The EdU incorporation method was originally developed for complementing known problems of S-phase population labeling by ^{3}H-thymidine and BrdU (19). In EdU, the methyl group on the 5-position of thymidine is replaced by an alkyne group, and this analogue is incorporated into DNA during replication. Subsequent fluorescent-azide coupling, termed 'click reaction' is rapid, sensitive and specific (19,20); because of this property, the sensitivity and background fluorescent signal were both dramatically improved, and hence, EdU can be used for the UDS assay.

We have presented data demonstrating the usefulness of EdU for the UDS assay. Although the sensitivity of the EdU-based method is not as high as the autoradiographic technique, we easily detected the defect in an XP-D cell line with ~40% residual UDS activity and based on our data we conservatively estimate that we can detect a level of 10–20% of normal UDS.

The EdU-based UDS assay may have an advantage in high-throughput XP diagnosis using flow cytometry. An attempt to use BrdU labeling and flow cytometry for UDS has been reported (14). Because of the improved sensitivity of EdU over BrdU, further introduction of multi-color labeling (i.e. normal internal control as well as specimen cells, with or without UV damage, all labeled with different fluorescent dyes) may provide a rapid and precise determination of the relative UDS levels.

In this report, we have demonstrated that: (i) the accuracy and resolution of the UDS assay based on EdU incorporation was comparable to the conventional autoradiographic method; we could distinguish between a normal and an XP-deficient primary fibroblast when the cells were co-cultured on a single coverslip. (ii) The assay was compatible with standard immunostaining and latex-bead-labeling, which provide proper internal controls. (iii) The time required for the assay was dramatically reduced; the entire UDS assay can be completed within half a day from the preparation of specimen coverslips. The UDS assay based on EdU could potentially become a standard technique in NER research as well as XP diagnosis.

ACKNOWLEDGEMENTS

We are grateful to Dr. Nicolaas Jaspers for helpful comments on the manuscript. We also thank Dr. Motohiro Yamauchi for critical advice on the EdU incorporation assay.

FUNDING

The KAKENHI from Japan Society for the Promotion of Science (grant number 20810021); a Special Coordination Fund for Promoting Science and Technology from Japan Science and Technology Agency; a Butterfield Award from the Great Britain Sasakawa Foundation (to T.O.); and a grant from the Thailand Research Fund and Commission on Higher Education (to S.L.); a Global COE Program from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan (to S.L., S.Y., and T.O.). Funding for open access charge: the KAKENHI.

Conflict of interest statement. None declared.

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Polymorphisms of DNA damage response genes in radiation-related and sporadic papillary thyroid carcinoma

Natallia M Akulevich[1], Vladimir A Saenko[2], Tatiana I Rogounovitch[1], Valentina M Drozd[3], Eugeny F Lushnikov[4], Victor K Ivanov[4], Norisato Mitsutake[1], Ryo Kominami[5] and Shunichi Yamashita[1,2]

Departments of [1]Molecular Medicine and [2]International Health and Radiation Research, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8523, Japan
[3]Department of Thyroid Disease Research, Belarusian Medical Academy for Postgraduate Education, Minsk 220013, Republic of Belarus
[4]Medical Radiological Research Center, Obninsk 249036, Russian Federation
[5]Department of Molecular Genetics, Graduate School of Medical and Dental Sciences, Niigata University, Niigata 951-8122, Japan

(Correspondence should be addressed to V A Saenko; Email: saenko@net.nagasaki-u.ac.jp)

Abstract

Papillary thyroid carcinoma (PTC) etiologically occurs as a radiation-induced or sporadic malignancy. Genetic factors contributing to the susceptibility to either form remain unknown. In this retrospective case–control study, we evaluated possible associations between single-nucleotide polymorphisms (SNPs) in the candidate DNA damage response genes (*ATM*, *XRCC1*, *TP53*, *XRCC3*, *MTF1*) and risk of radiation-induced and sporadic PTC. A total of 255 PTC cases (123 Chernobyl radiation-induced and 132 sporadic, all in Caucasians) and 596 healthy controls (198 residents of Chernobyl areas and 398 subjects without history of radiation exposure, all Caucasians) were genotyped. The risk of PTC and SNPs interactions with radiation exposure were assessed by logistic regressions. The *ATM* G5557A and *XRCC1* Arg399Gln polymorphisms, regardless of radiation exposure, associated with a decreased risk of PTC according to the multiplicative and dominant models of inheritance (odds ratio (OR) = 0.69, 95% confidence interval (CI) 0.45–0.86 and OR = 0.70, 95% CI 0.59–0.93 respectively). The *ATM* IVS22-77 T > C and *TP53* Arg72Pro SNPs interacted with radiation ($P = 0.04$ and $P = 0.01$ respectively). *ATM* IVS22-77 associated with the increased risk of sporadic PTC (OR = 1.84, 95% CI 1.10–3.24) whereas *TP53* Arg72Pro correlated with the higher risk of radiogenic PTC (OR = 1.80, 95% CI 1.06–2.36). In the analyses of *ATM*/*TP53* (rs1801516/rs664677/rs609429/rs1042522) combinations, the GG/TC/CG/GC genotype strongly associated with radiation-induced PTC (OR = 2.10, 95% CI 1.17–3.78). The GG/CC/GG/GG genotype displayed a significantly increased risk for sporadic PTC (OR = 3.32, 95% CI 1.57–6.99). The results indicate that polymorphisms of DNA damage response genes may be potential risk modifiers of ionizing radiation-induced or sporadic PTCs.

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Introduction

Thyroid cancer accounts for more than 90% of all endocrine malignancies. The incidence of thyroid cancer in the world is increasing during the past three decades, mainly due to the papillary thyroid carcinoma (PTC) which is the predominant type of malignant thyroid tumors (Davies & Welch 2006).

Most thyroid cancer patients do not have the history of radiation exposure, yet ionizing radiation (IR) is a recognized etiological factor of the disease. An increased risk of thyroid cancer has been documented after external irradiation (Ron *et al.* 1995) and after environmental exposure to ^{131}I, such as after the Chernobyl fallouts in Belarus, Ukraine, and Russia (Bennett *et al.* 2006).

Although radiation thyroid doses in Chernobyl PTC cases are generally greater than in controls in epidemiological studies (Cardis *et al.* 2005, Jacob *et al.* 2006, Likhtarev *et al.* 2006), thus confirming radiation to be a risk factor for thyroid cancer, those in

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1351–0088/09/016–491
DOI: 10.1677/ERC-08-0336
Online version via http://www.endocrinology-journals.org

controls are non-zero. Furthermore, there were some 14 million residents in the contaminated territories at the time of exposure (Bennett *et al.* 2006). Conceivably, at least some of them might have accumulated thyroid doses comparable with doses in diseased individuals. However, thyroid cancer developed only in a small fraction of irradiated population.

Among the variety of DNA damage types induced by radiation, double-strand DNA breaks are considered to be the most significant for chromosomal aberrations, mutagenesis, genetic instability, and carcinogenesis (Khanna & Jackson 2001). PTC is one of the rare human cancers of epithelial origin in whose oncogenesis gene rearrangements play a noticeable role. Several variants of rearrangements are described in PTC, with RET/PTC occurring most frequently (Nikiforov *et al.* 1997, Rabes *et al.* 2000).

While in the exposed individuals DNA damage could be attributed to IR, the origination of genetic alterations in sporadic cancers remains obscure. Nevertheless, the spectrum of oncogenic changes in radiation-related and sporadic PTCs is largely common. Such similarities imply the resemblance of molecular reactions on DNA damage in exposed and non-exposed thyrocytes. These reactions involve first of all DNA damage response factors, including DNA repair and checkpoint complexes.

The vast majority of Chernobyl thyroid malignancies were PTCs which displayed wide variations in clinical course, from highly aggressive tumors developing after the shorter latency to more indolent carcinomas with the longer latent period (Williams 2006). The randomness and multiplicity of forms of genetic alterations caused by IR can only partly explain these differences in the individual reactions on exposure as well as why cancer develops only in some of the exposed individuals.

It is attractive to hypothesize that inherited variability in the genes directly or indirectly involved in the maintenance of genome stability in response to environmental carcinogens such as IR or chemicals that may play a role in susceptibility for radiation-related or sporadic PTC or may be a marker of it. In this work, we tested the relation of genetic variants of some of such genes, namely *ATM*, *TP53*, *XRCC1*, *XRCC3*, and *MTF1* to PTC of different etiology.

The ataxia-telangiectasia mutated (*ATM*) gene plays a key role in the sensing and repair of DNA double-strand breaks. Activation of the ATM protein kinase by IR results in the subsequent initiation of several molecular pathways of DNA damage repair (Shiloh 2003). One of the ATM targets is the p53 pathway. Overexpression of *TP53* arrests the cell cycle and affects DNA repair and apoptosis.

