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Association of Genetic Polymorphisms in *CYP2E1*, *MPO*, *NQO1*, *GSTM1*, and *GSTT1* Genes with Benzene Poisoning

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Metabolic enzymes involved in benzene activation or detoxification, including NAD(P)H, quinone oxidoreductase 1 (NQO1), cytochrome P450 2E1 (CYP2E1), myeloperoxidase (MPO), glutathione-*S*-transferase mu-1 (GSTM1), and glutathione-*S*-transferase theta-1 (GSTT1), were studied for their roles in human susceptibility to benzene poisoning. The potential interactions of these metabolic enzymes with lifestyle factors such as cigarette smoking and alcohol consumption were also explored. We studied 156 benzene-poisoning patients and 152 workers occupationally exposed to benzene in South China. Sequencing, denaturing HPLC, restriction fragment-length polymorphism, and polymerase chain reaction were used to detect polymorphisms on the promoters and complete coding regions of *NQO1, CYP2E1, MPO,* and the null genotypes of *GSTM1* and *GSTT1*. Seventeen single nucleotide polymorphisms (SNPs) were identified in *NQO1, CYP2E1,* and *MPO* genes, including 6 novel SNPs in *CYP2E1* and *MPO*. Of the subjects who smoked and drank alcohol, an 8.15-fold [95% confidence interval (CI), 1.43–46.50] and a 21.50-fold (95% CI, 2.79–165.79) increased risk of benzene poisoning, respectively, were observed among the subjects with two copies of *NQO1* with a C-to-T substitution in cDNA at nucleotide 609 (c.609 C>T variation; i.e., *NQO1* c.609 T/T) compared to those with the heterozygous or wild (*NQO1* c.609 C/T and c.609 C/C) genotypes. Our data also indicated that individuals with *CYP2E1* c.–1293 C/C and c.–1293 G/C, and *NQO1* c.609 T/T, and *GSTT1* null genotypes tended to be more susceptible to benzene toxicity. Our results suggest that the combined effect of polymorphisms in *NQO1, CYP2E1,* and *GSTT1* genes and lifestyle factors might contribute to benzene poisoning. ***Key words:*** **benzene poisoning, polymorphisms, lifestyle, *NQO1, CYP2E1, MPO, GSTM1, GSTT1*.** ***Environ Health Perspect*** **110:1213–1218 (2002). [Online 15 October 2002]**
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Benzene is commonly used to synthesize organic chemicals and is an important component of many organic solvents. Workers exposed to benzene may potentially suffer chronic benzene poisoning (BP). Clinical reports have shown that exposure to benzene can result in a variety of blood and bone marrow disorders, including leukopenia, anemia, myelodysplastic syndrome, aplastic anemia, acute myeloid leukemia, and acute lymphocytic leukemia (Aksoy et al. 1972; Linet et al. 1996; Yin et al. 1987).

Previous studies have indicated that benzene toxicity mainly results from its intermediate reactive metabolites (Irons and Stillman 1996; Kolachana et al. 1993). Benzene is initially oxidized to benzene oxide by hepatic CYP2E1 in the liver (Koop et al. 1989; Valentine et al. 1996). Benzene oxide forms phenol spontaneously or conjugates with glutathione to form less toxic or nontoxic derivates via glutathione-*S*-transferases (GSTs). Phenol is catalyzed by CYP2E1 to potentially toxic di- or trihydroxybenzenes such as hydroquinone, catechol, and 1,2,4-benzentriol (Eastmond et al. 1987; Smith et al. 1989; Subrahmanyam et al. 1991). The di- or trihydroxy metabolites are further oxidized in the bone marrow by myeloperoxidase (MPO) to benzoquinones (Schattenberg et al. 1994), a potent hematotoxic and genotic agent, which can be detoxified by NAD(P)H:quinone oxidoreductase 1 (NQO1) to less harmful hydroxybenzenes (Joseph et al. 2000; Ross et al. 1996). Thus we hypothesized that the deficient or altered activity of enzymes involved in benzene metabolism such as CYP2E1, MPO, NQO1, and GSTs would significantly affect susceptibility to benzene toxicity.

Genetic polymorphisms in genes encoding CYP2E1, MPO, NQO1, and GSTs (Hirvonen et al. 1993; Piedrafita et al. 1996; Puga et al. 1997; Traver et al. 1997) might be responsible for human susceptibility to BP because they might have an effect on enzyme activity. *NQO1* with a C-to-T substitution in cDNA at nucleotide 609 (*NQO1* c.609C>T variation) causes reduced or lost enzyme activity (Traver et al. 1992). A case–control study revealed that workers exposed to benzene with both the higher CYP2E1 enzyme activity and the *NQO1* c.609 T/T genotype had a 7.6-fold increased risk of BP (Rothman et al. 1997). A single nucleotide polymorphism (SNP) in an Alu repeat in the *MPO* gene promoter, c.–463G>A, could decrease the expression of MPO by destroying an SP1 transcriptional factor binding site (Piedrafita et al. 1996). It has been reported that the *GSTT1* null genotype increased susceptibility to myelodysplastic syndrome [odds ratio (OR) = 4.3] (Chen et al. 1996), although confirmation of this finding is needed.

Most chronic and complex diseases are caused by interactions among environment, genes, and lifestyle (Mucci et al. 2001). We conducted a case–control study to explore the effects of polymorphisms in genes involved in benzene metabolism on human susceptibility to BP and to explore the potential effect of lifestyle modification on BP. Our results indicated that *NQO1* c.609C>T variation and lifestyle contributed to the risks of BP, and the combined effect of *NQO1* c.609 T/T, *CYP2E1* c.–1293 C/C and c.–1293 G/C, and the *GSTT1* null genotype significantly increased the risk of BP.

