Association of Genetic Polymorphisms, mRNA Expression of *p53* and *p21* with Chronic Benzene Poisoning in a Chinese Occupational Population

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Abstract

DNA damage induced by benzene reactive metabolites is thought of as an important mechanism underlying benzene hematotoxicity and genotoxicity, and genetic variation in cell-cycle control genes may contribute to susceptibility to chronic benzene poisoning (CBP). Using a case-control study that included 307 benzene-poisoned patients and 299 workers occupationally exposed to benzene in south China, we aimed to investigate the association between genetic polymorphisms of *p53* and *p21* and the odds of CBP. To investigate whether benzene exposure may influence mRNA expression of *p53* and *p21* in benzene-exposed workers, we also chose 39 CBP workers, 38 occupationally benzene-exposure workers, and 37 nonexposure workers in the same region of China. PCR-restriction fragment length polymorphism technique was applied to detect polymorphisms of *p53* (rs17878362, rs1042522, and rs1625895) and *p21* (rs1801270 and rs1059234), and real-time PCR was applied to detect the quantity of gene mRNA expression. We found that *p21* C98A variant genotypes (CA+AA) or C70T variant genotypes (CT+TT) were associated with decreased odds of CBP [odds ratio (OR), 0.51; 95% confidence interval (95% CI), 0.32-0.83, and OR, 0.53; 95% CI, 0.29-0.95, respectively. Further analysis showed the decreased odds of CBP in the subjects with *p21* CC/AT diplotype (OR, 0.51; 95% CI, 0.30-0.85). In addition, *p53* mRNA expression of CBP workers or benzene-exposure workers was significantly lower than that of nonexposure workers. Although these results require confirmation and extension, our results show that polymorphisms in *p21* may be protective against the risk of CBP in the Chinese occupational population. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1821–8)

Introduction

Benzene, an important industrial chemical, is widely recognized as a prototypical hematotoxic and genotoxic carcinogen. Exposure to benzene can induce chronic benzene poisoning (CBP) characterized by hematotoxicities, leading to pancytopenia, aplastic anemia, and myelodysplastic syndrome (1, 2).

In spite of many years of research, the mechanism of how benzene causes those toxic and carcinogenic effects remains elusive. Earlier studies have shown that benzene reactive intermediates can bind covalently to macromolecules including DNA, tubulin, histones, and topoisomerase II in the tissue, and therefore cause genotoxicity as a result of oxidative damage to DNA or DNA double-strand breaks caused by reactive oxygen species (3, 4). Thus, DNA damage following benzene exposure must be properly repaired to maintain genomic stability and to prevent proliferation of mutated cells and subsequent transformation into malignancies (5).

Besides DNA repair proteins, cell-cycle control proteins are also involved in DNA repair. P53, a well-known tumor suppressor protein, plays a central role in mediating cellular responses to DNA damage by acting as a transcription factor for genes involved in cell-cycle control, DNA repair, or apoptosis (6). In the presence of DNA damage after exposure to a carcinogen, P53 activated by specific phosphorylation events can increase expression levels of *p53*-responsive target genes, resulting in the arrest of the cell cycle in G_1 (7, 8) and preventing replication of damaged DNA. Previous studies have shown that loss of P53 function leads to genomic instability (9, 10), a common event in the development of human cancer (11). The *p53* gene is a known polymorphic, leading to variation in the change of gene expression and loss of this homeostatic control, inducing human carcinogenesis. So far, at least three *p53* polymorphisms have been reported to be involved in cancers: the 16-bp duplication in intron 3 (dbSNP rs17878362), a C-to-G transition at nucleotide 119 in exon 4 (C119G, codon 72, dbSNP rs1042522), and a G-to-A transversion at position 62 in intron 6 (IVS6 +62 G>A, dbSNP rs1625895; ref. 12).