The *ATM* and *TP53* genes play a significant role especially in the tumors that are induced by IR. A number of single-nucleotide polymorphisms (SNPs) in the *ATM* and *TP53* genes studied in populations of different ethnicities have been reported to associate with the risk of different radiogenic tumors (Hu *et al.* 2002, Angele *et al.* 2003, Thorstenson *et al.* 2003, Malmer *et al.* 2007). By contrast, studies of post-Chernobyl pediatric thyroid cancers demonstrated a low mutation and polymorphism rate in the *TP53* gene (Nikiforov *et al.* 1996, Hillebrandt *et al.* 1997). It, however, should be mentioned that after exposure to radiation p53 facilitates DNA repair in normal thyrocytes *in vitro* (Yang *et al.* 1997).

The base excision repair (BER) and homologous recombination repair (HRR) pathways are particularly important for genomic integrity restoration (Hoeijmakers 2001). The product of the X-ray repair cross complementing 1 (*XRCC1*) gene acts as a scaffold and a modulator of different enzymes involved in BER. The *XRCC1* Arg399Gln and Arg280His variants have been extensively investigated for their function and association with cancer risk; however, the results remain contradictory rather than conclusive (Hu *et al.* 2005). The *XRCC3* gene is a member of the *Rad51* DNA-repair gene family. Its product is a factor of the HRR. The *XRCC3* Thr241Met polymorphism has been controversially associated with different human malignancies (Han *et al.* 2006). Sturgis *et al.* (2005) reported 241Met allele association with the risk of differentiated thyroid cancer.

The metal-responsive transcription factor 1 (*MTF1*) gene has been implicated in tumor initiation and progression to malignant growth. MTF1 protein interacts with metallothioneins that are able to suppress cellular stresses generated by IR and other agents (Tamura *et al.* 2005). Polymorphism in murine *Mtf1* gene has been found to associate with the susceptibility to experimental γ-ray-induced thymic lymphomas. This observation points at possible involvement of human *MTF1* polymorphisms in the modulation of radiation-induced malignancies (Tamura *et al.* 2005).

To date no polymorphisms of the *ATM*, *XRCC1*, and *MTF1* genes have been studied neither in human sporadic or radiation-induced PTCs. Data on the *TP53* and *XRCC3* polymorphisms associations are quite limited (Hillebrandt *et al.* 1997, Boltze *et al.* 2002, Granja *et al.* 2004, Sturgis *et al.* 2005, Rogounovitch *et al.* 2006). Therefore, in this study, we addressed the relation of SNPs in aforementioned DNA damage response genes to the risk of PTCs of different etiology.

Materials and methods

Study population

A total of 255 histologically verified PTC cases and 596 healthy controls, all Caucasians, were included in the study. Among the patients, 123 individuals with PTC (24 males and 99 females) lived in the areas of the Russian Federation (38 patients) and Belarus (85 patients) contaminated with radionuclides from Chernobyl fallouts. At the time of the Chernobyl accident, these subjects were younger than 18 years old (mean age at exposure ± S.D., 9.8 ± 5.1 years old; 1–18 years old, range). The mean age at diagnosis was 24.4 ± 4.9 years old, range 19–37 years old (IR-induced PTCs). Information about individual radiation thyroid doses was available for PTC cases from Russia as reconstructed in previous studies (Davis *et al.* 2004, Stepanenko *et al.* 2004). The doses varied from 43 to 2640 mGy. Radiation thyroid doses for PTC patients and controls from Belarus evaluated in dosimetric investigations at the places of residence ranged 21–1500 mGy (Bouville *et al.* 2007). Among the controls, 198 individuals (65 males and 133 females, mean age at sampling 22.2 ± 3.2 years old; 19–35 years old, range) were residents of the Chernobyl areas (60 from the Russian Federation and 138 from Belarus). The averaged thyroid radiation dose in the exposed control subjects from Russia is 41 mGy (Bouville *et al.* 2007). All exposed control individuals were aged <18 years at the time of the accident (mean age at exposure 1.8 ± 3.2 years old; 1–16 years old, range) (IR-exposed controls). IR-exposed controls and patients with IR-induced PTCs not were individually matched; however, they were residents of the same settlements. This, given the uncertainty with individual radiation thyroid doses, was supposed to partly reduce exposure bias. Age of IR-exposed control subjects was set to be ±3 years of that of IR-induced PTC individuals.

One hundred and thirty-two PTC cases (21 males and 111 females, mean age at diagnosis 47.8 ± 11.4 years old; 19–76 years old, range) were adults without history of radiation exposure (sporadic PTCs). The remaining 398 control participants (180 males and 218 females, mean age at sampling 45.0 ± 10.3 years old; 16–65 years old, range) had no previous history of radiation exposure (non-exposed controls); their age was also set to be ±3 years of that of patients with sporadic PTC. Both sporadic PTCs and non-exposed controls originated from the European part of Russia not contaminated by the Chernobyl fallouts.

Thyroid tissues and/or blood samples were collected from patients during surgery or further follow-up. Blood samples and information from the controls were obtained during a routine health examination or complex screening for thyroid diseases.

Written informed consent was obtained from all participants. Protocols of the present study were approved by the Committee for Ethical Issues of Human Genome Analysis of Nagasaki University.

SNP selection

The candidate SNPs (Table 1) were selected based on their reported functional role (if available), associations with radiosensitivity or (thyroid) cancer risk. Accordingly, we did not search for tag SNPs or account for the genetic variability in the regions of SNP location. All SNPs are listed in a public database, dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), with validated status in ethnically diverse populations. To ensure sufficient power for calculations, only SNPs with minor allele frequency (MAF) of >1% were included.

SNP genotyping

DNA was extracted from normal thyroid tissues using proteinase K/phenol–chloroform method or from the whole blood lymphocytes with Puregene DNA Purification Kit (Gentra Systems, Inc., Minneapolis, PA, USA). All specimens were genotyped using various techniques (Table 1). Primers and probes (Table 2) were designed with Primer Express Version 1.0 (Applied Biosystems, Foster City, CA, USA) software.

Briefly, 25 μl PCR mixtures generally contained 50 ng DNA, 1.5 mM $MgCl_2$, 200 μM each dNTP, optimized concentrations of corresponding primers and 0.625 U AmpliTaq Gold (Applied Biosystems). All restriction endonucleases for PCR/RFLP were from New England BioLabs (Ipswich, MA, USA). TaqMan allelic discrimination assay for *TP53* variants was done essentially as described previously (Rogounovitch *et al.* 2006). Melting curve T_m-shift assay for *MTF1* genotyping was designed according to the described technology (Wang *et al.* 2005) and done in a Thermal Cycler Dice Real Time System TP800 (TaKaRa, Ohtsu, Japan). Technical details are available from the authors upon request.

For every SNP, some 20–30 randomly chosen DNA samples, unless otherwise specified, were also analyzed by direct sequencing with a Big Dye Terminator sequencing kit v 3.1 (Applied Biosystems) in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). A complete concordance between different techniques was observed.

Table 1 Single-nucleotide polymorphism (SNP), genotyping methods and possible functional role

SNP nucleotide/amino acid change	**Database ID**	**Genotyping method**	**Chromosome/exon or intron**	**MAF (%) in different populations** (NCBI dbSNP)	**SNP effects, minor allele versus wild-type** (reference)
ATM G5557A Asp1853Asn	rs1801516	PCR/RFLP[a] (AflII) GG (187, 30) GA (217, 187, 30) AA (217)	11/exon 39	European: 7–22 Asian: 0–2 Global: 5	Alters an exonic splicing enhancer, modulates correct splicing of exon 39 (Thorstenson *et al.* 2003) Decreases *ATM* expression level and capacity of DNA damage recognition (Heikkinen *et al.* 2005)
ATM IVS22−77 T>C T60136C	rs664677	PCR/RFLP (RsaI) TT (299) TC (299, 265, 34) CC (265, 34)	11/intron 22	European: 34–50 Asian: 44–70 Global: 35–36	No reports
ATM IVS48+238 C>G C113450G	rs609429	PCR/RFLP (KpnI) CC (172, 35) CG (207, 172, 35) GG (207)	11/intron 48	European: 60 Asian: 37 Global: 53	Generates a week additional donor splice site and decreases gene expression (Angele *et al.* 2003)
XRCC1 G25211A Arg280His	rs25489	PCR/RFLP (RsaI) GG (155, 123) GA (278, 155, 123) AA (278)	19/exon 9	European: 3–10 Asian: 0 Global: 7	Compromises DNA repair (reviewed by Hu *et al.* 2005)
XRCC1 G25897A Arg399Gln	rs25487	PCR/RFLP (MspI) GG (327, 107) GA (434, 327, 107) AA (434)	19/exon 10	European: 30–46 Asian: 27 Global: 23–26	Affects IR-induced mitotic delay and hypersensitivity to IR (Hu *et al.* 2002) Compromises single-strand DNA breaks repair (controversially) (reviewed by Taylor *et al.* 2002)
TP53 G640C Arg72Pro	rs1042522	TaqMan	17/exon 4	European: 23–27 Asian: 40–51 Global: 35	Lower efficiency in apoptosis induction; higher level of G1 arrest (Pim & Banks 2004)
XRCC3 C18067T Thr241Met	rs861539	TaqMan	14/exon 7	European: 41–45 Asian: 6–14 Global: 22	Decreased DNA repair capacity (reviewed by Han *et al.* 2006)
MTF1 T2193A	rs11488567	Melting curve T_m-shift	1/intron 1	Unknown	No reports
MTF1 G20433A	rs3912368	Melting curve T_m-shift	1/intron 5	European: 25–37 Asian: 21	No reports

[a]Restriction enzymes, genotypes and corresponding restriction fragments sizes (bp) are indicated for the SNPs analyzed by PCR/RFLP.

