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Biomarkers of benzene: Urinary metabolites in relation to individual genotype and personal exposure

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Abstract

This report is part of an extensive biomarker study conducted in a Chinese occupational population with benzene exposures ranging from 0.06 to 122 ppm (median exposure of 3.2 ppm). All urinary benzene metabolites measured in this study were significantly elevated after exposure to benzene at or above 5 ppm. Among these metabolites, however, only *S*-phenylmercapturic acid (*S*-PMA) and *trans*,*trans*-muconic acid (*t*,*t*-MA) showed a significant exposure-response trend over the exposure range from 0 to 1 ppm (for *S*-PMA, $p < 0.0001$ and for *t*,*t*-MA, $p = 0.006$). For benzene exposure monitoring, both *S*-PMA and *t*,*t*-MA were judged to be good and sensitive markers, which detected benzene exposure at around 0.1 and 1 ppm, respectively. Polymorphisms of the metabolic genes, including CYP2E1, quinone oxidoreductase (NQO1), GSTT1, and myeloperoxidase (MPO), were identified and did not show significant effects on the formation of metabolites, except GSTT1 on *S*-PMA. The production rate of *S*-PMA from benzene in exposed workers with GSTT1 null alleles (24.72 ± 32.48 μg/g creatinine/ppm benzene) was significantly lower than that in subjects with the wild type of GSTT1 (59.84 ± 47.66 μg/g creatinine/ppm benzene, $p < 0.0001$). Further regression analysis of *S*-PMA production rate on GSTT1 genotype with adjustment of sex, age, benzene exposure, and cotinine levels indicated that the genotype of GSTT1 plays a critical role in determining the inter-individual variations of *S*-PMA formation from benzene exposure. Therefore, the individual genotype of GSTT1 needs to be identified and considered while using *S*-PMA as a marker to estimate the personal exposure levels of benzene in future population studies.
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Keywords: Benzene; Urinary metabolites; *t*,*t*-MA; *S*-PMA; Gene polymorphisms; CYP2E1; NQO1; GSTT1; MPO

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1. Introduction

There is an increasing public concern about environmental exposure to ambient levels of benzene since it is among the components of mobile source emissions identified in the USA Clean Air Act Amendments of 1990 as toxic air pollutants that need to be reduced. At the present time, however, the risk assessment for benzene has been focused mainly on environmental exposure itself and has not adequately accounted for interindividual variation in human susceptibility to benzene induced adverse health effects. Recently, the National Institute of Environmental Health Sciences launched the Environmental Genome Project to focus research on understanding the roles of environmental responsive genes on human susceptibility to environmental agents [1]. One of its most interesting areas is the polymorphism of genes encoding for metabolism of toxicants. It is now clear that polymorphisms of the metabolic genes may result in significant variations in the enzymatic activities and may cause individuals to possess minimal (or, for certain polymorphisms, excess) levels of the enzyme activity as compared to the majority of people. These individuals when exposed to certain environmental agents may experience a pathologic response at exposure levels that do not affect the general population as a result of the variations in their enzyme activities.

In the past few years, a number of studies have been conducted to examine the roles that gene polymorphisms may play in determining individual susceptibility to benzene associated hematotoxicity [2–7]. However, discrepancies have been shown among the studies suggesting that further investigations for gene–environment interactions are needed in benzene population studies. Most recently, a multi-laboratory collaboration was conducted focusing on the validation of benzene biomarkers in a Chinese population with a broad range of benzene exposures. To extend this biomarker study, we further isolated DNA from each individual blood sample for identifying the polymorphisms of genes, including CYP2E1, myeloperoxidase (MPO), quinone oxidoreductase (NQO1), and GSTT1. We describe here in this report how gene polymorphisms interact with benzene exposures in the formation of urinary metabolites.