P21 (also known as Waf1/Cip1/CDKN1A) is one of the notable effectors of *p53* and is a general inhibitor of

Table 1. Characteristics of selected demographic and exposure variables in CBP cases and controls

	CBP cases	Healthy controls	P*
	No. (%)	No. (%)	
Total	307 (100.0)	299 (100.0)	
Age (y)			0.500
≤25	85 (27.7)	86 (28.8)	
26-35	117 (38.1)	97 (32.4)	
36-45	81 (26.4)	88 (29.4)	
>45	24 (7.8)	28 (9.4)	
Gender			0.870
Male	195 (63.5)	188 (62.9)	
Female	112 (36.5)	111 (37.1)	
Exposure duration (y)			0.252
≤5	150 (48.9)	160 (53.5)	
6-10	62 (20.2)	66 (22.1)	
11-15	37 (12.1)	35 (11.7)	
16-20	25 (8.1)	20 (6.7)	
>20	33 (10.7)	18 (6.0)	
Intensity of exposure (mg/m^3)			0.687
≤40	187 (60.9)	190 (63.5)	
40-100	91 (29.6)	86 (28.8)	
>100	29 (9.5)	23 (7.7)	
Smoking			0.046
Yes	154 (50.2)	137 (45.8)	
No	30 (9.8)	45 (15.1)	
No data	123 (40.0)	117 (39.1)	
Alcohol consumption			0.404
Yes	148 (48.2)	151 (50.5)	
No	38 (12.4)	31 (10.4)	
No data	121 (39.4)	117 (39.1)	

*χ^2 test for difference in the distributions between the two groups.

cyclin-dependent kinases. It functions to negatively control the cell cycle (13, 14). The expression of p21 is up-regulated by p53 in response to DNA damage, leading to cell-cycle arrest at the G$_1$ checkpoint (14). *In vitro* studies have shown that p21 expression can suppress tumor growth (15), and reduced p21 expression in tumor has been associated with poor prognosis in humans (16, 17). Thus, genetic variants in p21 may modulate its expression and thereby affect carcinogenesis. Among the single nucleotide polymorphisms (SNP) of p21, two SNPs were reported to be associated with cancers (18, 19): one is at nucleotide 98 in exon 2, which causes a nonsynonymous serine-to-arginine substitution at codon 31(C98A, dbSNP rs1801270), and the other locates the 3′ untranslated region and causes a single C-to-T substitution at nucleotide 70 in exon 3 (T70C, dbSNP rs1059234).

Because exposure to benzene can induce DNA damage and therefore activate the p53-regulated biological defense responses, we hypothesized that polymorphisms of p53 and p21 may contribute to the risk of CBP. In addition, *in vitro* studies indicated that inhaled benzene can influence the expression of the cell-cycle control genes including p53 and p21 (5, 20), but it is yet unclear that the expression of p53 and p21 in peripheral blood cells would be affected by exposure to benzene. In this study, therefore, we conducted a case-control study to investigate the effects of SNPs in p53 and p21 on human susceptibility to CBP and the alteration in mRNA level of p53 and p21 among CBP workers, benzene-exposed workers, and nonexposed workers in a Chinese occupational population.

Materials and Methods

Subjects. The details of this case-controls study have been described elsewhere (2, 21-23). Briefly, 267 of 307 CBP patients in this study came from six factories in Shanghai, Hangzhou, Maanshan, Wuhu, Cixi, and Guangzhou, where clusters of cases were reported. Another 40 patients, who returned to the hospital periodically for health examinations, were from 12 other small factories that had been closed down. The benzene poisoning was diagnosed from 1980 to 2005 by the local authorized Occupational Disease Diagnostic Team. The diagnostic criteria for occupational benzene poisoning, according to the Ministry of Health of China, have also been described previously (2, 21). A total of 299 healthy workers who had been occupationally exposed to benzene in the six major factories were selected as controls. Control subjects were frequency-matched to cases by gender, age within 5 y, employment duration of the same working environment within 3 y, and exposure level at the workplace. The calendar range for the start of employment of the cases and the controls was between 1958 and 2005 and between 1963 and 2005, respectively. To explore the effect of benzene on gene expression of p53 and p21, we chose 39 workers with CBP as cases (22 male and 17 female; mean age, 34.1 y) and 38 healthy workers occupationally exposed to benzene as benzene-exposed controls (22 male and 16 female; mean age, 33.9 y) from the same factory. We also chose 37 workers who were not exposed to benzene as unexposed controls (13 male and 24 female; mean age, 45.3 y) in the same region of China.