Table 2 Primers and probes for genotyping

SNP	Primer/probe sequences (5′–3′)[a]	Primer/probe concentration (μM)	Annealing temperature (°C)
ATM G5557A	F: CCATACTTGATTCATGATATTTTACcttAA	0.2	57
	R: TTCCATCTTAAATCCATCTTTCTC	0.2	
ATM IVS22−77 T>C	F: AGTTTAGCACAGAAAGACATATTGGAAGTAACgTA	0.2	57
	R: CGGGAAAAGAACTGTGGTTAAATATGAAA	0.2	
ATM IVS48+238 C>G	F: CTCAATTTCCTGGTTATAAAATGAGAAGgTAC	0.2	57
	R: TTAACTACTTGTCAGGGACTATCTTAAGGAC	0.2	
XRCC1 G25211A	F: GTCTGAGGGAGGAGGGTCTG	0.2	59
	R: TTCTGGAAGCCACTCAGCAC	0.2	
XRCC1 G25897A	F: CCACCAGCTGTGCCTTTG	0.2	55
	R: CCGGGACTCACTTTGAATGA	0.2	
TP53 G640C	F: CGTCCCAAGCAATGGATGATT	0.8	61
	R: CCGGTGTAGGAGCTGCTGG	0.8	
	w/t allele probe (FAM): CTCCCCGCGTGGCCCC	0.4	
	Variant allele probe (VIC): CTCCCCCCGTGGCCCC	0.4	
XRCC3 C18067T	F: AGGGCCAGGCATCTGCA	0.8	61
	R: CTTCCGCATCCTGGCTAA	0.8	
	w/t allele probe (FAM): TCACGCAGCGTGGCCCCCAG	0.5	
	Variant allele probe (VIC): TCACGCAGCATGGCCCCCAG	0.5	
MTF1 T2193A	F1: GCGGGCAGGGCGGCTTAACTTTAAAACCATCAAGTCATTTTTAgA	0.2	58
	F2: GCGGGCTTAACTTTAAAACCATCAAGTCATTTTTAAT	0.2	
	R: ACGCCCAGTCGGCATTGCT	0.2	
MTF1 G20433A	F1: GCGGGCAGGGCGGCCTAATTATGCTCACCTGAATATATACAGGG	0.075	63
	F2: GCGGGCCTAATTATGCTCACCTGAATATATACAGGA	0.2	
	R: GAGACCTGTAGAGCTAGGTGGATATACAGAGATAT	0.2	

[a]The bases shown in lowercase are mismatches introduced to generate restriction endonuclease sites (PCR/RFLP) or to optimize allelic specificity (T_m-shift). The underlined 5′ portions of primer sequences correspond to GC tails in the T_m-shift method.

Raw genotyping outputs were interpreted by at least two independent investigators. Missing results due to genotyping procedure failures accounted for <1% for any SNP tested.

Statistical analysis

Genotype frequencies in each group were determined by univariate analysis and evaluated for departure from Hardy-Weinberg equilibrium by the χ^2 test. SNP associations with PTC were assessed by multivariate logistic regression analysis for codominant, multiplicative, dominant, and recessive models to avoid assumptions regarding the mode of inheritance (see notes below Table 4). All analyses were adjusted for gender (male or female, nominal), age (years, continuous), and IR-exposure (yes or no, nominal). Besides all the parameters above, the full model included disease status (yes or no, nominal) and, depending on the mode of inheritance, genotype for each SNP (nominal variable in the codominant, dominant, and recessive models and ordinal in the multiplicative model).

Power calculations were done with the PS software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). With given sample size, the study had a power of 54–99% to detect an OR of 2.0 at the significance level of 5% with MAF ranging 4–45%.

Interaction between SNPs, cancer and radiation exposure were hypothesized *a priori* and evaluated by multivariate analysis with corresponding adjustments. Separate calculations of OR were done in irradiated and non-exposed case–control groups when *P* value for an interaction term did not exceed 0.05.

Statistical analysis was done using SPSS for Windows version 17.0 (SPSS, Inc., Chicago, IL, USA).

Results

The distribution of genotypes and MAF for each SNP in the four study groups is shown in Table 3. The observed distributions in the control groups were not statistically different from those expected from Hardy-Weinberg equilibrium for all SNP except for *ATM* G5557A and *ATM* IVS22-77 T>C in the non-exposed controls. Since such deviation might point at possible genotyping error (Hosking *et al.* 2004), we reanalyzed 96 non-exposed controls for these SNPs by direct sequencing. There were no inconsistencies between PCR/RFLP and sequencing results (data not shown) ruling out technical flaw. Furthermore, allelic frequencies determined in our study are in a good agreement

Table 3 Distribution of genotypes and minor allele frequencies by study groups

SNP, genotype	IR-induced PTC *n* (%)	IR-exposed controls *n* (%)	Sporadic PTC *n* (%)	Non-exposed controls *n* (%)
ATM G5557A	*n*=122	*n*=198	*n*=132	*n*=398
GG	95 (77.9)	138 (69.7)	105 (79.5)	293 (73.6)
GA	25 (20.5)	53 (26.8)	24 (18.2)	90 (22.6)
AA	2 (1.6)	7 (3.5)	3 (2.3)	15 (3.8)
P	0.24		0.36	
A, %	11.9	16.9	11.4	15.1
ATM IVS22−77 T>C	*n*=123	*n*=195	*n*=132	*n*=398
TT	35 (28.4)	62 (31.8)	45 (34.1)	135 (33.9)
TC	76 (61.8)	102 (52.3)	61 (46.2)	216 (54.3)
CC	12 (9.8)	31 (15.9)	26 (19.7)	47 (11.8)
P	0.17		0.06	
C, %	40.6	42.0	42.8	38.9
ATM IVS48+238 C>G	*n*=122	*n*=196	*n*=132	*n*=398
CC	37 (30.3)	68 (34.7)	41 (31.1)	131 (32.9)
CG	69 (56.6)	97 (49.5)	61 (46.2)	201 (50.5)
GG	16 (13.1)	31 (15.8)	30 (22.7)	66 (16.6)
P	0.47		0.28	
G, %	41.4	40.3	45.8	41.8
XRCC1 Arg280His[a]	*n*=123	*n*=195	*n*=132	*n*=398
GG	113 (91.9)	176 (90.3)	117 (88.6)	366 (92.0)
GA	10 (8.1)	19 (9.7)	15 (11.4)	32 (8.0)
P	0.63		0.24	
A, %	4.1	4.9	5.7	4.0
XRCC1 Arg399Gln	*n*=123	*n*=197	*n*=132	*n*=398
GG	55 (44.7)	75 (38.1)	65 (49.2)	158 (39.7)
GA	50 (40.7)	100 (50.7)	53 (40.2)	193 (48.5)
AA	18 (14.6)	22 (11.2)	14 (10.6)	47 (11.8)
P	0.20		0.15	
A, %	35.1	36.5	30.7	36.1
TP53 Arg72Pro	*n*=122	*n*=197	*n*=129	*n*=395
GG	53 (43.4)	115 (58.4)	69 (53.5)	196 (49.6)
GC	57 (46.7)	73 (37.0)	49 (38.0)	161 (40.8)
CC	12 (9.9)	9 (4.6)	11 (8.5)	38 (9.6)
P	0.02		0.74	
C, %	33.2	23.1	27.5	30.0
XRCC3 Thr241Met	*n*=120	*n*=198	*n*=132	*n*=398
CC	53 (44.2)	82 (41.4)	55 (41.7)	161 (40.5)
CT	51 (42.5)	89 (45.0)	65 (49.2)	192 (48.2)
TT	16 (13.3)	27 (13.6)	12 (9.1)	45 (11.3)
P	0.89		0.78	
T, %	34.6	36.1	33.7	35.4
MTF1 T2193A	*n*=122	*n*=198	*n*=131	*n*=397
TT	45 (36.9)	82 (41.4)	44 (33.6)	133 (33.5)
TA	64 (52.5)	91 (46.0)	67 (51.1)	188 (47.4)
AA	13 (10.6)	25 (12.1)	20 (15.3)	76 (19.1)
P	0.52		0.57	
A, %	36.8	35.6	40.8	42.8
MTF1 G20433A	*n*=123	*n*=198	*n*=132	*n*=398
GG	62 (50.4)	100 (50.5)	66 (50.0)	192 (48.2)
GA	53 (43.1)	88 (44.4)	56 (42.4)	151 (38.0)
AA	8 (6.5)	10 (5.1)	10 (7.6)	55 (13.8)
P	0.85		0.16	
A, %	28.0	27.3	28.8	32.8

NOTE. Total numbers of samples in each group vary slightly due to genotyping procedures failures.
[a]There was no homozygous (A/A) variant of XRCC1 Arg280His among all samples tested.

with those specified for Caucasians in the dbSNP (build 129, April 2008, Table 1) thus attesting to the appropriate data quality.

