ret/PTC Activation Is Not Associated with Individual Radiation Dose Estimates in a Pilot Study of Neoplastic Thyroid Nodules Arising in Russian Children and Adults Exposed to Chernobyl Fallout

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Background: Ionizing radiation is the strongest risk factor known for the development of thyroid neoplasia. While previous studies have demonstrated a high prevalence of ret/papillary thyroid cancer (PTC) activation in cohorts of patients developing thyroid nodules after childhood exposure to ionizing radiation, no study has directly compared ret/PTC activation with individual estimates of radiation dose to the thyroid. This study combines individual thyroid dosimetry data with molecular analysis of surgically removed thyroid nodules in order to determine if ret/PTC activation in thyroid nodules is associated with increasing estimated radiation dose from Chernobyl.

Methods: This pilot study included adults and children diagnosed with PTC ($n = 76$) and children diagnosed with follicular adenomas ($n = 24$) during May 1986 through December 1999, who were living in the Bryansk Oblast of the Russian Federation at the time of the Chernobyl accident, who had paraffin-embedded thyroid surgical samples available and for whom an individual dose to the thyroid could be estimated. The frequency of ret/PTC activation was determined using RT-PCR analysis. Individual radiation doses to the thyroid were estimated using a semiempirical model, and data were collected by detailed interview, primarily of the participant's mother.

Results: ret/PTC oncogene activation was detected in 23.8% (5/21) and 14.5% (8/55) of the childhood and adult PTC cases, respectively, and 8.3% (2/24) of the follicular adenoma cases. No statistically significant differences were noted in age at the time of exposure or diagnosis, gender, latency period, or estimated radiation dose between PTC patients with or without ret/PTC activation. Further, no significant dose-response relationship was detected among PTC patients with ret/PTC activation.

Conclusions: Factors other than individual thyroid radiation doses may influence the development and subsequent detection of ret/PTC oncogene activation in radiation related PTC arising in the Bryansk Oblast of the Russian Federation in the aftermath of the Chernobyl accident.

Introduction

ACTIVATION OF THE RET/PAPILLARY THYROID CANCER (PTC) oncogene is detected in 20–30% of sporadic PTCs (1). Analyses of radiation-associated PTC specimens usually find a higher rate of ret/PTC activation ranging from 30% to 86% (2). Even though ionizing radiation has been shown to induce ret/PTC rearrangements in thyroid cancer cell culture models,

other variables such as ethnic background, age at diagnosis, and latency period between radiation exposure and development of clinically evident PTC may also have a significant impact on the prevalence rate of ret/PTC activation reported in clinical samples (3–11).

Despite many studies that have described ret/PTC activation in PTC arising following thyroid radiation in childhood, very little information is available regarding the

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dose–response relationship between radiation dose to the thyroid and subsequent detection of ret/PTC activation in clinical samples. In cell culture models, a significant dose–response relationship has been demonstrated using radiation doses similar to thyroid radiation dose estimates in children exposed to the Chernobyl fallout (12–14). However, Collins *et al.* found that radiation dose to the thyroid from external beam irradiation of children for benign conditions was not significantly different between thyroid nodules that developed ret/PTC mutations (69 $\pm$ 12 cGy) and those without ret/PTC mutations (89 $\pm$ 29 cGy) (15).

Most studies of thyroid cancer following the Chernobyl accident reported to date are descriptive incidence and prevalence studies, are ecologic in design, and utilize population or aggregate estimates of thyroid radiation dose that do not take into account individual characteristics of exposure and factors related to disease risk. At present, there are only four published population-based case–control studies of thyroid cancer in children. One, based on 107 cases diagnosed in Belarus, found a strong relationship between estimated radiation dose and thyroid cancer, but thyroid doses were inferred for children from estimates for adults who lived in the same villages (16). Two studies, based on a total of 66 cases and individual estimates of thyroid radiation dose, found that the risk of thyroid cancer was significantly increased in a dose-dependent manner among residents of Bryansk Oblast of the Russian Federation who were exposed as children and adolescents to radiation fallout from the Chernobyl accident (17,18). Finally, a fourth case–control study of 276 patients less than 15 years of age at the time of the accident in Belarus and the Russian Federation matched with 1300 control subjects showed a strong dose–response relationship between childhood thyroid radiation dose and thyroid cancer risk (19). One cohort study using individual thyroid dose estimates has also been reported. That study found a significant association between estimated thyroid dose and thyroid cancer risk in a Ukrainian cohort of about 13,500 persons who were less than 18 years old at the time of the accident (20).

