The *ret*/PTC Mutations Are Common in Sporadic Papillary Thyroid Carcinoma of Children and Young Adults*

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ABSTRACT

The *ret*/PTC rearrangements (PTC-1, PTC-2, and PTC-3) are characteristic of papillary thyroid cancer (PTC). In adults, PTC-1 is common and may be associated with an aggressive clinical course. The incidence and significance of *ret*/PTC mutations are less well understood in children. We examined spontaneous PTC from 33 patients (23 females and 10 males) with a median age of 18 yr (range, 6–21 yr) and a median follow-up of 3.5 yr (range, 0–13.4 yr). The *ret*/PTC mutations were identified in 15 tumors (45%), including 8 PTC-1 (8 of 15, 53%), 2 PTC-2 (2 of 15, 13%), 2 PTC-3 (2 of 15, 13%), and 3 (3 of 15, 20%) combined PTC mutations (PTC-1 and PTC-2). This distribution is significantly different ($P = 0.001$, by χ^2 analysis) from that reported for children with radiation-induced PTC. There was no correlation between the presence or type of *ret*/PTC mutation and patient age, tumor size, focality, extent of disease at diagnosis, or recurrence. We conclude that *ret*/PTC mutations are 1) common in sporadic childhood PTC, 2) predominantly PTC-1, 3) frequently multiple, and 4) of different distribution than that reported for children with radiation-induced PTC. (*J Clin Endocrinol Metab* **85:** 1170–1175, 2000)

T HE THYROID-SPECIFIC oncogene *ret*/PTC was first described in 1987, when a rearranged form of the *RET* protooncogene was shown to transform NIH-3T3 cells (1, 2). *RET* rearrangements were subsequently identified in a subset of adult papillary thyroid carcinoma (PTC) and were recently identified as important features of PTC following the Chernobyl nuclear accident (3).

The frequency of *ret*/PTC rearrangements has varied according to the geographic area. The highest prevalence was reported in Italy (33–35%) and the lowest in Saudi Arabia (2.5%) and Japan (0–9%) (2, 4–8). However, most of these studies examined only a small number of adult cases and made no reference to childhood tumors (5). Motomura *et al.* recently reported that 71% of sporadic PTC from children in the United States and 87% of PTC from children living in radiation-contaminated areas of Belarus contain rearrangements of the *ret* oncogene (2, 9).

The majority of their cases (n = 57) were children with radiation-induced PTC that were found to contain predominantly PTC-3 mutations. There were only 17 children with spontaneous PTC, and in these PTC-1 was predominant. No correlation with clinical outcome was reported for either group, although it has been generally recognized that radiation-induced PTC following the Chernobyl nuclear accident is more aggressive (4, 6). These data indicate that mutations producing *ret*/PTC-3 rearrangements are common events in radiation-induced PTC in children (8). However, none of these previous studies correlated the presence of *ret*/PTC mutations to the clinical outcome of individual patients. Furthermore, the optimal control group of children who developed nonradiation-related, sporadic PTC remains small (n = 17). For these reasons, the current study was undertaken to examine the incidence of *ret*/PTC mutations, the type of *ret*/PTC mutations, and the clinical outcome for a cohort of 33 sporadic PTC that presented during childhood or adolescence.

Materials and Methods

This study received prior approval from the human use committee, Department of Clinical Investigation, Walter Reed Army Medical Center (Washington, DC).

Clinical data

The centralized tumor registry for the Department of Defense (ACTUR) was searched for all individuals diagnosed with thyroid cancer before 21 yr of age. Original medical records were abstracted to construct a clinical database maintained by the principal investigator (10). The extent of disease at diagnosis was defined according to the classification system of DeGroot *et al.* (11). Disease class I was confined to the thyroid gland. Disease class II involved the regional lymph nodes.

Disease class III had either direct extension beyond the thyroid capsule or inadequate surgical resection. Disease class IV involved distant metastasis to bone or lung. Recurrence was defined as the appearance of new disease (new radioactive iodine uptake or biopsy-proven disease) in any patient who had been free of disease (no disease detected by palpation or radioactive iodine scan) for at least 4 months. The clinical data for several of these patients [137 with PTC and 33 with follicular thyroid cancer (FTC)] have been previously reported (10).

