Scientific paper

Genetic Polymorphisms in Base Excision Repair in Healthy Slovenian Population and Their Influence on DNA Damage

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Abstract

Genetic polymorphisms in DNA repair genes may result in altered DNA damage. The aim of our study was to determine the frequencies of common functional single nucleotide polymorphisms (SNPs) in specific base-excision DNA repair (BER) genes in healthy Slovenian population and evaluate their influence on DNA damage established by comet assay. In total 141 unrelated healthy subjects were genotyped for *hOGG1* Ser326Cys, *XRCC1* Arg194Trp and Arg399Gln polymorphisms by real-time TaqMan assay. The frequencies of the *hOGG1* 326Ser/Ser, 326Ser/Cys and 326Cys/Cys genotypes were 63.8, 29.8, and 6.4%, respectively. The frequency distribution of *XRCC1* polymorphism was 88.7% for 194Arg/Arg, 9.2% for 194Arg/Trp, 2.1% for 194Trp/Trp and 46.8% for 399Arg/Arg 40.4% for 399Arg/Gln, 12.8% for 399Gln/Gln. The influence of selected BER polymorphisms on the percentage of comet tail DNA (% TD) was determined in a subgroup of 26 subjects. We found that% TD was significantly increased among individuals with *hOGG1* 326Ser/Cys heterozygous variant genotype as compared to 326Ser/Ser wild-type genotype (% TD = 8.9 ± 4.2 vs. % TD = 6.9 ± 1.4, P = 0.017). No significant associations between *XRCC1* Arg194Trp and Arg399Gln polymorphisms and% TD were found. Our results confirmed that DNA damage is modulated by *hOGG1* Ser326Cys polymorphism.

Keywords: DNA repair genes, base excision repair, genetic polymorphism, comet assay

1. Introduction

The large genomes of mammalian cells are vulnerable to an array of DNA-damaging agents, of both endogenous and environmental origin. If DNA damage is recognized by cell machinery, several responses may occur to prevent replication in the presence of genetic errors and to repair the damage. A deficiency in repair capacity due to the defects in genes involved in DNA repair is a major threat to the genetic integrity of cells and can lead to carcinogenesis. Several pathways of DNA repair exist that recognise and repair specific types of DNA damage and each pathway involves numerous enzymes.[1,2]

Three forms of excision repair are distinguished: mismatch repair (MMR), nucleotide excision repair (NER), and base excision repair (BER).[2] MMR corrects post-replicative errors caused by insufficient proof-reading activity of DNA polymerase.[3] The NER pathway mainly removes bulky adducts caused by environmental agents. One of its most important functions in humans is to repair UV-induced DNA damage.[4] The BER proteins excise and replace damaged bases and some of these enzymes are also involved in single-strand breaks (SSB) repair.[5] Repair of double-strand breaks (DSB) is intrinsically more difficult than other types of DNA damage because no undamaged template is available. Therefore DSB repair pathway utilizes either the non-homologous end-joining or homologous recombination.[6]

Continuous exposure of the cellular DNA to a multitude of agents in our living and working environment can cause damage to cellular macromolecules.[7–10] However, the majority of DNA damage has an endogenous origin and it is mainly due to oxidation of DNA by reactive oxygen species (ROS), which are generated in the cells by normal aerobic metabolism.[11] Oxidative stress may lead to DNA lesions, such as oxidized DNA bases, apurinic/apyrimidinic (AP) sites, SSBs, and DSBs. The BER pathway is the most important mechanism involved in re-

pair of oxidative lesions, as it recognises and repairs base modifications, as well as AP sites and DNA SSBs.[12,13]

The BER pathway is a multi-step process that is initiated with recognition and removal of the altered base by a DNA glycosilase, which catalyses the cleavage of the N-glycosil bond between the modified base and sugar moiety, thus generating an AP site.[14,15] After base excision an AP endonuclease (APE) nicks the phosphodiester backbone 5' to the AP site and also generates 3'-hydroxyl termini at SSBs,[16,17] which is necessary for gap-filling repair synthesis by a DNA polymerase β (POLB).[18] The final step is the sealing of the nicked DNA by DNA ligase I or III.[19]

