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RESEARCH ARTICLE

DNA excision repair and double-strand break repair gene polymorphisms and the level of chromosome aberration in children with long-term exposure to radon

Aleksey V. Larionov[a], Maxim Yu. Sinitsky[a,b,c], Vladimir G. Druzhinin[a,b], Valentin P. Volobaev[a], Varvara I. Minina[a,b], Maxim A. Asanov[b], Alina V. Meyer[a], Tatiana A. Tolochko[a] and Ekaterina E. Kalyuzhnaya[a]

[a]Department of Genetics, Biology Faculty, Kemerovo State University, Kemerovo, Russian Federation; [b]Federal State Budget Scientific Institution, The Federal Research Center of Coal and Coal Chemistry of Siberian Branch of the Russian Academy of Sciences, Kemerovo, Russian Federation; [c]Laboratory of Genome Medicine, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation

ABSTRACT

Purpose: To study polymorphic variants of repair genes in people affected by long-term exposure to radon. The chromosome aberration frequency in peripheral blood lymphocytes was used as the biological marker of genotoxicity.

Materials and methods: Genotyping of 12 single nucleotide polymorphisms in DNA repair genes (*APE*, *XRCC1*, *OGG1*, *ADPRT*, *XpC*, *XpD*, *XpG*, *Lig4* and *NBS1*) was performed in children with long-term resident exposure to radon. Quantification of the aberrations was performed using light microscopy.

Results: The total frequency of aberrations was increased in carriers of the G/G genotype for the *XpD* gene (rs13181) polymorphism in recessive model confirmed by the results of ROC-analysis ('satisfactory predictor', AUC = 0.609). Single chromosome fragments frequency was increased in carriers of the G/G genotype in comparison with the T/T genotype. In respect to the total frequency of aberrations, the G/G genotype for the *XpG* gene (rs17655) polymorphism was also identified as a 'satisfactory predictor' (AUC = 0.605). Carriers of the T/C genotype for the *ADPRT* gene (rs1136410) polymorphism were characterized by an increased level of single fragments relative to the T/T genotype.

Conclusion: The relationships with several types of cytogenetic damage suggest these three SNP (rs13181, rs17655 and rs1136410) may be considered radiosensitivity markers.

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Introduction

DNA repair is a critical function of cells because DNA is the crux of genetic information flow. Every spontaneous alteration of the original genetic sequence can lead to the expression of defective cell components that are unable to function. Furthermore, there is no reserve copy of DNA in mammals. Repair of damaged DNA to the original genetic sequence is the single solution to this problem. Errors in repair lead to cell death by necrosis and apoptosis. DNA damage can appear as a result of the influence of endogenous (reactive oxygen species [ROS]) and exogenous (ionizing and ultraviolet radiation) factors. Mammalian cells can develop 10^4–10^5 DNA single-strand breaks per day, and all of this damage must be quickly and accurately eliminated (Swenberg et al. 2011).

Several molecular mechanisms partly duplicate each other, and more than 150 proteins play roles in the elimination of DNA damage. Each method of repair includes sensory molecules, signal kinases and a set of molecules that are specific to a certain type of DNA damage. Excision DNA repair is a general specialized mechanism that corrects many types of DNA damage: cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts caused by UV radiation, various chemical DNA adducts formed by the influence of carcinogens and chemotherapeutic agents, intra- and inter-strand cross-links and damage caused by ROS. There are also mechanisms of base- and nucleotide-excision repair (BER and NER, respectively). BER includes a step giving rise to apurine-apyrimidine (AP) sites, which is removed from the sequence; the gap in the DNA is filled by DNA polymerases and ligases. NER includes the removal of the damaged chain fragment and its subsequent filling.

Double-strand breaks are a type of DNA damage that is caused by two radical hits involving hydroxyl radical and/or ionization. This type of damage can lead to new mutations, and a cell will be eliminated by apoptosis or malignant transformation if such damage is not repaired.

Non-repaired DNA damage can lead to chromosome aberrations (CA) (Obe et al. 2002). Damage, especially radiation-induced double-strand breaks, can initiate the loss of genetic material and intra- and inter-chromosome reconstructions. According to modern concepts, the dose-response curve, which reflects the number of double-strand breaks, is linear; thus, deflections from the linear model of the CA frequency or surviving cells primarily reflect the peculiarities of the process of reparation (Cornforth 2006). The effectiveness of the repair process determines the level of cytogenetic damage.

CONTACT Dr Aleksey V. Larionov alekseylarionov09@gmail.com Department of Genetics, Biology Faculty, Kemerovo State University, Krasnaya St. 6, Kemerovo, 650043, Russian Federation

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The level of DNA damage directly correlates with disturbances of cell division, DNA transcription and gene expression. These disturbances lead to cell death, apoptosis and malignant transformation. CA reflect an individual's susceptibility to a complex of endogenous and exogenous factors and can be markers of genotoxic risk (Mateuca et al. 2012; Vodenkova et al. 2015). Significant research indicates that cytogenetic indicators are useful biomarkers for genotoxic and carcinogenic responses (including the influence of radiation) in occupational and domestic conditions (Norppa 2004). The CA frequency in somatic cells has been suggested to act as a marker of overall carcinogenic risk (Bonassi and Au 2002). Some studies have reported a correlation between CA and cancer incidence (Bonassi et al. 2004; Norppa et al. 2006).

