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Research Article

DNA Damage and Nucleotide Excision Repair Capacity in Healthy Individuals

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Interindividual differences in DNA repair capacity (DRC) represent an important source of variability in genome integrity and thus influence health risk. In the last decade, DRC measurement has attracted attention as a potential biomarker in cancer prediction. Aim of the present exploratory study was to characterize the variability in DNA damage and DRC on 100 healthy individuals and to identify biological, lifestyle, or genetic factors modulating these parameters. The ultimate goal was to obtain reference data from cancer-free population, which may constitute background for further investigations on cancer patients. The endogenous DNA damage was measured as a level of DNA single-strand breaks and DRC, specific for nucleotide excision repair (NER), was evaluated using modified comet assay, following the challenge of peripheral blood mononuclear cells with benzo[a]pyrene diolepoxide. Additionally, genetic polymorphisms in NER genes (*XPA*, *XPC*, *XPD*, and *XPG*) were assessed. We have observed a substantial interindividual variability for both examined parameters. DNA damage was significantly affected by gender and alcohol consumption ($P = 0.003$ and $P = 0.012$, respectively), whereas DRC was associated with family history of cancer ($P = 0.012$). The stratification according to common variants in NER genes showed that DNA damage was significantly modulated by the presence of the variant T allele of *XPC* Ala499Val polymorphism ($P = 0.01$), while DRC was modulated by the presence of the A allele of *XPA* G23A polymorphism ($P = 0.048$). Our results indicate the range of endogenous DNA single-strand breaks and capacity of NER in healthy volunteers as well as the role of potentially relevant confounders. Environ. Mol. Mutagen. 52:511–517, 2011.

Key words: BPDE-induced DNA repair capacity; comet assay; interindividual variability; biological and lifestyle characteristics; genetic polymorphism

INTRODUCTION

DNA repair is a crucial mechanism in maintaining genomic stability. Defects in the DNA repair machinery increase cell vulnerability to DNA-damaging agents, accumulation of mutations in the genome, and finally lead to the development of various disorders. The importance of

Abbreviations: BPDE, benzo[a]pyrene diolepoxide, DRC, DNA repair capacity, GM, geometric mean, GSD, geometric standard deviation, MAF, minor allele frequency, NER, nucleotide excision repair, PBMC, peripheral blood mononuclear cells, RT, room temperature, SNPs, single-nucleotide polymorphisms, SSBs, single-strand breaks, TD, tail DNA %.

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DNA repair is clearly illustrated by monogenic DNA repair deficiency syndromes (such as Xeroderma pigmentosum or Cockayne syndrome etc.); however, mutations responsible for these syndromes are very rare. It is currently estimated that ~150 genes are directly involved in the DNA repair mechanisms [Friedberg, 2003; Hakem, 2008], many of them being polymorphic in the human population. This knowledge has stirred up enthusiastic research to determine whether different genotypes are associated with pathological phenotype, including cancer [Naccarati et al., 2007]. Functional consequences of the majority of DNA repair polymorphisms, mainly single-nucleotide polymorphisms (SNPs), have not been fully characterized yet [Goode et al., 2002; Xi et al., 2004].

Besides heritable polymorphisms in DNA repair genes, DNA repair capacity (DRC) represents an additional source of interindividual variability and therefore it has become attractive as a biomarker in human population studies [Benhamou and Sarasin, 2000; Vodicka et al., 2007]. DRC represents a complex marker comprising the sum of several factors such as gene variants, gene expression, stability of gene products, effect of inhibitors/stimulators, lifestyle and environmental factors [Paz-Elizur et al., 2007].

