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], Shunichi Yamashita[1,2]

Department 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

Corresponding author: Vladimir Saenko

Department of International Health and Radiation Research, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8523, Japan

 E-mail: saenko@net.nagasaki-u.ac.jp

Tel.: +81-95-819-7122

Fax: +81-95-819-7169

Abbreviated title: Genotypes of PTC of different etiology

Key words: papillary thyroid cancer, ionizing radiation, risk, SNP

1 **Abstract**

2 Papillary thyroid carcinoma (PTC) etiologically occurs as a radiation-induced or sporadic malignancy.

3 Genetic factors contributing to the susceptibility to either form remain unknown. In this retrospective

4 case-control study we evaluated possible associations between single nucleotide polymorphisms (SNPs)

5 in the candidate DNA damage response genes (*ATM*, *XRCC1*, *TP53*, *XRCC3*, *MTF1*) and risk of

6 radiation-induced and sporadic PTC. A total of 255 PTC cases (123 Chernobyl radiation-induced and 132

7 sporadic, all in Caucasians) and 596 healthy controls (198 residents of Chernobyl areas and 398 subjects

8 without history of radiation exposure, all Caucasians) were genotyped. The risk of PTC and SNPs

9 interactions with radiation exposure were assessed by logistic regressions. The *ATM* G5557A and *XRCC1*

10 Arg399Gln polymorphisms, regardless of radiation exposure, associated with a decreased risk of PTC

11 according to the multiplicative and dominant models of inheritance (OR=0.69, 95% CI 0.45-0.86 and

12 OR=0.70, 95% CI 0.59-0.93, respectively). The *ATM* IVS22-77 T>C and *TP53* Arg72Pro SNPs

13 interacted with radiation (*P*=0.04 and *P*=0.01, respectively). *ATM* IVS22-77 associated with the increased

14 risk of sporadic PTC (OR=1.84, 95% CI 1.10-3.24) whereas *TP53* Arg72Pro correlated with the higher

15 risk of radiogenic PTC (OR=1.80, 95% CI 1.06-2.36). In the analyses of *ATM*/*TP53*

16 (rs1801516/rs664677/rs609429/rs1042522) combinations, the GG/TC/CG/GC genotype strongly

17 associated with radiation-induced PTC (OR=2.10, 95% CI 1.17-3.78). The GG/CC/GG/GG genotype

18 displayed a significantly increased risk for sporadic PTC (OR=3.32, 95% CI 1.57-6.99). The results

19 indicate that polymorphisms of DNA damage response genes may be potential risk modifiers of IR-

20 induced or sporadic PTCs.

21

22

23

24

25

26

1 **Introduction**

2   Thyroid cancer accounts for more than 90% of all endocrine malignancies. The incidence of

3 thyroid cancer in the world is increasing during the past three decades, mainly due to the papillary thyroid

4 carcinoma (PTC) which is the predominant type of malignant thyroid tumors (Davis & Welch 2006).

5   Most thyroid cancer patients do not have history of radiation exposure, yet ionizing radiation (IR)

6 is a recognized etiological factor of the disease. An increased risk of thyroid cancer has been documented

7 after external irradiation (Ron *et al.* 1995) and after environmental exposure to ^{131}I, such as after the

8 Chernobyl fallouts in Belarus, Ukraine and Russia (Bennett *et al.* 2006).

9   Although radiation thyroid doses in Chernobyl PTC cases are generally greater than in controls in

10 epidemiological studies (Cardis *et al.* 2005; Jacob *et al.* 2006; Likhtarev *et al.* 2006), thus confirming

11 radiation to be a risk factor for thyroid cancer, those in controls are nonzero. Furthermore, there were

12 some 14 million residents in the contaminated territories at the time of exposure (Bennett *et al.* 2006).

13 Conceivably, at least some of them might have accumulated thyroid doses comparable to doses in

14 diseased individuals. However, thyroid cancer developed only in a small fraction of irradiated population.

15   Among the variety of DNA damage types induced by radiation, double-strand DNA breaks are

16 considered to be the most significant for chromosomal aberrations, mutagenesis, genetic instability and

17 carcinogenesis (Khanna & Jackson 2001). PTC is one of the rare human cancers of epithelial origin in

18 whose oncogenesis gene rearrangements play a noticeable role. Several variants of rearrangements are

19 described in PTC, with RET/PTC occurring most frequently (Nikiforov *et al.* 1997; Rabes *et al.* 2000).

20   While in the exposed individuals DNA damage could be attributed to ionizing radiation, the

21 origination of genetic alterations in sporadic cancers remains obscure. Nevertheless, the spectrum of

22 oncogenic changes in radiation-related and sporadic PTCs is largely common. Such similarities imply the

23 resemblance of molecular reactions on DNA damage in exposed and non-exposed thyrocytes. These

24 reactions involve first of all DNA damage response factors, including DNA repair and checkpoint

25 complexes.

1   The vast majority of Chernobyl thyroid malignancies were PTCs which displayed wide variations

2   in clinical course, from highly aggressive tumors developing after the shorter latency to more indolent

3   carcinomas with the longer latent period (Williams 2006). The randomness and multiplicity of forms of

4   genetic alterations caused by IR can only partly explain these differences in the individual reactions on

5   exposure as well as why cancer develops only in some of exposed individuals.

