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Association of DNA repair polymorphisms with DNA repair functional outcomes in healthy human subjects

Pavel Vodicka[1,*], Rudolf Stetina[2], Veronika Polakova[1], Elena Tulupova[1], Alessio Naccarati[1], Ludmila Vodickova[1,3], Rajiv Kumar[4,5], Monika Hanova[1], Barbara Pardini[1,6], Jana Slyskova[1], Ludovit Musak[7], Giuseppe De Palma[8], Pavel Soucek[3] and Kari Hemminki[4,5]

[1]Institute of Experimental Medicine, Videnska 1083, 14200, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Purkynje Military Medical Academy, Hradec Kralove, Czech Republic, [3]Center of Occupational Medicine, National Institute of Public Health, Prague, Czech Republic, [4]Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany, [5]Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden, [6]Department of Biology, University of Pisa, Italy, [7]Department of Medical Biology, Jessenius Medical Faculty, Comenius Univesity Martin, Slovak Republic and [8]Laboratory of Industrial Toxicology, Department of Clinical Medicine, Nephrology and Health Sciences, University of Parma, Italy

*To whom correspondence should be addressed. Tel: +420 2 41062694; Fax: +420 2 41062782;
Email: pvodicka@biomed.cas.cz

We investigated association between polymorphisms in DNA repair genes and the capacity to repair DNA damage induced by γ-irradiation and by base oxidation in a healthy population. Irradiation-specific DNA repair rates were significantly decreased in individuals with *XRCC1* Arg399Gln homozygous variant genotype (0.45 ± 0.47 SSB/10^9 Da) than in those with wild-type genotype (1.10 ± 0.70 SSB/10^9 Da, $P = 0.0006$, Mann–Witney *U*-test). The capacity to repair oxidative DNA damage was significantly decreased among individuals with *hOGG1* Ser326Cys homozygous variant genotype (0.37 ± 0.28 SSB/10^9 Da) compared to those with wild-type genotype (0.83 ± 0.79 SSB/10^9 Da, $P = 0.008$, Mann–Witney *U*-test). Investigation of genotype combinations showed that the increasing number of variant alleles for both *XRCC1* Arg399Gln and *APE1* Asn148Glu polymorphisms resulted in a significant decrease of irradiation-specific repair rates ($P = 0.008$, Kruskal–Wallis test). Irradiation-specific DNA repair rates also decreased with increasing number of variant alleles in *XRCC1* Arg399Gln in combination with variant alleles for two other *XRCC1* polymorphisms, Arg194Trp and Arg280His ($P = 0.002$ and $P = 0.005$, respectively; Kruskal–Wallis test). In a binary combination variant alleles of *hOGG1* Ser326Cys and *APE1* Asn148Glu polymorphisms were associated with a significant decrease in the capacity to repair DNA oxidative damage ($P = 0.018$, Kruskal–Wallis test). In summary, *XRCC1* Arg399Gln and *hOGG1* Ser326Cys polymorphisms seem to exert the predominant modulating effect on irradiation-specific DNA repair capacity and the capacity to repair DNA oxidative damage, respectively.

Abbreviations: BER, base excision DNA repair; SSB, single-strand breaks; SNP, single nucleotide polymorphism; PBL, peripheral blood lymphocytes.

Introduction

In the recent years, several studies have investigated polymorphisms in DNA repair genes and their possible links to the risk of various cancers. Sequence variants in DNA repair genes are assumed to modulate DNA repair capacity and, therefore, are associated with the altered cancer risk. As an example, the *hOGG1* Cys/Cys genotype has been associated with an increased lung cancer risk (1). In tobacco-related cancers a protective effect of *XRCC1* Arg194Trp variant allele was shown, while variant allele in *XRCC1* Arg399Gln polymorphism was associated with an increased risk among light smokers only (2). An increased risk of colorectal cancer was recently reported for *XRCC1* Arg399Gln variant allele (3). Statistically significant associations have been found between *XPD* polymorphisms and skin, breast and lung cancers [reviewed by ref. (4)]. Increasing number of studies relating genetic polymorphisms in DNA repair genes and various kinds of cancer in the past 5 years do not provide unambiguous consistent associations, mainly due to low statistical power for detecting a moderate effect, false-positive results, heterogeneity across study populations (5), failure to consider effect modifiers such as environmental exposures (6) and, most importantly, due to the virtually unknown relationship between the genotype and the functional outcome (phenotype) (7).

An analysis of SNPs in 88 DNA repair genes and their functional evaluation, based on the conservation of amino acids among the protein family members, shows that ~30% of variants of DNA repair proteins are likely to affect substantially the protein function. It applies particularly for polymorphisms in *XRCC1* Arg280His and Arg399Gln, and *XRCC3* Thr241Met (8). Susceptibility towards ionizing radiation, as measured by prolonged cell cycle G_2 delay, was determined in relation to *XRCC1* Arg194Trp, Arg399Gln and *APE1* Asn148Glu genotypes. Ionizing radiation sensitivity was significantly affected by amino acid substitution variants in both *XRCC1* and *APE1* genes (9). Using the cytogenetic challenge assay, *XRCC1* 399Gln and *XRCC3* 241Met alleles were associated with significant increase in chromosomal deletions as compared with the corresponding homozygous wild-types. Authors concluded that *XRCC1* 399Gln and *XRCC3* 241Met are significantly defective in base excision repair (BER), while *XPD* 312Asn and *XPD* 751Gln are significantly defective in nucleotide excision repair (NER) (10). Individuals with the wild-type Arg/Arg genotype in *XRCC1* Arg194Trp polymorphism exhibited significantly higher values of chromosomal breaks, as assessed by the mutagen sensitivity assay, than those with variant Trp allele, suggesting a protective effect of this allele. On the other hand, variant Gln allele in *XRCC1* Arg399Gln was significantly associated with an increase in chromosomal breaks per cell. These data are biologically plausible, since codon 399 is located within the *BRCA1* C-terminus