The human oxoguanine glycosylase 1 (hOGG1) and X-ray repair cross-complementing protein 1 (XRCC1) are key enzymes in the BER pathway, and they may play an important role in maintaining DNA repair capacity (DRC) and preventing cancer occurrence.[20] The hOGG1 directly removes 7,8-dihydro-8-oxoguanine (8-oxoG) from damaged DNA. 8-oxoG is one of the most stable deleterious products of oxidative DNA damage and represents a highly mutagenic miscoding lesion that can lead to G:C to T:A transversion mutations.[21] The XRCC1 protein serves as a scaffold protein for other proteins involved in DNA repair, such as DNA ligase III and POLB.[22,23] It is also involved in SSBs repair through interaction with the poly-ADP-ribose polymerase (PARP).[24]

As single nucleotide polymorphisms (SNPs) in the DNA repair enzymes alter enzymes' activity or its expression, they may modify the inter-individual variability in their DRCs, and can be consequently associated with the altered cancer risk. The aim of our study was to determine the frequencies of polymorphisms in *hOGG1* and *XRCC1* genes in healthy Slovenian population and to evaluate their influence on DNA damage established by comet assay.

2. Experimental

2. 1. Patients

The study population consisted of 141 unrelated healthy Central European Caucasian. A subgroup of 26 subjects was selected randomly and blind to any genotyping data for participation in comet assay. All subjects in the subgroup were asked to refrain of physical activities for at least 2 days before venepuncture. The study was approved by the Slovenian Ethics Committee for Research in Medicine and was carried out according to the Helsinki Declaration. Written informed consent was obtained from all individuals prior to participation.

2. 2. Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using a standard salting-out procedure and a Qiagen FlexiGene kit (Qiagen GmbH, Germany).[25] To determine the *hOGG1* Ser326Cys (rs1052133), *XRCC1* Arg194Trp (rs1799782) and Arg399Gln (rs25487) polymorphisms TaqMan SNP genotyping assays were used (C_3095552_1, C_11463404_10 and C_622564_10, respectively, AB assay, Applied Biosystems, Foster City, California, USA). Real time PCR was performed under universal conditions on ABI 7500 Real-Time PCR System (Applied Biosystems) and results were analysed with its System SDS software version 1.2.3. To ensure the data quality, 20% of samples were re-genotyped and genotypes for each subject were also checked manually.

2. 3. Comet Assay

Comet assay is an alkaline single-cell gel electrophoresis assay that has been optimized to analyse single- and double-stranded DNA damage in human lymphocytes. Cells were isolated from 1 ml of fresh whole blood[26] and separated in two aliquots; one aliquot was used immediately for comet assay and the other was processed for freezing. Cells were mixed with freezing mix (FCS:DMSO = 9:1), aliquoted in 200 µl per tube and slowly frozen in a cell freezing box with iso-propanol and stored at –70 °C. For the comet assay the cells were thawed at room temperature, pelleted by centrifugation at 3300 rpm for 15 min at 20 °C and washed with 1,5 ml of washing mix (RPMI :1 00 mM PBS = 2 : 1) per tube. Cells were again pelleted at 2800 rpm for 10 min at 4 °C and resuspended in 1% Low Melting Point (LMP) agarose.

Comet assay was performed according to the protocol by Tice et al.[27] Two slides were assayed in parallel for each sample and 50 randomly selected cells were analysed per slide within 24 hours after preparation. For visualization on epifluorescence microscope (Olympus CH 50) slides were stained with DAPI (1 µg mL^{-1}). Observations were made using 200 x final magnifications. Microscope was connected to the computer through CCD camera (Hamamatsu Orca 1) and DNA damage was quantified using Comet 5 software (Kinetic Imaging Ltd, 2000, UK). The level of DNA damage was evaluated by two parameters: the percentage of DNA in the tail (% TD) and the Olive Tail Moment (OTM, the product of the amount of DNA in the tail and the mean distance of migration in the tail).

