Changes in the clinicopathological characteristics and genetic alterations of follicular thyroid cancer

Young Shin Song^{1,*}, Jung Ah Lim^{2,*}, Hye Sook Min³, Min Joo Kim¹, Hoon Sung Choi⁴, Sun Wook Cho¹, Jae Hoon Moon⁵, Ka Hee Yi⁶, Do Joon Park^{1,7}, Bo Youn Cho⁸ and Young Joo Park¹

¹Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, Korea,

²Department of Internal Medicine, National Medical Center, Seoul, Korea, ³Department of Pathology, Seoul National University

College of Medicine, Seoul, Korea, ⁴Department of Internal Medicine, Kangwon National University Hospital,

Chuncheon, Korea, ⁵Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam,

Korea, ⁶Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea,

⁷Korea National Institute of Health, Cheongju, Korea, and ⁸Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea

*(Y S Song and J A Lim contributed to this work)

Correspondence
should be addressed
to Y J Park

Email
yjparkmd@snu.ac.kr

Abstract

Objective: Changes in the clinicopathological characteristics and genetic alterations of follicular thyroid cancer (FTC) over time have not been reported. Moreover, the prognostic effects of *RAS* and *TERT* promoter mutations in FTC have not been clearly elucidated. We investigated changes in the clinicopathological characteristics of patients with FTC over four decades, as well as the clinical significance of genetic mutations of FTC.

Design and methods: This retrospective study included 690 patients with FTC who underwent thyroidectomy between 1973 and 2015 at the Seoul National University Hospital. In 134 samples, genetic tests for *NIH/KRAS* and *TERT* promoter mutations and *PAX8/PPAR γ* rearrangement were performed.

Results: The age at diagnosis has increased ($P < 0.001$) in recent decades and extrathyroidal extension of the tumor has become less common ($P = 0.033$). Other clinicopathological characteristics and prognosis of FTC have not significantly changed. The prevalence of *RAS* mutations decreased ($P = 0.042$) over time, whereas that of *TERT* promoter mutations remained stable. *RAS* mutations were associated with distant metastasis and persistent disease, and *TERT* promoter mutations were associated with distant metastasis, advanced TNM stage, recurrence and disease-specific mortality. FTC patients with coexistent *RAS* and *TERT* promoter mutations showed a higher recurrence risk than those with only one mutation.

Conclusions: The age at diagnosis of FTC and the frequency of extrathyroidal extension have changed over four decades. Moreover, the prevalence of *RAS* mutations decreased. *RAS* and *TERT* promoter mutations may be associated with poor clinical outcomes in FTC, especially when the two mutations coexist.

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Introduction

Follicular thyroid cancer (FTC) is the second most common type of thyroid malignancy following papillary thyroid cancer (PTC), which accounts for 10%–32% of cases of differentiated thyroid cancer (DTC) (1, 2). In the last decades, the incidence of thyroid cancer has

sharply increased worldwide. This trend was especially predominant for small PTC, which shows less aggressive features and favorable outcomes (3, 4). However, long-term trends in the characteristics and outcomes of FTC, distinct from other types of thyroid cancer, have not been reported.

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Several previous studies have presented temporal changes in the mutational frequencies associated with PTC. In the United States, the overall prevalence of *BRAF* mutations was stable, but increased from 50.0% to 76.9% in conventional PTC over the last four decades (5). Moreover, *RAS* mutations increased from 2.7% to 24.9% due to an increase in follicular-variant PTC (FVPTC). In Europe, the frequency of *BRAF* mutations increased gradually from 28.0% to 58.1% over the last 15 years (6). The incidence of the *RET/PTC* rearrangement, in contrast, decreased from 33.0% to 9.8% over the same period. In Korea, which is a *BRAF* mutation-prevalent country, *BRAF*-mutated PTCs increased from 62.2% to 73.7% over the last two decades (7). However, no study has evaluated changes in the mutational frequencies of FTC over time.

