

**ret/PTC1 and ret/PTC3 in thyroid tumors from Chernobyl liquidators: comparison with sporadic tumors from Ukrainian and French patients**

**J Di Cristofaro**, V Vasko, V Savchenko, S Cherenko, A Larin, M D Ringel, M Saji, M Marcy, J F Henry, P Carayon, and C De Micco

1Institut National de la Sante´ et de la Recherche Médicale (U555), IPHM, Faculte´ de médecine, Mediterranean University, Marseille, France
2Department of Endocrine Surgery, Faculty of Medicine, Mediterranean University, Marseille, 13385 France
3Laboratory of Pathology, Faculty of Medicine, Mediterranean University, Marseille, 13385 France
4Hospital for Endocrine Surgery, Kiev, 03115 Ukraine
5Divisions of Endocrinology and Oncology, The Ohio State University and Arthur G. James Cancer Center, Colombus, OH 43210, USA

(Requests for offprints should be addressed to J Di Cristofaro; Email: julie.dicristofaro@medecine.univ-mrs.fr)

**Abstract**

Like children exposed to Chernobyl fallout, the workers who cleaned up after the accident, also known as liquidators, have exhibited an increased incidence of thyroid cancer. A high prevalence of ret/PTC3 rearrangement has been found in pediatric post-Chernobyl thyroid tumors, but this feature has not been investigated in liquidator thyroid tumors. In this study we analyzed the prevalence of ret/PTC1 and ret/PTC3 in thyroid tumors from 21 liquidators, 31 nonirradiated adult Ukrainian patients, and 34 nonirradiated adult French patients. ret rearrangements in carcinomas were found in 83.3% of liquidators, 64.7% of Ukrainian patients, and 42.9% of French patients. The prevalence of ret/PTC1 was statistically similar in the three groups. The prevalence of ret/PTC3 was significantly higher in liquidators than in French patients (P = 0.03) but it was also high in nonirradiated Ukrainian patients who exhibited values intermediate between liquidators and French patients. In adenomas the prevalence of rearrangement was significantly higher in all Ukrainians than in French patients (P = 0.004). Like children exposed to Chernobyl fallout, liquidators showed a high prevalence of ret/PTC3. This finding suggests that irradiation had the same effect regardless of age. However, given the high rate of ret/PTC3 in nonirradiated adult Ukrainians, the possibility of genetic susceptibility or low-level exposure to radiation in that group cannot be excluded.

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**Introduction**

For 10 days following the explosion of one reactor on 26 April 1986, the nuclear power plant in Chernobyl, Ukraine, released into the atmosphere $370 \times 10^{16} - 740 \times 10^{16}$ Bq of fission products containing huge amounts of radioactive iodine. The most contaminated zones were in southern Belarus, northern Ukraine, and the Bryansk and Kaluga regions of southern Russia (Kazakov et al. 1992, Stone 2001).

The people exposed to radiation were officially divided into four main groups: liquidators, i.e. workers involved in encasing the reactor in a concrete sarcophagus and in cleaning up the heaviest contamination in the 30 km zone around the plant from 1986 to 1989 (Kazakov et al. 1992, Demidchik et al. 2002); people evacuated from the restricted 30 km zone around the nuclear power plant; adult residents of contaminated territories in Belarus, Russia, and the Ukraine; and child residents of contaminated territories in Belarus, Russia, and the Ukraine. As expected, children proved to be more sensitive to radiation (Ron et al. 1995). As of 1990 a dramatic increase in the incidence of thyroid carcinoma,
especially the papillary thyroid carcinoma (PTC), was observed in children, particularly in the Gomel and Brest regions (Prisyazhiuk et al. 1991, Baverstock et al. 1992). In Belarus, the prevalence of pediatric cancer peaked in 1995 and thereafter decreased gradually while that of adolescent thyroid cancer increased (Demidchik et al. 2002). There are currently few data on thyroid carcinomas in the adult population of northern Ukraine.

