Genetic Polymorphisms in *XRCC1*, *APE1*, *ADPRT*, *XRCC2*, and *XRCC3* and Risk of Chronic Benzene Poisoning in a Chinese Occupational Population

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Abstract

DNA damage induced by benzene is an important mechanism of its genotoxicity that leads to chronic benzene poisoning (CBP). Therefore, genetic variation in DNA repair genes may contribute to susceptibility to CBP in the exposed population. Because benzene-induced DNA damage includes single- and double-strand breaks, we hypothesized that single-nucleotide polymorphisms in X-ray repair cross-complementing group 1 (*XRCC1*), apurinic/apyrimidinic endonuclease (*APE1*), ADP ribosyltransferase (*ADPRT*), X-ray repair cross-complementing group 2 (*XRCC2*), and X-ray repair cross-complementing group 3 (*XRCC3*) are associated with risk of CBP. We genotyped single-nucleotide polymorphisms at codons 194, 280, and 399 of *XRCC1*, codon 148 of *APE1*, codon 762 of *ADPRT*, codon 188 of *XRCC2*, and codon 241 of *XRCC3* in 152 CBP patients and 152 healthy workers frequency matched on age and sex among those who were occupationally exposed to benzene. The genotypes were determined by PCR-RFLP technique with genomic DNA. We found that no individuals had the *XRCC2* codon 188 variant alleles or *Met/Met* genotype of *XRCC3* codon 241 in this study population. However, individuals carrying the *XRCC1 194Trp* allele (i.e., *Arg/Trp+Trp/Trp* genotypes) had a decreased risk of CBP [adjusted odds ratio (OR_{adj}), 0.60; 95% confidence interval (95% CI), 0.37-0.98; $P = 0.041$] compared with subjects with the *Arg/Arg* genotype whereas individuals carrying the *XRCC1 280His* allele (i.e., *Arg/His+His/His* genotypes) had an increased risk of CBP compared with those with the *Arg/Arg* genotype (OR_{adj}, 1.91; 95% CI, 1.17-3.10; $P = 0.009$). The analysis of haplotypes of polymorphisms in *XRCC1* showed that there was a 2.96-fold (OR, 2.96; 95% CI, 1.60-5.49; $\chi^2 = 12.39$, $P = 0.001$) increased risk of CBP for subjects with alleles of *XRCC1 194Arg*, *XRCC1 280His*, and *XRCC1 399Arg* compared with those carrying alleles of *XRCC1 194Arg*, *XRCC1 280Arg*, and *XRCC1 399Arg*. Therefore, our results suggest that polymorphisms at codons 194 and 280 of *XRCC1* may contribute to CBP in a Chinese occupational population. (Cancer Epidemiol Biomarkers Prev 2005;14(11):2614–9)

Introduction

As an important component of many organic solvents, benzene is also commonly used to synthesize organic chemicals. The major industries using benzene are those involved in the production of rubber, paint, shoes, lubricants, dyes, detergents, drugs, and pesticides (1). Humans are exposed to benzene via either occupational exposure or environmental exposure to cigarette smoke, gasoline emissions, or products of incomplete combustion (2). Exposure to benzene may result in chronic benzene poisoning (CBP) characterized by hematotoxicities, leading to pancytopenia, aplastic anemia, myelodysplastic syndrome, and acute myeloid leukemia (3-7).

In biological systems, inhaled benzene is converted to benzene oxide, which is ultimately metabolized to phenol, catechol, hydroquinone, and benzoquinone (8, 9), and it is in this bioactivation process that substantive reactive oxygen species, such as hydroxyl radicals, are formed (10, 11). Benzene metabolites bind covalently to macromolecules, such as proteins and DNA in the tissue, and therefore cause genotoxicity (10, 12) as a result of oxidative damage to DNA or DNA double-strand breaks caused by reactive oxygen species.

Previous mechanistic studies of CBP showed that *p*-benzoquinone, a stable metabolite of benzene, formed two-ring benzetheno exocyclic base adducts with the bases C, A, and G of DNA, and the *p*-benzoquinone-C adducts were the substrate for apurinic/apyrimidinic endonuclease (APE1; refs. 13-15), the rate-limiting enzyme in base-excision repair (16). After excision of these adducts, other components involved in base-excision repair [i.e., X-ray repair cross-complementing group 1 (XRCC1), ADP ribosyltransferase (ADPRT), DNA polymerase β, and DNA ligase III] will complete this repair process (17). Strongly induced by the presence of single DNA strand breaks, ADPRT interacts with XRCC1 protein to participate in DNA repair and in the recovery of cells from DNA damage (18-21).