The corresponding original paraffin-embedded tissue blocks were available for 53 of these patients. Slides were stained with hematoxylin and eosin and were reviewed to establish the diagnoses. We were unable to extract ribonucleic acid (RNA) from 9 tissue blocks, leaving a final number of 44 cases in this study. This included 33 patients (75%) with sporadic PTC, 1 patient (2.3%) with radiation-associated PTC, 8 patients (18.1%) with FTC, 1 patient (2.3%) with medullary thyroid cancer (MTC), and 1 patient (2.3%) with intrathyroidal lymphoma (LYM).

Only 1 patient (no. 10) had a history of previous radiation exposure. This patient was treated with external beam radiation for a diagnosis of Hodgkin's lymphoma at the age of 8 yr. His thyroid cancer was first detected 11 yr later. A thorough review of the remaining cases revealed nothing to suggest radiation exposure in any other patient. Because the emphasis in this study is on sporadic PTC, the data from the single patient with radiation exposure are presented separately.

RNA extraction and complementary DNA (cDNA) preparation

Total RNA was isolated from five sections (1 μm thick) immediately adjacent to the diagnostic slides. Sections were pooled, deparaffinized using three extractions with xylene (1 ml, 65 C, 10 min), and collected by centrifugation (16,000 $\times$ g, room temperature, 5 min). The tissue was then rehydrated with graded ethanol washes (100%, 80%, and 50%, room temperature, 10 min) and diethyl pyrocarbonate (DEPC)-treated water. The DEPC-treated water was removed (16,000 $\times$ g, room temperature, 5 min), and the pellet was resuspended in 0.5 ml 0.1 mol/L Tris-HCl, 25 μmol/L ethylenediamine tetraacetate, and 1% SDS (pH 7.3) containing 500 μg proteinase K (Sigma, St. Louis, MO) and incubated with vigorous shaking (52 C, 24 h), with an additional 250 μg proteinase K added after 24 h (52 C, additional 24 h). After solubilization, 0.5 ml solution D [4 mol/L guanidinium isothiocyanate, 25 mmol/L sodium citrate (pH 7.0), 0.5% N-lauroylsarcosine, and 0.1 mol/L β-mercaptoethanol], 25 μL sodium acetate (pH 4.0), 0.25 mL DEPC-treated water-saturated phenol, and 50 μL chloroform/isoamyl alcohol (49:1) were added. The aqueous phase was removed, and the RNA was precipitated (100% ethanol, -20 C, 1 h). The pellet was redissolved in 0.3 mL solution D, precipitated with 100% ethanol (-70 C, 30 min), and washed (70% ethanol). The RNA was then treated with deoxyribonuclease I, ribonuclease free (20 U/200 μL incubation volume; Roche Molecular Biochemical, Indianapolis, IN) and extracted with phenol followed by chloroform/isoamyl alcohol (24:1), and precipitated (100% ethanol, -70 C, 1 h). The pellet was finally resuspended in DEPC-treated water containing RNase inhibitor (1 U/22 μL; Ambion, Inc., Austin, TX).

One microgram of total RNA was reverse transcribed with random hexamers (2.5 μmol/L) using Moloney murine leukemia virus reverse transcriptase at a final concentration of 2.5 U/μL, deoxy-NTPs (1 mmol/L each), MgCl$_2$ (5 mmol/L), PCR buffer II (1$\times$), and RNase inhibitor (1 U) in a final volume of 20 μL and thermocycled (22 C for 10 min, 42 C for 15 min, 99 C for 5 min, and a 5 C soak) for one cycle (PE Applied Biosystems, Foster City, CA). PCR amplification for each *ret*/PTC rearrangement contained 1 μL cDNA template in a final volume of 5 μL of a Perkin-Elmer Corp. (Foster City, CA) PCR reaction buffer mixture using TaqGold polymerase. The reaction was cycled for 10 min

at 95 C followed by 15 s at 95 C and 1 min at 60 C for a total of 43 cycles in a PE 9600 thermocyler (PE Applied Biosystems). The amplified product was separated either in a 4%, 3:1 NuSieve agarose gel (FMC, Rockland, ME) and detected using a 1:10,000 dilution of Syber Green stain (FMC) or a Novex (San Diego, CA) 20% Tris/borate/EDTA polyacrylamide gel and silver stained.

