The Increase in Thyroid Cancer Incidence During the Last Four Decades Is Accompanied by a High Frequency of *BRAF* Mutations and a Sharp Increase in *RAS* Mutations

Chan Kwon Jung, Mark P. Little, Jay H. Lubin, Alina V. Brenner, Samuel A. Wells, Jr., Alice J. Sigurdson, and Yuri E. Nikiforov

Department of Pathology (C.K.J., Y.E.N.), University of Pittsburgh, Pittsburgh, Pennsylvania 15261; Department of Hospital Pathology (C.K.J.), The Catholic University of Korea, Seoul 137–701, Republic of Korea; and Radiation Epidemiology and Biostatistics Branches (J.H.L., A.V.B., M.P.L., A.J.S.), Division of Cancer Epidemiology and Genetics and Cancer Genetics Branch (S.A.W.), National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Context: Thyroid cancer incidence rates in the United States and globally have increased steadily over the last 40 years, primarily due to a tripling of the incidence of papillary thyroid carcinoma (PTC).

Objective: The purpose of this study was to analyze trends in demographic, clinical, pathologic, and molecular characteristics of PTC from 1974 to 2009.

Design and Setting: We identified and histologically reviewed 469 consecutive cases of PTC from one US institution from 4 preselected periods (1974 to 1985, 1990 to 1992, 2000, and 2009) and assessed *BRAF* and *RAS* point mutations and *RET*/*PTC* rearrangements among 341 tumors ≥0.3 cm in size. Changes over time were analyzed using polytomous and binary logistic regression; all analyses were adjusted for age and sex.

Results: During this period, the median age of patients at diagnosis increased from 37 to 53 years ($P < .001$) and the percentage of microcarcinomas (≤1.0 cm) increased from 33% to 51% ($P < .001$), whereas extrathyroidal extension and advanced tumor stage decreased from 40% to 21% ($P = .005$) and from 43% to 28% ($P = .036$), respectively. Changes in tumor histopathology showed a decrease in classic PTC and an increase in the follicular variant ($P < .001$). The proportion of tumors with a *BRAF* mutation was stable (~46%) but increased from 50% to 77% ($P = .008$) within classic papillary PTCs. The proportion of tumors with *RAS* mutations increased from 3% to 25% and within follicular pattern tumors from 18% to 44% ($P < .001$). The proportion of *RET*/*PTC* rearrangements decreased from 11% to 2% ($P = .038$).

Conclusions: Similar to US national trends, we found an increasing age at diagnosis and greater detection of smaller-sized intrathyroidal PTCs. However, the overall proportion of *BRAF* mutations remained stable. Sharply rising percentages of the follicular variant histology and *RAS* mutations after 2000 suggest new and more recent etiologic factors. The increased incidence is not likely to be due to environmental or therapeutic radiation because the percentage of *RET*/*PTC* rearrangements decreased. **(*J Clin Endocrinol Metab* 99: E276–E285, 2014)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received June 11, 2013. Accepted November 5, 2013.
First Published Online November 18, 2013

Abbreviations: CI, confidence interval; OR, odds ratio; PTC, papillary thyroid carcinoma; SEER, Surveillance, Epidemiology and End Results.

The incidence of thyroid cancer has increased over the past 4 decades in many countries, including the United States (1–5). This increase is primarily due to the rise in the incidence of papillary thyroid cancer (PTC), whereas the rates of follicular, anaplastic, and medullary thyroid cancer have not changed markedly (2, 3, 6). Data from the Surveillance, Epidemiology and End Results (SEER) program indicate that the age-adjusted incidence rate of PTC in the United States in 2005 to 2006 was 9.25 per 100,000, a 3.2-fold increase since 1973 to 1974 when the incidence was 2.88 per 100,000 (7). Over the past 5 years, thyroid cancer rates in the United States have been growing faster than those of any other cancer type (8).

Reasons for the rising incidence of PTC remain unknown. About half of the increase in PTC rates occurred in tumors ≤1.0 cm, with another 30% for tumors of 1.1 to 2 cm and 20% for tumors of >2.0 cm (9), so the increase is not likely to be entirely due to a greater use of ultrasound and fine needle aspiration biopsy to detect small tumors. Because exposure to ionizing radiation during childhood is a well-established risk factor for thyroid cancer (10, 11), it is conceivable that the rising incidence may reflect increasing use of medical radiation procedures, such as diagnostic computed tomography scans (12, 13). Other possible temporally relevant risk factors include body mass index, smoking, environmental chemicals, and reproductive patterns (9). Finally, incidence rates may also be affected by changes in the histopathologic criteria for the encapsulated follicular variant of PTC, which have gradually become more permissive during the last 20 years (14).