As seen from Table 4, an association between *ATM* G5557A and PTC, regardless of radiation exposure, was found. The presence of the A allele significantly decreased PTC risk compared with wild-type G allele in the multiplicative model of inheritance (OR = 0.69, 95% CI 0.45–0.86, $P = 0.03$), which is useful for risk comparison between the groups based on the analysis of allelic frequencies in them.

Main effect on PTC risk appeared also significant for the *XRCC1* gene Arg399Gln polymorphism. The presence of the minor 399Gln allele decreased PTC risk compared with the Arg/Arg genotype (OR = 0.66, 95% CI 0.57–0.88, $P = 0.02$ and OR = 0.70, 95% CI 0.59–0.93, $P = 0.03$, in the co-dominant and dominant models respectively).

Analysis of combined *ATM* G5557A and *XRCC1* Arg399Gln genotypes demonstrated that increasing number of minor alleles (i.e. *ATM* 5557A and *XRCC1* 399Gln) significantly decreased PTC risk in corresponding individuals in comparison with those who do not carry minor alleles (Fig. 1).

No other SNP in any gene showed a significant main effect on PTC.

For *ATM* IVS22-77 T>C and *TP53* Arg72Pro, evidence for interaction between radiation exposure and PTC was found (P for interaction 0.04 and 0.01 respectively). As shown in Table 5, the analyses performed in IR-exposed and non-irradiated patients compared respectively, with irradiated and non-exposed controls revealed a significantly increased risk of sporadic PTC for the *ATM* IVS22-77 homozygous CC genotype carriers compared with the TC+TT genotypes (the recessive model of inheritance, OR = 1.84, 95% CI 1.10–3.24, $P = 0.03$), whereas in the irradiated group an insignificant inverse effect of these genotypes was observed (OR = 0.59, 95% CI 0.28–1.27, $P = 0.17$). For *TP53* codon 72 polymorphism, in all but the recessive models the increased risk of IR-induced PTC as compared with IR-exposed controls was observed. The highest risk of radiogenic PTC was in the co-dominant model (OR = 2.33, 95% CI 1.15–7.21, $P = 0.03$). A significant risk was also found in the multiplicative model of inheritance (OR = 1.70, 95% CI 1.17–2.46, $P = 0.006$). In addition, comparison between IR-exposed and non-exposed controls did not reveal statistically significant difference in adjusted distributions of these polymorphisms. In healthy subjects, the strongest association for the *ATM* IVS22-77 T>C was in the recessive model (OR = 1.38, 95% CI 0.84–2.26, $P = 0.21$) and in the multiplicative model for *TP53* Arg72Pro (OR = 0.70, 95% CI 0.52–1.19, $P = 0.11$) further emphasizing possible role of these SNPs in PTC of different etiology.

Considering multiple pathways for repairing diverse DNA damages induced by endogenous and exogenous carcinogens, genetic variants in different repair pathways may probably have a joint effect on cancer risk. In attempt to search for the stronger associations between PTC and studied SNPs, we performed the analyses of genotype combinations for the *ATM* and *TP53* polymorphisms as these genes are functionally related and three out of four SNPs included in our study showed effects on PTC. Among the possible *ATM/TP53* combinations (rs1801516/rs664677/rs609429/rs1042522) tested, two demonstrated significant differences in the subsets of both groups of PTCs (Fig. 2). Particularly, the combined *ATM/TP53* GG/TC/CG/GC genotype was strongly associated with the IR-induced PTC (OR = 2.10, 95% CI 1.17–3.78, $P = 0.015$). Another *ATM/TP53* combination, GG/CC/GG/GG, demonstrated a significantly increased risk for sporadic PTC (OR = 3.32, 95% CI 1.57–6.99, $P = 0.002$).

Discussion

Our study addressed possible associations between SNPs in the genes involved in DNA damage response and the risk of PTC of different etiology. The results demonstrated that the presence of the variant 5557A allele in exon 39 of *ATM* and *XRCC1* 399Gln allele, particularly in the heterozygous state, significantly associated with the decreased risk of PTC. The *ATM* IVS22-77 CC genotype in the non-exposed group and the *TP53* 72Pro allele in the radiation-related one associated with the increased risk of PTC. Moreover, two particular *ATM/TP53* combined genotypes were found with higher frequencies in the IR-induced or sporadic PTC when compared with the controls. Altogether, these data indicate that SNPs in the studied genes may likely modify PTC risk.

A significant association between the *ATM* G5557A and bilateral breast cancer in Caucasian patients has been shown before (Heikkinen *et al.* 2005). Also, this SNP has been reported as a possible modulator of clinical radiosensitivity in cancer. The *ATM* 5557A allele was associated with severe adverse effects of radiation therapy in prostate (Hall *et al.* 1998) and breast cancer patients (Angele *et al.* 2003). Later, an enhanced radiosensitivity of human fibroblasts in the presence of the *ATM* 5557A allele was demonstrated in an experimental work (Alsbeih *et al.* 2007). In contrast

Table 4 OR (95% CI) for papillary thyroid carcinoma (PTC) by gene polymorphism according to different models of inheritance (adjusted for age, gender and radiation exposure). $P < 0.05$ in bold

SNP	Genotype	OR (95% CI)	P
ATM G5557A	*GG*	1.00[a]	
	GA	0.75 (0.49–1.15)	0.31
	AA	0.61 (0.21–1.77)	0.45
	Risk per *A* allele[b]	0.69 (0.45–0.86)	**0.03**
	GA+*AA* versus *GG*[c]	0.73 (0.48–1.10)	0.13
	AA versus *GA*+*GG*[d]	0.65 (0.23–1.87)	0.41
ATM IVS22 −77 T>C	*TT*	1.00	
	TC	1.03 (0.70–1.50)	0.74
	CC	1.19 (0.70–2.04)	0.47
	Risk per *C* allele	1.08 (0.83–1.40)	0.57
	TC+*CC* versus *TT*	1.06 (0.74–1.53)	0.75
	CC versus *TC*+*TT*	1.17 (0.72–1.90)	0.52
ATM IVS48 +238 C>G	*CC*	1.00	
	CG	1.10 (0.75–1.62)	0.55
	GG	1.14 (0.69–1.89)	0.84
	Risk per *G* allele	1.07 (0.84–1.37)	0.57
	CG+*GG* versus *CC*	1.11 (0.77–1.60)	0.57
	GG versus *CG*+*CC*	1.08 (0.69–1.69)	0.74
XRCC1 Arg280His[e]	*GG*	1.00	
	GA	1.12 (0.62–2.01)	0.71
	Risk per *A* allele	1.15 (0.70–1.87)	0.61
XRCC1 Arg399Gln	*GG*	1.00	
	GA	0.66 (0.57–0.88)	**0.02**
	AA	0.88 (0.50–1.57)	0.56
	Risk per *A* allele	0.90 (0.69–1.17)	0.41
	GA+*AA* versus *GG*	0.70 (0.59–0.93)	**0.03**
	AA versus *GA*+*GG*	0.98 (0.57–1.69)	0.94
TP53 Arg72Pro	*GG*	1.00	
	GC	1.02 (0.70–1.47)	0.89
	CC	1.16 (0.63–2.14)	0.38
	Risk per *C* allele	1.05 (0.81–1.38)	0.70
	GC+*CC* versus *GG*	1.04 (0.74–1.48)	0.82
	CC versus *GC*+*GG*	1.15 (0.64–2.08)	0.64
XRCC3 Thr241Met	*CC*	1.00	
	CT	0.99 (0.69–1.44)	0.99
	TT	0.96 (0.54–1.70)	0.92
	Risk per *T* allele	0.99 (0.76–1.28)	0.92
	CT+*TT* versus *CC*	0.99 (0.70–1.41)	0.97
	TT versus *CT*+*CC*	0.96 (0.56–1.64)	0.88
MTF1 T2193A	*TT*	1.00	
	TA	1.07 (0.73–1.56)	0.61
	AA	0.83 (0.49–1.41)	0.46
	Risk per *A* allele	0.94 (0.73–1.21)	0.63
	TA+*AA* versus *TT*	1.00 (0.70–1.44)	0.99
	AA versus *TA*+*TT*	0.80 (0.49–1.29)	0.35
MTF1 G20433A	*GG*	1.00	
	GA	1.14 (0.79–1.63)	0.43
	AA	0.76 (0.40–1.43)	0.21
	Risk per *A* allele	0.97 (0.74–1.25)	0.80
	GA+*AA* versus *GG*	1.05 (0.76–1.49)	0.76
	AA versus *GA*+*GG*	0.71 (0.39–1.32)	0.27