Even though individual thyroid doses were not available, it has been noted that children residing in settlements that received the greatest radiation contamination after the Chernobyl accident appear to have higher prevalence of ret/PTC activation than subjects residing in lesser exposed areas (11). No published study has evaluated individual radiation dose estimates to the thyroid in relation to ret/PTC oncogene activation rates in a cohort of subjects developing thyroid cancer after the Chernobyl accident.

Therefore, the goal of this study was to investigate whether there is an association between estimated individual thyroid radiation dose from Chernobyl and the frequency of ret/PTC oncogene activation in benign or malignant thyroid nodules that developed in children and adults who were living in the Bryansk Oblast of the Russian Federation at the time of the Chernobyl accident. We hypothesized that ret/PTC activation would be associated with increasing radiation dose to the thyroid.

Materials and Methods

Identification of samples

Cases included adults and children diagnosed with thyroid cancer during May 1986 through December 1999 who were

living in the Bryansk Oblast of the Russian Federation at the time of the Chernobyl accident and who had paraffin-embedded thyroid surgical samples representative of the primary tumor available. The cancer cases were identified within two strata defined by age at the time of exposure to the Chernobyl fallout, with subjects 20 years old or less considered children at exposure, and subjects more than 20 years old considered adults at the time of exposure. The goal for the adults in this pilot study was to identify enough cases to result in successful RNA extraction from 50. RNA extraction was attempted for 90 adult thyroid cancer cases who were non-randomly selected for this pilot study and was successful for 76 (84%). Fifty-five of these 76 had their diagnosis confirmed by an independent review of a panel of expert pathologists, consented to participate, and had a dose estimate calculated. For the childhood cases, specimens were available for 44 confirmed cases of PTC. Of these 44, 21 (48%) were evaluable for ret/PTC, consented to participate, and had a dose estimate calculated.

In addition to the thyroid cancer cases analyzed, pathology archives in the major medical centers in Bryansk and Oboinsk were reviewed in order to identify follicular adenoma cases diagnosed during May 1986 through December 1999 in children exposed to the Chernobyl fallout. Forty-three potential cases were identified, and adequate RNA for analysis was recovered in 24 cases for whom the diagnosis was confirmed, a dose estimate was calculated, and consent was obtained.

All procedures and data collection instruments were approved by Institutional Review Boards at both the Fred Hutchinson Cancer Research Center and the National Center for Hematology in Moscow. All study participants provided written informed consent to participate in the study prior to data collection.

Histologic confirmation

Hematoxylin and eosin–stained slides were reviewed by a panel of three pathologists (E.L., V.T., and A.A.) to confirm the original clinical histological diagnosis. Cases for which the panel could not reach consensus were submitted to Professor Dillwyn Williams (Cambridge University) for a final diagnostic determination. This panel also selected paraffin blocks for molecular analysis in which the majority of the specimen within the block consisted of tumor tissue with minimal amounts of surrounding normal tissue.

Thyroid dose estimation

Individual thyroid dose estimates were calculated for each subject as previously described (17,21). In brief, an in-person interview using a standardized questionnaire was conducted with the subject's mother (first preference), father, or the subject by trained physician interviewers in the subject's home. These detailed questionnaires collected data for dose reconstruction, including milk consumption rate, milk and food types and sources, change in milk consumption rate after the accident, use of thyroid blocking agents, and relocation to less contaminated regions shortly after the accident (21).

Radiation doses to the thyroid were estimated using a semiempirical regression model that related the results of field measurements of ^{131}I activities in the thyroid glands of adults living in contaminated areas during May–June 1986 to deposition densities of ^{137}Cs in the soil averaged over the set-

TABLE 1. RET/PTC PRIMER DESIGN

	<i>Sense</i>	<i>Antisense</i>	<i>Expected size (bp)</i>
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
<i>Oligoprobes</i>			
ret/PTC-1		5'-GGGCACTGCAGGAGGAGAACC CGCA-3'	
ret/PTC-2		5'-GACCGAGACAGCTATAGAAGAATC-3'	
ret/PTC-3		5'-GGTCGGTGCTGGGTATGTAAGGA-3'	

PTC, papillary thyroid cancer.

tlement (village or city) and over the raion in which the subject resided at the time of the accident. To estimate the dose for children and adolescents, the estimated mean adult thyroid dose was multiplied by factors accounting for the individual's age, differences in thyroid mass, milk consumption rate, as well as the type and source of milk.