2. Materials and methods

2.1. Subject recruitment, benzene exposure, and sample collections

A total of 181 subjects, including 130 exposed and 51 unexposed workers, were recruited based on a three-step procedure from Chinese occupational populations as described previously [8]. Briefly, all exposed workers in participating factories were interviewed by our research staff according to a carefully designed questionnaire. The information provided by the workers was then given to the personnel officers of the plants for review and confirmation. Workers with at least a 3-year exposure history, who had no known diseases and were not currently taking medication, were then given physical examinations and laboratory tests for liver function and urinary protein levels. Subjects with any diagnosed diseases, especially abnormal liver function and unusual high protein levels in urine were excluded from further consideration. Those workers who passed the physical examination and laboratory tests were then monitored with 3M organic vapor monitor 3500 for their personal exposures to benzene, toluene, and xylene once a week for at least three times. The monitors were analyzed by gas chromatography for benzene, toluene, and xylene according to NIOSH standard procedure [9]. The average personal exposure levels of the three monitoring were used as criteria to make final recruitment. The unexposed subjects were recruited through frequency matching by age, sex, smoking and drinking histories. All recruited study subjects were informed of the nature of the study, the potential benefits and risks. Informed consent was obtained from each subject before study participation.

After recruitment, all subjects were first asked to provide about 50 ml of urine in the morning before starting their work and then monitored for exposures with 3 M vapor monitor 3500 during the workshift. They were asked to provide urine samples again at the end of workshift. All urine samples were stored at −20 °C until packed in dry ice and shipped to the United States for analyses of benzene metabolites in urine. The urinary metabolites reported in this paper are levels obtained in the samples collected at the end of workshift.

Because the daily ambient exposure levels to benzene, toluene, and xylene were less variable compared with occupational exposure, the participating unexposed subjects were monitored only once on the day of biological sample collection.

2.2. Measurements of benzene metabolites

Three urinary metabolites, *S*-phenylmercapturic acid (*S*-PMA), *trans*,*trans*-muconic acid (*t*,*t*-MA), and phenol were determined in all urine samples collected in this study.

S-PMA and *t*,*t*-MA were simultaneously measured by LC-ES–MS/MS according to the method developed originally by Melikian et al. [10]. The detailed description and results were reported elsewhere [11,12]. Phenol in urine was determined by GS/MS as previously described in our biomarker validation study [11].

2.3. Determination of creatinine and cotinine

Urinary creatinine was determined to provide a point of reference for adjusting the concentration of analytes in urine samples due to variations in liquid uptake between subjects. Creatinine was determined with a Kodak Ektachem 500 Computer-Directed Analyzer. Cotinine, one of the major metabolites of nicotine, was selected as an indicator of smoking status for the study subjects and was quantified by radioimmunoassay at the American Health Foundation's Clinical Biochemistry Facility.

2.4. Identification of gene polymorphisms

2.4.1. DNA isolation

Approximately 5 ml of frozen cells was first resuspended with equal volume of TBS (20 mM Tris–Cl at pH 7.5, 150 mM NaCl). Then the genomic DNA was prepared using the DNA isolation kit (Boehringer Mannheim) according to manufacturer's instructions. The isolated DNA was resuspended in TE (10 mM Tris–Cl at pH 7.5, 1 mM EDTA) for later use. The concentration and purity of the DNA samples was determined spectrophotometrically at 260 and 280 nm.

2.4.2. CYP2E1 polymorphism

The identification of the two *CYP2E1* polymorphisms, *Rsa*I and *Dra*I, was conducted using PCR based methods. All PCR reactions were carried out in a final volume of 50 μl. The reaction buffer contained 10 mM Tris–Cl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% (v/v) Triton X-100 with each primer at 0.25 μM, nucleotides at 0.2 mM, and Taq polymerase (1 unit/50 μl reaction).

The method developed by Hayashi et al. [13] was used to identify genotypes of *CYP2E1* using *Rsa*I digestion. In brief, the two primers that were used to amplify 5′ flanking region (−1408 to −996) are (a) 5′-CCAGTCGAGTCTACATTGTCA-3′ and (b) 5′-TTCATTCTGTCTTCTAACTGG-3′. The PCR conditions were 35 cycles of 1 min at 95 °C, 2 min at 45 °C and 2 min at 70 °C. 20 μl of each sample was digested with 10 units of *Rsa*I, electrophoresed on agarose gels, and DNA bands were visualized by ethidium bromide staining. Homozygous wild types showed a 360 bp fragment, whereas variants showed a 410 bp fragment. Heterozygous individuals had both bands.

The method described by Kim et al. [14] was used to determine the *CYP2E1* genotypes using *Dra*I digestion. The primers used for PCR are 5′-TCGTCAGTTCCTGAAAGCAGG-3′; and 5′-GAGCTCTGATGCAAGTATCGCA-3′. Amplified products were digested and electrophoresed on agarose gels. Bands were visualized by ethidium bromide staining. Homozygous wildtype showed 572, 302, 121 bp fragments and the variant showed 874 and 121 bp fragments. Heterozygous individuals, on the other hand, showed all four fragments.