Nucleotide excision repair (NER) is one of the major repair pathways in humans, responsible for the removal of helix-distorting base lesions produced by ultraviolet light (UV) and a variety of chemical agents [Gillet and Scharer, 2006]. At present, scant data are available on background DRC levels in healthy individuals [Gaivao et al., 2009; Wei et al., 2003; Zhu et al., 2008]. More than 10-fold interindividual differences in NER capacity have been reported, even though these data were collected from relatively small study groups (33 and 57 subjects) [Gaivao et al., 2009; Tyson et al., 2009]. Considering the important role of the DNA repair system, such variability may have a substantial influence on individual susceptibility to sporadic forms of cancer, characterized by gene-environment interactions. Thus, the assessment of DRC levels in healthy populations provides the essential background data for a comparison with potentially altered DRC in cancer patients. Cellular DRC can be measured by using several methods, as reviewed by [Benhamou and Sarasin, 2000; Spitz and Bondy, 2010]. One of the most straightforward approaches for evaluating DNA repair in human cells is the use of a challenge assay, i.e., the determination of the kinetics of DNA breaks or enzyme-sensitive sites disappearance during incubation of cells after treatment with specific damage-inducing agent [Dusinska and Collins, 2008].

We have employed a comet-based challenge assay to measure nucleotide excision DRC in peripheral blood mononuclear cells (PBMC), following chemical stress induced by benzo[a]pyrene diolepoxide (BPDE). The aim of this exploratory study was to analyze BPDE-induced DRC as well as endogenous DNA damage in a group of healthy individuals to assess the background variation in the population. Moreover, routinely interviewed biological and lifestyle characteristics and common variants in important NER genes (*XPA, XPC, XPD,* and *XPG*), considered to have a possible impact on DRC phenotype, have been concurrently investigated.

MATERIALS AND METHODS

Study Subjects and Data Collection

The study was conducted on biological material from healthy Caucasian volunteers who live and have been living in the area of Prague (Czech Republic). The condition for inclusion to the study was a healthy status and the absence of any manifest disease. Additionally, these individuals have not been exposed to any potentially genotoxic or carcinogenic agents from other than environmental sources. No other selection criteria have been applied.

Using detailed questionnaires, all volunteers provided information about gender, age, body weight and height (BMI index), occupational and medical history and lifestyle habits, such as smoking, alcohol consumption (intake of alcohol was calculated as a sum of drinks consumed per day, considering that 25 g of alcohol correspond to 4 mL of spirit, or 2 dL of wine, or 0.5 L of beer). The study population consisted of 52 women and 48 men with a mean age ± SD 41.6 ± 17.5 years, and with a range between 21 and 86 years. Twenty-eight individuals were smokers. Characteristics of the study population are shown in Table I. Each person included in the study signed an informed consent. The Ethics Committee of the Institute for Clinical and Experimental Medicine and Faculty Thomayer Hospital (Prague, Czech Republic) provided ethical approval, based on the Helsinki declaration.

Principle of the Assay

Endogenous DNA damage was determined as a basal level of single-strand breaks (SSBs) in DNA of untreated cells analyzed by alkaline comet assay based on routinely used protocol [Collins, 2004]. The reported parameter represents the mean value of five independent experimental points. BPDE-induced DRC was analyzed as a level of intermediate SSBs in DNA of BPDE-treated cells, originated during the repair of BPDE-DNA adducts by NER pathway. The increase in DNA breaks reflects the ability of NER machinery to recognize and incise corresponding adducts from DNA. We have employed a challenge assay, in which isolated PBMC, mitogen-stimulated in culture medium, were treated with BPDE. After challenge, PBMC were cultured in the medium and harvested after 1, 2, and 4 hr of culturing for being processed by comet assay analysis. Concentrations of genotoxic agent as well as repair time intervals were chosen according to our pilot experiments (data not shown) and [Zheng et al., 2005]. BPDE-induced DRC is reflecting the difference between the level of SSBs measured immediately after the treatment with BPDE at time 0, and the maximum level of SSBs detected within 4 hr of culturing. For each experimental point, the background SSBs level of untreated PBMC was subtracted.