functional domain and codon 194 is in the linker region of the *XRCC1* N-terminal functional domain (11). Three studies using different approaches have found a functional impact of *hOGG1* Ser326Cys polymorphism (12–14), but other studies [reviewed by ref. (1)] did not find any conclusive result for *hOGG1* genetic polymorphisms. *hOGG1* Ser326Cys polymorphism has also been described to affect the glycosylase function due to the localization and phosphorylation status (15). The results of such tests allow a more meaningful choice of genes for association studies, though they are still not sufficient for an accurate prediction for the DNA repair capacity.

In the present report we attempt to investigate associations between DNA repair genetic polymorphisms (*XPD* Lys751Gln, *XPG* Asn1104His, *XPC* Lys939Gln, *XRCC1* Arg194Trp, Arg280His and Arg399Gln, *APE1* Asn148Glu, *hOGG1* Ser326Cys *XRCC3* Thr241Met and *NBS1* Glu185Gln) and individual DNA repair activity in a general healthy population from the Central Europe, assessing *in vitro* the capacity to repair both irradiation-specific induced- and oxidative-induced DNA damage. In the former case, the comet assay (single cell alkaline gel electrophoresis) has been modified to measure the ability of lymphocytes to repair γ-irradiation induced single-strand breaks (SSBs) after 40 min of incubation (16), and in the latter, to measure the ability of a subcellular extract of lymphocytes to carry out the initial incision step of repair on a DNA substrate carrying specific lesions-namely, oxidized bases introduced by visible light in the presence of photosensitizer (17).

Materials and methods

Study population

The study was conducted on 244 healthy individuals (183 men and 61 women, mean age 41.3 ± 11.3 years, 90 individuals were smokers and 154 non-smokers) employed in local administration, medical centers and various branches of plastic industry. The investigated population was recruited in the regions of western Slovakia and eastern Bohemia, which exhibit close similarities in socio-economical conditions. Confounding factors, like X-rays, medical drug treatments, dietary (vitamins intake, particular diets) and lifestyle habits (smoking, alcohol and coffee consumptions) and possible exposure-related effects were recorded in detailed questionnaires and considered in the statistical analyses. Present cohort is representative, ethnically homogenous population and therefore suitable for the determination of relationships between DNA repair genetic polymorphisms and DNA repair rates. Lower number of observations for DNA repair rates, in comparison to that given in Table I, were due to methodological limitations (i.e. successful processing of the fresh material). The study design was approved by the local Ethical Committee and the participants provided their informed consent to be included in the study. The sampling of blood was carried out according to the Helsinki Declaration.

DNA repair polymorphisms

Single nucleotide polymorphisms (SNPs) in genes encoding DNA repair enzymes were determined by a PCR–RFLP based method. PCR products were generated using 50 ng of genomic DNA in 25 μl volume reactions containing 20 mM Tris–HCl, 50 mM KCl, 2.0 mM $MgCl_2$, 0.3 mM each dNTP, 0.3 μM each primer (Table I) and 0.2 U *Taq* DNA polymerase. The temperature conditions for PCR were established as denaturation at 94°C for 30 s, annealing (given in Table I) for 30 s, elongation at 72°C for 30 s and final extension at 72°C for 5 min. The amplified fragments were digested with appropriate restriction endonucleases (Table I) and analyzed. The digested PCR products were resolved on 3% agarose gels containing ethidium bromide and visualized under UV light. The genotype results were regularly confirmed by re-genotyping (10% of samples) and by TaqMan allelic discrimination assay (Assay-on-Demand', Applied Biosystems, Foster City, USA), using Real-Time Gene Amp PCR system on AB 7500 equipment (Applied Biosystems, Foster City, USA). The concordance rate was 100%.

γ-irradiation DNA repair test

Peripheral blood lymphocytes (PBL), isolated using Ficol gradient, were used to test individual DNA repair capacity as described previously (18,19). Briefly, cells embedded in agarose on slides were irradiated with 5 Gy of γ-rays (0.42 Gy/min) and either lysed immediately or incubated at 37°C for 40 min before the lysis. The DNA breaks induced by γ-rays are repaired during the 40 min of incubation period, according to the individual repair capacity. The results (i.e. the amount of repaired SSBs) are calculated as a difference between the initial levels of SSBs, measured immediately after irradiation, and the levels of SSBs detected after 40 min of incubation. The repaired DNA damage is subsequently expressed as SSB/10^9 Da. Consequently, higher values of repaired SSBs reflect higher DNA repair activity. The detailed description of the tentative origin γ-ray-induced DNA