Regarding the liquidators, many aspects remain unclear, beginning with their actual numbers, which have been estimated to be between 200,000 and 800,000 persons depending on sources. In 1997 Ivanov et al. reported a higher-than-expected incidence of thyroid morbidity and leukemia among liquidators (Ivanov et al. 1997). In 2002 the same investigators demonstrated that the incidence of thyroid carcinoma and leukemia in liquidators was significantly higher than in the general male population of Russia and that this difference could not be explained by a screening effect alone (Ivanov et al. 2002). The highest incidence of thyroid cancer was found among liquidators exposed during recovery work from April to July 1986, when the risk of internal irradiation of the thyroid with incorporated $^{131}$I was greatest.

Occurrence of ret oncogene rearrangements involving several different genes has been documented in PTC (Grieco et al. 1990, Santoro et al. 1992, Bongarzone et al. 1994). These rearrangements, which have been incriminated in PTC oncogenesis (Jhiang et al. 1996), involve the 5' region of ubiquitous genes and the 3' region of ret, which encodes the tyrosine kinase domain (Lanzi et al. 1992). Although more than 10 ret rearrangements have been described in PTC so far (Nikiforov 2002), the most frequent are ret/PTC1 and ret/PTC3 (Grieco et al. 1990, Santoro et al. 1992, Bongarzone et al. 1994), whose prevalence has varied greatly from one study to another. Variation may depend on age, genetic background, histological subtype, and environmental factors, especially exposure to external or internal irradiation (Bongarzone et al. 1996, Bounacer et al. 1997, Chua et al. 2000, Fenton et al. 2000, Basolo et al. 2002).

The specificity of the relationship between ret rearrangements and PTC has led many authors to investigate this aspect in post-Chernobyl patients. Most studies have focused on PTCs that developed rapidly in children from contaminated areas of Belarus and Ukraine (Fugazzola et al. 1995, Klugbauer et al. 1995, Nikiforov et al. 1997, Pisarchik et al. 1998a, 1998b, Smida et al. 1999, Thomas et al. 1999, Rabes et al. 2000, Santoro et al. 2000, Elisei et al. 2001). Only three studies included PTC from adults living in contaminated areas, but none attempted to compare with adults living in supposedly non-affected areas of the same regions (Klugbauer et al. 1995, Smida et al. 1999, Rabes et al. 2000). Current evidence suggests that the profiles of ret rearrangements in PTC from irradiated children are different from those of both irradiated adults and adults or children from other countries. According to one report, PTCs in children under 10 in 1986 showed an unusually high prevalence of ret/PTC3 (Klugbauer et al. 1995). Like people receiving external radiation for therapeutic purposes, PTCs in older children and adults exposed to Chernobyl fallout exhibited an increase in ret/PTC1 (Fugazzola et al. 1995, Klugbauer et al. 1995, Bounacer et al. 1997, Pisarchik et al. 1998b).

The aim of this study was to determine whether PTCs from Chernobyl liquidators show an increased prevalence in ret/PTC3 rearrangement similar to PTCs from children exposed to Chernobyl fallout. The prevalence of ret/PTC1 and ret/PTC3 was determined by reverse transcriptase PCR and hybridization with a radiolabeled probe in benign and malignant thyroid tumors from Chernobyl liquidators. To evaluate the respective roles of radiation exposure and genetic factors, similar testing was performed on benign and malignant tumors from adult patients living in uncontaminated areas of Ukraine and from nonirradiated (NI) adult patients living in south-eastern France.

**Material and methods**

**Thyroid tissue**

Thyroid tissue specimens were obtained from patients who underwent surgery between 1994 and 2003 at either the Center for Endocrine Surgery in Kiev, Ukraine, or the Timone University Hospital Center in Marseille, France.