The molecular pathogenesis of PTC involves several known mutational events, including point mutations in the *BRAF* and *RAS* genes and chromosomal rearrangements of the *RET* gene called *RET/PTC*. These genetic alterations lead to activation of the MAPK signaling pathway (15), and one of these mutations is found in approximately 70% of all PTCs (16). Studies have linked different mutational types with specific etiologic factors for thyroid cancer. *RET/PTC* chromosomal rearrangements have been associated with radiation exposure (Chernobyl, atomic bomb survivors in Japan, and radiotherapy), whereas *BRAF* and *RAS* point mutations are rare in radiation-associated cancer and are more likely to result from chemical carcinogenesis, possibly in association with high dietary iodine levels or other environmental exposures (15, 17, 18). Therefore, tracking changes in mutational profiles over time may provide clues for understanding the increase in thyroid cancer incidence. In addition, *BRAF* mutations are associated with invasive and clinically more aggressive PTCs (19). Thus, changes in the proportions of *BRAF* mutations may have important long-term clinical implications.

To our knowledge, this is the first study to examine temporal changes in mutational profiles and standardized histopathologic features of thyroid cancer in the United States over the last 4 decades, which provides novel insights into the increasing incidence of thyroid cancer.

Patients and Methods

Study patients and tumor samples

The study protocol was approved by the institutional review boards of the University of Pittsburgh and the National Cancer Institute. A total of 469 cases with a histologic diagnosis of PTC were retrieved from the Department of Pathology, University of Pittsburgh Medical Center in 4 preselected time periods: 1974 to 1985 (n = 127), 1990 to 1992 (n = 59), 2000 (n = 53), and 2009 (n = 230). More cases were selected from the earliest and latest periods to increase the power for identifying temporal trends. Cases were chosen consecutively within each of the preselected time periods until the numeric target based on a priori sample size calculations was fulfilled. Clinical and demographic information was abstracted from pathology reports. Two pathologists (C.K.J. and Y.E.N.) reviewed histologic slides to verify and standardize the histologic diagnosis, tumor variants, and growth patterns using the current World Health Organization criteria (20). Follicular variant PTCs were subclassified into encapsulated and infiltrative types (21). The TNM classification system was used for pathologic staging (22). Out of 469 selected cases, 38 had no paraffin blocks available and a further 70 had tumors <0.3cm. Of the 361 remaining cases with tumors ≥0.3 cm in size, 20 contained an insufficient amount of residual tumor tissue, and, therefore, molecular analyses were conducted for 341 tumors. The respective breakdown of cases by time period was as follows: 1974–1985 (n = 89), 1990–1992 (n = 41), 2000 (n = 40), and 2009 (n = 171).

DNA isolation

Five 5-μm unstained paraffin sections were used for manual microdissection of tumor tissue. Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (QIAGEN). For paraffin blocks stored for longer than 10 years, digestion with proteinase K was extended from 16 hours to 24 to 48 hours.

Mutational analysis for *BRAF* and *RAS* genes

Mutation screening for *BRAF* codons 599, 600, and 601, *NRAS* codon 61, *HRAS* codon 61, and *KRAS* codons 12 and 13 was performed using real-time LightCycler PCR (Roche) and fluorescence melting curve analysis as reported previously (23). PCR amplification of DNA from old archival tissues was extended from 40 to 45 cycles. All samples positive for mutations on melting curve analysis were confirmed by bidirectional sequencing on an ABI 3100 sequencer using the Big Dye Terminator Kit (Applied Biosystems) (23).

Detection of *RET/PTC* rearrangements

RET/PTC1 and *RET/PTC3* rearrangements were detected by dual-color interphase fluorescence in situ hybridization. BAC

clones RP11–351D16, RP11–369L1, and RP11–481A12 were used to generate probes for the *RET*, *CCDC6* (*H4*) and *NCOA4* (*ELE1*) genes, respectively. The *RET* probe was labeled with Green-dUTP using a nick translation kit (Vysis). Either the *CCDC6* or the*NCOA4* probe was labeled with Orange-dUTP (Vysis). Hybridization was performed, and slides were scored as described previously (24). Tumors with ≥9% of cells with 3 *RET* signals were considered positive for *RET/PTC* rearrangement (24). All positive cases were then hybridized with probes for either *RET* and *CCDC6* or *RET* and *NCOA4* to identify the type of *RET/PTC* rearrangement.

