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.

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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 derivatives via glutathione-S-transferases (GSTs). Phenol is catalyzed by CYP2E1 to potentially toxic di- or trihydroxybenzenes such as hydroquinone, catechol, and 1,2,4-benzenetriol (Eastmond et al. 1987; Smith et al. 1989; Subrahmanyan 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 genotoxic 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 > 150,000/µL.

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< 80,000/µL, with repeated confirmation of this count in a few months in a peripheral blood examination; the individual with documented benzene exposure has been employed for at least 6 months in the factory; and 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 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 MgCl₂, 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, 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 PstI (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 (Fritsch 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 the risk of BP.

< Table 1. PCR primer sequences.>

| AmpliCon | Primer sequences | Length (bp) |
|----------|-----------------|-------------|
| NQO1     | 5`GTACCTGGGACTACATGAGC3´ and 5`GAGGAAAAAGAGCCGATG3´ | 484 |
|         | 5`CAGGAAATGTGTTGTGATG3´ and 5`AAGCTAGAAGAAAATGCTCG3´ | 380 |
| Exon 1   | 5`TCTGTCAACACACACCCTAC3´ and 5`AGGAAAGAAAATGACGCGA3´ | 474 |
| Exon 2/3 | 5`CTGTTGTGAAATGACACCT3´ and 5`CAGGGAAATGTGTTGTGATG3´ | 502 |
| Exon 6   | 5`CAAGAATTAGAGACAGACCTG3´ and 5`GTAGTAGTGTGTGTGTGTG3´ | 453 |
| Exon 7   | 5`GCTGTTGACCTCATTAC3´ and 5`GAATATGAAAGGACGAGA3´ | 308 |
| Exon 8   | 5`GAATATGAAAGGACGAGA3´ and 5`GAATATGAAAGGACGAGA3´ | 425 |
| CYP2E1   | 5`AAGGAAATGTGTTGTGATG3´ and 5`AAGGAAATGTGTTGTGATG3´ | 563 |
|          | 5`CAAGGAAATGTGTTGTGATG3´ and 5`AAGGAAATGTGTTGTGATG3´ | 578 |
|          | 5`TCTGTCAACACACACCCTAC3´ and 5`AGGAAAGAAAATGACGCGA3´ | 495 |
| Exon 1   | 5`CACAACAGGTTGAGCATCG3´ and 5`CACAATTTGTTGTGATAATG3´ | 518 |
| Exon 2   | 5`ACTTCTGGAACACCGCTCT3´ and 5`GATTTCTGACCGCTCTCG3´ | 320 |
| Exon 3   | 5`GCCCTCTGCATCATTCTCT3´ and 5`GCCCTCTGCATCATTCTCT3´ | 304 |
| Exon 4   | 5`CTATCCTGATGACGCTT3´ and 5`GATTTCTGACCGCTCTCG3´ | 304 |
| Exon 5   | 5`AGCACAATCTAGTTTAGG3´ and 5`GAGGAGGACCCACCATGTG3´ | 305 |
| Exon 6   | 5`CGCTGCTGGCCCTGATC3´ and 5`GAGGACATCCTACCACCTC3´ | 298 |
| Exon 7   | 5`AATCTGAGCAACAAACTG3´ and 5`GCAAGGGAGGAGATGATG3´ | 300 |
| Exon 8   | 5`ATTCTTCTGGGCGTTCTCC3´ and 5`ACATGAGSAGGGAGATGATG3´ | 298 |
| Exon 9   | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 305 |
| Exon 10  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 308 |
| Exon 11  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 310 |
| Exon 12  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 312 |
| Exon 13  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 314 |
| Exon 14  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 316 |
| Exon 15  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 318 |
| Exon 16  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 320 |
| Exon 17  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 322 |
| Exon 18  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 324 |
| Exon 19  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 326 |
| Exon 20  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 328 |
| Exon 21  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 330 |
| Exon 22  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 332 |
| Exon 23  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 334 |
| Exon 24  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 336 |
| Exon 25  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 338 |
| Exon 26  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 340 |
| Exon 27  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 342 |
| Exon 28  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 344 |
| Exon 29  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 346 |
| Exon 30  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 348 |
| Exon 31  | 5`GTGCTGCTGGCCCTGATC3´ and 5`GAGGAGGACCCACCATGTG3´ | 350 |