[a]Codominant model of inheritance (wild-type homozygous genotype serves as the reference).
[b]Multiplicative model of inheritance (uses allele frequencies).
[c]Dominant inheritance model (combined heterozygous and homozygous for the minor allele versus wild-type homozygous).
[d]Recessive inheritance model (minor allele homozygous versus combined heterozygous and homozygous for the wild-type allele).
[e]The dominant and recessive models are not shown for *XRCC1* Arg280His because of the absence of homozygous (A/A) genotype among 848 samples tested.

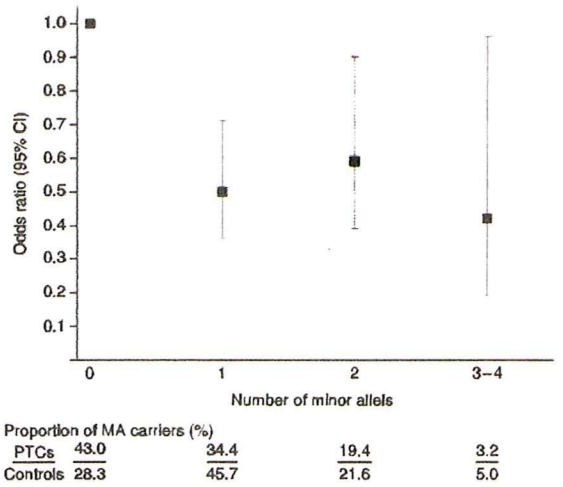

Figure 1 Effect of increasing number of minor alleles (MA) for *ATM* G5557A and *XRCC1* Arg399Gln (minor alleles, *ATM* 5557A, and *XRCC1* 399Gln) on PTC risk. The combined genotype with 0 MA was used as a reference. *P* values for genotypes with different MA number: $P_{1MA}<0.0001$; $P_{2MA}<0.01$; $P_{3-4MA}<0.05$. Carriers of three and four minor alleles were combined because of the exceedingly low number of 4 MA carriers in both PTC and control groups.

to these reports, Edvardsen *et al.* (2007) revealed an increasing rate of side effects of radiotherapy with decreasing frequency of this variant allele. Our data are rather in agreement with the latter report and favor the protective role of the *ATM* 5557A allele in PTC development.

The intronic *ATM* polymorphisms IVS22–77 T>C and IVS48+238 C>G in the homozygous state have been associated with increased breast cancer risk and in the heterozygous state with clinical radioprotection (Angele *et al.* 2003). These findings were confirmed in the *in vitro* experiments using lymphoblastoid cell lines established from corresponding patients. Our investigation demonstrated the association between the IVS22–77 CC genotype and increased risk of sporadic PTC in adult patients. By contrast, in the IR-induced PTC group, there was an inverse non-insignificant correlation for this genotype. At the same time, in the IR-induced PTCs, the number of patients heterozygous for IVS22–77 was somewhat, but insignificantly, higher as compared with sporadic PTCs (Table 3). The results for the IVS48+238 C>G tended to parallel those for the IVS22–77 T>C remaining below the threshold of significance. At present, the mechanistic and functional basis for the intronic *ATM* SNPs implications in cancer revealed in the previous studies and in ours as well is not fully understood. In a broader sense, however, they may be indicative of a role for the *ATM* gene (or its product) in the development of PTC.

As reviewed by Hu *et al.* (2005), the results of the *XRCC1* gene Arg399Glu investigations vary in different cancers for populations with different ethnicities. In relation to cancer and radiation, the 399Gln allele in combination with 280His was associated with breast cancer risk, and in pair with 194Trp with clinical radiosensitivity in Caucasian women with breast cancer. Also, the 399Gln allele was found to decrease

Table 5 OR (95% CI) for papillary thyroid carcinoma (PTC) of different etiology by *ATM* and *TP53* polymorphisms (adjusted for gender and age). $P<0.05$ in bold

		IR-induced PTC versus IR-exposed controls		Sporadic PTC versus non-exposed controls	
SNP	**Genotype**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
ATM IVS22−77 T>C	*TT*	1.00[a]		1.00	
	TC	1.38 (0.80–2.39)	0.19	0.82 (0.51–1.32)	0.50
	CC	0.73 (0.31–1.70)	0.44	1.63 (0.87–3.08)	0.09
	Risk per *C* allele[b]	0.97 (0.66–1.41)	0.86	1.18 (0.86–1.62)	0.32
	TC+*CC* versus *TT*[c]	1.23 (0.72–2.10)	0.44	0.97 (0.62–1.52)	0.88
	CC versus *TC*+*TT*[d]	0.59 (0.28–1.27)	0.17	1.84 (1.10–3.24)	**0.03**
TP53 Arg72Pro	*GG*	1.00		1.00	
	GC	1.68 (1.11–2.75)	**0.03**	0.84 (0.53–1.33)	0.52
	CC	2.33 (1.15–7.21)	**0.03**	0.84 (0.39–1.79)	0.73
	Risk per *C* allele	1.70 (1.17–2.46)	**0.006**	0.89 (0.64–1.23)	0.47
	GC+*CC* versus *GG*	1.80 (1.06–2.36)	**0.01**	0.84 (0.54–1.29)	0.43
	CC versus *GC*+*GG*	2.06 (0.79–5.41)	0.14	0.90 (0.44–1.88)	0.79

[a]Codominant model of inheritance (wild-type homozygous genotype serves as the reference).
[b]Multiplicative model of inheritance (uses allele frequencies).
[c]Dominant inheritance model (combined heterozygous and homozygous for the minor allele versus wild-type homozygous).
[d]Recessive inheritance model (minor allele homozygous versus combined heterozygous and homozygous for the wild-type allele).

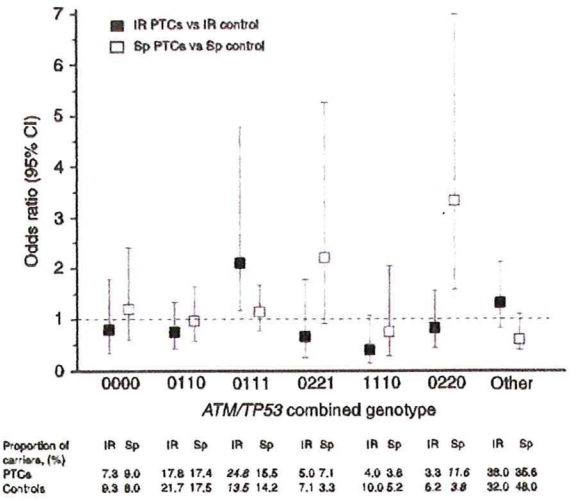

Figure 2 The combined *ATM/TP53* genotypes and risk of PTC of different etiology. The combined genotypes were analyzed separately in the IR-exposed and sporadic PTCs versus corresponding control. Six combinations of 3 *ATM* and 1 *TP53* SNPs (rs1801516/rs664677/rs609429/rs1042522) whose frequencies were higher than 5% at least in two of four subgroups are shown. In the numerical codes for any SNP, 0 – the genotype with no MA (i.e. homozygous wild-type); 1 – 1 MA presents (heterozygous genotype); 2 – 2 MA present (homozygous variant genotype); first three numbers correspond to 3 *ATM* SNPs and the last one to *TP53* polymorphism. In the figure, the *GG/TT/CC/GG* genotype is represented by the '0000' numerical code as it does not contain minor alleles; the *GG/TC/CG/GG* corresponds to 0110, *GG/TC/CG/GC* to 0111; *GG/CC/GG/GC* to 0221; *GA/TC/CG/GG* to 1110, and *GG/CC/GG/GG* to 0220. All combinations with frequencies <5% in three or more subgroups are pooled and indicated as 'other'.

the risk of bladder cancer and squamous cell carcinoma of the head and neck.