Statistical analysis

We assessed demographic, pathologic, and molecular changes, ie, trends, over the 4 time periods in several ways. We used standard polytomous regression analyses to evaluate changes over time for outcomes with multiple levels (tumor size, histopathology, and others). Because no obvious outcome level represents a referent "control," we adjusted parameter estimates (β) and resulting odds ratios (ORs) ([exp(β)]) to reflect relative deviations from no (ie, 0) trend across calendar years (Table 1). In addition, for presentation purposes, ORs were scaled to represent changes per 10 years over the study period. The ORs allow easier interpretation of trends; ORs >1.00 denote increasing trends over time, ORs <1.00 denote decreasing trends over time, and ORs equal to 1.00 denote consistency with no changes over time. OR confidence intervals (CIs) cannot be readily computed for the polytomous regression contrasts given in Table 1. However, as below, 95% CI are given for the polytomous regressions with assigned reference categories and for the bivariate logistic regressions reported in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). The *P* value for heterogeneity reflects a potential significant difference in trends over time across outcome levels. When the test of heterogeneity was borderline significant ($P \leq .10$), the selected outcome levels were compared, and the *P* values specific to each comparison were calculated (as in the analyses of tumor size, histopathology, and lymph node stage). All polytomous regression analyses were repeated by setting one arbitrarily chosen outcome level as a referent. In addition, we repeated regression analyses for outcomes with multiple levels by categorizing them into binary outcomes (one level vs all others combined). For binary outcomes, polytomous regression reduces to binary logistic regression. In this simplest case, if the proportion at one outcome level increases over time, then the proportion at the other outcome level must decrease. We present the results and details of polytomous regression models with assigned referent levels as well as bivariate logistic models in Supplemental Table 1. Tests of trend and heterogeneity of trends in the polytomous regression models and in the bivariate logistic models were derived from the likelihood ratio test (25). Epicure software (Hirosoft International) was used for binary regression analyses, whereas SPSS software (IBM SPSS version 21.0; IBM Corp) was used for polytomous logistic regression analyses and R software (R version 3.0.1, http://www.r-project.org/) was used for linear regression analyses of continuous outcomes, eg, age. Certain analyses with very small numbers of outcomes were analyzed also, using exact logistic regression methods, via LogXact 10 (Cytel, Inc). All tests were two-sided; unless otherwise noted, all analyses were adjusted for age and sex. See the Supplemental Methods for additional information.

Results

Clinical and pathologic changes during 4 time periods

The median age at diagnosis for PTC in female and male patients increased from 38 and 34 years in 1974–1985 to 51 and 56 years in 2009 ($P < .001$) (Table 1). Although there was no significant variation in tumor size over time overall ($P = .100$) (Table 1), the trends for tumors >1.0 cm vs ≤1.0 cm were significantly different ($P = .012$) (Supplemental Table 1). This finding is consistent with a significant increase in microcarcinomas (tumors ≤1.0 cm) from 33% to 51% and a corresponding significant decrease in larger tumors over time (Supplemental Table 1). The age trends by tumor size (adjusted for period and sex) are shown in Supplemental Table 2. As can be seen, these are borderline significant for the group of tumors ≤1.0 cm vs >1 cm ($P = .056$), with relative risk increasing with age (relative risk = 1.14/10 years; 95% CI, 1.01 to 1.30) and otherwise not. There was a significant relative variation in the histopathology of PTCs over time both when microcarcinomas were considered as a separate group ($P < .001$) (Table 1 and Supplemental Table 1) and when these were reclassified according to the histological growth pattern ($P < .001$) (Table 1 and Supplemental Table 1). In both instances, the variation observed was due to a highly significant relative decrease in the percentage of the classic type of PTC ($P < .001$) and a corresponding significant relative increase in the follicular variant of PTC ($P < .001$) (Figure 1, A and B). The increase in follicular variant PTC over time involved not only the encapsulated follicular variant ($P = .002$), for which diagnostic criteria changed over time, but also the infiltrative follicular variant ($P < .001$), for which diagnostic criteria remained unchanged (Figure 1C).

Other significant changes over time included a reduction in the percentage of extrathyroidal tumor extension ($P = .005$) (Table 1), an increase in the percentage of tumors of stage 1 to 2 ($P = .036$, Table 1), and an increase in the percentage of tumors with no lymph node metastases (N0) ($P < .001$, Table 1) with a corresponding decrease in the number of tumors with no information on lymph node metastases (NX). If changes were restricted to classic papillary or follicular growth pattern PTCs (irrespective of size), similar patterns of calendar year variation of N0 and NX stages were observed (data not shown). However, the frequency of tumor multifocality did not significantly vary over time ($P = .793$) (Table 1). Notably, the trends in histopathologic characteristics of PTCs were not due to a change in the extent of histopathologic analysis of thyroidectomy specimens over time, because the number of thyroid tissue blocks and sections examined per

Table 1. Trends in Clinical and Pathologic Characteristics of PTC Over Time, University of Pittsburgh Medical Center 1974–2009

