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Associations between *XRCC1* and *ERCC2* polymorphisms and DNA damage in peripheral blood lymphocyte among coke oven workers

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A wide variety of base damages and single-strand breaks formed by reactive oxygen species during metabolic activation of polycyclic aromatic hydrocarbons (PAHs) have been recognized to be involved in PAH carcinogenesis. In this study, alkaline comet assay was used to detect the DNA damage in peripheral blood lymphocytes among 143 coke-oven workers and 50 non-coke-oven workers, and the effects of genetic polymorphisms of *XRCC1* and *ERCC2* genes on DNA damage were evaluated. The olive tail moment was significantly higher in coke-oven workers than in non-coke-oven workers (2.6, 95% CI = 2.1–3.3 versus 1.0, 95% CI = 0.8–1.2, $p < 0.01$), and significant correlation between ln-transformed urinary 1-OHP and ln-transformed olive tail moment was found in total population ($n = 193$, Pearson's $r = 0.393$, $p < 0.001$) and in coke-oven workers ($n = 143$, Pearson's $r = 0.224$, $p = 0.007$). The olive tail moment was significantly higher in coke-oven workers with *GA* genotype of *G27466A* polymorphism of *XRCC1* than those with *GG* genotype (4.6, 95% CI = 2.5–8.7 versus 2.4, 95% CI = 1.9–2.9, $p < 0.01$ with adjustment for covariates). No significant associations between *C26304T*, *G28152A* and *G36189A* polymorphisms of *XRCC1* and *G23591A* and *A35931C* polymorphisms of *ERCC2* and olive tail moment were found in both groups. The study showed that the alkaline comet assay is a suitable biomarker in the detection of DNA damage among coke-oven workers and it suggested that the *A* allele of *G27466A* polymorphism of *XRCC1* may be associated with decreased DNA repair capacity toward PAH-induced base damage and strand breaks.

Keywords: polycyclic aromatic hydrocarbon, *XRCC1*, *ERCC2*, gene polymorphism, alkaline comet assay.

Introduction

An aetiological link between polycyclic aromatic hydrocarbon (PAH) exposure and lung cancer risk has been established in several occupational exposure circumstances, such as coke production (IARC 1984, 1987), and in cigarette smokers (Doll and Peto 1978, Flanders *et al.* 2003, Harris *et al.* 2004). Besides bulky DNA adducts of carcinogenic PAHs, such as benzo[a]pyrene (B[a]P) diol epoxide (BDPE)–DNA adduct, a wide variety of non-bulky base damage and single-strand breaks (SSB) formed by reactive oxygen species during metabolic activation of PAHs have been recognized to be involved in PAH carcinogenesis

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(Frenkel 1992, Bankson *et al.* 1993, Pryor 1997). Among the several major DNA repair pathways that operate on specific types of damaged DNA by PAHs, base excision repair (BER) is involved in repair of base damage and SSBs, and nucleotide excision repair (NER) is involved in the repair of bulky DNA adducts.

