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The effect of cigarette smoke and arsenic exposure on urothelial carcinoma risk is modified by glutathione S-transferase M1 gene null genotype

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ABSTRACT

Inter-individual variation in the metabolism of xenobiotics, caused by factors such as cigarette smoking or inorganic arsenic exposure, is hypothesized to be a susceptibility factor for urothelial carcinoma (UC). Therefore, our study aimed to evaluate the role of gene–environment interaction in the carcinogenesis of UC. A hospital-based case–control study was conducted. Urinary arsenic profiles were measured using high-performance liquid chromatography–hydride generator-atomic absorption spectrometry. Genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism technique. Information about cigarette smoking exposure was acquired from a lifestyle questionnaire. Multivariate logistic regression was applied to estimate the UC risk associated with certain risk factors. We found that UC patients had higher urinary levels of total arsenic, higher percentages of inorganic arsenic (InAs%) and monomethylarsonic acid (MMA%) and lower percentages of dimethylarsinic acid (DMA%) compared to controls. Subjects carrying the GSTM1 null genotype had significantly increased UC risk. However, no association was observed between gene polymorphisms of CYP1A1, EPHX1, SULT1A1 and GSTT1 and UC risk after adjustment for age and sex. Significant gene–environment interactions among urinary arsenic profile, cigarette smoking, and GSTM1 wild/null polymorphism and UC risk were observed after adjustment for potential risk factors. Overall, gene–environment interactions simultaneously played an important role in UC carcinogenesis. In the future, large-scale studies should be conducted using tag-SNPs of xenobiotic-metabolism-related enzymes for gene determination.

Introduction

Bladder cancer is the most common type of urothelial carcinoma (UC), which originates exclusively from the urothelium present throughout the urinary tract. It was documented that 2003 new cases of bladder cancer were diagnosed and 756 deaths occurred in Taiwan in 2009 (Department of Health, the Executive Yuan). One of the most important risk factors for bladder cancer is inorganic arsenic in drinking water (Styblo et al., 2002). A significant association between inorganic arsenic and bladder cancer existed whether in an arseniasis endemic area or non-obvious arsenic exposure area in Taiwan (Huang et al., 2008; Pu et al., 2007). In addition, cigarette smoking is also a well-known risk factor for bladder cancer and accounts for up to 50% of all new cases (Strope and Montie, 2008). Generally, cigarette smoke contains more than 60 carcinogenic compounds, which might induce proliferation of the bladder epithelium and induce carcinogenesis (Zaridze et al., 1991). Only a small proportion of people exposed to cigarette smoke or inorganic arsenic ultimately develop UC. The differential susceptibility might be due to polymorphisms in genes encoding biotransformation enzymes, which are associated with the metabolism of compounds in cigarette smoke or transform a procarcinogen into either a carcinogen or intermediate compound (Zhang et al., 2011). Among these genes are those encoding a number of xenobiotic metabolizing enzymes, including Phase I enzymes (cytochrome P450 1A1 (CYP1A1), microsomal epoxide hydrolase 1 (EPHX1)) and Phase II enzymes (glutathione-S-transferase T1 (GSTT1), GSTM1 and sulfotransferases 1A1 (SULT1A1)). The most widely studied SNP, in which single nucleotide polymorphisms (SNPs) are associated with changes in catalytic activity include CYP1A1 Msp I polymorphism (rs 4646903), SULT1A1 Arg213His (rs 9282861), EPHX1 His139Arg (rs 2234922), null genotypes of GSTT1 and GSTM1, (Chen et al., 2011; Ginsberg et al., 2010; Hassett et al., 1994; Mo et al., 2009). There is a

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growing body of evidence from human health risk assessments which have found a link to SNPs of CYP1A1, GSTT1, GSTM1, EPHX1, and SULT1A1 (Autrup, 2000; Dong et al., 2008; Park et al., 2005; Wang et al., 2002; Zheng et al., 2003). However, the conclusions were still controversial and needed to be explored further.