The primer pairs were designed to span the known breakpoints specific for each of the *ret*/PTC mutations in addition to the internal housekeeping gene glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH). Template negative controls and RNA extracted from nonpapillary thyroid cancers were included as negative controls along with each amplification. Reverse transcriptase-negative controls were included as well. Positive control clones for *ret*/PTC-1, *ret*/PTC-2, and *ret*/PTC-3 were provided by Dr. C. Jhiang, Ohio State University (Columbus, OH).

Table 1 shows the primer sequences for *ret*/PTC-1, *ret*/PTC-2, *ret*/PTC-3, and GAPDH in both the sense and antisense directions. The three primer pairs for the different *ret*/PTC mutations shared a common antisense primer, but unique sense primers. These were synthesized by Biotechnologies Ltd. (Laurel, MD) and were designed to amplify the region across the fusion point of the chimera using information reported by Klugbauer *et al.* (11). Based on this *ret*/PTC primer design, the amplified sequence for *ret*/PTC-1 would be 81 bp, that for *ret*/PTC-2 would be 108 bp, and that for *ret*/PTC-3 would be 134 bp. The GAPDH primers were purchased from Perkin-Elmer Corp.

Three of the samples (no. 1, 3, and 13) that were found to contain *ret*/PTC mutations by gel electrophoresis were sequenced to confirm the identity of the chimeric products for each of the *ret*/PTC rearrangements. Amplified PCR products of the appropriate size were excised from the agarose gel. The DNA was eluted by two successive freeze-thaw cycles (-70 C, room temperature) and centrifuged at 10,000 $\times$ g for 10 min, followed by a purification step (30-μm pore size filter; Millipore Corp., Bedford, MA) of the supernatant. Approximately 10 ng DNA template and 1.5 pmol of the appropriate template were added to BigDye Terminator Ready Reaction Mix (PE Applied Biosystems). Reactions were run according to the kit protocol for 25 cycles. Extension products were purified by ethanol precipitation and were separated by electrophoresis in a 4% acrylamide gel on an ABI Prism 377 DNA Sequencer (PE Applied Biosystems). Sequences from the tumors were aligned and compared to the reported sequences for *ret*/PTC-1, *ret*/PTC-2, and *ret*/PTC-3 using the Sequencer program (Genecode, Ann Arbor, MI). Sequences were confirmed with more than 99% probability for all three samples.

Data analysis and statistical significance

After RT-PCR amplification, data were only included if at least GAPDH or one of the three *ret*/PTC mutations was successfully amplified (44 of 53 attempted samples). Statistical analysis was performed using SPSS for Windows 95 (SPSS, Inc., Chicago, IL). Correlation was performed using Pearson correlation, and nonparametric analysis was performed using the χ^2 test.

Results

Sporadic PTC from 33 patients (23 females and 10 males) and 1 radiation-associated PTC (1 male) were examined. The details of tumor class, treatment, adjunctive therapy, and recurrence are shown in Table 2. The median age at diagnosis was 18 yr (range, 6–21 yr). Thirty-five percent of the patients were less than 17 yr of age, 18% were between the ages of 17–19 yr, and 47% were between 19–21 yr. The majority of

TABLE 1. RET/PTC primer design

	Sense	Antisense	Expected size (bp)
ret/PTC-1	5′-CAAAGCCAGCGTTACCATCG-3′	5′-CCTTCTCCTAGAGTTTTTCC-3′	81
ret/PTC-2	5′-GAAATTGTGGGGCATCGACC-3′	5′-CCTTCTCCTAGAGTTTTTCC-3′	108
ret/PTC-3	5′-CAAGCTCCTTACATACC-3′	5′-CCTTCTCCTAGAGTTTTTCC-3′	134
GAPDH	5′-GAAGGTGAAGGTCGGAGTC-3′	5′-GAAGATGGTGATGGGATTTC-3′	226