The most frequent genetic alterations in FTC are *RAS* mutations and the *PAX8/PPAR γ* rearrangement (8). The prognostic value of *RAS* mutations is still unclear, although some evidence suggests that *RAS*-mutated FTCs may be at risk for a poor prognosis (9, 10) or distant metastasis (11, 12). Moreover, no evidence exists linking the *PAX8/PPAR γ* rearrangement with clinical outcomes. However, recent studies have demonstrated that *TERT* promoter mutations, especially when they coexist with *BRAF* mutations, are associated with a poor prognosis in PTC (13). Regarding the prognostic effect of the coexistence of *TERT* promoter and *RAS* mutations, we recently demonstrated for the first time that their coexistence may increase the risk of disease-specific mortality and recurrence in DTC, including both PTC and FTC (14). However, the corresponding effect in FTC only has not been analyzed.

In this study, we investigated changes in the clinicopathological characteristics, prognosis and mutational profiles of FTC over four decades, as well as associations between genetic alterations and clinicopathological outcomes.

Patients and methods

Patients and tissue samples

This study included 690 consecutive patients with FTC (544 females and 146 males) who underwent thyroidectomy at Seoul National University Hospital (Seoul, Korea) between 1973 and 2015, and analyzed long-term trends in clinicopathological characteristics and prognoses. The median follow-up duration was 6.9 years (interquartile range, 3.6–11.6 years). The diagnosis of FTC was based on the postoperative pathology report, and clinicopathological data at baseline and during follow-up

were collected retrospectively from their electronic medical records.

Among the FTC patients who underwent surgery in 1997–2003 and 2009–2012, genetic analyses were performed of 134 patients (67 patients in each period) whose formalin-fixed paraffin-embedded (FFPE) DNA samples were available, including 119 patients from our previous report (14). For the mutational analysis, all tissue samples were reviewed by an expert pathologist who specializes in thyroid pathology, and the FTC tumor region of the samples was microdissected for subsequent DNA extraction. Pathological diagnoses were made according to the latest World Health Organization classifications for thyroid cancer (15). This study was conducted according to the guidelines of the Declaration of Helsinki. The research protocol was approved by the institutional review board committee of the Seoul National University Hospital (H-1207-124-420).

Mutational analyses

All samples of the FFPE tumor block were digested with proteinase K (Sigma) for more than 24 h at 56°C, and DNA was then isolated from the digested tissue using a Tissue SV Mini kit (General Biosystem Inc., Seoul, Korea). *NRAS* (codon 12/13 and codon 61), *HRAS* (codon 12/13 and codon 61), *KRAS* (codon 12/13 and codon 61) and *TERT* promoter (C228T and C250T) mutations were examined using polymerase chain reaction (PCR) and amplified using appropriate primers (Supplementary Table 1, see section on supplementary data given at the end of this article). PCR was performed using a BioMix kit (Bioline, Taunton, MA, USA). Purified PCR products obtained using a QIAquick Gel Extraction kit (Qiagen) were used for sequencing with a Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The sequences were analyzed using ABI Prism 3730 Genetic Analyzer (Applied Biosystems). Each DNA sample was assayed at least twice in order to confirm its *RAS* and *TERT* promoter mutation status by sequencing with both forward and reverse primers. *RAS* mutation status was assessed in all 134 samples, but *TERT* promoter mutation status was only assessed in 120 samples because of PCR failure in 14 samples. To evaluate the *PAX8/PPAR γ* rearrangement, we performed fluorescence *in situ* hybridization (FISH) analysis in FFPE tumor tissues using the *PPAR γ* (3p25) break probe (Kreatech Diagnostics, Amsterdam, The Netherlands). These commercially available probes are designed as a dual-color probe where the two regions cross the break-point (Supplementary Fig. 1). The FISH analysis

could only be done in 48 cases (2010–2012), depending on the staining and preservation status of FFPE.

