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Modulation of DNA damage/DNA repair capacity by *XPC* polymorphisms

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Abstract

XPC, a key protein in the nucleotide excision repair (NER) pathway, recognizes damaged DNA and initiates NER. Genetic variations in the *XPC* gene might be associated with altered DNA repair capacities (DRC). In this study, we genotyped three *XPC* polymorphisms, Ala499Val (C→T), PAT (-/+), and Lys939Gln (A→C), and measured the DNA damage/DRC by alkaline comet assay challenged by BPDE and gamma-radiation in 476 healthy subjects. We also evaluated the associations between DNA damage/DRC and genotypes of *XPC* polymorphisms. Compared with the *XPC* Lys939Gln homozygous wild type (AA) subjects, subjects with the variant alleles (AC and CC) had significantly higher DNA damages induced by BPDE (Median and 95% confidence interval [CI]: 3.16 (3.01-3.44) vs. 2.88 (2.51-3.05), $P=0.01$), and gamma-radiation (4.18(3.94-4.44) vs. 3.71 (3.49-4.04), $P=0.01$). However, subjects with the variant alleles (CT and TT) of Ala499Val exhibited a 8.6 % and 13.1% decrease in DNA damages induced by BPDE ($P=0.05$) and gamma-radiation ($P=0.001$), respectively. Significant correlations were found between genotypes and induced DNA damages in *XPC* Lys939Gln (For BPDE: $R=0.12$, $P=0.01$; for gamma-radiation: $R=0.094$, $P=0.046$) and Ala499Val (For BPDE: $R=-0.11$, $P=0.03$; for gamma-radiation: $R=-0.16$, $P=0.0009$). The haplotypes “T-A” (in the order of Ala499Val-PAT-Lys939Gln) was associated with the lowest DNA damages. Our results suggested that the DRC of host cells might be modulated by specific *XPC* polymorphisms.

Keywords

XPC; DNA repair capacity; comet assay; nucleotide excision repair

Introduction

The xeroderma pigmentosum complementation group C (*XPC*) protein plays a key role in nucleotide excision repair (NER) pathway. The functional DNA binding domains of *XPC*

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interact with HR23B to form a complex that recognizes and binds to the sites of DNA damage [1,2]. Upon binding, NER is initiated through the sequential recruitment of XPA, TFIIH, XPG, and RPA proteins [1]. Deficiency in XPC has been implicated in tumorigenesis. For example, *XPC*-defective mice were highly prone to skin cancer following exposure to UV radiation[3] and also susceptible to common cancers such as lung, esophageal, and bladder cancers when exposed to chemical carcinogens[4].

The *XPC* gene, which encodes a 940-amino acid protein[5-7], spans 33 kb on chromosome 3p25, and contains 16 exons and 15 introns (Genbank accession no. AC090645). More than a hundred polymorphic variants in the *XPC* gene have been identified and the three most common polymorphisms are Ala499Val (C→T), PAT (-/+), and Lys939Gln (A→C), which have been associated with risks of many human malignancies, including cancers of lung, bladder, breast, esophagus, skin, oral cavity, and head and neck[8-16]. The PAT+ polymorphism was associated with increased risks of skin[8,9], head and neck[11], and esophageal cancer [17]. The variant allele of Lys939Gln (A→C) was also associated with increased risks of skin[9], breast[14], and bladder cancers[12,18]. Although the variant allele of Ala499Val was reported to be a risk allele for lung cancer [19], it was found to be protective against cancers of colorectum [16] and endometrium[15]. A potential rationale behind these gene-cancer risk associations is that these genetic variants may result in alterations in phenotypes like DNA repair capacity (DRC).

To date, genotype-phenotype correlation has not been established for most polymorphisms, including those of *XPC* gene. Little is known about their functional impact on DRC. Polymorphisms in the coding and regulatory regions of DNA repair genes may modify gene expression, leading to altered DRC. Hence, study of genotype-phenotype correlation may help to gain more insights into the mechanism underlying the associations between genetic variants and cancer risk.