Materials and Methods

Subjects. The workers with BP who enrolled in this study came from Shanghai, Hangzhou, Maanshan, and Guangzhou, China, where clusters of cases were reported. Benzene poisoning was diagnosed from 1980 to 1998 by the local authorized Occupational Disease Diagnostic Team, and patients were registered in the hospitals of prevention and treatment for occupational diseases, which cooperated with us. The diagnostic criteria for occupational BP, according to the Ministry of Health, China, include *a*) total white blood cell count < 4,000/μL or white blood cell count between 4,000 and 4,500/μL and platelet count

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< 80,000/µL, with repeated confirmation of this count in a few months in a peripheral blood examination; *b*) the individual with documented benzene exposure has been employed for at least 6 months in the factory; and *c*) exclusion of other causes of abnormal blood counts such as chloromycetin use and ionizing radiation. The medical records of patients were independently reviewed, especially those with white blood cell counts > 3,500 to confirm the BP diagnosis. Of the 171 eligible patients, 156 (91%) agreed to participate in this study. About 72% of BP patients (112 patients) were from Shanghai Second Fabric Machine Factory (19 patients), Hangzhou Tool Machine Factory (13), Maanshan Iron & Steel Group (68), and Guangzhou Piano-Making Factory (12). Another 44 patients, who returned to the hospital periodically for health examinations, were from 12 other factories that had been closed down. BP cases from the factories registered in the hospitals were also clustered (more than five reports).

We chose 152 workers in the four major factories, who had been occupationally exposed to benzene, as controls. Control subjects were frequency-matched to cases by age within 5 years, exposure duration within 3 years, exposure level, and sex. All the eligible controls agreed to participate in this study.

The subjects were interviewed by 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 or industrial hygienists and long-term employees' evaluation considering historical changes (Dosemeci et al. 1996). 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 in controls was taken as the current level monitored by organic vapor passive dosimetry badges during collection of the blood samples from 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. The subjects were administrated a rigorous physical examination at a local occupational disease hospital. Alanine aminotransferase level in serum was examined to indicate liver function.

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° after collection and was sent to the laboratory later in dry ice.

Amplification of DNA samples. We extracted genomic DNA from blood samples by a routine phenol-chloroform method. We performed polymerase chain reaction (PCR) using 50 ng of genomic DNA, 0.2 µM of each primer, 100 µM dNTPs, 20 mM Tris-Cl (pH 8.8), 10 mM KCl, 1.0–1.5 mM $MgCl_2$, and 2.5 U *Pfu* polymerase (Stratagene, La Jolla, CA, USA) in a 25-µL reaction volume. DNA samples were amplified for 35 cycles at 94°C for 45 sec, 63°C for 1 min, and 72°C for 2 min. The primer sequences are listed in Table 1.

Detection of genetic polymorphisms. DNA sequencing was performed on an Automated DNA Sequencer ABI 377 (PE Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. We used the PolyPhred computer program (PolyPhred, available online) to indicate possible SNP loci. Verification of each candidate SNP was carried out by visual inspection.

Denaturing HPLC (DHPLC; Kuklin et al. 1997/1998) was carried out on an automated HPLC equipped with a DNA separation column (Transgenomic, San Jose, CA, USA). For *NQO1* exon4 and exon6, *MPO* promoter and exon 8, and *CYP2E1* exon 6, the temperature of the DHPLC column was 63°C, 60°C, 60°C, 63°C, and 58°C; the acetonitrile gradient was 53–61%, 54–62%, 63–69%, 58–68%, and 52–58%, respectively.

CYP2E1 c.–1293 G>C was analyzed by PCR-restriction length polymorphism (RFLP). PCR products were digested by *Pst*I (MBI, Hanover, MD, USA) at 37°C for 2 hr. Different length fragments were separated by polyacrylamide gel electrophoresis.

The null genotype of *GSTM1* and *GSTT1* (Arand et al. 1996) and 96-bp insertion in the promoter of *CYP2E1* (Fritsche et al. 2000) were detected by amplifying the target DNA regions and electrophoresis visualization on agarose gel. We used albumin as the internal control.

Statistical analyses. Our analysis was designed to examine the relationships of genotypes of *NQO1*, *CYP2E1*, *MPO*, *GSTM1*, and *GSTT1* with the risks of BP controlling potential confounding factors and evaluate whether lifestyle factors such as cigarette smoking and alcohol consumption modified

Table 1. PCR primer sequences.

