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Citation	Endocrine-related cancer, 16(2), pp.491-503; 2009
Issue Date	2009-06
URL	http://hdl.handle.net/10069/23254
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Polymorphisms of DNA damage response genes in radiation-related and sporadic papillary thyroid carcinoma

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Abbreviated title: Genotypes of PTC of different etiology

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

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 (OR=0.69, 95% 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 IR-induced or sporadic PTCs.

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 (Davis & Welch 2006).

Most thyroid cancer patients do not have 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 controls are nonzero. 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 to 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 ionizing radiation, 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.

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**

Genotype frequencies in each group were determined by univariate analysis and evaluated for departure from Hardy-Weinberg equilibrium by the chi-square 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 of all of 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 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 to 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 3 of 4 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 to 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 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

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 to 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 Arg72Pro polymorphism in PTC. Boltze *et al.* (2002) found a small number of heterozygotes and no Pro/Pro genotype in differentiated thyroid carcinomas from Germany. In 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

1 work, using an independent set of samples, confirm these findings suggesting the modifying role (or as of
2 a marker) of the TP53 Arg72Pro polymorphism in PTC developed after exposure to IR which is further
3 supported by the absence of significant difference in genotype distributions among our two control groups.
4 As shown in a genetic study, frequencies of the *C* allele (encoding 72Pro) do not generally differ
5 in populations of Belarus and Russia (Khrunin *et al.* 2005). However, East Slavs do not form a single
6 genetic cluster on multidimensional analysis. The 72Pro allele frequency in Belarus is about 0.3; in the
7 two different subpopulations from the Central and Northern regions of the European part of Russia it is
8 0.24 and 0.32, respectively. The study of healthy population from Poland (bordering with Belarus,
9 linguistically and culturally similar), reported the frequency of 0.28 for the 72Pro allele (Siddique *at al.*
10 2005). The 72Pro frequency reported by Rogounovitch *et al.* (2006) in Russian healthy controls is also
11 0.28. Thus, the effect of population admixtures in the controls in our investigation could not be
12 completely ruled out. Yet on the other hand, the ratio of Belarusian and Russian subjects in the IR-
13 exposed PTCs and controls was similar (2.24 and 2.30, respectively) suggestive of an unbiased estimate
14 and being an argument in support of *TP53* Arg72Pro polymorphism association with radiation-related
15 PTC.
16 While many studies established the effect of individual SNPs on cancer, the role of SNP
17 combinations has been less addressed. Several *ATM* and *TP53* haplotypes were associated with clinical
18 radiosensitivity in breast cancer (Angele *et al.* 2003) and brain tumor risk (Malmer *et al.* 2007). Recently,
19 the interactions of SNPs located on different chromosomes were investigated in various malignancies
20 (Yen *et al.* 2008; Yoon *et al.* 2008). One experimental study, in which *ATM* Asp1853Asn, *TP53*
21 Arg72Pro, *XRCC1* Arg399Gln and *XRCC3* Thr241Met were genotyped, demonstrated that the increasing
22 number of risk alleles enhanced radiosensitivity of human fibroblast cell lines and, potentially,
23 susceptibility to radiation-induced cancers (Alsbeih *et al.* 2007). So far no studies have investigated the
24 joint effect of gene polymorphisms on thyroid cancer. Our observations demonstrated that frequencies of
25 particular combined *ATM*/*TP53* genotypes were higher in patients with radiogenic or sporadic PTC
26 compared to corresponding control populations.

1 To some extent these results support the idea that genetic factors may possibly modify
2 predisposition to thyroid cancer. A recent study by Detours *et al.* (2007) reported difference in the
3 expression levels of some genes between Chernobyl PTCs from Ukraine and French sporadic PTCs.
4 Although the mentioned work and the present one used different molecular approaches, the results of both
5 are suggestive of a possible genetic "susceptibility signature" that may contribute to the individual
6 predisposition to IR and other carcinogens' effects. These findings are in favor of a "susceptibility model"
7 that may partly explain why only a minority of the large population exposed to the IR after the Chernobyl
8 disaster developed thyroid cancer (Yamashita & Saenko 2006; Detours *et al.* 2007; Detours *et al.* 2008).
9 It is necessary to note that even though 9 SNPs were analyzed in our study, no correction for
10 multiple comparisons was applied because of study design and techniques employed. The associations
11 were tested in a one-at-a-time fashion in a limited sample size in the difficult to access groups. The need
12 for correction in such circumstances is still debated (Rothman & Greenland 1998). Furthermore, since
13 data obtained in this work may be referred to as an initial screening result, non-adjusted presentation
14 enables their inclusion in future meta-analysis. Effects of candidate SNPs which we report need validation
15 in other studies.
16 In conclusion, the results presented here show that SNPs in *ATM* exon 39 and *XRCC1* exon 10
17 may be the markers of a decreased PTC risk in adults, whereas the *ATM* IVS22-77 and *TP53* codon 72
18 SNPs genes may associate with the risk of PTC development in non-irradiated and irradiated individuals.
19 To the best of our knowledge, presented here is the first study of this kind reporting the results of
20 genotyping of candidate DNA damage response genes in irradiated and non-irradiated PTC patients and
21 in corresponding healthy populations. Our data support the paradigm of genetic modifiers of radiation-
22 associated carcinogenesis and perhaps may contribute to genetic determination of PTC-prone subjects.
23 We believe such identification will allow future personalized cancer risk prediction which is of a
24 significant importance in view of the growing thyroid cancer incidence and also because of the relevance
25 to occupational and radiation emergency medicine issues.