In cultured HL-60 cells treated with *trans, trans*-muconaldehyde, hydroquinone, and their mixtures, the formation of DNA-protein cross-links and DNA strand breaks was observed (12), and increased homologous recombination repair was detected in cultured Chinese hamster ovary cells administrated with phenol, catechol, and benzoquinone (22). These results indicate that DNA repair genes involved in base-excision repair, such as *XRCC1, ADPRT*, and *APE1*, and in homologous recombination repair, such as X-ray repair cross-complementing group 2 (*XRCC2*) and X-ray repair cross-complementing group 3 (*XRCC3*) genes, play a role in repair of benzene-induced DNA damage. Therefore, genetic variation in these genes may be associated with risk of developing CBP in the exposed population.

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Table 1. Primer sequences, condition for amplification, restriction pattern, and restriction enzymes used

Polymorphisms	Primer sequences	Annealing temperature (°C)	Length (bp)	Restriction enzyme	Restriction pattern (bp)
XRCC1 Arg194Trp	F: 5′-GCCCCGTCCCAGGTA-3′ R: 5′-AGCCCCAAGACCCTTTCACT-3′	58	491	*Msp*I	*Arg/Arg*: 292, 178, 21; *Arg/Trp*: 313, 292, 178, 21; *Trp/Trp*: 313, 178
XRCC1 Arg280His	F: 5′-TGGGGCCTGGATTGCTGGGTCTG-3′ R: 5′-CAGCACCACTACCACACCCTGAAGG-3′	69.5	280	*Rsa*I	*Arg/Arg*: 280; *Arg/His*: 280, 140; *His/His*: 140
XRCC1 Arg399Gln	F: 5′-TTGTGCTTTCTCTGTGTCCA-3′ R: 5′-TCCTCCAGCCTTTTCTGATA-3′	56	615	*Msp*I	*Arg/Arg*: 377, 238; *Arg/Gln*: 615, 377, 238; *Gln/Gln*: 615
APE1 Asp148Glu	F: 5′-CTGTTTCATTTCTATAGGC*T* A-3′* R: 5′-AGGAACTTGCGAAAGGCTTC-3′	54	165	*Bfa*I	*Glu/Glu*: 146, 19; *Glu/Asp*: 165, 146, 19; *Asp/Asp*: 165
ADPRT Val762Ala	F: 5′-TTTTGCTCCTCCAGGCCAACG-3′* R: 5′-CCTGACCCTGTTACCTTAATGTCAGTTTT-3′	57	156	*Bsh*1236I	*Val/Val*: 156; *Val/Ala*: *156*, 135, 21; *Ala/Ala*: 135, 21;
XRCC2 Arg188His	F: 5′-GCGTCAATGGAGGAGAAAGTGTGAA-3′ R: 5′-TTGTGTCGTTGCAAAAAAACA*C*G-3′*	55	113	*Pma*cI	*Arg/Arg*: 113; *Arg/His*: 113, 89, 24; *His/His*: 89, 24
XRCC3 Thr241Met	F: 5′-GGTCGAGTGACAGTCCAAAC-3′ R: 5′-TGCAACGGCTGAGGGTCTT-3′	60	456	*Nla*III	*Thr/Thr*: 316, 140; *Thr/Met*: 316, 211, 140, 105; *Met/Met*: 211, 140, 105

*Underlined base modifies primer sequence, introducing a restriction site in the presence of the A or C nucleotide.

A number of single-nucleotide polymorphisms have been identified in *XRCC1, APE1, ADPRT, XRCC2*, and *XRCC3*, some of which are relatively common, including single-nucleotide polymorphisms at codons 194 (Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln) of *XRCC1* (23), codon 148 (Asp to Glu) of *APE1* (24), codon 762 (Ala to Val) of *ADPRT* (25, 26), codon 188 (Arg to His) of *XRCC2* (27), and codon 241 (Thr to Met) of *XRCC3* (27, 28). These single-nucleotide polymorphisms have been studied for their roles in the development of cancers of the breast, bladder stomach, and lung (29-33). However, whether or not these common genetic polymorphisms may affect risk of CBP is still unknown. Therefore, using the case-control design and PCR-RFLP technique, we investigated the associations between these genetic polymorphisms and risk of developing CBP in a Chinese occupational population who had been exposed to benzene in the workplace.