For descriptive purposes, the range of estimated doses for all cases was divided into approximate quartiles.

Molecular analysis

Paraffin-embedded tissue sections (20 microns in thickness) immediately adjacent to the diagnostic slides were rehydrated using techniques previously described (22,23). RNA was extracted from the rehydrated tissue pellets using the Qiagen RNeasy Mini kit with only a single modification. Cell lysis was done for 12 hours at room temperature in buffer RLT rather than the 30 minutes the manufacturer suggested.

RT-PCR reactions for each of the ret/PTC oncogenes and GAPDH were performed as previously described (22,23). Ret/PTC analysis was performed only on samples that provided adequate RNA for successful RT-PCR amplification of GAPDH mRNA.

Reverse transcriptase negative and template negative controls were included along with each amplification. Table 1 shows the primer and oligonucleotide probe sequences for ret/PTC-1, ret/PTC-2, ret/PTC-3, and GAPDH with expected amplification product sizes. Positive control clones for ret/PTC-1, ret/PTC-2, and ret/PTC-3 had been previously provided by Dr. C Jhiang, Ohio State University (Columbus, OH), and were used as previously described (23).

The amplified product was separated in a 2% agarose gel and detected with ethidium bromide (10 mg/mL, aqueous solution; Sigma, St. Louis, MO). The DNA was then transferred from the agarose gel to a solid support by the method of Southern. The membrane was then hybridized with previously published mutation-specific oligonucleotide probes that had been 3'-end labeled with digoxigenin-11-ddUTP using a DIG Oligonucleotide 3'-end labeling Kit (Roche Applied Science, Indianapolis, IN). The specific labeling was detected by chemiluminescence using antidigoxigenin antibody conjugated with alkaline phosphatase as previously described (23).

Statistical considerations

Frequency distributions of demographic characteristics and ret/PTC activation were calculated separately for each of

the three age-at-exposure and disease groups. For descriptive analyses, the distribution of estimated thyroid radiation doses of all 100 participants was categorized into quartiles. Comparisons of ret/PTC activation by demographic characteristics were based on Pearson's χ^2 -test for independence or Fisher's exact test where appropriate for categorical variables, and on the *T*-test for continuous variables. Logistic regression analysis was used to examine the dose-response relationship between estimated thyroid radiation dose from Chernobyl, treated as a continuous variable, and ret/PTC activation.

Results

Characteristics of the 21 individuals who developed PTC after exposure to Chernobyl fallout during childhood, the 55 individuals who developed PTC after exposure to Chernobyl fallout during adulthood, and the 24 individuals who developed follicular adenoma after exposure to Chernobyl fallout during childhood are shown in Table 2. As expected, the malignant cases were all classified as PTC, and there was a predominance of female gender in both the benign and malignant nodule cohorts.

The mean age at the time of exposure for the childhood thyroid cancer group was 4.5 years, for the adult thyroid cancer group was 32 years, and for the follicular adenoma group was 8 years. The mean age at the time of diagnosis for the childhood thyroid cancer group was 14.7 years, for the adult thyroid cancer group was 43 years, and for the follicular adenoma group was 18.4 years. The mean (median) estimated radiation dose to the thyroid for the childhood thyroid cancer group was 362.6 mGy (130.0 mGy), for the adult thyroid cancer group was 39.2 mGy (12.0 mGy), and for the follicular adenoma group was 44.8 mGy (5.8 mGy). The latency period between exposure and diagnosis exceeded 5 years in all of the PTC cases arising following childhood exposure as well as in the follicular adenoma group. However, 5.5% of the adult thyroid cancer group were diagnosed within 5 years of exposure.

Activation of the ret/PTC oncogene was detected by Southern blot analysis of RT-PCR amplified product in 24% (5/21; 95% confidence interval [CI] 8–47%) of the childhood thyroid cancer cases, 15% (8/55, CI 7–27%) of the adult thyroid cancer cases, and 8% (2/24, CI 1–27%) follicular adenoma cases (Fig. 1 and Table 2).