1 **Funding**

2 This work was supported in part by Grant-in-Aid for Scientific Research 19256003, 19510058 and
3 19790651 from Japan Society for the Promotion of Science.

4

5 **Declaration of interest**

6 Authors declare no potential conflict of interest.

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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	n = 122	n = 196	n = 132	n = 398
C>G	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)

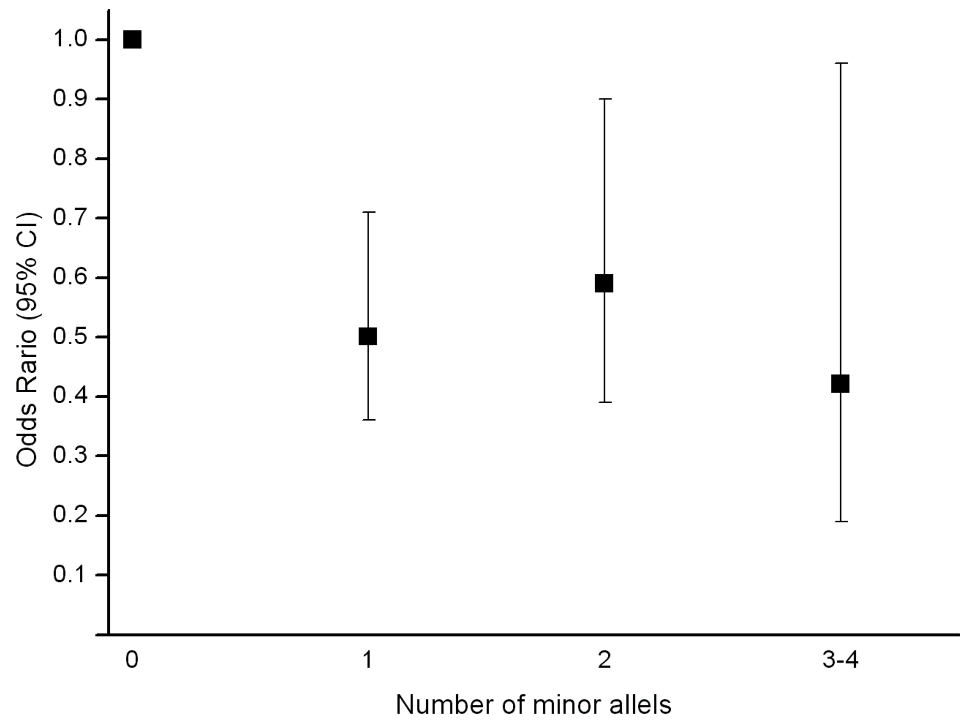
SNP	Genotype	IR-induced PTC *vs.* IR-exposed controls		Sporadic PTC *vs.* non-exposed controls	
		OR (95% CI)	***P***	**OR (95% CI)**	***P***
ATM IVS22-77	*TT*	1.00[a]		1.00	
T>C	*TC*	1.38 (0.80-2.39)	0.19	0.82 (0.51-1.32)	0.50
	CC	0.73 (0.31-1.70)	0.44	1.63 (0.87-3.08)	0.09
	Risk per *C* allele[b]	0.97 (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

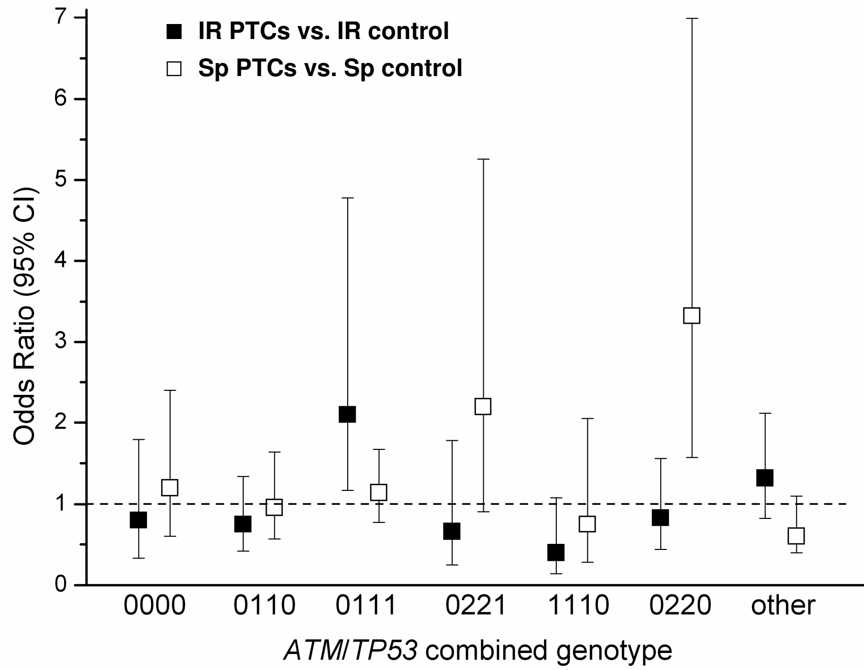
Fig. 1. Effect of increasing number of minor alleles (MA) for *ATM* G5557A and *XRCC1* Arg399Gln (minor alleles, *ATM* 5557A and *XRCC1* 399Gln) on PTC risk. The combined genotype with 0 MA was used as a reference. *P* values for genotypes with different MA number: $P_{1MA}<0.0001$; $P_{2MA}<0.01$; $P_{3\text{-}4\ MA}<0.05$. 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 (%)				
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