2.4.3. NQO1 polymorphism

The identification of $^{609}C \rightarrow T$ in this study was conducted using an RFLP assay [15]. Briefly, the sense (5′-TCCTCAGAGTGGCATTCTGC-3′) and antisense (5′-TCTCCTCATCCTGTACCTCT-3′) primers were used to amplify a 211 bp region, which includes the last seven bases of intron 5 and the first 204 bases of exon 6. The PCR products generated were then digested with 36 units of *Hin*fI for 5 h at 37 °C, separated on a 1.5% agarose gel and visualized by staining with ethidium bromide. A C to T point mutation at position 609 of the cDNA produced 151 and 44 bp products, whereas wild type alleles showed only a 195 bp band.

2.4.4. Identification of Sp/N alleles of MPO

Genomic DNA sequence comprising nucleotide positions −237 to −581 in the promoter re-

gion was amplified according to the method described by London et al. [16]. Briefly, amplification was carried out using forward primer MPOF (5′-CGGTATAGGCACACAATGGTGAG-3′) and reverse primer MPOR (5′-GCAATGGTTCAAGCGATTCTTC-3′). PCR was performed with 20 ng of genomic DNA in a 50 μl reaction mixture containing 1 μM of each of the primers, 200 μM of each of the dNTPs, 5 μl of 10× PCR buffer and 1 unit of Taq DNA polymerase (Boehringer Mannheim). Cycling conditions were 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min with a final cycle at 72 °C for 7 min. A 10 μl aliquot was digested with 20 units of *Aci*I restriction enzyme (Life technologies, Inc.,) according to manufacturer's instructions in a total volume of 25 μl. The restriction fragments was separated on agarose gels and visualized by ethidium bromide staining.

The G/A polymorphism at position −463 in the promoter region of the *MPO* gene destroys (A allele) or creates (G allele) an *Aci*I restriction site within the 350 bp amplified fragment. The three possible genotypes were defined by three distinct banding patterns: N/N (289 and 61bp fragments), Sp/N (289, 169, 120 and 61bp fragments) and Sp/Sp (169, 120, and 61 bp fragments).

2.4.5. GSTT1 polymorphism

The genotyping of the blood samples for GSTT1 was carried out essentially as described by Nelson et al. [17] using PCR procedures. Briefly, PCR of 100 ng of genomic DNA was carried out in 1× reaction buffer, 0.2 mM dNTPs, 1 unit of Taq DNA polymerase (Boehringer-Mannheim). Reaction mixture was incubated at 94 °C for 5 min followed by hold at 80 °C at which time dNTPs were added. Reactions were then kept for 40 cycles of 94 °C for 1 min, 63 °C for 1 min and 72 °C for 1 min, with a final incubation at 72 °C for 7 min. Two genes were co-amplified in each tube, one GSTT1 using primers of 5′-TTCCTTACTGGTCCTCACATCTC and 5′-TCACCGGATCATGGCCAGCA and the other CYP1A1 using primers of 5′-GAACTGCCACTTCAGCTGTCT and 5′-CAGCTGCATTTGGAAGTGCTC [18]. The amplified product from the GSTT1 gene was 480 bp in length and its absence in the PCR product is indicative of the deleted genotype. Co-amplification of CYP1A1 served as a positive control and generated a product of 312 bp.

2.5. Statistical analysis

Analyses were performed to examine whether there were any differences in the mean values for urinary benzene metabolites as a function of gene polymorphisms in the workers exposed to benzene. The urinary benzene metabolite variables (*S*-PMA, *t*,*t*-MA and phenol) were measured as continuous variables but proved to be right-skewed. A logarithmic transformation was, therefore, applied to these variables for analysis.

In order to evaluate whether exposed subjects manifested more response to benzene exposure than expected as a function of having particular genotypes, an initial analysis was performed using the levels of urinary metabolites according to the genotypes of the tested genes without considering the exposure levels of benzene. Furthermore, multiple regression analyses on genotypes were carried out for levels of *t*,*t*-MA, *S*-PMA, and phenol at the end of a workshift. Essentially, the rationale was to determine whether the variability in response to a given exposure of benzene (after controlling for other possible confounders) was associated with the genotypes. The regression analyses were conducted controlling for the potential confounder variables of sex, age, smoking (based on a combination of self-report and cotinine level in urine), and benzene exposure level.