PBMC Culture

Totally, 8 mL of peripheral venous blood from each subject were drawn into heparinised tubes, mixed 1:1 with RPMI 1640 medium (HEPES modification, containing 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum, 1.5% phytohemaglutinin and 0.2% penicillin/streptomycin, Sigma-Aldrich), layered over Histopaque-1077 (Sigma-Aldrich) and centrifuged at 320*g* for 40 min at room temperature (RT). Isolated PBMC were counted and checked for cell viability by trypan

TABLE I. Characteristics of the Study Population

Characteristic	Number of individuals
Gender	$n = 100$
Males	48
Females	52
Age	$n = 100$ (years)
Mean ± SD	41.6 ± 17.5
Median (25–75 percentile)	34 (27–56)
Range	21–86
Smoking status	$n = 100$ (%)
Non-smokers + Ex-smokers	72
Current smokers	28
Number of cigarettes/day[a]	$n = 26$ (%)
≤5	38.5
6–10	38.5
>10	23
Alcohol consumption	$n = 100$ (%)
No	36
Yes	64
Alcohol in grams/day[a]	$n = 98$ (%)
<25	67.2
25–49.9	20.3
50–74.9	10.9
75–100	1.6
Body Mass Index[a]	$n = 91$ (%)
Mean ± SD	24.5 ± 4.2
<18.5	4.4
18.5–24.9	60.4
25.0–29.9	28.6
30.0–39.9	6.6
>40	0
Family history of cancer[a]	$n = 99$ (%)
Positive	48.5
Negative	51.5
Place of residence[a]	$n = 95$ (%)
City	71.6
City + Country	12.6
Country	15.8
Education	$n = 100$ (%)
Basic school	7
High school	35
University	58

[a]Information was not provided by all participants.

blue exclusion. When viability was higher than 95%, cells were aliquoted into cultivation tubes with medium ($\sim 10^5$ cells per 5 mL medium). Tubes were incubated at 37°C. After a mitogen-stimulation period of 20 hr, PBMC were processed for further endogenous DNA damage analysis or challenge assay.

BPDE Challenge and Comet Assay

PBMC cultured in medium were treated by adding (+)-anti-Benzo[a]-pyrene-7,8-dihydrodiol-9,10-epoxide (from A. Seidel, BIU, Germany) in a 1 μM concentration for 30 min at 37°C. After treatment, the medium containing BPDE was removed by a centrifugation at 320*g*, 10 min, RT and fresh medium was added to the pellet. PBMC were further cultured at 37°C for up to 4 hr. After that, cells were separated from the medium by centrifugation (320*g*, 10 min, RT), rewashed with PBS, resuspended in low melting point agarose and layered on microscope slides, followed by lysis for ≥1 hr at 4°C (lysis solution: 2.5 M NaCl, 100 mM EDTA and 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10). In the next step, all slides were treated with alkaline buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 20 min. Ongoing electrophoresis was carried out at 25 V, 300 mA for 20 min. All slides were then washed twice with neutralizing buffer (0.4M Tris, pH 7.5). For the scoring, slides were stained with ethidium bromide (0.01 ng/μL, 20 μL per slide) and evaluated with fluorescence microscope (N-400 series, Optika microscopes, Italy) using an image analysis software (Lucia 4.82, Laboratory Imaging, Czech Republic). Data are reported as tail DNA% (TD), which was determined for 50 randomly selected cells from two parallel slides per experimental point.

Genotyping

All subjects were genotyped for six polymorphisms in four NER genes: *XPA, XPC, XPD,* and *XPG*. For genotyping *XPD* Lys751Gln (rs28365048), *XPG* Asn1104His (rs17655) and *XPC* Lys939Gln (rs2228001), PCR-RFLP procedure was carried out using primers and conditions described previously [Vodicka et al., 2007]. For *XPC* Ala499Val (rs2228000), primers and conditions are described in [Hu et al., 2005] and for *XPC*-PAT +/− (poly-AT insertion/deletion of 83 bases in intron 9; GenBank accession number AF076952) in [Shore et al., 2008]. *XPA* G23A (rs1800975) has been analyzed with TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA; Assay-on-demand, SNP genotyping products: C_482935_1). The results were regularly confirmed by random re-genotyping of more than 10% of the samples for each polymorphism and yielded concordant results. The genotypes with ambiguous and/or no results were excluded from the data set. The set of investigated SNPs was not feasible or successful to analyze for all individuals, the final number of observations for each SNP is shown in related table.