The effectiveness of the protection of cells is largely determined by widespread single nucleotide polymorphisms (SNP) in a population that change the activity of repair enzymes. Approximately 10–20% of the human population is believed to be characterized by a 20–35% reduction of repair efficiency, and this repair efficiency is primarily determined by hereditary factors (Cloos et al. 1999). Biochemical polymorphisms of repair systems largely determine cancer risk (lung, skin, breast, etc.) (Au et al. 2003; Batar et al. 2009; Jiang et al. 2009).

It should be noted that not all SNP in the genes of protective enzymes are studied adequately, and many are characterized by contradictory data concerning the activity or association with the frequency of cancer incidence. The aim of this work was the study of polymorphic variants of excision repair genes in people affected by significant genotoxic stress.

Materials and methods

Group description

Blood samples were obtained from 372 long-term resident children (from 8–18 years old; mean age = 12.24 ± 2.60 years) at a boarding school (Tashtagol city, Kemerovo Region, Russian Federation). Tashtagol is located in the south of the Kemerovo Region and is characterized by several industrial factories and a low level of chemical contamination. All examined children were long-term residents of the boarding school under standard conditions and followed a similar diet plan and daily routine. Many children living in remote towns and villages spend the majority of the year at school, thereby minimizing differences in lifestyle, social factors, bad habits and age.

The Ames test and the test for the induction of dominant lethal mutations in *Drosophila melanogaster* revealed no mutagenic activity in air or water samples (Druzhinin et al. 2015) from this city. Tashtagol is characterized by an increased concentration of radon. This fact was discovered as a result of long-term cytogenetic monitoring of the Kemerovo Region. The highest level of cytogenetic damage was discovered in boarding schools in Tashtagol. Thus, all children living and studying in this school are exposed to excessive concentrations of radon.

Radon is a carcinogen that belongs to the highest hazard class and can cause various types of genetic damage, as our previous articles demonstrated (Sinitsky and Druzhinin 2014; Druzhinin et al. 2015). Long-term exposure to radon in the boarding school in Tashtagol exposes children and adolescents (8–18 years old) to a relative increase in genotoxic stress.

As a control group we used blood samples obtained from children living in the settlements of Kemerovo Region (villages Krasnoye, Zarubino, Pacha) without increased radon level that are located at least 100 km from all industrial centers. The full description of the study groups is presented in Table 1.

Children who had received medical treatment or had X-ray examination 3 months prior to the study were not included in the study. For each person, informed consent was provided by the parents or persons with custody of the minors. The research was performed in accordance with the requirements of the Ethics Committee of the Kemerovo State University.

DNA extraction

DNA extraction was performed using the phenol-chloroform method. In the first stage, the fraction of nuclear cells obtained by the selective lysis of cell membranes in saccharose buffer was obtained from frozen venous blood samples (Agutter 1972). In the second stage, a routine phenol-chloroform extraction was performed (Miller et al. 1988). Three millilitres of venous blood was thawed and thoroughly mixed, and the samples were added to flasks with 10 ml of saccharose buffer and stored for 40 min at 0 °C. The flasks were then centrifuged, the pellet was resuspended in 300 μl of SE-buffer (EDTA 25 mM, NaCl 75 mM), and proteinase K (500 μg/ml) and SDS (1%) were added to each flask. After 12 h of incubation at 37 °C, 300 μl of phenol was added to each flask. All of the samples were extracted twice using the phenol-chloroform solution (25 portions of phenol, 24 portions of chloroform, one portion of isopropanol) and once with chloroform. Finally, the DNA samples were treated with 4 M NaCl and precipitated with 70% ethanol at −20 °C.

PCR

Lytech Company (Moscow, Russian Federation) products were used to perform PCR. This method is based on allele-specific

Table 1. Sex characteristics of the studied groups.

Group	Exposed			Control		
	Total	Male	Female	Total	Male	Female
Number	372	195	177	186	83	103
Age (mean ± SD)	12.24 ± 2.60	12.14 ± 2.63	12.36 ± 2.58	14.43 ± 2.58	13.90 ± 2.58	14.85 ± 2.51
Age (min–max)		8–18			8–19	

PCR for the key nucleotide. For each sample of DNA, two PCR tubes containing the two primer pairs ('Normal' and 'Pathology') were prepared. Each primer pair was specific to the key SNP. Each sample contained 17.5 μl of the solvent, 2.5 μl of the reaction mixture and 0.2 μl of Taq-polymerase. Approximately 5 μl of the DNA solution was added to the working mixture, and PCR was performed using the following algorithm suggested by the manufacturer: heating the mixture to 94 °C; 93 °C for 1 min; 35 cycles of 93 °C for 10 sec, 64 °C for 10 sec, and 72 °C for 20 sec; and a final elongation step at 72 °C for 1 min.

Twelve SNP were genotyped: *LIG4* Ala3Asp (C/T, rs1805389), *LIG4* Thr9Ile (C/T, rs1805388), *APE* Asp148Glu (T/G, rs1130409), *XRCC1* Arg194Trp (C/T, rs1799782), *XRCC1* Arg280His (G/A, rs25489), *XRCC1* Arg399Gln (G/A, rs25487), *OGG1* Ser326Cys (C/G, rs1052133), *ADPRT* Val762Ala (T/C, rs1136410), *XpC* Lys939Gln (A/C, rs2228001), *XpD* Lys751Gln (T/G, rs13181), *XpG* Asp1104His (C/G, rs17655), and *NBS1* Glu185Gln (C/G, rs1805794).