6   It is attractive to hypothesize that inherited variability in the genes directly or indirectly involved

7   in the maintenance of genome stability in response to environmental carcinogens such as IR or chemicals

8   may play a role in susceptibility for radiation-related or sporadic PTC or may be a marker of it. In this

9   work we tested the relation of genetic variants of some of such genes, namely *ATM*, *TP53*, *XRCC1*,

10   *XRCC3* and *MTF1*, to PTC of different etiology.

11   The *Ataxia-telangiectasia mutated* (*ATM*) gene plays a key role in the sensing and repair of DNA

12   double-strand breaks. Activation of the ATM protein kinase by IR results in the subsequent initiation of

13   several molecular pathways of DNA damage repair (Shiloh 2003). One of the ATM targets is the p53

14   pathway. Overexpression of *TP53* arrests the cell cycle and affects DNA repair and apoptosis.

15   The *ATM* and *TP53* genes play a significant role especially in the tumors that are induced by IR.

16   A number of single nucleotide polymorphisms (SNPs) in the *ATM* and *TP53* genes studied in populations

17   of different ethnicities have been reported to associate with the risk of different radiogenic tumors (Hu *et*

18   *al.* 2002; Angele *et al.* 2003; Thorstenson *et al.* 2003; Malmer *et al.* 2007). In contrast, studies of post-

19   Chernobyl pediatric thyroid cancers demonstrated a low mutation and polymorphism rate in the *TP53*

20   gene (Nikiforov *et al.* 1996; Hillebrandt *et al.* 1997). It, however, should be mentioned that after exposure

21   to radiation p53 facilitates DNA repair in normal thyrocytes *in vitro* (Yang *et al.* 1997).

22   The base excision repair (BER) and homologous recombination repair (HRR) pathways are

23   particularly important for genomic integrity restoration (Hoeijmakers 2001). The product of the *X-ray*

24   *repair cross complementing 1* (*XRCC1)* gene acts as a scaffold and a modulator of different enzymes

25   involved in BER. The *XRCC1* Arg399Gln and Arg280His variants have been extensively investigated for

26   their function and association with cancer risk; however, the results remain contradictory rather than

1   conclusive (Hu *et al.* 2005). The *XRCC3* gene is a member of the *Rad51* DNA-repair gene family. Its

2   product is a factor of the HRR. The *XRCC3* Thr241Met polymorphism has been controversially

3   associated with different human malignancies (Han *et al.* 2006). Sturgis *et al.* (2005) reported 241Met

4   allele association with the risk of differentiated thyroid cancer .

5   The *Metal-responsive transcription factor1* (*MTF1*) gene has been implicated in tumor initiation

6   and progression to malignant growth. MTF1 protein interacts with metallothioneins that are able to

7   suppress cellular stresses generated by IR and other agents (Tamura *et al.* 2005). Polymorphism in murine

8   *Mtf1* gene has been found to associate with the susceptibility to experimental γ-ray-induced thymic

9   lymphomas. This observation points at possible involvement of human *MTF1* polymorphisms in the

10   modulation of radiation-induced malignancies (Tamura *et al.* 2005).

11   To date no polymorphisms of the *ATM, XRCC1* and *MTF1* genes have been studied neither in

12   human sporadic or radiation-induced PTCs. Data on the *TP53* and *XRCC3* polymorphisms associations

13   are quite limited (Hillebrandt *et al.* 1997; Boltze *et al.* 2002; Granja *et al.* 2004; Sturgis *et al.* 2005;

14   Rogounovitch *et al.* 2006). Therefore, in this study we addressed the relation of SNPs in aforementioned

15   DNA damage response genes to the risk of PTCs of different etiology.

16

17   **Materials and methods**

18   **Study population**

19   A total of 255 histologically verified PTC cases and 596 healthy controls, all Caucasians, were

20   included in the study. Among the patients, 123 individuals with PTC (24 males and 99 females) lived in

21   the areas of the Russian Federation (38 patients) and Belarus (85 patients) contaminated with

22   radionuclides from Chernobyl fallouts. At the time of the Chernobyl accident these subjects were younger

23   than 18 years old (mean age at exposure ± SD, 9.8 ± 5.1 years old; 1-18 years old, range). The mean age

24   at diagnosis was 24.4 ± 4.9 years old, range 19-37 years old (IR-induced PTCs). Information about

25   individual radiation thyroid doses was available for PTC cases from Russia as reconstructed in previous

26   studies (Davis *et al.* 2004; Stepanenko *et al.* 2004). The doses varied from 43 to 2640 mGy. Radiation

1   thyroid doses for PTC patients and controls from Belarus evaluated in dosimetric investigations at the

2   places of residence ranged 21–1500 mGy (Bouville *et al.* 2007). Among the controls, 198 individuals (65

3   males and 133 females, mean age at sampling 22.2 ± 3.2 years old; 19-35 years old, range) were residents

4   of the Chernobyl areas (60 from the Russian Federation and 138 from Belarus). The averaged thyroid

5   radiation dose in the exposed control subjects from Russia is 41 mGy (Bouville *et al.* 2007). All exposed

6   control individuals were aged less than 18 years at the time of the accident (mean age at exposure 1.8 ±

7   3.2 years old; 1-16 years old, range) (IR-exposed controls). IR-exposed controls and patients with IR-

8   induced PTCs not were individually matched; however, they were residents of the same settlements. This,

9   given the uncertainty with individual radiation thyroid doses, was supposed to partly reduce exposure bias.