During the recovery phase following the Chernobyl accident, the government of the USSR sent workers from all parts of the country including Ukraine to carry out emergency containment operations and to clean up the 30 km zone around the nuclear power plant. These people were called liquidators. After the recovery work, they received an official certificate from the government and went home. Available dosimetric information concerning liquidators was recorded in the Russian National Medical and Dosimetric Registry (RNMDR; Pitkevitch et al. 1997). All patients in the liquidator group in this study were Ukrainians and presented an official certificate of recovery work. However, no information could be obtained concerning their individual irradiation status (Cherenko et al. 1992).
All subjects in the NI Ukrainian patient group lived outside the ‘officially contaminated’ areas and there is no evidence that they were exposed to contamination from Chernobyl. Similarly, the medical history of the NI French patient group includes no evidence of contact with radiation.

Histological diagnosis was established by three pathologists specializing in thyroid pathology (V S, V V and C D M) according to the World Health Organization classification (Hedinger 1988). A total of 36 benign nodules — 14 colloid nodules and 22 follicular adenomas — from liquidators (n = 9), NI Ukrainian patients (n = 14), and NI French patients (n = 13) were studied. Samples of normal thyroid tissue adjacent to colloid nodules were taken from 10 other French patients. A total of 50 PTCs were studied, including 12 from liquidators, 17 from NI Ukrainian patients, and 21 from NI French patients (Table 1). Histological subtyping of PTCs was performed according to current diagnostic criteria (Rosai et al. 1992).

Although no PTC was predominantly solid or entirely made of tall cells, both features were observed to some extent. Solid areas were found in PTCs from three NI Ukrainian patients, two NI French patients, and two liquidators. Tall cells were found in varying proportions (range, 20–40%) in PTCs from seven NI French patients, three NI Ukrainians patients, and eight liquidators. The study protocol was approved by the clinical research committee of Marseille Public Hospital System.

RNA extraction

The number of paraffin-embedded tissue slices available for each specimen ranged from one to 10. After dewaxing with xylene, tissue slices were washed twice with ethanol, air dried for 1 h, and digested overnight at 37°C using a solution of guanidine thiocyanate (1 M; Sigma-Aldrich, St Quentin Fallavier, France), sarcosyl (0.5%; Sigma-Aldrich), Tris/HCl (20 mM, pH 7.5), β-mercaptoethanol (25 mM; Sigma-Aldrich) and proteinase K (10 mg/ml; Roche Diagnostics, Meylan, France). The liquid phase was recovered by centrifugation at 13 000 r.p.m. and treated using 200 μl phenol/chloroform (70:30, v/v) and 200 μl phenol/chloroform/isoamylic acid (25:24:1, by vol.). RNA pellets were precipitated with 200 ml phenol/chloroform (70:30, v/v) and 200 ml phenol/chloroform/isoamylic acid (25:24:1, by vol.). RNA pellets were precipitated overnight at -20°C in 400 ml ethanol and 20 ml 3 M sodium acetate, pH 5.2. After centrifugation pellets were diluted in 20 ml diethylpolycarbonate (DEPC) treated water and stored at -80°C.

Reverse transcription

Using an RNA-amplification kit (Applied Biosystems, Applera France SA, Courtaboeuf, France), 7.5 μl RNA was reverse transcribed in a final volume of 50 μl 5 mM MgCl₂, 1× buffer, 1 U/μl RNase inhibitor, 1 mM of each dNTP, 2.5 μM random primers and 2.5 U/μl Moloney Murine Leukemia Virus (MuLV) transcriptase. Synthesis of cDNA was performed at 42°C for 45 min, followed by one denaturation step at 94°C for 5 min. Resulting cDNA was stored at -20°C.

Amplification

In order to check RNA extraction, the gapdh gene was amplified using a forward primer overlapping exons 2 and 3. Primer characteristics are detailed in Table 2. Methods and primers used to amplify ret/PTC1 and ret/PTC3 were similar to those used by M. Santoro (Santoro et al. 2000).