3. Results

3.1. Urinary metabolites as biomarkers of low levels of benzene exposure

S-PMA was found to correlate very well with personal benzene exposures across a broad range of exposures from 0.06 to 122 ppm. A clear-cut exposure-response relationship between benzene exposure and *S*-PMA was observed not only in the entire exposed group (Fig. 1A) but also in the subgroup with benzene exposure below 1 ppm (Fig. 1B). Further examination indicated that the after-work levels of *S*-PMA in workers exposed to benzene at concentrations below 0.1 ppm were significantly higher than in unexposed subjects (Fig. 1B, $p = 0.02$).

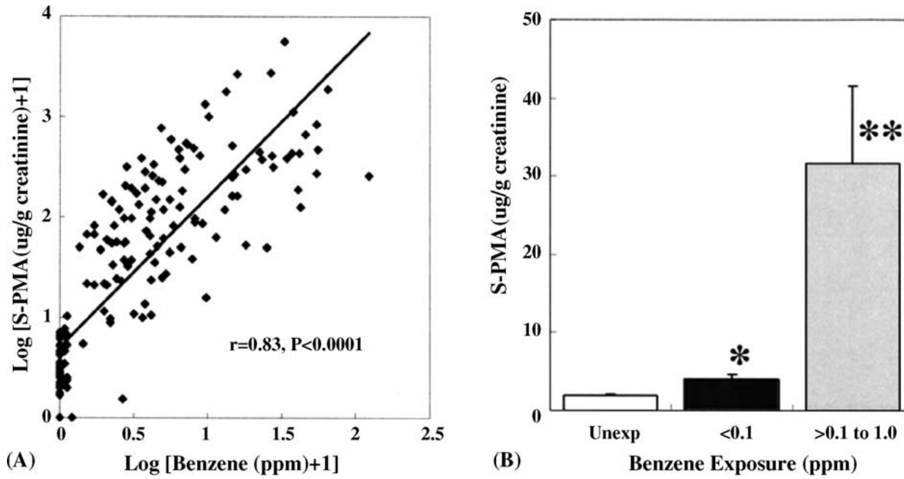

Fig. 1. Relationship between benzene exposure and *S*-PMA levels in urine.; With all study subjects (A) and subjects with exposure below 1 ppm (B); (*) compared with unexposed subjects, $p = 0.02$; (**) exposure trend, $p < 0.0001$.

Similar to *S*-PMA, the levels of *t,t*-MA were highly associated with exposure levels of benzene at a broad range (Fig. 2A, $r = 0.83$, $p < 0.0001$). A significant exposure-response trend was also observed in subjects exposed to benzene below 1 ppm (Fig. 2B, $p < 0.0001$). However, no significant difference in *t,t*-MA was detected between unexposed subjects and workers exposed to benzene below 0.1 ppm (Fig. 2B).

Table 1 displays the levels of biotransformation of each ppm benzene to urinary *S*-PMA and *t,t*-MA. The

Table 1
Efficiencies for biotransformation of benzene into *t,t*-MA and *S*-PMA[a]

	Mean ± S.D.	Median	Range
t,t-MA	1.57 ± 1.38	1.11	0.03–6.98
S-PMA	43.65 ± 44.65	25.17	0.32–203.16

[a] *t,t*-MA expressed as mg/g creatinine/ppm benzene; *S*-PMA as μg/g creatinine/ppm benzene.