Prior to the study, written informed consent was obtained from each subject and a questionnaire was used to determine the lifestyle of each subject, namely ethnic background, nutrition, cigarette smoking and 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 or industrial hygienists and long-term employees' evaluation considering historical changes (24). The intensity of benzene exposure (milligrams per cubic meter) for the patients was taken as the benzene level of workplaces while 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. Those who smoked at least 1 cigarette per day for >1 y were considered regular smokers. Alcohol intake 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 >6 mo. The subjects were administrated a rigorous physical examination at a local occupational disease hospital. Alanine aminotransferase level in serum was also examined for liver function evaluation.

Genotyping Assay. Genomic DNA was isolated from peripheral blood leukocytes using a standard method and stored at −80°C until analysis. p53 and p21 genotypes were determined using PCR and restriction fragment length polymorphism assays previously described (25, 26). The genotyping data were recorded by two independent readers who were blinded to the case-control status of the samples. At least of 10% of DNA randomly selected samples were run in duplicates to ensure quality control, and the results were 100% concordant.

Table 2. Genotypes for genetic polymorphisms in *p53* and *p21* and their effects on risk of CBP

Genotype	Cases (%)*	Controls (%)*	OR (95% CI)	OR$_{adj.}$ (95% CI)[†]
P53 intron 3[‡]				
WW	280 (94.6)	258 (92.8)	1.00	1.00
WM	16 (5.4)	20 (7.2)	0.74 (0.37-1.45)	% (0.22-1.58)
MM	0 (0.00)	0 (0.0)	–	–
WM+MM	16 (5.4)	20 (7.2)	0.74 (0.37-1.47)	% (0.22-1.58)
P53 C119G				
CC	56 (18.7)	51 (17.8)	1.00	1.00
CG	144 (48.0)	142 (49.5)	0.92 (0.59-1.44)	1.04 (0.58-1.87)
GG	100 (33.3)	94 (32.8)	0.97 (0.60-1.55)	0.87 (0.46-1.63)
CG+GG	244 (81.3)	236 (82.2)	0.94 (0.62-1.43)	0.97 (0.55-1.69)
P53 IVS6+62 G>A				
GG	272 (89.8)	265 (90.1)	1.00	1.00
GA	31 (10.2)	29 (9.9)	1.04 (0.61-1.78)	1.28 (0.63-2.57)
AA	0 (0.00)	0 (0.0)	–	–
GA+AA	31 (10.2)	29 (9.9)	1.04 (0.61-1.78)	1.28 (0.63-2.57)
P21 C98A				
CC	100 (33.0)	65 (22.5)	1.00	1.00
CA	158 (52.2)	173 (59.9)	0.59 (0.41-0.87)[§]	0.48 (0.29-0.79)[§]
AA	45 (14.9)	51 (17.6)	0.57 (0.35-0.95)[‖]	0.67 (0.33-1.35)
CA+AA	203 (67.0)	224 (77.5)	0.59 (0.41-0.85)[§]	0.51 (0.32-0.83)[§]
P21 C70T				
CC	66 (21.9)	45 (15.4)	1.00	1.00
CT	104 (34.6)	107 (36.6)	0.66 (0.42-1.06)	0.56 (0.29-1.06)
TT	131 (43.5)	140 (48.0)	0.64 (0.41-1.00)[‖]	0.51 (0.28-0.94)[‖]
CT+TT	235 (78.1)	247 (84.6)	0.65 (0.43-0.99)[‖]	0.53 (0.29-0.95)[‖]

*Data missing due to failure in DNA amplification.
[†]ORs were adjusted for potential confounders including gender, cigarette smoking, alcohol consumption, exposure duration, and intensity of benzene exposure.
[‡] WW, wild-type homozygous; WM, wild-type/mutant heterozygous; MM, mutant homozygous.
[§]$P < 0.01$.
[‖]$P < 0.05$.