bp, base pair.
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 hematoxicity. 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 relationships of genotypes 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 ≤ 0.05; criterion for removal: p ≥ 0.10). The screened covariates and intensity of benzene exposure were used to set up a saturated model.

Results

Demographics of cases and controls. The distribution of age, sex, smoking, 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 in 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 11.00 (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 (≤ 6, 6–10, 11–15, 16–20, > 25 years), and intensity of benzene exposure (≤ 10 mg/m^3, > 10–40, > 40–100, > 100 mg/m^3) and sex (p > 0.05). The percentage of female subjects was much higher in cases than in controls (48.0 vs. 38.5%). The percentage of male subjects was much higher in controls than in cases (52.0 vs. 62.0%). The percentage of male subjects was much higher in cases than in controls (48.0 vs. 38.5%).

Table 2. Characteristics of cases and controls.

| Characteristics | Cases | Controls |
|-----------------|-------|----------|
| Age (years)     | No.   | Percent  | No.   | Percent  |
| ≥ 25            | 115   | 71.9     | 110   | 73.0     |
| 26–35           | 42    | 26.9     | 40    | 26.5     |
| 36–45           | 25    | 15.6     | 22    | 14.5     |
| > 45            | 1     | 0.6      | 1     | 0.7      |
| Sex             |       |          |       |          |
| Male            | 121   | 73.5     | 116   | 77.0     |
| Female          | 47    | 26.5     | 36    | 23.0     |
| Exposure duration (years) |       |          |       |          |
| ≤ 5             | 26    | 16.4     | 28    | 18.2     |
| 6–10            | 48    | 30.1     | 44    | 29.0     |
| 11–15           | 30    | 18.6     | 28    | 18.4     |
| 16–20           | 25    | 15.6     | 25    | 16.2     |
| > 20            | 27    | 16.8     | 27    | 17.4     |
| Intensity of exposure (mg/m^3) |       |          |       |          |
| ≤ 10            | 48    | 30.1     | 48    | 30.8     |
| 11–20           | 43    | 27.0     | 41    | 27.4     |
| > 20            | 41    | 26.0     | 38    | 24.8     |
| Alcohol consumption |       |          |       |          |
| Yes             | 19    | 11.8     | 20    | 13.1     |
| No              | 130   | 82.2     | 130   | 85.7     |
| No data         | 7     | 4.5      | 9     | 5.6      |

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 1 c.–611C>T   | 0.52                 | 0.64                   |
|                | Exon 2 c.–609C>T   | 0.63                 | 0.37                   |
|                |                   | 0.93                 | 0.93                   |
| MPO            | Promoter c.–202T>A  | 0.79                 | 0.21                   |
|                | Exon 1 c.–609C>T   | 0.74                 | 0.26                   |
|                | Exon 2 c.–609C>T   | 0.74                 | 0.26                   |
|                |                   | 0.91                 | 0.91                   |
| GSTM1          | Promoter c.–330C>T  | 0.62                 | 0.38                   |
|                | Exon 1 c.–609C>T   | 0.63                 | 0.37                   |
|                | Exon 2 c.–609C>T   | 0.63                 | 0.37                   |
|                |                   | 0.93                 | 0.93                   |
| GSTT1          | promoter c.–1412C>T | 0.64                 | 0.36                   |
|                | Exon 1 c.–609C>T   | 0.63                 | 0.37                   |
|                | Exon 2 c.–609C>T   | 0.63                 | 0.37                   |
|                |                   | 0.93                 | 0.93                   |

Abbreviations: ins, insertion; UTR, untranslated region. Variation nomenclature is based on the principle described by de Dunnen and Antonarakis (2000).