Detection of amplification products

The detection of amplification products was performed using separation by horizontal electrophoresis in a 3% agarose gel. TAE (Tris, Acetate, EDTA) was used as both the gel and electrode buffer. Approximately 10 μl of a 1% solution of ethidium bromide was added to 100 ml of melted agarose and mixed. The melted agarose was cooled to 50–60 °C and poured into the plate to solidify. Next, 8–10 μl of the amplified PCR product was placed into the gel well according to a sequence that corresponded to the sample number. The electrophoretic chamber was connected to a power supply at a voltage corresponding to an intensity of the electric field of 10–15 V per cm of gel for 15 min. Following electrophoresis, the gel was removed from the casting tray and placed on the glass of a UV-transilluminator. Image capture was performed by a photo camera and the computer software GelImager.

Cytogenetic investigation

The CA frequency in peripheral blood lymphocytes was used as the biological marker of genotoxicity. For the detection CA, 500 μl of venous blood was added to flasks containing RPMI-1640 (PanEco, Moscow, Russian Federation), embryonic veal serum and 100 U/ml ampicillin. Phytohaemagglutinin (100 μl/flask) was added to each culture to stimulate cell division. After 48-h incubation period, 0.5 μg/ml of colchicine was poured into each culture. Subsequently, each culture was treated with a hypotonic solution (KCl, 0.55%) and fixed using Carnoy's fixer. The detailed description of this method was presented in our previous study (Druzhinin et al. 2015).

Quantification of the aberrations was performed using light microscopy at 1000× magnification (oil immersion) without karyotyping. The selection of metaphases included in the analysis and the criteria for cytogenetic abnormalities conforming to the generally accepted recommendations (Savage 1976; Brøgger et al. 1990).

Statistical analysis

Statistical analysis was performed using the program StatSoft STATISTICA 10.0. We used the Kolmogorov–Smirnov test to verify the compliance of the data with the normal distribution. The data analysis was performed using the non-parametric statistics block. Group comparisons were performed using the *U*-rank Mann–Whitney test. We used ROC analysis (calculation of the AUC index) modified by DeLong (DeLong et al. 1988) using the package MedCalc 14.8.1 to assess the predictive significance of dominant and recessive models. Pearson's correlation coefficient for non-parametric data was used to calculate the correlation. If the cohorts contained more than 50 donors, we calculated Student's criterion based on the value of Pearson's correlation (Glantz 2001). To avoid the effect of multiple comparisons, FDR (False Discovery Rate) correction was applied (Benjamini and Yekutieli 2001).

Results

In our previous research, we reported increased levels of all of the studied cytogenetic indicators (chromosome and chromatid-type aberrations (CSA and CTA), including single and acentric fragments, rings and dicentric chromosomes) in the exposed group. We ascertained that exposure to radon and its daughter products leads to the appearance of cytogenetic abnormalities. The measurement of radon concentrations revealed a supernormal value, whereas the air and water probes did not demonstrate mutagenic activity. The full results of the radiological and cytogenetic investigations are presented in our previous article (Druzhinin et al. 2015).

We used the *U*-rank Mann–Whitney test to compare the CA frequency among various genotypes (Table 2) because the distribution of all of the quantitative variables (CA) was not normal.

The frequency of cytogenetic abnormalities and genotype frequencies did not differ between males and females in the exposed and control groups; thus, the analysis of the influence of polymorphisms on the frequency of cytogenetic damage was not divided by gender. We observed an increase in the total CA frequency in the *ADPRT* (rs1136410) C/C genotype and an increased frequency of single fragments in the heterozygous genotype for *ADPRT* (rs1136410) T/C carriers with respect to the homozygous genotype T/T ($p < 0.01$) and a decreased frequency of chromosomal changes relative to the C/C genotype ($p < 0.01$).

Genotypes G/G and T/G for the *XpD* (rs13181) polymorphism were characterized by a similar tendency ($p < 0.001$ and $p < 0.01$, respectively) relative to the T/T genotype. In addition, G/G and G/G genotypes for the *XpG* (rs17655) polymorphism were associated with an increased frequency of single fragments compared with genotype C/C. On the contrary, homozygotes for the minor allele (G/G) for the *OGG1* (rs1052133) polymorphism were characterized by a decreased frequency of chromosomal aberrations relative to the C/C genotype. Adjustments for multiple comparisons ($p_{corr} = 0.05/128 = 0.00039$, where $128 =$ total number of comparisons in the Mann–Whitney test) revealed only one significant result:

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Table 2. Chromosome aberrations (CA) frequency in the examined participants from the exposed group in respect to their allelic variants of DNA repair genes.