10 Age of IR-exposed control subjects was set to be ± 3 years of that of IR-induced PTC individuals.

11   One hundred and thirty-two PTC cases (21 males and 111 females, mean age at diagnosis 47.8 ±

12 11.4 years old; 19-76 years old, range) were adults without history of radiation exposure (sporadic PTCs).

13 The remaining 398 control participants (180 males and 218 females, mean age at sampling 45.0 ± 10.3

14 years old; 16-65 years old, range) had no previous history of radiation exposure (non-exposed controls);

15 their age was also set to be ± 3 years of that of patients with sporadic PTC. Both sporadic PTCs and non-

16 exposed controls originated from the European part of Russia not contaminated by the Chernobyl fallouts.

17   Thyroid tissues and/or blood samples were collected from patients during surgery or further

18 follow-up. Blood samples and information from the controls were obtained during a routine health

19 examination or complex screening for thyroid diseases.

20   Written informed consent was obtained from all participants. Protocols of the present study were

21 approved by the Committee for Ethical Issues of Human Genome Analysis of Nagasaki University.

22

23 **SNP selection**

24 The candidate SNPs (Table 1) were selected based on their reported functional role (if available),

25 associations with radiosensitivity or (thyroid) cancer risk. Accordingly, we did not search for tag SNPs or

26 account for the genetic variability in the regions of SNP location. All SNPs are listed in a public database,

1 dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), with validated status in ethnically diverse populations. To

2 ensure sufficient power for calculations, only SNPs with minor allele frequency (MAF) of >1% were

3 included.

4

5 **SNP genotyping**

6 DNA was extracted from normal thyroid tissues using proteinase K/phenol-chloroform method or

7 from the whole blood lymphocytes with Puregene DNA Purification Kit (Gentra Systems, Inc.,

8 Minneapolis, PA, USA). All specimens were genotyped using various techniques (Table 1). Primers and

9 probes (Table 2) were designed with Primer Express Version 1.0 (Applied Biosystems, Foster City, CA,

10 USA) software.

11 Briefly, 25 μl PCR mixtures generally contained 50 ng DNA, 1.5 mM $MgCl_2$, 200 μM each dNTP,

12 optimized concentrations of corresponding primers and 0.625 U of AmpliTaq Gold (Applied Biosystems,

13 Foster City, CA, USA). All restriction endonucleases for PCR/RFLP were from New England BioLabs

14 (Ipswich, MA, USA). TaqMan allelic discrimination assay for *TP53* variants was done essentially as

15 described before (Rogounovitch *et al.* 2006). Melting curve T_m-shift assay for *MTF1* genotyping was

16 designed according to the described technology (Wang *et al.* 2005) and done in a Thermal Cycler Dice

17 Real Time System TP800 (TaKaRa, Ohtsu, Japan). Technical details are available from the authors upon

18 request.

19 For every SNP, some 20-30 randomly chosen DNA samples, unless otherwise specified, were

20 also analyzed by direct sequencing with a Big Dye Terminator sequencing kit v3.1 (Applied Biosystems,

21 USA) in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA). A complete concordance

22 between different techniques was observed.

23 Raw genotyping outputs were interpreted by at least two independent investigators. Missing

24 results due to genotyping procedure failures accounted for <1% for any SNP tested.

25

26 **Statistical analysis**

1   Genotype frequencies in each group were determined by univariate analysis and evaluated for

2   departure from Hardy-Weinberg equilibrium by the chi-square test. SNP associations with PTC were

3   assessed by multivariate logistic regression analysis for codominant, multiplicative, dominant and

4   recessive models to avoid assumptions regarding the mode of inheritance (see notes below Table 4). All

5   analyses were adjusted for gender (male or female, nominal), age (years, continuous) and IR-exposure

6   (yes or no, nominal). Besides of all of the parameters above, the full model included disease status (yes or

7   no, nominal) and, depending on the mode of inheritance, genotype for each SNP (nominal variable in the

8   codominant, dominant and recessive models and ordinal in the multiplicative model).

9   Power calculations were done with the PS software

10   (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). With given sample size, the

11   study had a power of 54-99 % to detect an OR of 2.0 at the significance level of 5% with MAF ranging 4-

12   45%.

13   Interaction between SNPs, cancer and radiation exposure were hypothesized *a priori* and

14   evaluated by multivariate analysis with corresponding adjustments. Separate calculations of OR were

15   done in irradiated and non-exposed case-control groups when *P* value for an interaction term did not

16   exceed 0.05.

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

18   USA).

19

20   **Results**

21   The distribution of genotypes and MAF for each SNP in the four study groups is shown in Table

22   3. The observed distributions in the control groups were not statistically different from those expected

23   from Hardy-Weinberg equilibrium for all SNP except for *ATM* G5557A and *ATM* IVS22-77 T>C in the

24   non-exposed controls. Since such deviation might point at possible genotyping error (Hosking *et al.* 2004),

25   we reanalyzed 96 non-exposed controls for these SNPs by direct sequencing. There were no

26   inconsistencies between PCR/RFLP and sequencing results (data not shown) ruling out technical flaw.

1   Furthermore, allelic frequencies determined in our study are in a good agreement with those specified for

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

3   As seen from Table 4, an association between *ATM* G5557A and PTC, regardless of radiation

4   exposure, was found. The presence of the A allele significantly decreased PTC risk compared with wild-

5   type G allele in the multiplicative model of inheritance (OR=0.69, 95% CI 0.45-0.86, *P*=0.03), which is

6   useful for risk comparison between the groups based on the analysis of allelic frequencies in them.