PCR were carried out using an RNA-amplification kit (Applied Biosystems). 10 μl cDNA was amplified in a final volume of 25 μl 5 mM MgCl₂, 1× buffer, 4 ng/μl each primer and 0.25 U/μl Taq polymerase. Nested

### Table 1: Epidemiological data of patients in this study

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Females/males</th>
<th>Age at diagnosis (years) Mean Range</th>
<th>Age in 1986 (years) Mean Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary carcinomas (n = 50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquidators (n = 12)</td>
<td>4/8</td>
<td>46.8 35-67</td>
<td>33.3 18-50</td>
</tr>
<tr>
<td>NI Ukrainian patients (n = 17)</td>
<td>7/10</td>
<td>46.8 34-63</td>
<td>33.1 21-47</td>
</tr>
<tr>
<td>NI French patients (n = 21)</td>
<td>15/6</td>
<td>41.7 24-77</td>
<td>30.8 10-67</td>
</tr>
<tr>
<td>Benign tumors (n = 36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquidators (n = 9)</td>
<td>5/4</td>
<td>57.0 49-72</td>
<td>42.1 36-54</td>
</tr>
<tr>
<td>NI Ukrainian patients (n = 14)</td>
<td>5/9</td>
<td>46.1 24-70</td>
<td>30.4 8-56</td>
</tr>
<tr>
<td>NI French patients (n = 13)</td>
<td>7/6</td>
<td>50.2 31-70</td>
<td>39.1 18-63</td>
</tr>
<tr>
<td>Normal tissues (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI French patients (n = 10)</td>
<td>8/2</td>
<td>52.8 30-79</td>
<td>42.4 19-69</td>
</tr>
</tbody>
</table>
PCR were performed with 2 μl PCR products. Amplification consisted of the following steps: denaturation at 94°C for 5 min, amplification by 35 cycles of denaturation at 94°C for 30 s, hybridization at the melting point of the particular primer (see Table 2 for details) for 1 min, and elongation at 72°C for 7 min. Amplification was carried out in a MasterCycler Eppendorf (Dominique Dutscher, Brumath, France). PCR products were stored at −20°C.

Detection of gapdh, ret/PTC1 and ret/PTC3 by hybridization with a specific radiolabeled probe

Electrophoresis of 15 μl PCR products was done in a 2% agarose gel. After 10×SSC capillary transfer to a nylon membrane (Hybond N+; Amersham Bioscience Europe, Orsay, France), blotted products were hybridized either with a ret probe covering the tyrosine kinase domain or a gapdh probe (Table 2). Probes were radiolabeled using an exo-free Kleenow enzyme (Roche Diagnostics) and [α-32P]dCTP. After exposure on an imaging plate radioative energy sensor (BAS-IP.MP 2040S; Fuji Photo Film Co., Kanagawa, Japan) for 24 h at room temperature, membranes were scanned (Fujix BAS1000 IP reader; Fuji) and analyzed with the TINA 2.09 image program (Raytest Isotopenmes- seräte, Courbevoie, France).

Controls

RNA from TPC1 cells and RNA from a PTC harboring ret/PTC3 were used as positive controls for ret/PTC1 and ret/PTC3 respectively. They were provided kindly by Professor A. Fusco (University Federico II, Naples, Italy). Normal thyroid tissue was used as a negative control in addition to the usual no-template controls.

Data analysis and statistical significance

Correlations were analyzed using Fisher’s or χ² tests. For small samples, the Yates correction was applied and exact probability was calculated. Results were considered significant if P was less than 0.05.