Statistical analyses

Data are presented as frequencies and percentages, as means and standard deviations or as medians and interquartile range. Categorical variables were compared with the Pearson chi-square test or the Fisher exact test (if the number was <5). The independent *t* test or the Wilcoxon–Mann–Whitney test was used for continuous variables. In order to compare trends of the clinicopathological characteristics of FTC over three periods, the linear-by-linear association test was used for categorical variables and analysis of variance for continuous variables. The log-rank test was used to compare variables based on the time of events. Cox proportional hazards regression was used to assess the risk of recurrence. Statistical significance was defined as two-sided *P* values <0.05. All statistical analyses were performed with SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Results

Changes in clinicopathological characteristics and outcomes of FTC patients between 1973 and 2015

The 690 patients with FTC were classified into three groups by the calendar year of diagnosis: 1973–1995 (*n*=68), 1996–2005 (*n*=231), and 2006–2015 (*n*=391; [Table 1](#)). The mean age of patients in 1973–1995 was significantly younger than those in other periods (*P*<0.001), but there was no difference in age after 1995. Most of histological characteristics, including the size and proportion of widely invasive FTC, vascular invasion, multiplicity and lymph node metastasis, did not change over time. However, extrathyroidal extension, especially gross invasion, has decreased recently (*P*=0.033). Although the overall incidence of distant metastasis decreased from 14.7% to 5.1%, no significant difference was found in the survival analysis using the time from the beginning to the occurrence of an event (log-rank *P*=0.346). Moreover, the percentage of patients with distant metastasis at the initial presentation did not significantly vary over time. The most frequent sites of distant metastasis were lung and bone throughout the period. Unilateral lobectomy alone was common in 1973–1995, after which total thyroidectomy or lobectomy with completion thyroidectomy became more frequent, although there was no significant temporal trend (*P*=0.179). The proportion

of patients who received radioactive iodine (RAI) therapy, especially as a remnant ablation, increased over time (*P*<0.001), but the total dose of RAI significantly decreased (*P*<0.001). During the median follow-up of 6.9 years, 9.0% of the patients experienced persistent disease or tumor recurrence, and 2.2% died from FTC. No significant differences were found among time periods in the proportion of patients with no evidence of disease, persistence, recurrence or disease-specific mortality.

Prevalence of *RAS* and *TERT* promoter mutations and the *PAX8/PPAR γ* rearrangement in FTC

None of the characteristics of the 134 patients who underwent genetic analysis were significantly different from the overall samples of the corresponding period ([Supplementary Table 2](#)). Among them, 43 (32.1%) and 7 (5.8%) patients had tumors with *RAS* and *TERT* promoter mutations respectively ([Table 2](#)). The prevalence of *RAS* mutations decreased from 40.3% to 23.9% (*P*=0.042), and the *HRAS* codon 61 mutation mainly contributed to this decrease. The *NRAS* codon 61 mutation was most common in both periods. The codon 12/13 mutations of *NRAS*, *HRAS* and *KRAS* were not found. *TERT* promoter mutations in each period were found in three (4.5%) and four (7.4%) cases respectively (*P*=0.699). The major type of *TERT* promoter mutation was *TERT* C228T in both periods, although *TERT* C250T was observed in one case in 2009–2012. The *PAX8/PPAR γ* rearrangement was found in one (2.1%) case in 2009–2012.

Clinicopathological characteristics and outcomes of FTC according to mutational status

Comparing the clinicopathological features of FTC according to *RAS* mutational status ([Table 3](#)), distant metastasis occurred more frequently in *RAS*-mutated FTCs (log-rank *P*=0.030), even though other features did not differ between *RAS* wild-type and *RAS*-mutated FTCs. *RAS* mutations were significantly associated with a lower frequency of no evidence of disease (log-rank *P*=0.011) and a higher proportion of persistent disease (*P*=0.037). However, no differences in recurrence or disease-specific mortality were found.

