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Association between nucleotide excision repair gene polymorphisms and chromosomal damage in coke-oven workers

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Abstract
The associations between several genetic polymorphisms of nucleotide excision repair genes (NER) and chromosome damage level were studied among 140 coke-oven workers exposed to a high level of polyaromatic hydrocarbons (PAHs) and 66 non-exposed workers. Seven polymorphisms with functional potential in five NER genes (*ERCC1*, *ERCC2*, *ERCC4*, *ERCC5* and *ERCC6*) were genotyped in the 206 study subjects. Multivariate analysis of covariance revealed that coke-oven workers with the *ERCC1* 19007 CC genotype had significantly higher cytokinesis-block micronucleus frequency (CBMN) ($10.5 \pm 6.8‰$) than those with CT ($8.1 \pm 6.6‰$, $p = 0.01$) or TT ($6.6 \pm 3.7‰$, $p = 0.05$) or CT+TT genotypes ($7.5 \pm 6.3‰$, $p = 0.004$). The *ERCC6* A3368G polymorphism was also associated with CBMN frequency among coke-oven workers. Subjects with the AA genotype have a significantly higher CBMN frequency ($10.0 \pm 6.9‰$) than those with AG ($6.7 \pm 4.2‰$, $p = 0.05$) or AG+GG genotypes ($6.6 \pm 4.1‰$, $p = 0.02$). Stratification analysis revealed the significant associations between *ERCC1* C19007T and *ERCC6* A3368G, and the CBMN frequencies were only found among older workers. In addition, a significant association between *ERCC2* G23591A polymorphism and CBMN frequencies was also found among older coke-oven workers. The results suggest that polymorphisms of *ERCC1* C19007T, *ERCC6* A3368G and *ERCC2* G23591A are associated with the CBMN frequencies among coke-oven workers.

Keywords: *Nucleotide excision repair, gene polymorphisms, chromosomal damage, coke-oven workers.*

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Introduction

Coke production has been classified as class I carcinogens by the International Agency for Research on Cancer (IARC) (1984), but only a small fraction of workers exposed to coke emission eventually develop lung cancer, which suggests that individual genetic variation may influence their susceptibility to polycyclic aromatic hydrocarbons (PAH) carcinogenesis. In humans, carcinogenic PAHs in coke emission were

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metabolized to form ultimate carcinogens that could damage DNA to exert their mutagenic and carcinogenic effects (Miller & Miller 1981). Three DNA repair pathways, including base excision repair (BER), nucleotide excision repair (NER) and double-strand break repair (DSBR), were involved in repair of the PAH-induced DNA damages (Braithwaite et al. 1998). If not correctly repaired before DNA synthesis, the left DNA damages may be fixed as mutations in the daughter cells. Mutations in some important genes may initiate carcinogenic process (Fenech 2002a). It could be postulated that inter-individual difference in metabolic activation and detoxification of carcinogens and DNA repair capacity (DRC) could influence individual risk of lung cancer in occupational exposed workers (Shields 2000). Chromosome damage has been recognized as an important event in chemical carcinogenesis (Fenech 2002a,b). In our previous study, polymorphisms of *mEH* and *XRCC1* were found to be associated with altered chromosome damage level assessed by cytokinesis-block micronucleus (CBMN) assay among coke-oven workers, suggesting that both metabolic and BER pathways play an important role in genetic damage induced by PAHs (Leng et al. 2004, 2005).