Comet assay, also known as single cell gel electrophoresis (SCGE), is a well-established functional assay to measure DNA damage at the level of individual cells. It has been widely used to quantify DNA damages in peripheral blood lymphocytes (PBLs) challenged by mutagens such as BPDE, NNK, H₂O₂, and gamma-radiation[20]. Comet assay is also used to evaluate the in vitro host DRC[21]. In this study, to test the hypothesis that three common polymorphisms in *XPC* gene, Ala499Val (C→T), PAT (-/+), and Lys939Gln (A→C), modulate DRC, we determined the DNA damage/repair capacity in PBLs challenged by BPDE and gamma-radiation using alkaline comet assay in 476 healthy subjects. We then examined the effects of genotypes, haplotypes, and diplotypes of the *XPC* gene on host DRC.

Materials and Methods

Population characteristics

The study population consisted of healthy control subjects recruited from an ongoing molecular epidemiologic study of bladder cancer described previously[22]. All subjects were recruited from the Kelsey Seybold Clinic, the largest multi-specialty physician group in the Houston metropolitan area. The subjects were identified by reviewing short survey forms distributed to individuals coming to the clinic for annual health check-ups or for addressing health-related issues. The short survey form elicited willingness to participate in the study. The subjects were subsequently contacted by telephone to confirm their willingness to participate, and an appointment was scheduled at a Kelsey Seybold clinic site convenient to the participant. On the day of the interview, the subjects visited the clinic specifically for the purpose of participating in this study but not for any treatment purposes. All subjects had no prior history of cancer (except for non-melanoma skin cancer). After signing an informed consent form, all participants were interviewed using a structured questionnaire to collect information on

demographic characteristics, smoking history, family history, and occupation history. Immediately after the interview, a blood sample of 40 ml was collected into coded heparinized tubes and delivered to the laboratory for immediate DNA isolation and molecular analysis. The study has been approved by the Institutional Review Boards of both M.D. Anderson and Kelsey Seybold Clinic.

Genotyping

Genotyping for the Lys939Gln and Ala499Val polymorphisms were performed as described previously using the TaqMan assay [23]. PCR-RFLP was conducted to genotype the PAT polymorphism according to a previous protocol with minor modifications[24]. 5% of the samples were randomly selected and run in duplicate with 100% concordance.

Blood culture

Blood cultures were prepared immediately after the samples were delivered to the laboratory. Whole blood (0.4 ml) was cultured in 60-mm×15-mm Petri dishes using 1.6 ml RPMI 1640 (JRM Biosciences, Lenexa, Kansas) with 15% fetal calf serum and 1.25% phytohemagglutinin (Wellcome Research Laboratories, Research Triangle Park, North Carolina) and incubated at 37°C for 96 hours. For each study subject, three separate cultures were established to measure the baseline (untreated), BPDE-induced, and γ -radiation-induced DNA damage using alkaline comet assay. For the BPDE treatment, a final concentration of 2 μ mol/L BPDE [25] was added 24 hours prior to the comet assay. For the γ -radiation treatment, after exposed to 1.5-Gy γ -radiation from a cesium-137 source (cesium irradiator Mark 1, Model 30; J.L. Shepherd and Associates, Glendale, CA) under room temperature, the cultures were placed on ice immediately to minimize DNA repair. The comet assay was performed within 15 minutes after radiation.

Comet assay

To quantify DNA damage/repair capacity, we used a modified alkaline comet assay (pH>13), as originally described by Singh et al[26]. 50 μ L of the blood culture were mixed with 150 μ L of 0.5% low melting point agarose (Life Technologies), dropped, and covered with a glass coverslip on fully-frosted microscope slides precoated with 1% regular agarose. After the mixture was allowed to solidify at 4°C for 10 minutes, a third layer of 0.5% low melting point agarose was placed on top of the cell suspension/low melting point agarose layer on the slide, covered with a new glass coverslip, and placed on a 4°C metal plate for an additional 10 minutes. Embedded peripheral blood lymphocytes (PBLs) were lysed for one hour at 4°C in freshly prepared 1 $\times$ lysis buffer (2.5 mol/L NaCl, 100 mmol/L EDTA, 1% sodium sarcosinate, 10 mmol/L Tris, adjusted to pH 10 with NaOH, and completed with 10% DMSO and 1% Triton prior to use). Following cell lysis, the slides were washed three times in 0.4 mol/L Tris-HCl (pH 7.4) buffer and then placed in a horizontal electrophoresis box without power and filled with freshly prepared alkali buffer (300 mmol/L NaOH, 1mmol/L EDTA, pH >13). The slides were then left at 4°C for 30 minutes to allow for DNA denaturing, unwinding, and exposing of the alkali-labile sites. Electrophoresis was performed using a constant electric current of 295 to 300 mA for 23 minutes at 4°C. After electrophoresis, the slides were neutralized with three 5-minute washes in 0.4 mol/L Tris-HCl (pH 7.4). Finally, the slides were stained with ethidium bromide and stored in a moisture dark box at 4°C. To minimize extraneous DNA damage from ambient ultraviolet radiation, all steps were performed in the dark room. The average inter-assay coefficient variation (CV) for our comet assay was 9.8%. The assays were performed and read by the same individual for this study who was blinded of the case-control status.