The X-ray repair cross-complementing group 1 (XRCC1) protein acts as a facilitator or coordinator through its interaction with poly (ADP-ribose) polymerase, DNA polymerase β and DNA ligase III in BER and SSB repair (Thompson and West 2000). Three coding polymorphisms in the *XRCC1* gene, including C26304T ($Arg^{194}Trp$), G27466A ($Arg^{280}His$) and G28152A ($Arg^{399}Gln$) have been identified (Shen *et al.* 1998), among which the genotype/phenotype relationship of $Arg^{399}Gln$ polymorphisms was studied most extensively, however, with inconsistent results. The Gln^{399} allele was associated with decreased DNA repair capacity (Lunn *et al.* 1999, Abdel-Rahman and El-Zein 2000, Duell *et al.* 2000, Hu *et al.* 2001, Matullo *et al.* 2001a, Lei *et al.* 2002, Au *et al.* 2003, Li *et al.* 2003, Wong *et al.* 2003, Casse *et al.* 2003). While, other studies failed to show any such correlations (Matullo *et al.* 2001b, Palli *et al.* 2001). Further, an *in vitro* expression experiment found the Arg^{399} and Gln^{399} alleles equally completed both the SSB repair defect and the sensitivity to methyl methanesulfonate in *XRCC1*-deficient EM9 cells (Taylor *et al.* 2002). The excision repair cross-complementing group 2 (ERCC2) protein is involved in the NER pathway by functioning as an ATP-dependent DNA helicase with its 5′–3′ activity joint to the basal transcription factor IIH (TFIIH) (Weber *et al.* 1988). Contrary results have also been reported on its functional significance (Lunn *et al.* 2000, Seker *et al.* 2001, Spitz *et al.* 2001, Matullo *et al.* 2001a,b, Palli *et al.* 2001, Qiao *et al.* 2002, Hou *et al.* 2002, Pastorelli *et al.* 2002, Tang *et al.* 2002, Au *et al.* 2003, Gao, *et al.* 2003).

Alkaline comet assay (pH > 13) could detect DNA strand breaks, alkali-labile sites (ALS) and incomplete excision repair sites (Tice *et al.* 2000). In alkaline electrophoresis condition (pH > 13), the expression of SSBs and conversion of ALS, mainly consisted of modified sugar and base residues (Kohn 1991), to strand breaks were maximized. Now, the alkaline comet assay has been widely used in biomonitoring study in human populations occupationally or environmentally exposed to mutagenic and carcinogenic chemicals. In occupational PAH exposure populations, elevated DNA strand breaks and unstable base damages have been found (Popp *et al.* 1997, Marczynski *et al.* 2002), however, their associations with DNA repair gene polymorphisms have not been characterized.

In this study, the relationships between genetic polymorphisms of *XRCC1* and *ERCC2* and DNA damage detected by alkaline comet assay in coke-oven workers were investigated. The working hypothesis is that genetic variants in DNA repair gene could modify the PAH-induced base damage and strand breaks.

Materials and methods

Study population and sample collection

The 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 this cross-sectional study have been described previously (Leng *et al.* 2004). In brief, 143 coke-oven workers as the exposure group and 50 medical staffs as non-exposure controls were recruited in this study. Exclusion

criteria for participation in the study included recent treatment with mutagenic agents (such as X-ray), chronic conditions (such as autoimmune diseases), and recent acute infections that required medications such as antibiotics. After an informed consent was obtained, all participants were interviewed by an occupational physician using a detailed questionnaire including demographic information, lifestyle matters including smoking history and alcohol consumption, history of occupational exposure and personal medical history. Individuals who had smoked more than 100 cigarettes in their lifetime were considered as smokers and asked for information on a variety of smoking-related questions. Among these smokers, individuals who still smoked when interviewed were classified as current smokers; the others were classified as former smokers. Biological samples, including 4-day-shift-end urine and venous blood, were obtained from each subject and coded after collection.

PAH exposure assessment

The air concentrations of BSM and particulate-phase B(a)P in the working environment of coke-oven workers and non-coke-oven workers were sampled about one and a half month before urine and blood sample collection and analysed according to OSHA Method No. 58 (1986). The excretion of urinary 1-hydroxypyrene (1-OHP) as the internal dose of personal recent PAH exposure was measured according to the method of Jongeneelen *et al.* (1986) with minor modifications (Leng *et al.* 2004). Measurements below the limit of detection (LOD) were replaced with LOD / $\sqrt{2}$ before statistical analysis (Hornung and Reed 1990). The urinary 1-OHP concentrations were corrected by urinary creatinine and presented as μmol mol^{-1} creat.