Statistical Analysis

DRC net data (background level of SSBs was subtracted at each experimental point) are expressed as geometric mean (GM) ± geometric standard deviation (GSD). The asymmetric distribution of the investigated DNA damage and repair parameters in the study population was normalized by logarithmic transformation. Genotype frequencies for each polymorphism were tested for compliance with Hardy-Weinberg equilibrium. The relationships between variables of interest at the bivariate level were studied by means of T-test, ANOVA and Pearson correlation. The multivariate linear regression was employed to study the simultaneous effect of genotypes, age, gender, smoking habit, alcohol consumption, BMI and family history of cancer on the logarithmically transformed endogenous DNA damage and DRC. All statistical tests were performed at 5% level of statistical significance. The SPSS analytical package version 16.0 (Chicago, IL, USA) and SAS JMP 8 (NC, USA) were employed for all statistical analyses.

RESULTS

Interindividual Variability in the Study Population

We have observed a large interindividual variability of both analyzed parameters within the study group. For endogenous DNA damage, the level of SSBs ranged from 0.3 to 26.5 TD, with GM ± GSD being 7.4 ± 6.5 TD. Individual levels of SSBs, reflecting BPDE-induced DRC, ranged from 0 to 76.0 TD, the GM ± GSD values were 19.2 ± 14.0 TD (Fig. 1).

Relations to Biological and Lifestyle Characteristics

The stratification of endogenous DNA damage for main biological and lifestyle characteristics (Table II) showed a

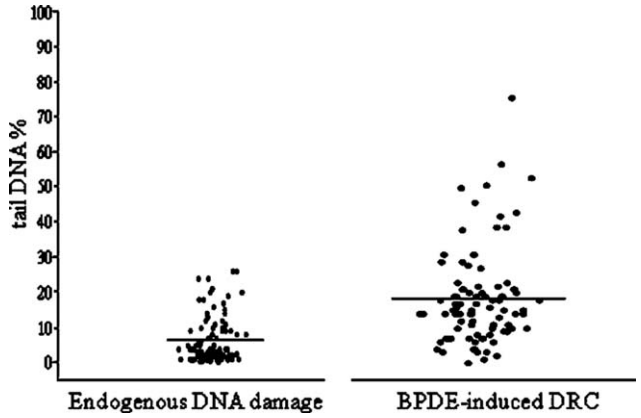

Fig. 1. Interindividual variability of endogenous DNA damage and BPDE-induced DNA repair capacity of 100 individuals. The lines represent geometric mean in the study population.

significant difference between men and women ($P = 0.003$): the GM for DNA damage in women (4.6 ± 5.0 TD) was lower than that in men (8.4 ± 7.2 TD). A significant difference was detected between alcohol consumers and teetotalers (GM 7.8 ± 7.0 TD vs. 4.0 ± 4.6 TD; $P = 0.012$). Moreover, there was a positive correlation between alcohol intake (in grams per day) and DNA damage ($R = 0.225$, $P = 0.023$). Distribution of gender among groups characterized for alcohol consumption was as follows: among alcohol consumers ($N = 64$), 37.5% were women and 62.5% men, whereas among teetotalers ($N = 36$), 77.8% were women and 22.2% men. Gender and alcohol contributed as independent predictors of the outcome, as proved by multivariate regression model. There was no significant difference of DNA damage between smokers and nonsmokers (GM 6.9 ± 6.0 TD vs. 6.3 ± 6.7 TD, respectively) and no association with age ($R = -0.078$, $P = 0.448$).

BPDE-induced DRC was neither affected by age ($R = -0.051$, $P = 0.137$) nor was associated with any of the investigated factors, except for family history of cancer (FHC). Individuals with a positive FHC exhibited higher BPDE-induced DRC than individuals without any cancer in the family (positive FHC: GM 21.8 ± 15.3, negative FHC: 15.1 ± 12.5; $P = 0.012$).

