Original Article

Detection of CYP2E1, a Genetic Biomarker of Susceptibility to Benzene Metabolism Toxicity in Immortal Human Lymphocytes Derived from the Han Chinese Population*

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Abstract

Objective Cytochrome P450 2E1 (CYP2E1) is an important metabolizing enzyme involved in oxidative stress responses to benzene, a chemical associated with bone marrow toxicity and leukemia. We aimed to identify the CYP2E1 genetic biomarkers of susceptibility to benzene toxicity in support of environmental and occupational exposure prevention, and to test whether a model using immortal human lymphocytes might be an efficient tool for detecting genetic biomarkers.

Methods Immortalized human lymphocyte cell lines with independent genotypes on four CYP2E1 SNP sites were induced with 0.01% phenol, a metabolite of benzene. CYP2E1 gene function was evaluated by mRNA expression and enzyme activity. DNA damage was measured by Single-Cell Gel Electrophoresis (SCGE).

Results Among the four SNPs, cells with rs2070673TT and rs2030920CC showed higher levels of CYP2E1 transcription and enzymatic activity than the other genotypes in the same SNP site. Cells with higher gene expression genotypes also showed higher comet rates compared with lower gene expression genotypes.

Conclusion These results suggest that CYP2E1 rs2070673 and rs2030920 might be the genetic biomarkers of susceptibility to benzene toxicity and that the immortalized human lymphocytes model might be an efficient tool for the detection of genetic biomarkers of susceptibility to chemicals.

Key words: Cytochrome P450 2E1; Single-nucleotide polymorphism; Genetic biomarker; Human immortalized B lymphocytes; Benzene; Phenol

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INTRODUCTION

Benzene is a ubiquitous environmental pollutant associated with aplastic anemia and acute leukemia because of its hematotoxic, genotoxic, and carcinogenic properties[1-2]. Occupational exposure of benzene can occur in the work place of certain industries, such as the production of paint and organic chemicals[3]. Meanwhile, daily environmental exposure to benzene is caused by gasoline fumes, automobile exhaust fumes and both first- and second-hand tobacco smoke[4]. In recent years, benzene exposure has become more common as more people have moved into newly constructed apartments, with the improvement of living conditions in China[5]. A number of studies indicate that benzene toxicity is dependent upon metabolic activation and detoxification[6].

The CYP2E1 gene is an important enzyme in the metabolic activation of chemicals including benzene, acetaminophen (APAP), acetone, halothane and

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ethanol[7]. Benzene is metabolized to epoxy benzene by CYP2E1, which spontaneously forms phenol. Phenol is then catalyzed to hydroquinone and catechol by CYP2E1, which are more toxic than phenol. Oxidation of benzene by CYP2E1 to reactive intermediates is a prerequisite of cellular toxicity. Sheets and Carlson observed decreased CYP2E1 activity and a decreased risk of benzene-induced hematotoxicity[8]. Mice deficient in CYP2E1 activity did not demonstrate genotoxicity or cytotoxicity, which would have been detected in bone marrow, spleen, or blood[9]. Single-nucleotide polymorphisms (SNP) in genes coding for metabolizing enzymes, can modulate gene function and cellular toxicity in combination with specific exposure[10-11]. Some polymorphisms seem to affect expression of the protein, but for most, the influence on gene function and cellular response induced with a substrate is still unknown. Identification of SNPs may be valuable as a tool that can be used in support of environmental and occupational disease prevention. Recently, advances in molecular genetics have provided some methods for the assessment of the influence of gene function on the response to chemicals. However, these protocols are often bottlenecked in the discovery process[12]. The link between genetic polymorphisms, exposure biomarkers and benzene toxicity has also been studied by comparing the statistical differences between a control group and workers who were exposed[13]. However, the effect was unclear as the effect of the polymorphism was modest and the influence of CYP2E1 SNPs on gene expression could be influenced by several substrates, including alcohol[14]. Moreover, studies assessing the genetic contribution to chemical exposure are hindered due to limited blood samples, uncertain chemical exposure and the requirement for large sample sizes to gain statistical significance. Reporter gene detection systems have also been used to detect the expression of different genotypes[15]. But the alteration of the expression of a reporter gene may not accurately reflect the true impact of the original gene mutation. Therefore, models for evaluating SNPs, gene function and phenotype are being developed to identify the genetic markers of susceptibility to toxicity of chemicals. In Watter’s study[16], lymphoblast cells derived from the Centre d’Etude du Polymorphisme Humain (CEPH) reference pedigrees were used to identify the genetic determinants of chemotherapy cytotoxicity. This provides a possible model for hypothesis testing in human pharmacogenetic studies. Immortalization of human B lymphocytes by Epstein-Barr Virus (EBV) is an effective procedure for inducing long-term growth of certain B lymphocytes with normal karyotypes[17]. The immortalized B lymphocytes was an easily accessible collection of individuals with genetic variations, in which cells lines with different genotypes were freely available[18]. This cell line platform provides a possible opportunity for detection of multiple functional SNP strategies to chemical response, in specific ethnic or disease populations.

