Carcinogenesis, 2015, Vol. 36, No. 11, 1299–1306

doi:10.1093/carcin/bgv127
Advance Access publication September 8, 2015
Original Manuscript

ORIGINAL MANUSCRIPT

Interactions of DNA repair gene variants modulate chromosomal aberrations in healthy subjects

Pavel Vodicka[1,2,3,*], Ludovit Musak[4], Christoph Frank[5], Alena Kazimirova[6], Veronika Vymetalkova[1,2], Magdalena Barancokova[6], Bozena Smolkova[7], Zuzana Dzupinkova[8], Katerina Jiraskova[1,2], Sona Vodenkova[1,2,9], Michal Kroupa[1,10], Oto Osina[4], Alessio Naccarati[1,11], Fabrizio Palitti[12], Asta Försti[5,13], Maria Dusinska[14], Ludmila Vodickova[1,2,3] and Kari Hemminki[5,13]

[1]Department of Molecular Biology of Cancer, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 14220 Prague, Czech Republic, [2]Institute of Biology and Medical Genetics, 1st Faculty of Medicine, Charles University in Prague, 12800 Prague, Czech Republic, [3]Biomedical Centre, Faculty of Medicine in Pilsen, Charles University in Prague, 32300 Pilsen, Czech Republic, [4]Clinic of Occupational Medicine and Toxicology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava and University Hospital in Martin, 03601 Martin, Slovak Republic, [5]Division of Molecular Genetic Epidemiology, German Cancer Research Centre (DKFZ), 69120 Heidelberg, Germany, [6]Faculty of Medicine, Slovak Medical University in Bratislava, 83303 Bratislava, Slovak Republic, [7]Department of Cancer Genetics, Cancer Research Institute of Slovak Academy of Sciences, 83391 Bratislava, Slovak Republic, [8]Molecular Biology Service, Research Institute for Molecular Pathology, 1030 Vienna, Austria, [9]Department of General Biology and Genetics, 3rd Faculty of Medicine, Charles University in Prague, 10000 Prague, Czech Republic, [10]Faculty of Medicine in Pilsen, Charles University in Prague, 30605 Pilsen, Czech Republic, [11]Molecular and Genetic Epidemiology Research Unit, HuGeF–Human Genetics Foundation, 10126 Torino, Italy, [12]Department of Agrobiology and Agrochemistry, University of Tuscia, 011000 Viterbo, Italy, [13]Center for Primary Health Care Research, Lund University, 20506 Malmö, Sweden and [14]Health Effects Laboratory MILK, NILU–Norwegian Institute for Air Research, 2027 Kjeller, Norway

*To whom correspondence should be addressed. Tel: +420 241 062 694; Fax: +420 241 062 782; Email: pvodicka@biomed.cas.cz

Abstract

Human cancers are often associated with numerical and structural chromosomal instability. Structural chromosomal aberrations (CAs) in peripheral blood lymphocytes (PBL) arise as consequences of direct DNA damage or due to replication on a damaged DNA template. In both cases, DNA repair is critical and inter-individual differences in its capacity are probably due to corresponding genetic variations. We investigated functional variants in DNA repair genes (base and nucleotide excision repair, double-strand break repair) in relation to CAs, chromatid-type aberrations (CTAs) and chromosome-type aberrations (CSAs) in healthy individuals. Chromosomal damage was determined by conventional cytogenetic analysis. The genotyping was performed by both restriction fragment length polymorphism and TaqMan allelic discrimination assays. Multivariate logistic regression was applied for testing individual factors on CAs, CTAs and CSAs. Pair-wise genotype interactions of 11 genes were constructed for all possible pairs of single-nucleotide polymorphisms. Analysed individually, we observed significantly lower CTA frequencies in association with *XPD* Lys751Gln homozygous variant genotype [odds ratio (OR) 0.64, 95% confidence interval (CI) 0.48–0.85, $P = 0.004$; $n = 1777$]. A significant association of heterozygous variant genotype in *RAD54L* with increased CSA frequency (OR 1.96, 95% CI 1.01–4.02, $P = 0.03$) was determined in 282 subjects with available genotype. By addressing gene–gene interactions, we discovered 14 interactions significantly modulating CAs, 9 CTAs and 12 CSAs frequencies. Highly significant interactions included always pairs from two different pathways. Although individual variants in genes encoding DNA repair proteins modulate CAs only modestly, several gene–gene interactions in DNA repair genes

Received: June 17, 2015; **Revised:** August 18, 2015; **Accepted:** August 20, 2015

evinced either enhanced or decreased CA frequencies suggesting that CAs accumulation requires complex interplay between different DNA repair pathways.

Abbreviations

BER	base excision repair
CA	chromosomal aberration
CI	confidence interval
CSA	chromosome-type aberration
CTA	chromatid-type aberration
DSB	double-strand break
NER	nucleotide excision repair
OR	odds ratio
PBL	peripheral blood lymphocytes
SNP	single-nucleotide polymorphism

Introduction

Human DNA is constantly exposed to physical (ultraviolet, ionizing radiation) and chemical (reactive oxygen species, alkylating and aralkylating) damaging agents. Efficient DNA repair machinery, comprising several distinct pathways, maintains effectively genomic integrity. Alterations in the DNA repair increase the vulnerability of the cells, resulting in an accumulation of mutations in the genome, which may ultimately lead to tumorigenesis (1). Structural chromosomal aberrations (CAs) arise as a consequence of direct DNA damage (e.g. ionizing radiation, free radicals) or due to replication on a damaged DNA template (2), in both cases DNA repair represents a key player (3). The lesions causing double-strand breaks (DSBs) are mainly responsible for chromosome-type aberrations (CSAs), whereas chromatid-type aberrations (CTAs) arise as a consequence of DNA lesions generated by genotoxic damage during G0 phase, which are insufficiently repaired prior the entering of the cell into S-phase (4). CAs in peripheral blood lymphocytes (PBL) thus reflect inter-individual sensitivity to many genotoxic substances and serve as biomarkers of an early effect of genotoxic carcinogens and carcinogenic risk (5–10).