Amplicon	Primer sequences	Length (bp)
NQO1		
Promoter	5´GTAGCTGGGACTTACAGGCG3´ and 5´GAGCAGAAAAAGAGCCGATG3´	484
Promoter	5´CAGGGAAGTGTGTTGTATGG3´ and 5´AAGTCAAGAAAAGTCTCTCGG3´	380
Exon 1	5´TCTGTCACACACACCCCTACA3´ and 5´AGGAACAAAATTCAGGCCAAG3´	474
Exon 2/3	5´CTGGTTGGTAATGGGTTTTC3´ and 5´CACGCAAATGTCCCTGACAC3´	502
Exon 4	5´AAAGTGCTAACTCCCCA3´ and 5´GGAAGCTCCATCTCAAACAA3´	453
Exon 5	5´CAGCAAATAGGACAGACTTGTG3´ and 5´GTAGTGAACTAAGATGGTG3´	308
Exon 6	5´GAATTGGTTGACTTACCTC3´ and 5´AACTAAAGCAAGTCAGGGA3´	425
CYP2E1		
Promoter	5´AAGCCAAGGCTTCAATTTCA3´ and 5´GATGAGAAATGAAGAAAATAAAAGTCA3´	563
Promoter	5´CAAGTGATTTGGCTGGATTG3´ and 5´TCGGAATTCGATTCCACTTT3´	578
Promoter	5´TTGGTTGACTCACTCTTTCCTTT3´ and 5´CCATCGTTTCAAAGGCTGAT3´	495
Exon 1	5´CAACCAGGGTGTTGACACAG3´ and 5´CCACAATTTGTCTGCAAATGA3´	518
Exon 2	5´ACTTCTAGCCACGGGTCTCC3´ and 5´GATCTCATCCTTCGGATGCT3´	320
Exon 3	5´GCCCCCTCTGTCACTTCTTT3´ and 5´CCCTGCTAGGCAGCAGATAC3´	295
Exon 4	5´CATCTTCTGGTTGCCCTGAC3´ and 5´TGTTGTTTGGCTTTTGCAGT3´	304
Exon 5	5´AGCCACAAATTCAGGTTTGG3´ and 5´GGAGAGCCCACATCATGG3´	305
Exon 6	5´CGGTCTGTCTCCGGTATCAC3´ and 5´CAGCCATCTCACCACATCAC3´	298
Exon 7	5´AACTGGCCAGGAACCAATC3´ and 5´GCAGGAAGGCGATTAGTGAT3´	300
Exon 8	5´ATTCTTCACTGGGGGTTTCC3´ and 5´ACATGTGGAGGGGAGATGAG3´	289
Exon 9	5´CGCTTCCCCTAGTCTCACTG3´ and 5´GTTCAGGGTGTCCTCCACAC3´	288
96 insertion	5´GTGATGGAAGCCTGAAGAACA3´ and 5´CTTTGGTGGGGTGAGAACAG3´	586
*Pst*I	5´ACATTGTCAGTTCTCACCTC3´ and 5´ATACCTATGGACTACCTTC3´	453
MPO		
Promoter	5´ATTTCCTGTCCCCTTAGCC3´ and 5´AGCCTCTAGCCACATCATCA3´	555
Promoter	5´GCTTCTTGCCTAAGGAAAAATACA3´ and 5´AGAGGGCCCTGTCTATGGAT3´	513
Exon 1/2	5´CCTCAAGGAGGTCTGGCTTT3´ and 5´GTGAAAGGCCTGGGACATC3´	534
Exon 3/4	5´CCTCACTTATGGCTCCAACG3´ and 5´TCTCTGAGCCCGGTTCCT3´	551
Exon 5/6	5´GTGTCAGCGCCCTGTCCT3´ and 5´ACAATCTCCCCTCTCCCAAC3´	494
Exon 7	5´GGTGAGCCAGTCTAGCCTCT3´ and 5´TCCAACAGGGAACATCTCCT3´	404
Exon 8	5´GCAAATCTTTTCTGGGATGG3´ and 5´CAGGAGCGTTAGGAACTTGC3´	309
Exon 9	5´AGGCCATTCCAATGACTTGT3´ and 5´TCTGGCCTAGGTCCTGCTTA3´	304
Exon 10	5´GCCAGATACTTCCCCTGACC3´ and 5´AGGGACCCTAGAGTGGGAAG3´	300
Exon 11	5´AGCAGAGAGACCTGCCCATA3´ and 5´GGCTCCAAGAGAGTCAAGGA3´	349
Exon 12	5´CACAGTGTCCATGGGTGTTC3´ and 5´TGAAACACTCCCCATGTTCA3´	448
Exon 12	5´CTGGGTGCAGCTGAGAAAAT3´ and 5´CTGGTCCAGCTCTGCTAACC3´	484
Exon 12	5´GGCGTGAGAAGCATATAGAGG3´ and 5´AGAGTGGAGGTCCCCATCAC3´	298
GSTM1	5´GAACTCCCTGAAAAGCTAAAGC3´ and 5´GTTGGGCTCAAATATACGGTG3´	215
GSTT1	5´TTCCTTACTGGTCCTCACATCTC3´ and 5´TCACCGGATCATGGCCAGCA3´	480
Albumin	5´GCCCTCTGCTAACAAGTCCTAC3´ and 5´GCCCTAAAAAGAAAATCGCCAATC3´	350

bp, base pair.

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the relationships of genotypes with risks of BP. SPSS 8.0 software (SPSS Inc., Chicago, IL, USA) was used to set up a database and analyze data. We used chi-square tests to examine the association between genetic polymorphisms and individual susceptibility to benzene hematotoxicity. If the value for a cell was < 5 in the chi-square test, we applied Fisher's exact test. To evaluate whether the lifestyle modified the relationship between genetic polymorphisms and susceptibility to BP, we examined the associations by applying chi-square tests after stratification according to cigarette smoking or alcohol consumption. The test for homogeneity of ORs was examined by the Breslow-Day method. The heterogeneity of ORs indicated there could be interaction ($p < 0.05$). To control potential confounding factors such as sex, intensity of benzene exposure, and exposure duration, we applied unconditional logistic regression to examine the relations of genetic polymorphisms with BP. Two-tailed p-values < 0.05 were considered statistically significant. ORs and 95% confidence intervals (95% CIs) were calculated to estimate the individual risk of BP. ORs adjusted for the potential confounding factors are also reported. We also used multiple-variables unconditional logistic regression analysis to analyze the data. We applied stepwise forward logistic regression selection to screen the covariates including sex, exposure duration, genetic polymorphisms, and interactions among them (criterion for acceptance: $p \le 0.05$; criterion for removal: $p \ge 0.10$). The screened covariates and intensity of benzene exposure were used to set up a saturated model.

Table 2. Characteristics of cases and controls.