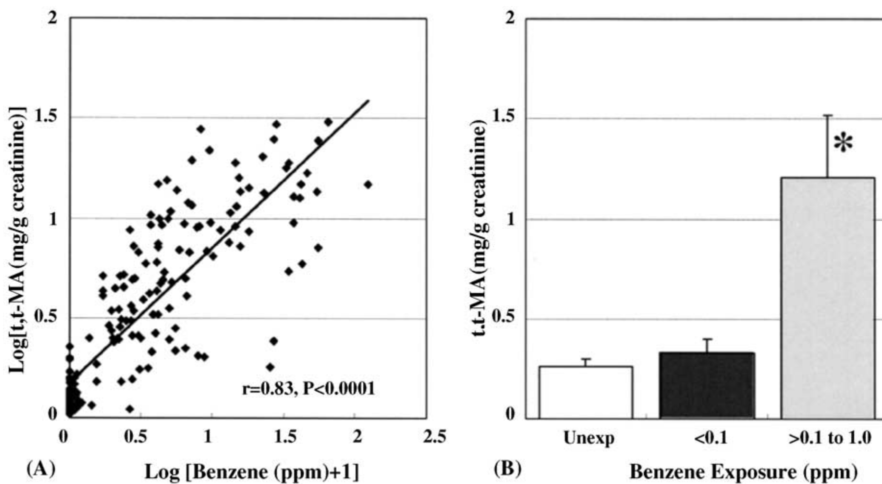

Fig. 2. Relationship between benzene exposure and levels of *t,t*-MA in urine. With all study subjects (A) and subjects with exposure below 1 ppm (B); (*) exposure trend, $p < 0.0001$.

Table 2
Levels of urinary metabolites according to CYP2E1 genotypes (mean ± S.D.)[a]

	CYP2E1 *Rsa*I[b]			CYP2E1 *Dra*I[b]		
	W/W	W/V	V/V	W/W	W/V	V/V
t,t-MA (mg/g creatinine)	6.26 ± 6.77	6.17 ± 6.06	5.41 ± 6.52	6.77 ± 6.90	4.42 ± 4.71	5.42 ± 6.78
S-PMA (μg/g creatinine)	253.1 ± 672.0	380.7 ± 669.1	227.8 ± 372.4	300.0 ± 720.6	276.4 ± 549.7	199.7 ± 338.3
Phenol (mg/g creatinine)	34.3 ± 66.1	28.4 ± 37.8	11.36 ± 18.0	33.0 ± 66.3	23.0 ± 37.7	26.5 ± 59.8
Benzene exposure (ppm)	10.2 ± 15.5	10.5 ± 21.1	4.2 ± 5.0[c]	11.3 ± 19.3	4.0 ± 5.4[d]	9.1 ± 12.5

[a] Means and standard deviations reported are raw variables, but the statistical tests were performed on the log transformed data and showed no significant difference.
[b] W/W (wild type), W/V (heterozygous), V/V (homozygous variant).
[c] Benzene exposure levels in subjects with homozygous variant alleles were significantly lower than that in subjects with either W/W or W/V alleles ($p < 0.01$).
[d] Benzene exposure levels in subjects with W/V alleles were significantly lower than that in subjects with W/W alleles ($p < 0.01$).

metabolic efficiencies of benzene biotransformation into urinary *S*-PMA and *t,t*-MA varied greatly from subject to subject ranging from 0.32 to 203.16 μg/g creatinine/ppm benzene and 0.03 to 6.98 mg/g creatinine/ppm benzene, respectively (Table 1).

3.2. Urinary metabolites in relation to individual genotypes of metabolic genes

Due to the extremely low background frequency, only one subject was identified to have homozygous variant alleles of MPO. Therefore, genotype data of MPO was not analyzed in relation to the formation of urinary metabolites.

3.3. CYP2E1 polymorphisms and their relation to the formation of urinary metabolites

The mean values of all urinary metabolites were similar between subjects with wild type and homozygous variant alleles of CYP2E1 (Table 2). The lack of statistical difference might be due to the variations in benzene exposure levels among subjects with different genotypes as indicated in Table 2. To test this hypothesis, stratifications need to be made according to benzene exposure levels. However, because the number of subjects with homozygous variant alleles is small (about 10% of 130 exposed workers), we cannot make any statistically meaningful stratification with benzene exposure levels. In order to control for the potential effects of benzene exposure, multiple regressions of the urinary metabolites on CYP2E1 genotypes, with controlling for benzene exposure, gender and smoking, were carried out. Results suggest that there was no significant difference in the response of any urinary metabolites with respect to CYP2E1 polymorphisms.

3.4. GSTT1 genotypes and its relation to the formation of urinary metabolites

The results of initial data analysis were given in Table 3. The mean level of *S*-PMA in urine collected after work was significantly lower in subjects with GSTT1 null allele than in GSTT1 positive workers ($p < 0.001$). Further analysis indicated that the efficiency of the biotransformation of benzene into *S*-PMA (production rate per ppm benzene exposure) was much lower in subjects with GSTT1 null alleles than that in the workers with GSTT1 positive alleles ($p < 0.0001$, Table 4). Furthermore, a multiple regression of *S*-PMA on GSTT1 genotype, with controlling for benzene exposure, gender and smoking, were calculated and showed that GSTT1 genotype and benzene exposure levels were the key factors in determining the biotransformation efficiency of benzene into *S*-PMA (Table 5).