TERT promoter mutations were significantly associated with distant metastasis (log-rank *P*=0.001), in particular to the lung (log-rank *P*=0.006), and advanced TNM stage (*P*=0.045; [Table 4](#)). Furthermore, the patients with a *TERT* promoter mutation tended to have a poor

Table 1 Clinicopathological characteristics of patients with follicular thyroid cancer, 1973–2015.

	Year			P ^a
	1973–1995	1996–2005	2006–2015	
Cases	68	231	391	
Follow-up duration, years ^b	21.4 (13.2–26.4)	11.7 (6.6–14.0)	4.9 (2.7–7.2)	<0.001
Age at diagnosis, years	39.0 ± 11.1	45.2 ± 14.2 ^c	46.3 ± 14.3 ^c	0.516
Male sex, <i>n</i> (%)	16 (23.5)	41 (17.7)	89 (22.8)	0.972
Body mass index, kg/m ²	23.9 ± 5.1	23.8 ± 3.6	23.7 ± 6.2	0.467
Tumor size, cm	3.4 ± 2.2	3.4 ± 1.8	3.2 ± 1.8	0.729
Widely invasive FTC, <i>n</i> (%)	7 (10.3)	37 (16.0)	48 (12.3)	0.419
Vascular invasion, <i>n</i> (%)	14 (20.6)	39 (16.9)	63 (16.1)	0.221
Multiplicity, <i>n</i> (%)	11 (16.2)	41 (17.7)	52 (13.3)	0.033
Extrathyroidal extension, <i>n</i> (%)	5/29 (17.2)	32/179 (17.9)	42/382 (11.0)	<0.001
Microscopic/gross	1/4 (3.4/13.8)	1/31 (0.6/17.3)	33/9 (8.6/2.4)	0.823
Resection margin, <i>n</i> (%)	1 (1.5)	6 (2.6)	7 (1.8)	0.574
Lymph node metastasis, <i>n</i> (%)	0 (0.0)	8 (3.5)	10 (2.6)	0.346 ^d
Distant metastasis, <i>n</i> (%)	10 (14.7)	26 (11.3)	20 (5.1)	0.420
At initial presentation	4 (5.9)	12 (5.2)	16 (4.1)	
During follow-up	6 (8.8)	14 (6.1)	4 (1.0)	
5 years/10 years	4/5 (5.9/7.4)	18/22 (7.8/9.5)	18/NA (4.6/NA)	
Site of distant metastasis, <i>n</i> (%)				
Lung	9 (90.0)	17 (65.4)	12 (60.0)	
Bone	5 (50.0)	19 (73.1)	15 (75.0)	
Mediastinum	0 (0.0)	3 (11.5)	2 (10.0)	
Brain	1 (10.0)	2 (7.7)	0 (0.0)	
Others	2 (20.0)	4 (15.4)	4 (20.0)	0.179
Type of thyroidectomy, <i>n</i> (%)				
Total thyroidectomy	6 (8.8)	90 (39.0)	152 (38.9)	
Subtotal thyroidectomy	10 (14.7)	27 (11.7)	11 (2.8)	
Lobectomy	46 (67.6)	48 (20.8)	100 (25.6)	
Lobectomy and completion	6 (8.8)	66 (28.6)	128 (32.7)	
RAI treatment, <i>n</i> (%)	22 (32.4)	111 (48.1)	233 (59.6)	<0.001
Remnant ablation	14 (20.6)	86 (37.2)	213 (54.5)	
For distant metastasis	8 (11.8)	25 (10.8)	20 (5.1)	
Total dose of RAI, mCi ^b	200 (110–858)	120 (60–390) ^c	60 (60–130) ^c	<0.001
No evidence of disease, <i>n</i> (%)	57 (83.8)	202 (87.4)	369 (94.4)	0.317 ^d
Persistence, <i>n</i> (%)	3 (4.4)	12 (5.2)	15 (3.8)	0.568
Recurrence, <i>n</i> (%)				
Overall	8 (11.8)	17 (7.4)	7 (1.8)	0.399 ^d
5 years/10 years	0/1 (0.0/1.5)	7/14 (3.0/6.1)	4/NA (1.0/NA)	
Disease-specific mortality, <i>n</i> (%)				
Overall	5 (7.4)	9 (3.9)	1 (0.3)	0.177 ^d
5 years/10 years	0/0 (0.0/0.0)	5/8 (2.2/3.5)	1/NA (0.3/NA)	