SNPs may fall within the locus regions, the coding sequences of genes, the untranslated regions (UTRs) of mRNA or in the intron regions, according to the National Centre for Biotechnology Information (NCBI) database. The CYP2E1 gene shows genetic polymorphisms that vary markedly in their frequency among different ethnic populations[19]. Locus control regions are defined by their ability to enhance the expression of linked genes, to physiological levels. Polymorphisms of the gene for CYP2E1 may cause alterations in enzyme activity, ultimately affecting cellular toxicity and thus susceptibility to leukemia that is etiologically associated with exposure to benzene. Several case-control studies describe how CY2E1-5B (rs2031920 andrs3813867) polymorphisms influenced the risk of developing leukemia[20-21], but could not demonstrate the influence with any certainty.

In this paper, we explored the CYP2E1 SNPs as biomarkers of susceptibility to benzene metabolism toxicity in the Han Chinese population using immortalized B cells. Four SNPs in locus regions were investigated, based on the SNP database (dbSNP) from the NCBI. The multiple SNPs were genotyped by the adapter-ligation mediated allele specific amplification (ALM-ASA) and PCR-RFLP methods. The variations in CYP2E1 mRNA, enzyme activity, and DNA damage induced with 0.01% phenol were measured in immortalized B lymphocytes with different genotypes on every CYP2E1 SNP site.

MATERIALS AND METHODS

Multiplex SNP Genotyping

Ninety-six EBV-transformed immortalized B lymphocyte cell lines derived from the Han Chinese population with no known occupational exposure to organic solvents or family history of genetic disease, ranging in age from 16 to 60 yr (male 51, female 45),

were used in our study. Both SNPs and haplotypes were considered during the selection of the cell lines for each genotype. Five SNPs (rs3813866, rs2031921, rs2070673, rs3813867, and rs2031920) representing polymorphism in the Chinese population located in the locus region (upstream of code area) of the CYP2E1 gene were chosen from dbSNP (NCBI). Among the five SNPs, rs3813867, and rs2031920 were located in one haplotype and had complete Linkage Disequilibrium (LD). Therefore, SNPs rs3813866, rs2031921, rs2070673, and rs2031920 were selected for the study. Information on the four selected SNP is shown in Table 1.

The genotypes of, rs2070673, rs2031921, and rs3813866 were detected with adapter-ligation mediated allele specific amplification (ALM-ASA) method. First, a pre-amplification was carried out to produce a long target containing all SNPs of interest. using the following method: 0.6 μmol/L CYP2E1-FP primer (5’- CT GACTCTGCTGCTCTCAAG-3’) and CYP2E1-RP primer (5’- AGATTCCAGCTGCTGTGCAC -3’), 0.5 mmol/L-1 dNTP, 3 mmol/L-1 $MgCl_2$, and 1.25 U Taq DNA polymerase (TaKaRa, Co., Ltd). The following temperature profile was used: 95 °C for 1 min, followed by 29 cycles at 95 °C for 1 min, 62 °C for 30 s, 68 °C for 5 min and a final elongation at 70 °C for 7 min. The pre-amplification PCR product was stored at -20 °C.

Table 1. Information of SNP in Locus Region in Asian Population from dbSNP NCBI

RefSNPID	Position in Gene	Function Class	Ref SNP Alleles	Ancestral Allele	Alleles (Ancestral)	Alleles (Mutant)
Rs2070673	-298	Locus region	A/T	A	0.440-0.500	0.500-0.560
Rs2031921	-992	Locus region	C/T	T	0.812	0.188
Rs2031920	-1020	Locus region	C/T	C	0.711-0.812	0.167-0.289
Rs3813866	-1531	Locus region	A/T	T	0.717	0.283

Second, the pre-amplified DNA fragments were digested with restriction endonuclease SmaI, to form sticky ends to which an adapter was ligated to either end of the digested fragment with T4 DNA ligase. One end of the adapter was designed as a sequence sticky to the ends of enzymatically digested fragments with the other end having a common sequence. The final reaction volume was 10 μL, with 10 U SmaI endodigested enzyme, 1 μL pre-amplification product and 2 μL 10 × reaction buffer. Reaction condition was 37 °C for 2 h, 15 min enzyme inactivation at 70°C and then at 16°C for 2 h with 50 pmol adapter 1 (5’-CCCCACTTCTTG TTCTCTCTCCATCAGGCGCATCACTCGCCC-3') and ada pter 3 (5’- gatccgagtgat gcgctaag -3’), 2.5 μL 10×rea ction buffer, 50 U T4 DNA ligase, and water up to a total volume of 25 μL. The product was stored at 4 °C.