Fifty consecutive cells from each sample (twenty-five from each side of one slide) were selected and automatically quantified under a fluorescent microscope at 20 $\times$ objective (Nikon, Melville,

New York) with Komet 4.0.2 software (Kinetic Imaging Ltd., Bromborough, U.K.). The average olive tail moment[27] was calculated for the baseline, BPDE-, and γ -radiation-induced DNA damage in each subject. We used the raw value after mutagen-challenge to calculate DNA damage/repair without subtracting the baseline values.

Statistical Analysis

The Hardy-Weinberg equilibrium for the *XPC* genotypes was tested by a goodness-of-fit χ^2 test. The level of linkage disequilibrium (LD) between polymorphisms was represented using the standardized pairwise disequilibrium coefficient D' [28]. Frequencies of haplotypes and diplotypes (a haplotype pair) were analyzed using the HelixTree Genetics Analysis Software (version 4.1.0, Golden Helix, Bozeman, MT). The non-parametric Mann-Whitney and Kruskal-Wallis test was used to compare the differences in DNA damage/DRC among different genotypes while adjusting for multiple comparisons. Simple Spearman rank-order correlation was used to calculate the correlation between age and genotypes (wild-type genotype was assigned as 0, heterozygote type as 1 and homozygous variant as 2). All the statistical analyses were performed using Stata software 8.0 (Stata Corp., College Station, TX). All statistical tests were two-sided. A P value < 0.05 was considered statistically significant.

Results

Characteristics of study population and distribution of *XPC* polymorphisms

The analyses included 476 healthy subjects. All subjects were Caucasians and 78% were males. The mean age ($\pm$ standard deviation) was 63($\pm$ 11) years. Genotype distribution of all three polymorphisms, Ala499Val, PAT, and Lys939Gln, were in Hardy-Weinberg equilibrium (all values of $P > 0.05$). The PAT polymorphism was in linkage LD with both Lys939Gln and Ala499Val ($D' = 0.968, 0.833$, respectively, both values of $P < 0.001$). Lys939Gln was also in LD with Ala499Val ($D' = 0.863, P < 0.001$). The common haplotypes C-A, T-A, C-C, C+C, and T+C (in the order of Ala499Val-PAT-Lys939Gln) accounted for 95.5% of all haplotypes. The most common haplotype was C-A, in which all three alleles were wild- types. The five most common diplotypes (C-A/C-A, C-A/C-A, C-A/C+C, C-A/T+C, and C+C/C+C) accounted for 77.4% of all diplotypes.

The associations between selected host characteristics (age, gender, and smoking status) and DNA damage/repair capacity are shown in Table 1. The DNA damage/repair capacity induced by both BPDE and γ -radiation were not modified by age or smoking status. However, females exhibited higher γ -radiation-induced DNA damage than males (4.57 vs. 3.94, $P=0.03$).