Interestingly, not only variant but also wild-type allele (i.e. *XRCC1* 399Arg) demonstrated possible role in cancer. High-dose radiation to the chest was more strongly associated with breast cancer among white American women with *XRCC1* Arg399Arg genotype (Duell *et al.* 2001). Looking for potential biological explanations for these findings, the authors found a higher prevalence of *TP53* deletions in the Arg399Arg cases exposed to occupational radiation compared with exposed patients with the Gln399Gln genotypes or unexposed cases of either genotype. Figueiredo *et al.* (2004) observed an increased risk of disease among wild-type homozygous (Arg/Arg) and heterozygous Canadian Caucasian women with a family history of breast cancer compared with the individuals without such.

The described above data may be explained, at least in part, by the results of functional study of this polymorphism in which an equal ability for both alleles to suffice single strand break repair by *XRCC1* has been found (Taylor *et al.* 2002). The results of our study, taken together with those reported previously, suggest that *XRCC1* polymorphism in particular the Arg399Gln genotype may influence PTC risk, perhaps by modifying the effects of environmental exposure and/or through interaction with other genetic factors.

The *TP53* Arg72Pro polymorphism affects the biological activity of p53. The Arg72 form is more efficient at inducing apoptosis while the Pro72 appears to induce a higher level of G1 arrest (Pim & Banks 2004). Based on these findings, a number of studies have attempted to assess a correlation between *TP53* codon 72 polymorphism and risk of certain types of cancer, however, with inconsistent results, as reviewed by Pietsch *et al.* (2006). This inconsistency may possibly be explained in part by the coexistence of the codon 72 polymorphism and gain of function mutations in *TP53* in some tumors (Pietsch *et al.* 2006, Soussi & Wiman 2007).

Several groups have investigated the TP53 Arg72-Pro polymorphism in PTC. Boltze *et al.* (2002) found a small number of heterozygotes and no Pro/Pro genotype in differentiated thyroid carcinomas from Germany. By contrast, in ethnically heterogeneous Brazilian population, the Pro/Pro genotype was associated with the higher risk of differentiated thyroid cancer (Granja *et al.* 2004). The study of codon 72 polymorphism in thyroid tumors from Russian and Ukrainian patients demonstrated a significantly lower frequency of wild-type homozygotes (i.e. Arg/Arg) among adults with IR-induced PTC when compared with sporadic PTC cases and general population (Rogounovitch *et al.* 2006). Data obtained in the present work, using an independent set of samples, confirm these findings suggesting the modifying role (or as of a marker) of the TP53 Arg72Pro polymorphism in PTC developed after exposure to IR which is further supported by the absence of significant difference in genotype distributions among our two control groups.

As shown in a genetic study, frequencies of the *C* allele (encoding 72Pro) do not generally differ in populations of Belarus and Russia (Khrunin *et al.* 2005). However, East Slavs do not form a single genetic cluster on multidimensional analysis. The 72Pro allele frequency in Belarus is about 0.3; in the two different subpopulations from the Central and Northern regions of the European part of Russia it is 0.24 and 0.32 respectively. The study of healthy population from Poland (bordering with Belarus, linguistically and culturally similar), reported the frequency of 0.28 for the 72Pro allele (Siddique *et al.* 2005). The 72Pro frequency reported by

Rogounovitch *et al.* (2006) in Russian healthy controls is also 0.28. Thus, the effect of population admixtures in the controls in our investigation could not be completely ruled out. Yet on the other hand, the ratio of Belarusian and Russian subjects in the IR-exposed PTCs and controls was similar (2.24 and 2.30 respectively) suggestive of an unbiased estimate and being an argument in support of *TP53* Arg72Pro polymorphism association with radiation-related PTC.

While many studies established the effect of individual SNPs on cancer, the role of SNP combinations has been less addressed. Several *ATM* and *TP53* haplotypes were associated with clinical radiosensitivity in breast cancer (Angele *et al.* 2003) and brain tumor risk (Malmer *et al.* 2007). Recently, the interactions of SNPs located on different chromosomes were investigated in various malignancies (Yen *et al.* 2008, Yoon *et al.* 2008). One experimental study, in which *ATM* Asp1853Asn, *TP53* Arg72Pro, *XRCC1* Arg399Gln, and *XRCC3* Thr241Met were genotyped, demonstrated that the increasing number of risk alleles enhanced radiosensitivity of human fibroblast cell lines and, potentially, susceptibility to radiation-induced cancers (Alsbeih *et al.* 2007). So far no studies have investigated the joint effect of gene polymorphisms on thyroid cancer. Our observations demonstrated that frequencies of particular combined *ATM/TP53* genotypes were higher in patients with radiogenic or sporadic PTC compared with corresponding control populations.

To some extent these results support the idea that genetic factors may possibly modify predisposition to thyroid cancer. A recent study by Detours *et al.* (2007) reported difference in the expression levels of some genes between Chernobyl PTCs from Ukraine and French sporadic PTCs. Although the mentioned work and the present one are different in molecular approaches, the results of both are suggestive of a possible genetic 'susceptibility signature' that may contribute to the individual predisposition to IR and other carcinogens' effects. These findings are in favor of a 'susceptibility model' that may partly explain why only a minority of the large population exposed to the IR after the Chernobyl disaster developed thyroid cancer (Yamashita & Saenko 2006, Detours *et al.* 2007, 2008).

It is necessary to note that even though nine SNPs were analyzed in our study, no correction for multiple comparisons was applied because of study design and techniques employed. The associations were tested in a one-at-a-time fashion in a limited sample size in the difficult to access groups. The need for correction in such circumstances is still debated (Rothman & Greenland 1998). Furthermore, since data obtained in this work may be referred to as an initial screening result, non-adjusted presentation enables their inclusion in future meta-analysis. Effects of candidate SNPs that we report need validation in other studies.

In conclusion, the results presented here show that SNPs in *ATM* exon 39 and *XRCC1* exon 10 may be the markers of a decreased PTC risk in adults, whereas the *ATM* IVS22-77 and *TP53* codon 72 SNPs genes may associate with the risk of PTC development in non-irradiated and irradiated individuals. To the best of our knowledge, presented here is the first study of this kind reporting the results of genotyping of candidate DNA damage response genes in irradiated and non-irradiated PTC patients and in corresponding healthy populations. Our data support the paradigm of genetic modifiers of radiation-associated carcinogenesis and perhaps may contribute to genetic determination of PTC-prone subjects. We believe such identification will allow future personalized cancer risk prediction which is of a significant importance in view of the growing thyroid cancer incidence and also because of the relevance to occupational and radiation emergency medicine issues.

Declaration of interest

The authors declare no potential conflict of interest.

Funding

This work was supported in part by Grant-in-Aid for Scientific Research 19256003, 19510058, and 19790651 from Japan Society for the Promotion of Science.

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THYROID
Volume 19, Number 7, 2009

DOI: 10.1089/thy.2008.0238

The Usual Ultrasonographic Features of Thyroid Cancer Are Less Frequent in Small Tumors That Develop After a Long Latent Period After the Chernobyl Radiation Release Accident

Valentina M. Drozd,[1] Maxim L. Lushchik,[1] Olga N. Polyanskaya,[1] Michail V. Fridman,[1] Yuri E. Demidchik,[1] Andrej P. Lyshchik,[2] Johannes Biko,[3] Christoph Reiners,[3] Yoshisada Shibata,[4] Vladimir A. Saenko,[5] and Shunichi Yamashita[4,5]

Background: The Chernobyl accident resulted in an unprecedented number of radiation-induced thyroid cancers in young individuals as detected by national and international screening programs. The vast majority of thyroid malignancies were papillary carcinomas that, despite being similar by histopathology, displayed large variability in clinical course. The correlations between ultrasound (US) and clinicopathological features in young patients with radiation-induced thyroid cancer, however, have not been well studied. Because of the importance of US for deciding which subjects should have fine-needle aspiration biopsy, we assessed the US features of papillary thyroid carcinoma in patients exposed to Chernobyl fallouts.

Design: We performed a retrospective multivariate logistic regression analysis of US features, clinicopathological data, and the latency period between radiation exposure and the diagnosis of cancer in 94 patients who were 10.6–34.3 years old (16.5 ± 6.2, mean $\pm$ standard deviation) at the time of diagnosis and 0.1–18.0 years old (5.6 ± 4.2) at the time of the Chernobyl accident.

Results: Nodules greater than 10 mm were associated with the higher frequency of irregular margins ($p = 0.001$), longer period of latency ($p = 0.016$), and bilateral lymph node involvement ($p = 0.025$). Irregular tumor margins correlated with the shorter period of latency ($p = 0.009$) and unilateral nodal disease ($p = 0.010$). Hypoechoic nodules were observed more frequently in female patients ($p = 0.012$), in the absence of halo ($p = 0.003$) or calcifications ($p = 0.005$). Hypoechogenicity also correlated with the shorter latency ($p = 0.015$) and younger age of patients ($p = 0.048$).