Relations to the Genotype Background

We examined whether endogenous DNA damage and DRC were modulated by any of six polymorphisms in four NER genes: *XPA* G23A, *XPC* Lys939Gln, *XPC* Ala499Val, *XPC*-PAT +/−, *XPD* Lys751Gln and *XPG* Asn1104His (Table III). Considering the size of our study population, SNPs were chosen according to the minor allele frequency (MAF > 0.25), and according to the expected effect on DRC phenotype based on [Friedberg, 2006]. Distribution of the genotypes was in agreement with the Hardy-Weinberg equilibrium. We have observed

TABLE II. Endogenous DNA Damage and BPDE-Induced DNA Repair Capacity Stratified for Biological and Lifestyle Characteristics

	Endogenous DNA damage[a]				BPDE-induced DRC[a]			
Characteristic	*N*	GM	GSD	P^b	*N*	GM	GSD	P^b
Gender								
Women	52	4.6	5.0		52	18.0	14.0	
Men	48	8.4	7.2	0.003	48	18.3	14.5	0.857
Age								
≤34 (below median)	51	7.9	7.7		51	19.8	12.6	
>34 (above median)	49	6.9	7.3	0.422	49	18.5	17.3	0.654
Smoking status								
Smokers	28	6.9	6.0		28	21.3	15.3	
Nonsmokers	72	6.3	6.7	0.445	72	17.0	13.4	0.147
Alcohol consumption								
Consumers	64	7.8	7.0		64	19.2	13.6	
Teetotalers	36	4.0	4.6	0.012	36	16.4	15.0	0.258
Family history of cancer								
Positive	48	7.4	7.0		48	21.8	15.3	
Negative	47	5.7	6.2	0.256	47	15.1	12.5	0.012

[a]Data are expressed as geometric mean (GM) and geometric standard deviation (GSD) of tail DNA% values.
[b]ANOVA for differences in mean values in studied variables.

a significant association between endogenous DNA damage and *XPC* Ala499Val (C→T) polymorphic site. Individuals with variant TT genotype exhibited the highest DNA damage ($P = 0.042$), but even the presence of one copy of the T allele was significantly associated with an increased DNA damage ($P = 0.011$). This finding was also supported by a regression model, where *XPC* Ala499Val significantly affected the DNA damage along with gender ($P = 0.03$ and $P = 0.0007$, respectively). BPDE-induced DRC was associated with *XPA* G23A genotype; the presence of the variant A allele was associated with reduced DRC ($P = 0.048$) (Table III).

DISCUSSION

DRC can be considered as a useful marker for studying the maintenance of DNA integrity in biomonitoring studies, as well as a transient susceptibility marker in carcinogenesis. Several approaches have been conducted to explore DNA repair process in relation to certain diseases, exposure to environmental or occupational pollution or in healthy populations. However, there are still scant data addressing substantial questions, such as how much healthy individuals vary in their repair capacity, what range of variability can still be considered as a normal distribution, and whether the variation is influenced by genetic polymorphisms [Collins and Gaivao, 2007].

In this study, we report an evaluation of DNA damage and NER capacity in 100 healthy individuals, characterized for major biological and lifestyle factors and genetic background. Our experimental protocol, based on a modi-

TABLE III. Endogenous DNA Damage and BPDE-Induced DNA Repair Capacity Stratified for Analyzed NER Gene Polymorphisms

