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Association of genetic polymorphisms in ERCC1 and ERCC2/XPD with risk of chronic benzene poisoning in a Chinese occupational population

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ABSTRACT

DNA damage induced by benzene and its metabolites is thought of as an important mechanism underlying benzene genotoxicity in chronic benzene poisoning (CBP). Therefore, genetic variation in DNA repair genes may contribute to susceptibility to CBP in the exposed population. Since benzene-induced DNA damages include DNA adducts, we hypothesized that the polymorphisms of ERCC1 (Excision repair cross complementation group 1) and ERCC2/XPD (Excision repair cross complementation group 2/xeroderma pigmentosum group D) are associated with the risk of CBP. A case-control study involving 102 benzene-poisoned patients and 204 none-benzene-poisoned controls occupationally exposed to benzene was carried out in the Northeast region of China. The polymorphisms of codon 118 (rs11615) and C8092A (rs3212986) of ERCC1, codon 751 (rs13181), 312 (rs1799793) and 156 (rs238406) of ERCC2/XPD were genotyped by TaqMan® Real-time PCR. The results showed that individuals carrying the ERCC1 codon 118 TT genotype had an increased risk of CBP (OR_{adj} = 3.390; 95%CI: 1.393–8.253; P = 0.007) comparing with its CC genotype. After stratified by smoking, gender and exposure duration we found that the increased risk of CBP associated with the ERCC1 codon 118 TT genotype confined to nonsmokers (OR = 3.214; 95% CI: 1.359–7.601; P = 0.006), female (OR = 3.049; 95% CI: 1.235–7.529; P = 0.013) and exposure duration> 12 years (OR = 3.750; 95% CI: 1.041–13.513; P = 0.035). Since ERCC1 and ERCC2/XPD are both located on chromosome 19q13.3, haplotype analysis of all 5 SNPs was also conducted. However no correlations between the risks of CBP and other genotypes or haplotypes were found. Therefore, our findings suggest an important role of ERCC1 codon 118 polymorphisms for a biomarker to CBP in the Chinese occupational population.

1. Introduction

Benzene is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) in 1982 and National Toxicology Program (NTP) in 2005 [1,2]. Its most prominent target organ is the bone marrow, leading to hematoxicity and leukemia [3]. Although there are many forms of benzene exposure, such as industrial emissions through gasoline vapors, motor exhaust fumes, cigarette smoke and water source contamination, occupational exposures are considered to be of a higher level [4,5]. In China, the number of workers occupationally exposed to benzene during the production of rubber, paint, shoes, lubricants, dyes, detergents, drugs, and pesticides is up to 500,000, thus benzene is consider as No. 1 occupational poison compared with other chemicals [6].

Benzene causes toxicity to the hematopoietic system (hematotoxicity), can result in chronic benzene poisoning (CBP), pancytopenia, aplastic anemia, myelodysplastic syndrome, and acute myeloid leukemia [7,8]. Several studies on benzene toxicities have demonstrated that benzene reactive intermediates can bind covalently to DNA in the target tissue and cause genotoxicity. Benzene can form DNA adducts through its metabolites such as p-benzoquinone (p-BQ) and hydroquinone (HQ) [9], and then induce single- or double-strand breaks that damages the structure and function of biological macromolecules such as DNA and protein [10]. The covalent binding of reactive metabolites to DNA is proposed as an important step in tumor initiation [11]. DNA damage induced by benzene exposure must be properly repaired to maintain genomic stability and to prevent proliferation of mutated cells and subsequent transformation into malignancies [12].

DNA repair capacity in excising DNA adducts induced by Benzene and its metabolites are crucial for human. Generally spoken, there are at least 6 main DNA repair pathways known to correct DNA damage: Direct Repair (DR), Base Excision Repair (BER), Nucleotide Excision Repair (NER), Mismatch Repair (MMR),

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Table 1
The primers and probes of ERCC1 and ERCC2/XPD SNPs.