Table I. Details on investigated SNPs in DNA repair genes

Genetic polymorphism	Exon	Primer sequence	Annealing temp. (°C)	Restriction enzyme	Genotype distribution	Frequency of variant allele
Base-excision repair						
XRCC1 Arg194Trp	6	F GCC CCG TCC CAG GTA R AGC CCC AAG ACC CTT TCA CT	63	*Msp*I	CC TC TT 184 30 2	qT = 0.078
XRCC1 Arg280His	9	F TTG ACC CCC AGT GGT GCT R CCC TGA AGG ATC TTC CCC AGC	57	*Rsa*I	GG GA AA 202 13 1	qA = 0.035
XRCC1 Arg399Gln	10	F GCC CCT CAG ATC ACA CCT AAC R CAT TGC CCA GCA CAG GAT AA	65	*Msp*I	GG GA AA 104 112 18	qA = 0.316
hOGG1 Ser326Cys	7	F AGT GGA TTC TCA TTG CCT TCG R GGT GCT TGG GGA ATT TCT TT	59	*Fnu*4HI	CC CG GG 154 75 13	qG = 0.209
APE1 Asn148Glu	5	F CTG TTT CAT TTC TAT AGG CTA R AGG AAC TTG CGA AAG GCT TC	59	*Bfa*I	TT TG GG 88 112 35	qG = 0.387
Nucleotide-excision repair						
XPD Lys751Gln	23	F CCC CTC TCC CTT TCC TCT GTT R GCT GCC TTC TCC TGC GAT TA	60	*Pst*I	AA AC CC 65 138 36	qC = 0.439
XPG Asn1104His	15	F TGG ATT TTT GGG GGA GAC CT R CGG GAG CTT CCT TCA CTG AGT	56	*Hsp*92II	GG GC CC 114 102 11	qC = 0.273
XPC Lys939Gln	15	F GAT GCA GGA GGT GGA CTC TCT R GTA GTG GGG CAG CAG CAA CT	61	*Pvu*II	AA AC CC 83 110 41	qC = 0.410
Double-strand break repair						
XRCC3 Thr241Met	7	F GCT CGC CTG GTG GTC ATC R CTT CCG CAT CCT GGC TAA AAA	59	*Hsp*92II	CC CT TT 71 121 36	qT = 0.423
NBS1 Glu185Gln	5	F GGA TGT AAA CAG CCT CTT G R CAC AGC AAC TAT TAC ATC CT	59	*Hinf*I	GG GC CC 89 122 25	qC = 0.364

damage as well as the calibration and optimization of the repair test have already been described in details elsewhere (17).

Oxidative DNA repair test

The repair capacity of PBL extracts towards repairing 8-oxoguanine was determined as previously described (20). Briefly, isolated lymphocytes from each individual were collected and divided into aliquots and stored in liquid nitrogen at −80°C, until experiment. Before an assay, a frozen aliquot was thawed and washed with 1% Triton X-100 in a lysis buffer (45 mM HEPES, 0.4 M KCl, 1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol, pH 7.8) and the lysate was centrifuged to remove nuclei and cell debris. The supernatant was mixed with a reaction buffer (45 mM HEPES, 0.25 mM EDTA, 2% glycerol, 0.3 mg/ml BSA pH 7.8) and kept on ice until use.

A substrate of HeLa cells (2×10^5 per dish) was prepared and pretreated with 2 ml 0.1 μM phosphosensitizer Ro 19-8022 (Hoffmann-La Roche, Basel, Switzerland) and PBSG, and irradiated with a fluorescent lamp (2 min on ice from a 1000 W tungsten halogen lamp, to induce 8-oxoguanines). HeLa cells were successively washed, removed from dishes by gentle trypsinization and embedded in agarose on slides and placed in lysis solution (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris made to pH 10 with NaOH, and 1% Triton X-100) for 1 h at 4°C. After lysis slides are incubated either with individual PBL extracts or with buffer alone at 37°C for 45 min, followed by electrophoresis and neutralization according to comet assay standard protocol (20).

The results (i.e. the amount of repaired oxidative DNA damage, reflecting the removal of 8-oxoguanines) are calculated as a difference between the levels of SSBs, measured in slides with PBL extract and the levels of SSBs measured in slides with buffer only. The level of SSBs, is expressed as SSB/10^9 Da.

Statistical analyses

Statistical calculations were performed using Statgraphics, version 7 (Manugistics Inc., Cambridge, MA). Hardy–Weinberg equilibrium was tested using the chi-square 'goodness-of-fit' test. The data for both DNA repair assays, given in Tables II–VI, are expressed as mean ± SD. For testing significant differences between groups, specifically Table II, the non-parametrical Mann–Whitney *U*-test was applied. Associations between the combined genotypes and DNA repair rates were tested by Kruskal–Wallis test (as shown in Tables III–VI). Simple linear regression analysis was used to estimate the correlation between confounder and DNA repair rates, whereas multifactorial regression analysis was applied to discern the major influencing factors on the DNA repair rates (i.e. analyzing main confounding factors and DNA repair polymorphisms simultaneously).

For statistical analyses non-smokers as well as males were assigned as '0', while smokers and females as '1', age was calculated as continuous variable. Similarly, for statistical analyses the wild-type genotype was assigned as '0', heterozygous variant allele bearers as '1' and homozygous variant allele bearers as '2'.