TABLE 2. Demographics of patients with papillary thyroid carcinoma

Case no.	Age (yr)/sex	Class	Surgery	^{131}I post-op	Tumor size (cm)/focality	Recurrence	F/U (months)	Clinical status	ret/PTC rearrangement
1	14 /M	I	Total	Yes	2.0/Unifocal	Yes/11 months	63	NED	ret/PTC-1
2	21/F	I	Total	Yes	1.2/Multifocal	Yes/12 months	107	Alive, N/A	ret/PTC-1
									ret/PTC-2
3	6/F	I	Total	N/A	2.6/Unifocal	No	0	N/A	ret/PTC-2
4	11/F	I	Lobectomy	N/A	2.0/Unifocal	No	17	Alive, N/A	
5	11/F	I	Subtotal	No	1.5/Unifocal	No	18	NED	
6	14/M	I	Total	No	0.5/Multifocal	No	10	NED	
7	17/F	I	Total	No	2.4/Unifocal	No	50	NED	ret/PTC-3
8	18/F	I	Total	Yes	1.5/Unifocal	No	2	NED	ret/PTC-2
9	18/F	I	Total	Yes	2.9/Unifocal	No	100	NED	
10	19/M^a	I	Subtotal	N/A	1.1/Unifocal	No	77	Alive, N/A	ret/PTC-1
									ret/PTC-3
11	19/F	I	Subtotal	Yes	0.7/Unifocal	No	106	NED	ret/PTC-1
									ret/PTC-2
12	20/M	I	Total	Yes	5.0/multifocal	No	24	NED	
13	20/F	I	Total	Yes	0.7/unifocal	No	39	NED	ret/PTC-3
14	21/F	I	Total	N/A	2.3/multifocal	No	18	NED	ret/PTC-1
15	21/F	I	Total	N/A	1.8/unifocal	No	68	NED	ret/PTC-1
									ret/PTC-2
16	13/M	II	Subtotal	Yes	4.2/multifocal	Yes/159 months	161	AD	
17	14/F	II	Total	Yes	3.0/multifocal	Yes/67 months	86	NED	
18	10/F	II	Total	Yes	0.8/unifocal	No	15	NED	ret/PTC-1
19	13/F	II	Subtotal	N/A	5.5/multifocal	N/A	0	N/A	
20	15/F	II	Total	Yes	0.7/multifocal	No	85	NED	
21	17/F	II	Total	Yes	2.0/multifocal	No	116	NED	ret/PTC-1
22	18/F	II	Total	Yes	1.8/multifocal	No	27	Alive, N/A	
23	19/M	II	Total	Yes	0.5/multifocal	No	36	NED	
24	19/F	II	Total	Yes	2.3/multifocal	No	37	NED	ret/PTC-1
25	19/M	II	N/A	N/A	1/multifocal	No	104	Alive, N/A	
26	20/F	II	Total	Yes	N/A	No	92	NED	ret/PTC-1
27	20/F	II	Total	Yes	1/unifocal	No	101	NED	ret/PTC-1
28	21/F	II	Total	N/A	1/multifocal	No	22	Alive, N/A	ret/PTC-1
29	19/F	III	Total	Yes	2.0/multifocal	No	86	AD	
30	15/M	III	Total	Yes	N/A	Yes/40 months	60	AD	
31	10/M	III	Subtotal	Yes	5.0/unifocal	Persistent	27	AD	
32	17/M	III	Total	Yes	2.6/multifocal	No	3	AD	
33	20/M	III	Total	Yes	N/A	No	46	Alive, N/A	
34	20/F	IV	Total	Yes	0.5/multifocal	No	63	NED	