Genotype		n	Total CA, % (95% CI)	Single fragments, % (95% CI)	Acentric fragments, % (95% CI)	Chromosome interchanges, % (95% CI)
APE Asp148Glu	T/T	113	4.50 (4.03–4.97)	3.06 (2.65–3.46)	1.18 (1.00–1.35)	0.25 (0.17–0.33)
	T/G	150	4.43 (4.02–4.83)	3.03 (2.68–3.28)	1.15 (1.00–1.30)	0.23 (0.16–0.30)
	G/G	86	4.66 (4.12–5.19)	3.48 (3.01–3.94)	1.00 (0.80–1.20)	0.18 (0.09–0.27)
XRCC1 Arg194 Trp	C/C	317	4.41 (4.12–4.69)	3.06 (2.82–3.30)	1.10 (1.00–1.21)	0.23 (0.18–0.28)
	C/T	47	4.21 (3.47–4.95)	2.93 (2.30–3.56)	1.11 (0.84–1.38)	0.17 (0.05–0.29)
XRCC1 Arg280His	G/G	324	4.40 (4.12–4.69)	3.05 (2.81–3.29)	1.11 (1.01–1.21)	0.23 (0.18–0.23)
	G/A	41	4.16 (3.36–4.94)	2.98 (2.30–3.66)	1.00 (0.72–1.29)	0.15 (0.01–0.28)
XRCC1 Arg399Gln	G/G	172	4.63 (4.26–5.00)	3.20 (2.87–3.52)	1.22 (1.07–1.36)	0.24 (0.18–0.30)
	G/A	109	4.60 (4.12–5.07)	3.35 (2.93–3.76)	1.06 (0.88–1.24)	0.18 (0.11–0.26)
	A/A	43	5.10 (4.35–5.85)	3.68 (3.02–4.33)	1.22 (0.93–1.50)	0.20 (0.08–0.32)
OGG1 Ser326 Cys	C/C	107	4.18 (3.69–4.68)	2.83 (2.41–3.25)	1.08 (0.90–1.26)	**0.28 (0.20–0.35)**
	C/G	178	4.60 (4.21–4.98)	3.21 (2.89–3.54)	1.14 (1.00–1.28)	0.21 (0.16–0.27)
	G/G	65	4.35 (3.71–4.99)	3.21 (2.66–3.75)	1.05 (0.82–1.29)	**0.09** (0.00–0.19)**
ADPRT Val762 Ala	T/T	146	**3.97 (3.55–4.38)**	**2.65 (2.30–3.01)**	1.09 (0.93–1.24)	0.20 (0.14–0.26)
	T/C	157	4.55 (4.15–4.95)	**3.34* (3.00–3.69)**	1.04 (0.89–1.19)	**0.17 (0.11–0.23)**
	C/C	53	**5.15* (4.46–5.84)**	3.37 (2.77–3.96)	1.39 (1.14–1.64)	**0.37* (0.26–0.47)**
XpC Lys939Gln	A/A	130	4.85 (4.42–5.29)	3.44 (3.05–3.82)	1.20 (1.03–1.36)	0.21 (0.14–0.28)
	A/C	116	4.70 (4.24–5.36)	3.27 (2.87–3.68)	1.19 (1.01–1.36)	0.23 (0.16–0.30)
	C/C	62	4.74 (4.11–5.36)	3.43 (2.87–3.98)	1.07 (0.84–1.31)	0.22 (0.12–0.32)
XpD Lys751Gln	T/T	121	**4.26* (3.82–4.69)**	**2.77 (2.39–3.14)**	1.21 (1.04–1.37)	0.26 (0.18–0.34)
	T/G	136	4.78 (4.37–5.18)	**3.43* (3.07–3.78)**	1.13 (0.98–1.29)	0.21 (0.14–0.29)
	G/G	61	**5.29 (4.68–5.90)**	**3.91** (3.39–4.44)**	1.14 (0.90–1.37)	0.22 (0.11–0.33
XpG Asp1104His	C/C	151	4.23 (3.82–4.64)	**2.93 (2.58–3.28)**	1.10 (0.95–1.25)	0.19 (0.13–0.25)
	C/G	151	4.32 (3.91–4.73)	3.02 (2.68–3.37)	1.03 (0.88–1.18)	0.25 (0.18–0.31)
	G/G	41	5.28 (4.49–6.07)	**3.82* (3.15–4.49)**	1.29 (1.00–1.58)	0.16 (0.04–0.28)
NBS1 Glu185Gln	C/C	133	4.80 (4.36–5.24)	3.35 (2.97–3.73)	1.26 (1.10–1.41)	0.20 (0.12–0.27)
	C/G	151	4.22 (3.81–4.64)	2.93 (2.57–3.29)	1.02 (0.87–1.17)	0.26 (0.19–0.33)
	G/G	54	4.41 (3.71–5.10)	3.39 (2.69–3.88)	0.90 (0.65–1.15)	0.19 (0.08–0.31)
LIG4 Thr9Ile	C/C	281	4.41 (4.13–4.75)	3.14 (2.88–3.40	1.08 (0.97–1.19)	0.21 (0.16–0.26)
	C/T	66	4.24 (3.61–4.87)	2.79 (2.25–3.33)	1.16 (0.93–1.39)	0.28 (0.17–0.38)
	T/T	4	4.63 (2.05–7.20)	3.13 (0.93–5.32)	1.25 (0.32–2.18)	0.25 (−0.67–0.68)
LIG4 Ala 3Asp	C/C	302	4.37 (4.08–4.67)	3.10 (2.84–3.35)	**1.05* (0.94–1.15)**	0.21 (0.16–0.26)
	C/T	45	4.87 (4.11–5.64)	3.14 (2.49–3.80)	**1.47 (1.20–1.75)**	0.27 (0.14–0.39)
	T/T	4	1.88 (−0.67–4.43)	1.13 (−1.05–3.31)	0.25 (−0.67–1.17)	0.38 (−0.05–0.80)

Note: The association of genotypes with the frequency of aberrations (Mann–Whitney test) at the following levels are highlighted in bold type: $*p < 0.01$; $**p < 0.001$.