7   Main effect on PTC risk appeared also significant for the *XRCC1* gene Arg399Gln polymorphism.

8   The presence of the minor 399Gln allele decreased PTC risk compared with the Arg/Arg genotype

9   (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

10   dominant models, respectively).

11   Analysis of combined *ATM* G5557A and *XRCC1* Arg399Gln genotypes demonstrated that

12   increasing number of minor alleles (i.e. *ATM* 5557A and *XRCC1* 399Gln) significantly decreased PTC

13   risk in corresponding individuals in comparison with those who do not carry minor alleles (Fig. 1).

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

15   For *ATM* IVS22-77 T>C and *TP53* Arg72Pro, evidence for interaction between radiation

16   exposure and PTC was found (*P* for interaction 0.04 and 0.01, respectively). As shown in Table 5, the

17   analyses performed in IR-exposed and non-irradiated patients compared, respectively, with irradiated and

18   non-exposed controls revealed a significantly increased risk of sporadic PTC for the *ATM* IVS22-77

19   homozygous CC genotype carriers compared with the TC+TT genotypes (the recessive model of

20   inheritance, OR=1.84, 95% CI 1.10-3.24, *P*=0.03), whereas in the irradiated group an insignificant

21   inverse effect of these genotypes was observed (OR=0.59, 95% CI 0.28-1.27, *P*=0.17). For *TP53* codon

22   72 polymorphism, in all but the recessive models the increased risk of IR-induced PTC as compared to

23   IR-exposed controls was observed. The highest risk of radiogenic PTC was in the co-dominant model

24   (OR=2.33, 95% CI 1.15-7.21, *P*=0.03). A significant risk was also found in the multiplicative model of

25   inheritance (OR=1.70, 95% CI 1.17-2.46, *P*=0.006). In addition, comparison between IR-exposed and

26   non-exposed controls did not reveal statistically significant difference in adjusted distributions of these

1   polymorphisms. In healthy subjects the strongest association for the *ATM* IVS22-77 T>C was in the

2   recessive model (OR=1.38, 95% CI 0.84-2.26, *P*=0.21) and in the multiplicative model for *TP53*

3   Arg72Pro (OR=0.70, 95% CI 0.52-1.19, *P*=0.11) further emphasizing possible role of these SNPs in PTC

4   of different etiology.

5   Considering multiple pathways for repairing diverse DNA damages induced by endogenous and

6   exogenous carcinogens, genetic variants in different repair pathways may probably have a joint effect on

7   cancer risk. In attempt to search for the stronger associations between PTC and studied SNPs, we

8   performed the analyses of genotype combinations for the *ATM* and *TP53* polymorphisms as these genes

9   are functionally related and 3 of 4 SNPs included in our study showed effects on PTC. Among the

10   possible *ATM/TP53* combinations (rs1801516/rs664677/rs609429/rs1042522) tested, two demonstrated

11   significant differences in the subsets of both groups of PTCs (Fig.2). Particularly, the combined

12   *ATM/TP53* GG/TC/CG/GC genotype was strongly associated with the IR-induced PTC (OR=2.10, 95%

13   CI 1.17-3.78, *P*=0.015). Another *ATM/TP53* combination, GG/CC/GG/GG, demonstrated a significantly

14   increased risk for sporadic PTC (OR=3.32, 95% CI 1.57-6.99, *P*=0.002).

15

16   **Discussion**

17   Our study addressed possible associations between SNPs in the genes involved in DNA damage

18   response and the risk of PTC of different etiology. The results demonstrated that the presence of the

19   variant 5557A allele in exon 39 of *ATM* and *XRCC1* 399Gln allele, particularly in the heterozygous state,

20   significantly associated with the decreased risk of PTC. The *ATM* IVS22-77 CC genotype in the non-

21   exposed group and the *TP53* 72Pro allele in the radiation-related one associated with the increased risk of

22   PTC. Moreover, two particular *ATM/TP53* combined genotypes were found with higher frequencies in the

23   IR–induced or sporadic PTC when compared to the controls. Altogether, these data indicate that SNPs in

24   the studied genes may likely modify PTC risk.

25   A significant association between the *ATM* G5557A and bilateral breast cancer in Caucasian

26   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 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 to 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 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

1   among white American women with *XRCC1* Arg399Arg genotype (Duell *et al.* 2001). Looking for

2   potential biological explanations for these findings, the authors found a higher prevalence of *TP53*

3   deletions in the Arg399Arg cases exposed to occupational radiation compared with exposed patients with

4   the Gln399Gln genotypes or unexposed cases of either genotype. Figueiredo *et al.* (2004) observed an

5   increased risk of disease among wild-type homozygous (Arg/Arg) and heterozygous Canadian Caucasian

6   women with a family history of breast cancer compared to the individuals without such.

7   The described above data may be explained, at least in part, by the results of functional study of

8   this polymorphism in which an equal ability for both alleles to suffice single strand break repair by

9   *XRCC1* has been found (Taylor *et al.* 2002). The results of our study, taken together with those reported

10 previously, suggest that *XRCC1* polymorphism, in particular the Arg399Gln genotype, may influence

11 PTC risk, perhaps by modifying the effects of environmental exposure and/or through interaction with

12 other genetic factors.