Class, Disease class according to DeGroot *et al*. (10); Surgery, extent of initial operative procedure; ^{131}I, documented patient received ^{131}I after initial surgery; Tumor size, greatest diameter of the primary lesion as noted on pathology report at diagnosis; Focality, multifocal vs. unifocal lesions at diagnosis; Recurrence, recurrence and/months after initial therapy when recurrence was first detected; F/U, length of follow-up in months. Clinical status: N/A, not available; AD, alive with disease; NED, no evidence of disease.
^{a} Single patient with radiation exposure.

patients (82%) were defined as having DeGroot disease class I or disease class II (12). The median postoperative follow-up time was 3.6 yr (range, 0–13.4 yr). Forty-four percent were followed for 5 yr or more. This cohort is similar to that of most series, in which the majority of young patients with PTC are female and have either class I or class II disease (9).

FTC from eight patients (seven females and one male) were also examined. The median age at diagnosis was 19.5 yr (range, 16–21 yr). MTC from one patient and an intra-thyroidal LYM from one patient were also examined.

Figure 1 shows the results of the PCR amplification. The expected 226-bp GAPDH product was identified in 42 samples (Fig. 1A). Figure 1, B, C, and D, shows representative gel electrophoretograms for the 3 different *ret*/PTC oncogenes.

We sequenced three cases to confirm the identity of the *ret*/PTC mutation identified on gel. In case 1, gel electrophoresis identified *ret*/PTC-1 mutation, which was confirmed by the sequence data. In case 3, *ret*/PTC-2 was identified by gel electrophoresis and confirmed by sequence

determination. In case 13, *ret*/PTC-3 was identified by gel electrophoresis and sequence confirmation.

As shown in Table 3, 15 subjects had *ret*/PTC mutations (15 of 33, 45%). Eight of the 15 mutations (53%) were identified as *ret*/PTC-1, 2 (13%) were *ret*/PTC-2, 2 (13%) were *ret*/PTC-3, and 3 (20%) contained both *ret*/PTC-1 and *ret*/PTC-2. Nine patients with *ret*/PTC mutations were classified as having disease class I, and 6 were classified as having disease class II. None was identified with either disease class III or IV. Two of the 15 patients with *ret*/PTC mutations (13.3%) developed recurrent PTC within 12 months. The single patient (case 10) with radiation-associated PTC contained both *ret*/PTC-1 and *ret*/PTC-3, was classified as disease class I, and did not develop recurrence. As expected, none of the patients with FTC or MTC or the single patient with LYM was positive for *ret*/PTC mutations.

In contrast, of the 18 patients who were negative for *ret*/PTC mutations, 3 (of 18, 16.6%) developed recurrent PTC.