		Frequency		Endogenous DNA damage[a]			BPDE-induced DRC[a]		
Polymorphism	Genotype	*N*	%	GM	GSD	*P*[b]	GM	GSD	*P*[b]
XPD Lys751Gln (*N* = 94)	*AA*	23	24.5	6.5	7	0.546	15.0	10.1	0.373
	AC	48	51	5.5	5.3		18.5	14.8	
	CC	23	24.5	7.9	7.3		22.7	16.1	
	AC+CC	71	75.5	6.3	6.1	0.820	19.8	15.2	0.347
XPG Asn1104His (*N* = 98)	*GG*	54	55	6.9	6.2	0.378	20.5	15.9	0.492
	GC	39	40	5.8	6.6		15.7	11.7	
	CC	5	5	8.8	8.7		14.7	7.8	
	GC+CC	44	45	6.2	6.5	0.476	15.6	11.3	0.239
XPC Lys939Gln (*N* = 98)	*AA*	38	38.8	6.6	6.3	0.538	19.1	14.7	0.893
	AC	46	47	6.9	6.8		18.8	15.3	
	CC	14	14.2	5.3	6.0		14.5	7.6	
	AC+CC	60	61.2	6.5	6.6	0.767	17.8	13.9	0.806
XPC Ala499Val (*N* = 91)	*CC*	57	62.6	5.3	5.2	0.042	16.6	12.4	0.369
	CT	32	35.2	8.6	7.6		22.7	17.4	
	TT	2	2.2	10.0	11.3		16.7	3.6	
	CT+TT	34	37.4	8.7	7.6	0.011	22.3	16.9	0.156
XPC-PAT +/− (*N* = 88)	+/+	48	54.6	7.4	7.1	0.396	19.9	14.9	0.306
	+/−	32	36.4	6.4	5.9		17.7	13.6	
	−/−	8	9	4.0	3.9		11.0	6.3	
	+/− and −/−	40	45.4	7.1	5.6	0.405	16.4	12.7	0.395
XPA G23A (*N* = 98)	*GG*	47	48	6.9	6.8	0.720	19.9	14.0	0.084
	GA	40	41	6.3	6.2		16.6	15.3	
	AA	11	11	6.1	6.6		17.6	10.9	
	GA+AA	51	52	6.2	6.2	0.420	16.8	14.3	0.048

[a]Data are expressed as geometric mean (GM) and geometric standard deviation (GSD) of tail DNA% values.
[b]ANOVA for differences in mean values among studied genotypes.

fied version of the comet assay, introduces some positive aspects. DRC was analyzed from fresh blood, thus the impact of cryopreservation does not interfere with the results, and was examined within an intact cell system. Furthermore, a rather low intraindividual variability in DRC was detected, when assayed for eight individuals sampled twice within six months period ($R = 0.762$, $P = 0.028$).

We have observed a substantial interindividual variability in both examined parameters, in DNA damage as well as DRC. The levels of SSBs that represents NER-mediated breaks originated from BPDE-adducts removal varied from 0 to 76 TD. To our knowledge, there is no study using similar experimental conditions reporting DNA repair variability on sufficiently large population to be confronted with our findings. However, even if some variability may arise from interexperimental discrepancies, observed differences in BPDE-induced DRC may reflect the normal variation in healthy population. In such a case, DNA adducts recognition and removal may be expected to play a serious role in maintaining the homeostasis of the organism.

The mean level of SSBs reported as endogenous DNA damage was 7.4 ± 6.5 TD. By expressing TD value in SSBs/10^6 nucleotides [Collins et al., 1996; Collins et al., 2008], we may assume that healthy individuals in our study group bear 0.1 SSBs/10^6 nucleotides in their DNA (~300 breaks/cell). These breaks, measured by alkaline comet assay, are of different origin and might represent single-strand breaks, alkali-labile DNA adducts, oxidized bases, abasic sites, repair intermediates or breaks associated with replication [Dusinska and Collins, 2008]. Moller [2006] pooled results from 125 studies to assess reference level of SSBs in healthy populations, measured by comet assay in human blood cells, and estimated the median being 8.6 TD. As DNA damage was found to be different according to the geographical latitude, the comparison with data obtained from the Czech population alone is more relevant to our study and is in accordance with those reported (6.5 TD; 0.09 SSBs/10^6 nucleotides; 270 breaks/cell) [Moller, 2006].

In our study, the level of endogenous DNA damage was significantly affected by gender. A lower DNA damage in women, reported also by [Bajpayee et al., 2002; Hofer et al., 2006], can partially be explained by action of estrogens which bind to estrogen receptors and increase the expression of various genes, including those encoding antioxidant enzymes. As a result, mitochondria in females produce fewer reactive oxygen species [Baltgalvis et al., 2010; Strehlow et al., 2003; Vina et al., 2005].