Human cancers are often associated with chromosomal instability (both numerical and structural CAs) in the cells (11–13); these aberrations are also considered as causative events in malignant transformation (14). Frequencies of CA in PBL are predictive for cancer risk in prospective epidemiological studies (15–17), and patients with many types of cancer show elevated CAs at the time of diagnosis (10,18,19).

Individual DNA repair capacity in response to DNA damage, effectively preventing an accumulation of CAs, is often modulated by the gene variants in different DNA repair pathways (20–22). Indeed, a correlation between gene variants involved in base excision repair (BER) and the corresponding BER repair capacity has been documented (23). However, single-nucleotide polymorphisms (SNPs) in non-coding regions and even changes in wobble bases, which do not affect amino acid sequence, may be important as well (24). The investigation of DNA repair gene variants in association with DNA repair capacity or DNA damage, often performed on small study groups, does not usually bring consistent results, with the exception of the BER gene 8-oxoguanine DNA glycosylase, *OGG1* (23–26) and the nucleotide excision repair (NER) gene *XPA* (27–29). Few reports analysed effects of genetic predispositions on inter-individual variability in CAs by studying variants in genes encoding xenobiotic-metabolizing enzymes, enzymes of DNA repair or folate metabolism and DNA repair capacity (8,30–33). Most recently, by investigating SNPs in metabolic genes, modulations of DNA damage and CAs by gene–gene interactions were reported (34,35). In our recent study, we have described the significant association of rs9344 polymorphism in *Cyclin D1* at a splice site with non-specific CAs in healthy individuals (36). Interestingly, Cyclin D1 participates in DNA DSB repair pathway by binding to RAD51 that is a main recombinase involved in homologous recombination (37).

In the present study, we examined the hypothesis that functionally relevant SNPs in the BER, NER and DSB repair pathways and their gene–gene interactions may modulate frequencies of structural CAs in a large set of healthy subjects.

Materials and methods

Study population

The group of studied subjects (>2100) with measured frequencies of CAs has been very recently described in Hemminki *et al.* (35). The above healthy subjects were recruited between 2002 and 2011 in eastern Bohemia and 1997–2006 in Slovakia and consisted of unexposed controls as well as subjects with defined occupational exposures, such as small organic compounds, cytostatics, anaesthetics, metals, asbestos, mineral fibres and ionizing radiation. Peripheral blood sampling and data collection were carried out simultaneously only in the subjects apparently healthy at the time of sampling. Likewise, individuals with close relatives with any malignant diseases were excluded from the study. Otherwise, no other exclusion criteria were applied. All individuals completed a questionnaire regarding the job category, mode and duration of exposure, various exogenous factors (such as smoking, drug usage, exposure to X-ray radiation, alcohol consumption and dietary habits) prior to blood collection and provided a written consent.

The present study adheres to all principles of the Helsinki Declaration and its design was approved by the Local Ethical Committees of the Jessenius Medical Faculty (Martin, Slovakia) and of the Slovak Medical University (Bratislava, Slovakia).

Cytogenetic analysis

Cytogenetic analysis was performed in PBL stimulated to grow by phytohaemagglutinin and cultured for 48 h. Two scores conducted microscopically analysis (each evaluating half of the 100 mitoses scored per subject) in a double-blind fashion on coded slides. The frequency of CAs and the constituent CTAs and CSAs were evaluated as stated in refs. 6–9,19,32,38. The subjects were classified according to the median of CA distributions into either low-frequency (<2%) or high-frequency (≥2%) groups. Regarding CTAs and CSAs, the cut-off was set up at 1%. These arbitrary cut-off levels were introduced on the basis of the long-term experience with human biological monitoring in the Czech and Slovak Republics (9,10,39).

Genotyping

SNPs in DNA repair genes were taken into the study on the basis of predicted functional effects (SIFT and PolyPhen databases) and relevant published literature. Genotyping of DNA repair gene polymorphisms *XPD* Lys751Gln (rs13181; T > G), *XPG* Asp1104His (rs17655; C > G), *XPC* Lys939Gln (rs2228001; A > C), XPA 5′UTR (rs1800975; G > A), *XRCC1* Arg194Trp (rs1799782; C > T), Arg280His (rs25489; G > A) and Arg399Gln (rs25487G > A), *OGG1* Ser326Cys (rs1052133; C > G), *XRCC2* Arg188His (rs3218536; G > A), *RAD54L* Ala730Ala (rs1048771; C > T) and *XRCC3* Thr241Met (rs861539; C > T) was carried out using primers and conditions described previously (23,40). The amplified fragments were digested with appropriate restriction endonucleases and the digested PCR products resolved on 2% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. Genetic polymorphisms in *APE1* Asn148Glu (rs1130409; G > T) and *NBS1* Glu185Gln (rs1805794; C > G) were analysed using the TaqMan allelic

discrimination assay (Applied Biosystems, Foster City, CA, Assay-on-demand, SNP Genotyping products: C 26470398 10 for *NBS1* and C 8921503 10 for *APE1*). The TaqMan genotyping reaction was amplified on a 7500 Real-Time PCR System (Applied Biosystems) using the following cycling conditions: 95°C for 10 min and 40 cycles at 92°C for 15 s and 60°C for 60 s. The results were regularly confirmed by random re-genotyping of more than 10% of the samples for each polymorphism analysed (40).