FIG. 1. Results of PCR amplification. RNA was reverse transcribed, and the cDNA products were separated by electrophoresis. A, GAPDH amplification products from 7 representative samples. The 226-bp GAPDH product was identified on 4% agarose gel stained with Syber Green. B, *ret*/PTC-1 amplification products from 10 subjects and a positive control clone resolved over a 4% agarose gel and stained with Syber Green. The positive control (lane 11) and 3 tumor samples (cases 1, 14, and 24; lanes 4, 6, and 9, respectively) reveal the expected 81-bp amplification product. C, *ret*/PTC-2 amplification products from 15 subjects and a positive control resolved over polyacrylamide gel and stained with silver. The positive control (lane 16) and 2 tumor samples (case 3, lane 5; case 8, lane 10) reveal the expected 108-bp product. D, *ret*/PTC-3 amplification products from 14 subjects and a positive control resolved over polyacrylamide gel and stained with silver. The positive control (lane 15) and 1 tumor sample (case 13, lane 8) reveal the expected 134-bp product.
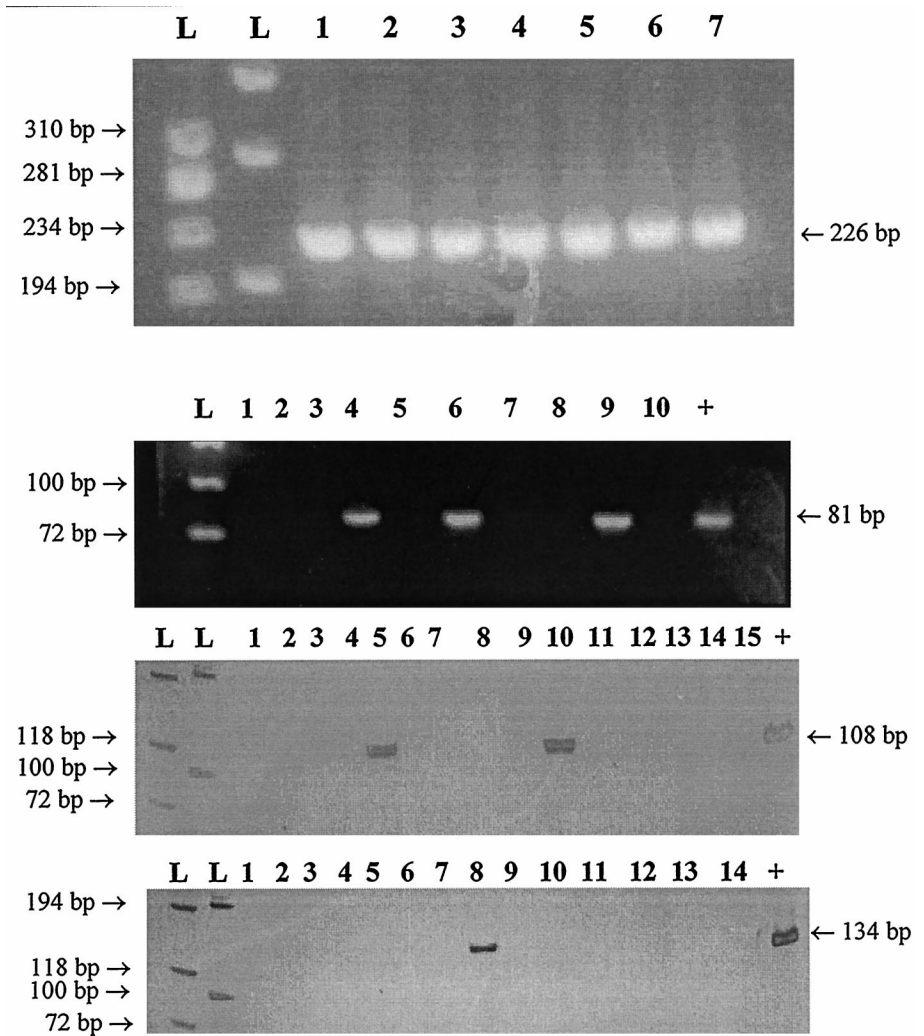
TABLE 3. Distribution of RET/PTC mutations

	Number of patients (n = 34)	Disease class I (n = 16)	Disease class II (n = 13)	Disease class III (n = 4)	Disease class IV (n = 1)	Recurrence (n = 5)	Time to recur (months)	Median follow-up (yr)
ret/PTC-positive patients								
PTC-1	8	2	6			1	11	4.7
PTC-2	2	2						
PTC-3	2	2						
PTC-1/PTC-2	3	3				1	12	
PTC-1/PTC-3[a]	1	1						
ret/PTC-negative patients								
ret/PTC (−)	18	6	7	4	1	3	40,67,159	3.4

[a] Patient previously treated with external beam radiation therapy.

Recurrence developed slightly later in the patients who were *ret*/PTC negative (40–160 months); however, this difference only approached statistical significance ($P = 0.08$, by independent sample two-tail t test).

There was no correlation between the presence of *ret*/PTC mutations and patient age, tumor size, tumor focality (unifocal *vs.* multifocal disease), extent of disease at diagnosis, treatment (extent of surgery and/or use of adjunctive radioactive iodine ablation), recurrence, or disease-free survival.