Endogenous DNA damage also positively correlated with alcohol consumption and this is in agreement with previous studies [Weng et al., in press; Zhu et al., 2000]. Interestingly, the levels of DNA damage increased with the increasing quantity of consumed alcohol, as estimated in grams per day. This might be the effect of the highly

reactive, oxygen-containing molecules, generated during alcohol metabolism, e.g., acetaldehyde. Acetaldehyde is a possible human carcinogen, acting through multiple mechanisms, such as induction of DNA damage and interference with DNA replication [IARC, 1999; Seitz and Becker, 2007]. The role of polymorphisms in alcohol metabolizing genes (*ALDH* and *ADH* families) would also be of interest in this context. However, in this study with a limited number of volunteers consuming alcohol, screening of variations in above genes would have inevitably resulted in reduction of statistical power of the outcome. Therefore, study remained focused on genetic variation in NER genes in particular.

The levels of endogenous DNA damage were not affected by smoking habit, as reported also by [Hecht, 1999]. On the contrary, several recent studies reported an effect of smoking on the levels of DNA damage [Hoffmann et al., 2005; Kopjar et al., 2006]. The lack of association in our population could be caused by a low number of strong smokers, whereas sporadic and moderate smokers prevailed.

DRC was not significantly affected by any of the investigated biological and lifestyle factors. However, individuals with cancer family anamnesis exhibited significantly higher DRC. Only scarce and contradictory data are available investigating this parameter along with DNA repair, at present. In contrast with our findings, Li and colleagues [Li et al., 2009] reported lower DRC in individuals with positive FHC, suggesting that this subgroup may in particular be susceptible to cancer. Interestingly, in our study population neither DNA damage, nor DRC were affected by age. Although the influence of age on investigated parameters is often inconclusive, several studies have suggested inverse relation between age and DRC [Moller, 2006; Weng et al., 2009]. Even if the age range in our study population was large, 50% of individuals were 34 years old or younger, and such distribution might have obscured an effect of age on the DRC.

Significant associations have emerged between DNA damage, BPDE-induced DRC and variants in *XPC* and *XPA* genes, both encoding enzymes involved in the preincision complex of NER [Nouspikel, 2009]. In our study, carriers of variant T allele in *XPC* Ala499Val polymorphism exhibited higher DNA damage, but no significant association of this SNP with DRC was observed. However, a more efficient DRC in association with the T allele of this particular polymorphism was found by other groups [Shen et al., 2006; Zhu et al., 2008]. The variant A allele of the *XPA* G23A SNP was related with a reduced DRC and the same observation was reported by other investigators [Langie et al., 2010; Lin et al., 2007; Wu et al., 2003]. Moreover, homozygous AA genotype for this SNP was associated with an increased risk for lung cancer [Butkiewicz et al., 2004; Kiyohara and Yoshimasu, 2007]. All these observations may define the *XPA* 23A allele as a low activity allele. The associations between NER polymorphisms and DRC are still difficult to interpret at present, since few DRC studies have been carried out on sufficiently large populations, enabling detection of subtle (if any) effects of individual SNPs. Besides, different approaches of addressing DRC have been employed so far, hampering a straight comparison of results among studies. The involvement and relevance of genetic variation in NER phenotype remain to be further explored.

CONCLUSIONS

We have observed a relevant variability in response of PBMC to BPDE-induced DNA damage, most likely reflecting interindividual differences in DRC among individuals, which deserves to be further investigated. The level of endogenous DNA damage found in our study population is in agreement with results reported by other laboratories and might be considered as a reference background. Possible modulating effects of biological factors, lifestyle habits and SNPs in NER genes have been addressed. Interestingly, the conformity of available data investigating *XPA* G23A polymorphism along with DRC may determine this particular SNP as a predictor for NER capacity. We postulate that BPDE-induced NER capacity may serve as a useful complex biomarker for providing background data on healthy individuals. This may represent a starting point for assessing DRC in association with cancer risk and, finally, for an estimation of the efficiency of the anticancer therapy and prognosis. In this context, the question whether DRC in surrogate tissue reflects DRC in target tissue should also be addressed.

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