Results

Results are summarized in Table 3 and illustrated in Fig. 1. Typical rearrangement-positive cases are shown in Fig. 2. Rearrangement was never observed in normal thyroid tissue. In carcinomas the overall prevalence of ret rearrangement, i.e. ret/PTC1 and ret/PTC3, was 83.3% in liquidators, 64.7% in NI Ukrainian patients, and 42.9% in NI French patients. The prevalence of rearrangement in liquidators was significantly higher than in NI French patients (P = 0.05) but it was not higher than in NI Ukrainian patients. Prevalence of rearrangement in NI Ukrainians was not significantly higher than in NI French patients. In adenomas ret rearrangements were found in 33.3% of liquidators, 57.1% of NI Ukrainian patients, and in no NI French patients. In French patients ret rearrangements were significantly more frequent in carcinomas than adenomas (P = 0.01). This difference was not significant in NI Ukrainian patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’→3’)</th>
<th>Size (bp)</th>
<th>Tm (°C)</th>
<th>Probe (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gapdh</td>
<td>Forward</td>
<td>AGT CAA CGG ATT TGG TCG</td>
<td>167</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GCA AAT TCC ATG GCA CGG</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>ret/PTC1</td>
<td>Forward</td>
<td>ATT GTC ATC TCG CTT TCC</td>
<td>306</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTT TCA GCA TCT TCA CGG</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>ret/PTC3</td>
<td>Forward</td>
<td>AAG CAA ACC TGC CAG TGG</td>
<td>244</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTT TCA GCA TCT TCA CGG</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>ret/PTC1 Nested</td>
<td>Forward</td>
<td>GCA AAG CCA GCG TTA CC</td>
<td>85</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TTC GCC TTC TCC TAG AGT</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>ret/PTC3 Nested</td>
<td>Forward</td>
<td>CCC CAG GAC TGG CTT ACC</td>
<td>113</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TTC GCC TTC TCC TAG AGT</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>

Tm, melting temperature.
but it was almost significant in liquidators ($P=0.055$). In benign nodules the prevalence of \( ret \) rearrangements was significantly higher in all Ukrainians than in NI French patients ($P=0.004$). As in carcinomas, the overall rate of \( ret \) rearrangements in benign nodules from liquidators and NI Ukrainians was not statistically different.

The prevalence of \( ret/PTC1 \) in carcinomas was 58.3% in liquidators, 47.0% in NI Ukrainian patients, and 38.1% in NI French patients. In adenomas \( ret/PTC1 \) was found in 22.2% of liquidators, 35.7% of NI Ukrainian patients, and 0% of NI French patients. In carcinomas no significant difference was found between the three groups. In adenomas the difference was significant only between NI Ukrainian and French patients ($P=0.05$).

The prevalence of \( ret/PTC3 \) in carcinomas was 41.7% in liquidators, 23.5% in NI Ukrainian patients,
and 5% in NI French patients. In adenomas ret/PTC3 was found in 11.1% of liquidators, 28.6% of NI Ukrainian patients, and no NI French patients. In carcinomas the difference was not significant between liquidators and NI Ukrainian patients but it was significant between liquidators and NI French patients (P = 0.03). Although the rate of ret/PTC3 was not statistically higher when only carcinomas from NI Ukrainian patients and carcinomas from NI French patients were compared, a significant difference did appear when all tumors from NI Ukrainian patients (25.8%) were compared with all tumors from NI French patients (2.9%; P = 0.02). The difference was even more significant between all tumors from Ukrainian patients (26.9%) and all tumors from NI French patients (P = 0.005). These data indicate that ret/PTC3 rearrangement in Ukrainian patients was the main source of difference between French and Ukrainian patients.

The relative prevalence of ret/PTC1 and ret/PTC3 was similar in tumors from liquidators and NI Ukrainian patients while the prevalence of ret/PTC1 was higher than that of ret/PTC3 in carcinomas from NI French patients (P = 0.02). Two carcinomas from liquidators, one carcinoma from a NI Ukrainian patient and one adenoma from another NI Ukrainian patient presented both ret/PTC1 and ret/PTC3 rearrangements. A metastasis in the NI Ukrainian patient presenting a carcinoma with both ret/PTC1 and ret/PTC3 expressed only ret/PTC1. Classical PTC and follicular variants showed no significant difference in the prevalence of either ret/PTC1 or ret/PTC3. Carcinomas containing tall-cell areas were more frequent in liquidators than in NI Ukrainian patients (P < 0.01) but the prevalence of ret rearrangements in these tumors was not statistically different, i.e. 8/8 compared with 3/5.