Comet assay in peripheral blood lymphocyte

The blood samples for alkaline comet assay were stored at 4°C after venipuncture for no more than 1 h before separation of lymphocytes. Lymphocytes were separated from approximately 1 ml heparinized whole blood and suspended in 500 μl ice-cold PBS. Cells were routinely checked for variability by Trypan Blue exclusion test and the variability was always >90%. The comet assay was performed immediately after lymphocyte separation according to Singh *et al.* (1988) with minor modifications. The experimental protocol was as follows. Slides were first layered with 100 μl 1% normal melting agarose in PBS, then covered immediately with a cover slip and stored at 4°C for about 20 min to allow the agarose to solidify. After solidification, the cover lip was removed carefully and 10 μl PBS containing 10^5 cells was mixed with 100 μl 1% low melting agarose at 37°C was pipetted on the first layer. The slides were covered with cover lip again and kept at 4°C for 20 min. After removal of cover lips, the slides were immersed in a lyses solution (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris, with freshly added 1% Triton X-100 and 10% DMSO, pH 10) for 1 h at 4°C. The slides were removed from the lyses solution, washed with alkaline electrophoresis buffer three times and were then placed in a horizontal gel electrophoresis tank with freshly prepared alkaline buffer (300 mM NaOH, 1 mM Na_2-EDTA, pH 13.0) at 4°C for 40 min to allow DNA unwinding and expression of alkali-labile site. Electrophoresis was conducted for the next 20 min at 1.25 V cm^{-1} using an electrophoresis compact power supply (Beijing Liuyi Instrument Factory, Beijing, China), and electrophoresis current was adjusted to 300 mA by raising or lowering the buffer level. After electrophoresis, the slides were washed gently to remove the alkaline and detergents by placing them horizontally and flooding them slowly with 0.4 M Tris buffer at pH 7.5. After neutralization, the agarose gel was desiccated by immersing slides in 100% ice-cold ethanol for no more than 10 min and left in room temperature for dry. All operations were finished within about 6 h after blood sampling in a darkroom. The slides were stored in airproof boxes with desiccant and shipped back laboratory. The slides were stained by ethidium bromide and examined at 200× magnification with Olympus IX 50 microscope equipped with a 100-W mercury lamp and WG filter block. Measurements were made using an image analysis system (version 1.0, IMI comet analysis software, China; Zhu *et al.* 2001). Over 100 cells per subject were scored (50 cells per each of two replicate slides). Tail length (TL) and olive tail moment (olive TM) were calculated. As the two indexes were closely correlated ($r=0.977$, $n=193$ for all subjects), only olive TM was used as measurement of DNA migration in this paper. For each subject, the arithmetic mean of olive TM of 100 cells was presented as DNA damage level in the following statistical analysis.

XRCC1 *and* ERCC2 *genotyping*

DNA was isolated from whole blood using the standard method (Miller *et al.* 1988). Four SNPs in *XRCC1* gene, including *C26304T* ($Arg^{194}Trp$), *G27466A* ($Arg^{280}His$), *G28152A* ($Arg^{399}Gln$) and *G36189A* ($Gln^{632}Gln$) were detected using method of PCR-RFLP (Shen *et al.* 1998, Butkiewicz *et al.* 2001, Mort *et al.* 2003). The *G23591A* ($Asp^{312}Asn$) and *A35931C* ($Lys^{751}Gln$) polymorphisms of *ERCC2* gene were determined according to published protocols (Shen *et al.* 1998). All genotypes were evaluated and agreed upon by at least two persons independently. Ten percent of DNA samples were genotyped a second time and the concordance was 100%.