Statistical analysis

Statistical calculations have been conducted essentially as recently described in Hemminki *et al.* (35). Briefly, odds ratios (ORs) from the multivariate logistic regression analysis were used to investigate simultaneous effects of occupational exposures and main confounders on the frequencies of CAs, CTAs and CSAs. For each SNP, adjusted ORs were calculated to discern their effect on chromosomal damage. Additionally, all possible pairs of two SNPs were evaluated in binary interaction analysis. In particular, the following genetic models were tested for each pair. 'Three genotypes' assumed the SNP to be a categorical covariate comprising of genotypes AA, AB and BB. For the dominant mode of inheritance, AB and BB were merged as one group, whereas AA and AB together represented the reference group for recessive models. Moreover, genotypes were converted into zero, one or two risk alleles for the log-additive 'allele number' model. Likelihood ratio tests were performed to assess whether including the SNP–SNP interaction term yielded a significantly better fit of the data. If both SNPs significantly interacted with each other for various modes of inheritance for the same pair of SNPs, the model with the lowest Akaike information criterion was chosen. For each best model, the corresponding ORs and the Wald estimates for their confidence intervals (CIs) and *P*-values were calculated. To assess the contribution of all genetic components (both SNPs and interaction term) to the model, likelihood ratio-based *P*-values were computed. Considering multiple comparisons performed in the present study, we have also highlighted significant results after applying Dunn–Bonferroni correction. After correction, the new threshold of *P*-value significance results is 0.004.

Results

Gene polymorphisms in major DNA repair pathways included into the study with respect to their functional relevance are shown in Table 1. Distribution of subjects with high and low CA, CTA and CSA frequencies, also in relation to their main confounders (age, gender, smoking and occupational exposure), is summarized in Table 2. The frequency of individuals with particular CAs, CTAs and CSAs percentage is illustrated in Figure 1a–c. In the whole set, the mean (± standard deviation) frequencies of CAs, CTAs and CSAs were 1.54 ± 1.54%, 0.74 ± 0.98% and 0.80 ± 1.16%, respectively, with median and range being 1 (0–11), 0 (0–6) and 0 (0–10). Chromatid-type exchanges (mean ± standard deviation 0.02 ± 0.14%) were substantially less abundant than chromosome-type exchanges (mean ± standard deviation 0.09 ± 0.35%), which included mainly dicentrics and centric rings. Chromatid-type exchanges occurred in 2% of subjects with CTAs, whereas chromosome-type exchanges were detected in 7.7% of individuals with CSAs. Based on the data from 2196 investigated individuals, CAs as well as the constituent CTAs and CSAs were significantly increased in occupationally exposed subjects (ORs 2.36, 1.73 and 1.64, respectively). Age was only moderately associated with increasing CAs, whereas its association with CSAs was borderline significant (OR 1.07, 95% CI 1.00–1.13, $P = 0.04$). Smoking did not affect significantly frequencies of either CAs or the constituent CTAs and CSAs.

Results for individual genotypes, including the numbers of subjects with the particular allele in relation to the frequency of CAs, are shown in Table 3. By assessing individual DNA repair gene polymorphisms, we observed a strong association between variant GG genotype in rs13181 of *XPD* gene and decreased CTA frequency (OR 0.64, 95% CI 0.48–0.85, $P = 0.004$; $n = 1777$ subjects). This association was strong enough to withstand a correction for multiple testing (*P*-value after correction for multiple comparison is 0.004). The effect of homozygous variant G allele in *XPD* on CTAs is additionally documented in Figure 2, where this allele was associated with the lowest CTA frequencies. Further, a significant association of CT genotype in rs1048771 of *RAD54L* with increased CSAs was also observed (adjusted OR 1.96, 95% CI 1.01–4.02, $P = 0.03$; determined in 282 subjects with available genotype). None of the other studied DNA repair gene variants modulated the frequencies of CAs, CTAs and CSAs.

We performed pair-wise interactions for each of the 11 genes, which were tested for association with CA frequencies. Various genetic models were examined, and if both SNPs significantly interacted with each other for various modes of inheritance for the same pair of SNPs, the model with the lowest Akaike information criterion was chosen. The data based on the interaction term analysis and the likelihood ratio test

Table 1. DNA repair polymorphisms evaluated in this study

Gene	SNP ID	Amino acid substitution	Alleles (major/minor)	Chromosome	Location	MAF (NCBI)	MAF in controls	Function (SIFT, PolyPhen-2 algorithms)
BER								
XRCC1	rs1799782	Arg194Trp	G/A	19q13.2	Missense	0.13	0.06	Deleterious
XRCC1	rs25489	Arg280His	C/T	19q13.2	Missense	0.07	0.04	Possibly deleterious
XRCC1	rs25487	Arg399Gln	A/G	19q13.2	Missense	0.26	0.37	Benign
OGG1	rs1052133	Ser326Cys	C/G	3p26.2	Missense	0.30	0.20	Deleterious
APEX1	rs1130409	Asp148Glu	T/G	14q11.2	Missense	0.38	0.46	Ambiguous
NER								
XPA	rs1800975	—	G/A	9q22.3	5′UTR	0.35	0.34	—
XPD	rs13181	Lys751Gln	T/G	19q13.3	Missense	0.24	0.41	Deleterious
XPG	rs17655	Asp1104His	G/C	13q33	Missense	0.36	0.22	Deleterious
XPC	rs2228001	Lys939Gln	T/G	3p25	Missense	0.32	0.41	Benign
DSB								
XRCC2	rs3218536	Arg188His	C/T	7q36.1	Missense	0.05	0.06	Benign
XRCC3	rs861539	Thr241Met	G/A	14q32.3	Missense	0.22	0.36	Deleterious
NBN	rs1805794	Glu185Gln	C/G	8q21	Missense	0.36	0.33	Benign
RAD54L	rs1048771	Ala730=	C/T	1p32	Synonymous	0.19	0.10	Splicing regulation

MAF, minor allele frequency.