Evaluation of DNA repair rates in relation to gene–gene interactions, when three and more polymorphisms were considered, was based on the construction of arbitrary score for variant allele. Wild-type allele was assigned as '0', heterozygous variant allele as '1' and homozygous variant allele as '2'. The above approach does not discriminate all possible genotype combinations (i.e. 27 theoretically possible outcomes in ternary and 81 in quaternary combinations), but takes into account a number of variant alleles in particular combination and the higher score reflects the higher number of variant alleles in either genes.

Results

Genotype distribution

The genotype distributions for individual DNA repair genes are shown in Table I. Allelic frequencies in *XPD* Lys751Gln, *XPG* Asn1104His, *XPC* Lys939Gln, *XRCC1* Arg399Gln, *XRCC3* Thr241Met, *NBS1* Glu185Gln and *APE1* Asn148Glu are in agreement with those earlier described for the central European population (21,22), while allelic frequencies in *hOGG1* Ser326Cys, *XRCC1* Arg194Trp and Arg280His for the same population are shown for the first time (Table I). The genotype distribution for all investigated polymorphisms, except for *XPD* Lys751Gln ($\chi^2 = 7.0$, $P = 0.01$), was in the Hardy–Weinberg equilibrium.

Table II. Irradiation-specific DNA repair rates and oxidative DNA damage repair rates stratified for individual DNA repair polymorphisms

Genotypes	Irradiation-specific DNA repair rates (SSNs/10^9 Da)	Oxidative DNA damage repair rates (SSBs/10^9 Da)
XRCC1 Arg399Gln		
GG	1.10 ± 0.70[a] ($n = 92$)	0.75 ± 0.69 ($n = 88$)
GA	0.76 ± 0.69[a] ($n = 103$)	0.76 ± 0.87 ($n = 95$)
AA	0.45 ± 0.47 ($n = 17$)	0.75 ± 0.41 ($n = 14$)
XRCC1 Arg280His		
GG	0.86 ± 0.74 ($n = 183$)	0.72 ± 0.69 ($n = 169$)
GA	0.90 ± 0.65 ($n = 12$)	0.44 ± 0.49 ($n = 12$)
AA	1.34 ($n = 1$)	0.5 ($n = 1$)
XRCC1 Arg194Trp		
CC	0.88 ± 0.73 ($n = 167$)	0.68 ± 0.63 ($n = 155$)
CT	0.87 ± 0.74 ($n = 27$)	0.77 ± 0.95 ($n = 26$)
TT	0.89 ± 0.72 ($n = 2$)	0 ($n = 1$)
APE1 Asn148Glu		
TT	0.95 ± 0.82 ($n = 75$)	0.79 ± 0.85 ($n = 72$)
TG	0.83 ± 0.65 ($n = 100$)	0.73 ± 0.68 ($n = 96$)
GG	0.86 ± 0.61 ($n = 32$)	0.68 ± 0.69 ($n = 26$)
hOGG1 Ser326Cys		
CC	0.88 ± 0.68 ($n = 143$)	0.83 ± 0.79[a] ($n = 130$)
CG	0.90 ± 0.78 ($n = 63$)	0.61 ± 0.67[a] ($n = 64$)
GG	0.66 ± 0.76 ($n = 12$)	0.37 ± 0.28 ($n = 13$)
XPD Lys751Gln		
AA	0.87 ± 0.63 ($n = 57$)	0.61 ± 0.66 ($n = 55$)
AC	0.86 ± 0.72 ($n = 123$)	0.86 ± 0.84 ($n = 119$)
CC	0.91 ± 0.81 ($n = 35$)	0.52 ± 0.47 ($n = 29$)
XPG Asn1104His		
GG	0.83 ± 0.70 ($n = 98$)	0.79 ± 0.80 ($n = 90$)
GC	0.94 ± 0.73 ($n = 95$)	0.72 ± 0.72 ($n = 93$)
CC	0.83 ± 0.45 ($n = 11$)	0.76 ± 1.01 ($n = 10$)
XPC Lys939Gln		
AA	0.82 ± 0.70 ($n = 77$)	0.86 ± 0.87 ($n = 67$)
AC	0.86 ± 0.65 ($n = 96$)	0.68 ± 0.69 ($n = 94$)
CC	1.00 ± 0.86 ($n = 36$)	0.73 ± 0.72 ($n = 38$)
XRCC3 Thr241Met		
CC	0.89 ± 0.66 ($n = 65$)	0.79 ± 0.69 ($n = 57$)
CT	0.85 ± 0.70 ($n = 105$)	0.76 ± 0.79 ($n = 101$)
TT	0.77 ± 0.74 ($n = 34$)	0.73 ± 0.84 ($n = 35$)
NBS1 Glu185Gln		
GG	0.89 ± 0.60 ($n = 79$)	0.82 ± 0.75 ($n = 73$)
GC	0.81 ± 0.72 ($n = 111$)	0.66 ± 0.69 ($n = 102$)
CC	1.12 ± 0.99 ($n = 23$)	0.68 ± 0.72 ($n = 25$)

The results are presented as mean ± SD.
[a]$P < 0.05$. The comparison between individual genotypes was performed by Mann–Whitney *U*-test.