Third, an allele-specific amplification was performed using allele-specific primers and a universal primer in one tube by using the adapter-ligated fragments as templates. The three SNPs were amplified in two groups, wild and mutant, for easier separation. Conditions for the multiplex PCRs were as follows: 40 pmol allele-specific primer mixture (sequences in Table 2), 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 1μL 10 × PCR reaction buffer, 1 μL adapter-ligated products, and 0.6 U of Taq DNA polymerase. The final reaction volume was 10 μL. Cycling conditions were 94 °C for 3 min, 30 cycles of melting at 94 °C for 30 s, 30 s of annealing at 60 °C, 30 s of extension at 72 °C, followed by a final extension at 72 °C for 10 min.

Finally, the multiplex allele-specific amplification products were separated by 2% agarose gel electrophoresis. Each reaction tube corresponds to one kind of allele-specific primer and so the genotype of each SNP can be easily discriminated by the special amplified products in the wild and mutated reactions. The specific product was only amplified in the wild type reaction for genotype of the homozygous wild-type (W/W); only in the mutated type special reaction for the homozygous mutated-type (M/M) and in both reactions for heterozygosis (W/M).

The PCR-RFLP method was used for genotyping the CYP2E1 rs2031920 polymorphism, since the percentage of GC bases near the rs2030920 site is too low, and therefore not suitable for detection by ALM-ASA. The 25 μL reaction volume consisted of 2 mmol/L $MgCl_2$, 200 μmol dNTP, 25 pm of each primer (F: 5'-CAAGTCGAGTCTACATTGTCA-3', R: 5’-GCAATGGTTCAAGCGATTCTTC-3’), 1.5U Tag DNA polymerase with 1 x PCR reaction buffer (Promega Biotec Co., Ltd) and 50 ng genomic DNA. The amplification steps were performed as follows: 94 °C for 3 min, followed by 34 cycles of 94 °C for 40 s, 58 °C

for 40 s and 72 °C for 40 s and a final elongation at 72 °C for 7 min. The PCR product was digested with 10 U RasI at 37 °C for 4 h, resulting in 360 bp and 50 bp fragments for the CC genotype, 410 bp, 360 bp, and 50 bp fragments for the CT genotype and 410 bp fragments for the TT genotype.

Table 2. Sequence of Alleles-specific Primers used for Genotyping 3 SNP in CYP2E1 Gene and Thelength of Allele-specific Amplification Fragments

NCBI code	Location	Primer (5'→3') wild (mutant)	Fragment length (bp)
rs2070673	-298	AACCAGTGCCAAAGGGCAcGA（T）	364
rs2031921	-992	ATTGCAACCTATGAATTAAGAtCC(T)	245
rs3813866	-1531	GAGGAAACTTGTGGACCCgAA(T)	781

Note. The capital letters (in bold) in the 3'of the primer represent the base specific to the SNP type, and the lower cases represent the artificially mismatched base.

Proliferation Inhibition Toxicity Assay of Phenol

Immortalized B lymphocytes were maintained in RPMI 1 640(Invitrogen) containing 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, and 100 U/mL penicillin and 100 μg/L streptomycin (all Invitrogen, Ltd). The maximal proliferation inhibition concentration of human lymphocytes to phenol was measured by cell proliferation at 0.05, 0.01, and 0.005% phenol in triplicate. The proliferation toxicity of human immortalized B lymphocytes to phenol was measured by the MTT [3-(4,5-dim ethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were seeded into 96-well plates (1x105/well in 100 μL), and grown in RPMI 1 640 medium supplemented with 10% FBS, at 37 °C. Cells were harvested after 24, 48, and 72 h exposure and washed once with RPMI 1 640 medium. The cells were then incubated for 4 h in RPMI 1 640 medium containing 1 mmol/L MTT (Sigma Chemical Co., Ltd). After incubation, the medium was discarded and 100μL dimethylsulfoxide was added to each well. The absorbance of formazan was determined using a Microplate Reader (Berthold LB940) at 570 nm. The relative growth rate (RGR) was used to evaluate proliferation rate induced by phenol. RGR (%) = (OD570 treated group / OD570 control group) × 100%

Phenol Treatment

Six cell lines of each genotype group were resuspended at 1 × 10^6 cells mL^{-1} of RPMI 1 640 medium and then incubated at 37 °C for 48 h with or without 0.01% phenol. At the end of the incubation period, the cells were isolated by centrifugation.