Table 2. Numbers of subjects with high and low frequency of CAs, CSAs and CTAs and their distribution according to basic variables

Variable	Persons	CAs			CSAs			CTAs		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
CAs (high/low)	951/1245									
CTAs (high/low)	1041/1154									
CSAs (high/low)	983/1213									
Age (minimum, maximum, mean)	18, 88, 43	1.06[a]	1.00–1.13	0.07	**1.07**	**1.00–1.13**	**0.04**	0.95	0.90–1.01	0.11
Occupational exposure (exposed/unexposed)	1207/989	**2.36**	**1.97–2.83**	**<0.01**	**1.73**	**1.45–2.06**	**<0.01**	**1.64**	**1.38–1.96**	**<0.01**
Gender (male/female)	1171/1025	1.03	0.86–1.23	0.77	1.05	0.88–1.26	0.59	**0.83**	**0.69–0.99**	**0.04**
Smoking (smokers/non-smokers)	614/1557	1.19	0.97–1.45	0.09	0.95	0.78–1.16	0.63	1.13	0.93–1.38	0.23

Significant results are highlighted in bold.
[a]ORs for age were calculated for 10 years age difference (unit = 10).
P-value was considered significant if it was <0.05.

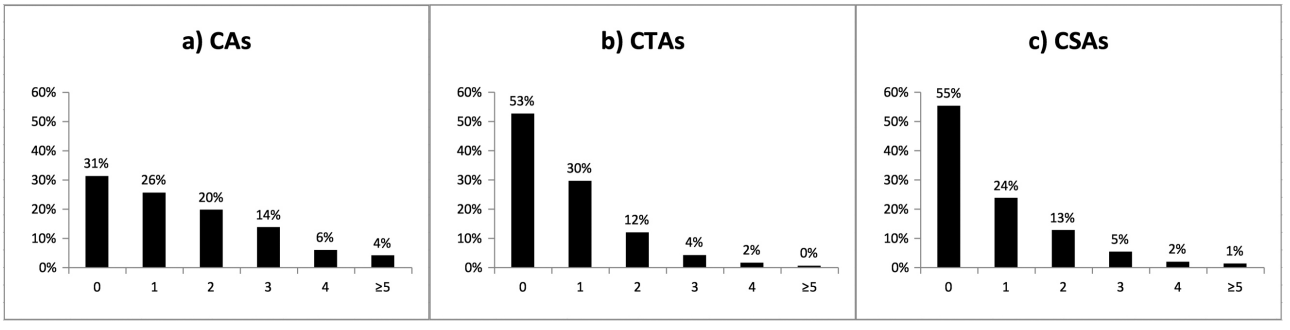

Figure 1. The frequency of individuals with particular percentage of CAs (**a**), CTAs (**b**) and CSAs (**c**). x-axis represents the percentage of chromosomal aberrations, y-axis represents the percentage of subjects with corresponding chromosomal aberrations.

are shown in Table 4. We have discovered several significant interactions, involving gene variants from BER, NER and DSB pathways: 14 interactions modulated CA, 9 CTA and 12 CSA frequencies. The overall genotype effect, considering individual SNPs, their interactions and adjustment variable combinations are shown by the global null hypothesis test. Tests for the global null hypothesis provided mostly highly significant outcomes. The ORs and the significances of the models for each combination of genotypes are showed in Supplementary Material, available at *Carcinogenesis* Online, and partially presented in Table 4.

For CAs, significant gene–gene interactions were observed mainly for genes participating in BER (*APE1*, *hOGG1*), NER (*XPC*, *XPD*) and DSB repair (*XRCC3*) together with (*NBS1*, *XRCC2* and *XPG1*). Rs1805794 in *NBS1* gene appeared most often in these interactions; although interactions with BER gene variants resulted in the higher CA frequency, opposite effect was recorded for the interactions with NER variants. Individuals both with homozygous variant GG and GG genotypes in *OGG1* and *XPG* genes, respectively, resulted in significant decrease of CAs (OR 0.59, 95% CI 0.37–0.96, P = 0.03).

For CTAs, the combinations of homozygous variant genotypes of *XPD* and *XPG* (OR 0.54) and/or *XRCC1* (OR 0.68) genes showed decreased frequencies of CTAs. So did the combination of variant alleles in *OGG1* and *XRCC3* (OR 0.52). On the contrary, a combination of variant alleles in DSB repair genes (*XRCC3* and *XRCC2*) resulted in the significant increase of OR to 2.56.

For CSAs, again variant alleles in *XRCC1* and *OGG1* (both BER genes) in combination with homozygous variant genotype in *XPG* resulted in significantly decreased frequencies of CSAs (OR 0.22 and 0.72, respectively).