DNA repair rates and confounders

Both irradiation-specific DNA repair rates and the capacity to repair of oxidative DNA damage were not affected by age and there was no significant difference in both DNA repair rates between men and women. Irradiation-specific DNA repair rates were significantly higher among smokers (1.05 ± 0.81 SSB/10^9 Da) as compared to non-smokers (0.77 ± 0.62 SSB/10^9 Da, $P = 0.014$, Mann–Whitney *U*-test), while the capacity to repair of oxidative DNA damage was not affected by smoking habit. By investigating simultaneous influence of genotypes in DNA repair and recorded confounders (age, sex, exposure status and smoking), irradiation-specific DNA repair rates were mainly affected by polymorphism in *XRCC1* Arg399Gln gene ($t = -4.54$, $P < 0.001$), and also by smoking ($t = 2.92$, $P = 0.004$, $R^2 = 0.132$; multiple regression analysis). Figure 1 shows the lowest irradiation-specific DNA repair rates being associated with homozygous variant *AA XRCC1* Arg399Gln genotype both in smokers and non-smokers, although only in non-smokers the difference in

Table III. Effect of selected binary combinations of SNPs of BER genes on irradiation-specific DNA repair rates (expressed as SSBs/10^9 Da)

	APE1 Asn148Glu genotype			
	TT	TG	GG	χ^2, *P*
(A) *XRCC1* Arg399Gln genotype				
GG	1.14 ± 0.77 (46)	1.00 ± 0.67 (30)	1.10 ± 0.59 (14)	0.77, 0.682
GA	0.81 ± 0.86 (28)	0.78 ± 0.64 (58)	0.68 ± .059 (17)	0.37, 0.831
AA	0.57 ± 0.43 (6)	0.42 ± 0.47 (12)	0.28 ± 0.28 (2)	0.96, 0.619
χ^2, *P*	6.98, 0.03	8.35, 0.015	3.76, 0.154	Overall $\chi^2 = 20.87$, $P = 0.0008$
	XRCC1 Arg280His genotype			
	GG	GA	AA	χ^2, *P*
(B) *XRCC1* Arg399Gln genotype				
GG	1.11 ± 0.73 (74)	0.98 ± 0.80 (7)	1.34 (1)	0.43, 0.807
GA	0.75 ± 0.72 (91)	0.84 ± 0.47 (4)	— (0)	0.20, 0.652
AA	0.44 ± 0.48 (16)	0.60 ± 0.06 (2)	— (0)	0.34, 0.563
χ^2, *P*	18.32, 0.0001	0.94, 0.627	—	Overall $\chi^2 = 14.91$, $P = 0.002$
	XRCC1 Arg194Trp genotype			
	CC	CT	TT	χ^2, *P*
(C) *XRCC1* Arg399Gln genotype				
GG	1.12 ± 0.73 (71)	1.00 ± 0.83 (10)	1.40 (1)	0.68, 0.711
GA	0.74 ± 0.73 (91)	0.81 ± 0.64 (18)	— (0)	0.20, 0.651
AA	0.49 ± 0.47 (16)	0.00 (1)	0.38 (1)	1.22, 0.543
χ^2, *P*	16.90, 0.0001	2.27, 0.321	1.00, 0.317	Overall $\chi^2 = 15.00$, $P = 0.005$

The results are presented as mean ± SD; in parentheses are reported the number of individuals with the particular genotype combination. Comparisons were performed by Kruskal–Wallis test.

comparison to wild-type *GG* genotype was statistically significant.

DNA repair rates and genotype analyses

Irradiation-specific DNA repair rates were significantly decreased in individuals with the homozygous variant (*AA*) in *XRCC1* Arg399Gln than those with the wild-type (*GG*) and heterozygous (*GA*) genotypes, (Mann–Whitney *U*-test: $P = 0.0006$ and $P = 0.002$, respectively; Table II).

We did not observe any significant influence on irradiation-specific DNA repair rates in the *XRCC1* Arg194Trp and Arg280His and *APE1* Asn148Glu polymorphisms. Similarly, no association between the genetic polymorphism in *hOGG1* Ser326Cys and irradiation-specific DNA repair rates was observed (Table II).

Our results did not show any effect on irradiation-specific DNA repair rates by genetic polymorphisms in genes involved either in NER (*XPD* Lys751Gln, *XPG* Asn1104His and *XPC* Lys939Gln) or DNA recombination repair (*XRCC3* Thr241Met and *NBS1* Glu185Gln) (Table II).

Combinations of different polymorphisms in BER genes were investigated in relation to irradiation-specific DNA repair rates. By testing all genotype combinations of *XRCC1* Arg399Gln and *APE1* Asn148Glu the irradiation-specific repair rates significantly decreased with increasing number of variant (*A*) allele in *XRCC1* Arg399Gln, whereas *APE1* Asn148Glu genotype contributed moderately (Kruskal–Wallis test: $\chi^2 = 20.87$, $P = 0.008$, Table III).

A significant decrease in irradiation-specific DNA repair rates was also constantly observed in association with variant allele (*A*) in *XRCC1* Arg399Gln, whereas no contribution of the two other investigated polymorphisms in *XRCC1* gene (Arg280His and Arg194Trp) was observed [Kruskal–Wallis test: $\chi^2 = 14.91$, $P = 0.002$ and $\chi^2 = 15.00$, $P = 0.005$, respectively; (Table III, B and C)].