SNPs	Primers or probes	Sequence
ERCC1 C19007T(rs11615)	Forward primer	5′-CCT TCG TCC CTC CCC AGA-3′
	Reverse primer	5′-CCC AGC ACA TAG TCG GGA AT-3′
ERCC1 C8092A(rs3212986)	Forward primer	5′-GCT TTC TTT AGT TCC TCA GTT TCC C-3′
	Reverse primer	5′-CAG TGC CCC AAG AGG AGA TG-3′
ERCC2/XPD Lys751Gln(rs13181)	Forward primer	5′-CAG GAG TCA CCA GGA ACC GT-3′
	Reverse primer	5′-CTC AGC CTG GAG CAG CTA GAA T-3′
ERCC2/XPD Asp312Asn(rs1799793)	Forward primer	5′-CCG CAG GAT CAA AGA GAC AGA-3′
	Reverse primer	5′-CCT CTG CGA GGA GAC GCT AT-3′
ERCC1 C19007T(rs11615)	Probes of C-allele	5′-FAM-CGT GCG CAA CGT GCC CTG-MGB NFQ-3′
	Probes of T-allele	5′-VIC-TCG TGC GCA ATG TGC CCT G-MGB NFQ-3′
ERCC1 C8092A(rs3212986)	Probes of C-allele	5′-FAM-TGC TGC TGC TGC TTC CGC TTC-MGB NFQ-3′
	Probes of A-allele	5′-VIC-CTG CTG CTG CTT CTT CCG CTT CTT-MGB NFQ-3′
ERCC2/XPD Lys751Gln(rs13181)	Probes of A-allele	5′-FAM-ATC CTC TTC AGC GTC T-MGB NFQ-3′
	Probes of C-allele	5′-VIC-TCC TCT GCA GCG TC-MGB NFQ-3′
ERCC2/XPD Asp312Asn(rs1799793)	Probes of G-allele	5′-FAM-CCG TGC TGC CCG ACG AAGT-MGB NFQ-3′
	Probes of A-allele	5′-VIC-CGT GCT GCC CAA CGA AGT GC-MGB NFQ-3′

Homologous Recombination Repair (HRR), Non-Homologous End Joining (NHEJ) [13]. NER is an important and versatile repair system which can remove DNA adducts induced by benzene [14]. Excision repair cross-complementing group 1 (ERCC1) and Excision repair cross-complementing group 2/xeroderma pigmentosum group D (ERCC2/XPD) both are located on chromosome 19q13.3 that participate in the key steps of NER such as the damage recognition and removal of the lesion [15,16]. Single nucleotide polymorphisms (SNPs), as important genetic biomarkers, have been reported to be related with altered gene expression and changed phenotype's function. There are two common SNPs in ERCC1: one is rs11615 at exon 4 without amino acid change (Asn118Asn) and the other is rs3212986 located at the 3′-untranslated region of ERCC1 (C8092A). Several SNPs have been identified in ERCC2/XPD and three of them are explored in our current study: ERCC2/XPD rs13181 at exon23; rs1799793 at exon10 result in amino acid change (Lys751Gln and Asp312Asn, respectively) and rs238406 at exon6 is a silent polymorphism (Arg156Arg). Chen et al. [17] reported that ERCC1 is one of DNA repair genes associated with benzene poisoning. Epidemiological studies suggest that ERCC2/XPD polymorphisms were associated with the risk of CBP [18–20]. Although some population-based studies may predict individual's susceptibility to CPB by modulating host DNA repair capacity, the conclusions are not entirely consistent and it is imperative to explore whether there is a potential relationship between polymorphisms of ERCC1 and ERCC2/XPD and the risk of CBP in a Chinese occupational population.