Discussion

We amplified GAPDH and the *ret*/PTC oncogenes (*ret*/PTC-1, *ret*/PTC-2, and *ret*/PTC-3) from 34 PTC removed from children and young adults. Only 1 patient had a history of prior radiation exposure. Fifteen of the 33 spontaneous PTCs (45.5%) contained at least 1 *ret*/PTC mutation.

The prevalence of *ret*/PTC rearrangements in childhood thyroid cancer is not well known. Several researchers have

studied *ret*/PTC rearrangements in childhood thyroid cancers that developed after the Chernobyl nuclear disaster (13). However, reports of *ret*/PTC rearrangements in sporadic pediatric thyroid cancers are rare. As shown in Table 4, previous studies reported variable prevalence of *ret*/PTC rearrangements in young patients with sporadic PTC. Bongazone *et al.* examined 9 PTC from Italian children (4–19 yr of age) and found that 67% contained *ret*/PTC mutations (2, 14). Motomura *et al.* examined 8 Japanese children (9–14 yr of age) and found that 37.5% contained *ret*/PTC mutations (2). Finally, Nikiforov *et al.* examined 17 children (5–18 yr of age) from the United States and found that 71% contained *ret*/PTC rearrangements (2, 9). In all 3 studies *ret*/PTC-1 was predominant. This pattern is similar to that reported in adult populations (2, 15). None of these studies, however, correlated the presence of *ret*/PTC rearrangements with the clinical outcome for individual children.

The overall frequency of *ret*/PTC mutations in our series (45.5%) is similar to that determined by all 3 previous studies and almost identical to that found by Williams *et al.,* who studied children in the United Kingdom with spontaneous PTC. In their study, 10 of 21 (47%) tumors had *ret*/PTC mutations (5). However, they did not identify the different types of *ret*/PTC rearrangements, nor did they provide any clinical correlation.

The distribution of *ret*/PTC rearrangements in childhood thyroid cancer is based predominantly on PTC that developed after exposure to the Chernobyl nuclear accident. Initial studies of Chernobyl-associated PTC identified *ret*/PTC-3 as the most common form of *RET* rearrangement in radiation-induced childhood PTC (3, 10–12, 14). However, Pisarchik *et al.* found that 29% of adult and childhood PTC in Belarus (n = 31) actually contained *ret*/PTC-1 rearrangements (3). Pisarchik hypothesized the increase in frequency of the *ret*/PTC-1 rearrangements could be related to the longer latency period in their cases, which developed PTC later than those in the initial reports. The patients who had *ret*/PTC-3 rearrangements had been detected much earlier after the Chernobyl incident (3).

The distribution of *ret*/PTC mutations in our series (53% *ret*/PTC-1, 13% *ret*/PTC-2, and 13% *ret*/PTC-3) is significantly different from the distribution reported for radiation-associated PTC ($P = 0.001$, by χ^2 analysis) (8, 9, 11). There are

several possible reasons for this difference. First, it is possible that *ret*/PTC-3 could be specifically induced by radiation exposure. Only 1 patient in our series had prior radiation exposure, and his tumor was found to contain both *ret*/PTC-1 and *ret*/PTC-3. Second, previous reports contained a greater number of children with extensive disease. For example, the series by Pisarchik *et al.* included 15 children with disease class III (3). In contrast, only 6 patients in our series had disease class III or IV. It is therefore possible that *ret*/PTC-3 mutations could be associated with more invasive disease. Finally, the children in this study were all from the United States, and the differences seen in the distribution of *ret*/PTC mutations may reflect a difference in either ethnic background or geographic regions.

Overall, our data are most consistent with previous studies of spontaneous adult PTC, in which *ret*/PTC-1 mutations have been most common (2, 15). Sugg *et al.* found that *ret*/PTC-1 mutations were most frequent in adult PTC, followed by *ret*/PTC-3 and finally by *ret*/PTC-2 (1).