Total RNA Extraction and Reverse Transcription-PCR and Real Time PCR. Lymphocytes were isolated from 2 mL anticoagulated peripheral blood using a standard method. Trizol (Gibco BRL Products) was added to the cells and was agitated between three and five times. The mixture was then stored at −80°C until use.

Total RNA was isolated from the lymphocytes and quantified by measurement of the absorbance at 260 nm; purity was assessed from the 260/280 nm absorbance ratio. Reverse transcription-PCR was done using the one-step SuperScript kit (Gibco BRL) according to the manufacturer's instructions. A total reaction mixture volume of 25 μL contained 3 μg of RNA.

The quantity of *p53* and *p21* gene mRNA expression was detected by real-time PCR (SYBR Green I) with PRISM ABI 7900HT Sequence Detection System (Applied Biosystem). Expression of the housekeeping gene glycer-

aldehyde 3-phosphoric acid dehydrogenase (*GAPDH*) was used for normalization of *p53* and *p21* mRNA to enable cross-comparisons among the samples. All the primers were designed by using the Primer premier software 5.0 (PREMIER Biosoft International). Specificity of primers was confirmed by homology search. The *p53* primers were 5′-TGACTGTACCACCATCCACTA-3′ (sense) and 5′-AAACACGCACCTCAAAGC-3′ (antisense; GI:20407067), giving rise to a 146 bp PCR production. The *p21* primers were 5′-TGGACCTGT-CACTGTCTTGT-3′ (sense) and 5′-TCCTGTGGGCGGAT-TAG-3′ (antisense; GI: 7978496), giving rise to a 176 bp PCR production. The *GAPDH* primers were 5′-AGGGCTGCTTTTAACTCTG-3′ (sense) and 5′-CTGGAAGATGGTGATGGG-3′ (antisense; GI: 3641890), giving rise to a 177 bp PCR production. The real-time PCR was done using 50 ng of cDNA, 0.25 μL of each

Table 3. Haplotypes/diplotypes of *p53* and the odds of chronic benzene poisoning

Haplotype/Diplotypes*	Cases (%)[†]	Controls (%)[†]	OR (95% CI)	χ^2	P
Haplotype					
WCC	233 (38.1)	222 (37.6)	1.00		
WGC	345 (56.4)	335 (56.8)	0.98 (0.77-1.24)	0.02	0.876
MCA	13 (2.1)	16 (2.7)	0.77 (0.36-1.65)	0.44	0.505
WCA	14 (2.3)	12 (2.0)	1.11 (0.50-2.46)	0.07	0.794
Diplotypes					
WCC/WCC	45 (15.1)	44 (15.2)	1.00		
WCC/WGC	132 (44.3)	126 (43.5)	1.02 (0.63-1.66)	0.01	0.922
WGC/WGC	95 (31.9)	92 (31.7)	1.01 (0.61-1.67)	0.00	0.970
Other diplotypes[‡]	26 (8.7)	28 (9.6)	0.91 (0.46-1.79)	0.08	0.780

*The allele order is intron3, C119G, and IVS6+62 C>A from left to right.
[†]Data missing due to failure in DNA amplification.
[‡]All subgroups with frequencies <5% were combined as one group.

Table 4. Haplotypes/diplotypes of *p21* and the odds of chronic benzene poisoning

Haplotype/Diplotypes*	Cases (%)[†]	Controls (%)[†]	OR (95% CI)	χ^2	P
Haplotype					
CC	224 (37.2)	184 (31.5)			
AT	235 (39.0)	267 (45.7)			
AC	131 (21.8)	120 (20.6)			
CT	12 (2.0)	13 (2.2)			
Diplotypes					
CC/CC	60 (19.9)	37 (12.7)	1.00		
CC/AT	75 (24.9)	91 (31.2)	0.51 (0.30-0.85)	6.81	0.009
CC/CT	26 (8.6)	15 (5.1)	1.07 (0.50-2.28)	0.30	0.863
AT/AT	39 (13.0)	48 (16.4)	0.50 (0.28-0.90)	5.35	0.021
AT/CT	79 (26.3)	79 (27.1)	0.62 (0.37-1.03)	3.41	0.065
Other diplotypes[‡]	22 (7.3)	22 (7.5)	0.62 (0.30-1.27)	1.75	0.186