Materials and Methods

Subjects. The detailed information of the study population has been reported previously (34). Briefly, 112 of 152 CBP patients in this study were from four factories where clusters of the cases were reported in Shanghai, Hangzhou, Maanshan, and Guangzhou, China. Another 40 patients, who returned to the hospital periodically for health examination, were from other 12 small factories that had been closed down. The benzene poisoning diagnoses were made between 1980 and 1998 by the Authorized Local Occupational Disease Diagnostic Team. The diagnostic criteria for occupational CBP, according to the Ministry of Health, China, include (*a*) total WBC counts <4,000/μL or WBC counts between 4,000 and 4,500/μL and platelet counts <80,000/μL, with repeated confirmation of these counts in a few months in a peripheral blood examination; (*b*) documented benzene exposure as a result of an employment for at least 6 months in the factory; and (*c*) exclusion of other known causes of abnormal blood counts such as chloromycetin use and ionizing radiation. The medical records of these patients were independently reviewed by at least two hemopathologists, especially for those with WBC counts >3,500 to confirm the CBP diagnosis. One hundred fifty-two healthy workers that had been occupationally exposed to benzene in the four factories were selected as controls. The cases and controls were frequency matched on age within 5 years, sex, exposure duration within 3 years, and exposure level at the workplace. The calendar range for start of employment of the cases and the controls was from 1958 to 1998 and from 1963 to 1998, respectively. All the eligible individuals agreed to participate in this study after an informed consent was orally explained and obtained.

The subjects were interviewed by a trained personnel and a questionnaire was used to obtain general information including ethnic background, nutrition, cigarette smoking, alcohol consumption, protective measures, self-reported symptoms, medical history, and occupational history, such as work unit (department), type of work, and exposure duration. Exposure estimation was based on monitoring data obtained by industrial hygienists and long-term employees' evaluation considering historical changes in the workplace (35). The intensity of benzene exposure (milligrams per cubic meter) for the patients was taken as the benzene level of workplaces when diagnoses were made; the intensity of benzene exposure for the controls was taken as the current level monitored by organic vapor passive dosimetry badges during collection of the blood samples from the controls. Those who smoked at least one cigarette per day for more than 1 year were considered regular smokers. Alcohol consumption was defined as drinking at least 7 standard units of alcohol on average per week [1 standard unit = 10 g of alcohol equivalent; e.g., a glass/can/bottle (330 mL) of regular beer (5%), a measure (40 mL) of liquor, a glass (120 mL) of wine] for more than 6 months. Under the criteria, the smokers and alcohol users in cases satisfied it when they were diagnosed CBP whereas those in controls meant current status when they enrolled in the study. The subjects were administrated a rigorous physical examination at a local occupational disease hospital. Alanine aminotransferase level in serum was also examined to assess liver functions.

Collection of Blood Samples. Blood samples of the subjects enrolled in this study were collected only after informed consent was obtained. Blood was immediately frozen at −80°C after collection and was sent to the laboratory later on dry ice.

PCR-RFLP Genotyping Assays. Genomic DNA was extracted from blood samples by a routine phenol-chloroform method (36). The genotypes were determined by the PCR-RFLP method and primers, annealing temperature, length of amplified fragments, restriction pattern, and restriction enzymes used are listed in Table 1. The PCR was done using 50 ng of genomic DNA, 0.2 μmol/L of each primer, 1 × PCR buffer, 0.2 μmol/L of each deoxynucleotide triphosphate, 2.0 mmol/L $MgCl_2$, and 0.625 units of Taq in a 25-μL reaction volume. PCR program was a 5-minute denaturation step at 95°C followed by 35 cycles of 94°C for 50 seconds, 55°C to 69.5°C for 30 to 50 seconds, 72°C for 30 to 50 seconds

(according to the length of fragment amplified), respectively, and a final extension step at 72°C for 10 minutes.