	Cases		Controls	
	No.	Percent	No.	Percent
Total	156	100.0	152	100.0
Age (years)				
≤ 25	11	7.0	6	3.9
26–35	58	37.2	50	32.9
36–45	66	42.3	69	45.4
> 45	21	13.5	27	17.8
Sex				
Male	62	39.7	57	37.5
Female	94	60.3	95	62.5
Exposure duration (years)				
≤ 5	26	16.7	33	21.7
6–10	48	30.8	50	32.9
7–15	30	19.2	29	19.1
16–20	25	16.0	26	17.1
> 20	27	17.3	14	9.2
Intensity of exposure (mg/m^3)				
≤ 40	28	17.9	33	21.7
41–100	94	60.3	94	61.8
> 100	34	21.8	25	16.5
Smoking				
Yes	19	12.2	34	22.4
No	130	83.3	117	77.0
No data	7	4.5	1	0.6
Alcohol consumption				
Yes	18	11.5	17	11.2
No	133	85.3	134	88.2
No data	5	3.2	1	0.6
Type of work				
Painting	63	40.4	58	38.2
Spraying	25	16.0	28	18.4
Painting and spraying	20	12.8	16	10.5
Printing	4	2.6	7	4.6
Mechanic	14	9.0	10	6.6
Warehouseman	3	1.9	5	3.3
Other	27	17.3	28	18.4
Self-reported symptoms				
Dizziness	135	86.5	73	48.0
Weariness	133	85.3	42	27.6
Dreaminess	80	51.3	28	18.4
Inappetence	46	29.5	8	5.3
Ecchymosis	86	55.1	19	12.5
Bleeding while brushing teeth	96	61.5	28	18.4

Results

Demographics of cases and controls. The distribution of age, sex, exposure duration, intensity of benzene exposure, type of work, self-reported symptoms, cigarette smoking, and alcohol consumption in the cases and controls is shown in Table 2. The median age and exposure duration in 156 BP cases was 36.00 (range: 21.00–61.00) and 11.00 (range: 1.00–38.00); 38.50 (range: 19.00–57.00) and 9.00 (range: 1.00–36.00), respectively, in 152 controls. 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, > 25 years), intensity of benzene exposure (≤ 40 mg/m^3, 41–100 mg/m^3, > 100 mg/m^3), and sex ($p > 0.05$). The percentage of female subjects was much higher than that of males (60.26% vs. 39.74% in BP cases and 62.50% vs. 37.50% in controls). The higher female ratio may also explain the relatively low frequency of cigarette smoking (17.67%) and alcohol consumption (11.59%) in the subjects. Exposure duration was highly correlated with age (Spearman rank correlation, $p < 0.05$).

Genetic polymorphisms of NQO1, CYP2E1, MPO, GSTM1, and GSTT1. We initially screened 24 cases and 24 controls randomly for possible genetic variations in *NQO1*, *CYP2E1*, and *MPO* genes by direct sequencing. More than 99% of SNPs with frequencies ≥ 5% will be observed among the normal population $[1 - (1 - 5\%)^{48 \text{ individuals} \times 2 \text{ chromosomes}} = 99.27\%]$. We identified 17 SNPs by sequencing in the screened regions of *NQO1*, *CYP2E1*, and *MPO* and 6 SNPs were genotyped by DHPLC in all subjects. Two SNPs in *MPO* promoter, *MPO* c.–581T>C and *MPO* c.–463G>A were completely linked; thus only the *MPO* c.–463G>A was analyzed. The SNPs on the *CYP2E1* promoter region were too complicated (10 SNPs in 1,000 bp) to be detected by DHPLC (Table 3). We only determined 1 of 10 SNPs, *CYP2E1* c.–1293G>C, because the G-to-C transition forms a *Pst*I site. Table 3 shows the allele and genotype frequencies of polymorphisms on *NQO1*, *CYP2E1*, *MPO*, *GSTM1*, and *GSTT1* genes. Genotype frequencies of these genetic polymorphisms calculated from the control group were in Hardy-Weinberg equilibrium, making selection bias less likely (chi-square test, $p > 0.05$).

Table 3. Allele and genotype frequencies of genetic polymorphisms on *NQO1, CYP2E1, MPO, GSTM1,* and *GSTT1.*

Location	Sequence variation	Allele frequency (%)	Genotype frequency (%)
NQO1			
Exon 4	c.415C>T[a]	C: 98.7; T: 1.3	T/T: 0.3; C/T: 2.0; C/C: 97.7
Exon 6	c.609C>T[b]	C: 50.4; T: 49.6	T/T: 22.6; C/T: 54.1; C/C: 23.3
MPO			
Promoter	c.–581T>C[a]	T: 90.8; C: 9.2	T/T: 81.7; T/C: 18.3
Promoter	c.–463G>A	G: 90.8; A: 9.2	G/G: 81.7; G/A: 18.3
5′UTR	c.–25G>A[a]	G: 98.9; A: 1.1	G/G: 97.8; G/A: 2.2[c]
Intron 8	IVS8 + 19G>A[a]	G: 90.4; A: 9.6	G/G: 80.7; G/A: 19.3
CYP2E1			
Promoter	c.–1563T>A[a]	T: 70.7; A: 29.3	T/T: 46.3; T/A: 48.8; A/A: 4.9[c]
Promoter	c.–1513T>G[a]	T: 51.2; G: 48.8	T/T: 28.6; T/G: 45.2; G/G: 26.2[c]
Promoter	c.–1412C>T[a]	T: 97.6, C: 2.4	C/C: 95.2; C/T: 4.8[c]
Promoter	c.–1293G>C[a]	G: 78.4, C: 21.6	G/G: 59.2; G/C: 38.5; C/C: 2.3
Promoter	c.–1053C>T	C: 68.5; T: 31.5	C/C: 42.8; C/T: 51.4; T/T: 5.8[c]
Promoter	c.–1025T>C	T: 69.1; C: 30.9	T/T: 44.1; T/C: 50.0; C/C: 5.9[c]
Promoter	c.–929A>G	A: 80.9; G: 19.1	A/A: 61.8; A/G: 38.2[c]
Promoter	c.–806T>C	T: 64.2; C: 35.8	T/T: 38.2; T/C: 52.0; C/C: 9.8[c]
Promote	c.–352A>G	A: 78.9; G: 21.1	A/A: 57.8; A/G: 42.2[c]
Promoter	c.–333T>A	T: 50.0; A: 50.0	T/T: 27.3; T/A: 45.4; A/A: 27.3[c]
Exon 8	c.1263C>T[a]	C: 87.6; T: 12.4	C/T: 24.9; C/C: 75.1
Promoter	96-bp ins	+: 19.8; –: 80.2[d]	+/+: 4.3; +/– : 30.9; –/– : 64.8
GSTM1		Null: 47.5	Non-null: 52.5
GSTT1		Null: 54.0	Non-null: 46.0