Determination of CYP2E1 mRNA

Total cellular RNA was isolated using Trizol (Invitrogen) and mRNA levels were determined by a two-step real-time RT-PCR analysis. First, cDNA was synthesized under reverse transcription conditions at 42 °C for 45 min in a 20 μL reaction mixture containing 1μg total RNA and 100 U MMLV reverse transcriptase. Heating at 95°C for 5 min to allow denaturing was followed by storage at 5 °C after which the cDNA was used for amplification. Second, real-time quantitative PCR analyses for CYP2E1 cDNAs were performed using the SYBR Green I Kit (Toyobo Co., Ltd) on a real-time fluorescence quantitative PCR system (ABI7300). β-actin was used as a housekeeping gene and used for normalization of data. The sequences of PCR primer pairs are as follows: CYP2E1 F: 5'-ACCTGCCCCATGAAGCAACC-3'; CYP2E1 R: 5'-GAAACAACTCCATGCGAGCC-3; β-actin F: 5'-CAACTCCATCATGAAGTGTGAC-3', β-actin R: 5'-CCACACGGAGTACTTGCGCTC-3'. PCR reactions contained 12.5 μL SYBR Green I Master Mix, 10 μmol/L of both the forward and reverse primer and 1 μL cDNA template with a final volume of 25 μL. Cycling conditions were melting at 94 °C for 3 min, followed by 34 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s, followed by signal collection of SYBR fluorescence and ending with a melting curve analysis in the range of 75-95 °C. Relative copies were calculated using the $2^{-\Delta\Delta CT}$ method. Ratio values were used to evaluate the regulation of mRNA induced by phenol. (Ratio of mRNA level = relative copies of sample / relative copies of control).

Determination of CYP2E1 Enzyme Activity[22]

CYP2E1 enzyme activity was determined by measuring the hydroxylation of aniline. The cell pellets were suspended in 5 mmol/L aniline, 0.15 mmol/L NADPH, 5 mmol/L isocitrate, 5 mmol/L $MgCl_2$ in 100 mmol Tris–HCl buffer (pH 7.4) and

were then incubated at 37 °C for 30 min. The reaction was stopped by adding 1 mL TCA and the samples centrifuged for 10 min at 3 000 rpm. 1 mL 10% Na_2CO_3 and 1 mL 1% phenol reagent was added to 1 mL of the supernatant. This mixture was vortexed and incubated for 30 min at 37°C. The amount of hydroxylated aniline was determined by measuring absorbance using an ELISA at 630 nm. 4-aminophenol was applied to make the standard curve, where the slope (k) was calculated. The protein concentration was measured using the Bradford method[23].

Enzyme activity (nmol·min^{-1}·g^{-1} protein) = ΔD / (k × time × quantity of protein)

Where ΔD = D sample – D blank; k = slope of standard curve.

Ratio values were used to evaluate the regulation of enzyme activity induced by phenol. Ratio of enzyme activity = Enzyme activity of sample / Enzyme activity of control

Determination of DNA Lesions

The DNA lesions were evaluated by Single-Cell Gel Electrophoresis (SCGE)[24-25]. The comet rates were calculated in 300 cells in each sample. The comet image analysis calculated the percentage DNA in the comet tail for each sample. Twenty randomly selected cells were photographed and scanned. The images were auto-analyzed by the Comet Assay Software Project (CASP) designed by Konca K.

Statistical Analysis

The independent effect on CYP2E1 mRNA expression, enzyme activity and DNA lesions by each SNP was assessed by the Student's *t*-test. The criteria for significance was set at $P<0.05$.

RESULTS

Genotyping Analysis of the CYP2E1 rs2070673, rs2031920, rs2031921, and rs3813866 SNP Sites

The rs2070673, rs2031921, and rs3813866 SNPs were genotyped using the ALM-ASA method and the rs2031920 was genotyped using the PCR-RFLP method. The 3-plex amplification and PCR-RFLP products are depicted in Figure 1. The mutant allele frequencies were 37.0%, 21.7%, 29.1%, and 25.0% in CYP2E1 rs2070673, rs2031921, rs2031920, and rs3813866 SNPs, respectively. The frequencies of homozygous wild-type, heterozygote and homozygous mutated-type are 39.7%, 46.0%, and 14.3% in the rs2070673 SNP; 21.7%, 66.7%, and 23.3 % in the rs2031921 SNP; 61.5%, 29.7%, and 8.8% in the rs2031920 SNP; and 58.8%, 32.5%, and 8.8% in the rs3813866 SNP.

Inhibition of Immortalized Lymphocyte Proliferation by Phenol

The inhibitory effect of phenol on cell proliferation was assayed by the MTT test in a time and concentration dependent manner. The inhibitory effect of phenol was evident at concentrations higher than 0.01%, and obvious after only 24 h exposure (Table 3) .