Discussion

The onset and development of human cancer are associated with genome instability (41,42), resulting in both numerical and structural chromosomal abnormalities in cancer cells (11–13). CAs in PBL have been employed as biomarkers reflecting individual sensitivity to exogenous and endogenous genotoxic substances for many years (4,5). There are several reports pointing to the occupational exposure as a causative of enhanced CA frequencies for small molecular chemicals in plastics industry (32,43,44), for anaesthetic gases and antineoplastic drugs (9), for heavy metals (45) and for mineral fibres (6,7,38). In the present study, individuals that were occupationally exposed mainly to small molecular organic chemicals, anaesthetic gases, heavy metals and fibres (1207 subjects) indeed exhibited higher CAs, CTAs and CSAs in comparison with 989 unexposed individuals. Frequencies of chromatid- or chromosome-type exchanges contributed only marginally to the total CTAs and CSAs irrespective of exposure, as shown by us earlier (9). We recorded a borderline effect of smoking on CAs. The exposure of human DNA to above-listed agents inevitably leads to DNA damage. The capacity of DNA repair machinery to cope with these DNA alterations preserves the genomic integrity and prevents carcinogenesis (46,47). Thus, we investigated the role of functional variants in various DNA repair pathways, comprising BER (represented by *XRCC1*, *hOGG1* and *APE1*), NER (*XPA*, *XPC*, *XPD* and *XPG*) and DSB repair (*XRCC2*, *XRCC3*, *NBN* and *RAD54L*) genes. Interestingly, we observed a strong association between *XPD* Lys751Gln homozygous variant genotype and decreased CTA frequency. Our study on 1777 subjects confirmed our earlier observations on 225 healthy subjects (30) and later report on 140 subjects with higher age (8). *XPD* represents an important helicase involved in NER

Table 3. Odds ratios for high and low frequency groups by gene polymorphisms

	Cas					CTAs					CSAs				
Variables (SNP)	HF	LF	OR	95% CI	P[a]	HF	LF	OR	95% CI	P[a]	HF	LF	OR	95% CI	P[a]
XPD rs13181															
TT	271	358			0.98	301	328			**0.004**	287	342			0.87
TG	367	484	1.02	0.83–1.26		399	452	0.99	0.80–1.21		377	474	0.95	0.77–1.17	
GG	128	169	1.00	0.75–1.33		112	185	**0.64**	**0.48–0.85**		135	162	1.01	0.76–1.33	
XPG rs17655															
CC	460	601			0.67	477	584			0.43	485	576			0.57
CG	270	350	1.04	0.85–1.27		293	327	1.12	0.92–1.37		273	347	0.95	0.78–1.17	
GG	30	45	0.83	0.51–1.35		32	43	0.88	0.54–1.42		30	45	0.78	0.48–1.26	
XPC rs2228001															
AA	251	368			0.17	284	335			0.65	271	348			0.06
AC	398	467	1.20	0.97–1.49		403	462	0.99	0.81–1.23		409	456	1.12	0.91–1.38	
CC	117	171	1.00	0.75–1.33		124	164	0.88	0.66–1.17		112	176	0.81	0.61–1.08	
XRCC1 rs1799782															
CC	361	201			0.47	331	231			0.67	357	205			0.99
CT	49	25	1.09	0.66–1.85		40	34	0.80	0.49–1.31		47	27	1.02	0.62–1.71	
TT	1	2	0.25	0.01–2.66		2	1	0.97	0.09–21.1		2	1	1.04	0.10–22.7	
XRCC1 rs25489															
GG	212	120			0.70	200	132			0.18	209	123			0.13
GA	14	7	1.26	0.50–3.48		15	6	1.74	0.67–5.10		13	8	1.09	0.44–2.86	
AA	1	1	0.37	0.01–9.61		2	0	—	—		0	2	—	—	
XRCC1 rs25487															
GG	310	374			0.18	316	368			0.69	321	363			0.26
GA	352	507	0.86	0.70–1.02		385	474	0.96	0.78–1.18		384	475	0.93	0.76–1.14	
AA	98	119	1.07	0.78–1.45		102	115	1.09	0.80–1.49		86	131	0.77	0.56–1.05	
OGG1 rs1052133															
CC	495	623			0.44	526	592			0.31	511	607			0.32
CG	237	337	0.89	0.73–1.10		251	323	0.89	0.72–1.09		247	327	0.90	0.73–1.10	
GG	28	46	0.81	0.49–1.32		29	45	0.76	0.46–1.22		28	46	0.74	0.45–1.19	
XRCC3 rs861539															
CC	302	395			0.51	308	389			0.86	321	376			0.34
CT	355	454	0.98	0.79–1.21		375	434	1.06	0.86–1.30		358	451	0.91	0.74–1.11	
TT	83	126	0.83	0.60–1.14		95	114	1.04	0.76–1.42		86	123	0.80	0.58–1.09	
APE1 rs1130409															
GG	179	195			0.82	192	182			0.60	188	186			0.71
GT	316	338	1.03	0.78–1.35		313	341	0.89	0.69–1.16		321	333	0.94	0.72–1.22	
TT	144	127	1.11	0.79–1.55		145	126	1.00	0.72–1.38		146	125	1.06	0.77–1.47	
NBS1 rs1805794															
CC	245	189			0.85	242	192			0.47	249	185			0.89
CG	243	177	1.07	0.81–1.42		238	182	1.05	0.80–1.38		234	186	0.94	0.71–1.24	
GG	63	49	0.98	0.63–1.51		57	55	0.81	0.53–1.23		63	49	0.93	0.61–1.43	
XPA rs1800975															
GG	246	389			0.69	260	375			0.29	262	373			0.96
GA	236	406	0.92	0.73–1.15		274	368	1.08	0.86–1.35		262	380	0.98	0.79–1.23	
AA	57	109	0.89	0.61–1.27		59	107	0.81	0.56–1.16		68	98	1.03	0.72–1.46	
XRCC2 rs3218536															
GG	177	533			0.36	231	479			0.14	203	507			0.34
GA	23	77	0.88	0.52–1.43		39	61	1.30	0.84–2.00		31	69	1.13	0.70–1.78	
AA	0	3	—	—		0	3	—	—		0	3	—	—	
RAD54L s1048771															
CC	134	93			0.16	114	113			0.84	138	89			**0.03**
CT	30	2	0.95	0.51–1.79		26	26	1.04	0.57–1.93		39	13	**1.96**	**1.01–4.02**	
TT	3	0	—	—		2	1	2.03	0.19–44.5		3	0	—	—	

Significant results are highlighted in bold. HF, high-frequency group—cases; LF, low-frequency group—controls.