Abbreviations: ins, insertion; UTR, untranslated region. Variation nomenclature is based on the principle described by den Dunnen and Antonarakis (2000).
[a]SNPs were deposited into HGVbase (Human Genome Variation database, available online; Fredman et al. 2002); among the SNPs, *MPO* c.–581T>C, *MPO* c.–25G>A, *MPO* IVS8 + 19G>A, *CYP2E1* c.–1563T>A, *CYP2E1* c.–1513T>G, and *CYP2E1* c.–1412C>T were novel. [b]*NQO1* c.609C>T is located at the 559 position from the start codon of *NQO1* mRNA; we used *NQO1* c.609C>T in this study to be consistent with other papers. [c]Allele frequency and genotype frequency were calculated in 48 subjects; others were calculated in all subjects. [d]+ indicates a 96-bp insertion in one chromosome; – indicates no 96-bp insertion in the chromosome.

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Effect of genetic polymorphisms of NQO1, MPO, CYP2E1, GSTM1, and GSTT1 on the risks of BP. The distribution of eight independent polymorphisms of the studied genes was compared in cases and controls (Table 4). Due to their small number, some genotypes were grouped with other genotypes according to previous reported research on the function on these genes. No association of genetic polymorphisms and susceptibility to risks of BP was found between BP cases and benzene-exposed workers ($p > 0.05$). Although the frequency of the cases with two copies of *NQO1* c.609C>T variation (*NQO1* c.609 T/T genotype) was slightly higher than that of controls (25.71% vs 19.58%), there was no statistical difference between them. There was little variation in OR values when adjusted for sex, exposure duration, and intensity of benzene exposure.

Relations of genetic polymorphisms of NQO1, MPO, CYP2E1, GSTM1, and GSTT1 with the risks of BP modified by lifestyle. The test for homogeneity (H) of ORs indicated a possible interaction between *NQO1* c.609 C>T and cigarette smoking/alcohol consumption ($\chi_H^2 = 5.969$, $p = 0.015$; $\chi_H^2 = 6.492$, $p = 0.011$, respectively; χ^2 and p-values adjusted by sex, exposure duration, and intensity of benzene exposure). The subjects were stratified according to cigarette smoking. The frequency of regular smokers with *NQO1* c.609 T/T genotypes in BP cases and benzene-exposed controls was 44.44% and 9.68%, respectively (Fisher's exact test, $p = 0.01$; Table 5). Our data showed a 7.73-fold increased risk of BP for smokers carrying *NQO1* c.609 T/T compared with the those with the heterozygous or wild type gene (*NQO1* c.609 C/T or C/C; OR = 7.73; 95% CI, 1.71–34.97; Table 5). Adjustment for sex, exposure duration, intensity of benzene exposure, and alcohol consumption had a minimal impact on the results (*NQO1* c.609C>T; OR = 8.15; 95% CI, 1.43–46.50; Table 5). Compared with the individuals with the *CYP2E1* c.–1293G>C wild genotype (*CYP2E1* c.–1293 G/G), the smokers carrying *CYP2E1* c.–1293 G/C or C/C genotypes had a 3.30-fold increased risk of BP (OR = 3.30; 95% CI, 1.02–10.65; Table 5), but no significant difference was observed after adjustment for sex, exposure duration, and alcohol consumption ($p = 0.07$). Due to the small number of smokers in this study (19 cases and 34 controls), the association of the combined effect of *CYP2E1* c.–1293G>C variation and cigarette smoking with BP should be explored in a larger sample size.

Among the alcohol drinkers, the frequency of BP cases with *NQO1* c.609 T/T was 61.11%, which was about five times as much as that of controls (Table 6). Compared with those of *NQO1* c.609 C/T and C/C genotypes, the subjects with *NQO1* c.609 T/T had an 11.00-fold increase for BP (OR = 11.00; 95% CI, 1.89–63.86; Fisher's exact test, $p = 0.005$), and this risk increased even higher, to 21.50-fold, after adjustment for sex, exposure duration, intensity of benzene exposure, and cigarette smoking (OR = 21.50; 95% CI, 2.79–165.79). The frequency of the BP cases with *GSTM1* null genotype among the alcohol drinkers was higher than that of the controls (66.67% vs. 33.33%), but no significant difference between them was observed in this study. There was, however, a 4.21-fold increased risk of BP for the alcohol drinkers with *GSTM1* null genotype compared with those with *GSTM1* non-null genotype (Table 6).