Statistical analysis

Urinary 1-OHP and olive TM were natural logarithm (ln)-normally distributed and geometric means and 95% confidence interval (CI) were presented. Student's *t*-test or ANOVA test followed by Bonferroni correction for multiple comparisons was used to compare the ln-transformed olive TM by age, sex, smoking status and coking history as appropriate in two study groups separately. Pearson correlation was used to evaluate the relationship between ln-transformed olive TM and ln-transformed urinary 1-OHP. Multiple analysis of covariance followed by Bonferroni correction for multiple comparisons when the overall *F*-test was significant was used to study the association between *XRCC1* and *ERCC2* genotypes and the ln-transformed olive TM with adjustment for ln-transformed urinary 1-OHP, age (coking history for coke-oven workers), sex and cigarettes per day. All statistical tests were two-sided ($\alpha = 0.05$) and performed using Statistical Analysis System software (version 8.0; SAS Institute, Inc., Cary, NC, USA).

Results

The demographic data of coke-oven workers and non-coke-oven workers is summarized in table 1. The distributions of age, sex and prevalence of drinking were not significantly different between the two exposure groups, but the percentages of current smokers and number of cigarettes smoked per day were higher in coke-oven workers than in non-coke-oven workers ($p < 0.05$). The medians of air BSM and particulate-phase B[a]P were significantly higher in coke-oven (BSM, 328.6 μg m^{-3} and B[a]P, 926.9 ng m^{-3}, $n = 30$) than in non-coke-oven working environment (BSM, 97.8 μg m^{-3} and B[a]P, 49.1 ng m^{-3}, $n = 10$) ($p < 0.01$) with the highest at the top-of-oven (BSM, 1233.3 μg m^{-3} and B[a]P, 2634.9 ng m^{-3}, $n = 10$) (data not shown). The geometric mean of urinary 1-OHP was significantly higher in coke-oven workers than in non-coke-oven workers (11.3 versus 0.6 μmol mol^{-1} creat, $p < 0.01$) (table 1).

The olive TM was significantly higher in coke-oven workers (2.6, 95% CI = 2.1–3.3) than in non-coke-oven workers (1.0, 95% CI = 0.8–1.2). Because there was statistically significant difference for the percentages of current smokers and number of cigarettes smoked per day between the two exposure populations,

Table 1. Demographic data of coke-oven and non-coke-oven workers in a Chinese occupational population.

Variables	Non-coke-oven workers	Coke-oven workers	*p*
Number	50	143	
Age (years, mean ± SD)	40 ± 6	39 ± 7	0.75[a]
Sex (men/women, % of men)	45/5 (90.0)	131/12 (91.6)	0.73[b]
Current smokers (yes/no, %)	19/31 (38.0)	92/51 (64.3)	<0.01[b]
Cigarettes/day (mean ± SD)	5.1 ± 7.6	8.2 ± 7.2	0.01[c]
Alcohol user (yes/no, %)	20/30 (40.0)	62/81 (43.4)	0.68[b]
Coking history (years, mean ± SD)	–	18.4 ± 7.2	–
BSM (μg m^{-3}, median (range))	97.8 (2.6–149.1)	328.6 (61.8–2677.8)	<0.01[c]
Particulate-B[a]P (ng m^{-3}, median (range))	49.1 (43.0–51.5)	926.9 (250.7–3723.1)	<0.01[c]
Urinary 1-OHP (μmol mol^{-1} creatinine, geometric mean (95% CI))	0.6 (0.5–0.7)	11.3 (9.8–13.1)	<0.01[d]

[a]Student's *t*-test for difference between coke-oven workers and non-coke-oven workers.
[b]Chi-square tests for differences in distributions between coke-oven workers and non-coke-oven workers.
[c]Mann–Whitney *U*-tests for differences between coke-oven workers and non-coke-oven workers.
[d]Student's *t*-test for ln-transformed data between coke-oven workers and non-coke-oven workers.