The NER pathway mainly eliminates the bulky DNA adducts formed by metabolites of PAHs (Asami et al. 1997). Several important proteins, including ERCC1, ERCC2/XPD, ERCC4/XPF, ERCC5/XPG, and ERCC6, work together to remove the DNA bulky adducts (Wood 1997, Mallery et al. 1998, Ura & Hayes 2002). In literatures, several single nucleotide polymorphisms (SNPs) in these genes were found to be associated with decreased DRC and/or increased cancer risks. For example, the common polymorphism at codon 118 (C19007T, Asn/Asn, exon 4) of the *ERCC1* gene has been associated with altered mRNA levels and cancer risk (Yu et al. 1997, Lee et al. 2005a). Three SNPs in the *ERCC2* gene, including C22541A at codon 156, G23591A at codon 312, A35931C at codon 751, Lys/Gln, exon 23) have been found to be associated with the reduced repair capacity and increased DNA or chromosome damage and cancer risk (Spitz et al. 2001, Benhamou & Sarasin 2002, Goode et al. 2002, Hou et al. 2002, Hu et al. 2004, Terry et al. 2004, Vodicka et al. 2004). A Korean breast cancer study observed an association between the variant allele C of T30028C polymorphism (Ser835Ser, exon 11) of *ERCC4* and increased cancer risk (Lee et al. 2005b). Several studies have documented that the *ERCC5* codon 1104 polymorphism (G3507C, Asp/His, exon 15) contributed to high risk of primary lung cancer, breast cancer, squamous cell carcinomas of the oropharynx, larynx and esophagus, and could influence single-strand DNA break levels (Goode et al. 2002, Kumar et al. 2003, Jeon et al. 2003, Vodicka et al. 2004, Cui et al. 2006). A study on NER gene polymorphisms and recurrence after treatment for superficial bladder cancer revealed that the G allele of the common polymorphism of *ERCC6* codon 1097 (A3368G, Met /Val, exon 18) is associated with better DNA repair capacity (Gu et al. 2005).

Therefore, we hypothesized that these SNPs with functional potential in these five NER genes including *ERCC1*, *ERCC2*, *ERCC4*, *ERCC5*, *ERCC6*, are associated with chromosome damage in occupational PAH-exposed population. To test this hypothesis, we genotyped and investigated the effect of the polymorphisms of these genes on the chromosome damage in peripheral blood lymphocytes from 140 coke-oven workers and 66 non-coke-oven controls.

Materials and methods

Subjects and sample collection

This study was approved by the Research Ethic Committee of the National Institute for Occupational Health and Poison Control, Chinese Centre for Disease Control and Prevention. Details of the population have been described previously (Leng et al. 2004, 2005). In brief, the exposure group consisted of 140 workers from the same coke-oven and the non-exposed control group comprised 66 medical staffs. Exclusion criteria for participation in the study included recent treatments with mutagenic agents (such as X-ray), chronic conditions (such as autoimmune disease), and recent acute infections that required medications such as antibiotics. The two groups are in the same economic conditions. Detailed data on age, gender, smoking and drinking status, history of occupational exposure were derived from questionnaires. After informed consent was obtained from each subject 4-day shift-end urine and venous blood.

PAH exposure information

The air levels of benzene-soluble matter (BSM) and particulate-phase benzo[*a*]pyrene (B[*a*]P) in the working environment of coke-oven workers and controls were measured about one and a half month before urine and blood sample collection and analysed according to the OSHA Method No. 58 (Organic Methods Evaluation Branch, OSHA Analytical Laboratory 1986). The excretion of urinary 1-hydroxypyrene (1-OHP) was measured as the internal dose of personal recent PAH exposure. Measurements below the limit of detection (LOD) were replaced with $\mathrm{LOD}/\sqrt{2}$ before statistical analysis (Hornung & Reed 1990). The urinary 1-OHP concentrations were corrected by urinary creatinine and presented as $\mu\mathrm{mol\ mol}^{-1}$ Creat.

Cytokinesis-block micronucleus (CBMN) assay using peripheral blood lymphocytes

The CBMN assay was done according to the standard method as described previously (Fenech 1993, 2000). Duplicate lymphocyte cultures were set up. A total of 1000 binucleated lymphocytes were examined for detection of micronuclei for each subject. The CBMN frequencies were estimated as number of micronuclei per 1000 binucleated lymphocytes. The slides' scorer is blind to the exposure status of the 206 participants.