Multiple-variables unconditional logistic regression analysis. The covariates and cross-product terms examined in the logistic regression model included intensity of exposure, alcohol, *NQO1* c.609C>T, *CYP2E1* c.–1293G>C, and *GSTT1* genotypes (Table 7). The model suggested there was a joint action between alcohol consumption and *NQO1* c.609 C>T variation ($p = 0.007$) and among *NQO1* c.609 C>T, *CYP2E1* c.–1293G>C, and *GSTT1* null genotypes ($p = 0.019$). We examined the combined effects by stratification according to *NQO1*, *CYP2E1*, and *GSTT1* genotypes. The result showed individuals with *NQO1* c.609 T/T, *CYP2E1* c.–1293 C/C or C/G, and *GSTT1* null genotypes were more susceptible to BP with a 5.64-fold increased risk compared with individuals carrying *NQO1* c.609 C/T or C/C, *CYP2E1* c.–1293 G/G, and *GSTT1* non-null genotypes.

Discussion

By examining the polymorphisms of the promoter and coding regions of *NQO1*, *CYP2E1*, and *MPO* and the null genotype of *GSTM1* and *GSTT1* genes, we studied the relationship between genetic polymorphism and the human susceptibility to risks of BP. The interaction of genetic diversities of these genes with lifestyle on BP was also explored.

Though no association was suggested between genetic polymorphisms of these genes and risks of BP from our study, Rothman et al. (1997) reported a 2.6-fold increased risk of BP in the workers with *NQO1* c.609 T/T genotype. There was, however, an 8.15-fold or 21.50-fold increased risk of BP in the individuals with *NQO1* c.609 T/T genotype compared with *NQO1* C/C or C/T genotypes after stratification by cigarette smoking and alcohol consumption, respectively (Tables 5 and 6). Cigarette smoking and alcohol consumption were considered risk factors contributing to many diseases such as lung cancer and bladder cancer. Moreover, because benzene is a component of cigarette smoke, the cumulative exposures of regular smokers should be higher than those of nonsmokers who never smoked while exposed to benzene. Our results suggested that there might be an association between *NQO1* c.609 T/T genotype and the risks of BP with modification by cigarette smoking and alcohol consumption. The C-to-T point variation of this SNP, which causes a proline

Table 4. Effect of *NQO1*, *CYP2E1*, *MPO*, *GSTT1*, and *GSTM1* genotypes on the risks of BP in benzene-exposed workers.

	Case[a]		Control[a]			
	No.	Percent	No.	Percent	OR (95% CI)	OR_{adj} (95% CI)[b]
Total	156	100.00	152	100.00		
NQO1						
c.609C>T						
T/T	36	25.71	28	19.58	1.42 (0.81–2.49)	1.43 (0.81–2.51)
C/T and C/C	104	74.29	115	80.42	1.00	1.00
CYP2E1						
96 bp insertion						
Ins_{96}–/+ and +/+	53	36.30	45	34.09	1.10 (0.67–1.80)	1.08 (0.66–1.79)
Ins_{96}–/–	93	63.70	87	65.91	1.00	1.00
c.–1293G>C						
G/C and C/C	65	41.67	59	39.33	1.10 (0.70–1.74)	1.12 (0.71–1.76)
G/G	91	58.33	91	60.67	1.00	1.00
c.1263C>T						
C/T	31	24.80	37	25.00	0.99 (0.57–1.72)	0.99 (0.57–1.73)
C/C	94	75.20	111	75.00	1.00	1.00
MPO						
c.–463G>A						
G/A	28	18.67	27	18.00	1.05 (0.58–1.88)	1.09 (0.60–1.97)
G/G	122	81.33	123	82.00	1.00	1.00
IVS8 + 19G>A						
G/A	29	20.42	26	18.18	1.15 (0.64–2.08)	1.11 (0.61–2.02)
G/G	113	79.58	117	81.82	1.00	1.00
GSTM1						
Null	72	50.35	63	44.68	1.26 (0.79–2.00)	1.25 (0.78–2.00)
Non-null	71	49.65	78	55.32	1.00	1.00
GSTT1						
Null	82	53.59	79	54.48	0.96 (0.61–1.52)	0.95 (0.60–1.50)
Non-null	71	46.41	66	45.52	1.00	1.00

[a]Data missing due to inability to amplify DNA. [b]ORs were adjusted (adj) for potential confounding variables including exposure duration, intensity of benzene exposure, and sex.

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to serine change, is associated with a loss activity of NQO1 (Traver et al. 1992). Moran et al. (1999) demonstrated that benzene metabolite hydroquinone induced high levels of NQO1 activity in bone marrow $CD34^+$ cells with the wild genotype (*NQO1* c.609 C/C), and noncytotoxic doses of hydroquinone induced intermediate levels of NQO1 activity in heterozygous (*NQO1* c.609 C/T) cells but exerted no effect on cells with the *NQO1* c.609 T/T genotype. It is possible that failure to induce functional NQO1 enzyme activity in *NQO1* c.609 T/T individuals might be responsible for BP.

In addition to the interaction between genetic polymorphisms and lifestyle, different genetic polymorphisms could contribute to the risk of BP. We speculated that individuals with

Table 5. Effects of genotypes of *NQO1*, *CYP2E1*, *MPO*, *GSTT1*, and *GSTM1* modified by smoking on the risks of BP in benzene-exposed workers.