we compared the olive TM between the two exposure populations in current smokers and in non-current smokers respectively and found similar results. Significant correlation between ln-transformed urinary 1-OHP and ln-transformed olive TM was found in total population ($n=193$, Pearson's $r=0.393$, $p<0.001$) (figure 1) and in coke-oven workers ($n=143$, Pearson's $r=0.224$, $p=0.007$) (figure 3), while in non-coke-oven workers, no significant correlation was found (figure 2). We further classified the coke-oven workers into three groups according to their coking history, including 0.5–16, 16–23 and 23–32 years. Significant association between coking history and olive TM was found in coke-oven workers (ANOVA test, $p=0.02$, table 2). Pair wise tests with Bonferroni correction found significant difference between 23–32 and 0.5–16 years ($p=0.03$). In consideration of the confounding effect of age, analysis of covariance was used to adjust the effect of age and similar result was found (data not shown). We did not find significant influences of age, sex and smoking status on olive TM either in coke-oven workers or in non-coke-oven workers (table 2).

The frequencies for variant alleles of *C26304T*, *G27466A*, *G28152A* and *G36189A* polymorphisms of *XRCC1* were 0.33, 0.08, 0.27 and 0.07, respectively, and those of *G23591A* and *A35931C* polymorphisms of *ERCC2* gene were 0.07 and 0.07 in the 193 study subjects. All the six genotypes' distributions were in Hardy–Weinberg equilibrium and were similar between coke-oven workers and non-coke-oven workers (data not shown).

The effects of *XRCC1* and *ERCC2* genotypes on olive TM in two study groups were presented in table 3. In coke-oven workers, significant association between *G27466A* polymorphism and olive TM was found, the olive TM was significantly higher in subjects with *GA* genotype (4.6, 95% CI = 2.5–8.7) than those with *GG* genotype (2.4, 95% CI = 1.9–2.9, $p<0.01$) with adjustment for ln-transformed urinary 1-OHP, coking history, sex and cigarettes per day. The associations between *C26304T*, *G28152A* and *G36189A* polymorphisms and olive TM were not

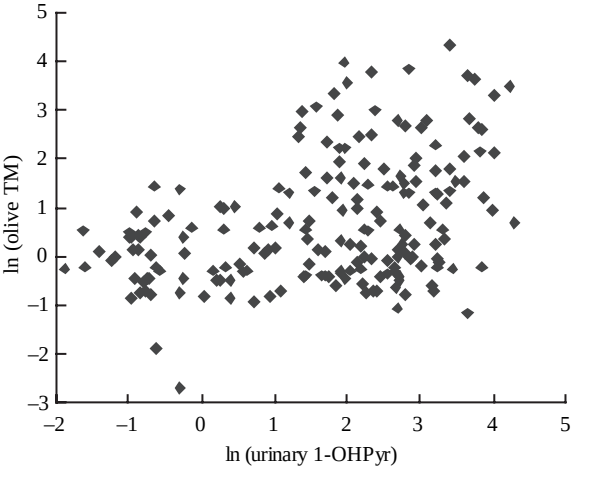

Figure 1. Scatter plot between ln-transformed olive tail moment and ln-transformed urinary 1-OHP levels in coke-oven workers and non-coke-oven workers. Pearson Correlation $n=193$, Pearson's $r=0.393$, $p<0.001$.

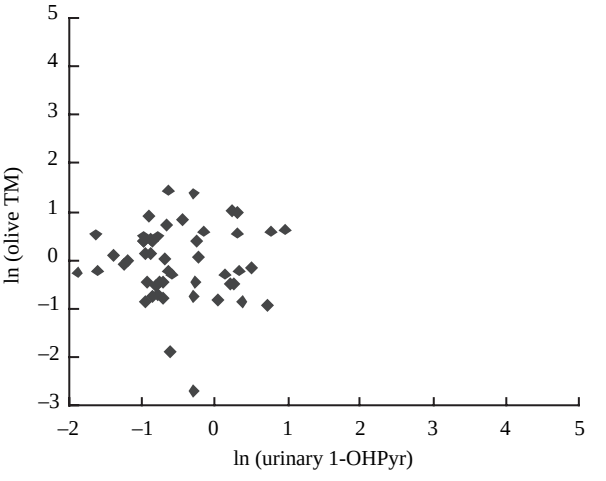

Figure 2. Scatter plot between ln-transformed olive tail moment and ln-transformed urinary 1-OHP levels in non-coke-oven workers. Pearson Correlation $r = -0.005$, $p = 0.972$.

significant among coke-oven workers. No overall associations between four *XRCC1* polymorphisms and olive TM were found in non-coke-oven workers. We could not find any significant influences of *ERCC2* genotypes on olive TM in both exposure populations.