*The allele order is C98A and C70T from left to right.
[†]Some data were missing due to inability to amplify DNA.
[‡]All subgroups with frequencies <5% were combined as one group.

primer (10 μmol/L), and 2 × ABsoluteTM QPCR SYBR Green Mix (including 500 nmol/L ROX, ABgene) in a 10 μL reaction volume. PCR program was a 15-min activation step at 95°C, followed by 40 cycles of 95°C for 15 s, and finally 60°C for 1 min. Every sample was done by three parallels. The results were analyzed by PRISM ABI 7900HT Sequence Detection System (Applied Biosystem). The threshold cycle (C_t) values for the triplicate reactions were averaged and the average *GAPDH* C_t value for each sample was subtracted from the average C_t value of interest to obtain a normalized C_t value. The mRNA expression of objective gene was shown by $2^{-\Delta Ct}$ value. To simplify the presentation of the data, the relative expression values were multiplied by 10^3.

Statistical Analysis. All analyses were done using SPSS 10.0 software (SPSS Inc.). Each SNP was tested in controls to ensure fitting with Hardy-Weinberg equilibrium. Frequencies of the different genetic polymorphisms were compared between cases and controls by Fisher's exact test or χ^2 test, when appropriate. To evaluate whether the lifestyle factor modified the association between genetic polymorphisms and odds of CBP, χ^2 test was applied after stratification according to cigarette smoking or alcohol consumption. The test for homogeneity of odds ratios (OR) was examined by the Breslow-Day method. The OR and 95% confidence interval (95% CI) for estimating the associations of genetic polymorphisms with the odds of CBP were obtained from unconditional logistic regression models without and with adjustment for potential confounders including gender, cigarette smoking, alcohol consumption, exposure duration, and intensity of benzene exposure. Linkage disequilibrium analysis was tested by the method described by Shi and He (27). Haplotype and diplotype frequencies were estimated using a Bayesian approach implemented with PHASE (28, 29). The frequency distribution of the haploytpes and diplotypes was calculated by χ^2 analysis. Two-tailed $P < 0.05$ was considered statistically significant. To account for multiple testing, we applied the Bonferroni correction.

Due to the nonnormal distribution of gene expression data, values of *p53* and *p21* mRNA expression were reported as median and interquartile range. In order to adjust for the influence of demographic characteristics such as gender, cigarette smoking, and alcohol consumption, we did a multiple factor analysis using robust multiple regression, with PROC ROBUSTREG under SAS Version 9.1 (SAS Institute Inc.). Two-tailed $P < 0.05$ was considered statistically significant.

Results

Demographics of Cases and Controls. The distributions of age, gender, exposure duration, cigarette smoking, and alcohol consumption in the cases and controls are shown in Table 1. The median age and exposure duration was 31 years (range, 18-57) and 4.5 years (range, 1-38) in 307 CBP cases, respectively, and 32 years (range, 18-68) and 4 years (range, 1-36), in 299 controls, respectively. There was no significant difference in the distribution of age (≤25, 26-35, 36-45, >45 years), exposure duration (≤5, 6-10, 11-15, 16-20, >20 years), intensity of benzene exposure (≤40 mg/m³, 40-100 mg/m³, >100 mg/m³), and gender ($P > 0.05$ for all), suggesting that frequency-matching was adequate. Because the data were in part coming from historical records, some records were incomplete for data on cigarette smoking or alcohol consumption. For those who had historical data, the percentage of females was 60.0% in the cases and 60.9% in the controls for those who had cigarette smoking data, and 60.6% in cases and 60.9% in controls who had alcohol consumption data. The range for total WBC counts in the controls was from 4,500/μL to 5,900/μL; the mean was 4,838 ± 259/μL.