Characteristic	Time Period				Scaled OR[a]	*P*[b]
	1974–1985 (n = 127)	1990–1992 (n = 59)	2000 (n = 53)	2009 (n = 230)		
Median age, y (range)						
All	37 (10–77)	51 (12–78)	50 (20–82)	53 (15–89)	NA	<.001
Female	38 (10–77)	41 (12–78)	50 (20–82)	51 (19–89)		<.001
Male	34 (18–66)	56 (23–77)	52 (28–66)	56 (15–78)		<.001
Sex, n (%)						
Female	92 (72.4)	43 (72.9)	46 (86.8)	176 (76.5)	1.08	.113[c]
Male	35 (27.6)	16 (27.1)	7 (13.2)	54 (23.5)	0.93	
Tumor size, n (%)						
≤1.0 cm	42 (33.1)	26 (44.1)	22 (41.5)	118 (51.3)	1.16	
1.1–2.0 cm	48 (37.8)	13 (22.0)	13 (24.5)	59 (25.7)	0.94	
2.1–3.0 cm	21 (16.5)	9 (15.3)	9 (17.0)	21 (9.1)	0.86	.100
3.1–4.0 cm	7 (5.5)	6 (10.2)	4 (7.5)	16 (7.0)	1.06	
>4.0 cm	9 (7.1)	5 (8.5)	5 (9.4)	16 (7.0)	0.99	
Histologic variants, n (%)						
Classic papillary	66 (52.0)	25 (42.4)	20 (37.7)	43 (18.7)	0.72	
Follicular	13 (10.2)	2 (3.4)	7 (13.2)	58 (25.2)	1.53	
Microcarcinoma[d]	42 (33.1)	26 (44.1)	22 (41.5)	118 (51.3)	1.12	<.001
Tall cell	1 (0.8)	5 (8.5)	0 (0.0)	8 (3.5)	1.06	
Others	5 (3.9)	1 (1.7)	4 (7.5)	3 (1.3)	0.76	
Histologic growth pattern, n (%)						
Classic papillary	97 (76.4)	31 (58.5)	29 (56.9)	82 (36.3)	0.79	
Follicular	23 (18.1)	16 (30.2)	13 (25.5)	128 (56.6)	1.51	<.001
Microcarcinoma[e]	0 (0.0)	6 (10.2)	2 (3.8)	4 (1.7)	0.88	
Tall cell	1 (0.8)	5 (8.5)	4 (7.5)	12 (5.2)	0.79	
Others	6 (4.7)	1 (1.7)	5 (9.4)	4 (1.7)	1.21	
Tumor multifocality, n (%)						
Single	71 (55.9)	34 (57.6)	32 (60.4)	124 (53.9)	0.99	.793
Multiple	56 (44.1)	25 (42.4)	21 (39.6)	106 (46.1)	1.01	
Extrathyroidal extension, n (%)						
Negative	76 (59.8)	43 (72.9)	38 (73.1)	178 (79.1)	1.14	.005
Positive	51 (40.2)	16 (27.1)	14 (26.9)	47 (20.9)	0.88	
Tumor stage, n (%)[f]						
T1–2	72 (56.7)	40 (67.8)	34 (65.4)	164 (71.9)	1.09	
T3–4	55 (43.3)	19 (32.2)	18 (34.6)	64 (28.1)	0.91	.036
Node stage, n (%)						
N0	21 (16.5)	9 (15.3)	14 (26.4)	88 (38.3)	1.39	
N1	34 (26.8)	17 (28.8)	11 (20.8)	42 (18.3)	0.91	<.001
NX	72 (56.7)	33 (55.9)	28 (52.8)	100 (43.5)	0.79	

Abbreviation: NA, not applicable.

[a] The scaled OR ([exp(β)]) represents the changes per 10 years over the study period. The ORs for the trends represent relative departures from no trend with calendar time. ORs and CIs for other logistic models with slightly different assignments for referent groups are provided in Supplemental Table 1.

[b] Unless otherwise described, *P* for linear trend is adjusted for age and gender.

[c] Adjusted for age only.

[d] Microcarcinoma is a tumor ≤1.0 cm in size.

[e] The growth pattern for these microcarcinomas could not be determined.

[f] Three tumors of unknown stage were excluded.

sample were similar in all time periods (mean, 10.0–12.8 sections per case).

Based on the review of surgical pathology reports, a clinical history of ionizing radiation exposure was more frequently recorded in 1974–1985 (19%) than in other time periods (eg, in 2009 [1.3%]) ($P < .001$, data not shown). There was a significant increase in the percentage of patients with a recorded clinical history of hyperparathyroidism over time (0.8%–8.3%, $P = .006$, data not shown).

Mutational analysis

A total of 341 tumors (72.7%) were ≥0.3 cm in size and had sufficient residual tumor tissue for mutational anal-