Polymorphism analysis

DNA was extracted from whole peripheral blood samples as described previously (Miller et al. 1988). Seven common SNPs described in literatures were selected in our present study (Table I). The polymorphisms of *ERCC1* C19007T, *ERCC2* C22541A, *ERCC2* G23591A *ERCC2* A35931C, *ERCC4* T30028C and *ERCC5* G3507C were detected by the published methods (Vogel et al. 2001, Liang et al. 2003, Mort et al. 2003, Zhou et al. 2004). The *ERCC6* A3368G (SNP database ID:rs2228526) genotype was determined by the primer introduced restriction analysis-PCR (PIRA-PCR) approach (Ke et al. 2001), with the primer sequences of 5′-GTG ATC CTT TGA AAG ATG ACC CTC AC-3′ and 5′-CCT GAA GAA TTT GAA CAT TCC

Table I. Polymorphisms in the excision repair cross-complementing group genes and the effects of amino acid and functional effects.

SNP	ID	Amino acid	Chromosomal location	Functional effects	References
ERCC1 C19007T	rs11615	Asn118Asn	19q13.2-q13.3	unknown	
ERCC2 C22541A	rs238406	Arg156Arg	19q13.2-q13.3	unknown	
ERCC2 G23591A	rs1799793	Asp312Asn	19q13.2-q13.3	no detectable difference in DNA repair proficiency	Seker et al. (2001)
ERCC2 A35931C	rs13181	Lys751Gln	19q13.2-q13.3	no detectable difference in DNA repair proficiency	Seker et al. (2001)
ERCC4 T30028C	rs1799801	Ser835Ser	16p13.3-p13.13	unknown	
ERCC5 G3507C	rs17655	Asp1104His	13q33	unknown	
ERCC6 A3368G	rs2228526	Met1097Val	10q11	unknown	

CCA-3′. PCR reactions were performed in a 30-μl volume containing 75 mmol l^{-1} Tris-HCl (pH = 8.8), 200 mmol l^{-1} $(NH_4)_2SO_4$, 0.1% Tween 20, dNTPs 166 μmol l^{-1}, 2.0 mmol l^{-1} $MgCl_2$, 0.25 μmol l^{-1} each primer, 0.75 UTaq polymerase (Fermentas, Lithuania) and 50 ng genomic DNA. The cycling conditions were initial denaturation at 95°C for 5 min, followed by 32 cycles of 30 s at 95°C, 30 s at 58°C and 30 s at 72°C. The 139-bp PCR products were then digested overnight by HpyCHIV enzyme (New England BioLabs, Ipswich). The G allele but not the A allele has a HpyCHIV restriction site, so the three possible genotypes are defined by three distinct banding patterns: AA (139 bp), AG (24, 115, 139 bp), and GG (24, 115 bp) genotypes. Ten per cent of DNA samples were genotyped a second time and the concordance rate was 100%.

Statistical methods

Urinary 1-OHP and CBMN frequencies were natural logarithm-normally distributed. Spearman rank correlation test was used to evaluate the relationship between urinary 1-OHP and CBMN frequencies. Mann–Whitney *U*-tests were used to compare the air levels of PAH, cigarettes per day, and CBMN frequencies between controls and coke-oven workers and to compare the CBMN frequencies between coke-oven workers and controls and to compare the CBMN frequencies between the categories of selected variables (age, gender, smoking and drinking status, coking history). A chi-square test was used to compare the frequencies of current smokers and alcohol consumers between two study populations. The goodness-of-fit chi-square test was used to detect whether the allele frequencies were in Hardy–Weinberg equilibrium. Multivariate analysis of covariance model was used to analyse the associations between all genotypes and ln-transformed CBMN frequencies. In the multivariate models, the urinary 1-OHP levels and the mEH phenotypes were adjusted to remove the effects of PAH exposure and metabolism on micronuclei formation and to assess the effects of DNA repair gene polymorphisms (Leng et al. 2005). Because age was significantly related to CBMN frequencies among coke-oven workers, we stratified the data by the mean age of coke-oven workers (39.1 years) to analyse the associations between all genotypes and ln-transformed CBMN frequencies in younger and older workers separately. In the linkage disequilibrium analysis, the program PHASE (version 2.1) was used to calculate the haplotype frequencies (Stephens et al. 2001, Stephens & Donnelly 2003). Values of $p \leq 0.05$ were considered

significant. All statistics are two-sided and performed with Statistical Analysis System software (version 8.0; SAS Institute, Inc., Cary, NC, USA).