DNA damage/repair capacity and *XPC* Ala499Val, PAT, and Lys939Gln polymorphisms

BPDE-induced DNA damage was not modified by polymorphisms of the *XPC*-PAT genotype. However, compared to the wild-type genotype (AA) of Lys939Gln, both the heterozygous (AC) and the homozygous variant (CC) genotypes showed significantly higher BPDE-induced damages (3.19 vs. 2.88, $P=0.02$; and 3.06 vs. 2.88, $P=0.04$, respectively) (Table 2). When the heterozygous (AC) and the homozygous variant (CC) genotypes were combined, the BPDE-induced DNA damage was also significantly higher than that of wild type (3.16 vs. 2.88, $P=0.01$) (Table 2). Similarly, subjects carrying at least one variant C allele (AC and CC combined) exhibited significantly higher γ -radiation-induced DNA damage than the wild-type genotype (4.18 vs. 3.71, $P=0.01$) (Table 2). Moreover, significant correlations were found between genotypes of Lys939Gln and DNA damages induced by BPDE ($R=0.12, P=0.01$) and γ -radiation ($R=0.094, P=0.046$). The BPDE-induced DNA damage was 3.15, 2.95 and 2.42 for the wild-type (CC), heterozygous variant (CT) and homozygous variant (TT) of Ala499Val, respectively, with significantly lower damage in homozygous variant (TT) than the wild type (2.42 vs. 3.15, $P=0.01$) (Table 2). Both the heterozygous and the homozygous variant genotypes

of *XPC* Ala499Val conferred significantly lower γ -radiation induced DNA damage than the wild type (3.72 vs. 4.26, $P=0.003$; and 3.47 vs. 4.26, $P=0.04$, respectively) (Table 2). Significant correlations were also identified between genotypes of Ala499Val and DNA damages induced by BPDE ($R=-0.11$, $P=0.03$) and γ -radiation ($R=-0.16$, $P=0.0009$).

DNA damage/repair capacity in *XPC* haplotypes and diplotypes

Table 3 shows the BPDE and γ -radiation induced DNA damages in the common haplotypes of the *XPC* gene. When compared with the most common “C-A” haplotype, the “T-A” haplotype was associated with significantly decreased DNA damage (for BPDE-induced assay, 2.63 vs. 3.15, $P=0.009$; for γ -radiation induced assay, 3.49 vs. 4.02, $P=0.0009$).

Among 10 common diplotypes, the “T-A/T-A” diplotype had the lowest BPDE- and γ -radiation-induced DNA damages (Table 4). However, no significant differences were observed in BPDE-induced DNA damage among the various *XPC* diplotypes. Compared to the “C-A/C-A” diplotype, which contains of all wild-type alleles, the diplotype “C-A/T-A” was associated with significantly lower γ -radiation DNA damage (3.59 vs. 4.28, $P=0.02$) (Table 4).

Discussion

The association between *XPC* polymorphisms and cancer risk has been studied extensively but the results have been inconsistent. A potential rationale behind these gene-cancer risk associations is that these genetic variants may result in alterations in phenotypes (i.e., DRC). In the current study, we evaluated the associations of DNA damage/repair capacities with *XPC* Ala499Val, PAT, and Lys939Gln polymorphisms. In our study, all three polymorphisms were in high level of LD with each other, consistent with previous findings[24,29,30]. We found that the variant alleles of Lys939Gln were associated with reduced DRC, while the variant allele of Ala499Val conferred increased DRC. Furthermore, the haplotype “T-A” exhibited significantly low DNA damage. These results demonstrated that DRC may be modulated by genetic variants of *XPC*. In addition, we also observed differences between *XPC* genotypes in baseline comet assays (data not shown). These differences could possibly reflect the repair of either the cumulative lifetime DNA damage or the oxidative stress-induced damages in the incubation period (96 hours).

Considerable variations in DRC exist in the general population[31] and genetic polymorphisms of DNA repair genes may contribute to these variations[11,32,33]. Polymorphisms in the coding and regulatory regions of *XPC* gene may alter the gene expression and thereby modulate the DNA repair function. Our finding, based on the mutagen-challenged comet assay, indicated that the subjects with variant allele (C) of Lys939Gln had lower DRC as compared to those with wild type allele (T). Consistent with our result, Kuricova et al.[34] found that styrene-exposed workers with the homozygous variant allele of *XPC* Lys939Gln had higher DNA single-strand breaks than those with wild-type allele. Higher frequency of chromosomal aberrations[34,35] was also found in subjects with the variant allele of Lys939Gln when compared with the wild-type. Subjects with the variant allele of Lys939Gln have lower irradiation-specific DRC[35]. To date, four studies[9,12,14,18] have reported that the variant allele of Lys939Gln was associated with an increased risk of malignancies including cancers of bladder, skin and breast. The associations between the variant C allele of Lys939Gln and impaired host DRC observed in our study provide biological plausibility for the increased cancer risk.