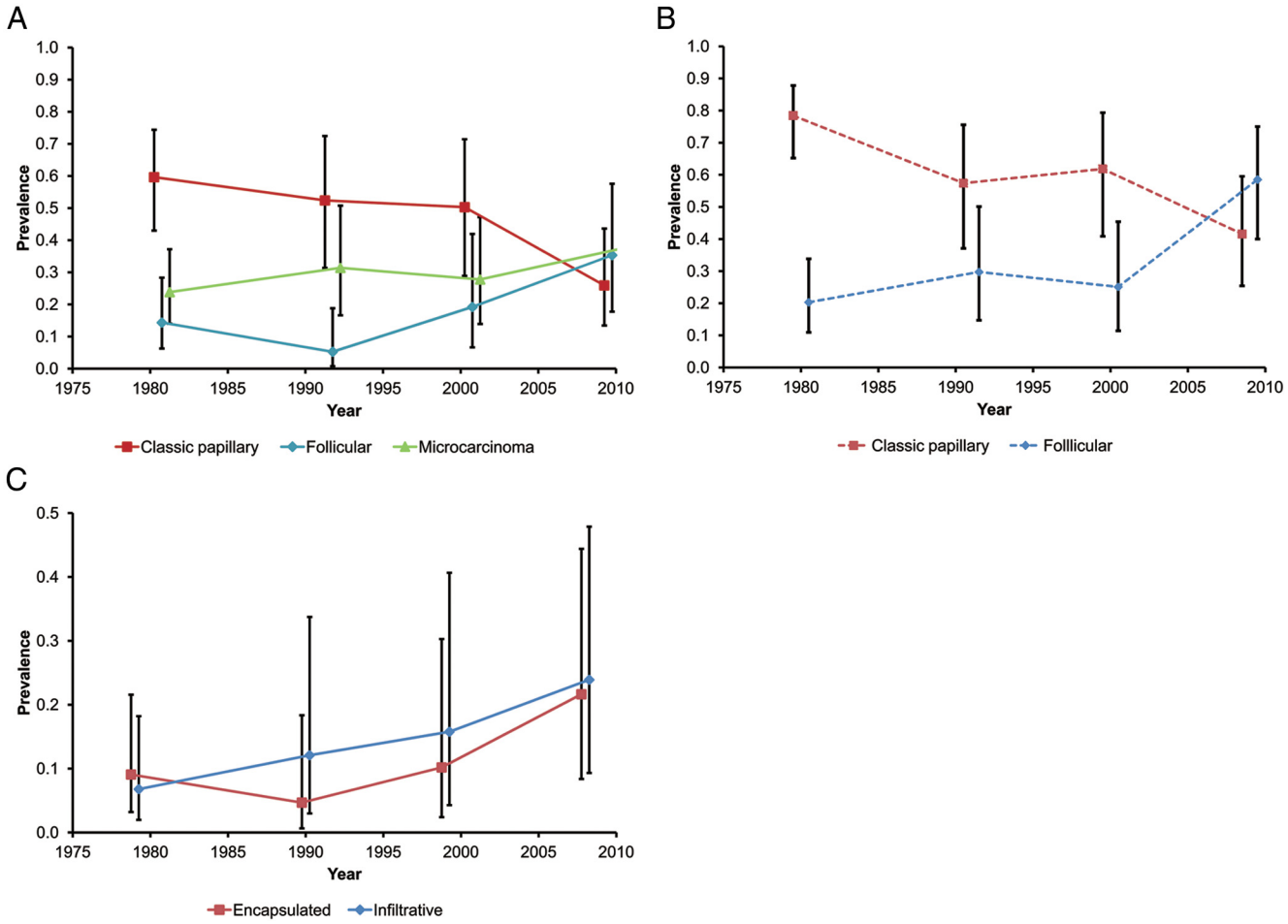

Figure 1. Time trends in the age- and sex-adjusted prevalence (with 95% CI) of specific histopathologic types of PTC. A, Time trends for the most common types of PTC: classic papillary, follicular variant, and microcarcinoma defined as tumor ≤1 cm in size. B, Time trends for classic papillary and follicular variant of PTC irrespective of tumor size, ie, with microcarcinomas reclassified based on their growth pattern. C, Time trends for encapsulated and infiltrative types of the follicular variant of PTC (University of Pittsburgh Medical Center, 1974–2009).

ysis, including 89 (70.1%) from 1974 to 1985, 41 (69.5%) from 1990 to 1992, 40 (75.5%) from 2000, and 171 (74.3%) from 2009. In the 1974–1985 time period, the analysis was successful in 92% for *HRAS*, 89% for *BRAF*, 78% for *NRAS*, 46% for *KRAS*, and 79% for *RET/PTC*. In the more recent time periods, >95% of cases were successfully analyzed for all mutations. All mutations detected in PTCs were mutually exclusive and nonoverlapping.

Trends in *BRAF* mutations

We identified 151 *BRAF* mutations in 329 cases (Table 2), including 149 V600E and 2 cases with double mutations, one T599R and V600E and one T599I and K601E. The percentage of *BRAF* mutations overall was stable over time, fluctuating between 41% and 58% (Table 2 and Figure 2). Further analysis revealed that the percentage of *BRAF* mutations in classic PTCs significantly increased from 50% to 77% ($P = .008$) (Table 3), whereas there was no change in the percentage of *BRAF* mutations in the follicular variant of PTC ($P = .827$) (Table 3). When classic variant *BRAF*-positive PTCs were separated by size, there was no evidence that the percentage of microcarcinomas within this tumor type changed significantly over time ($P = .583$) (Table 3).

Trends in *RAS* mutations

RAS mutations occurred in 46 of 329 cases, including 33 *NRAS*, 11 *HRAS*, and 2 *KRAS* mutations (Table 2). The *RAS* mutation prevalence increased sharply in the most recent calendar period ($P < .001$) (Table 2 and Figure 2), due primarily to the increase in *NRAS* mutations. Overall, 43 of 46 PTCs (93.5%) with *RAS* mutations had a follicular growth pattern. Over time, the percentage of *RAS* mutations increased exclusively in the follicular variant ($P < .001$) (Table 3). Although based on small numbers, there was a suggestion that the proportion of microcarcinomas in the follicular variant *RAS*-positive PTCs decreased over time ($P = .036/P_{exact} = 0.093$) (Table 3), whereas the proportion of infiltrative tumors increased ($P = .039/P_{exact} = 0.075$) (Table 3).