2. 4. Statistical Analysis

To verify that allele frequencies were in Hardy-Weinberg equilibrium a chi-square statistic was used. Multiple linear regression analysis with the adjustment of age and sex was used to determine the influence of studied genetic polymorphisms on % TD. In this analysis logarithmic transformation of non-normally distributed % TD was used and normality of its standardized residuals was evaluated by Kolmogorov-Smirnov test. Arithmetic mean was used to measure central tendency, while standard deviation was a measure of variability. The level of signifi-

cance was set to $P < 0.05$. All statistical analyses were done using SPSS for Windows version 14.0.1 software (Statistical Package for the Social Sciences, Chicago, IL).

3. Results and Discussion

In our study we first determined the frequencies of common functional single nucleotide polymorphisms (SNPs) in specific BER genes in healthy Slovenian population. In total, 141 subjects, 60 male (42.6%) and 81 female (57.4%), median age 23 (range 18–36) years, were analysed for *hOGG1* Ser326Cys, *XRCC1* Arg194Trp and Arg399Gln polymorphisms. The distribution of polymorphism frequencies is summarized in Table 1.

The frequencies of the *hOGG1* 326Ser/Ser, 326Ser/Cys and 326Cys/Cys genotypes in the Slovenian population were 63.8, 29.8, and 6.4%, respectively. The frequency distribution of *XRCC1* polymorphism was 88.7% for 194Arg/Arg, 9.2% for 194Arg/Trp, 2.1% for 194Trp/Trp and 46.8% for 399Arg/Arg 40.4% for 399Arg/Gln, 12.8% for 399Gln/Gln. The genotype distributions for investigated polymorphisms, except for *XRCC1* 194Arg/Trp (P = 0.007), were consistent with Hardy-Weinberg equilibrium.

Table 1 Allele frequencies of *hOGG1* Ser326Cys, *XRCC1* Arg194Trp and Arg399Gln polymorphisms in healthy unrelated subjects (N = 141)

Gene	Genotype	N (%)
hOGG1	326Ser/Ser	90 (63.8)
	326Ser/Cys	42 (29.8)
	326Cys/Cys	9 (6.4)
XRCC1	194Arg/Arg	125 (88.7)
	194Arg/Trp	13 (9.2)
	194Trp/Trp	3 (2.1)
XRCC1	399Arg/Arg	66 (46.8)
	399Arg/Gln	57 (40.4)
	399Gln/Gln	18 (12.8)

Ethnic and inter-individual differences in BER genetic polymorphisms have been observed in various populations. As compared to other studies, we found that the observed genotype frequencies of *hOGG1* Ser326Cys polymorphism were mostly in agreement with values published for other Caucasian populations (Table 2).

The variations in the distribution of *XRCC1* Arg399Gln polymorphism showed higher prevalence of the *XRCC1* 399Arg allele in Slovenian population in comparison to other Caucasian populations (Table 3).

We also observed that *XRCC1* 194 Trp/Trp genotype was slightly but not significantly more frequent in Slovenian population than in other Caucasian population, as other studies mostly found less than 1% of subjects with Trp/Trp genotype (Table 4).

It is important to obtain data for the observed frequencies of these polymorphisms comparable to those available for Caucasian populations, since there was no information on the inter-individual variability of these polymorphisms in the Slovenian population before our study.

Further on, we investigated the influence of SNPs in the genes coding for the BER enzymes on DNA damage

Table 2 The distribution of genotype frequencies for *hOGG1* Ser326Cys polymorphism in different Caucasian populations

Individuals	N	Genotype frequency (%)			References
		Ser326Ser	Ser326Cys	Cys326Cys	
Czech	532	62	34	4	Pardini et al.[28]
Hungarian	149	64	33	3	Sugimura et al.[29]
German	105	57	41	2	Wikman et al.[30]
Irish	247	57	39	4	Ferguson et al.[31]
Italian	146	66	30	4	Coppede et al.[32]
Slovenian	141	64	30	4	present study
Turkish	206	50	41	9	Karahalil et al.[33]

Table 3 The distribution of genotype frequencies for *XRCC1* Arg399Gln polymorphism in different Caucasian populations