Similar statistical analyses were conducted in regard to the potential effects of GSTT1 genotype on the production of *t,t*-MA and phenol. No differences in either metabolite level or production rate were detected between subjects with GSTT1 null and positive alleles.

3.5. NQO1 genotype and its relation to the formation of urinary metabolites

The preliminary data analysis without considering benzene exposure levels revealed that there was no significant difference of urinary metabolite excretion be-

Table 3
Levels of urinary metabolites according to GSTT1 and NQO1 genotypes (mean ± S.D.)[a]

	GSTT1		NQO1[b]		
	Null	Positive	W/W	W/V	V/V
t,t-MA (mg/g creatinine)	4.99 ± 5.37	7.16 ± 7.31	6.74 ± 7.23	6.23 ± 6.44	4.09 ± 4.38
S-PMA (μg/g creatinine)	237.6 ± 811.5	330.4 ± 469.5[c]	295.0 ± 453.5	294.9 ± 772.5	222.8 ± 252.6
Phenol (mg/g creatinine)	19.1 ± 28.3	39.6 ± 71.0	33.0 ± 66.3	23.0 ± 37.7	26.5 ± 59.8
Benzene exposure (ppm)[d]	7.5 ± 9.1	11.7 ± 20.6	12.1 ± 23.6	8.4 ± 11.8	10.3 ± 11.8

[a] Means and standard deviations reported are raw variables, but the statistical tests were performed on the log transformed data.
[b] W/W (wild type), W/V (heterozygous), V/V (homozygous variant).
[c] $p < 0.001$.
[d] No significant differences were detected in benzene exposure levels between groups of subjects with GSTT1 positive and null alleles or among subjects with different NQO1 genotypes.

Table 4
Efficiencies of biotransformation into urinary metabolites according to GSTT1 genotype (mean ± S.D.)[a]

	GSTT1 null	GSTT1 positive
t,t-MA	1.51 ± 1.53	1.64 ± 1.27
S-PMA	24.72 ± 32.48	59.84 ± 47.66[b]
Phenol	6.96 ± 17.62	8.86 ± 18.05

[a] Urinary metabolites expressed as mg/g creatinine/ppm benzene, except *S*-PMA as μg/g creatinine/ppm benzene.
[b] $p < 0.0001$ between subjects with GSTT1 positive and null alleles.

tween subjects with wild type alleles and workers with variant alleles of NQO1 (Table 3). Multiple regression analysis of urinary metabolites on NQO1 genotype, adjusted for sex, age, benzene exposure, and cotinine levels, also failed to show any significant effects of NQO1 genotype on the production of the urinary metabolites (data not shown).

4. Discussion

A number of studies indicated that the susceptibility to benzene hematotoxicity is determined by the metabolism and processing of benzene in both the liver and the bone marrow [19–22]. Following exposure, benzene is metabolized in the body through a variety of enzymatic systems, including CYP2E1, MPO, NQO1, and GSTT1 as outlined in Fig. 3 [19,23–25]. The resulted metabolites may either react with the macromolecules in the target organ or be excreted primarily through urine. Therefore, several urinary metabolites of benzene have long been recognized and studied as useful biomarkers of benzene exposure. In particular, urinary *t,t*-MA and *S*-PMA have been demonstrated to be sensitive markers for the most current benzene exposure at low levels [12,26,27]. The main purpose of this study is to investigate the potential role that the polymorphisms of responsive genes may play in benzene metabolism and excretion of metabolites from urine.