When *XRCC1* Arg399Gln polymorphism was not taken into consideration, binary genotype combinations in BER genes did not significantly affect the level of irradiation-specific DNA repair rates (data not shown).

Similar results were observed for combinations of 3 and 4 polymorphisms in BER genes assessed using a score system that reflects the number of variant alleles in particular combination (the data are shown in Table V), revealing again that the predominant effect on the irradiation-specific DNA repair rates is associated with variant (*A*) allele in *XRCC1* Arg399Gln, and this significant tendency persists in spite of increasing number of genes analyzed in combination.

The capacities to repair oxidative DNA damage were significantly decreased in individuals with the homozygous variant (*GG*) genotype in *hOGG1* Ser326Cys as compared to those with wild-type (*CC*) and heterozygous (*CG*) genotypes (Mann–Whitney *U*-test: $P = 0.008$ and $P = 0.041$, respectively; Table II).

A significant decrease in the capacity to repair DNA oxidative damage was also associated with combination of variant alleles in *hOGG1* Ser326Cys and *APE1* Asn148Glu, (Kruskal–Wallis test: $\chi^2 = 8.84$, $P = 0.018$, Table IV). As evident from Table IV, the predominant effect is due to the variant *G* allele in *hOGG1* Ser326Cys.

Table IV. Effect of a selected binary combination of SNPs of BER genes on the capacity to repair oxidative DNA damage (expressed as SSBs/10^9 Da)

	APE1 Asn148Glu genotype			
	TT	TG	GG	χ^2, *P*
(A) *hOGG1* Ser326Cys genotype				
CC	0.84 ± 0.83 (48)	0.81 ± 0.75 (59)	0.63 ± 0.46 (19)	0.62, 0.734
CG	0.60 ± 0.78 (22)	0.61 ± 0.54 (30)	0.51 ± 0.39 (8)	0.85, 0.654
GG	0.54 ± 0.20 (5)	0.48 ± 0.36 (6)	0.23 ± 0.23 (2)	1.25, 0.535
χ^2, P	4.43, 0.097	4.10, 0.129	1.80, 0.408	Overall $\chi^2 = 8.84$, $P = 0.018$
	XRCC1 Arg194Trp genotype			
	CC	CT	TT	χ^2, *P*
(B) *hOGG1* Ser326Cys genotype				
CC	0.71 ± 0.59 (95)	0.96 ± 1.00 (20)	0.00(1)	2.96, 0.227
CG	0.66 ± 0.31 (50)	0.35 ± 0.48 (6)	— (0)	1.21, 0.271
GG	0.38 ± 0.31 (10)	0.28 ± 0.21 (2)	— (0)	0.06, 0.830
χ^2, *P*	4.02, 0.094	3.37, 0.186	—	Overall $\chi^2 = 8.85$, $P = 0.045$
	XRCC1 Arg280His genotype			
	GG	GA	AA	χ^2, *P*
(C) *hOGG1* Ser326Cys genotype				
CC	0.78 ± 0.69 (108)	0.26 ± 0.23 (7)	0.50 (1)	6.09, 0.048
CG	0.63 ± 0.71 (51)	0.68 ± 0.69 (5)	— (0)	0.01, 0.920
GG	0.36 ± 0.29 (12)	— (0)	— (0)	—
χ^2, *P*	7.71, 0.021	0.83, 0.370	—	Overall $\chi^2 = 8.13$, $P = 0.017$

The results are presented as mean ± SD; in parentheses are reported the number of individuals with the particular genotype combination. Comparisons were performed by Kruskal–Wallis test.

Binary combinations of polymorphisms in *hOGG1* Ser326Cys and *XRCC1* Arg194Trp, and Arg280His showed that the predominance of the variant *G* allele in *hOGG1* Ser326Cys is associated with the lower capacity to repair DNA oxidative damage (Tables IV and IV).

By testing the effect of all analyzed polymorphisms in BER genes, the capacity to repair DNA oxidative damage decreased with increasing number of variant alleles in *hOGG1* Ser326Cys in combination with the increasing number of variant alleles in the other investigated polymorphisms (Kruskal–Wallis test: $\chi^2 = 11.07$, $P = 0.050$, Table VI).

Discussion

Age-related decrease has been observed for hOGG1 activity in PBL from healthy individuals (13) as well as for irradiation-specific repair rates (16), whereas both irradiation-specific DNA repair rates and the capacity for the repair of oxidative DNA damage were not affected by age in our study. Interestingly, irradiation-specific DNA but not oxidative DNA damage-related DNA repair rates were significantly higher in smokers than non-smokers. In previous studies xenobiotic exposure-related increase in BER capacities has been recorded in individuals occupationally exposed to styrene (16) and xenobiotics in the tire plant, suggesting possible induction of DNA repair (23). Approximately 2-fold higher irradiation-specific DNA repair rates were found in smokers than in non-smokers (23). These findings may have be consequential, since exposure to potentially carcinogenic industrial chemicals as well as to the complex mixture of carcinogens in cigarette smoke seems to result in an increased BER capacity in healthy, cancer-free population. Whether this increase is due to an induction, or to a process of adaptation, remains to be clarified.