Results

The demographic information for this studied population has been described in detail in previously published papers (Leng et al. 2004, 2005). In brief, the distributions of age, gender and alcohol consumption were not significantly different between coke-oven workers and controls, except smoking status. Coke-oven workers have significantly higher PAH exposure levels than controls (BSM, 328.6 versus 97.8 $\mu g\, m^{-3}$; particulate-phase B[a]P, 926.9 versus 49.1 $ng\, m^{-3}$; urinary 1-OHP, 11.8; 95% CI, 10.2–13.6 versus 0.7; 95% CI, 0.6–1.3 $\mu mol\, mol^{-1}$ Creat, $p < 0.01$). Age and coking history were significantly correlated in coke-oven workers ($n = 140$, Pearson correlation, $r = 0.813$, $p < 0.001$). The CBMN frequency was significantly higher in coke-oven workers ($9.6 \pm 6.6‰$) than in controls ($4.0 \pm 3.6‰$, $p < 0.01$) and age was significantly correlated with CBMN frequencies among all the subjects ($n = 206$, Pearson correlation, $r = 0.159$, $p = 0.02$) and coke-oven workers ($n = 140$, Pearson correlation, $r = 0.188$, $p = 0.02$), but controls ($n = 66$, Pearson correlation, $r = 0.078$, $p = 0.53$).

The variant allele frequencies of *ERCC1* C19007T, *ERCC2* C22541A, *ERCC2* G23591A, *ERCC2* A35931C, *ERCC4* T30028C, *ERCC5* G3507C, and *ERCC6* A3368G were 0.25, 0.45, 0.07, 0.07, 0.17, 0.50, and 0.07, respectively, in the 206 subjects. The distributions of all these eight polymorphisms were in Hardy–Weinberg equilibrium and were similar between coke-oven workers and controls (data not shown).

Multivariate analysis of covariance with adjustment for ln-transformed urinary 1-OHP, age, and mEH phenotypes revealed that among coke-oven workers, the *ERCC1* CC genotype carriers exhibited significantly higher CBMN frequency ($10.5 \pm 6.8‰$) than did the CT ($8.1 \pm 6.6‰$, $p = 0.01$) or TT ($6.6 \pm 3.7‰$, $p = 0.05$) or CT+TT genotypes carriers ($7.5 \pm 6.3‰$, $p = 0.004$). For the *ERCC6* A3368G polymorphism, AA genotype carriers exhibited significantly higher CBMN frequency ($10.0 \pm 6.9‰$) than did the AG ($6.7 \pm 4.2‰$, $p = 0.05$) or AG+GG genotypes carriers ($6.6 \pm 4.1‰$, $p = 0.02$). No such associations were observed in controls. No other association between the polymorphisms of *ERCC2* C22541A, *ERCC2* G23591A, *ERCC2* A35931C, *ERCC4* T30028C, *ERCC5* G3507C and CBMN frequencies were found in both coke-oven workers and controls (Table II). Stratification analysis revealed that the significant association between the two polymorphisms, *ERCC1* C19007T and *ERCC6* A3368G, and the CBMN frequencies was most pronounced in older workers. In addition, we also observed that for the polymorphism of *ERCC2* G23591A, GA carriers had significantly higher CBMN frequency ($14.0 \pm 6.3‰$) than those GG carriers ($9.8 \pm 5.9‰$, $p = 0.01$) in older coke-oven workers (Table III).

Because *ERCC1* C19007T, *ERCC2* G23591A and *XRCC1* C26304T (Arg194Trp), an important polymorphism which had an effect on CBMN frequencies in our previous study, were located in chromosome 19q13.2–3, we performed linkage disequilibrium analysis between these three SNPs. Polymorphisms of *ERCC1* C19007T and *ERCC2* G23591A were in strong linkage disequilibrium ($D' = 0.94$; $p < 0.01$), *ERCC1* C19007T and *XRCC1* C26304T were in linkage equilibrium ($D' = 0.05$; $p > 0.1$) in our study population.