Table 2. Changes in Prevalence of Somatic Mutations in PTC Over Time, University of Pittsburgh Medical Center, 1974–2009

	Time Period				
Mutation	**1974–1985**	**1990–1992**	**2000**	**2009**	***P*[a]**
BRAF, n (%)	34/79 (43.0)	24/41 (58.5)	23/40 (57.5)	70/169 (41.4)	.273
RAS (total), n (%)	2/68 (2.9)	1/41 (2.4)	1/40 (2.5)	42/169 (24.9)	<.001
HRAS	1/82 (1.2)	1/41 (2.4)	1/40 (2.5)	8/169 (4.7)	.102[b]
NRAS	1/69 (1.4)	0/40 (0.0)	0/40 (0.0)	32/169 (18.9)	<.001
KRAS	0/41 (0.0)	0/39 (0.0)	0/40 (0.0)	2/169 (1.2)	.099[b]
RET/PTC (total), n (%)	8/70 (11.4)	2/33 (6.1)	2/38 (5.3)	4/169 (2.4)	.038
RET/PTC1	6/70 (8.6)	1/33 (3.0)	2/38 (5.3)	3/169 (1.8)	.127
RET/PTC3	2/70 (2.9)	1/33 (3.0)	0/38 (0.0)	1/169 (0.6)	.153

[a] *P* value for linear trend adjusted for age and sex. Results were obtained by fitting binomial logistic models via maximum likelihood. Comparisons are for mutation present vs all others.

[b] Adjusted for age only.

Trends in *RET/PTC* rearrangement

RET/PTC rearrangements occurred in 16 of 310 tumors, including 12 tumors with *RET/PTC1* and 4 tumors with *RET/PTC3* rearrangements (Table 2). The prevalence of *RET/PTC* rearrangement decreased over time ($P = .038$) (Table 2 and Figure 2). Stratifying by histologic growth pattern (Table 3), 10 (62.5%) tumors with *RET/PTC* rearrangements had a classic papillary growth pattern, whereas 4 (25%) had the follicular growth pattern.

Discussion

The incidence of PTC, the most common type of thyroid cancer, has been increasing in the United States and in many other countries since the early 1970s (1–5). Consistent with US national trends (2, 3, 6), we found a continuously increasing age at thyroid cancer diagnosis and a greater prevalence of low-stage, smaller-sized, intrathyroidal PTCs. Specifically, we observed a significant increase in tumors ≤1.0 cm in size, which now represents the most common presentation of thyroid cancer in the United States (26). The reduction in tumor size correlates with an increase in the percentage of intrathyroidal tumors, which lack direct invasion outside of the thyroid gland and metastatic spread to lymph nodes. The latter trend may be partially attributed to the increased rate of lymph node dissection among patients without clinical node involvement.

This study included careful histopathologic evaluation of glass slides from >450 tumors from different time periods. The analysis revealed a sharp decrease in the proportion of classic papillary type and an increase in the follicular variant. This finding is consistent with a previous US report based on the SEER database, which found a significant, progressive increase in the incidence of the follicular variant of PTC over time (3, 27). However, our finding differs from the study of PTC diagnosed in several regions of Italy that found no change in the proportions of PTC variants over time (28). The reasons for the different patterns of trends in the two studies are unclear but may be related to the fact that the Italian study included cases diagnosed during a later period, 1996 to 2000 (28). Microscopic criteria for the noninvasive, encapsulated follicular variant of PTC became less stringent during the early1990s, so that some tumors previously diagnosed as benign follicular adenomas would be now classified as the follicular variant of PTC

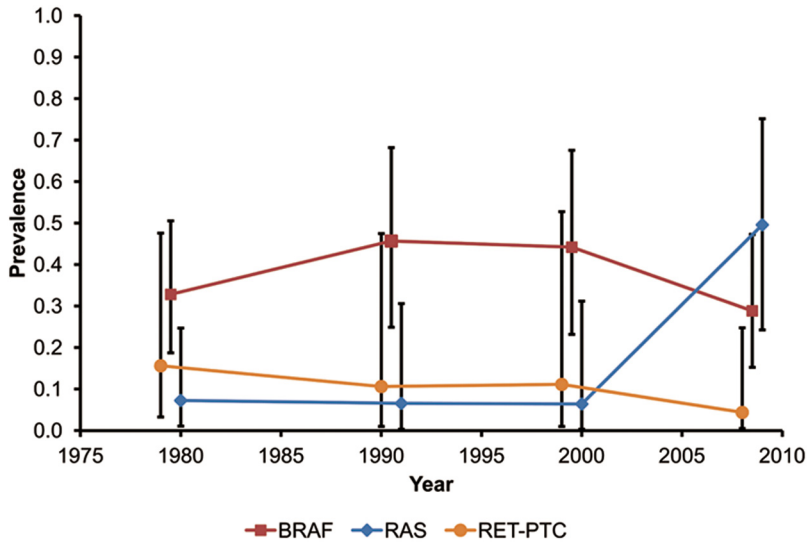

Figure 2. Time trends in the age- and sex-adjusted prevalence (with 95% CI) of the three mutation types in papillary thyroid carcinoma (University of Pittsburgh Medical Center, 1974–2009).