Irradiation-specific DNA repair rates were significantly higher among individuals with the wild-type genotype in *XRCC1* Arg399Gln as compared to those with homozygous variant genotype. Because most of the DNA damage induced by γ-irradiation is repaired in a short time (<1 h), the measured DNA repair activity is attributable mainly to the BER pathway (16,18), in agreement with the role of the *XRCC1* gene. An observation of the decreased DNA repair capacity in individuals bearing variant *A* allele in *XRCC1* exon 10 (codon 399) is additionally supported by the cytogenetic challenge assay (10), protein conservation analysis (8) and by increased irradiation sensitivity (9). These data seem to be biologically plausible, as XRCC1 protein acts as a coordinator of single strand break repair proteins in the base excision repair pathway with polymorphic codon 399 located within the *BRCA1* C-terminus functional domain (11). By testing the effect of other genetic polymorphisms in individual genes involved in BER, i.e. *XRCC1* Arg194Trp and Arg280His and *APE1* Asn148Glu, we did not observe any significant influence on irradiation-specific DNA repair rates. Although the highest DNA repair rate was seen in just one individual with homozygous variant genotype in *XRCC1* Arg280His, no conclusion may be drawn on the base of our present study. On the contrary, irradiation hypersensitivity was observed in 135 women with homozygous variant Glu/

Table V. Irradiation-specific DNA repair rates in relation to combinations of SNPs in BER genes[a]

Score	*n*	Irradiation-specific DNA repair rates (SSBs/10^9Da)
(A) *XRCC1* Arg399Gln, Arg280His and Arg194Trp		
0	66	1.14 ± 0.71
1	90	0.77 ± 0.75
2	36	0.72 ± 0.60
3	3	0.40 ± 0.35
4	1	0.38
$\chi^2 = 15.29$, $P = 0.004$		
(B) *XRCC1* Arg399Gln, Arg280His and *APE1* Asn148Glu		
0	37	1.20 ± 0.81
1	51	0.83 ± 0.79
2	75	0.87 ± 0.65
3	33	0.55 ± 0.52
4	2	0.18 ± 0.18
5	1	0.38
$\chi^2 = 16.81$, $P = 0.005$		
(C) *XRCC1* Arg399Gln, Arg194Trp and *APE1* Asn148Glu		
0	33	1.22 ± 0.82
1	55	0.92 ± 0.82
2	71	0.82 ± 0.60
3	28	0.55 ± 0.60
4	7	0.55 ± 0.45
5	1	0.38
$\chi^2 = 14.94$, $P = 0.011$		
(D) *XRCC1* Arg399Gln, Arg280His, Arg194Trp and *APE1* Asn148Glu		
0	29	1.23 ± 0.83
1	52	0.88 ± 0.84
2	71	0.87 ± 0.62
3	34	0.60 ± 0.57
4	7	0.54 ± 0.50
5	2	0.47 ± 0.13
$\chi^2 = 12.77$, $P = 0.026$		

The results are presented as mean ± SD. Comparisons were performed by Kruskal-Wallis test.
[a]Combinations were constructed on the base of an arbitrary score for variant alleles (See Materials and Methods for details). The higher score means the higher number of variant alleles in combined SNPs.

Glu genotype in *APE1* (9). It becomes more apparent in the light of the occurrence of the variant allele in the general population, which slightly exceeds 3%. The functional significance of *XRCC1* Arg280His polymorphism is not yet known (2). The data from the literature indicate that individuals with the wild-type Arg/Arg genotype in *XRCC1* Arg280His exhibit significantly higher chromosomal breaks per cell than those with variant His allele. We did not observe any association of polymorphism in codon 194 in *XRCC1* Arg194Trp, probably due to low occurrence of the variant allele in our studied population (29 individuals with at least one variant allele). The lack of observed effect of *APE1* polymorphism on BER is in agreement with the outcome of computational functional test, which suggested that this SNP is unlikely to exhibit an effect on the protein function (8).

Table VI. The capacity to repair oxidative DNA damage in relation to combinations of SNPs in BER genes[a]

Score	*n*	Oxidative DNA repair rates (SSBs/10^9 Da)
hOGG1 Ser326Cys, *XRCC1* Arg194Trp, Arg280His, Arg399Gln and *APE1* Asn148Glu		
0	14	1.05 ± 0.70
1	45	0.65 ± 0.59
2	58	0.72 ± 0.69
3	41	0.67 ± 0.69
4	17	0.49 ± 0.45
5	4	0.25 ± 0.22
$\chi^2 = 11.07$, $P = 0.050$		

The results are presented as mean ± SD. Comparisons were performed by Kruskal–Wallis test.
[a]Combinations were constructed on the base of an arbitrary score for variant alleles (See Materials and Methods for details). The higher score means the higher number of variant alleles in combined SNPs.