Conclusions: Irregular nodule margins, a usual sign of malignancy, are less useful in detecting thyroid cancers in radiation-exposed patients with tumors less than 10 mm. Thyroid cancers that are detected after longer latent periods display less of the US features characteristic of a malignant process, while benign US features are observed more frequently. Therefore, we recommend fine-needle aspiration biopsy to ensure early diagnosis of thyroid cancer for patients with a history of radiation exposure, even if their nodules are less than 10 mm.

Introduction

CHILDHOOD THYROID CANCER is rare, but early diagnosis is important in pediatric practice. Real-time ultrasound (US) examination is the most useful means of screening for thyroid cancer. US is widely available, has a low cost, requires short examination time, is not painful, and does not expose the patient to radiation (1). When US is combined with US-guided fine-needle aspiration biopsy (FNAB) this can provide early diagnosis of thyroid disease in high-risk individuals exposed to radiation (2–5).

Among the two million children exposed to Chernobyl fallout in Belarus, over 2000 cases of thyroid cancer have been diagnosed in young patients during the 20-year period

[1]Department of Thyroid Disease Research, Belarusian Medical Academy for Postgraduate Education, Minsk, Republic of Belarus.
[2]Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, Tennessee.
[3]Clinic and Polyclinic of Nuclear Medicine, University of Wuerzburg, Wuerzburg, Germany.
Departments of [4]Molecular Medicine and [5]International Health and Radiation Research, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

TABLE 1. ULTRASOUND FEATURES SUGGESTIVE OF A MALIGNANT OR BENIGN THYROID NODULE

	Malignant (references)	*Benign (references)*
Number of nodules		
Solitary	Frates *et al.* (8)	
Multiple	Frates *et al.* (8)	
Structure		
Homogeneous		
Heterogeneous	Hegedus (1), Drozd *et al.* (3)	
Echogenicity		
Hypoechoic	Hegedus (1), Drozd *et al.* (3), Cooper *et al.* (9), Shimura *et al.* (10), Tae *et al.* (11)	Wienke *et al.* (12)
Isoechoic	Drozd *et al.* (3)	Hegedus (1)
Mixed		
Margins		
Regular	Drozd *et al.* (3)	Hegedus (1), Shimura *et al.* (10), Tae *et al.* (11)
Irregular	Hegedus (1), Drozd *et al.* (3), Cooper *et al.* (9), Shimura *et al.* (10), Tae *et al.* (11), Papini *et al.* (13), Koike *et al.* (14), Rago *et al.* (15), Chan *et al.* (16)	
Halo		
Absent	Hegedus (1), Peccin *et al.* (17)	
Present	Drozd *et al.* (3)	Hegedus (1)
Calcifications		
Absent		Tae *et al.* (11)
Present	Hegedus (1), Cooper *et al.* (9)	Hegedus (1), Shimura *et al.* (10), Koike *et al.* (14)
Lymphadenopathy		
Absent		Hegedus (1)
Present	Hegedus (1), Drozd *et al.* (3), Pellegriti *et al.* (18)	
Hypoechoic		
Isoechoic	Drozd *et al.* (3)	

(1986–2006) after the accident (6). The mortality rate has been extremely low. This is probably due, at least in part, to US screening. Had the disaster occurred a decade before US was available, the mortality would probably have been substantially higher (7).

In adults the US features associated with malignancy are hypoechogenicity, mostly nonhomogenous structure, calcifications, and irregular (microlobulated and blurred) margins (1,3,8–18) as summarized in Table 1. In the pediatric population the potential usefulness of these features is less well studied. Most of the data relating to the US features of thyroid malignancy are based on studies of sporadic cases. Less is known about the US features of radiation-associated thyroid cancer, particularly as it relates to the interval between radiation exposure and US.

In this study we retrospectively analyzed young patients with thyroid cancer who had been exposed to the accidental release of radiation from the Chernobyl reactor in 1986. We found that the US features of malignant nodules in this group were more characteristic of what has been described for thyroid cancer with increasing tumor size and less characteristic of thyroid cancer with increasing duration between radiation exposure and surgical diagnosis.

Materials and Methods

Patients

The study group included 94 patients (47 males and 47 females, sex ratio 1:1) from Belarus with papillary thyroid carcinoma for whom pathological findings, US imaging, and demographic data were available at the database of Belarusian Medical Academy for Postgraduate Education. Seven additional cases in the database distributed evenly over the period of data acquisition (1990–2003) were not included because of excessively missing clinicopathological information.

The patients' age in the study ranged 0.1–18.0 years with the mean ± standard deviation (SD) of 5.6 ± 4.2 at the time of the Chernobyl accident, and 10.6–34.3 years with the mean ± SD of 16.5 ± 6.2 years at diagnosis. The latent period, defined as the interval between radiation exposure (April 26, 1986) and the first thyroid surgery, ranged from 45.6 to 207.6 months with the mean ± SD of 128.4 ± 49.2 months.

US-guided FNAB followed by cytological analysis was routinely done for all thyroid nodules measuring 5 mm or greater on US. In all cases in this study the preoperative cytological diagnosis was papillary thyroid carcinoma or suspected thyroid carcinoma. All the patients were surgically treated at the Thyroid Cancer Center, Minsk. The cytological diagnosis of papillary thyroid carcinoma was confirmed by pathological examination after surgery.

Clinicopathological data were retrieved from medical records. The study protocol was approved by the Belarusian Medical Academy for Postgraduate Education and Nagasaki University Graduate School of Biomedical Sciences institutional review boards.

US examination

Thyroid nodules were initially detected during annual screenings in most radiocontaminated regions of Belarus.

TABLE 2. CLINICAL, ULTRASONOGRAPHIC, AND PATHOLOGICAL PARAMETERS BY NODULE SIZE

Parameters	*Group*		p-*Value*[a]
	Nodule size ≤10 mm n = 25	*Nodule size >10 mm* n = 69	
Clinical characteristics			
Sex			
Male	11 (44.0)[b]	36 (52.2)	
Female	14 (56.0)	33 (47.8)	0.64
Age at the time of accident (years)	5.0 ± 3.9[c]	5.7 ± 4.9	0.88
Age at the time of diagnosis (years)	14.8 ± 5.9	17.11 ± 6.6	0.14
Latent period (months)	117.9 ± 46.6	133.6 ± 48.7	0.089
Ultrasonographic characteristics			
Nodule volume (mL)	0.2 ± 0.1	1.6 ± 2.5	<0.001
Number of nodules			
Solitary	21 (84.0)	59 (85.5)	
Multiple	4 (16.0)	10 (14.5)	1.00
Structure			
Homogeneous	14 (58.3)	26 (37.7)	
Heterogeneous	10 (41.7)	43 (62.3)	0.097
Unknown	1	0	
Echogenicity			
Hypoechoic	18 (75.0)	36 (52.9)	
Isoechoic	4 (16.7)	18 (26.5)	
Mixed	2 (8.3)	14 (20.6)	0.18
Unknown	1	1	
Margins			
Regular	10 (43.5)	10 (14.7)	
Irregular	13 (56.5)	58 (85.3)	0.009
Unknown	2	1	
Halo			
Absent	22 (88.0)	56 (85.5)	
Present	3 (12.0)	10 (14.5)	1.00
Unknown	0	3	
Calcifications			
Absent	25 (100.0)	59 (85.5)	
Present	0 (0.0)	10 (14.9)	0.058
Lymphadenopathy			
Absent	15 (60.0)	39 (56.5)	
Present	10 (40.0)	30 (43.5)	0.82
Hypoechoic	9 (36.0)	21 (30.4)	
Isoechoic	1 (4.0)	8 (11.6)	0.61
Unknown	0	1	
Pathological characteristics			
Tumor size (mm)[d]	7.8 ± 2.6	13.2 ± 5.3	<0.001
T stage			
T1	24 (96.0)	63 (91.3)	
T2	0 (0.0)	4 (5.8)	
T3	1 (4.0)	2 (2.9)	0.64
N stage			
N0	13 (52.0)	20 (29.4)	
N1a	10 (40.0)	18 (26.5)	
N1b	2 (8.0)	30 (44.1)	0.003
Unknown	0	1	
Histopathological variant			
Solid	3 (12.0)	6 (8.9)	
Papillary and follicular	22 (88.0)	61 (91.1)	0.99
Unknown	0	2	
Invasiveness			
Intrathyroidal	21 (84.0)	60 (86.9)	
Extrathyroidal	4 (16.0)	9 (13.1)	0.74

[a]Based on Wilcoxon rank-sum or Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables; patients without available information were excluded.
[b]Number of patients with percentage within parentheses.
[c]Mean ± standard deviation.
[d]Largest measurement.