Discussion

In the present study, the coke-oven workers appeared to be exposed to a higher level of PAHs based on the stationery environmental monitoring, including air

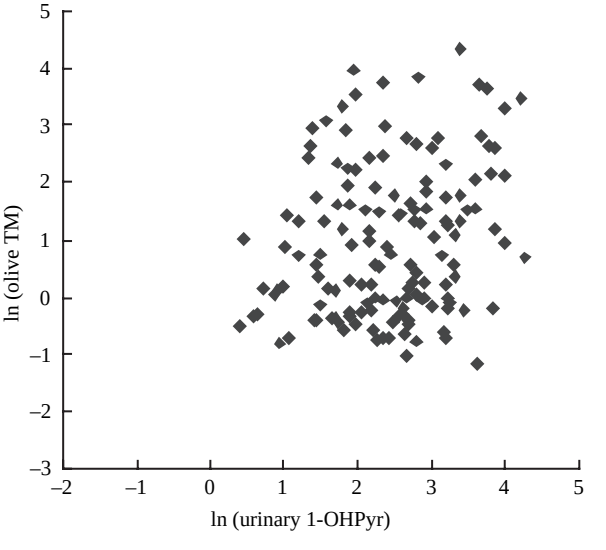

Figure 3. Scatter plot between ln-transformed olive tail moment and ln-transformed urinary 1-OHP levels in coke-oven workers. Pearson Correlation $r = 0.224$, $p = 0.007$.

Table 2. Olive tail moment by age, sex, smoking status and coking history.

	Non-coke-oven workers			Coke-oven workers			
Variables	*n*	Geometric mean (95% CI)	p^a	*n*	Geometric mean (95% CI)	p^a	p^b
All	50	1.0 (0.8–1.2)		143	2.6 (2.1–3.3)		<0.01
Age (years)			0.99			0.17	
<39	24	1.0 (0.7–1.4)		62	2.2 (1.6–3.0)		<0.01
≥39	26	1.0 (0.7–1.3)		81	3.0 (2.2–4.0)		<0.01
Sex			0.78			1.00	
Men	45	0.9 (0.8–1.2)		131	2.6 (2.1–3.3)		<0.01
Women	5	1.1 (0.4–2.6)		12	2.6 (1.1–6.5)		0.23
Smoking status			0.19			0.53	
Current smokers	19	1.2 (0.8–1.6)		92	2.8 (2.1–3.7)		0.01
Non-current smokers	31	0.9 (0.6–1.1)		51	2.4 (1.7–3.4)		<0.01
Coking history (years)						0.02	
0.5–16				43	1.7 (1.2–2.4)		
16–23				52	3.0 (2.1–4.4)		
23–32				48	3.4 (2.3–5.0)[c]		

[a]Student's *t*-tests for the differences in ln-transformed olive tail moment between the categories of selected variables.

[b]Mann–Whitney *U*-tests for the differences in olive tail moment between the coke-oven workers and non-coke-oven workers in each category of selected variables.

[c]$p = 0.03$, post-hoc (Bonferroni) comparison with 0.5–16-year workers.