Individuals	N	Genotype frequency (%)			References
		Arg399Arg	Arg399Gln	Gln399Gln	
American	549	42	45	13	Applebaum et al.[34]
Czech	532	41	46	14	Pardini et al.[28]
Irish	247	40	39	14	Ferguson et al.[31]
Italian	121	44	50	6	Improta et al.[35]
Polish	124	40	43	18	Kowalski et al.[36]
Slovenian	141	47	40	13	present study

Table 4 The distribution of genotype frequencies for *XRCC1* Arg194Trp polymorphism in different Caucasian populations

Individuals	N	Genotype frequency (%)			References
		Arg194Arg	Arg194Trp	Trp194Trp	
American	549	88	11	1	Applebaum et al.[34]
Czech	532	88	11	1	Pardini et al.[28]
Italian	121	86	14	0	Improta et al.[35]
Polish	124	82	18	0	Kowalski et al.[36]
Slovenian	141	89	9	2	present study

level quantified as % TD and OTM, established by the comet assay in a subgroup of 26 randomly selected subjects, 6 male (23.1%) and 20 female (76.9%), mean age 24 (range 20–29) years. In its basic form the comet assay is a sensitive method for detecting DNA strand breaks. Additionally, the alkaline conditions of the comet assay are sufficient to convert the alkali-labile sites (i.e. AP-sites) to breaks, and thus it is possible to detect the inefficient activity of hOGG1.[37] Consequently, variable DNA content in the comet tail may directly reflect genetic variability in DNA repair genes. For the evaluation of DNA repair by comet assay we used % TD rather than OTM, as % TD is linearly related to the DNA break frequency.[26] Additionally, several studies recommended the use of % TD for regulatory studies.[38,39]

Selected genetic polymorphisms, age and sex were used as predictors in a multivariate analysis of the logarithmically transformed % TD and OTM. We observed that % TD was influenced by age (P = 0.01) but not by sex (P = 0.964). When we assessed the influence of *hOGG1* Ser326Cys and *XRCC1* Arg194Trp and Arg399Gln polymorphisms on % TD and OTM in the subgroup of 26 subjects, we found an association of *hOGG1* Ser326Cys polymorphism with % TD (P = 0.032), but no significant influence of *XRCC1* genotypes on % TD (data not shown). For further statistical analysis we excluded three smokers, 1 male and 2 female, from this subgroup, because some studies observed an effect of smoking on DNA migration in the comet assay.[40,41] The new subgroup consisted of 23 subjects, 5 male (21.7%) and 18 female (78.3%), mean age 23 (range 20–29) years. The influence of the studied polymorphisms on% TD and OTM in this subgroup is presented in Table 5.

In the group of 23 subjects we clearly demonstrated that *hOGG1* Ser326Cys polymorphism has an important impact on DNA damage. We observed that the individuals with the homozygous wild-type *hOGG1* 326Ser/Ser genotype had significantly lower mean % TD values as compared to the subjects with heterozygous variant 326Ser/Cys genotype (% TD = 6.9 ± 1.4 vs. % TD = 8.9 ± 4.2, P = 0.017) (Fig 1a). The *XRCC1* Arg399Gln polymorphism had no significant influence on % TD, although the subjects with *XRCC1* 399Arg/Arg genotype tended to have lower mean % TD values as compared to those with at least one 399Gln allele (% TD = 6.9 ± 1.4 vs. % TD = 7.7 ± 3.0, P = 0.064) (Fig 1b). We observed no significant influence of *hOGG1* Ser326Cys (P = 0.087) and *XRCC1* Arg399Gln (P = 0.087) polymorphism on OTM. Only two subjects with *XRCC1* 194Arg/Trp genotype were detected, so the association of this SNP with % TD and OTM was not assessed.