[a]Based on likelihood ratio test.

P-value was considered significant if it was <0.05.

and communicates with other DNA repair gene products in dealing with exogenous DNA damage (48), but the functional role of *XPD* Lys751Gln remains unclear. The assumption that functional SNPs in *XPD* gene modulate CTA frequency is consistent with mechanistic understanding, since CTAs arise as a consequence of DNA lesions generated by genotoxic compounds during G0 phase, which are insufficiently repaired prior to S-phase. On the other hand, scarce reports suggest increased DNA repair capacity associated with variant G allele of *XPD* gene (49,50) or are inconclusive (22). Unless the function of variant G allele in

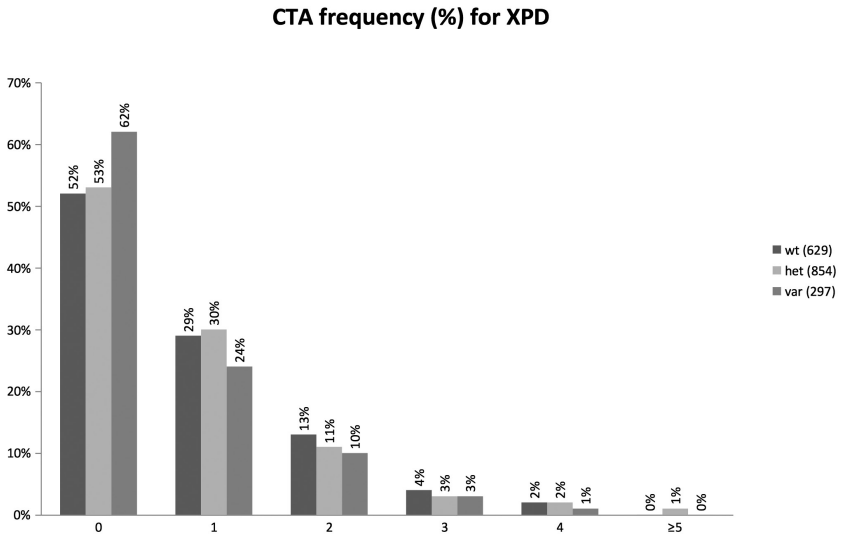

Figure 2. The frequency of individuals by CTA percent in relation to the *XPD* genotype. *x*-axis represents the frequency of individuals by CTA percent in relation to *XPD* genotype (TT, black bar; TG, light grey and GG, dark grey). *y*-axis represents the percentage of subjects with corresponding CTA frequency per particular genotype.

Table 4. Pair-wise interactions of genotypes with cases and controls

					Interaction term analysis			Global null hypothesis test for significance of all covariates		
	SNP 1	Mode for SNP 1	SNP 2	Mode for SNP 2	df	χ^2	*P*	df	χ^2	*P*
CAs	*APE1*	Three genotypes	*NBS1*	Allele number	2	11.80	0.003	9	67.45	<0.001
	hOGG1	Dominant	*XPG*	Allele number	1	7.58	0.005	7	65.78	<0.001
CSAs	*APE1*	Three genotypes	*XPD*	Recessive	2	9.78	0.008	9	94.40	<0.001
	XPD	Dominant	*XRCC3*	Allele number	1	8.33	0.004	7	31.31	<0.001
	XPG	Recessive	*NBS1*	Three genotypes	2	11.04	0.004	9	47.20	<0.001
	XRCC1	Allele number	*XPG*	Recessive	1	6.04	0.01	7	30.84	<0.001
	hOGG1	Allele number	*XPC*	Three genotypes	2	8.85	0.01	9	35.24	<0.001
	hOGG1	Dominant	*XPG*	Dominant	1	9.93	0.002	7	31.41	<0.001
CTAs	*XPG*	Allele number	*XRCC2*	Dominant	1	6.81	0.009	7	20.90	0.004
	XRCC3	Allele number	*XRCC2*	Dominant	1	6.71	0.01	7	21.06	0.003

Only the most significant genetic models for each interaction are shown ($P < 0.01$ in interaction term analysis). To assess the contribution of all genetic components (both SNPs and interaction term) to the model, likelihood ratio-based *P*-values were computed. df, degrees of freedom.
P-value was considered significant if it was <0.05.

XPD gene is clarified, the assumptions that higher level of DNA damage blocks replication fork and CTAs cannot be propagated remain speculative. An association of variant T allele in RAD54L with increased frequency of CSAs is a novel observation. *RAD54L* exhibits a DNA-dependent ATPase and supercoiling activities and plays a role in homologous recombination related repair of DSBs (51,52). However, this association is less robust due to the number of subjects with available genotype. Individually, a small risk is irrelevant, but the combination of several low-risk alleles can add up to substantial risks, even in the absence of multiplicative statistical interactions (53).