13   The *TP53* Arg72Pro polymorphism affects the biological activity of p53. The Arg72 form is more

14 efficient at inducing apoptosis while the Pro72 appears to induce a higher level of G1 arrest (Pim &

15 Banks 2004). Based on these findings, a number of studies have attempted to assess a correlation between

16 *TP53* codon 72 polymorphism and risk of certain types of cancer, however, with inconsistent results, as

17 reviewed by Pietsch *et al.* (2006). This inconsistency may possibly be explained in part by the coexistence

18 of the codon 72 polymorphism and gain of function mutations in *TP53* in some tumors (Pietsch *et al.*

19 2006; Soussi & Wiman 2007).

20   Several groups have investigated the TP53 Arg72Pro polymorphism in PTC. Boltze *et al.* (2002)

21 found a small number of heterozygotes and no Pro/Pro genotype in differentiated thyroid carcinomas

22 from Germany. In contrast, in ethnically heterogeneous Brazilian population, the Pro/Pro genotype was

23 associated with the higher risk of differentiated thyroid cancer (Granja *et al.* 2004). The study of codon 72

24 polymorphism in thyroid tumors from Russian and Ukrainian patients demonstrated a significantly lower

25 frequency of wild-type homozygotes (i.e. Arg/Arg) among adults with IR-induced PTC when compared

26 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 *at 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 to 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 used different 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; Detours *et al.* 2008).

It is necessary to note that even though 9 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 which 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.

4

5 **Declaration of interest**

6 Authors declare no potential conflict of interest.

1 **References**

2 Alsbeih G, El-Sebaie M, Al-Harbi N, Al-Buhairi M, Al-Hadyan K & Al-Rajhi N 2007 Radiosensitivity of

3   human fibroblasts is associated with amino acid substitution variants in susceptible genes and

4   correlates with the number of risk alleles. *International Journal of Radiation Oncology, Biology*

5   *and Physics* **68** 229-235.

6 Angele S, Romestaing P, Moullan N, Vuillaume M, Chapot B, Friesen M, Jongmans W, Cox DG, Pisani

7   P, Gerard JP *et al.* ATM haplotypes and cellular response to DNA damage: association with breast

8   cancer risk and clinical radiosensitivity. *Cancer Research* **63** 8717-8725.

9 Boltze C, Roessner A, Landt O, Szibor R, Peters B & Schneider-Stock R 2002 Homozygous proline at

10   codon 72 of p53 as a potential risk factor favoring the development of undifferentiated thyroid

11   carcinoma. *International Journal of Oncology* **21** 1151-1154.

12 Bouville A, Likhtarev IA, Kovgan L, Minenko VF, Shinkarev SM & Drozdovitch V 2007 Radiation

13   dosimetry for highly contaminated Belarusian, Russian and Ukranian populations, and for less

14   contaminated populations in Europe. *Health Physics* **93** 487-501.

15 Bennett B, Repacholi M & Carr Z 2006 *Health Effects of the Chernobyl Accident and Special Health*

16   *Care Programmes.* Geneva: WHO Press.

17 Cardis E, Kesminiene A, Ivanov V, Malakhova I, Shibata Y, Khrouch V, Drozdovitch V, Maceika E,

18   Zvonova I, Vlassov O *et al.* 2005 Risk of thyroid cancer after exposure to 131I in childhood.

19   *Journal of the National Cancer Institute* **97** 724-732.

20 Davies L & Welch HG 2006 Increasing incidence of thyroid cancer in the United States, 1973-2002.

21   *Journal of the American Medical Association* **295** 2164-2167.

22 Davis S, Stepanenko V, Rivkind N, Kopecky KJ, Voilleque P, Shakhtarin V, Parshkov E, Kulikov S,

23   Lushnikov E *et al.* 2004 Risk of thyroid cancer in Bryansk oblast of the Russian Federation after

24   the Chernobyl Power Station accident. *Radiation Research* **162** 241-248.

Detours V, Delys L, Libert F, Weiss Solis D, Bogdanova T, Dumont JE, Franc B, Thomas G & Maenhaut C 2007 Genome-wide gene expression profiling suggests distinct radiation susceptibility in sporadic and post-Chernobyl papillary thyroid cancers. *British Journal of Cancer* **97** 818-825.

Detours V, Versteyhe S, Dumont JE & Maenhaut C 2008 Gene expression profiles of post-Chernobyl thyroid cancers. *Current Opinion in Endocrinology, Diabetes and Obesity* **15** 440-445.

Duell EJ, Millikan RC, Pittman GS, Winkel S, Lunn RM, Tse CK, Eaton A, Mohrenweiser HW, Newman B & Bell DA 2001 Polymorphisms in the DNA repair gene XRCC1 and breast cancer. *Cancer Epidemiology Biomarkers and Prevention* **10** 217-222.

Edvardsen H, Tefre T, Jansen L, Vu P, Haffty BG, Fossa SD, Kristensen VN & Borresen-Dale AL 2007 Linkage disequilibrium pattern of the ATM gene in breast cancer patients and controls; association of SNPs and haplotypes to radio-sensitivity and post-lumpectomy local recurrence. *Radiation Oncology* **2**:25.

Figueiredo JC, Knight JA, Briollais L, Andrulis IL & Ozcelik H 2004 Polymorphisms XRCC1-R399Q and XRCC3-T241M and the risk of breast cancer at the Ontario site of the Breast Cancer Family Registry. *Cancer Epidemiology Biomarkers and Prevention* **13** 583-591.

Granja F, Morari J, Morari EC, Correa LA, Assumpcao LV & Ward LS 2004 Proline homozygosity in codon 72 of p53 is a factor of susceptibility for thyroid cancer. *Cancer Letters* **210** 151-157.