The variant alleles at codons 194, 280, and 399 of *XRCC1* create *Msp*I, *Rsa*I, and *Msp*I sites, respectively (37, 38); the variant alleles at codon 148 of *APE1*, codon 762 of *ADPRT*, codon 188 of *XRCC2*, and codon 241 of *XRCC3* create *Bfa*I, *Bsh*1236I (*Fnu*DII, *Bst*I), *Pma*cI (*Eco*721), and *Nla*III sites (29), respectively. After digestion at 37°C for 16 hours (*Msp*I, *Rsa*I, *Bfa*I, *Bsh*1236I, and *Pma*cI are from Fermentas, Inc., Vilnius, Lithuania; *Bfa*I and *Nla*III are from New England Biolabs, Inc., Beverly, MA) and resolved on 2% to 3% Metaphor agarose gels (BBI, Toronto, Canada). The digested PCR products were observed under UV image system (Gel Doc 2000, Segrate, Milan, Italy). All genotypes were evaluated and agreed on by at least two persons independently. Ten percent of DNA samples were selected randomly for repeat and the concordance was 100%.

Statistical Analysis. Student's *t* test was used to compare differences between continuous variables, such as age and exposure duration, and χ^2 test was used to examine the differences in the distribution of genetic polymorphisms between cases and controls. The odds ratio (OR) and 95% confidence interval (95% CI) for estimating the associations between genotypes of *XRCC1, APE1, ADPRT, XRCC2*, and *XRCC3* and risk of CBP were obtained from both univariate and multivariate unconditional logistic regression models with adjustment for potential confounding factors, such as age, sex, exposure duration, and intensity of benzene exposure (adjusted OR and 95% CI). Haplotype analysis was conducted with PHASE 2.0.2 software (University of Washington, Seattle, WA). All tests were done by using SPSS 10.0 software (SPSS, Inc., Chicago, IL).

Results

Characteristics of Cases and Controls. The distributions of age, sex, exposure duration, intensity of benzene exposure, type of work, cigarette smoking, and alcohol consumption in cases and controls are presented in Table 2. Overall, there was slightly difference in the distributions of these variables between cases and controls but none of them were statistically significant. For example, the median age and exposure duration were 36.78 (range, 21.00-61.00) and 12.97 (range, 1.00-38.00) in the 152 CBP cases, respectively, and 38.25 (range: 19.00-57.00) and 11.10 (range: 1.00-36.00) in the 152 controls (data not shown), respectively. There was no significant difference in the distribution of age groups (≤25, 26-35, 36-45, >45 years), exposure duration (≤5, 6-10, 11-15, 16-20, >25 years), and intensity of benzene exposure (≤40, 41-100, >100 mg/m^3; $P > 0.05$ for all). However, the percentage of female subjects (59.9%) was higher than that of males (40.1%) in both cases and controls (62.5% versus 37.5%). This higher female ratio may explain the relatively low frequency of cigarette smoking (17.4%) and alcohol consumption (11.5%) in the subjects than expected from the general population because the frequency of cigarette smoking and alcohol consumption among men is much higher than among women (39-41). The range for total WBC counts in the controls was from 4,500/μL to 5,900/μL; the mean was 4,800 ± 260/μL.

Genetic Polymorphisms of *XRCC1, APE1, ADPRT, XRCC2*, and *XRCC3*. The frequencies of *XRCC1, APE1, ADPRT, XRCC2*, and *XRCC3* variant alleles and genotypes in cases and controls are presented in Table 3. No *XRCC2* codon 188 variant genotype was detected, the frequency of *XRCC3 241Met* allele was low (0.06), and no *Met/Met* genotype was detected. However, the variant allele and genotype frequency of *XRCC1 Arg280His* polymorphism were significantly different between cases and controls ($P < 0.01$) but the variant allele and genotype frequencies of *XRCC1 Arg194Trp, XRCC1 Arg399Gln, APE1 Asp148Glu, ADPRT Val762Ala*, and *XRCC3 Thr241Met* were not ($P > 0.05$ for all). The distributions of the genotypes of these genetic polymorphisms in the controls were all in Hardy-Weinberg equilibrium ($P > 0.05$, χ^2 test).