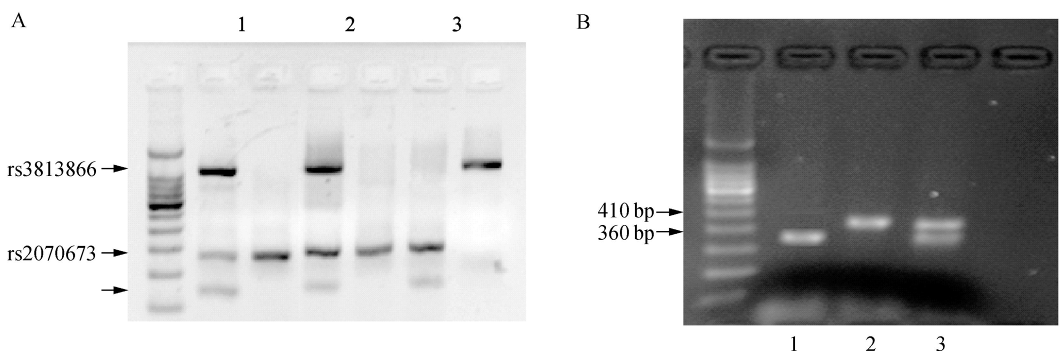

Figure 1. A) Agarose gel electrophoretograms of 3-plex genotyping of 3 genomic samples on rs3813866, rs2070673, rs2031921. Each pair of lanes contains the products from mutant primers (left lane) and wild type primers (right lane); B) Agarose gel electrophoretograms of CYP2E1 rs2031920 genotypes. lane1: TT, lane 2: CT, lane 3: CC.

Table 3. Time and Concentration Dependent Proliferation Rate of Human Immortalized B Lymphocytes Treated with Phenol

Dose	RGR (%)		
	24 h	48 h	72 h
Control	100.00±0.00	100.00±0.00	100.00±0.00
0.005%	100.38±3.13	100.99±1.18	97.96±2.61
0.01%	99.56±2.75	101.56±2.16	96.40±2.98
0.05%	75.62±3.43*	73.97±4.27*	69.05±3.53*

Note. *Significant different with control, P<0.05.

Induction of CYP2E1 mRNA expression by phenol in cells with rs2070673, rs2031921, rs2031920, and rs3813866 SNPs

Six cell lines were assigned to each genotype group (homozygous wild-type, heterozygous, homozygous mutant) in every SNP site and treated with 0.01% phenol for 48h to explore how phenol induces transcriptional expression of human CYP2E1 in immortalized B lymphocytes (Figure 2). CYP2E1 mRNA expression was significantly higher following phenol treatment compared with control samples in all genotypes of each SNP site, with comparable variations of 1.28 to 3.86 fold. Among the three genotypes in the rs2070673 SNP sites, the mRNA expression in cell lines with the TT genotype was significantly more up-regulated than those with the CC and TC genotypes. Among the three genotypes in the rs2031921 SNP sites, no significant difference was observed in the regulated mRNA expression in cell lines with TT, TA, and AA genotypes. Among the three genotypes in the rs2031920 SNP sites, the mRNA expression in cell lines with the CC genotype could be significantly more up-regulated than those with TT and TC genotypes. Among the three genotypes in the rs3813866 SNP sites, the mRNA expression in cell lines with TT and TA genotypes could be significantly more up regulated than those with the AA genotype. Therefore, the CYP2E1 rs2070673, rs2030920, and rs3813866 SNPs might influence CYP2E1 mRNA expression induced by phenol.