Potential interactions between ret/PTC oncogene activation rates and important demographic characteristics and

TABLE 2. CHARACTERISTICS OF 100 PATIENTS WITH PTC OR FOLLICULAR ADENOMA FOLLOWING EXPOSURE TO FALLOUT FROM THE CHERNOBYL ACCIDENT

Characteristic	Childhood cancer (21 cases)		Adult cancer (55 cases)		Follicular adenoma (24 cases)	
	n	%	n	%	n	%
Sex						
Male	8	38	6	11	4	17
Female	13	62	49	89	20	83
Histology						
Papillary cancer	21	100	55	100	—	—
Follicular adenoma	—	—	—	—	24	100
Age at exposure (years)						
<1	5	24	0	—	3	12
1	6	29	0	—	2	8
2–5	4	19	0	—	7	29
6–10	1	5	0	—	3	12
11–15	4	19	0	—	4	17
16–20	1	5	2	4	5	21
21–25	0	—	8	15	0	—
26–30	0	—	11	20	0	—
31–35	0	—	19	35	0	—
36–40	0	—	12	22	0	—
41–52	0	—	3	5	0	—
Age at diagnosis (years)						
9–15	16	76	0	—	12	50
16–20	1	5	0	—	3	12
21–25	4	19	0	—	4	17
26–30	0	—	1	2	5	21
31–35	0	—	7	13	0	—
36–40	0	—	11	20	0	—
41–45	0	—	17	31	0	—
46–50	0	—	13	24	0	—
51–62	0	—	6	11	0	—
Latency (years)						
1–5	0	—	3	5	0	—
6–10	13	62	16	29	12	50
11–13	8	38	36	65	12	50
Ret/PTC activation						
Ret/PTC-1	3	14	7	13	2	8
Ret/PTC-2	0	—	0	—	0	—
Ret/PTC-3	2	10	2	4	0	—
Any ret/PTC	5	24	8 ^a	15	2	8
Estimated thyroid dose (mGy)						
0.17–3.44	2	10	14	25	9	38
3.45–10.94	5	24	13	24	7	29
10.95–70.9	3	14	19	35	3	12
71.0–1260	11	52	9	16	5	21
N	21		55		24	
Mean	362.6		39.2		44.8	
Median	130.0		12.0		5.8	
Minimum, maximum	0.8, 1260		0.6, 370		0.2, 490	

^aOne patient had both ret/PTC-1 and ret/PTC-3 mutations.
PTC, papillary thyroid cancer.

radiation dose quartiles are presented in Table 3. Ret/PTC activation rates did not differ significantly based on quartile of radiation exposure, gender, or age at diagnosis. However, ret/PTC activation rates appeared to be higher in children less than 2 years old at time of exposure as compared to activation rates seen in adults at the time of the exposure. For all cancer cases and follicular adenomas combined, a higher rate of ret/PTC activation was seen in subjects with a latency period

of 11–13 years (21%) as compared to subjects with a latency period of 1–10 years (7%; $p = 0.04$). Among the papillary cancer cases, the corresponding ret/PTC activation rates were 23% and 9%, respectively, although this difference was not statistically significant ($p = 0.13$).

When considered as continuous variables, age at the time of exposure, age at diagnosis, latency period, and estimated radiation dose did not differ significantly between PTC patients

FIG. 1. The left panels present representative images of agarose gel electrophoresis of RT-PCR products for positive controls (GAPDH primers, reverse transcriptase included), negative controls (GAPDH primers, no reverse transcriptase added). The right panels show representative agarose gel electrophoresis of ret/papillary thyroid cancer (PTC)-1 (upper) and ret/PTC-3 (lower). The numbered lanes in the left-hand panels do not correspond to the numbered lanes in the right-hand panels since only samples that were GAPDH positive were submitted for ret/PTC analysis. L, DNA sizing ladder.

with ret/PTC activation compared to those without ret/PTC activation (Table 4). Further, no significant dose-response relationship was detected for ret/PTC activation among papillary thyroid patients (Table 5). The estimated dose-response was essentially unchanged in analyses that adjusted for age at diagnosis (results not shown).

Discussion

Activation of the ret/PTC oncogene was detected by RT-PCR amplification and Southern blotting in 24% (CI 8–47%) of childhood PTCs, and 15% (CI 7–27%) of adult PTCs developing after exposure to Chernobyl fallout in the most heavily contaminated raions of the Bryansk Oblast in the Russian Federation. Activation of the ret/PTC oncogene was not significantly associated with individual estimates of Chernobyl-derived radiation dose to the thyroid in the 76 PTC samples analyzed. However, this pilot study had only modest statistical power to detect such an association, so these results alone do not rule out the possibility that ret/PTC activation may be associated with radiation exposure.