The higher prevalence of ret rearrangements in benign tumors from Ukrainian patients than French patients prompted us to re-examine the histological slides of all benign tumors. Fine analysis of the nuclei was performed to search for isolated or small foci of cells possessing limited nuclear features of papillary cancers. Such areas were found at varying degrees in four adenomas from liquidators, eight from NI Ukrainian patients, and none from French patients. ret rearrangements were found in two adenomas from liquidators and six from NI Ukrainian patients. In two NI Ukrainian patients poor tissue preservation prevented fine analysis of the nuclei in adenomas possessing ret/PTC.

No correlation was found between ret rearrangements and patient gender, extent of the disease according to the TNM classification (International Union Against Cancer 2002), age, or age at exposure concerning irradiated patients.

**Discussion**

The well-documented risk of developing thyroid tumors after radiation exposure was illustrated dramatically by the wave of thyroid carcinomas that occurred in children exposed to fallout from the Chernobyl disaster (Prisyazhiuk et al. 1991, Baverstock et al. 1992, Ron
et al., 1995, Schlumberger et al., 1999). Another documented effect of irradiation was direct induction of ret rearrangements in thyroid cells (Ito et al., 1993, Mizuno et al., 2000). Ret rearrangements have been reported in 84% of thyroid tumors developing after external radiation therapy (Bounacer et al., 1997, de Vathaire et al., 1999) and up to 76% of carcinomas occurring in children after the Chernobyl accident (Nikiforov et al., 1997, Elisei et al., 2001).

Long-term follow up of large cohorts of Chernobyl liquidators has demonstrated a significant increase in the incidence of thyroid carcinoma. Following the break-up of the USSR, the centralized registry of Chernobyl liquidators from all over Soviet Union, including Ukraine, was discontinued and the RNMDR pursued long-term follow up only on liquidators residing in Russia. As reported by Ivanov et al. (2002) individual liquidator data are limited and concern only external radiation. There are no data about thyroid exposure to $^{131}$I. Data from the RNMDR showed a significant increase in thyroid cancer incidence starting from 1992 with a standardized incidence ratio of 5.24. Ivanov et al. consider that the liquidator group described in their study was a representative sample, and that the excess relative risk of thyroid cancer is the same for all liquidators regardless of nationality (Ivanov et al., 1997).

It has been reported previously that thyroid cancer in liquidators was significantly more aggressive than in adults with no history of irradiation (Cherenko et al., 2004). However little is known about the pathological and molecular characteristics of Chernobyl liquidators' thyroid carcinoma. This study presents the first evidence of a high prevalence of ret rearrangements, especially of the ret/PTC3 subtype. Unexpectedly, however, our results also showed high rates of ret/PTC1 and ret/PTC3 in the NI Ukrainian patient control group. In NI French patients the prevalence of ret/PTC1 in carcinomas was higher than previously reported in France but ret/PTC3 prevalence remained low and no rearrangement was found in benign tumors.

Most specimens analyzed were paraffin-embedded in Ukraine and the quality of preservation as well as the integrity of nucleic acids was poor. This could explain the difficulty of detecting ret rearrangements and the necessity of using a highly sensitive technique. Since the detection method used here is qualitative and gives no information about the number of cells affected by ret rearrangements, it is not possible, however, to rule out the possibility that tumors were heterogeneous and contained only a small proportion of affected cell populations. In this regard two studies using the fluorescence in situ hybridization (FISH) method to analyze ret rearrangements in pediatric post-Chernobyl tumors and in adult sporadic tumors showed that only a small proportion of cells in positive cases harbored the ret rearrangement (maximum, 46%; Cinti et al., 2000, Unger et al., 2004). These results suggest that thyroid tumors could be either of polyclonal origin or that ret rearrangement is a late subclonal event. As discussed in a recent editorial by J. A. Fagin, understanding the role of ret rearrangements in thyroid carcinogenesis will require further molecular study (Fagin 2004).