The clinical significance of *ret*/PTC mutations is debated, even in adults. Sugg *et al.* correlated the presence of several oncogenes with clinical outcome for 20 adults with PTC (16). They found *ret*/PTC mutations in 7 of 20 (35%) of their cases. Five of the 7 patients with metastatic disease had *ret*/PTC rearrangements. The researchers hypothesize that *ret*/PTC rearrangements may have a potential role in metastasis. In a follow-up to their initial study, Bongarzone *et al.* examined the clinicopathological features of adults and children with *ret*/NTRK1 rearrangements (6). They examined 76 patients (4–80 yr of age), of whom 34.2% (26 of 76) were positive for *ret*/PTC mutations. With respect to children less than 20 yr of age, 60% (6 of 10) had a *ret*/NTRK1 rearrangement. There was a positive correlation between *ret*/NTRK1 mutations and young age (<30 yr) as well as locally advanced disease (6). However, the study did not separate *ret*/PTC rearrangements from NTRK1 rearrangements for all age groups.

In contrast, Mayr *et al.* examined 58 PTC from patients aged 17–84 yr of age and found *ret*/PTC mutations in 13.8%, with *ret*/PTC-1 being most frequent (17). The patients with *ret*/PTC mutations commonly had lymph node involvement at diagnosis. In contrast, distant metastatic spread and death from disease were not observed in pa-

TABLE 4. RET/PTC rearrangements in pediatric papillary thyroid cancer

Authors (ref.)	Age range of patients (yr)	No. of children	Radiation exposure	Geographic area	% with ret/PTC rearrangements	Predominant type of ret/PTC rearrangement
Bongarzone (14)	4–19	9	No	Italy	67	ret/PTC-1
Motomura (2)	9–14	8	No	Japan	38	ret/PTC-1
Williams (5)	7–14	21	No	United Kingdom	47	ret/PTC-1
Nikiforov (9)	5–18	23	No	United States	48	ret/PTC-1
Lee (4)	31–81	0	No	China	54	ret/PTC-2
Pisarchik (3)	12–21	14	Yes	Belarus	43	ret/PTC-1
Fuggazzola (8)	6–14	6	Yes	Belarus	67	ret/PTC-3
Nikiforov (9)	5–18	38	Yes	Belarus	76	ret/PTC-3
Klugbauer (10)	8–39	12	Yes	Belarus	67[a]	ret/PTC-3
Pisarchik (13)	10–28	35	Yes	Belarus	23[b]	ret/PTC-1
Delvincourt (7)	19–70	2	N/A	France	50[a]	N/A

[a] Percentage of children with ret/PTC rearrangements.
[b] Percentage of all patients with ret/PTC rearrangements.
N/A, Information not available.

tients with *ret*/PTC mutations. Delvincourt *et al.* examined 16 PTC from patients aged 19–68 yr and found *ret*/PTC mutations in 12.5% (2 of 16). They found no correlation between the presence or absence of *ret*/PTC mutations and distant metastasis (7).

The data from our study reveal no correlation between the presence or absence of *ret*/PTC mutations and clinical outcome. Furthermore, there was no relationship between any specific type of *ret*/PTC mutation and clinical outcome. However, our median follow-up for this cohort is relatively short (3.6 yr) and may not be long enough to detect all recurrences. This is in direct contrast to the results reported by Sugg *et al.* (1), but is consistent with the findings of Mayr *et al.* (17).

Our study found double *ret*/PTC mutations in 12% of the PTC examined. This frequency is similar to that identified by Sugg *et al.* (1). They examined 36 PTC and found a total of 16 *ret*/PTC mutations. Two of the 16 samples contained multiple *ret*/PTC rearrangements (12.5%). In addition, 9 of 39 occult cancers (23%) contained 2 forms of *ret*/PTC mutations.

In summary, we found that *ret*/PTC mutations are present in spontaneous childhood PTC and that they occur more frequently than in adults. The distribution of mutations is similar to that seen in adults, with *ret*/PTC1 being the most common. However, the distribution is significantly different from that seen in PTC from the Chernobyl population. Double *ret*/PTC mutations were common, occurring in 12% of the samples examined. The presence of *ret*/PTC mutations does not correlate to the extent of disease at diagnosis or short term clinical outcome for children and young adults.

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