BSM (328.6, range 61.8–2677.8 μg m^{-3}) and particulate-phase B[a]P (926.9, range 250.7–3723.1 ng m^{-3}), and levels of urinary 1-OHP (11.3, 95% CI = 9.8–13.1 μmol mol^{-1} Cr), compared with several studies using alkaline comet assay (Carstensen *et al.* 1999, Marczynski *et al.* 2002, Crebelli *et al.* 2002) or alkaline filer elution assay (Popp *et al.* 1997) to detect the oxidative DNA damage in occupational PAH exposure populations. In our study, the olive TM was significantly higher in coke-oven workers than in non-coke-oven workers, and dose–response relationship was found between occupational PAH exposure (expressed as urinary 1-OHP and coking history) and olive TM in coke-oven workers. Although there was statistically significant difference for the percentages of current smokers and number of cigarettes smoked per day between the two exposure populations, we believed that the genotoxic effects seen in this study were very much driven by PAH exposure parameters because of the lack of association between smoking and DNA damage in the two exposure populations and because we compared the olive TM between the two exposure populations in current smokers and in non-current smokers respectively and found significant difference. The association between PAH exposure and olive TM suggested that alkaline comet assay is a potential biomarker to detect the PAH-induced DNA damage in coke-oven workers exposed to high levels of PAHs.

The distributions of *XRCC1* and *ERCC2* genotypes in coke-oven workers were similar to those in non-coke-oven workers and the genotype's frequencies were consistent with those described in the literature for the Chinese population (Ratnasinghe *et al.* 2001, Liang *et al.* 2003), which suggested no selection bias for the subjects' enrolments in terms of genotypes.

Table 3. Olive tail moment relative to *XRCC1* and *ERCC2* genotypes.

Genotypes	Non-coke-oven workers			Coke-oven workers			
	n	Geometric mean 95% CI	p^a	*n*	Geometric mean 95% CI	p^b	p^c
XRCC1 genotypes							
C26304T			0.12			0.65	
CC	28	1.0 (0.7–1.3)		55	2.3 (1.7–3.2)		<0.01
CT	18	1.0 (0.8–1.5)		76	2.8 (2.1–3.8)		<0.01
TT	4	0.5 (0.1–4.7)		12	3.4 (1.3–9.0)		0.04
G27466A			0.22			<0.01	
GG	42	0.9 (0.7–1.2)		119	2.4 (1.9–2.9)		<0.01
GA	8	1.3 (0.9–1.8)		24	4.6 (2.5–8.7)		0.05
AA	0			0			
G28152A			0.19			0.30	
GG	31	0.9 (0.7–1.3)		71	3.0 (2.1–4.2)		<0.01
GA	15	0.9 (0.6–1.2)		63	2.4 (1.8–3.3)		<0.01
AA	4	1.8 (0.5–6.5)		9	1.7 (0.4–3.4)		0.85
G36189A			0.48			0.37	
GG	42	0.9 (0.7–1.2)		123	2.7 (2.2–3.4)		<0.01
GA	8	1.2 (0.7–2.1)		20	2.1 (1.2–3.8)		0.12
AA	0			0			
ERCC2 genotypes							
G23591A			0.78			0.17	
GG	46	1.0 (0.8–1.2)		120	2.5 (2.0–3.2)		<0.01
GA	4	0.8 (0.3–2.1)		23	3.5 (1.9–6.4)		0.05
AA	0			0			
A35931C			0.62			0.37	
AA	45	0.9 (0.7–1.2)		120	2.8 (2.2–3.5)		<0.01
AC	5	1.1 (0.5–2.3)		23	2.0 (1.3–3.2)		0.32
CC	0			0			

[a]Multiple analysis of covariance tests for differences in ln-transformed olive tail moment between genotypes with adjustment for ln-transformed urinary 1-OHP, age, sex and cigarettes per day in non-coke-oven workers.

[b]Multiple analysis of covariance tests for differences in ln-transformed olive tail moment between genotypes with adjustment for ln-transformed urinary 1-OHP, coking history, sex and cigarettes per day in coke-oven workers.

[c]Mann–Whitney *U*-tests for the differences in olive tail moment between the coke-oven workers and non-coke-oven workers in each genotype.