Hall EJ, Schiff PB, Hanks GE, Brenner DJ, Russo J, Chen J, Sawant SG & Pandita TK 1998 A preliminary report: frequency of A-T heterozygotes among prostate cancer patients with severe late responses to radiation therapy. *The Cancer Journal from Scientific American* **4** 385-389.

Han S, Zhang HT, Wang Z, Xie Y, Tang R, Mao Y & Li Y 2006 DNA repair gene XRCC3 polymorphisms and cancer risk: a meta-analysis of 48 case-control studies. *European Journal of Human Genetics* **14** 1136-1144.

Heikkinen K, Rapakko K, Karppinen SM, Erkko H, Nieminen P & Winqvist R 2005 Association of common ATM polymorphism with bilateral breast cancer. *International Journal of Cancer* **116** 69-72.

1   Hillebrandt S, Streffer C, Demidchik EP, Biko J & Reiners C 1997 Polymorphisms in the p53 gene in
2   thyroid tumours and blood samples of children from areas in Belarus. *Mutation Research* **381** 201-
3   207.

4   Hoeijmakers JH 2001 Genome maintenance mechanisms for preventing cancer. *Nature* **411** 366-374.

5   Hosking L, Lumsden S, Lewis K, Yeo A, McCarthy L, Bansal A, Riley J, Purvis I & Xu CF 2004
6   Detection of genotyping errors by Hardy-Weinberg equilibrium testing. *European Journal of*
7   *Human Genetics* **12** 395-399.

8   Hu JJ, Smith TR, Miller MS, Lohman K & Case LD 2002 Genetic regulation of ionizing radiation
9   sensitivity and breast cancer risk. *Environmental and Molecular Mutagenesis* **39** 208-215.

10   Hu Z, Ma H, Chen F, Wei Q & Shen H 2005 XRCC1 polymorphisms and cancer risk: a meta-analysis of
11   38 case-control studies. *Cancer Epidemiology Biomarkers and Prevention* **14** 1810-1818.

12   Jacob P, Bogdanova TI, Buglova E, Chepurniy M, Demidchik Y, Gavrilin Y, Kenigsberg J, Meckbach R,
13   Schotola C, Shinkarev S *et al.* 2006 Thyroid cancer risk in areas of Ukraine and Belarus affected by
14   the Chernobyl accident. *Radiation Research* **165** 1-8.

15   Khanna KK & Jackson SP 2001 DNA double-strand breaks: signaling, repair and the cancer connection.
16   *Nature Genetics* **27** 247-254.

17   Khrunin AV, Tarskaia LA, Spitsyn VA, Lylova OI, Bebyakova NA, Mikulich AI & Limborska SA 2005
18   p53 polymorphisms in Russia and Belarus: correlation of the 2-1-1 haplotype frequency with
19   longitude. *Molecular Genetics and Genomics* **272** 666-672.

20   Likhtarev I, Bouville A, Kovgan L, Luckyanov N, Voilleque P & Chepurny M 2006 Questionnaire- and
21   measurement-based individual thyroid doses in Ukraine resulting from the Chornobyl nuclear
22   reactor accident. *Radiation Research* **166** 271-286.

23   Malmer BS, Feychting M, Lonn S, Lindstrom S, Gronberg H, Ahlbom A, Schwartzbaum J, Auvinen A,
24   Collatz-Christensen H, Johansen C *et al.* 2007 Genetic variation in p53 and ATM haplotypes and
25   risk of glioma and meningioma. *Journal of Neurooncology* **82** 229-237.

Nikiforov YE, Nikiforova MN, Gnepp DR & Fagin JA 1996 Prevalence of mutations of ras and p53 in benign and malignant thyroid tumors from children exposed to radiation after the Chernobyl nuclear accident. *Oncogene* **13** 687-693.

Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H & Fagin JA 1997 Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Research* **57** 1690-1694.

Pietsch EC, Humbey O & Murphy ME 2006 Polymorphisms in the p53 pathway. *Oncogene* **25**:1602-1611.

Pim D & Banks L 2004 p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. *International Journal of Cancer* **108** 196-199.

Rabes HM, Demidchik EP, Sidorow JD, Lengfelder E, Beimfohr C, Hoelzel D & Klugbauer S 2000 Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. *Clinical Cancer Research* **6** 1093-1103.

Rogounovitch TI, Saenko VA, Ashizawa K, Sedliarou IA, Namba H, Abrosimov AY, Lushnikov EF, Roumiantsev PO, Konova MV, Petoukhova NS *et al.* S 2006 TP53 codon 72 polymorphism in radiation-associated human papillary thyroid cancer. *Oncology Reports* **15** 949-956.

Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, Schneider AB, Tucker MA & Boice JD, Jr. 1995 Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. *Radiation Research* **141** 259-277.

Rothman KJ & Greenland S 1998 *Modern Epidemiology.* 2nd ed. Philadelphia: Lipincott-Raven.

Shiloh Y 2003 ATM and related protein kinases: safeguarding genome integrity. *Nature Reviews Cancer* **3** 155-168.

Siddique MM, Barlam C, Fiszer-Maliszewska L, Aggarwal A, Tan A, Tan P, Soo KC & Sabapathy K 2005 Evidence for selective expression of the p53 codon 72 polymorphs: implications in cancer development. *Cancer Epidemiology Biomarkers and Prevention* **14** 2245-2252.

Soussi T & Wiman KG 2007 Shaping genetic alterations in human cancer: the p53 mutation paradigm. *Cancer Cell* **12** 303-312.