Table II. Cytokinesis-block micronucleus frequency (‰) in controls and coke-oven workers by genotypes of DNA repair genes.

	Non-coke-oven controls			Coke-oven workers			
Genotypes	*n*	Mean±SD	p^1	*n*	Mean±SD	p^1	p^2
ERCC1 C19007T							
CC	37	4.2±4.1	reference[3]	81	10.5±6.8	reference[3]	<0.01
CT	23	4.2±3.0	0.67	49	8.1±6.6	0.01	<0.01
TT	6	1.3±1.0	0.07	10	6.6±3.7	0.05	<0.01
CT+TT	29	3.6±2.9	0.77	59	7.5±6.3	0.004	<0.01
ERCC2 C22541A							
CC	14	5.1±3.0	reference[3]	49	8.8±5.8	reference[3]	0.04
CA	39	3.5±3.5	0.11	60	10.0±6.9	0.73	<0.01
AA	13	4.2±3.7	0.43	31	10.3±7.2	0.33	<0.01
CA+AA	52	3.7±3.7	0.13	91	10.0±7.0	0.50	<0.01
ERCC2 G23591A							
GG	60	3.8±3.5	reference[3]	119	9.3±6.6	reference[3]	<0.01
GA	5	5.0±4.8	0.59	21	11.3±6.7	0.14	0.05
AA	1	10.0±	0.19	0	–	–	–
GA+AA	6	5.8±4.8	0.31	21	11.3±6.7	0.14	0.08
ERCC2 A35931C							
AA	59	4.1±3.6	reference[3]	119	9.4±6.6	reference[3]	<0.01
AC	7	3.1±3.1	0.46	21	10.6±6.4	0.22	<0.01
CC	0	–	–	0	–	–	–
ERCC4 T30028C							
TT	38	3.8±3.2	reference[3]	101	9.6±6.2	reference[3]	<0.01
TC	27	4.1±4.1	0.79	35	9.7±7.6	0.60	<0.01
CC	1	10.0±	0.17	4	8.0±8.0	0.41	0.80
TC+CC	28	4.3±4.1	0.62	39	9.6±7.6	0.48	<0.01
ERCC5 G3507C							
GG	20	3.9±3.2	reference[3]	28	9.5±7.0	reference[3]	<0.01
GC	29	4.1±3.9	0.94	82	10.0±7.2	0.81	<0.01
CC	17	3.8±3.8	0.76	30	8.7±4.1	0.76	<0.01
GC+CC	46	4.0±3.9	0.82	112	9.6±7.0	0.79	<0.01
ERCC6 A3368G							
AA	60	3.7±3.1	reference[3]	118	10.0±6.9	reference[3]	<0.01
AG	6	6.0±6.8	0.62	21	6.7±4.2	0.05	<0.01
GG	0	–	–	1	2.0	0.06	–
AG+GG	6	6.0±6.8	0.62	22	6.6±4.1	0.02	<0.01

[1]Multiple analysis of covariance tests for differences in ln-transformed CBMN frequencies between genotypes with adjustment for ln-transformed urinary 1-OHP, age and mEH phenotypes.
[2]Mann–Whitney *U*-tests for the differences in ln-transformed CBMN frequencies between the coke-oven workers and non-coke-oven controls in each genotype.
[3]Reference group for comparisons of ln-transformed CBMN frequencies between genotypes.

Discussion

The present study has investigated the role of NER gene polymorphisms in chromosomal damage measured by CBMN assay using peripheral blood lymphocytes among coke-oven workers. It was observed that three polymorphisms, *ERCC1* C19007T, *ERCC6* A3368G and *ERCC2* G23591A, could influence the CBMN

Table III. Cytokinesis-block micronucleus frequency (‰) in coke-oven workers by genotypes of DNA repair genes stratified by age.