^aP values from chi-square test for trend (categorical variables) and from analysis of variance (continuous variables); ^bValues presented as median (interquartile range); ^cSignificantly different from subjects in 1973–1995; ^dLog-rank P values.

FTC, follicular thyroid cancer; NA, not applicable; RAI, radioactive iodine.

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Table 2 Changes of mutational frequencies in follicular thyroid cancer between 1997–2003 and 2009–2012.

	Year		P
	1997–2003	2009–2012	
Cases	67	67	
RAS mutation, n (%)	27 (40.3)	16 (23.9)	0.042
NRAS codon 61	19 (28.4)	14 (20.9)	
HRAS codon 61	8 (11.9)	1 (1.5)	
KRAS codon 61	0 (0.0)	1 (1.5)	
N/H/KRAS codon 12, 13	0 (0.0)	0 (0.0)	
TERT promoter mutation, n (%)	3/66 (4.5)	4/54 (7.4)	0.699
C228T	3/66 (4.5)	3/54 (5.6)	
C250T	0/66 (0.0)	1/54 (1.9)	
PPAR γ rearrangement, n (%)	NA	1/48 (2.1)	NA

NA, not applicable.

prognosis and were less likely to remain disease-free (log-rank $P=0.001$). The percentage of patients with persistent disease was higher in the TERT promoter mutation group, although this was not statistically significant due to its low incidence. Moreover, the overall recurrence and disease-specific mortality rates in TERT promoter wild-type vs mutant samples were significantly different (2.7% vs 28.6% and 0.9% vs 14.3%, respectively) (log-rank P , for recurrence=0.002; for disease-specific mortality=0.007).

The only patient who showed the PAX8/PPAR γ rearrangement was a 39.1-year-old man at diagnosis who had an 8.0-cm minimally invasive FTC without lymph node or distant metastasis. He underwent a lobectomy with completion thyroidectomy following RAI therapy (120mCi) for remnant ablation and showed no tumor recurrence during 7.4 years of follow-up.

Table 3 Clinicopathological characteristics and outcomes of follicular thyroid cancer according to the mutational status of RAS.

	RAS		P
	Wild type	Mutant	
Cases, n (%)	91 (67.9)	43 (32.1)	
Follow-up duration, years ^a	6.5 (4.5–13.3)	6.1 (4.5–13.4)	
RAS mutation type, n (%)			
N/H/KRAS	–	33/9/1 (24.6/6.7/0.7)	0.002
Age at diagnosis, years	45.9 $\pm$ 14.4	42.7 $\pm$ 13.0	0.223
Male sex, n (%)	21 (23.1)	9 (20.9)	0.781
Body mass index, kg/m ²	24.4 $\pm$ 3.6	23.9 $\pm$ 3.9	0.470
Tumor size (cm)	3.6 $\pm$ 2.0	3.2 $\pm$ 1.3	0.267
Widely invasive FTC, n (%)	16 (17.6)	7 (16.3)	0.852
Vascular invasion, n (%)	20 (22.0)	7 (16.3)	0.443
Multiplicity, n (%)	11 (12.1)	5 (11.6)	0.939
Extrathyroidal extension, n (%)	15 (17.9)	6 (15.8)	0.779
Microscopic/gross	6/9 (7.1/10.7)	1/5 (2.6/13.2)	
Resection margin, n (%)	2 (2.2)	2 (4.7)	0.593
Lymph node metastasis, n (%)	1 (1.1)	2 (4.7)	0.241
Distant metastasis, n (%)	3 (3.3)	6 (14.0)	0.030 ^b
At initial presentation	0 (0.0)	3 (7.0)	0.031
During follow-up	3 (3.3)	3 (7.0)	
5 years/10 years	2/2 (2.2/2.2)	3/4 (7.0/9.3)	
Site of distant metastasis, n (%)			
Lung	3 (3.3)	3 (7.0)	0.385
Bone	1 (1.1)	3 (7.0)	0.097
Other sites	1 (1.1)	1 (2.6)	
TNM stage, n (%)			0.644
I–II/III–IV	73/18 (80.2/19.8)	33/10 (76.7/23.3)	
No evidence of disease, n (%)	88 (96.7)	36 (83.7)	0.011 ^b
Persistence, n (%)	1 (1.1)	4 (9.3)	0.037
Recurrence, n (%)			
Overall	2 (2.2)	3 (7.0)	0.195 ^b
5 years/10 years	1/2 (1.1/2.2)	1/2 (2.3/4.7)	
Disease-specific mortality, n (%)			
Overall	1 (1.1)	1 (2.3)	0.572 ^b
5 years/10 years	1/1 (1.1/1.1)	1/1 (2.3/2.3)	