The rate of ret/PTC activation in the children in this study is clearly lower than the 84–87% activation rates detected in PTC cases arising following external beam irradiation (15,24,25), and may be lower than the 30–86% activation rates detected in PTCs developing after exposure to Chernobyl fallout during childhood that have been reported to date (3–11). However,

TABLE 3. RET/PTC ACTIVATION BY DEMOGRAPHIC CHARACTERISTICS

Characteristic	Papillary cancer			Follicular adenoma			All		
	No.	Ret/PTC activation		No.	Ret/PTC activation		No.	Ret/PTC activation	
	No.	No.	%	No.	No.	%	No.	No.	%
Sex									
Male	14	2	14	4	0	0	18	2	11
Female	62	11	18	20	2	10	82	13	16
Histology									
Papillary cancer	76	13	17	0	—	—	76	13	17
Follicular adenoma	0	—	—	24	2	8	24	2	8
Age at exposure (years)									
<1	5	1	20	3	1	33	8	2	25
1	6	2	33	2	0	0	8	2	25
2	2	1	50	4	1	25	6	2	33
3–5	2	0	0	3	0	0	5	0	0
6–20	8	1	12	12	0	0	20	1	5
22–52	53	8	15	0	0	0	53	8	15
Age at diagnosis (years)									
9–19	16	5	31	15	2	13	31	7	23
20–29	6	0	0	9	0	0	15	0	0
30–39	16	1	6	0	—	—	16	1	6
40–49	29	7	24	0	—	—	29	7	24
50–62	9	0	0	0	—	—	9	0	0
Latency (years)									
1–10	32	3	9	12	0	0	44	3	7
11–13	44	10	23	12	2	17	56	12	21
Dose quartile (mGy)									
0.17–3.44	16	2	12	9	0	0	25	2	8
3.45–10.94	18	2	11	7	0	0	25	2	8
10.95–70.9	22	4	18	3	0	0	25	4	16
71.0–1260	20	5	25	5	2	40	25	7	28

PTC, papillary thyroid cancer.

TABLE 4. CHARACTERISTICS OF 76 PTC CASES, BY RET/PTC ACTIVATION STATUS

	Ret/PTC	n	Mean	SD	Median	Minimum	Maximum	p-value ^a
Age at exposure (years)	Yes	13	20.5	15.8	28.0	0.0	37.0	0.27
	No	63	25.2	13.4	29.0	0.0	52.0	
Age at diagnosis (years)	Yes	13	31.8	15.9	41.0	11.0	48.0	0.34
	No	63	35.9	13.9	39.0	9.0	62.0	
Latency (years)	Yes	13	11.4	2.6	12.1	3.6	13.5	0.36
	No	63	10.7	2.4	11.1	1.1	13.6	
Dose (mGy)	Yes	13	158.0	250.3	39.0	1.0	690.0	0.66
	No	63	122.5	268.5	13.0	0.6	1260.0	

^ap-value for difference in mean for each variable by ret/PTC activation.
PTC, papillary thyroid cancer.

this low activation rate is very similar to the 35% activation rate detected following low dose external beam irradiation in a cohort of Israeli children treated for tinea capitis approximately 30 years prior to detection of thyroid cancer (23), the 29% activation rate described in a cohort of children developing thyroid cancer in Belarus after a latency period of approximately 10 years (9), and the 33% activation rate detected in post-Chernobyl Ukrainian children (26). The rate of ret/PTC activation in subjects who were adult at the time of the accident is very similar to the 20–30% reported in sporadic PTC cases (1) and the 5% rate reported by Sugg *et al.* (27). This finding, coupled with the rather short latency period of less than 6 years in three of the cases, suggests that some of these cases may be part of the background sporadic rate of PTC and not directly related to radiation exposure. In children, the background rate of thyroid cancer is very low, making it much more likely that a thyroid cancer arising following significant radiation exposure would in fact be related to the radiation exposure. However, in adults, the background rate of sporadic thyroid cancer is much higher, therefore making it much less likely that an individual case of thyroid cancer can be attributed to the radiation exposure.

Often differences in ret/PTC activation rates are secondary to differences in the sensitivity of detection techniques. However, in this study we used techniques that are highly sensitive that are likely to overestimate the prevalence of clinically significant ret/PTC rearrangements. It seems unlikely that the low rate of ret/PTC activation detected in this cohort is a result of insensitive detection techniques. How-

ever, the starting material for RNA extractions in this study was paraffin-embedded routinely prepared thyroid surgical specimens. It is likely that significant RNA degradation occurred during the paraffin-embedding process, long-term storage, and recovery of the RNA from the paraffin blocks. RNA degradation limited the number of samples we had available for ret/PTC analysis and could have resulted in false-negative findings in the samples in which RNA was deemed acceptable for ret/PTC analysis. Indeed a recent quantitative RT-PCR-based analysis of ret/PTC levels in PTCs showed marked variations in mRNA levels between samples (28). Only comparative studies using fresh frozen tissues can quantify the potential significance of these preservation and storage issues related to paraffin embedding.