	Age <39.1 (years)			Age ≥39.1 (years)		
Genotypes	*n*	Mean±SD	p^1	*n*	Mean±SD	p^1
ERCC1 C19007T						
CC	39	9.7±7.0	reference[2]	42	11.8±6.1	reference[2]
CT+TT	27	7.1±6.4	0.14	32	8.7±6.2	0.005
ERCC2 C22541A						
CC	28	7.9±6.0	reference[2]	23	9.8±5.5	reference[2]
CA	26	8.4±6.4	0.95	32	11.3±7.5	0.67
AA	12	11.0±9.9	0.30	19	9.8±5.1	0.93
CA+AA	38	9.2±7.4	0.74	51	10.8±6.6	0.77
ERCC2 G23591A						
GG	56	8.7±7.2	reference[2]	63	9.8±5.9	reference[2]
GA	10	8.4±6.0	0.99	11	14.0±6.3	0.01
ERCC2 A35931C						
AA	56	8.3±7.2	reference[2]	63	10.3±6.0	reference[2]
AC	10	9.9±6.0	0.22	11	11.2±7.0	0.48
ERCC4 T30028C						
TT	46	8.7±6.1	reference[2]	54	10.5±6.3	reference[2]
TC+CC	20	9.1±8.6	0.35	20	10.1±6.4	0.48
ERCC5 G3507C						
GG	14	6.4±3.7	reference[2]	14	12.6±8.3	reference[2]
GC	41	9.9±8.2	0.21	40	10.0±6.1	0.30
CC	10	6.4±3.7	0.12	20	9.9±4.1	0.96
GC+CC	51	9.4±7.3	0.23	60	9.8±5.6	0.36
ERCC6 A3368G						
AA	54	9.2±7.4	reference[2]	64	11.0±6.2	reference[2]
AG+GG	12	6.2±3.9	0.22	10	7.1±4.5	0.04

[1]Multiple analysis of covariance tests for differences in ln-transformed CBMN frequencies between genotypes with adjustment for ln-transformed urinary 1-OHP and mEH phenotypes.
[2]Reference group for comparisons of ln-transformed CBMN frequencies between genotypes.

frequencies in the coke-oven workers, suggesting variants of these three genes might have an effect on DNA repair capacity among the population exposed to a high level of PAHs.

The distribution of *ERCC1*, *ERCC2*, *ERCC4*, *ERCC5* and *ERCC6* genotypes had no difference between coke-oven workers and controls and the frequencies of variant alleles were similar to those reported in the literature of the Chinese population, which suggest no selection bias for the subject's enrolments in terms of genotypes (Liang et al. 2003, Yin et al. 2005).

ERCC1 is the leading component of the NER process. It forms a tight complex with ERCC4, and this heterodimer has the primary function of incising the DNA strand, at a site 5′ to the covalent DNA base damages. In animal experiments, cells from *ERCC1*-deficient mice showed increased genomic instability and a reduced frequency of S-phase-dependent illegitimate chromosome exchange, which lead to the accumulation of double-strand breaks following replication, suggesting that decreased ERCC1 activity may have effect on increased levels of unrepaired lesions and double-strand breaks (Melton et al. 1998). Furthermore, it has been shown that

ERCC1-deficient hepatocyte nuclei from mice were already enlarged and polyploid at birth, by 3 weeks of age increasingly polyploid and aneuploid nuclei were evident (McWhir et al. 1993). Griffin et al. found that in embryonic stem cells, loss of *ERCC1* leads to increased fragment formation (Griffin et al. 2005). Micronuclei (MN) originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division (Fenech et al. 2002a, b), therefore ERCC1 may be vital in the formation of micronuclei in the population, and our finding of strong association between polymorphism of *ERCC1* C19007T and altered CBMN frequency among coke-oven workers is biologically plausible. There are two association studies between *ERCC1* C19007T polymorphism and lung cancer risk in Chinese population, which did not observe any significant association (Zhou et al. 2005, Yin et al. 2006). Chromosome damage is early biological event of PAH carcinogenesis, therefore our finding should be further verified in prospective study of coke-oven workers' lung cancer.