We further investigated the relationship between polymorphisms in the BER gene *XRCC1* and the NER gene *ERCC2* and DNA damage detected by alkaline comet assay in peripheral blood lymphocytes among coke-oven workers. We found that *XRCC1* gene *G27466A* variant allele was associated with high olive TM levels among coke-oven workers. Because of the relative low distribution frequency for variant allele of *G27466A* polymorphism of *XRCC1* in Caucasians and Asians, there were few studies concerning the effect of this polymorphism with phenotypic biomarker or cancer risk. The variant in *G27466A* polymorphism of *XRCC1* has been found to be associated with significant increase of bleomycin-induced chromatid-type breaks in 80 healthy Caucasian smokers (Tuimala *et al.* 2002), an increased lung cancer risk (adjusted OR = 1.8, 95% CI = 1.0–3.4) in a nested case-control study within a Chinese cohort of tin miners with adjustment for radon and tobacco exposure (Ratnasinghe *et al.* 2001), the evaluated DNA damage levels in a Chinese population with occupational formaldehyde exposure (unpublished

data) and an increased risk for occupational chronic benzene poisoning (adjusted OR = 1.9, 95% CI = 1.2–3.1) in a Chinese case-control study (Zhang *et al.* 2004). These four studies supported our findings and suggested that *A* allele in *G27466A* polymorphism of *XRCC1* gene may be associated with decreased DNA repair capacity, although the functional significance of this polymorphism has not been clearly characterized.

We could not find any significant associations between *ERCC2* polymorphisms and olive TM, suggesting that the NER pathway was not important or of a backup role for repair of PAH-induced DNA damage detected by alkaline comet assay. Several studies simultaneously investigated the associations between *XRCC1* and *ERCC2* and DNA damage with unambiguous repair mechanisms. For DNA damages, such as BPDE–DNA adducts quantified by acid hydrolysis combined with high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selected ion recording after immunoaffinity purification (Seker *et al.* 2001) and ultraviolet light-induced chromatid breaks (Au *et al.* 2003), which were mainly repaired by NER pathway, only *ERCC2* polymorphisms were found to significantly influence the genetic damages. Qiao *et al.* (2002) also found that it was *ERCC2* but *XRCC1* polymorphisms that significantly influenced the DNA repair capacity evaluated by a host-cell reactivation assay of repair of ultraviolet damage to DNA. While, for DNA damages, such as polyphenol DNA adducts (Duell *et al.* 2000) and X-ray induced chromosome deletions (Au *et al.* 2003), which were mainly repaired by BER pathway, only *XRCC1* polymorphisms were found to significantly influence these genetic damages. Using appropriate biomarkers in association studies of DNA repair gene polymorphisms is important for delineating the exact role of the genes and gene polymorphisms. The SSBs and unstable base damage detected by alkaline comet assay are mainly repaired by BER pathway. While the bulky DNA adducts mainly repaired by NER pathway, such as BPDE–DNA adduct, were stable even under alkali treatment, it is difficult for alkaline comet assay to detect them unless with method modification (Crebelli *et al.* 2002). Therefore, no significant association between *ERCC2* polymorphisms and olive TM in our study is of biological possibility.

In conclusion, this is the first report to investigate the associations between *XRCC1* and *ERCC2* polymorphisms and DNA damage in occupational PAH exposed population. Our finding suggests that variant allele of *G27466A* polymorphism of *XRCC1* is associated with increased level of DNA damage in high-level PAH exposed workers. However, the relative low frequency for *G27466A* variant of *XRCC1* in Chinese population makes us cautious in extrapolating our results and that whether the *G27466A* polymorphism of *XRCC1* itself is the causal variant needs to be validated in future study because the *G27466A* polymorphism may be in linkage disequilibrium with the really causal SNPs in *XRCC1* gene or in adjacent genes nearby.

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