Other possible reasons for the wide range of ret/PTC activation rates reported in studies of sporadic and radiation-related thyroid cancer may be related to ethnic differences, detection methods, gender, age at exposure, age at diagnosis, and latency period that vary widely between studies (3–11).

One possible explanation of the lower rates of ret PTC activation in this study might be the lower thyroid radiation doses received by many subjects exposed in the Bryansk Oblast compared to heavily contaminated regions of Belarus and persons exposed to external beam irradiation. As noted above, while our pilot data show no significant effect of estimated thyroid dose on activation of ret/PTC oncogene in individual patients, they do not conclusively rule out this possibility. Nevertheless, the results reported here are consistent with the findings of Collins *et al.* in a cohort of children treated with external beam irradiation for benign disease in which no difference in thyroid radiation dose was detected between patients with or without ret/PTC activation (15). Therefore, factors other than radiation dose may be responsible for the relatively low rate of ret/PTC activation in the present study.

Estimated thyroid doses from Chernobyl fallout in the present study ranged from 0.17 to 1260 mGy, but were skewed toward lower doses, reflecting the geographical distribution of the population of Bryansk Oblast. The mean estimated dose was about 363 mGy for the 21 childhood cancer cases and about 39 mGy for the adult cancer cases. Doses from external beam irradiation as low as 10–50 cGy (100–500 mGy) have been associated with subsequent increase in the development of PTC. Further, these thyroid doses are similar to the external beam irradiation doses used by Caudill *et al.* in studies demonstrating a dose-response effect on develop-

TABLE 5. ESTIMATED DOSE-RESPONSE FOR RET/PTC ACTIVATION AMONG 76 PATIENTS WITH PAPILLARY CANCER

Cases	Odds ratio ^a	95% Confidence interval	p-value
All	1.05	0.85, 1.29	0.66
Males	0.89	0.53, 1.47	0.64
Females	1.17	0.89, 1.53	0.25

^aMultiplicative change in the odds of ret/PTC activation associated with increase of 100 mGy in estimated thyroid radiation dose, that is, the estimated value of $\exp(\beta \times 100)$ where β is the slope coefficient in the logistic dose-response model $\log [P_d/(1 - P_d)] = \alpha + \beta d$ for the probability of ret/PTC activation (P_d) as a function of estimated dose d (in mGy, treated as a continuous variable). Nuisance parameter α is the logarithm of the odds ratio for $d \neq 0$.

ment of ret/PTC translocations in cell culture studies (12). In cell culture, it may be possible to detect ret/PTC activation in cells that may never become clinically significant. If additional growth factors or additional mutations are required to develop a clinically apparent thyroid cancer, the dose-response relationship detected in cell culture studies may not be apparent in clinical samples.

Activation of the ret/PTC oncogene was also detected in 2/24 (5%) of follicular adenoma samples analyzed. Even though ret/PTC was initially thought to be present only in PTC, some (3,23–25,29,30), but not all (31–33), studies document detection of ret/PTC transcripts in benign nodules, follicular adenomas, and other benign thyroid conditions. Since ret/PTC activation was observed in only two of the follicular adenoma patients, it was not possible to test whether activation was related to radiation dose. However, it is interesting that both of the follicular adenoma samples that had ret/PTC activation were in the highest quartile of estimated radiation dose to the thyroid and had latency periods of 11 and 12 years. In fact, the prevalence of ret/PTC was found to differ by latency period when the two follicular adenomas were combined with the cancer cases, with a higher ret/PTC rate among those with a longer (11–13 years) versus shorter latency period (1–10 years).

In summary, ret/PTC activation rates are much lower in childhood and adult PTC cases arising following exposure to Chernobyl fallout in the most heavily contaminated regions of Bryansk Oblast of Russia compared with previously published reports of children exposed to Chernobyl fallout in Belarus, or children exposed to external beam irradiation. The rates of ret/PTC activation do not appear to be significantly influenced by estimated thyroid radiation doses. Therefore, factors other than individual thyroid radiation doses may influence the development and subsequent detection of ret/PTC oncogene activation in radiation-related PTC arising at the radiation doses experienced in the Bryansk Oblast of the Russian Federation in the aftermath of the Chernobyl accident.

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