Table 3. Trends in Prevalence of *BRAF* and *Ras* Mutations by PTC Growth Pattern, University of Pittsburgh Medical Center, 1974–2009

	Time Period				
	1974–1985	1990–1992	2000	2009	*P*[a]
BRAF, n (%)					
Classic papillary[b]	31/62 (50.0)	16/22 (72.7)	17/24 (70.8)	50/65 (76.9)	.008
Microcarcinoma[c]	10/31 (32.3)	3/16 (18.8)	4/17 (23.5)	19/50 (38.0)	.583
Follicular[b]	0/12 (0.0)	3/13 (23.1)	0/9 (0.0)	9/88 (10.2)	.827
RAS (total), n (%)					
Classic papillary	0/52 (0.0)	0/21 (0.0)	0/24 (0.0)	1/65 (1.5)	NA
Follicular	2/11 (18.2)	1/13 (7.7)	1/9 (11.1)	39/88 (44.3)	<.001
Microcarcinoma[c]	2/2 (100.0)	1/1 (100.0)	0/1 (0.0)	15/39 (38.5)	.036/.093[e]
Infiltrative[d]	1/2 (50.0)	0/3 (0.0)	0/3 (0.0)	18/29 (62.1)	.039/.075[e]
RET/PTC (total), n (%)					
Classic papillary	6/52 (11.5)	1/18 (5.6)	2/23 (8.7)	1/65 (1.5)	.141
Follicular	0/12 (0.0)	1/9 (11.1)	0/8 (0.0)	3/88 (3.4)	NA

Abbreviation: NA, not applicable.

[a] *P* value for linear trend adjusted for age and sex. Results were obtained by fitting binomial logistic models via maximum likelihood. Comparisons are for mutation present vs absent.

[b] Histologic types were either classic papillary or follicular. The microcarcinomas have been assigned to their respective type; for 7 microcarcinomas the histologic type could not be determined so some numbers do not exactly match those in Table 2.

[c] Microcarcinomas (≤1.0 cm) within *BRAF*-positive classic papillary PTCs and within *RAS*-positive follicular variant PTCs were analyzed separately.

[d] *RAS*-positive tumors within infiltrative type follicular variant PTCs were analyzed separately.

[f] Mid *P* value for exact linear trend.

(14). Applying uniform criteria to all time periods, we found an increase in both infiltrative and encapsulated follicular variants over time. This finding suggests that, at least for infiltrative follicular variant of PTC, the increase cannot be totally explained by changes in histological criteria and that new etiologic factors may have influenced the rising incidence of tumors with the follicular growth pattern.

Our molecular analyses revealed three important trends in the mutational composition of PTCs over time: (1) the overall proportion of *RAS* point mutations increased significantly after 2000, and this was entirely due to increases in the follicular variant of PTC, (2) the proportion of *BRAF* mutations was largely stable over the entire time period, although it increased significantly within the classic papillary type of PTC, particularly after 1985, and (3) the proportion of *RET/PTC* rearrangements significantly decreased. These findings provide etiologic insights into the increasing incidence of thyroid cancer. In thyroid cancer, critical genes are known to be altered via two distinct mutational mechanisms: point mutation and chromosomal rearrangement. Whereas the former derives from a single nucleotide change within the DNA chain, the latter represents a large-scale genetic abnormality, with breakage and fusion of parts of the same or different chromosomes. These two mutational mechanisms are associated with different etiologic factors. Specifically, thyroid cancer–specific chromosomal rearrangements have a strong association with ionizing radiation exposure. As many as 80% of PTCs in persons exposed to radiation either accidentally (mostly radioiodine) or therapeutically (mostly external beam) carry *RET/PTC* (29–31). In contrast, point mutations of *BRAF* and *RAS* are less common in radiation-related tumors (32). For PTCs found in atomic bomb survivors in Japan, the *RET/PTC* prevalence increased monotonically with increasing radiation dose, whereas *BRAF* point mutations showed an inverse relation (33, 34). Childhood exposure to external ionizing radiation is a well-established risk factor for thyroid cancer (10). During the 1940s and 1950s, ionizing radiation was sometimes used for medical treatment of tinea capitis, enlarged tonsils, and an enlarged thymus gland. In our study, patients from the time period 1974 to 1985 were younger at diagnosis (median age, 37 years) than those in other time periods, and 19% had a history of childhood radiation exposure recorded in pathology reports compared with less than 2% in 2009. The decreasing frequency of *RET/PTC* over time suggests that the increasing incidence of thyroid cancer was not likely to be due to ionizing radiation exposure, at least in the form of environmental and therapeutic radiation known to be associated with *RET/PTC* rearrangement.