Genetic Polymorphisms of *p53* and *p21*. The frequency distributions of genotypes of *p53* and *p21* in this study population revealed that no mutant homozygous (MM) genotype of *p53* intron 3 and AA genotype of *p53* IVS6 +62 G>A were detected (Table 2). The variant genotype frequency of *p21* C98A polymorphism was significantly different between cases and controls ($P < 0.05$), but the

Table 5. Demographic characteristics of the study subjects

Variables	Nonexposed	Benzene-exposed	CBP
Number	37	38	39
Age (y)	45.4 ± 5.9*	33.0 ± 7.3	33.4 ± 7.8
Male (%)	15 (34.9)	22 (57.9)	22 (56.4)
Current smoker (%)	14 (32.6)	14 (36.8)	12 (30.8)
Current alcohol user (%)	12 (27.9)	14 (36.8)	19 (48.7)

*$P < 0.05$.

Table 6. $p53$ and $p21$ mRNA expression ($2^{-\Delta Ct} \times 10^3$) in the study subjects

Gene	Nonexposed ($n = 37$)	Benzene-exposed ($n = 38$)	CBP ($n = 39$)
$p53$	16.40 (9.62, 26.83)	2.16 (0.92, 4.03)*	1.61 (0.33, 4.07)*
$p21$	4.02 (2.39, 5.05)	4.05 (1.79, 7.49)	3.90 (1.32, 8.52)

*$P < 0.001$ (robust multiple regression analysis).

other variant genotypes frequencies were not ($P > 0.05$ for all). The distributions of the genotypes of these genetic polymorphisms in the controls were in Hardy-Weinberg equilibrium ($P > 0.05$) except the genotypes of $p21$ C98A and $p21$ C70T.

Effect of Genetic Polymorphism in $p53$ and $p21$ on the Risk of CBP. For the $p53$ gene, no statistical difference was found in the distribution of genotypes of $p53$ intron 3, C119G, and IVS6+62 G>A ($P > 0.05$) in this population. However, the proportion of individuals carrying $p21$ 98 CA+AA, or $p21$ 70 CT+TT was lower in cases (67.0% and 78.1%, respectively) than in controls (77.5% and 84.6%, respectively). Correspondingly, the odds of CBP for individuals carrying $p21$ 98 CA+AA genotypes or $p21$ 70 CT+TT genotypes was 0.51-fold lower (OR$_{adj}$, 0.51; 95% CI, 0.32-0.83; $P = 0.006$) or 0.53-fold lower (OR$_{adj}$, 0.53; 95% CI, 0.29-0.95; $P = 0.032$) compared with those carrying the $p21$ 98 CC genotype or the $p21$ 70 CC after adjustment for potential confounders such as gender, cigarette smoking, alcohol consumption, exposure duration, and intensity of benzene exposure.

Haplotypes of Polymorphisms in $p53$ and $p21$ on the Risk of CBP. To examine the linkage among the three loci (intron 3, exon 4, and intron 6) of $p53$, we did linkage disequilibrium analyses. The D' values were 0.831 (intron 3 with exon 4), 0.796 (intron 3 with intron 6), and 0.599 (exon 4 with intron 6). We also examined the linkage between the two loci of $p21$, and the D' value was 0.834.

Based on the observed genotypes of $p53$ and $p21$ in this study population, we reconstructed their haplotypes and diplotypes (Tables 3 and 4). For $p53$, four haplotpyes was reconstructed applied by the Phase software. However, no significant differences were observed in the distribution of their haplotypes in this population, and further analysis showed that there were no significant differences in the distribution of their diplotypes either (Table 3). For $p21$, four haplotypes were found in this population. Because the polymorphisms of $p21$ in this present study departed from Hardy-Weinberg disequilibrium, we only investigated the role of $p21$ diplotypes on the odds of CBP. The results showed that there were statistically significant differences in the distributions of those diplotypes in cases and those in controls. Using the Bonferroni correction with five tests (requiring $P < 0.01$), a statistically significant difference was observed in the distribution of CC/AT diplotypes ($P = 0.009$; Table 4). Compared with those carrying the CC/CC diplotpes, subjects with CC/AT diplotype had a decreased odds of CBP (OR, 0.51; 95% CI, 0.30-0.85).