^aValues presented as median (interquartile range).

^bLog-rank P values.

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Table 4 Clinicopathological characteristics and outcomes of follicular thyroid cancer according to the mutational status of *TERT* promoter.

	<i>TERT</i>		<i>P</i>
	Wild type	Mutant	
Cases, <i>n</i> (%)	113 (94.2)	7 (5.8)	
Follow-up duration, years ^a	6.7 (4.9–13.5)	6.4 (4.5–14.3)	
<i>TERT</i> mutation type, <i>n</i> (%)			
C228T/C250T	–	6/1 (5.0/0.8)	0.002
Age at diagnosis, years	44.6 ± 14.2	51.9 ± 15.7	0.191
Male sex, <i>n</i> (%)	24 (21.2)	2 (28.6)	0.644
Body mass index, kg/m ²	24.3 ± 3.8	22.6 ± 3.5	0.290
Tumor size (cm)	3.5 ± 1.8	4.8 ± 2.6	0.071
Widely invasive FTC, <i>n</i> (%)	22 (19.5)	1 (14.3)	1.000
Vascular invasion, <i>n</i> (%)	22 (19.5)	3 (42.9)	0.157
Multiplicity, <i>n</i> (%)	13 (11.5)	1 (14.3)	0.590
Extrathyroidal extension, <i>n</i> (%)	21 (20.8)	0 (0.0)	0.341
Microscopic/gross	7/14 (6.9/13.9)	0/0 (0.0/0.0)	
Resection margin, <i>n</i> (%)	4 (3.5)	0 (0.0)	1.000
Lymph node metastasis, <i>n</i> (%)	1 (0.9)	1 (14.3)	0.114
Distant metastasis, <i>n</i> (%)	6 (5.3)	3 (42.9)	0.001 ^b
At initial presentation	2 (1.8)	1 (14.3)	0.166
During follow-up	4 (3.5)	2 (28.6)	
5 years/10 years	4/4 (3.5/3.5)	1/2 (14.3/28.6)	
Site of distant metastasis, <i>n</i> (%)			
Lung	4 (3.5)	2 (28.6)	0.039
Bone	3 (2.7)	1 (14.3)	0.216
Other sites	1 (0.9)	1 (14.3)	
TNM stage, <i>n</i> (%)			0.045
I–II/ III–IV	90/23 (79.6/20.4)	3/4 (42.9/57.1)	
No evidence of disease, <i>n</i> (%)	106 (93.8)	4 (57.1)	0.001 ^b
Persistence, <i>n</i> (%)	4 (3.5)	1 (14.3)	0.263
Recurrence, <i>n</i> (%)			0.002 ^b
Overall	3 (2.7)	2 (28.6)	
5 years/10 years	2/2 (1.8/1.8)	0/1 (0.0/14.3)	
Disease-specific mortality, <i>n</i> (%)			0.007 ^b
Overall	1 (0.9)	1 (14.3)	
5 years/10 years	1/1 (0.9/0.9)	1/1 (14.3/14.3)	

^aValues presented as median (interquartile range).