	Smoking				Nonsmoking			
	Case (%)[a]	Control (%)[a]	OR (95% CI)	OR_{adj} (95% CI)[b]	Case (%)[a]	Control (%)[a]	OR (95%CI)	OR_{adj} (95% CI)[b]
Total	19 (100.00)	34 (100.00)			130 (100.00)	117 (100.00)		
NQO1								
c.609C>T								
T/T	8 (44.44)	3 (9.68)	7.73 (1.71–34.97)**	8.15 (1.43–46.50)*	27 (23.48)	25 (22.73)	1.04 (0.56–1.94)	1.01 (0.54–1.89)
C/T and C/C	10 (55.56)	29 (90.32)	1.00	1.00	88 (76.52)	85 (77.27)	1.00	1.00
CYP2E1								
96 bp insertion								
Ins96–/+ and +/+	7 (36.84)	12 (42.86)	0.78 (0.24–2.57)	0.70 (0.18–2.66)	44 (36.67)	32 (31.07)	1.28 (0.73–2.25)	1.23 (0.70–2.17)
Ins96–/–	12 (63.16)	16 (57.14)	1.00	1.00	76 (63.33)	71 (68.93)	1.00	1.00
c.–1293G>C								
G/C and C/C	11 (57.89)	10 (29.41)	3.30 (1.02–10.65)*	3.24 (0.90–11.71)	49 (37.69)	48 (41.74)	0.84 (0.51–1.41)	0.87 (0.51–1.47)
G/G	8 (42.11)	24 (70.59)	1.00	1.00	81 (62.31)	67 (58.26)	1.00	1.00
c.1263C>T								
C/T	3 (18.75)	8 (23.53)	0.75 (0.17–3.31)	0.74 (0.15–3.79)	28 (27.45)	29 (25.66)	1.10 (0.60–2.01)	1.07 (0.58–1.97)
C/C	13 (81.25)	26 (76.47)	1.00	1.00	74 (72.55)	84 (74.34)	1.00	1.00
MPO								
c.–463G>A								
G/A	5 (26.32)	4 (12.12)	2.59 (0.60–11.16)	2.51 (0.49–13.02)	22 (17.74)	23 (19.83)	0.87 (0.46–1.67)	0.93 (0.48–1.80)
G/G	14 (73.68)	29 (87.88)	1.00	1.00	102 (82.26)	93 (80.17)	1.00	1.00
IVS8 + 19G>A								
G/A	4 (21.05)	9 (30.00)	0.62 (0.16–2.40)	0.55 (0.13–2.34)	24 (20.69)	17 (15.18)	1.46 (0.74–2.89)	1.36 (0.68–2.72)
G/G	15 (78.95)	21 (70.00)	1.00	1.00	92 (79.31)	95 (84.82)	1.00	1.00
GSTM1								
Null	10 (52.63)	12 (40.00)	1.67 (0.52–5.31)	1.33 (0.40–4.46)	59 (50.43)	51 (46.36)	1.18 (0.70–1.98)	1.12 (0.66–1.92)
Non-null	9 (47.37)	18 (60.00)	1.00	1.00	58 (49.57)	59 (53.64)	1.00	1.00
GSTT1								
Null	10 (55.56)	17 (53.13)	1.10 (0.3–4.12)	1.13 (0.33–3.83)	68 (53.13)	62 (55.36)	0.91 (0.55–1.52)	0.88 (0.53–1.48)
Non-null	8 (44.44)	15 (46.87)	1.00	1.00	60 (46.87)	50 (44.64)	1.00	1.00

[a]Data missing due to inability to amplify DNA. [b]ORs were adjusted (adj) for potential confounding variables including sex, exposure duration, intensity of benzene exposure, and alcohol consumption. *$p < 0.05$, **$p < 0.01$.

Table 6. Effects of genotypes of *NQO1*, *CYP2E1*, *MPO*, *GSTT1*, and *GSTM1* modified by alcohol on the risks of BP in benzene-exposed workers.

	Alcohol				No alcohol			
	Case (%)[a]	Control (%)[a]	OR (95% CI)	OR_{adj} (95% CI)[b]	Case (%)[a]	Control (%)[a]	OR (95%CI)	OR_{adj} (95% CI)[b]
Total	18 (100.00)	17 (100.00)			133 (100.00)	134 (100.00)		
NQO1								
c.609C>T								
T/T	11 (61.11)	2 (12.50)	11.00 (1.89–63.86)*	21.50 (2.79–165.79)*	23 (19.66)	26 (20.63)	0.94 (0.50–1.76)	0.90 (0.47–1.72)
C/T and C/C	7 (38.89)	14 (87.50)	1.00	1.00	94 (80.34)	100 (79.37)	1.00	1.00
CYP2E1								
96 bp insertion								
Ins96–/+ and +/+	9 (50.00)	6 (40.00)	1.50 (0.38–6.00)	1.99 (0.44–8.95)	42 (34.15)	38 (32.76)	1.06 (0.62–1.82)	1.06 (0.61–1.84)
Ins96–/–	9 (50.00)	9 (60.00)	1.00	1.00	81 (65.85)	78 (67.24)	1.00	1.00
c.–1293G>C								
G/C and C/C	5 (27.78)	4 (23.53)	1.25 (0.27–5.73)	3.43 (0.56–21.00)	55 (41.35)	54 (40.91)	1.02 (0.62–1.66)	0.97 (0.58–1.60)
G/G	13 (72.22)	13 (76.47)	1.00	1.00	78 (58.65)	78 (59.09)	1.00	1.00
c.1263C>T								
C/T	6 (37.50)	5 (29.41)	1.44 (0.37–6.16)	1.24 (0.23–6.74)	25 (24.04)	32 (24.62)	0.97 (0.53–1.77)	0.99 (0.54–1.83)
C/C	10 (62.50)	12 (70.59)	1.00	1.00	79 (75.96)	98 (75.38)	1.00	1.00
MPO								
c.–463G>A								
G/A	5 (27.78)	2 (12.50)	2.69 (0.44–16.37)	2.10 (0.30–14.87)	22 (17.32)	25 (18.80)	0.91 (0.48–1.70)	0.97 (0.50–1.85)
G/G	13 (72.22)	14 (87.50)	1.00	1.00	105 (82.68)	108 (81.20)	1.00	1.00
IVS8 + 19G>A								
G/A	6 (33.33)	3 (21.43)	1.83 (0.37–9.17)	3.19 (0.40–25.19)	23 (19.33)	23 (17.97)	1.09 (0.58–2.08)	0.97 (0.50–1.90)
G/G	12 (66.67)	11 (78.57)	1.00	1.00	96 (80.67)	105 (82.03)	1.00	1.00
GSTM1								
Null	12 (66.67)	5 (33.33)	4.00 (0.93–17.11)	4.21 (0.84–21.04)	57 (47.50)	58 (46.40)	1.05 (0.63–1.73)	1.01 (0.61–1.70)
Non-null	6 (33.33)	10 (66.67)	1.00	1.00	63 (52.50)	67 (53.60)	1.00	1.00
GSTT1								
Null	7 (41.18)	8 (53.33)	0.61 (0.15–2.49)	0.54 (0.11–2.63)	71 (54.20)	71 (55.04)	0.97 (0.59–1.58)	0.97 (0.58–1.59)
Non-null	10 (58.82)	7 (46.67)	1.00	1.00	60 (45.80)	58 (44.96)	1.00	1.00