Previous investigations have shown a high prevalence of ret rearrangements in thyroid carcinoma from adult patients exposed to Chernobyl fallout or external radiation as compared with sporadic cancers (Bounacer et al., 1997, Smida et al., 1999, Rabes et al., 2000, Elisei et al., 2001). The significant increase in the rate of ret rearrangements in carcinoma from liquidators in this study is consistent with these results. However, most previous studies indicated that in adults both external and Chernobyl radiation induced mainly ret/PTC1 rearrangements (Ito et al., 1993, Bounacer et al., 1997, Mizuno et al., 2000, Elisei et al., 2001), whereas in children external radiation induced ret/PTC1 and Chernobyl radiation induced mainly ret/PTC3 rearrangements (Klugbauer et al., 1995, Nikiforov et al., 1997, Santoro et al., 2000). Thus our results showing a high prevalence of ret/PTC3 in liquidators as well as in NI adult Ukrainians are unexpected.

The preponderance of ret/PTC3 in children with post-Chernobyl carcinomas has been linked to various factors including the effects of radioactive iodine, short latency period for tumor onset, and a special sensitivity of thyroid tissue in young children to radiation (Fugazzola et al., 1995, Klugbauer et al., 1995). The shift from ret/PTC3 to ret/PTC1 in adolescents and adults from Belarus and Ukraine has been attributed to older age at time of exposure and longer latency time between exposure to Chernobyl fallout and tumor onset (Nikiforov 2002). In agreement with the findings of Elisei et al. (2001), our data showing a high rate of ret/PTC3 in liquidators refute the hypothetical relationship of ret/PTC3 with age at exposure or latency time to tumor onset. All but one liquidator were older than 23 years in 1986 and the tumor in the youngest, who was 18 years old in 1986, exhibited ret/PTC1. The latency period between clean-up work and tumor onset ranged from 12 to 17 years. These findings suggest that the high rate of ret/PTC3 in the thyroid tumors from both liquidators and irradiated children could result from a special effect of Chernobyl fallout.

A novel aspect of this study was to compare the prevalence of ret rearrangements in thyroid cancers from liquidators with that of sporadic cancers from NI
adult Ukrainian patients living outside contaminated areas, i.e. people having a similar genetic background. No such comparisons were described in previous studies on adult patients exposed to Chernobyl fallout (Klugbauer et al. 1995, Smida et al. 1999, Rabes et al. 2000). We found a higher prevalence of rearrangements in carcinomas from liquidators than from NI Ukrainian patients. This difference was especially pronounced for \( \text{ret}/\text{PTC3} \), i.e. 41.7\% for liquidators compared with 23.5\% for NI Ukrainian patients, but did not reach statistical significance, possibly due to small sample size. The higher rate of \( \text{ret}/\text{PTC3} \) in all tumors from NI Ukrainian patients than from French patients suggests that Ukrainians may also have a genetic propensity for \( \text{ret}/\text{PTC3} \) rearrangement.

Geographical differences in the prevalence of \( \text{ret} \) rearrangements are probably not attributable solely to differences in methodology. Such differences have been observed in studies like ours in which the same methodology was used to analyze tumor specimens from different countries (Santoro et al. 1992). Ethnic susceptibilities and environmental factors have been put forth as possible explanations for geographical differences. Genetic background might play a significant role since high prevalence rates ranging from 43 to 63\% have been reported, especially in the Asian-Pacific countries, independently of any known environmental risk factor (Lee et al. 1998, Chua et al. 2000, Mussholt et al. 2000, Lam et al. 2002). However, all speculation in this regard is currently hazardous since little is known about the environmental factors, other than documented radiation exposure, that might induce \( \text{ret} \) rearrangements.

An alternative explanation for the relative higher rate of \( \text{ret} \) rearrangements that we observed in so-called sporadic Ukrainian tumors would be uneven exposure to Chernobyl fallout. A study of genetic damage in wildlife from contaminated areas and control regions of Belarus and Ukraine yielded similar results, i.e. increased mutation rates in control regions. Based on this finding it has been suggested that some presumably ‘clean’ regions in Belarus and Ukraine may have been contaminated to some degree and that low-level exposure to radiation might be harmful to chromosomes (Stone 2001). A recent study on thyroid tumors from patients treated by low-dose external radiation found 49.9\% of \( \text{ret} \) rearrangements in carcinoma and 46.2\% in adenoma (Sadetski et al. 2004). As the patients in the NI Ukrainian control group originated from Kiev, which is 100 km from Chernobyl, there is a high likelihood that some of them were exposed to some radiation. To clarify this point, we tried to evaluate tumors from Ukrainian patients operated on before 1986 but could not complete the study because tissue samples were either unavailable or too poorly preserved to allow RNA analysis.