Table 5
Multiple regression of *S*-PMA on GSTT1 genotype controlling for sex, age, benzene exposures and cotinine levels

	Estimate	Standard error	*T*-value	*p*-value
Intercept	63.9612	21.5039	2.9744	0.0035
Sex	1.5499	9.0186	0.1719	0.8638
Age	−0.4039	0.5247	−0.7697	0.4430
Benzene	−0.6860	0.2272	−3.0190	0.0031
GSTT1	−16.9932	3.8405	−4.4248	0.0000
Cotinine	−0.0021	0.0033	−0.6316	0.5288

4.1. CYP2E1 polymorphisms

A recent case-control study of benzene exposed workers in China revealed a 2.6-fold increased risk of benzene poisoning in workers with high CYP2E1 enzyme activity versus those with low activity. In contrast, the genotype of CYP2E1 did not influence the risk of benzene poisoning suggesting that this allele did not significantly affect enzyme activity or benzene metabolism [2]. Similar findings were observed in the most recent two studies conducted among bus drivers and policemen, indicating that no association was found between excretion of urinary metabolites and CYP2E1 polymorphism [28,29]. It is, therefore, not surprising that CYP2E1 polymorphism was found in this study to have no influence on the levels of any

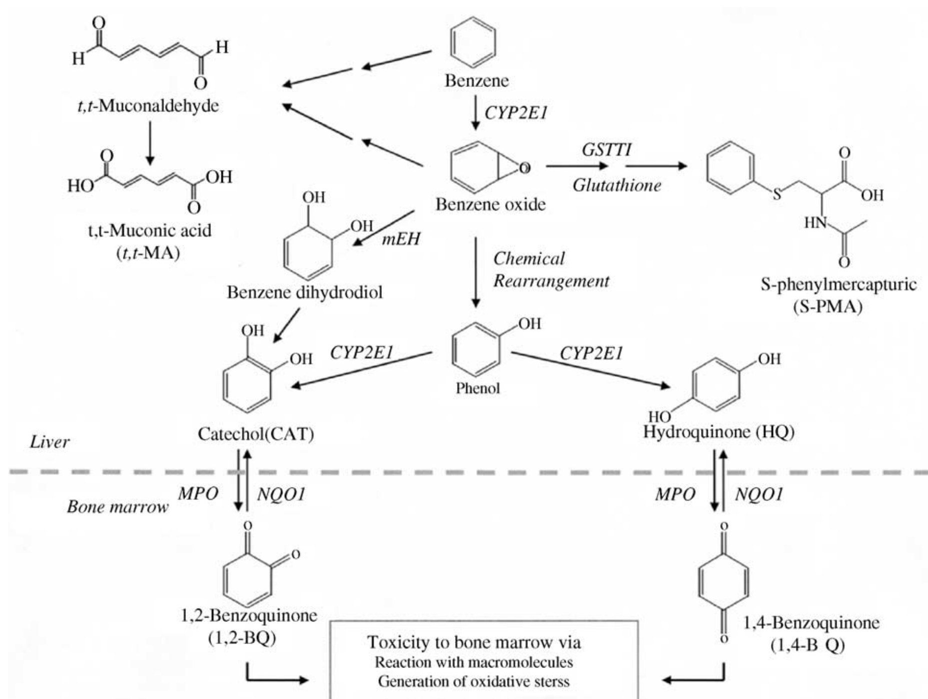

Fig. 3. Benzene: metabolic pathways and toxicity.

observed urinary metabolites no matter how data were analyzed. Therefore, it appears unlikely that the identified polymorphism of CYP2E1 plays a substantial role in benzene metabolism and individual variations of susceptibility to benzene hematotoxicity.

These negative results can be well explained by studies that explored the relationships between genotype and phenotype of CYP2E1. It was reported that the CYP2E1 *Rsa*I variant alleles may be associated with reduced CYP2E1 activity. However, the association between *Rsa*I variant allele and decreased enzyme activity was fairly weak compared with the effects of both pathophysiological determinants and environmental factors [30–32]. To the best of our knowledge, the functional and biochemical characterization of *Dra*I variant alleles has not been fully conducted yet. There is no evidence whether or not the rare allele affects CYP2E1 enzyme activity and contributes to individual susceptibility to benzene hematotoxicity. In order to demonstrate that differences in the regulatory region of CYP2E1 are partially responsible for the interindividual variation in CYP2E1 enzyme activity, McCarver et al. recently identified an estimated 100-bp insertion mutation at a region from positions −2270 to −1672 [33]. Their data further revealed that the presence of the insertion mutation was associated with greater CYP2E1 metabolic ability, but only among individuals who either were obese or had recently consumed ethanol. These findings may again suggest that CYP2E1 polymorphism, at least the known variant alleles, may not be that important in modulating its enzyme activity compared with pathophysiological and other factors.