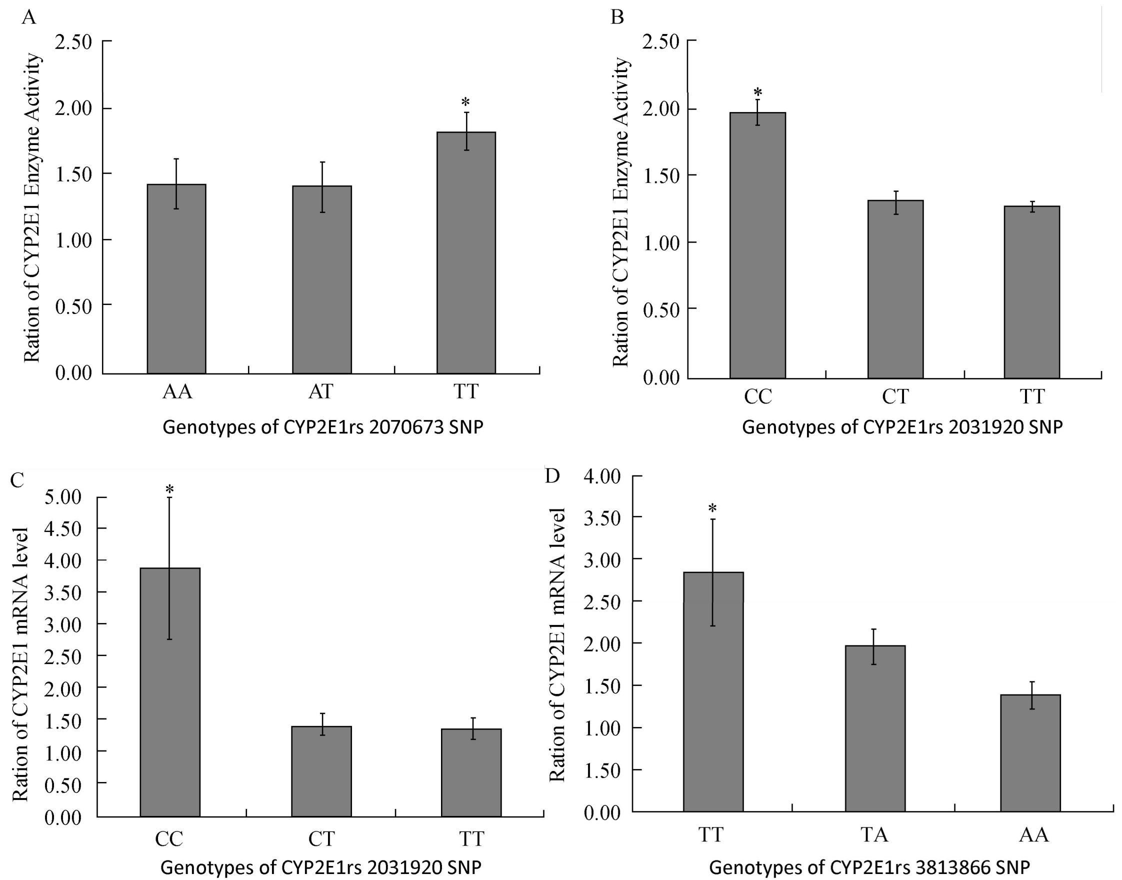

Figure 2. Induction of mRNA expression in cells with four SNP of CYP2E1 after 48 h treatment with 0.01% phenol compared with the untreated control. Figures a, c, and d show mRNA level expression with phenol induction influenced significantly by CYP2E1 rs2070673, rs2031920, and rs3813866 respectivel (P<0.05).
Note. *significant difference compared with the other genotypes (P<0.05).

Induction of Enzyme Activity by Phenol in Cells with CYP2E1 rs2070673, rs2031920, and rs3813866SNP

We investigated whether rs2070673, rs2030920, and rs3813866, which influenced transcriptional expression, could also influence CYP2E1 enzyme activity. CYP2E1 enzyme activities were detected in human immortalized lymphocytes with different genotypes (Figure 3).

Enzyme activity was significantly higher in phenol treated samples than control samples, in all genotypes of each SNP site, with comparable variations of 1.26 to 1.96 fold. Among the three genotypes in the rs2070673 SNP sites, enzyme activity in cell lines with the TT genotype was significantly more upregulated than others with AA and TA genotypes. The enzyme activity was 1.82-fold upregulated by phenol in cells with the TT genotype compared with untreated cells. Among the three genotypes in the rs2031920 SNP sites, enzyme activity in cell lines with the CC genotype was significantly more up-regulated than others with TT and TC genotypes. Enzyme activity was 1.96 fold upregulated by phenol in cells with the CC genotype compared with untreated cells.

Among the three genotypes in the rs3813866 SNP sites, there was no significant difference in the regulated enzyme activity in cell lines with TT, TA or AA genotypes. These results show that rs2070673TT and rs2030920CC can influence gene function and enzyme activity.

DNA Damage by Phenol in Cells with rs2070673 and rs2031920 SNPs

DNA damage to cells with different genotypes on rs2070673 and rs2031920 SNP sites were evaluated by the Comet Assay. This was to investigate whether the higher DNA damage could be induced by high enzyme activity following exposure to phenol (Table 4). Cells with the rs2030920CC genotype could induce a higher comet rate and DNA tail percentage compared with cells with the CT/TT genotypes. Meanwhile cells with the rs2070673TT genotype only induced a higher DNA tail percentage compared with cells of TA and AA. This suggested that the rs2070673 and rs2030920 SNPs might be genetic biomarkers for predicting benzene toxicity, and that the rs2030920 SNP might be more sensitive to the genotoxic effect of benzene.