Similarly as in our recent study (35), we have addressed pair-wise interactions of the genotypes of each of the 11 DNA repair genes, which were tested for association with CA frequencies. We have discovered several highly significant interactions, involving gene variants from BER, NER and DSB pathways: 14 interactions modulated CA, 9 CTA and 12 CSA frequencies. For CAs, gene variants participating in BER (*APE1*, *hOGG1*), NER (*XPC*, *XPD*) and DSB repair (*XRCC3*) were mainly involved in significant interactions with other DNA repair gene variants (*NBS1*, *XRCC2* and *XPG1*). Interestingly, *NBS1* gene variant appears most often in these interactions. However, in interaction with their BER variants the CA frequency increases, whereas the interaction with NER gene variants shows the opposite. *NBS1* plays a relevant role in the maintenance of genomic integrity by being involved in the cellular response to DNA damage. The contradictory effect on CA frequencies in the interplay of *NBS1* variants with either BER or NER polymorphisms is certainly interesting and may reflect the specificity of these two excision repair pathways towards different kinds of DNA damage. For CTAs, the homozygous variant combinations in *XPD* with *XPG* or *XRCC1* decrease the frequencies. So does the combination of variant alleles in *OGG1* and *XRCC3*. On the contrary, a combination of variant alleles in DSB repair genes results in the significant increase. These results point again to an effect of the G allele of *XPD* Lys751Gln on CTA frequency modulation as stated above. For CSAs, variant alleles in BER genes *XRCC1* and *OGG1* in combination with homozygous variant genotype in *XPC* or *XPG* result

in significantly decreased frequencies. Interestingly, variant G allele in rs1052133 of *OGG1* in combination with variant alleles in genes involved in NER or DSB repair resulted in decreased frequencies of CAs, CTAs and CSAs, despite the fact that variant G allele is associated with the lower capacity to repair oxidative DNA damage (24). This phenomenon may be connected with the fact that 8-hydroxy-deoxyguanine adducts may block replication fork, thus preventing accumulation of CAs.

CAs arise as a consequence of the interaction between occupational exposure to various genotoxicants. In this study, we tested the impact of functional gene variants in DNA repair pathways on the frequency of CAs. Although individuals with homozygous variant genotype GG for *XPD* gene showed an association with CTAs, several gene–gene combinations in DNA repair genes evinced either enhanced or decreased frequencies of CAs, CTAs and CSAs. As suggested by Melis *et al.* (54) and now confirmed by us, the complex mechanism of CAs accumulation requires complex interplay between different DNA repair pathways. However, the mechanism may not be tracked without the knowledge of the experimentally proven functional impact of DNA repair gene variants.

Supplementary material

Supplementary Material can be found at http://carcin.oxfordjournals.org/

Funding

Projects 15-14789S (Czech Science Foundation), P27/2012 (Charles University of Prague, Czech Republic), CZ.1.05/2.1.00/03.0076, 26220220111, co-financed by EU sources and the European Regional Development Fund, 2012/25-UKMA-2 (Ministry of Health of the Slovak Republic) and APVV-0412-11.
Conflict of Interest Statement: None declared.

References

1. Hakem, R. (2008) DNA-damage repair; the good, the bad, and the ugly. EMBO J., 27, 589–605.
2. Bignold, L.P. (2009) Mechanisms of clastogen-induced chromosomal aberrations: a critical review and description of a model based on failures of tethering of DNA strand ends to strand-breaking enzymes. Mutat. Res., 681, 271–298.
3. Natarajan, A.T. et al. (2008) DNA repair and chromosomal alterations. Mutat. Res., 657, 3–7.
4. Durante, M. et al. (2013) From DNA damage to chromosome aberrations: joining the break. Mutat. Res., 756, 5–13.
5. Mateuca, R.A. et al. (2012) Cytogenetic methods in human biomonitoring: principles and uses. Methods Mol. Biol., 817, 305–334.
6. Dusinská, M. et al. (2004) Does occupational exposure to mineral fibres cause DNA or chromosome damage? Mutat. Res., 553, 103–110.
7. Dusinská, M. et al. (2004) Genotoxic effects of asbestos in humans. Mutat. Res., 553, 91–102.
8. Kazimírová, A. et al. (2009) Micronuclei and chromosomal aberrations, important markers of ageing: possible association with XPC and XPD polymorphisms. Mutat. Res., 661, 35–40.
9. Musak, L. et al. (2013) Chromosomal damage among medical staff occupationally exposed to volatile anesthetics, antineoplastic drugs, and formaldehyde. Scand. J. Work Environ. Health, 39, 618–630.
10. Vodenkova, S. et al. (2015) Structural chromosomal aberrations as potential risk markers in incident cancer patients. Mutagenesis, in 30, 557–563.
11. Futreal, P.A. et al. (2004) A census of human cancer genes. Nat. Rev. Cancer, 4, 177–183.
12. Rajagopalan, H. et al. (2004) Aneuploidy and cancer. Nature, 432, 338–341.
13. Burrell, R.A. et al. (2013) Replication stress links structural and numerical cancer chromosomal instability. Nature, 494, 492–496.
14. Mitelman, F. et al. (2007) The impact of translocations and gene fusions on cancer causation. Nat. Rev. Cancer, 7, 233–245.
15. Rossner, P. et al. (2005) Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environ. Health Perspect., 113, 517–520.
16. Boffetta, P. et al. (2007) Chromosomal aberrations and cancer risk: results of a cohort study from Central Europe. Am. J. Epidemiol., 165, 36–43.
17. Rossi, A.M. et al. (2009) Association between frequency of chromosomal aberrations and cancer risk is not influenced by genetic polymorphisms in GSTM1 and GSTT1. Environ. Health Perspect., 117, 203–208.
18. Bonassi, S. et al. (2008) Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis, 29, 1178–1183.
19. Vodicka, P. et al. (2010) Chromosomal damage in peripheral blood lymphocytes of newly diagnosed cancer patients and healthy controls. Carcinogenesis, 31, 1238–1241.
20. Naccarati, A. et al. (2007) Sporadic colorectal cancer and individual susceptibility: a review of the association studies investigating the role of DNA repair genetic polymorphisms. Mutat. Res., 635, 118–145.
21. Vineis, P. et al. (2009) A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility. J. Natl. Cancer Inst., 101, 24–36.
22. Abdel-Rahman, S.Z. et al. (2011) Evaluating the effects of genetic variants of DNA repair genes using cytogenetic mutagen sensitivity approaches. Biomarkers, 16, 393–404.
23. Vodicka, P. et al. (2007) Association of DNA repair polymorphisms with DNA repair functional outcomes in healthy human subjects. Carcinogenesis, 28, 657–664.
24. Simonelli, V. et al. (2012) Gene susceptibility to oxidative damage: from single nucleotide polymorphisms to function. Mutat. Res., 731, 1–13.
25. Wu, L.L. et al. (2004) Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin. Chim. Acta, 339, 1–9.
26. Loft, S. et al. (2012) Urinary excretion of 8-oxo-7,8-dihydroguanine as biomarker of oxidative damage to DNA. Arch. Biochem. Biophys., 518, 142–150.
27. Friedberg, E.C. (2003) DNA damage and repair. Nature, 421, 436–440.
28. Hoelzl, C. et al. (2009) Use of single cell gel electrophoresis assays for the detection of DNA-protective effects of dietary factors in humans: recent results and trends. Mutat. Res., 681, 68–79.
29. Dolara, P. et al. (2012) Antioxidant vitamins and mineral supplementation, life span expansion and cancer incidence: a critical commentary. Eur. J. Nutr., 51, 769–781.
30. Vodicka, P. et al. (2004) Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. Carcinogenesis, 25, 757–763.
31. Naccarati, A. et al. (2006) Genetic polymorphisms and possible gene-gene interactions in metabolic and DNA repair genes: effects on DNA damage. Mutat. Res., 593, 22–31.
32. Musak, L. et al. (2008) Chromosomal aberrations in tire plant workers and interaction with polymorphisms of biotransformation and DNA repair genes. Mutat. Res., 641, 36–42.
33. Skjelbred, C.F. et al. (2011) Influence of GSTM1, GSTT1, GSTP1, NAT1, NAT2, EPHX1, MTR and MTHFR polymorphism on chromosomal aberration frequencies in human lymphocytes. Carcinogenesis, 32, 399–405.
34. Dusinska, M. et al. (2012) Are glutathione S transferases involved in DNA damage signalling? Interactions with DNA damage and repair revealed from molecular epidemiology studies. Mutat. Res., 736, 130–137.
35. Hemminki, K. et al. (2015) Metabolic gene variants associated with chromosomal aberrations in healthy humans. Genes Chromosomes Cancer, 54, 260–266.
36. Hemminki, K. et al. (2014) Cyclin D1 splice site variant triggers chromosomal aberrations in healthy humans. Leukemia, 28, 721–722.