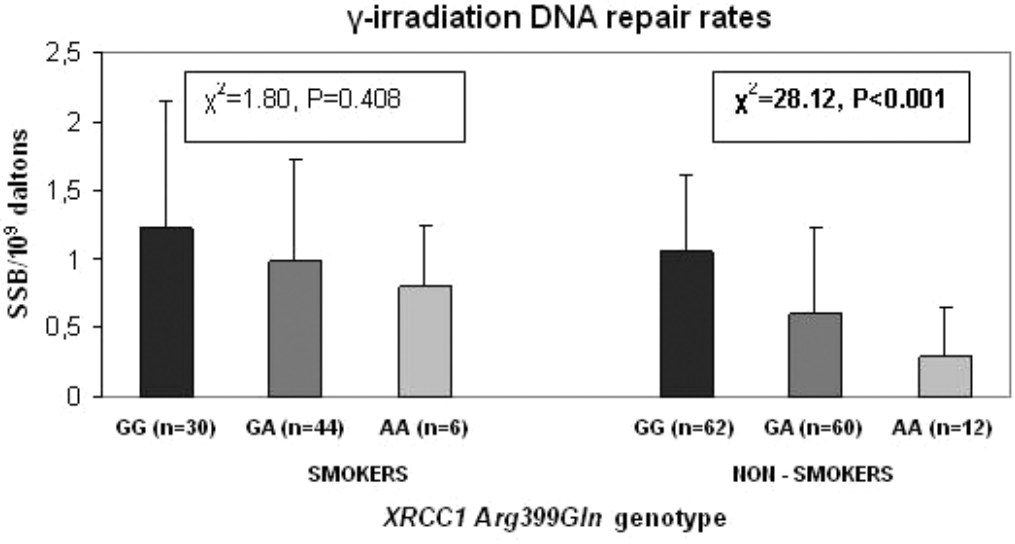

Fig. 1. The γ-irradiation DNA repair rates (expressed as SSB/10^9 Da) in individuals stratified for smoking habit (smokers $n = 80$, non-smokers $n = 134$) and for *XRCC1* Arg399Gln polymorphism. The results are presented as mean ± SD, statistical comparison was performed by Kruskal–Wallis test.

A significant decrease in irradiation-specific DNA repair rates was apparently associated with the binary combination of variant alleles in *XRCC1* Arg399Gln and *APE1* Asn148Glu polymorphisms. Although polymorphism in *APE1* Asn148Glu has no significant effect on irradiation-specific DNA repair, it seems to augment the effect exerted by *XRCC1* Arg399Gln polymorphism. An effect of various BER gene polymorphisms in combination on irradiation-specific DNA repair rates was also tested using an attributed score, reflecting a number of variant alleles in the particular combination, since all existing allele combinations could not be tested due to the low frequency of variant allele, particularly in *XRCC1* Arg194Trp and Arg280His. Apparently, the highest irradiation-specific DNA repair rates were associated with the lowest score, i.e. with the predominance of wild-type alleles in particular combinations. The results suggest that the main effect is due to the *XRCC1* Arg399Gln variant allele.

The capacity to repair oxidative DNA damage was 2-fold higher among individuals with the wild-type genotype (*CC*) in *hOGG1* Ser326Cys as compared to those with homozygous variant genotype. Although the larger functional studies also suggest reduced repair function with variant alleles in *hOGG1* (13,24), the evidence is generally inconclusive. On the other hand, variant *G* allele in *hOGG1* Ser326Cys was suggested to affect the glycosylase function due to the localization and phosphorylation status (15). Our data on the *hOGG1* Ser326Cys polymorphism and the capacity to repair oxidative DNA damage may provide more quantitative data on the decrease of oxidative damage repair in association with the variant allele in the above gene.

A significant decrease in the capacity to repair DNA oxidative damage was also associated with variant alleles in *hOGG1* Ser326Cys and *APE1* Asn148Glu polymorphisms, when this binary gene–gene interaction was investigated. Our data suggest that *APE1* Asn148Glu polymorphism contributes to highlight an effect of variant *G* allele in *hOGG1* Ser326Cys, although *APE1* Asn148Glu polymorphism itself did not influence the oxidative DNA damage repair capacity.

Binary combinations of polymorphisms in *hOGG1* Ser326Cys and *XRCC1* Arg194Trp, and Arg280His showed that the predominance of wild-type *C* allele in *hOGG1* Ser326Cys is associated with the higher capacity to repair DNA oxidative damage. The proper investigation of gene–gene interactions should be based on substantially larger population and the present data should be cautiously interpreted. Additionally, some other polymorphisms, such as those involved in nucleotide excision repair, may modulate levels of DNA damage as well as activity of OGG1 repair enzyme [higher activity was reported to be associated with the wild-type A allele in *XPA* gene, (25)].

By investigating simultaneous influence of genotypes in genes coding for BER enzymes and recorded confounders (age, sex, exposure status and smoking), irradiation-specific DNA repair rates were significantly affected by polymorphism in *XRCC1* Arg399Gln and by smoking. These data suggest the importance of gene–environment interactions and the research in this direction should be continued. Similarly, the capacity to repair DNA oxidative damage was significantly modulated by tentative exposure status and by *hOGG1* Ser326Cys polymorphism. A participation of environmental and occupational exposure factors on both irradiation-specific DNA repair rates as well as on the capacity to repair oxidative DNA damage has been reported earlier, suggesting that the particular DNA repair pathways may be induced by the exposure to xenobiotics (16,23).

An understanding of the relationships between DNA repair polymorphisms and corresponding functional reflections may contribute to the interpretation of results obtained from case–control association studies on various types of cancer. In order to clarify the roles of DNA repair polymorphisms and DNA repair capacities, as important susceptibility factors affecting the onset of cancer, both markers need to be analyzed first in general healthy population (background levels) and subsequently compared with those found in newly diagnosed, untreated cancer patients.

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