Effect of Genetic Polymorphisms in *XRCC1, APE1, ADPRT*, and *XRCC3* on Risk of CBP. No statistical difference was found in the distribution of genotypes of *XRCC1 Arg399Gln, APE1 Asp148Glu, ADPRT Val762Ala*, and *XRCC3 Thr241Met* ($P > 0.05$). However, the proportion of individuals carrying *XRCC1 194Arg/Trp+Trp/Trp* was lower in cases (50.7%) than in controls (62.2%; $P = 0.041$) and the proportion for individuals carrying *XRCC1 280Arg/His+His/His* genotypes was higher in cases (52.1%) than in controls (36.0%; $P = 0.009$). Correspondingly, the risk of CBP for individuals carrying of *XRCC1 194Arg/Trp+Trp/Trp* genotypes was 1.67-fold lower (OR_{adj}, 0.60; 95% CI, 0.37-0.98; $\chi^2 = 4.18$, $P = 0.041$) compared with those carrying the *Arg/Arg* genotype after adjustment for age, sex, exposure duration, and intensity of benzene exposure, respectively. In contrast, individuals with *XRCC1 280Arg/His* and *XRCC1 280Arg/His+His/His* genotypes had 1.67-fold (OR_{adj}, 1.67; 95% CI, 1.02-2.74; $\chi^2 = 4.18$, $P = 0.04$) and 1.91-fold (OR_{adj}, 1.91; 95% CI, 1.17-3.10; $\chi^2 = 6.74$, $P = 0.009$) increased risk of CBP, respectively, compared with those with the *Arg/Arg* genotype (Table 3). The risk of CBP stratified by smoking and alcohol use suggested that significantly increased risk associated with the *XRCC1 280Arg/His+His/His* genotypes was confined to nonsmokers (OR_{adj}, 1.96; 95% CI,

Table 2. Characteristics of selected demographic and exposure variables in CBP cases and healthy controls

Variables	Cases	Controls	*P**
	n (%)	*n* (%)	
Total	152 (100.0)	152 (100.0)	
Age (y)			0.36
≤25	11 (7.2)	6 (3.9)	
26-35	58 (38.2)	50 (32.9)	
36-45	62 (40.8)	69 (45.4)	
>45	21 (13.8)	27 (17.8)	
Sex			0.64
Male	61 (40.1)	57 (37.5)	
Female	91 (59.9)	95 (62.5)	
Exposure duration (y)			0.25
≤5	26 (17.1)	33 (21.7)	
>5 and ≤10	47 (30.9)	50 (32.9)	
>10 and ≤15	30 (19.7)	29 (19.1)	
>16 and ≤20	22 (14.5)	26 (17.1)	
>20	27 (17.8)	14 (9.2)	
Intensity of exposure (mg/m^3)			0.59
≤40	28 (18.4)	33 (21.7)	
41-100	93 (61.2)	94 (61.8)	
>100	31 (20.4)	25 (16.5)	
Smoking			0.04
Yes	19 (12.5)	34 (22.4)	
No	126 (82.9)	117 (77.0)	
No data	7 (4.6)	1 (0.6)	
Alcohol consumption			0.79
Yes	18 (11.8)	17 (11.2)	
No	129 (84.9)	134 (88.2)	
No data	5 (3.3)	1 (0.6)	
Type of work			0.91
Painting	62 (40.8)	58 (38.2)	
Spraying	25 (16.4)	28 (18.4)	
Painting and spraying	18 (11.8)	16 (10.5)	
Printing	4 (2.6)	7 (4.6)	
Mechanic	13 (8.6)	10 (6.6)	
Warehouseman	3 (2.0)	5 (3.3)	
Other	27 (17.8)	28 (18.4)	

*Pearson χ^2 test for difference in distributions between the case and control groups.

1.14-3.38) and nonalcohol users (OR_{adj}, 1.78; 95% CI, 1.05-3.03) whereas reduced risk of CBP associated with the *APE1 148Asp/Glu+Glu/Glu* genotypes was confined to alcohol users (OR_{adj}, 0.11; 95% CI, 0.02-0.69). However, homogeneity test of ORs for these effects stratified by smoking and alcohol consumption suggested that there was potential interaction merely between polymorphism of *APE1Asp148Glu* and alcohol consumption ($\chi_H^2 = 6.93$, $P = 0.01$).