The interpretation of our results is biologically plausible since there are evidences that all the three polymorphisms have a functional effect. In hOGG1 enzyme, polymorphic amino acid 326Cys is located outside the conserved domain. Nevertheless, recent data showed that the variant hOGG1 326Cys protein has lower ability to prevent mutagenesis by 8-OHdG than hOGG1 326Ser *in vivo.* [42] This polymorphism was described to affect the glycosylase function due to the localization and phosphorylation status.[43] The XRCC1 is a multi-domain protein that interacts with many other proteins involved in DNA repair. It was shown that *XRCC1* Arg194Trp and Arg399Gln polymorphisms have impact on DRC, since amino acid 194 is in the linker region of the XRCC1 N-terminal functional domain and 399 is located within the BRCA1 C-terminus functional domain.[44]

Table 5 The influence of *hOGG1* and *XRCC1* genotypes on the mean % TD and OTM values

Gene	Genotype	N (%)[a]	Mean % TD ± SD	P	Mean OTM ± SD	P
hOGG1	326Ser/Ser	17 (73.9)	6.895 ± 1.392	0.017	0.924 ± 0.295	0.087
	326Ser/Cys	6 (26.1)	8.862 ± 4.220		1.298 ± 1.073	
XRCC1	194Arg/Arg	21 (91.3)	7.566 ± 2.554		1.034 ± 0.620	
	194Arg/Trp	2 (8.7)	5.750 ± 0.863		0.895 ± 0.233	
XRCC1	399Arg/Arg	9 (39.1)	6.896 ± 1.359	0.062	0.863 ± 0.241	0.087
	399Arg/Gln + Gln/Gln	14 (60.9)	7.738 ± 3.020		1.124 ± 0.730	

[a] Genotype frequencies in the subgroup (N = 23)

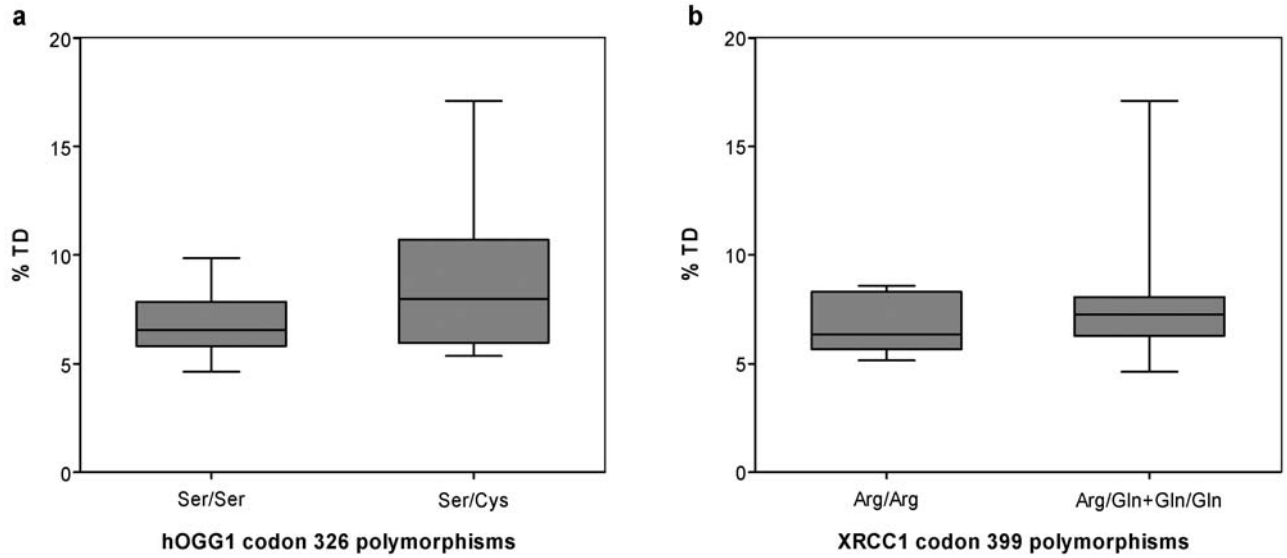

Fig. 1 *hOGG1* Ser326Cys polymorphism and % TD in 23 subjects. Subjects with *hOGG1* 326Ser/Ser genotype had significantly lower mean % TD as compared to subjects with *hOGG1* 326Ser/Cys and/or 326Cys/Cys genotype (a). *XRCC1* Arg399Gln polymorphism and % TD in 23 subjects. The *XRCC1* polymorphism had no significant influence on the % TD, although the subjects with *XRCC1* 399Arg/Arg genotype tended to have lower mean % TD values as compared to those with *XRCC1* 399Arg/Gln + Gln/Gln genotype (b).