37. Jirawatnotai, S. et al. (2011) A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. Nature, 474, 230–234.
38. Kazimirova, A. et al. (2007) Cytogenetic analysis of lymphocytes of workers occupationally exposed to rockwool and glass fibres. Molecular-epidemiologic study. Chem. Listy, 101, 192–193.
39. Srám, R.J. et al. (2004) Cytogenetic analysis and occupational health in the Czech Republic. Mutat. Res., 566, 21–48.
40. Pardini, B. et al. (2008) DNA repair genetic polymorphisms and risk of colorectal cancer in the Czech Republic. Mutat. Res., 638, 146–153.
41. Shen, Z. (2011) Genomic instability and cancer: an introduction. J. Mol. Cell Biol., 3, 1–3.
42. Abbas, T. et al. (2013) Genomic instability in cancer. Cold Spring Harb. Perspect. Biol., 5, a012914.
43. Vodicka, P. et al. (2004) Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant. Environ. Mol. Mutagen., 44, 283–292.
44. Vodicka, P. et al. (2006) Styrene metabolism, genotoxicity, and potential carcinogenicity. Drug Metab. Rev., 38, 805–853.
45. Halasova, E. et al. (2012) Evaluating chromosomal damage in workers exposed to hexavalent chromium and the modulating role of polymorphisms of DNA repair genes. Int. Arch. Occup. Environ. Health, 85, 473–481.
46. Curtin, N.J. (2012) DNA repair dysregulation from cancer driver to therapeutic target. Nat. Rev. Cancer, 12, 801–817.
47. Abbotts, R. et al. (2014) DNA repair in cancer: emerging targets for personalized therapy. Cancer Manag. Res., 6, 77–92.
48. Spies, M. (2014) Two steps forward, one step back: determining XPD helicase mechanism by single-molecule fluorescence and high-resolution optical tweezers. DNA Repair (Amst.), 20, 58–70.
49. Wlodarczyk, M. et al. (2012) XPD gene rs13181 polymorphism and DNA damage in human lymphocytes. Biochem. Genet., 50, 860–870.
50. Gdowicz-Klosok, A. et al. (2013) The influence of XPD, APE1, XRCC1, and NBS1 polymorphic variants on DNA repair in cells exposed to X-rays. Mutat. Res., 755, 42–48.
51. Mjelle, R. et al. (2015) Cell cycle regulation of human DNA repair and chromatin remodeling genes. DNA Repair (Amst.), 30, 53–67.
52. Eppink, B. et al. (2011) The response of mammalian cells to UV-light reveals Rad54-dependent and independent pathways of homologous recombination. DNA Repair (Amst.), 10, 1095–1105.
53. Vineis, P. et al. (2008) Expectations and challenges stemming from genome-wide association studies. Mutagenesis, 23, 439–444.
54. Melis, J.P. et al. (2013) Oxidative DNA damage and nucleotide excision repair. Antioxid. Redox Signal., 18, 2409–2419.