2. Materials and methods

2.1. Study subjects

One-hundred two CBP patients in the present study came from several major factories (including Rubber Research Institute of Shenyang, Tian Yuan Petrochemical Factory, Hose Factory, Shenyang First Leather shoes Factory and Shenyang Tool Machine Factory, etc.), where clusters of the cases were reported in Shenyang, China. The benzene poisoning was diagnosed from 1986 to 2011 by the local authorized Occupational Disease Diagnostic Team. The diagnostic criteria for occupational CBP, according to the Ministry of Health, China, include (a) total WBC counts <4000/μL or WBC counts between 4000 and 4500/μL and platelet counts <80,000/μL, with repeated confirmation of these counts in a few months in a peripheral blood examination; (b) documented benzene exposure as a result of an employment for at least 6 months in the factory; and (c) exclusion of other known causes of abnormal blood counts such as chloromycetin use and ionizing radiation. The medical records of these patients were independently reviewed by at least two hemopathologists, especially for those with WBC counts >3500 to confirm the CBP diagnosis. Of the 112 eligible patients, 102(90%) agreed to participate in this study. Two-hundred four healthy workers who had been occupationally exposed to benzene in the same factories with similar benzene exposure environment to cases were selected as controls. The cases and controls were frequency matched on age, sex and exposure duration. All the eligible controls agreed to participate in this study. The protocol and consent form were approved by the Institutional Review Board of China Medical University prior to the study. All activities involving human subjects were done under full compliance with government policies and the Helsinki Declaration. Each participant donated 2 mL venous blood only after informed consent was obtained and their demographic data (ethnic background, smoking status, alcohol consumption, protective measures, medical history and occupational history such as work unit, type of work and exposure duration) were recorded in questionnaires in detail and the study participant consents were obtained. The intensity of benzene exposure (milligrams per cubic meter) for the patients was taken as the benzene level of workplaces while diagnoses were made; the intensity of benzene exposure for the controls was taken as the current level monitored by organic vapor passive dosimetry badges during collection of the blood samples. Those who smoked at least 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. Under the criteria, the smokers and alcohol users in cases satisfied it when they were diagnosed CBP whereas those in controls meant current status when they enrolled in the study. The subjects were administrated a rigorous physical examination at a local occupational disease hospital. Alanine aminotransferase level in serum was also examined to assess liver functions.

2.2. Genotyping assay

Venous blood (2 mL) was placidly drawn from apiece per capita and dealt with folic acid sodium anticoagulation, DNA routinely was extracted by phenol chloroform extraction which had been described elsewhere [21]. Two SNPs in ERCC1 (rs11615 and rs3212986) and three SNPs in ERCC2/XPD (rs13181, rs1799793 and rs238406) were analyzed by TaqMan® on ABI 7500 Real-time PCR system (ABI, US, Stagapore). The primers and probes of ERCC1 and ERCC2/XPD SNPs are shown in Table 1 and all were purchased from ABI Company. The primers and probes of ERCC2/XPD Arg156Arg (rs238406) were purchased from ABI company (Assay ID: C_8714009_10 Part number: 4351375). PCR reactions were run in a 20 μl final volume including Premix Ex Taq™ 10.0 μl, 0.4 μl of each probe and primer, 2 μl DNA (10 ng/μl). Cycling conditions were 95 °C for10 min, and 40 cycles of 95 °C for 5 s and 60 °C for 34 s. Data analysis for allele discrimination was performed with the SDS software.

2.3. Statistical analysis

All statistical analyses were performed with SPSS 16.0. Each SNP was tested in controls to ensure fitting with Hardy-Weinberg equilibrium. Frequencies of the different genetic polymorphisms were compared between cases and controls by Fisher's exact test or χ^2 test, when appropriate. To evaluate whether the lifestyle factor modified the association between genetic polymorphisms and odds of CBP, χ^2 test was applied after stratification according to cigarette smoking or alcohol consumption. The test for homogeneity of odds ratios (OR) was examined by the Breslow–Day method. The OR and 95% confidence interval (95% CI) for estimating the associations of genetic polymorphisms with CBP were obtained from unconditional multinomial logistic regression models without and with adjustment for