TABLE 3. PARAMETERS ASSOCIATED WITH THE FREQUENCY OF LARGE NODULE SIZE (>10 MM), LOGISTIC REGRESSION ANALYSIS

Parameters	*Comparison*	*Odds ratio*	*95% CI*	p-*Value*[a]
Number of nodules	Singular vs. multiple	0.24	0.05–1.12	0.071
Latent period	By 1-month increment	1.02	1.00–1.03	0.016
Margins	Irregular vs. regular	12.75	2.99–69.3	0.001
N stage				
	N1a vs. N0	0.51	0.13–1.92	0.331
	N1b vs. N0	7.08	1.48–48.7	0.025

[a]Based on likelihood ratio test.
CI, confidence interval.

Individuals with US thyroid abnormalities underwent health examination, including thyroid ultrasonography, at the hospital. US examination was done with a Toshiba SSA 240A (7.5 MHz sector probe) ultrasonic real-time scanner, with patient in the supine position with the neck slightly hyperextended.

For the purpose of this study, archived US images were reanalyzed by experienced radiologists (V.M.D., M.L.L., and A.P.L.) using identical criteria. Sonograms were interpreted in terms of nodule size (largest dimension), number of nodules (solitary and multiple), echogenicity (hypoechoic, isoechoic, or mixed), echostructure (homogeneous or heterogeneous), margins (regular or irregular), presence or absence of a halo (rim), calcifications, and regional lymphadenopathy. A consensus estimate was reached for every parameter in all cases under study.

Thyroid and nodule volumes (mL) were estimated, using the height (H), width (W), and length (L) of the thyroid lobes or of a nodule measured in the transverse and longitudinal scans (cm) by the formula for ellipsoid volume: $V = H \times W \times L \times \pi/6$ (19).

Histopathology

All cases were pathologically diagnosed as papillary carcinomas. Solid variant of papillary thyroid carcinoma (less mature histotype) was observed in nine (9.6%) cases. Typical papillary morphology and follicular variant of papillary thyroid carcinoma (more differentiated histotypes) were documented in 85 (90.4%) cases. Tumor size varied from 5 to 30 mm with the mean ± SD of 11.7 ± 3.9 mm. The presence or absence of capsular and extrathyroidal invasion was recorded. Tumor staging was according to the International Union Against Cancer (UICC) TNM Classification of Malignant Tumors (20).

Statistical analysis

All patients were divided into subgroups according to tumor size on ultrasonography (≤10 mm and >10 mm), type of margins (regular or irregular), and echogenicity (hypoechoic, isoechoic, or mixed). Subgroups thus defined were compared with respect to clinical, ultrasonographic, or pathological characteristics by Fisher exact test for categorical data, and by Wilcoxon rank-sum test or Kruskal-Wallis test for quantitative measurements. Association of these characteristics with tumor size, type of margins, or echogenicity was evaluated by logistic regression analysis, where the most appropriate model was selected by Akaike information criterion starting from the full model. The FREQ, NPAR1WAY, and LOGISTIC procedures in the SAS system (21) were used for the calculations. A p-value less than 0.05 was regarded as indicating statistical significance.

Results

Clinical, ultrasonic, and pathological characteristics with respect to nodule size by US

The tumor size on ultrasonography was ≤10 mm in 25 patients and >10 mm in 69 patients (Table 2). No significant difference was observed between the two groups in age at the time of accident, age at diagnosis, or sex ratio. Although not significant, the latent period tended to be longer in the patients with larger nodules.

Irregular margins were detected more frequently in the patients with larger nodules ($p = 0.009$). There was a very strong trend toward the frequency of calcifications being higher in the patients with larger nodules ($p = 0.058$). No significant differences were observed in other US characteristics between the two subgroups. By definition, tumors smaller than 1 cm are classified as pT1. However, 91% of the larger tumors in this series also were classified as pT1 (with diameter smaller than 2 cm). There were no pT4 tumors; the low prevalence of advanced stage tumors is most probably due to extensive screenings. Lymph node involvement (on pathology) significantly correlated with the larger ultrasonic nodule size ($p = 0.003$). No significant difference was observed between the two subgroups regarding the frequency of histopathological variants or tumor invasiveness.

Three factors—latent period, margins, and N stage—were found by logistic regression analysis to be associated with the frequency of larger tumor size (Table 3). The frequency of larger tumors was significantly increased with the latency ($p = 0.016$), was significantly higher among the nodules with irregular margins ($p = 0.001$), and was significantly higher in the patients with bilateral lymph node involvement ($p = 0.025$).

Clinical, ultrasonic, and pathological characteristics with respect to nodule margins

Tumor margins were regular or irregular in 20 and 72 patients, respectively (Table 4). The age at diagnosis was significantly lower ($p = 0.006$) and the latency period was significantly shorter ($p = 0.021$) in the patients with irregular margins of cancer nodules.

A heterogeneous US structure was observed more frequently in nodules with irregular margins ($p = 0.022$). US signs of lymph node involvement as well as N1a tumor stage were significantly more frequent in the patients with nodules with irregular margins ($p = 0.022$ and $p = 0.025$, respectively). The frequency of isoechoic lymph nodes, not considered a suspicious sign, also tended to correlate with the irregular margins of thyroid nodules, although the significance was marginal ($p = 0.064$). An isoechoic pattern and the presence of

TABLE 4. CLINICAL, ULTRASONOGRAPHIC, AND PATHOLOGICAL PARAMETERS BY TUMOR MARGIN TYPE

Parameters	*Group*		p-*Value*[a]
	Regular margins n = *20*	*Irregular margins* n = *72*	
Clinical characteristics			
Sex			
Male	11 (55.0)[b]	36 (50.0)	
Female	9 (45.0)	36 (50.0)	0.80
Age at the time of accident (years)	7.2 ± 6.1[c]	5.1 ± 4.2	0.27
Age at the time of diagnosis (years)	20.2 ± 7.5	15.3 ± 5.9	0.006
Latent period (months)	155.9 ± 34.3	122.7 ± 49.9	0.021
Ultrasonographic characteristics			
Nodule size (mm)[d]	12.6 ± 5.3	15.4 ± 6.7	0.052
Nodule volume (mL)	0.7 ± 0.8	1.4 ± 2.5	0.019
Number of nodules			
Solitary	17 (85.0)	60 (83.3)	
Multiple	3 (15.0)	12 (16.7)	1.00
Structure			
Homogeneous	13 (65.0)	25 (34.7)	
Heterogeneous	7 (35.0)	47 (65.3)	0.022
Echogenicity			
Hypoechoic	9 (45.0)	44 (61.1)	
Isoechoic	9 (45.0)	13 (18.1)	
Mixed	2 (10.0)	15 (20.8)	0.051
Halo			
Absent	14 (70.0)	65 (90.3)	
Present	6 (30.0)	7 (9.7)	0.029
Calcifications			
Absent	18 (90.0)	64 (88.9)	
Present	2 (10.0)	8 (11.1)	1.00
Lymphadenopathy			
Absent	16 (80.0)	37 (51.4)	
Present	4 (20.0)	35 (48.6)	0.022
Hypoechoic	4 (20.0)	26 (36.1)	
Isoechoic	0 (0.0)	8 (11.1)	0.064
Unknown	0	1	
Pathological characteristics			
Tumor size (mm)[d]	10.3 ± 3.5	12.4 ± 5.6	0.15
T stage			
T1	20 (100.0)	64 (89.0)	
T2	0 (0.0)	4 (5.5)	
T3	0 (0.0)	4 (5.5)	0.50
N stage			
N0	12 (60.0)	20 (28.2)	
N1a	2 (10.0)	24 (33.8)	
N1b	6 (30.0)	27 (38.0)	0.025
Unknown	0	1	
Histopathological variant			
Solid	1 (5.0)	9 (12.5)	
Papillary and follicular	19 (95.0)	63 (87.5)	0.70
Invasiveness			
Intrathyroidal	18 (90.0)	60 (83.3)	
Extrathyroidal	2 (10.0)	12 (16.7)	0.72

[a]Based on Wilcoxon rank-sum or Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables; patients without available information were excluded.
[b]Number of patients with percentage within parentheses.
[c]Mean ± standard deviation.
[d]Largest measurement.

halo were more frequent in the nodules with regular margins ($p = 0.051$ and $p = 0.029$, respectively).

Three factors, that is, latent period, nodule size, and N stage, were found by logistic regression analysis to be associated with the frequency of irregular margins (Table 5). Irregular margins were decreased in frequency with longer latency ($p = 0.009$), were significantly frequent in the larger nodules (>10 mm) as compared to smaller ones (≤10 mm; $p = 0.042$), and were observed significantly more frequently in the patients with unilateral lymph node involvement ($p = 0.010$).