ERCC2 works as an ATP-dependent 5′–3′ helicase joining the basal transcription factor IIH (TFIIH) complex (Benhamou & Sarasin 2002). The G → A mutation at position 23591 in codon 312 of *ERCC2* results in the amino acid exchange from Asp to Asn. Codon 312 of *ERCC2* locates in the domain of interaction with p44, a constituent of the TFIIH complex, which is the activator of ERCC2 helicase activity, so this substitution may alternate activity and influence DRC (Benhamou & Sarasin 2002). Spitz et al. reported that the variant Asn/Asn genotype of *ERCC2* 312 was associated with less optimal DRC assessed by the host cell reactivation assay (Spitz et al. 2001). Several reports showed that variant in *ERCC2* 312 was a risk factor for lung cancer, including a population-based case-control study in Xuanwei Chinese exposed to indoor smoky coal emissions that contain very high levels of PAHs (Hou et al. 2002, Benhamou & Sarasin 2002, Shen et al. 2005). Stratified analysis result in the present study that polymorphism of *ERCC2* G23591A had an effect on CBMN frequencies only in the older coke-oven workers, suggested the effect of *ERCC2*312 genotypes on DNA repair capacity was age-dependent among the population exposed to high level of PAHs.

The *ERCC1* and *ERCC2* gene are in the same chromosomal region 19q13.2–3, and they are separated only by <250 kbp, suggesting a close link in DNA repair function (Dabholkar et al. 1995). Linkage disequilibrium analysis showed that these two SNPs, *ERCC1* C19007T and *ERCC2* G23591A were linked with each other, so we could not exclude the possibility that the effect of *ERCC2* G23591A on CBMN frequencies among older coke-oven workers might due to the really causal SNP within the *ERCC1* gene. In the same region 19q13.2-3, another DNA repair gene *XRCC1* is located, which was found previously could modulate chromosome damage among the same study population (Leng et al. 2005). *ERCC1* C19007T and *XRCC1* C26304T were in linkage equilibrium in our study population, suggesting that BER and NER pathways may be involved in repair of chromosome damage in workers occupationally exposed to high level of PAHs.

To date, few studies have addressed the impact of *ERCC6* A3368G polymorphism on phenotype biomarkers or cancer risk. Gu et al. reported that superficial bladder cancer patients with variant allele G had worse clinical outcomes than those A allele carriers after the Bacille Calmette-Guerin treatment, indicating the G allele is associated with a better DNA repair capacity (Gu et al. 2005). This result is consistent with our finding that G allele was associated with lower CBMN frequencies among

coke-oven workers, although the functional significance of this polymorphism has not been reported to date. Due to the relative low distribution frequency of variant allele G, this association needs to be verified in larger studies.

The stratification analysis results that the associations between genotypes and CBMN frequencies were more pronounced in subjects who were $\geq$ 39.1 years might due to that DRC is lower in older workers than younger workers. However, in our study, coking history was correlated with age, so older subgroup might have a relative long and more accumulative environmental exposure. Therefore, whether age itself or together with cumulative genomic insults could explain the association between genotypes and chromosome damage in older workers needs to be further evaluated.

With regard to *ERCC2* C22541A, *ERCC2* A35931C, *ERCC4* T30028C and *ERCC5* G3507C, we did not find any statistically significant association between these polymorphisms and chromosome damage in the present study, indicating that these polymorphisms might not functionally important towards chromosomal damage detected by CBMN assay among our study population. Another possible explanation may be that our study did not have enough power to detect small differences in CBMN frequencies among genotypes of these DNA repair genes in coke-oven workers exposed to high level of PAH.

In conclusion, this is the first report to investigate the association between NER gene polymorphisms and chromosome damage, an important event in chemical carcinogenesis, among occupational population exposed to high level of PAHs. Our findings suggest that C allele of *ERCC1* codon 118, A allele of *ERCC6* codon 1097, and A allele of *ERCC2* codon 312 were associated with higher CBMN frequencies among coke-oven workers. The major limitation of our present study is the small number of subjects, so these findings should be verified in further larger studies. Combined with results in our previous study that the polymorphisms of *XRCC1*, which plays a critical role in the BER pathway, could modulate the CBMN frequencies among the same study population, our findings revealed that both NER and BER pathways may be involved in repair of DNA damage that lead to chromosome damage in workers occupationally exposed to high level of PAHs.

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