Haplotypes of Polymorphisms in *XRCC1* on Risk of CBP. Because we genotyped for three single-nucleotide polymorphisms of *XRCC1* and they are reportedly in linkage disequilibrium with each other, we calculated their haplotypes based on the observed genotypes. As shown in Table 4, there were seven haplotypes in this study population. There were a statistically significant difference for the distribution of haplotypes of HAP3 [i.e., (*XRCC1 194Arg, 280His*, and *399Arg*), HAP4 (*XRCC1 194Trp, 280Arg*, and *399Arg*), and HAP7 (*XRCC1 194Trp, 280His*, and *399Gln*); $P < 0.05$ for all]. Compared with those carrying the HAP1 haplotype, there was a 2.96-fold increased risk of CBP for subjects with the HAP3 haplotype (OR, 2.96; 95% CI, 1.60-5.49; $\chi^2 = 12.39$, $P = 0.001$; Table 4).

Discussion

Previous studies have shown that benzene toxicity mainly resulted from its intermediate reactive metabolites that cause DNA damage by directly oxidative damage and covalently binding to DNA. Previous study with the comet assay showed that the damage levels of lymphocyte and granulocytes were significantly higher in peripheral blood of benzene-exposed group than in the unexposed control group (42). It is postulated that DNA damage induced by benzene exposure and resultant cellular dysfunction, apoptosis, and lethality, which are correlated with the intensity of DNA damage and an individual's susceptibility, may explain the manifestations of CBP such as pancytopenia (43). In the present study, our data further suggested that genetic polymorphisms in DNA repair genes, such as *XRCC1*, may have played a role in benzene-induced CBP in the workplace in this Chinese population.

The XRCC1 protein is an important component involved in base-excision repair. After excision of a damaged base, it stimulates endonuclease action and acts as a scaffold in the subsequent restoration of the site by complexing with DNA ligase III via a BRCT domain in its COOH terminus and with DNA polymerase β via the NH_2-terminal domain (44, 45) Both

Table 3. Genotypes for genetic polymorphisms in *XRCC1*, *XRCC2*, and *XRCC3* and their effects on risk of CBP