^bLog-rank *P* values.

Association between the *RAS* and *TERT* promoter mutations and the effect of their coexistence on FTC recurrence

A significant association of *RAS* mutations with *TERT* promoter mutations was observed (*P*=0.045; [Supplementary Table 3](#)). *TERT* promoter mutations were found in 2.5% of *RAS* wild-type cases vs 12.2% of

RAS-mutated cases, and conversely, *RAS* mutations were found in 31.9% of *TERT* wild-type cases vs 71.4% of *TERT*-mutated cases.

The patients with a *RAS* mutation, especially with coexistent *RAS* and *TERT* promoter mutations, showed higher rates of recurrence or persistence than those without any mutations ([Table 5](#)). Two patients had a *TERT*

Table 5 Hazard ratios of *RAS* and *TERT* promoter mutations for recurrence of follicular thyroid cancer.

Mutation status	Cases	Recurrence/persistence, <i>n</i> (%)	Recurrence, <i>n</i> (%)	HR	Unadjusted		<i>P</i>	Adjusted ^a		<i>P</i>
					95% CI			HR	95% CI	
<i>RAS</i> (–) <i>TERT</i> (–)	77	3 (3.9)	2 (2.6)	1				1		
<i>RAS</i> (+) <i>TERT</i> (–)	36	4 (11.1)	1 (2.8)	1.13	0.10–12.46	0.922		2.86	0.21–39.57	0.433
<i>RAS</i> (–) <i>TERT</i> (+)	2	0 (0.0)	0 (0.0)	–				–		
<i>RAS</i> (+) <i>TERT</i> (+)	5	3 (60.0)	2 (40.0)	13.59	1.88–98.55	0.010		6.27	0.76–51.68	0.088

^aAdjustment for age at diagnosis and sex; HR, hazard ratio.

promoter mutation alone, and FTC recurrence was not observed in them. The coexistence of two mutations had the highest recurrence rate and increased the recurrence risk 6.27-fold compared to the absence of mutations, although statistical significance was lost after adjustment for age at diagnosis and sex.

Discussion

The age at the diagnosis of FTC increased in the late 1990s, and the extrathyroidal extension of tumors significantly decreased recently. Moreover, the prevalence of *RAS* mutations also decreased in recent years, while that of *TERT* promoter mutations did not change. *RAS* mutations were associated with distant metastasis, persistent disease and frequent *TERT* promoter mutations. The coexistence of *RAS* and *TERT* promoter mutations was associated with a higher rate of recurrence, suggesting that they had additive effects on the prognosis, similarly to *BRAF* and *TERT* promoter mutations (13, 14).

The increase in small and low-risk PTCs has been attributed to the recent increase in thyroid cancer screening with ultrasonography (3, 16). In our study, the older age at the diagnosis of FTC after the late 1990s might be attributed to the introduction of a cancer screening program in the late 1990s. However, most other clinicopathological characteristics, including tumor size, were unchanged over time. This is because FTC usually does not have specific suspicious features of malignancy on ultrasonography (17), and in such cases, fine-needle aspiration (FNA) is usually performed when the nodular size is larger than 1–2 cm (18, 19). Furthermore, because FNA cannot distinguish between follicular adenoma and carcinoma on the basis of cytology, surgery is not immediately performed in some wait-and-see cases. However, a possible improvement in the aggressiveness of FTC was found; the total frequency of extrathyroidal extension decreased despite recent advances in pathologic evaluation. Recent increases in microscopic extensions might be related to more cautious pathologic examinations; however, gross extensions became markedly less common in the most recent decade, which may suggest a prognostic improvement in recently diagnosed FTCs. We propose more long-term observations to clarify this.