Table 3. AUC index (*ADPRT*, *XpD*, *XpG*, *OGG1* genes) for the total chromosome aberrations (CA) number and single fragments in dominant and recessive models.

	Dominant model		Recessive model	
Genotype	AUC	*p*-Value	AUC	*p*-Value
Total CA				
XpD (T/G)	0.537	0.296	0.609	0.0006
XpG (C/G)	0.571	0.045	0.605	0.0041
OGG1 (C/G)	0.537	0.25	0.507	0.87
ADPRT (T/C)	0.578	0.01	0.581	0.065
Single fragments				
XpD (T/G)	0.586	0.014	0.597	0.0029
XpG (C/G)	0.577	0.03	0.584	0.022
OGG1 (C/G)	0.542	0.2	0.535	0.37
ADPRT (T/C)	0.59	0.003	0.541	0.35

the increasing frequency of single fragments in the carriers of the G/G genotype for the *XpD* gene ($p = 0.00038$).

Four SNP (*ADPRT*, *XpD*, *XpG* and *OGG1*) that exhibited a trending association with cytogenetic markers were tested as predictors of the risk of increased CA in dominant and recessive models of gene interactions. The results are presented in Table 3.

The AUC index revealed no good predictors among the surveyed substitutions, except for *XpD* (rs13181) and *XpG* (rs17655), for the total frequency of CA in a recessive model. According to the formal criteria (AUC >0.6), these SNP correspond to the category of 'satisfactory predictor', and homozygous genotypes for the minor allele *XpD* 751 G/G and *XpG* 1104 G/G are likely to represent risk factors that may increase the frequency of CA. There were no satisfactory predictors among other types of SNP or other cytogenetic abnormalities.

Based on the obtained results, the combined action of several minor alleles (*XpD* G/G, *XpG* G/G, *ADPRT* C/C) may cause an increase in the frequency of aberrations. For calculation, two SNP were chosen: *XpD* (rs13181) and *XpD* (rs17655), which were identified as 'satisfactory predictors' of the total frequency of CA, and *ADPRT* (rs1136410) was correlated with an increase in the frequency of CA in homozygous carriers for the minor allele genotype C/C.

To evaluate this hypothesis, the correlation coefficient for the frequency of single, acentric fragments and chromosome interchanges was calculated (Figure 1). Non-parametric Spearman correlation (r_s) was selected to identify the correlation between the total number of minor alleles and the level of aberrations because the distribution of the frequency of cytogenetic abnormalities was different from normal. A total of 304 donors were examined. The total number of cases exceeded 50, so on the basis of the coefficient r_s, the *t*-value (*t*-test) was calculated (Glantz 2001). The value of the *t*-test for the single fragments frequency ($r_s = 0.2251$) was $t = 4.015$ (t critical $= 4.000$, $p = 0.001$).

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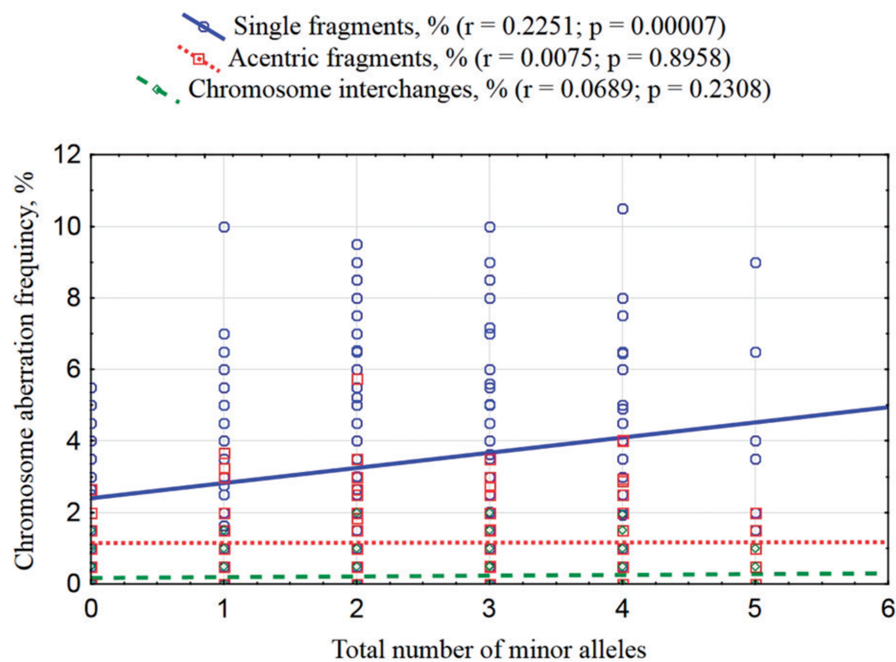

Figure 1. The frequency of chromosomal aberrations (CA) of the total number of minor alleles of the three single nucleotide polymorphisms (SNP): *XpD* Lys751Gln (T/G), *XpG* Asp1104His (C/G), and *ADPRT* Val762Ala (T/C).