Polymorphisms	Cases*	Controls*	OR (95% CI)	OR_{adj} (95% CI)†	χ^2	P‡
	n (%)	*n* (%)				
XRCC1 Arg194Trp					3.84	0.15
Arg/Arg	71 (49.3)	51 (37.8)	1.00	1.00		
Arg/Trp	56 (38.9)	66 (48.9)	0.61 (0.37-1.01)	0.60 (0.36-1.01)	3.68	0.06
Trp/Trp	17 (11.8)	18 (13.3)	0.68 (0.32-1.44)	0.76 (0.51-1.13)	1.85	0.17
Arg/Trp+Trp/Trp	73 (50.7)	84 (62.2)	0.62 (0.39-1.01)	0.60 (0.37-0.98)§	4.18	0.04
Trp allele frequency	0.31	0.38			2.63	0.11
XRCC1Arg280His					7.31	0.001
Arg/Arg	69 (47.9)	87 (64.0)	1.00	1.00		
Arg/His	65 (45.1)	49 (36.0)	1.67 (1.03-2.72)§	1.67 (1.02-2.74)§	4.18	0.04
His/His	10 (7.0)	0 (0.0)	—	—	—	—
Arg/His+His/His	75 (52.1)	46 (36.0)	1.93 (1.20-3.12)‖	1.91 (1.17-3.10)‖	6.74	0.009
His allele frequency	0.30	0.18			6.33	0.01
XRCC1 Arg399Gln					0.96	0.62
Arg/Arg	60 (41.7)	56 (39.7)	1.00	1.00		
Arg/Gln	76 (52.8)	73 (51.8)	0.97 (0.60-1.58)	0.99 (0.60-1.60)	0.01	0.93
Gln/Gln	8 (5.6)	12 (8.5)	0.62 (0.24-1.64)	0.80 (0.49-1.33)	0.73	0.39
Arg/Gln+Gln/Gln	84 (58.3)	85 (60.3)	0.92 (0.58-1.48)	0.93 (0.58-1.50)	0.08	0.78
Gln allele frequency	0.32	0.34			0.39	0.53
APE1 Asp148Glu					0.61	0.74
Asp/Asp	52 (35.9)	49 (36.0)	1.00	1.00		
Asp/Glu	76 (52.4)	67 (49.3)	1.07 (0.64-1.78)	0.98 (0.58-1.64)	0.01	0.93
Glu/Glu	17 (11.7)	20 (14.7)	0.80 (0.38-1.70)	0.79 (0.37-1.70)	0.36	0.55
Asp/Glu+Glu/Glu	93 (64.1)	87 (64.0)	1.01 (0.62-1.64)	0.97 (0.59-1.59)	0.01	0.91
Asp allele frequency	0.38	0.39			0.12	0.73
ADRPT1 Val762Ala					0.23	0.89
Val/Val	74 (51.4)	66 (48.5)	1.00	1.00		
Val/Ala	59 (41.0)	59 (43.4)	0.89 (0.55-1.46)	0.88 (0.53-1.44)	0.29	0.60
Ala/Ala	11 (7.6)	11 (8.1)	0.89 (0.36-2.19)	0.91 (0.37-2.28)	0.04	0.84
Val/Ala+Ala/Ala	70 (48.6)	70 (0.0)	0.89 (0.56-1.43)	0.86 (0.54-1.39)	0.37	0.54
Asp allele frequency	0.28	0.30			0.10	0.75
XRCC2 Arg188His					—	—
Arg/Arg	143 (100.0)	137 (100.0)	1.00	1.00		
Arg/His	0 (0.0)	0 (0.0)	—	—	—	—
His/His	0 (0.0)	0 (0.0)	—	—	—	—
Arg/His+His/His	0 (0.0)	0 (0.0)	—	—	—	—
His allele frequency	0.00	0.00			—	—
XRCC3 Thr241Met					0.54	0.46
Thr/Thr	129 (90.8)	119 (88.1)	1.00	1.00		
Thr/Met	13 (9.2)	16 (11.9)	0.75 (0.35-1.62)	0.75 (0.34-1.65)	0.50	0.48
Met/Met	0 (0.0)	0 (0.0)	—	—	—	—
Thr/Met+Met/Met	13 (9.2)	16 (11.9)	0.75 (0.35-1.62)	0.75 (0.34-1.65)	0.50	0.48
Met allele frequency	0.05	0.06			0.51	0.48

*Some data were missing due to inability to amplify DNA.
†ORs were adjusted for potential confounding variables including age, sex, exposure duration, and intensity of benzene exposure.
‡Pearson χ^2 test for difference in distributions between the case and control groups.
§$P < 0.05$,
‖$P < 0.01$.

Table 4. Combined effects of haplotypes derived from the observed genotypes of *XRCC1 194, 280*, and *399* on risk of CBP

Haplotypes*	Cases †	Controls †	χ^2	P ‡	OR (95% CI)	χ^2 for OR	P ‡
	n (%)	*n* (%)					
HAP1 (111)	81 (26.9)	83 (27.8)	0.02	0.88	1.00	—	—
HAP2 (112)	78 (25.9)	82 (27.4)	0.10	0.75	0.97 (0.63-1.51)	0.01	0.91
HAP3 (121)	52 (17.3)	18 (6.0)	16.51	0.00005	2.96 (1.60-5.49)	12.39	0.001
HAP4 (211)	54 (17.9)	80 (26.8)	5.04	0.02	0.69 (0.44-1.10)	2.46	0.12
HAP5 (212)	6 (2.0)	10 (3.3)	1.00	0.31	0.61 (0.21-1.70)	0.83	0.37
HAP6 (221)	25 (8.3)	26 (8.7)	0.02	0.89	0.99 (0.53-1.85)	0.002	0.96
HAP7 (222)	5 (1.7)	0 (0.0)	5.00	0.03	—	—	—

*The allele order is *XRCC1 Arg194Trp, Arg280His*, and *Arg399Gln* from left to right. Wild-type allele is indicated by 1 and variant alleles by 2.
†Some data were missing due to inability to amplify DNA.
‡Person χ^2 test for difference in distributions between the case and control groups.

XRCC2 and XRCC3 proteins are essential for homologous recombination to recognize and repair the double-strand breaks on DNA strands; lack of *XRCC2* or *XRCC3* gene will result in decreased function of homologous recombination (46-48).