Stepanenko VF, Voilleque PG, Gavrilin YuI, Khrouch VT, Shinkarev SM, Orlov MYu, Kondrashov AE, Petin DV, Iaskova EK & Tsyb AF 2004 Estimating individual thyroid doses for a case-control study of childhood thyroid cancer in Bryansk oblast, Russia. *Radiation Protection Dosimetry* **108** 143-160.

Sturgis EM, Zhao C, Zheng R & Wei Q 2005 Radiation response genotype and risk of differentiated thyroid cancer: a case-control analysis. *Laryngoscope* **115** 938-945.

Tamura Y, Maruyama M, Mishima Y, Fujisawa H, Obata M, Kodama Y, Yoshikai Y, Aoyagi Y, Niwa O, Schaffner W *et al.* 2005 Predisposition to mouse thymic lymphomas in response to ionizing radiation depends on variant alleles encoding metal-responsive transcription factor-1 (Mtf-1). *Oncogene* **24** 399-406.

Taylor RM, Thistlethwaite A & Caldecott KW 2002 Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair. *Molecular and Cellular Biology* **22** 2556-2563.

Thorstenson YR, Roxas A, Kroiss R, Jenkins MA, Yu KM, Bachrich T, Muht D, Wayne TL, Chu G, Davis RW *et al.* 2003 Contributions of ATM mutations to familial breast and ovarian cancer. *Cancer Research* **63** 3325-3333.

Wang J, Chuang K, Ahluwalia M, Patel S, Umblas N, Mirel D, Higuchi R & Germer S 2005 High-throughput SNP genotyping by single-tube PCR with Tm-shift primers. *Biotechniques* **39** 885-893.

Williams ED 2006 Chernobyl and thyroid cancer. *Journal of Surgical Oncology* **94** 670-677.

Yamashita S & Saenko V 2006 Mechanisms of Disease: molecular genetics of childhood thyroid cancers. *Nature Clinical Practice Endocrinology and Metabolism* **3** 422-429.

Yang T, Namba H, Hara T, Takamura N, Nagayama Y, Fukata S, Ishikawa N, Kuma K, Ito K & Yamashita S 1997 p53 induced by ionizing radiation mediates DNA end-jointing activity, but not apoptosis of thyroid cells. *Oncogene* **14** 1511-1519.

1   Yen CY, Liu SY, Chen CH, Tseng HF, Chuang LY, Yang CH, Lin YC, Wen CH, Chiang WF, Ho CH *et*
2   *al.* 2008 Combinational polymorphisms of four DNA repair genes XRCC1, XRCC2, XRCC3, and
3   XRCC4 and their association with oral cancer in Taiwan. *Journal of Oral Pathology and Medicine*
4   **37** 271-277.

5   Yoon YJ, Chang HY, Ahn SH, Kim JK, Park YK, Kang DR, Park JY, Myoung SM, Kim do Y, Chon CY
6   *et al.* 2008 MDM2 and p53 polymorphisms are associated with the development of hepatocellular
7   carcinoma in patients with chronic hepatitis B virus infection. *Carcinogenesis* **29** 1192-1196.

Table 1. 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 vs. wild-type (reference)
ATM G5557A Asp1853Asn	rs1801516	PCR/RFLP [a] (*Afl*II) 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 (*Rsa*I) 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 (*Kpn*I) 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 (*Rsa*I) 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 (*Msp*I) 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.

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
	37 (30.3)	68 (34.7)	41 (31.1)	131 (32.9)
CC	69 (56.6)	97 (49.5)	61 (46.2)	201 (50.5)
CG	16 (13.1)	31 (15.8)	30 (22.7)	66 (16.6)
GG	0.47		0.28	
P	41.4	40.3	45.8	41.8
G, %				
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

[a] There was no homozygous (A/A) variant of XRCC1 Arg280His among all samples tested.

NOTE. Total numbers of samples in each group vary slightly due to genotyping procedures failures.

Table 4. OR (95% CI) for PTC by gene polymorphism according to different models of inheritance (adjusted for age, gender and radiation exposure)

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 vs. *GG* [c]	0.73 (0.48-1.10)	0.13
	AA vs. *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 vs. *TT*	1.06 (0.74-1.53)	0.75
	CC vs. *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 vs. *CC*	1.11(0.77-1. 60)	0.57
	GG vs. *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 vs. *GG*	0.70 (0.59-0.93)	**0.03**
	AA vs. *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 vs. *GG*	1.04 (0.74-1.48)	0.82
	CC vs. *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 vs. *CC*	0.99 (0.70-1.41)	0.97
	TT vs. *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 vs. *TT*	1.00 (0.70-1.44)	0.99
	AA vs. *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 vs. *GG*	1.05 (0.76-1.49)	0.76
	AA vs. *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 vs. wild-type homozygous).
[d] Recessive inheritance model (minor allele homozygous vs. 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.

Table 5. OR (95% CI) for PTC of different etiology by *ATM* and *TP53* polymorphisms (adjusted for gender and age)

SNP	Genotype	IR-induced PTC *vs.* IR-exposed controls		Sporadic PTC *vs.* non-exposed controls	
		OR (95% CI)	*P*	OR (95% CI)	*P*
ATM IVS22-77	*TT*	1.00[a]		1.00	
T>C	*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 vs. *TT*[c]	1.23 (0.72-2.10)	0.44	0.97 (0.62-1.52)	0.88
	CC vs. *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 vs. *GG*	1.80 (1.06-2.36)	**0.01**	0.84 (0.54-1.29)	0.43
	CC vs. *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 vs. wild-type homozygous).
[d] Recessive inheritance model (minor allele homozygous vs. combined heterozygous and homozygous for the wild-type allele).