Recently, next-generation sequencing (NGS) has improved our understanding of the genetics and biology of thyroid cancer, including FTC and PTC (20, 21, 22), and *RAS* point mutations are the most representative

driver mutations in FTC. As the results of our study, *RAS* mutations have been reported to be associated with distant metastasis (11, 12) and poor prognoses in FTC (9, 10). This prognostic impact of *RAS* mutations on FTC seems to be in contrast to the favorable prognosis of PTC with *RAS* mutations, which is usually FVPTC without aggressive tumor behavior (23, 24). Moreover, a similar frequency of *RAS* mutations was also observed in follicular adenoma (20, 25). Therefore, we suggest that the same *RAS* mutations might play different prognostic roles depending on the type of cancer.

Interestingly, as was found for *BRAF* mutations in PTC, *RAS* mutations were also associated with *TERT* promoter mutations in FTC, and their coexistence was associated with worse prognoses. *TERT* promoter mutations have not been detected in benign thyroid nodules, and have only uncommonly been found in cancer with a benign nature (26). In this study, patients with *TERT*-mutated FTC showed more frequent instances of distant metastasis, and higher recurrence and mortality. Although we could not demonstrate the effects of *TERT* promoter mutations alone, when they coexisted with a *RAS* mutation, the hazard ratio of recurrence was increased by more than 6 times (Table 5). Thus, *TERT* could be a useful prognostic marker of malignancy, especially for *RAS*-mutated follicular neoplasm.

The prevalence of the *PAX8/PPAR γ* rearrangement has been found to vary geographically, ranging from 0% to 57% of FTCs (27); notably, a Japanese study failed to identify a single *PAX8/PPAR γ* rearrangement in FTC (28). Its prevalence in Asia has been reported to be lower than that in other regions, but this is not conclusive because of the small number of studies in Asia. In this study, we observed a similarly low frequency of the *PAX8/PPAR γ* rearrangement in FTC (2.1%).

Regarding other oncogenes of FTC not tested in this study, Nikiforova *et al.* (29) tested 12 cancer genes using targeted sequencing panel in 36 FTCs and identified that the second most common mutations after *RAS* ($n=12$) were *TSHR* ($n=4$) and *TP53* ($n=4$) mutations in conventional and oncocytic FTCs, respectively. However, in the two recently published studies of FTC genomics in Korea using a NGS approach, including a study of our institute (20, 22), the prevalence of *TSHR* and *TP53* mutations were relatively very low: there was no FTC patient harboring *TSHR* mutation in both studies, and only one of 30 patients (3.3%) with *TP53* mutation who had a favorable prognosis in our study (20). Moreover, according to the recent NGS studies on anaplastic or poorly differentiated thyroid cancer (30, 31), *TP53*, unlike *TERT*, might be

related to the aggressiveness of undifferentiated thyroid cancer rather than well-differentiated thyroid cancer.

Our study had some limitations. Genetic testing was not performed in consecutive patients because of the limited availability of FFPE samples (14). Nevertheless, the characteristics of FTC samples performed genetic testing were not significantly different from the overall samples of FTC in the corresponding periods of our study, which could be representative. Regarding the histologic report, some FVPTC cases could have been included in the past, because FVPTC was first described in 1953 (32) and broadly recognized in the mid-1970s (33, 34), and more detailed examinations have been performed in recent decades.

Notwithstanding these limitations, to the best of our knowledge, this study is the first comprehensive study of the long-term changes in the characteristics and prognosis of FTC. In addition, the clinicopathological variables were extensively examined in all subjects, which might be useful for evaluating the effects of *RAS* and *TERT* promoter mutations on the clinical outcomes of FTC.

In conclusion, the age at diagnosis of FTC and the frequency of extrathyroidal extension have changed over the last four decades. Moreover, the prevalence of *RAS* mutations decreased. *RAS* mutations may be associated with poor clinical outcomes in FTC, especially in coexistence with *TERT* promoter mutations.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-17-0456>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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