40, 2006.
27. Mitsutake N, Fukushima T, Matsuse M, Rogounovitch T, Saenko V, Uchino S, et al. BRAFV600E mutation is highly prevalent in thyroid carcinomas in the young population in Fukushima: a different oncogenic profile from Chernobyl. Sci Rep 16976, 2015
28. Nagataki S, Takamura N. A review of the Fukushima nuclear reactor accident: radiation effects on the thyroid and strategies for prevention. Curr Opin Endocrinol Diabetes Obes 21:384–393, 2014.
29. LiVolsi VA, Albores-Saavedra J, Asa SL, Baloch ZW, Sobrinho-Simões M, Wenig B et al. Papillary carcinoma, in DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds): Pathology and genetics of tumours of endocrine organs. Lyon: IARC; 2004. pp 57–66.
30. Harach HR, Williams ED. Childhood thyroid cancer in England and Wales. Br J Cancer 72:777–783, 1995.
31. Warden DW, Ondrejka S, Lin J, Durkin L, Bodo J, Hsi ED. Phospho-ERK(THR202/Tyr214) is overexpressed in hairy cell leukemia and is a useful diagnostic marker in bone marrow trephine sections. Am J Surg Pathol 37:305–308, 2013
32. Oishi N, Kondo T, Mochizuki K. Localized Langerhans cell histiocytosis of the thymus with BRAF V600E mutation : a case report with immunohistochemical and genetic analyses. Hum Pathol 45:1302–1305, 2014
33. Xu B, Yoshimoto K, Miyauchi A, Kuma S, Mizusawa N, Hirokawa M, et al. Cribriform-morular variant of papillary thyroid carcinoma: a pathological and molecular genetic study with evidence of frequent somatic mutations in exon 3 of the beta-catenin gene. J Pathol. 199:58–67, 2003.
34. Kwon MJ, Rho Y-S, Jeong JC, Shin HS, Lee JS, Cho SJ, et al. Cribriform-morular variant of papillary thyroid carcinoma: a study of 3 cases featuring the PIK3CA mutation. Hum Pathol 46:1180–1188, 2015
35. Song YS, Lim JA, Park YJ. Mutation Profile of Well-Differentiated Thyroid Cancer in Asians. Endocrinol Metab 30:252–262, 2015.
36. Finkelstein A, Levy GH, Hui P, Prasad A, Virk R, Chhieng DC, et al. Papillary thyroid carcinomas with and without BRAF V600E mutations are morphologically distinct. Histopathology 60:1052–1059, 2012.
37. Koperek O, Kornauth C, Capper D, Berghoff AS, Asari R, Niederle B, et al. Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma. Am J Surg Pathol 36:844–850, 2012.
38. Virk RK, Theoharis CG a, Prasad A, Chhieng D, Prasad ML. Morphology predicts BRAFV600E mutation in papillary thyroid carcinoma: an interobserver reproducibility study. Virchows Arch 464:435–442, 2014.
39. Li C, Lee KC, Schneider EB, Zeiger MA. BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: a meta-analysis. J Clin Endocrinol Metab. 97:4559–4570, 2012.
40. Lim JY, Hong SW, Lee YS, Kim B-W, Park CS, Chang H-S, et al. Clinicopathologic implications of the BRAF(V600E) mutation in papillary thyroid cancer: a subgroup analysis of 3130 cases in a single center. Thyroid 23:1423–1430, 2013
41. Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA 309:1493–1501, 2013
42. Ito Y, Yoshida H, Maruo R, Morita S, Takano T, Hirokawa M, et al. BRAF mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. Endocr J 56:89–97, 2009
43. Gouveia C, Can NT, Bostrom A, Grenert JP, van Zante A, Orloff LA. Lack of association of BRAF mutation with negative prognostic indicators in papillary thyroid carcinoma: the University of California, San Francisco, experience. JAMA Otolaryngol Head Neck Surg 139:1164–1170, 2013
44. Li C, Aragon Han P, Lee KC, Lee LC, Fox AC, Beninato T, et al. Does BRAF V600E Mutation Predict Aggressive Features in Papillary Thyroid Cancer? Results From Four Endocrine Surgery Centers. J Clin Endocrinol Metab. 98:3702–3712, 2013
45. Cheng S, Serra S, Mercado M, Ezzat S, Asa SL. A High-Throughput Proteomic Approach Provides Distinct Signatures for Thyroid Cancer Behavior. Clin Cancer Res 17:2385–2394, 2011