The increase in the frequency of *RAS* mutations within the follicular variant PTC suggests the potential for new non-radiation–associated etiologies for this tumor type. *RAS* mutation–driven carcinogenesis has been associated with exposure to environmental chemical carcinogens in experimental animals and in human tumors (35–40). Ex-

amples of the latter include *RAS* mutations in hepatocellular carcinomas in workers exposed to vinyl chloride (41), in lung adenocarcinomas associated with tobacco smoking (42), and in acute leukemias in patients with occupational exposure to chemical agents or in their children (43, 44). In our data, the increase in *RAS* mutations began after 2000, which suggests a more recent etiologic factor, distinct from those factors affecting the increase starting in the early 1970s, but coinciding in time with an acceleration in the rate of increases in PTCs in US women and men aged 45 years and older (1).

An important finding in this study was the overall stable prevalence of *BRAF* mutations and the increase in *BRAF* among tumors of the classic papillary type of PTC, particularly after 1985. A similar increasing trend in *BRAF* prevalence in classic PTCs during 1991 to 2005 was observed in another region of the United States (45) and during 1996 to 2010 in several regions of Italy (28), suggesting a universal trend, although in our study this trend started earlier. The significance of this finding is 2-fold. First, the continuously high percentage of *BRAF* point mutations overall, together with the increasing percentage of *BRAF* mutations within classic PTCs during the period of increasing incidence of thyroid cancer, supports a role for environmental factors, perhaps dietary or chemical influences. These factors may be distinct from those associated with the increase in *RAS*-positive follicular variant PTCs. Indeed, a recent study of *BRAF* mutations in PTCs in China showed a 2-fold increased risk of *BRAF* V600E mutations in regions with high iodine levels in drinking water compared with that in regions with normal iodine content in drinking water (46). Another recent study reported a significantly higher incidence of *BRAF* mutations in PTCs from the volcanic region of Sicily, where there are high concentrations of boron, iron, vanadium, manganese, and other chemical elements in drinking water, compared with that in neighboring regions with normal concentrations of these elements (47). It is important to note that the Hawaiian Islands, another volcanic region, has one of the highest incidence rates of thyroid cancer in the United States (48), although high fish and iodine intake may also be factors. Second, the stable occurrence of *BRAF* mutations coupled with the stable proportion of microcarcinomas within *BRAF*-positive classic variant PTCs suggests that the growing incidence of thyroid cancer is not derived solely from better detection of incidental, nonprogressing tumors because *BRAF* V600E mutations are known to occur in association with aggressive histopathologic features of PTCs, increased tumor recurrence, and tumor-related mortality (49, 50). The stable prevalence of *BRAF* mutations raises the possibility that the increase in cancer incidence probably involves clinically relevant tumors, which are detected and removed at early stages, preventing the progression to more aggressive disease.

This study has several limitations. First, 25% to 30% of tumors in each time period were ≤0.3 cm and therefore had insufficient material for mutation analysis. However, the demographic and clinical characteristics of these patients were generally similar to those of patients with larger tumors. Second, our case series was derived from one institution, and therefore our results may not be representative of the entire United States. However, demographic and tumor features (age at diagnosis, sex, and tumor size) of these patients with PTCs were generally consistent with SEER thyroid cancer cases, and some of the molecular findings were similar to those reported previously in other regions of the United States (45). This study was based on cases selected consecutively within noncontiguous time periods. Therefore, it is possible that some fluctuations could have been missed, although we believe that such a design was adequate to reveal major time trends. Only cases with an original diagnosis of PTC were selected for this study; consequently, the proportion of follicular variant PTCs, particularly of the encapsulated type that in the early calendar period could have been classified as follicular adenoma or carcinoma, may be underestimated and the trend overestimated. However, because the increase in follicular variant PTCs was apparent for both encapsulated and infiltrative type tumors (not affected by changes in diagnostic criteria over time) and increases in *RAS*-positive follicular PTCs were also evident in infiltrative type tumors, the observed time trends are likely to be real.

In conclusion, our results suggest that the increase in the incidence of PTC over the last 40 years was characterized by increased age at diagnosis and detection of smaller-sized, intrathyroidal PTCs, which nevertheless possesses the potential for progression evident from the common, stable occurrence of *BRAF* mutations. A rapid increase in tumors with the follicular growth pattern and in the prevalence of *RAS* mutations suggests new or more recent etiologic factors, probably of a chemical or dietary nature. In contrast, the trends for *RET/PTC* rearrangements suggest that the role of ionizing radiation, at least as related to environmental exposures and historical patterns of radiation treatment for benign conditions, may be diminishing.

Acknowledgments

We are grateful to Dr. Elaine Ron (1943–2010) for her vision and untiring work in launching this project. Her efforts and her mem-

ory were with us throughout and were integral in bringing it to fruition.

Address all correspondence and requests for reprints to: Dr. Yuri Nikiforov, Department of Pathology, University of Pittsburgh, 200 Lothrop Street, PUH, Room C-606, Pittsburgh, PA 15213. E-mail: nikiforovye@upmc.edu.

This work was supported by the Intramural Research Program of the National Cancer Institute (NCI) Division of Cancer Epidemiology and Genetics and NCI contract HSSN261200900017C/N02CP-90017 with subcontract 5–312-0212208 to the University of Pittsburgh.

Disclosure Summary: The authors have nothing to disclose.

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