Demographic Characteristics of the Study Subjects for the Study of mRNA Expression. The three groups matched on the basis of gender as well as smoking and drinking habits, but the CBP and benzene-exposed groups were significantly younger than the unexposed group ($P < 0.05$; Table 5).

mRNA Expression of $p53$ and $p21$. Robust multiple regression indicated that $p53$ mRNA expression of cases or benzene-exposure controls was significantly lower than that of the nonexposure controls ($P < 0.001$), but the difference of $p21$ mRNA expression between these three groups was not significant ($P = 0.695$; Table 6).

Discussion

In the present study, we studied the effects of polymorphisms in the $p53$ and $p21$ genes on the risk of CBP among 307 CBP workers and 299 benzene-exposed workers in China. We also investigated the effect of benzene exposure on the $p53$ and $p21$ mRNA expression in the peripheral blood lymphocytes among 39 CBP workers, 38 benzene-exposed controls, and 43 nonexposure controls. Although no significant association was found among three polymorphisms (intron 3, C119G, and IVS6+62 G>A) in $p53$ and the odds of CBP, our results showed that benzene exposure decreased the mRNA expression of $p53$ in human peripheral blood. In contrast to the $p53$ gene, our results showed that two SNPs in $p21$ (C98A and C70T) were associated with the decreased odds of CBP, but no significant differences in the $p21$ gene mRNA expression were observed among CBP workers, benzene-exposed controls, and nonexposure controls.

In response to a variety of stress signals, including genotoxic stress, $p53$ is activated, leading to the initiation of a range of biological defense pathways. Through $p53$-mediated cell-cycle arrest, cells are allowed sufficient time to repair DNA damage and replication errors. Different studies suggest that $p53$ seems to play a key role in benzene-induced hematotoxicity, and its dysfunction is a potential factor in benzene-induced carcinogenesis (30, 31).

Altering the amino-acid sequence of its product, the C119G/R72P polymorphism in $p53$ can affect its biochemical properties and biological functions (12). Several epidemiologic studies have found that this polymorphism was associated with the risk of different types of cancer, but the results are conflicting (32-34). Recently, Shen et al. (35) reported that the $p53$ C119G polymorphism was associated with a statistically significant decrease in total WBC counts among exposed workers. In the current study, however, we failed to observe an association between this SNP and the susceptibility to CBP.

In addition, we investigated two polymorphisms in $p53$ noncoding regions. Among all of the polymorphisms identified in $p53$, the most frequently studied have been the polymorphism in intron 3 (16 bp duplication) and the codon 72 polymorphism. Some investigators reported that the +16 allele (mutant allele) was associated with decreased apoptotic and DNA repair capacity in

lymphoblastoid cell lines (25). The other *p53* polymorphism we studied was a G-to-A transversion at position 62 in intron 6. This SNP was found to be associated with altered *p53* function (36). Epidemiologic studies have correlated the intron 3 duplication or the intron 6 polymorphism to increased risk of various cancers (25, 37-39). The present study, by contrast, failed to observe the effect of these two polymorphisms of *p53* on the susceptibility to CBP.

In order to confirm the effects of *p53* polymorphisms on individual susceptibility to CBP, we applied the Phase software to reconstruct its haplotypes and diplotypes. Our results showed no association between *p53* haplotypes or diplotypes and the odds of CBP. There are some plausible explanations for our findings. First, the variant genotypes of intron 3 or intron 6 are uncommon in Chinese populations. The low prevalence of these two genotypes would make it difficult to detect their association with the odds of CBP in our study. Second, *p53* polymorphisms combined with either environmental factors such as smoking (40), or certain conditions such as disease types, disease stages, and age of onset (41-43), are likely to contribute to the risk of cancers. Women seldom smoked cigarettes. Yet in this study, most of those who had cigarette smoking were female, potentially introducing bias in our results.

In the present study, we also detected *p53* mRNA expression in peripheral blood lymphocytes between benzene-exposed workers and nonexposure workers. Earlier *in vitro* studies (30, 31) showed that *p53* was a central regulator of the bone marrow response to inhaled benzene. Our finding that there was a significant difference in the mRNA expression among CBP workers, benzene-exposed controls, and nonexposure controls, confirmed prior findings in those studies. Our result indicated that by decreasing the *p53* mRNA expression in peripheral blood lymphocytes, exposed benzene might affect cell-cycle arrest, leading to CBP and leukemia. But this mechanism is unclear. Considering the bone marrow is the main target tissue of benzene, further study is needed to confirm this hypothesis.