Figure legends

Fig. 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; $P_{3-4 MA}$<0.05. Carriers of 3 and 4 minor alleles were combined because of the exceedingly low number of 4 MA carriers in both PTC and control groups.

Fig. 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 vs. 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 3 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 less than 5% in three or more subgroups are pooled and indicated as "other".
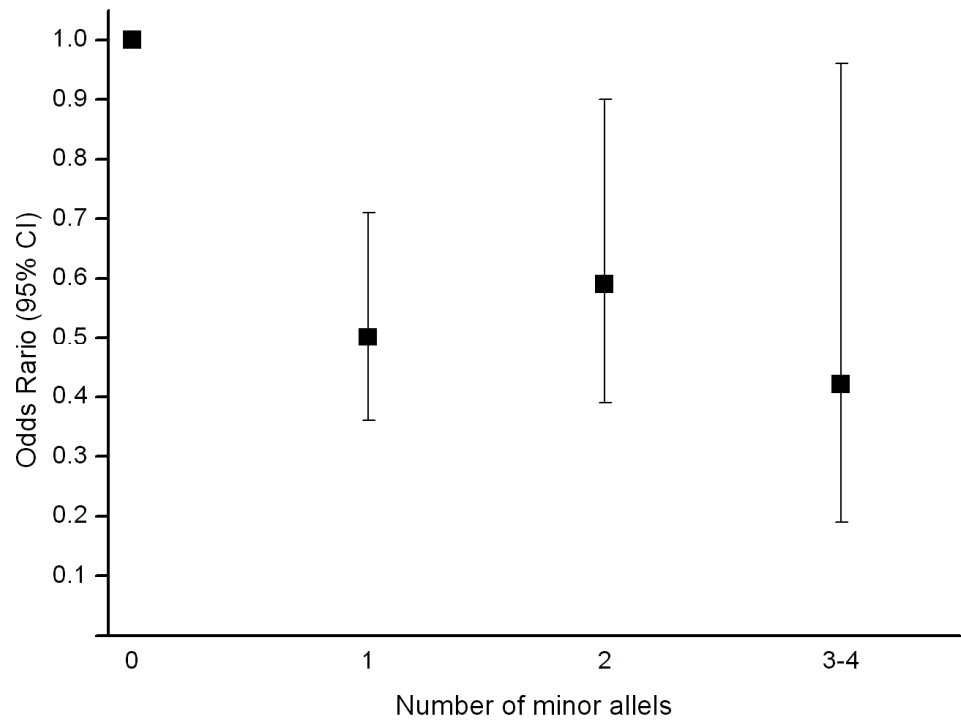
Proportion of MA carriers (%)

	0	1	2	3-4
PTCs	43.0	34.4	19.4	3.2
Controls	28.3	45.7	21.6	5.0

Fig. 1
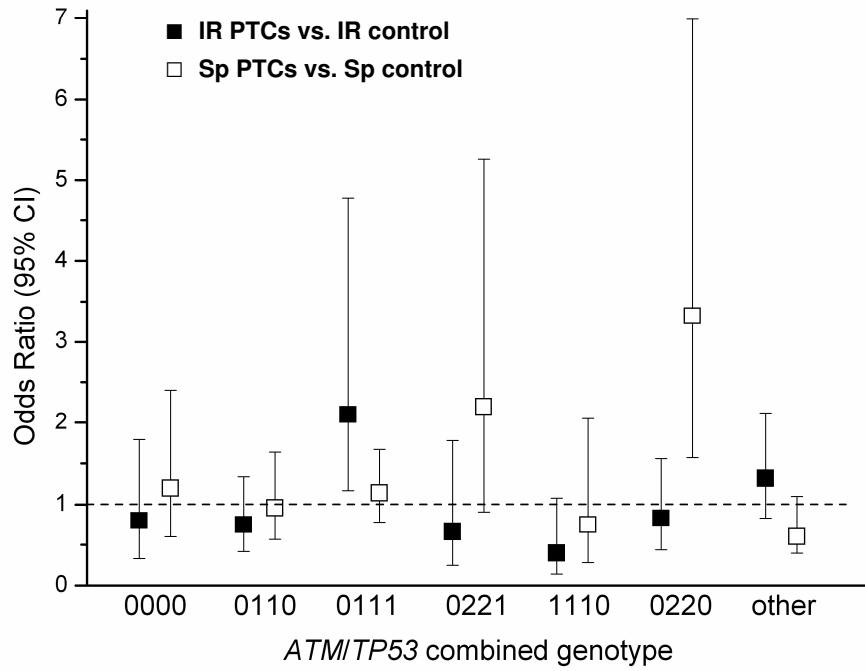
Proportion of carriers, (%)	IR	Sp	IR	Sp	IR	Sp	IR	Sp	IR	Sp	IR	Sp	IR	Sp
PTCs	7.3	9.0	17.8	17.4	**24.8**	15.5	5.0	7.1	4.0	3.8	3.3	**11.6**	38.0	35.6
Controls	9.3	8.0	21.7	17.5	**13.5**	14.2	7.1	3.3	10.0	5.2	6.2	**3.8**	32.0	48.0

Fig. 2