Additionally, the observed influences of individual genotypes on DNA damage are in good agreement with other studies. Similar to our study, decreased activity of variant hOGG1 326Cys enzyme has already been established.[20,45] Our results are also consistent with findings that *XRCC1* 399Arg/Arg genotype is associated with reduced DRC[9,46] and increased number of chromosomal breaks per cell.[20] Due to the known biological function of amino acid 194 in XRCC1 protein, an effect of this polymorphism on DNA damage is expected. So far several studies have evaluated the influence of *XRCC1* Arg194Trp polymorphism on DNA integrity, yet their findings are conflicting. Some investigators observed higher values of DRC in the carriers of 194Arg allele,[7] while others found no functional significance of this SNP.[20,46,47] Similarly to our study, low significance of XRCC1 Arg194Trp polymorphism may be a consequence of low frequency of 194Trp allele in Caucasian population.

The main limitation of our study was that it included a relatively small number of individuals, albeit comparable to some other studies.[9,48–50] Another bias was that not all the polymorphic genes involved in the BER were tested. Although studies of polymorphisms in the BER enzymes are mostly focused on the SNPs in the *hOGG1* and *XRCC1* gene, other SNPs in the enzymes of BER pathway, that might have an effect on DRC, were previously studied but not investigated in our study. In addition, our study was not biased by genetic heterogeneity, since all the subjects were recruited in a geographic area with an ethnically homogeneous population.[51]

To our knowledge, no study on the association of genetic polymorphisms in BER pathway with DNA repair in healthy individuals, unexposed to any agents in working environment, has been reported to date. Other similar studies have been performed on individuals, who had been occupationally exposed to various DNA-damaging agents, such as pesticide,[8] styrene,[9] mineral fibres,[52] cobalt, hard metal dust,[49] and polycyclic aromatic hydrocarbons.[10] Although, our study was established on relatively small group of healthy subject we managed to show a major impact of *hOGG1* Ser326Cys polymorphism on % TD. However, further prospective studies with larger sample sizes are required to support our observations.

4. Conclusions

Our results confirmed that DNA damage is modulated by *hOGG1* Ser326Cys polymorphism. This study may thus contribute to the interpretation of the association between functional DNA repair gene polymorphisms and cancer risk, cancer progression and treatment response in Slovenian cancer patients.

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Povzetek

Genetski polimorfizmi mehanizmov za popravljanje DNA lahko vplivajo na stopnjo poškodb DNA. Namen raziskave je bil določiti pogostnost polimorfizmov posameznih nukleotidov (SNP) v izbranih genih mehanizma za popravljanje DNA z izrezovanjem baz (BER) v zdravi slovenski populaciji ter opredeliti njihov vpliv na stopnjo poškodb DNA, ki je bila določena s kometnim testom. Polimorfizme *hOGG1* Ser326Cys, *XRCC1* Arg194Trp in Arg399Gln smo pri 141 nesorodnih zdravih odraslih osebah določali z metodami genotipizacije, ki temeljijo na verižni reakciji s polimerazo (PCR) v realnem času. Frekvence genotipov *hOGG1* 326Ser/Ser, 326Ser/Cys in 326Cys/Cys so bile 63,8 %, 29,8 % in 6,4 %. Porazdelitev genotipov *XRCC1* je bila: 194Arg/Arg 88,7 %, 194Arg/Trp 9,2 %, 194Trp/Trp 2,1 % in 399Arg/Arg 46,8 %, 399Arg/Gln 40,4 %, 399Gln/Gln 12,8 %. Na skupini 26 preiskovancev smo določili vpliv izbranih polimorfizmov BER na odstotek DNA v kometnem repu (% TD). Ugotovili smo, da imajo heterozigoti *hOGG1* 326Ser/Cys statistično značilno višjo vrednost % TD kot osebe z genotipom 326Ser/Ser (% TD = 8.9 ± 4.2 proti % TD = 6.9 ± 1.4, P = 0.017). Polimorfizma *XRCC1* Arg194Trp in Arg399Gln nista imela vpliva na % TD. Rezultati naše raziskave potrjujejo vpliv polimorfizma *hOGG1* Ser326Cys na stopnjo poškodb DNA.