Two previously reported SNPs in *p21* were found in the subjects enrolled in our study. Between the two SNPs, the codon 31 polymorphism (C98A) is the only one located in the coding region. Su et al. (44) reported that this polymorphism may alter mRNA expression of *p21*. Interestingly, the other polymorphism of *p21* tested in our study (*p21* C70T) is linked to the C98A polymorphism (12). The C70T polymorphism is located in the 3′ untranslated region, and in turn, the 3′untranslated region is a region that controls mRNA stability and degradation. It has been suggested that the C70T polymorphism may prevent timely degradation of the *p21* mRNA, therefore influencing the cellular DNA damage-induced cell cycle arrest response (26, 45). Although a number of published studies have examined the effect of *p21* polymorphisms on human cancers, as far as we know, this is the first study to explore the role of *p21* polymorphisms in the susceptibility to CBP. In the present study, we observed that after adjustment for potential confounders, individuals carrying *p21* 98A variant genotypes or *p21* 70T variant genotypes decreased the odds of CBP. Further analysis indicated that workers carrying *p21* CC/AT diplotype are less susceptible to CBP, a finding consistent with a number of studies in which these two SNPs of *p21* correlate

with cancer risk (26, 45-48). Therefore, these two SNPs may play a role in susceptibility to CBP.

Although P53 does not actively repair DNA, it functions to activate *p21* to regulate cell-cycle checkpoints that can pause the cell cycle to allow for DNA repair. Therefore, it is expected that *p21* plays an important role in the *p53*-mediated cell cycle arrest in response to DNA damage. In contrast to *p53*, we failed to find that benzene exposure altered the *p21* mRNA expression in the peripheral blood lymphocytes. Faiola et al. (5) found that in mice, *p21* mRNA was not altered in hematopoietic stem cells following benzene exposure. This may explain, in part, why no apparent difference of *p21* mRNA expression in the peripheral blood lymphocytes among the three groups was found in this study.

Although genetic polymorphisms play an important role in the expression of diseases, most chronic and complex illnesses are likely caused by interactions among environmental exposure, genetic polymorphisms, and lifestyle behaviors (21), such as tobacco and alcohol consumption, all of which are known risk factors for CBP (49, 50). This study found reduced odds of CBP for non-alcohol users with the *p21* 98A or 70T allele and nonsmokers with the *p21* 98A allele. However, the test for homogeneity of odds ratios suggested no potential interactions between those polymorphisms and tobacco/alcohol consumption. In addition, the relative low frequency of alcohol and tobacco consumption may have introduced some bias in our results; the majority of subjects with relevant information available were females who also were less likely than males to have indulged in alcohol and cigarette use. As such, replication of our findings in other benzene-exposed populations is critical.

Furthermore, accurate exposure estimation is quite important for evaluating exposure levels between the case and control groups. Because not all cases and controls are in the same exposure environment due to different types of work, we were limited to evaluating the intensity of benzene exposure according to methods described by Dosemeci et al. (24). That is, exposure levels were measured differently between cases and controls; it may not completely reflect individual variation in exposure. To minimize the potential for bias, more accurate methods for exposure estimation must be introduced, including individual sampling, which is more accurate than the traditional area sampling used in this study. Benzene metabolites reflecting the exposure levels also are needed to be applied in accurate exposure estimation.

We found for the first time that genetic variants in *p21* may contribute to the development of CBP in occupational exposure to benzene in a Chinese population. This finding may have important implications for the prevention of CBP in susceptible workers. Although this study included a homogeneous ethnic background of the subjects, well-documented workplace exposure history to benzene, and a frequency-matching design, it is possible that some associations will be false-positives, particularly those based on small numbers of subjects carrying at-risk alleles after adjustment for potential confounders. In addition, joint action between genetic polymorphisms and lifestyle risk factors on special diseases such as CBP is complicated, and small studies like the present one 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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