Table 4. The frequency of chromosomal aberrations (CA) in respect to the number of minor alleles of three SNP: *XpD* (rs13181) G/G, XpG (rs17655) G/G, and *ADPRT* (rs1136410) C/C.

		Mean (95% CI)			
Minor allele count	*n*	Total CA, %	Single fragments	Acentric fragments	Chromosome interchanges
0	23	3.69 (2.69–4.68)	**2.29 (1.42–3.16)**	1.16 (0.77–1.54)	0.20 (0.03–0.36)
1	57	4.14 (3.50–4.77)	**2.88 (2.33–3.42)**	1.09 (0.84–1.33)	0.14 (0.04–0.24)
2	109	4.74 (4.28–5.19)	3.30 (2.90–3.70)	1.21 (1.04–1.39)	0.23 (0.15–0.30)
3	78	4.91 (4.37–5.44)	**3.56* (3.09–4.03)**	1.07 (0.86–1.28)	0.26 (0.17–0.35)
4	31	5.68 (4.82–6.54)	**4.23* (3.49–4.98)**	1.25 (0.92–1.59)	0.19 (0.05–0.33)
5	6	5.92 (3.97–7.87)	4.42 (2.72–6.11)	1.08 (0.33–1.84)	0.33 (0.01–0.65)

Note: Marked differences compared with '0' and '1' in the Mann–Whitney test. $^{*}p < 0.01$.

The frequency of aberrations in each group according to the total amount of minor alleles is shown in Table 4. In the control group, we observed no significant association of the frequency of cytogenetic damage with the studied genotypes. Furthermore, the distribution of all genotypes was in accordance with the Hardy–Weinberg equilibrium in both groups.

Discussion

Exposure to radiation

Radon is presently recognized as one of the most dangerous environmental carcinogens. Radon is released at a low speed due to the natural decay of the isotope uranium-238. The U-238 isotope is widespread throughout the Earth's crust; the U-238 content is higher in mountainous igneous rock than in sedimentary rock. Radon radiation does not play a significant role in nature because radon is dispersed in a large volume of air and quickly breaks down (half-life = 3.82 days for isotope Rn^{222}). However, residential and industrial buildings can accumulate radon (up to 10-fold excess relative to the open air), which leads to serious radiation exposure for people living or working in such buildings. Areas with high concentrations of radon are often located sporadically, depending on the form of micro-relief, parent rock, defects in design, building materials and other factors. Considering the leading contribution of radon to the mean summary radiation dose, radon exposure is likely to lead to harmful consequences, including some genotoxic effects. Radon is a leading cause of the increased frequency of CA and micronuclei in the exposed group from Tashtagol, especially chromosomal interchanges.

Radon, being electrically neutral, is not itself a potential health threat, but its decay daughter products, ^{218}Po, ^{214}Po, ^{214}Pb, and ^{214}Bi, are electrically charged and can affix themselves to tiny dust or smoke particles in indoor air. These particles can be inhaled into the lung where they may penetrate the epithetlial cells that cover the bronchi and alveoli. These short-lived, unstable decay daughter products (especially ^{218}Po and ^{214}Po) emit alpha particles that can interact with biological tissues in the lungs and induce DNA damage (Bilban and Bilban 2005; Rafique et al. 2010). Inhaled radon in the lung dissolves in the blood-stream and travels to all parts of the body, acquiring equilibrium in tissues according to its

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relative solubility. Therefore, the presence of radon in fat in hemopoietically active marrow provides an enhanced source of a-radiation from radon and its daughter nuclei (Allen et al. 1995). Thus, both mature lymphocytes in the blood stream caused by dissolved radon and blood cells in the step of forming caused by radon deposited in bone marrow fat fraction may be exposed.

The average volume radon activity in the residential areas of the children of the exposed group was 468 ± 77 Bq/m^3 during all investigation. It exceeds the similar parameter for the control group (94 ± 23 Bq/m^3). Gamma background (from natural sources of radiation) in the exposed group was 11–18 microroentgen/h (control group: 12–14 microroentgen/h). The individual effective dose inhalation exposure due to isotopes of radon and its short-lived decay products was ~27 mSv/year. These results were described in detail in our previous article (Druzhinin et al. 2015).

According to recent investigations ionizing radiation induces the clustered DNA damage, among which SSB-complexes with opposed base damage or abasic sites and complex SSB with adjacent base damage and adjacent base damage are presented more than 80% (Georgakilas et al. 2013). Low- and high-LET radiation cause same number of individual DNA lesions per unit of absorbed dose but in a case of high-LET (α-particles) these lesions are distributed within a smaller number of sites (segments of DNA), which implies a higher level of cluster complexity, i.e. the average number of lesions per cluster tends to increase with increasing LET (Georgakilas et al. 2000, 2013).

As one of the form of DSB, caused by ionizing radiation considered clusters of DSB. This form of DNA damage disrupts the continuity of the DNA in the same general way as simpler forms of DSB do. Several DSB in close proximity (DSB clusters), it severely undermines local chromatin stability and thus overall processing in a location- and composition-dependent manner. On the basis of its constitution, this form of damage can also be considered as a form of highly local chromothripsisa – a phenomenon whereby as of yet undefined processes cause extensive local genomic fragmentation (thripsis), which invokes inaccurate rejoining that feeds carcinogenesis (Schipler and Iliakis 2013).