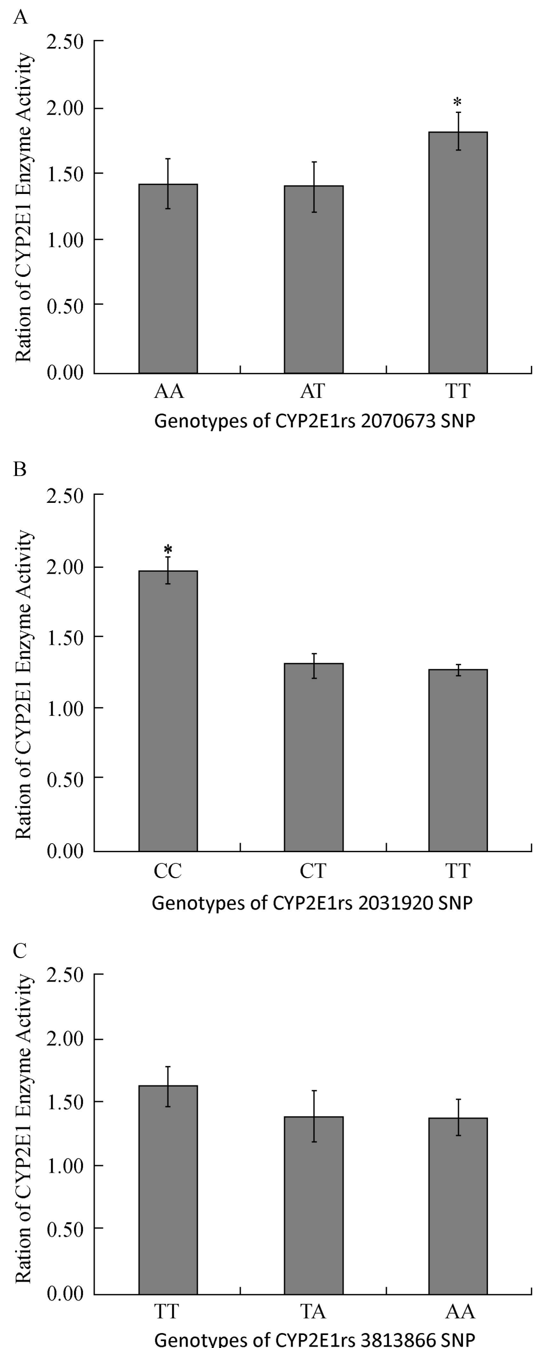

Figure 3. Induction of enzyme activity expression in cells with three SNP of CYP2E1 after 48 h treatment with 0.01% phenol compared with the untreated control. Figures a and b shows enzyme activity with phenol induction influenced by rs2070673 and CYP2E1 rs2031920 respectively (P<0.05). ***Note.*** *significant difference compared with the other genotypes (P<0.05).

DISCUSSION

The cytochrome P-450 enzyme, CYP2E1, has been found to play significant roles in the metabolism of benzene[26-27]. In the Han Chinese population, there are several SNP sites in the locus regions of CYP2E1. In this paper, immortalized B lymphocyte hinese population, based on the NCBI databases, were explored for their influence on gene expression and cellular toxicity induced with phenol. The rs2070673 and rs2030920 SNPs of CYP2E1 could influence gene expression and enzyme activity.

Locus control regions are defined by their ability to enhance the expression of linked genes, to physiological levels. The SNPs falling within the locus region may regulate the transcription of mRNA which might change the protein expression. However, the regulation of mRNA does not always leads to changes in the expression of protein. So, the enzyme activity was used as the ultimate evaluation of gene function in this study. We did not check the effect on enzyme activity for rs2031921 as it failed to alter the mRNA transcription. However, if a SNP is located in a coding region, the enzyme activity still needs to be checked even if the mRNA was not changed as SNPs in coding regions might influence the enzyme activity without altering the mRNA expression.

Table 4. DNA Damage by Phenol in Cells with CYP2E1 rs2070673 and rs2031920 SNP

Genotype	Groups	Comet rate (%)	Ratio of comet rate	Tail DNA (%)	Ratio of tail DNA
rs2031920	Control	3.22±5.09	7.26±1.04*	1.96±0.38	6.19±2.2*
CC	0.01%	22.28±4.02*		11.54±2.34*	
rs2031920	Control	3.56±1.17	4.39±1.38	2.18±0.50	3.95±1.79
CT/TT	0.01%	13.17±3.19*		8.02±1.92	
rs2070673	Control	3.33±0.86	5.23±1.53	2.15±0.53	5.34±1.77*
TT	0.01%	16.50±2.16*		11.01±2.56	
rs2070673	Control	3.44±1.06	4.23±1.58	1.92±0.52	3.82±1.23
AA/AT	0.01%	13.56±2.93*		6.93±1.22	

Note. *Significant different with control, *P*<0.05.