4.2. GSTT1 polymorphism

As illustrated in Fig. 3, GSTT1 is involved the minor pathway of benzene metabolism into *S*-PMA, and therefore, should play a significant role in modulating the biotransformation efficiency of benzene into *S*-PMA. As expected, the present study showed that there was indeed a significant interaction between GSTT1 genotype and benzene exposure in *S*-PMA excretion from urine no matter how the data were analyzed.

Especially, when a multiple regression analysis of *S*-PMA on GSTT1 genotype was conducted with controlling for sex, age, benzene exposures and cotinine levels, GSTT1 showed even a greater effect on individual *S*-PMA biotransformation efficiency than benzene exposure at the observed levels (Table 5). This effect of GSTT1 polymorphism may, at least, partially account for the wide interindividual variations in biotransformation efficiency of *S*-PMA observed in this study (Table 1). However, discrepancies are found between the present study and a number of studies conducted recently by others. Rossi and co-workers [29] examined the role of genetic polymorphism in modulating urinary excretions of *t*,*t*-MA and *S*-PMA in 59 non-smoking bus drivers and demonstrated that no evidence was found for a possible role of GSTT1 polymorphism in determining the wide differences observed in the biotransformation rate of benzene into *S*-PMA. Surprisingly, however, a significantly higher *t*,*t*-MA in urine was observed to be associated with GSTT1 null genotype. As exposure biomarkers, *t*,*t*-MA and *S*-PMA could reliably detect benzene exposure levels at about 1 ppm and 0.1 ppm, respectively with the most sensitive methods employed currently [12,26,27]. In Rossi's study, the levels of benzene exposures were approximately 0.082 ppm ($0.082 \pm 0.026\ \text{mg/m}^3$), which are far below the concentrations that *t*,*t*-MA and *S*-PMA can detect. Therefore, conclusions should be carefully drawn from this study, especially for *t*,*t*-MA due to potential effects of sources other than benzene exposure, such as sidestream exposure to tobacco smoke. Another study conducted by Verdina et al. indicated that in a population exposed to very low benzene concentrations, urinary *t*,*t*-MA and *S*-PMA levels were affected to only a very limited extent by metabolic gene polymorphisms, whereas other factors, such as gender, lifestyle, or other confounders, might account for a larger fraction of the interindividual variability of these biomarkers [28]. A most recent study conducted in volunteers exposed to benzene showed that the urinary levels of *S*-PMA were relatively low ($3.79 \pm 3.23\ \mu\text{g/24 h}$) in the subjects with GSTT1 null alleles and higher ($6.71 \pm 5.24\ \mu\text{g/24 h}$) in the volunteers with GSTT1 positive alleles although there was no significant difference [6]. The small number of the volunteers (32 with GSTT1 null and 8 with positive genotypes) may account for the lack of statistical significance.

4.3. NQO1 polymorphism

For benzene metabolism, NQO1 was claimed to detoxify the reactive metabolites of benzene formed in bone marrow, and therefore, was proposed to be an important genetic susceptibility factor [25,34]. This hypothesis was confirmed by the investigation conducted by scientists from NCI and Chinese Academy of Preventive Medicine [2] and our findings in the current study (to be reported elsewhere). However, the findings currently available regarding the roles of NQO1 in determining the formations of urinary metabolites were contradictory. In the study with 50 volunteers [6], Sørensen and co-workers found that excretions of *t*,*t*-MA and *S*-PMA were significantly lower in subjects with wild type NQO1 compared with subjects who have the heterozygous genotype ($p = 0.004$ and $p = 0.011$, respectively). Conversely, a significantly higher *S*-PMA excretion was detected in the subjects with wild type of NQO1 among the 59 recruited bus drivers [29]. The results in this present study did not show any associations of *t*,*t*-MA and *S*-PMA with NQO1 genotype. The roles of NQO1 in this regard need to be further examined.

In conclusion, among genetic factors, the CYP2E1 *Rsa*I and *Dra*I polymorphisms do not appear substantially to influence benzene metabolism or formation of benzene exposure markers, such as *t*,*t*-MA and *S*-PMA. The GSTT1 null allele significantly decreases individuals' ability to convert benzene into *S*-PMA, but has no effect on the production of other urinary exposure markers of benzene, including *t*,*t*-MA and phenol. No significant interactions were observed between the NQO1 genotype and formation of urinary metabolites measured in this study.

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