[a]Data missing due to inability to amplify DNA. [b]ORs were adjusted (adj) for potential confounding variables including sex, exposure duration, intensity of benzene exposure, and smoking. *$p < 0.01$.

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Table 7. Multiple-variables unconditional logistic regression analysis.

Variables	β	*p*-Value	OR (95% CI)
Intensity of benzene exposure			
40–100 mg/m^3	–0.341	0.312	0.711 (0.368–1.377)
> 100 mg/m^3	0.099	0.762	1.104 (0.581–2.099)
Alcohol (yes vs. no)	–0.735	0.146	0.4795 (0.178–1.292)
NQO1[a]	–0.150	0.791	0.8607 (0.283–2.620)
CYP2E1[b]	0.094	0.820	1.0995 (0.485–2.493)
GSTT1[c]	–0.232	0.525	0.7928 (0.387–1.624)
NQO1[a] × *CYP2E1*[b]	–0.906	0.328	0.4040 (0.065–2.490)
NQO1[a] × *GSTT1*[c]	–0.404	0.622	0.6673 (0.134–3.333)
CYP2E1[b] × *GSTT1*[c]	–0.558	0.332	0.5723 (0.185–1.769)
Alcohol (yes vs. no) × *NQO1*[a]	3.271	0.007	26.3425 (2.379–291.688)
GSTT1[c] × *NQO1*[a] × *CYP2E1*[b]	3.281	0.019	26.6020 (1.703–415.478)
Constant	0.262	0.456	

[a]*NQO1* c.609 T/T versus C/T and C/C. [b]*CYP2E1* c.–1293 G/C and C/C versus G/G. [c]*GSTT1* null versus non-null.

Table 8. Combined effect of *CYP2E1*, *GSTT1*, and *NQO1* genetic polymorphisms on risks of benzene poisoning.

CYP2E1 c.–1293 G>C	*GSTT1*	*NQO1* C.609 C>T	Case[a] (*n*)	Control[a] (*n*)	OR (95% CI)	OR_{adj} (95% CI)[b]
G/C and C/C	Null	T/T	11	2	5.32 (0.97–38.19)	5.64 (1.22–26.10)*
G/C and C/C	Null	C/T and C/C	19	28	0.66 (0.34–1.28)	0.65 (0.34–1.26)
G/C and C/C	Non-null	T/T	4	7	0.50 (0.14–1.89)	0.51 (0.14–1.85)
G/C and C/C	Non-null	C/T and C/C	23	19	1.17 (0.53–2.59)	1.19 (0.53–2.68)
G/G	Null	T/T	9	9	0.99 (0.37–2.63)	0.99 (0.37–2.68)
G/G	Null	C/T and C/C	32	35	0.80 (0.39–1.66)	0.88 (0.44–1.78)
G/G	Non-null	T/T	11	8	1.33 (0.47–3.78)	1.35 (0.46–3.96)
G/G	Non-null	C/T and C/C	30	29	1.00	1.00

[a]Data missing due to inability to amplify DNA. [b]ORs were adjusted (adj) for potential confounding variables including sex, exposure duration, and intensity of benzene exposure. *$p < 0.05$.

high-level activity of metabolic enzymes involved in oxidizing benzene to more toxic metabolites such as CYP2E1 and low-level activity of metabolic enzymes participated in detoxification pathway as GSTT1 and GSTM1 were less resistant to benzene toxicity. There was no detectable enzyme activity in the individuals with *GSTT1* null genotypes. Using an *in vitro* transfection system with CAT as a reporter gene, it has been observed that the *Ras*I polymorphism, which was completely linked with *Pst*I in the 5´-flanking sequence of the *CYP2E1* gene, caused a 10-fold increase in transcription activity compared with the wild type sequence (Hayashi et al. 1991). However, another study showed the decreased oral clearance of chlorzoxazone with *Ras*I homozygous variants in Japanese residents of Hawaii (Marchand et al. 1999). The higher inducible enzyme activity was also observed in individuals with a 96 bp insertion (Ins_{96}+/– and Ins_{96}+/+) on the *CYP2E1* promoter region (McCarver et al. 1998). Analysis of the combined effects of various genotype on risks of BP showed that the individuals with the *NQO1* c.609 T/T, *CYP2E1* c.–1293 G/C and C/C, and *GSTT1* null genotype had an increased risk of BP with exposure to benzene (Table 8). The results suggest that individuals with lower NQO1 and GSTT1 activity and higher CYP2E1 activity tend to be more susceptible to benzene toxicity.

Joint action between genetic polymorphisms and environment on specific diseases is complicated. A more comprehensive, larger scale study should be warranted to confirm the gene–environment interaction on susceptibility to BP.

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