Our prevalence of \( \text{ret} \) rearrangements in PTCs of patients from south-eastern France (42.9\%) was the highest ever reported in France. Previous figures from more northerly French regions have ranged from 11 to 15\% (Santoro et al. 1992, Delvincourt et al. 1996, Bounacer et al. 1997). This difference was due solely to \( \text{ret}/\text{PTC1} \) since the low prevalence of \( \text{ret}/\text{PTC3} \) in our study was the same as reported in the Paris region (Bounacer et al. 1997). Two previous studies suggest that the highly sensitive detection method used in this study might not fully explain the higher prevalence of \( \text{ret} \) rearrangements in our patients. Cinti et al. (2000), who used the FISH method considered as one of the most sensitive, also found a low prevalence of rearrangements in carcinoma from Lyon and Poitiers in more northerly parts of France. Santoro et al. (1992), who used the same method as us, found a significantly higher rate of rearrangements in carcinoma from Milan than from Lyon (33 vs 11\%, \( P<0.01 \)). Thus it can be speculated that the high rates found in Marseille, a major port city on the Mediterranean coast, are linked to differences in geographical factors rather than methodology. Two studies focused on Mediterranean populations have found \( \text{ret} \) rearrangements in 50\% of sporadic carcinomas from Italy and 66.7\% of sporadic carcinomas from North Africa (Elisei et al. 2001, Sadetski et al. 2004). Marseille has known several waves of immigration from these countries.

Conflicting results have been reported concerning the presence and frequency of \( \text{ret} \) rearrangements in benign thyroid nodules (Ishizaka et al. 1991, Bounacer et al. 1997, Cheung et al. 2001, Elisei et al. 2001, Sadetski et al. 2004). According to PCR-based studies the prevalence in naturally occurring adenoma has ranged from 0\% (Bounacer et al. 1997, Cheung et al. 2001) to 50\% (Sadetski et al. 2004). In radiation-associated adenoma the highest prevalences, i.e. 52.4\%, have been observed in post-Chernobyl adenomas (Elisei et al. 2001). Our results showing \( \text{ret} \) rearrangements in 33.3\% of benign nodules from liquidators and none from French patients are in keeping with most previous findings. Several arguments support the idea that thyroid adenoma is a heterogeneous tumor class and that some are composed of cell populations with different molecular oncogenic processes (Ishizaka et al. 1991, Vasko et al. 2003). In thyroid nodules with incomplete morphological features of papillary carcinoma, \( \text{ret} \) rearrangements occur in foci possessing nuclei with papillary nuclear features, suggesting that
these foci may be precursors of papillary cancers (Fusco et al. 2002, Vasko et al. 2004). In our study such foci were found in 60% of adenomas from Ukrainian patients and corresponded with the presence of ret rearrangements in 72.7% of the cases (nuclear features could not be analyzed reliably in two cases). The absence of such foci in adenomas from French patients further supports the correlation of nuclear morphology with ret activation.

This study confirms the role of ret rearrangements and in particular of ret/PTC3 in the development of thyroid tumors after exposure to Chernobyl fallout. Indeed the high level of ret/PTC3 observed in liquidators shows that this feature is not restricted to pediatric cases or rapidly developing carcinoma. It is also found thyroid carcinoma developing in adults more than 10 years after exposure. However, the baseline prevalence of ret rearrangement was also high in tumors from NI Ukrainian patients occurring after 1986 in comparison with tumors from NI French patients. Although the implication of genetic background cannot be ruled out, it is reasonable to speculate that uneven exposure to radiation accounts for the high rate of ret/PTC3 rearrangement observed in Ukrainian patients.

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