Genetic polymorphisms are common in the general population. Most polymorphisms are located outside gene boundaries and thus thought not to have any apparent effects on protein functions. If a polymorphism is within the coding region of a gene, amino acid substitution may occur and result in change of protein activity whereas polymorphisms in the promoter will alter the efficiency of transcription and polymorphisms located at an intron/exon boundary in a gene may produce incorrect mRNA splicing, which results in incomplete or inactive proteins (49). Thus, in this study, although no association of polymorphisms in *XRCC1 Arg399Gln, APE1 Asp148Glu, ADPRT Val762Ala, XRCC2 Arg188His*, and *XRCC3 Thr241Met* with risk of CBP was found, other polymorphisms in the promoter and coding region of these genes need to be identified, which may also contribute to risk of CBP.

The *XRCC1* codon 194 and 280 polymorphisms are located in the vicinity of two sequences mediating the protein-protein interactions with ADPRT and polymerase β (19-21). Thus, the variant alleles may encode a twisted XRCC1 protein, resulting in increased or decreased affinity to other proteins, which may affect normal DNA repair capability. Our results indicated that there was significant difference in distribution of genotypes of *XRCC1 Arg194Trp* and *XRCC1 Arg280His* and the risk was reduced in those with *XRCC1 194Arg/Trp+Trp/Trp* genotypes but increased in those with *XRCC1 280Arg/His* and *XRCC1 280Arg/His+His/His* genotypes. These data were consistent with previous reports on risk of cancers of the breast (50), bladder (51), and lung (52, 53). In the analysis of haplotypes of polymorphisms in *XRCC1*, we also found that workers carrying genotypes of *XRCC1 194Arg/Arg, XRCC1 280Arg/His+His/His*, and *XRCC1 399Arg/Arg* simultaneously are more susceptible to CBP. Thus, variants at codons 194 and 280 of *XRCC1* may be associated with altered function of the coding products and thus contribute to the individual's genetic susceptibility to CBP, and more attention should be paid on workers occupying jobs with benzene exposure and carrying genotypes of *XRCC1 194Arg, XRCC1 280His*, and *XRCC1 399Arg* simultaneously, especially in the health surveillance work. This hypothesis needs to be tested in larger studies.

Most chronic and complex diseases are likely caused by interactions among environmental exposure, genetic polymorphism, and lifestyles (34) such as smoking and alcohol consumption, which have been identified as risk factors for CBP (54, 55). In this study, reduced risk of CBP for alcohol users with *APE1 148Asp/Glu+Glu/Glu* genotypes was found; however, the matching design may have masked the effect of smoking and alcohol consumption on risk of CBP. The relative low frequency of alcohol consumption and smoking may contribute to results in this study for the majority of subjects involved in this study were females who were less likely than men to be indulged in alcohol and cigarette use, and further study with larger sample is needed to elucidate interactions between polymorphisms in these DNA repair genes and smoking or alcohol use. Furthermore, accurate exposure estimation is quite important for evaluating exposure levels between the case and control groups; however, because not all cases and controls are in the same exposure environment and due to difference in types of work, we evaluated intensity of benzene exposure according to method described by Dosemeci et al. (34) and the exposure levels were measured differently between cases and controls that may not completely reflect personal exposure. Thus, more accurate methods for exposure estimation are needed to be introduced in further study such as individual sampling, which is more accurate than traditional area sampling. Those benzene metabolites reflecting the exposure levels are also needed to be applied in accurate exposure estimation.

In summary, we found for the first time that genetic variants in *XRCC1* may contribute to the development of CBP in occupational exposure to benzene in a Chinese population. This finding, once verified by large studies, will have important implication in the prevention of CBP in the susceptible workers. The strengths of this study include a homogeneous ethnic background of the subject, well-documented exposure history to benzene in the workplace, and a frequency-matching design. However, joint action between genetic polymorphisms and environmental exposure on special diseases such as CBP is complicated and small studies like the present study do not have enough statistical power to detect gene-environment interactions. Thus, a more comprehensive, larger-scale study is needed to further explore the effects of gene-environment interaction on genetic susceptibility to CBP.

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Genetic Polymorphisms in *XRCC1, APE1, ADPRT, XRCC2*, and *XRCC3* and Risk of Chronic Benzene Poisoning in a Chinese Occupational Population

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