Since oxidation of benzene is a prerequisite of cellular toxicity activated by CYP2E1, DNA lesions induced with benzene might be used as a phenotypic marker. SCGE is a rapid method with high sensitivity for the detection of DNA damage in individual cells, which contribute to the etiology of cancer[28]. Here, cells with the rs2030920CC genotype could induce a higher comet rate and DNA tail percentage, compared with cells of the CT/TT genotypes. Meanwhile cells with rs2070673TT only induced the higher percentage DNA tails compared with cells with TA and AA. The variation in benzene-induced toxicity was also investigated in transgenic CYP2E1 knockout mice (CYP2e1−/−)[9]. Benzene-induced cytotoxicity or genotoxicity was not observed in CYP2e1−/− mice. In contrast, benzene exposure resulted in severe genotoxicity and cytotoxicity in wild-type mice. This demonstrated that CYP2E1 was the major enzyme determining phenol metabolism and thus higher enzyme activity would result in higher toxicity in mice. In this study, the higher comet rates were also observed in cell lines with higher gene expression and special genotypes. It was indicated that genetic polymorphisms in the locus regions of the human P4502E1 gene might affect binding of the transacting factor and change transcriptional regulation[29]. This suggests that rs2070673 and rs2030920 SNPs of CYP2E1 might be the genetic susceptibility markers of benzene toxicity since both SNPs influence gene expression and DNA damage. SNP rs2030920 might be more predictive for benzene toxicity as it may cause significantly higher comet rates and percentage DNA tails.

The polymorphism of genes encoding for benzene metabolism may play a key role in individual susceptibility to benzene associated hematotoxicity and leukemia. Recently, several studies focused on the genetic biomarkers of susceptibility to benzene with a broad range of benzene exposures. rs2030920 is the most frequently studied polymorphism of CYP2E1, namely CYP2E1*5B (RsaI/PstI RFLP; position: C-1053T/ G-1293C, rs3813867/rs2031920). In previous studies, rs2030920 was proven to alter enzyme expression in vitro and also in liver cancer samples[30]. Gene expression of CYP2E1 could be enhanced by the rs2030920CC genotype. The effect of the rs2030920CC genotype on benzene metabolism and

toxicity was also investigated in occupational workers since Trans, transmuconic acid (tt-MA) metabolites of benzene are excreted in the urine, they were commonly identified as a biomarkers of benzene exposure[31]. Wild-type homozygous subjects with rs2030920 had significantly increased excretion of tt-MA[32]. Chanvaivit et al also showed that biomarkers of internal doses of benzene following occupational exposure were slightly influenced by genetic polymorphisms in rs2030920[33]. Kim et al studied 250 benzene-exposed and 136 control workers in Tianjin, China, and demonstrated an association between polymorphisms in rs2030920 and benzene hematotoxicity, which provided evidence that CYP2E1 rs2030920 could affect metabolism of benzene in the human liver[34]. However, data are conflicting as several studies have found a negative effect on benzene metabolism[35]. Our results here support the finding that the rs2030920 CC genotype could increased CYP2E1 gene expression. There have been no reports on the relationship between rs2070673, rs2031921, and rs3813866 SNP variation and CYP2E1 gene function. However, the SNP distribution had been deduced in different nationalities based on the NCBI SNP database. Although a functional test was still absent for the variant allele of CYP2E1 rs2070673, some studies showed women who carried the variant allele appeared to have a higher risk of non-Hodgkin lymphoma (NHL) associated with hair dye use[36]. In our study, the rs2070673 SNP of CYP2E1 was shown to influence the enzyme activity, which suggests that the susceptibility to NHL associated with hair dye use may be due to the variation in enzyme activity. These findings suggest that rs2030920 and rs2030673 SNPs of CYP2E1 may be genetic markers that can be used to support of environmental and occupational benzene exposure prevention in the Han Chinese population.

Considering that the enzyme activity only changed when stimulated by substrates[37] and the moderate effect of the SNPs, the immortalized lymphocyte model was used in this study to screen for genetic markers. The results demonstrated that human immortalized B lymphocytes, derived from different populations, might be used as a tool for uncovering the CYP2E1 genetic biomarkers related to benzene exposure. It was possible to observe the gene expression and cellular toxicity in each cell line with different genotypes, with exact chemical exposure. In previous studies, the CEPH lymphoblastic cells had been used to identify genomic loci and candidate genes influencing natural variation in gene expression[38] and transcriptional response to ionizing radiation[39]. Therefore, the immortalized lymphocyte model might be an efficient tool for screening of genetics biomarkers to some chemicals. This also resolves the dilemma of ethical issues that may arise in population studies with chemical exposure.

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