We observed lower DRC in the subjects with the variant allele (+) of the PAT polymorphism, although this association did not reach statistical significance. Increased cancer risk in *XPC* PAT+ was observed in three studies on skin[9], head and neck[11], and esophageal cancers

[17]. Qiao et al.[33] studied *XPC* genotype-related DRC using a host-cell reactivation assay and found that healthy subjects with the homozygous variant genotype of the PAT polymorphism (+/+) exhibited lower DRC as compared to wild-type carriers (-/-). In another study, the PAT +/+ genotype was associated with higher levels of anti-BPDE-DNA adducts than those with the -/- genotype in coke oven workers[36].

We also found that subjects with the variant allele (T) of the Ala499Val had higher DRC than those with the wild-type allele (C). Consistent with our results, Shen et al.[37] measured the DRC in healthy subjects using *in vitro*-induced BPDE-DNA adducts assay and also found that subjects with the variant allele (CT or TT) had significantly higher DRC compared with subjects carrying the wild-type CC genotype. There are at least three previous studies indicating a protective effect of the variant allele of the *XPC* Ala499Val polymorphism on cancer risk. We found a decreased risk of bladder cancer in subjects carrying the variant allele of this polymorphism[38]. In an endometrial cancer study, Weiss et al.[15] observed a borderline protective effect of the variant T allele. Huang et al. [16] also reported a protective effect of the variant allele on the risk of advanced colorectal cancer. However, there were also contradictory studies suggesting that the variant allele was associated with an increased cancer risk [19,32]. The evidence of genotype-phenotype correlation in this study indicated that the variant allele of the Ala499Val might be associated with higher DRC and therefore have protective effect on cancer risk.

The haplotype “T-A”, composed of three favorable alleles in terms of conferring better DRC (i.e., the T allele of Ala499Val, the “-” allele of PAT, and the A allele of Lys939Gln), was found to have the lowest DNA damages induced by BPDE and gamma-radiation. The diplotypes that included at least one “T-A” haplotype also conferred lower gamma-radiation-induced DNA damage. In our previous study[38], the haplotype “T-A” was borderline protective against bladder cancer. Weiss et al.[15] found a marginally reduced risk of endometrial cancer in subjects with the variant allele (T) of Ala499Val and the wild-type allele (A) of the Lys939Gln. Huang et al.[16] reported that ever smokers carrying the 499T and 939A alleles exhibited a decreased colorectal cancer risk. Our results of genotype-phenotype correlation provide biological plausibility for these epidemiologic studies.

BPDE does not induce DNA strand breaks directly. However, under our experimental setting using alkaline condition, the BPDE-induced DNA adducts and oxidative damages can be converted into DNA strand breaks that are detectable using the alkaline comet assay[25,39, 40]. Recognition and incision are the rate-limiting steps in NER while rejoining of DNA strand breaks is a rapid process with a halftime of a few minutes in most cell types[41]. DNA breaks are only transiently present in the NER process[42]. In our experiments, after BPDE treatment, the peripheral blood lymphocytes were incubated for an additional 24 hours before the comet assay was performed. During this 24-hour period, cells were continuously removing and repairing BPDE-induced DNA damages. Therefore, the level of DNA breaks in our study after 24-hour incubation should reflect the overall host NER capacity[21,25]. In support of this notion, we previously performed experiments using various XP cells deficient in *XPC*, *XPA*, or *XPB* genes. We found that significant higher level of DNA breaks, as evidenced by the longer olive tail moments in the alkaline comet assay, were present in all XP cells compared with wild-type cell lines after mutagen challenge (data not shown), supporting that a high level of DNA breaks detected using BPDE-challenged comet assay correlated with impaired NER capacity of the host cell.

The major lesions of gamma-radiation treatment are DNA breaks. The rapid component of DNA repair, but not the slow component, occurs within 5 minutes after gamma-radiation [43]. In our experiment, gamma-radiation was performed at room temperature. Cells were then placed on ice blocks to slow DNA repair (mainly to inhibit the slow component of repair),

embedded in agarose, and attached to the slides approximately 15 minutes after gamma-radiation. Under this procedure, the fast component of DSB repair was not inhibited and was unavoidable. Consequently, our gamma-radiation-challenged comet assay reflects the net results of DNA damage and fast repair. There have been some reports that XPC may be involved in repairing DSBs [35,39,40]. Despras et al. recently reported that in XPC-proficient cells, XPC protein is released from nuclear structures after induction of DSBs and long-term XPC silencing reduces DSB repair [40]. *XPC* Lys939Gln was also found to be associated with the gamma-radiation-specific repair rates [35]. Additionally, other NER gene variations, such as *XPD* and *XPG*, have also been associated with the repair of gamma-radiation-induced DNA damage[35,43]. These lines of evidence support our observation that specific *XPC* polymorphisms may be associated with gamma-radiation-induced DNA damage. Further studies are warranted to elucidate the biological mechanisms underlying these associations.