The generation of DSB clusters and their contribution to the adverse effects of ionizing radiation has also been the subject of extensive mathematical modeling (Friedland et al. 2011). Ostashevsky (2000) analyzed in this manner the consequences of chromatin fragmentation and ultimately of cell death. The assumption of the developed model is that DSB generate small and, therefore, unstable DNA fragments (terminal or interstitial) that can be lost from the chromatin context, thus compromising repair of the constituent DSB. The probability that such fragments will be lost from their chromatin context is thought to increase with decreasing fragment length. A more specialized induction of DSB clusters within chromatin loop. The satisfactory fitting achieved under these assumptions of cell survival and DSB repair results suggests that DSB clusters represent a precarious form of DNA damage. Notably, all these models also offer a plausible explanation for the increased biological efficacy of high-LET radiation, as the yields of clustered DSBs are expected to increase, and the length of the associated fragments to decrease with increasing LET. Repair complications from DSB clustering will mainly derive from the instability of the generated DNA fragments, whose loss from the higher-order chromatin context is likely to impair the function of all DSB repair pathways and to cause thus chromosome aberrations (Schipler and Iliakis 2013).

Associations of studied SNP in repair genes with cytogenetic indicators

In our research, an increased frequency trend of the total CA and single fragments was observed in carriers of minor alleles for the *XpD* (rs13181) T/G polymorphism, the *XpG* (rs17655) C/G polymorphism and the *ADPRT* (rs1136410) T/C polymorphism. After adjusting for multiple comparisons, only one result for the *XpD* gene was statistically significant. Furthermore, the combination of minor variants for three SNP (*XpD* Lys751Gln, *XpG* Asp1104His and *ADPRT* Val762Ala) led to a more significant increase in the frequency of single fragments. Our results demonstrate a probable relationship between this minor allelic variant that leads to a decrease in excision repair protein activity and an increase in the frequency of single fragments.

Despite the fact that the frequency of CA in the exposed group was significantly higher than in the control group (Druzhinin et al. 2015), we found no association of genetic features with markers of radiation exposure (chromosome-type exchanges, including dicentric and ring chromosomes) in this study. All of the established tendencies were related to chromatid-type aberrations. This may be due to the limited size of the exposed sample; despite the increasing frequency of chromosomal aberrations, the total number of chromosome-type exchanges may not have been sufficient to detect statistically significant trends. Most SNP in our study were related to genes with broad specificity, which largely eliminates DNA double-strand breaks that can induce the origin of chromatide-type aberrations (single fragments).

These three genes encode excision repair proteins. NER and BER have been studied in detail, and the major components of these processes are DNA glycosylases, AP endonucleases, DNA polymerases and DNA ligases. These repair mechanisms are some of the most important ways to repair nucleotide and base damage. Nucleotide excision repair removes bulkier base modifications including derivatives of nucleotides, such as ethenopurine (formed due the action of vinyl chloride), C8 aminofluorene adducts and other damages formed due to the action of chemical mutagens while base excision repair glycosylases repair small single base lesions.

The *XpD* gene is located on chromosome 19q32.2 and encodes an ATP-independent helicase. Amino acid substitution Lys751Gln (T/G) changes the protein conformation and possibly affects its interaction with the p44 helicase. As a part of the TFIIH complex, *XpD* unwinds the DNA strand, providing endonucleases with access to the damaged area (Coin et al. 1999). Several studies describe the relationship of this SNP with cancer risk. In a meta-study (Mocellin et al. 2009), a significantly increased risk of cutaneous melanoma in carriers of

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the minor variant G/G was described. The result of this substitution may be the significant reduction of the functional activity of the XpD enzyme. Carriers of the minor allele in combination with another SNP (the *XpD* gene Asp312Asn polymorphism) are characterized by a decrease in the ability to repair cyclobutane pyrimidine dimers in skin cells (Hemminki et al. 2001). Research using the DNA comet assay also demonstrated an increase in the frequency of DNA damage in carriers of the G/G genotype (Wlodarczyk and Nowicka 2012).

Investigation in tire production workers (Vodicka et al. 2004) exposed to various xenobiotics, such as polycyclic aromatic hydrocarbons (PAH) containing soot, 1,3-butadiene and other organic compounds, found that the level of CA was lower in the carriers of homozygotes (T/T) compared with the G/G genotype. Our results for the *XpD* gene Lys751Gln polymorphism conform to the data, indicating a decrease repair activity in the carriers of the G/G genotype. Notably, this trend is observed only for single fragments and only in the group exposed to radon, but not in the control group.

Direct effects of ionizing radiation led to ionization of the 2-deoxyribose moieties that can induce DNA strand breaks. Indirect effects led to water radiolysis accompanied by the generation of highly reactive hydroxyl radical (•OH). A strand breaks forming from these types of DNA strand breaks are the origin of radiation-induced formation of 'single fragments'.

The *XpG* gene, located on chromosome 13q33, encodes a protein that contains 1186 amino acids. The protein is an endonuclease that cuts the damaged site at the 3′-end of the DNA chain. *XpG* interacts with the TFIIH complex, which provides helicase binding and the correct unwinding of DNA strands, making them available to the action of endonucleases. Disruption of the interaction of the XpG-TFIIH complex leads to the dissociation of helicase and the breaking of the protein complex, which interrupts the repair process (Ito et al. 2007). The natural variant of the *XpG* gene, the Asp1104His polymorphism (C/G), is located near the COOH-terminal protein domain at the site of interaction with other NER proteins (XpB, XpD, subunits of TFIIH complex) (Wakasugi and Sancar 1999). The COOH-terminal domain of the XpG protein contains many alkaline amino acids and changes the alkaline acid amino to an acid (His – Asp), which can significantly alter its ability to interact with other proteins. The results of a meta-analysis indicate that the replacement may be a risk factor for different types of cancer (He et al. 2014).