*SNPs were deposited into HGVSbase (Human Genome Variation database, available online; Fredman et al. 2002) among the SNPs, 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 2). We only determined 1 of 10 SNPs, CYP2E1 c.–1293G>C, because the G-to-C transition forms a Pal site. Table 3 shows the allele and genotype frequencies of polymorphisms on NQO1, CYP2E1, MPO, GSTM1, and GSTT1 genotypes. 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).
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/alkohol consumption ($\chi^2 = 5.466, p = 0.015; \chi^2 = 6.692, p = 0.011$, respectively; $\chi^2 p$-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.609 C>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.609 C>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 to leucine change.
to scirn 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 | Control | OR (95% CI) | OR adj (95% CI) | OR (95% CI) | OR adj (95% CI) |
|---------|---------|-------------|----------------|-------------|----------------|
| Smoking | 19 (100.0) | 34 (100.0) | 130 (100.0) | 117 (100.0) |
| C/T and C/C | 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 |

**Data missing due to inability to amplify DNA. *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 | Smoking | Control | OR (95% CI) | OR adj (95% CI) | OR (95% CI) | OR adj (95% CI) |
|---------|---------|---------|-------------|----------------|-------------|----------------|
| Smoking | 18 (100.0) | 17 (100.0) | 133 (100.0) | 134 (100.0) |
| C/T and C/C | 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 (80.54) | 100 (79.37) | 1.00 | 1.00 |

**Data missing due to inability to amplify DNA. *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 7. Multiple-variables unconditional logistic regression analysis.

| Variables | β | p-Value | OR (95% CI) |
|-----------|---|---------|-------------|
| Intensity of benzene exposure | | | |
| 40–100 mg/m³ | -0.341 | 0.312 | 0.711 (0.368–1.371) |
| > 100 mg/m³ | 0.099 | 0.762 | 1.104 (0.581–2.099) |
| Alcohol (yes vs. no) | | | |
| NQO1a | -0.150 | 0.791 | 0.8067 (0.283–2.620) |
| CYP2E1b | 0.094 | 0.920 | 1.0959 (0.925–2.493) |
| GSTT1c | -0.232 | 0.525 | 0.7232 (0.387–1.824) |
| NQO1a × CYP2E1b | -0.906 | 0.328 | 0.4040 (0.065–2.490) |
| NQO1a × GSTT1c | -0.404 | 0.622 | 0.6673 (0.133–3.333) |
| CYP2E1b × GSTT1c | -0.558 | 0.332 | 0.5723 (0.185–1.769) |

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

| CYP2E1 | GSTT1 | NQO1 | Case† | Control‡ | OR (95% CI) | OR adj (95% CI) |
|--------|-------|------|-------|---------|-------------|----------------|
| G/C and C/C | Null | T/T | 11 | 2 | 5.32 (0.97–28.19) | 5.64 (1.22–26.10)* |
| G/C and C/C | Null | T/C and C/C | 19 | 28 | 0.66 (0.34–1.26) | 0.65 (0.34–1.26) |
| G/C and C/C | Non-null | T/T | 4 | 7 | 1.50 (0.14–14.95) | 0.51 (0.14–1.95) |
| G/C and C/C | Non-null | T/C and C/C | 23 | 19 | 1.17 (0.53–2.59) | 1.19 (0.53–2.62) |
| G/G | Null | T/T | 9 | 9 | 0.99 (0.37–2.63) | 0.99 (0.37–2.68) |
| G/G | Null | T/C 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 (0.47–7.38) | 1.35 (0.46–3.96) |
| G/G | Non-null | T/C and C/C | 30 | 29 | 1.00 | 1.00 |

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

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NQO1 c.609 T/T vs. C/T and C/C. CYP2E1 c.1293 G/C and C/C versus G/G. GSTT1 null versus non-null.

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