Although our results were consistent with many studies, there are also some discrepant reports. For instance, using a post-UV host cell reactivation assay, Khan et al. found similar DNA repair capacity between the two alleles of the *XPC* Lys939Gln SNP[24]. It has also been reported that a potentially functional variant in the *XPC* intron11 splicing site leads to skipping of exon 12 as well as reduced DNA repair capacity[29]. This SNP is tightly linked to the PAT polymorphism which could explain the findings with regard to PAT. We could not distinguish between effects of our measured polymorphisms and other linked genes or polymorphisms that might be responsible for the functional differences observed. A comprehensive genotyping of all the functional SNPs and tagging SNPs in and surrounding *XPC* genes is necessary to determine the causative variations.

To the best of our knowledge, this is the largest population study on the genotype-phenotype correlations of the three most commonly studied *XPC* polymorphisms. However, the statistical power of the analyses of the low-frequency genotypes, haplotypes, and diplotypes may not be adequate. For example, limited statistical power was achieved when testing the differences between the DNA damages/DRC associated with the diplotypes “C-A/C-A” and “T-A/T-A”. Future studies with larger sample sizes are needed to verify our observations.

In summary, we found that the variant alleles of the *XPC* Lys939Gln polymorphism was associated with lower DRC and the variant allele of Ala499Val with higher DRC. We also observed that carriers of the haplotype “T-A” exhibited higher DRC. These results provide biological plausibility for previous epidemiologic studies linking *XPC* polymorphisms with altered cancer risk. Our results support that genetic variations in DNA repair genes modulate DRC and contribute to cancer etiology.

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Associations of age, gender and smoking status with DNA damage/repair capacity

Table 1

	N	BPDE-induced		γ -radiation-induced	
		Median (95% CI) ^d	<i>P</i> ^b	Median (95% CI) ^d	<i>P</i> ^b
Gender					
Male	339	3.00 (2.77-3.17)		3.94 (3.74-4.10)	
Female	104	3.27 (3.06-3.52)	0.21	4.57 (4.04-5.10)	0.03
Smoking status					
Never	198	3.05 (2.81-3.22)		3.97 (3.76-4.28)	
Ever	244	3.16 (2.92-3.41)	0.28	4.07 (3.77-4.39)	0.36
Age	442	$R^c = -0.07, P^c = 0.15$		$R^c = -0.05, P^c = 0.40$	

^a Confidence interval.

^b Non-parametric Mann-Whitney test, representing the statistical significance of the difference between the values of males and females, or between the values of never smokers and ever smokers.

^c Spearman coefficient of correlation.

Table 2
DNA damage/repair capacity in different genotypes of XPC

	BPDE-induced			γ -radiation-induced		
	N	Median (95% CI ^d)	<i>p</i> ^b	N	Median (95% CI ^d)	<i>p</i> ^b
Intron 9 PAT (-/+) [*]						
-/-	207	3.00 (2.72-3.24)	Ref.	217	3.91 (3.68-4.28)	Ref.
-/+	162	3.21 (3.04-3.46)		177	4.07 (3.89-4.43)	
+/+	60	3.08 (2.82-4.04)		65	4.26 (3.85-4.70)	
		$R^c = 0.08, P^c = 0.12$			$R^c = 0.06, P^c = 0.21$	
-/+ & +/+	222	3.17 (3.03-3.45)	0.13	242	4.17 (3.94-4.42)	0.19
Lys939Gln (A→C) [*]		Baseline			BPDE-induced	
AA	171	2.88 (2.51-3.05)	Ref.	160	3.71 (3.49-4.04)	Ref.
AC	208	3.19 (3.03-3.45)		219	4.17 (3.93-4.57)	
CC	66	3.06 (2.75-4.05)		74	4.22 (3.71-4.47)	
		$R^c = 0.12, P^c = 0.01$			$R^c = 0.094, P^c = 0.046$	
AC&AC	274	3.16 (3.01-3.44)	0.01	293	4.18 (3.94-4.44)	0.01
Ala499Val (C→T) [*]						
CC	242	3.15 (2.98-3.40)	Ref.	259	4.26 (3.99-4.52)	Ref.
CT	155	2.95 (2.60-3.32)		169	3.72 (3.45-3.99)	
TT	20	2.42 (1.93-2.98)		21	3.47 (2.72-4.82)	
		$R^c = -0.11, P^c = 0.03$			$R^c = -0.16, P^c = 0.0009$	
CT&TT	175	2.88 (2.55-3.13)	0.05	190	3.70 (3.45-3.94)	0.001