The observed increase in the frequency of single chromosome fragments may be the result of reduced effectiveness for the minor variant of the *XpG* gene. This enzyme is involved in the NER, its main function is the elimination of bulky DNA modifications that in some cases can induced double-strand breaks and chromosome aberrations. It is known that NHEJ, HR and other mechanisms play the most important role in the DSB repair. At the same time it has been suggested that NER enzymes can prevent the formation of DSBs by removing bulky lesions and modulate CA frequency. In a number of cases bulky DNA modifications can lead to stalled replication forks that can be a reason of DSB formation (Kifer 2007). DNA damage in the presynthetic period can lead to a formation of DSB after S-phase. Consequently deficiencies in NER lead to increased numbers of cells carrying chromosomal aberrations (Chipchase and Melton 2002). Furthermore, the genotype G/G is associated with the increased frequency of binucleated lymphocytes with micronuclei, as shown in our previous article (Sinitsky et al. 2015).

ADPRT belongs to the family of poly(ADP-ribose) polymerases (PARP1), which (with XRCC1) is a key protein in BER. The *ADPRT* gene, localized on chromosome 1q41-q42, encodes a protein of 113 kDa (Wieler et al. 2003). ADPRT specifically binds to a damaged DNA strand and provides an attachment site for the XRCC1 and Lig3α complex. Moreover, ADPRT is the energy source for DNA ligase. Binding to the broken DNA strand, ADPRT initiates a polymerization reaction of ADP-ribose to form long chains that are required for the modification of nuclear proteins (geteromodification) and the protein ADPRT protein (automodification) (Caldecott 2003).

The *ADPRT* Val762Ala (T/C) polymorphism is associated with increased susceptibility to the development of some forms of cancer. In a meta-analysis performed on the results of 39 studies, Asian carriers of the C/C genotype were shown to exhibit an increased risk of cancer, whereas Caucasians of the same genotype did not (Qin et al. 2014). This SNP is located in the catalytic domain of the protein and is accompanied by the loss of a methyl group. This loss may be the reason for the disruption of the connection of one of the protein loops with the active site of the enzyme, which may cause a reduction in the functional activity of the protein (Cottet et al. 2000). The ADPRT knockout mouse is characterized by high levels of genomic instability, including an increased CA frequency (d'Adda di Fagagna et al. 1999).

The increased single fragment frequency in the carriers of the C/C genotype (the *ADPRT* gene Val762Ala polymorphism), as was shown in our work, coincides with the previously discovered increase in the frequency of binucleated lymphocytes with nucleoplasmic bridges (Sinitsky et al. 2015). This finding may also indicate a decrease in the repair function, expressed as an increase in the frequency of different types of cytogenetic damage (CA and MN).

The frequency of protrusions of the 'bubble' type registered using micronuclear assays in exfoliated cells was lower in carriers of the His/His genotype for the *XpG* Asp1104His polymorphism (C/G, rs17655). These protrusions are the result of eliminations from cell DNA repair complexes; therefore, it is possible that this genotype is associated with the insufficient activity of DNA repair under conditions of radon exposure.

Increasing frequency of cytogenetic markers in radon exposed groups was also shown in another article. Investigation of radiation exposure in radon spa personnel in Czech Republic has shown the increasing frequency of micronucleus-containing lymphocytes in this group in comparison with control. Obtained average dose, calculated on the basis of a percentage of centromere-free micronuclei, for exposed group was around 30 mSv. Micronucleus frequency per 100 binucleate cells was 0.95 ± 0.83 in the spa personnel and 0.50 ± 0.42 in the control (Zolzer et al. 2013).

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Conclusions

The results of this work, as well as the results of our previously studies, confirm the hypothesis of the leading role of high radon concentrations in the formation of cytogenetic abnormalities. In the exposed group (children and teenagers with long-term exposure to radon), we observed a 2-fold increase in the frequency of CA and MN in peripheral blood lymphocytes.

According to the results of our investigation of the polymorphic variants of repair genes, we observed a tendency to increase the total CA frequency and single fragment frequency in the carriers of the *XpD* gene (rs13181) G/G, the *XpG* gene (rs17655) G/G, and the *ADPRT* gene (rs1136410) C/C minor alleles in the exposed group. The *ADPRT* gene (rs1136410) C/C genotype was also a risk factor for the formation of MN and lymphocytes with nucleoplasmic bridges. Furthermore, a minor variant for the *XpG* gene (rs17655) G/G was associated with an increased frequency of single fragments and MN in binucleated lymphocytes.

The relationships with several types of cytogenetic damage suggest that these three SNP (*ADPRT* Val762Ala (T/C, rs1136410), *XpG* Asp1104His (C/G, rs17655) and *XpD* Lys751Gln (T/G, rs13181)) may be considered radiosensitivity markers.

Disclosure statement

The authors report no conflicts of interest. The authors alone are response for the content and writing of the paper.

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