^a Confidence interval.

^b *p* value of multiple comparison to reference group if overall significance was detected after non-parametric Kruskal-Wallis H test.

^c Spearman coefficient of correlation.

^{*} The minus PAT, A, and C, was the wild-type allele of the PAT, Lys939Gln, and Ala499Val polymorphisms.

Table 3

XPC* haplotypes and DNA damage /repair capacity

	BPDE-induced			γ -radiation		
	N	Median (95% CI ^d)	<i>p</i> ^b	N	Median (95% CI ^d)	<i>p</i> ^b
C-A**	407	3.15 (2.98-3.26)	Ref.	433	4.02 (3.87-4.29)	Ref.
C+C	206	3.24 (3.02-3.68)	0.058	222	4.25 (4.09-4.44)	0.30
T-A	119	2.63 (2.40-2.94)	0.009*	126	3.49 (3.32-3.76)	0.0009*
T+C	58	2.80 (2.25-3.47)	0.84	63	3.90 (3.22-4.80)	0.47
C-C	58	3.02 (2.52-3.48)	0.40	62	3.97 (3.65-4.85)	0.67
Others	36	3.30(3.08-3.77)	0.16	42	4.00(3.57-5.11)	0.45

^a Confidence interval.

^b *p* value of multiple comparison with reference group (C-A) if overall significance was detected after non-parametric Kruskal-Wallis H test.

* The minus PAT, A, and C, was the wild-type allele of the PAT, Lys939Gln, and Ala499Val polymorphisms.

** In the order of Ala499Val (C→T), PAT (-/+), Lys939Gln (A→C)

Table 4

XPC* diplotypes and DNA damage /repair capacity

	BPDE-induced			γ-radiation		
	N	Median (95% CI ^d)	P ^b	N	Median (95% CI ^d)	P ^b
C-A/C-A**	54	3.19 (2.37-3.52)	Ref.	57	4.28 (3.53-4.86)	Ref.
C-A/C+C	90	3.28 (3.08-3.88)	0.18	96	4.24 (3.94-4.63)	0.67
C-A/T-A	79	2.92 (2.40-3.16)	0.45	84	3.59 (3.37-3.91)	0.02
C-A/T+C	58	2.80 (2.25-3.47)	0.68	63	3.90 (3.22-4.80)	0.37
C+C/C+C	57	3.16 (2.79-4.12)	0.31	62	4.25 (3.83-4.65)	0.60
C-A/C-C	41	3.04 (2.44-3.52)	0.88	39	4.58 (3.72-5.29)	0.57
C-A/T-C	18	3.62 (2.84-5.28)	0.07	20	4.29 (3.21-6.09)	0.89
T-A/T-A	20	2.42 (1.93-2.98)	0.10	21	3.47 (2.72-4.82)	0.086
C-A/C+A	13	3.24 (2.82-3.86)	0.61	15	4.01 (3.59-6.14)	0.39
C-C/C-C	8	2.81 (2.26-5.59)	0.58	11	3.71 (3.16-5.41)	0.60
Others	4	2.80 (1.33-3.11)	0.38	6	3.24 (2.58-5.00)	0.93

^a Confidence interval.
^b P value of multiple comparison with reference group (C-A/C-A) if overall significance was detected after non-parametric Kruskal-Wallis H test.
* The minus PAT, A, and C, was the wild-type allele of the PAT, Lys939Gln, and Ala499Val polymorphisms.
** In the order of Ala499Val (C→T), PAT (-/+), Lys939Gln (A→C)