Inorganic arsenic metabolism in the human body has generally been considered a detoxification pathway through methylation (Styblo et al., 2002). Methylation of inorganic arsenic (InAs: $As^{3+} + As^{5+}$) to monomethylarsenic acid (MMA^{+5}) and dimethylarsenic acids (DMA^{+5}) occurs by the one-carbon metabolism pathway, with S-adenosylmethionine (SAM) serving as the methyl donor (Marafante and Vahter, 1984). Increasingly, epidemiology studies suggest that subjects with poor arsenic methylation capability, including higher InAs% or MMA% or lower DMA% had an increased risk of cancers, including UC and bladder cancer, skin lesions and vascular diseases (Leonardi et al., 2012; Pu et al., 2007; Wang et al., 2007). The related enzymes CYP1A1, SULT1A1, EPHX1, GSTT1, and GSTM1 are needed to catalyze metabolism of both cigarette smoke and inorganic arsenic. Polymorphisms of these enzymes are potential sources of inter-individual variability in internal dose of cigarette smoking or inorganic arsenic metabolism and thus may affect the risk of UC incidence.

Although previous studies concentrated on the significance of genetic polymorphism in these xenobiotic metabolism genes and UC risk, the lack of urinary arsenic profile data limited the ability to determine the relationships among gene susceptibility, urinary arsenic and UC risk. Therefore, we conducted a hospital-based case–control study to evaluate whether the gene polymorphisms of CYP1A1, SULT1A1, EPHX1, GSTT1, and GSTM1 modified the risk of UC by affecting urinary arsenic metabolites or exposure of cigarette smoke.

Methods

Study participants. A detailed protocol of recruitment in this present study has been previously described (Pu et al., 2007). Briefly, we conducted a hospital-based, case–control study and collected 191 UC cases and 364 age- and sex-matched healthy participants as controls from September 2007 to October 2011. All subjects were recruited from the National Taiwan University Hospital and the Taipei Municipal Wan Fang Hospital. All UC cases diagnosed by histological confirmation were outpatients at the Department of Urology. Matched controls were recruited from those receiving adult health examinations or senior citizen health examinations at the Department of Family Medicine. All study subjects provided informed consent before a questionnaire interview and biological specimen collection. The Research Ethics Committee of the National Taiwan University Hospital in Taipei of Taiwan approved the study and the study was performed in accordance with the World Medical Association Declaration of Helsinki.

Questionnaire interview. Structural questionnaires were administered through interviews in the clinics. The questionnaire acquired information on demographics, socioeconomic characteristics, lifestyle factors (such as cigarette smoking and environmental smoke exposure), as well as personal and family medical histories.

Biological specimen collection. Spot urine samples were collected at the time of recruitment and immediately transferred to a −20 °C freezer and stored until the analysis of arsenic species. Concurrently, we used ethylene-diamine-tetraacetic acid (EDTA) vacuumed syringes to collect peripheral blood samples and extracted DNA for the identification of enzyme gene polymorphisms.

Urinary arsenic species assessment. Urinary arsenic profiles of As^{3+}, DMA^{5+}, MMA^{5+} and As^{5+} were analyzed by high-performance liquid chromatography equipped with a hydride generator and atomic absorption spectrometer (HPLC-HG-AAS). The analysis protocol for determination of the arsenic species has been described in a previous study (Hsueh et al., 1998). Recovery rates of the four arsenic species were calculated using the following formula: ([(sample spiked standard solution concentration) − sample concentration]/standard solution concentration) × 100. The recovery rates of As^{3+}, DMA^{5+}, MMA^{5+}, and As^{5+} were from 93.8 to 102.2%, with detection limits of 0.02, 0.08, 0.05 and 0.07 μg/L, respectively. For quality control of the measurements, we purchased freeze-dried SRM 2670 urine from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA),which contained 480 ± 100 μg/L arsenic and the urine control was analyzed along with the urine specimens of study subjects. The detected value of arsenic in the SRM 2670 standard was 507 ± 17 μg/L ($n = 4$). To ensure the stability of urinary arsenic profiles, the detection of arsenic species was performed within 6 months after collection (Chen et al., 2002).

Genotyping of SNPs in CYP1A1, SULT1A1, EPHX1, GSTT1, and GSTM1. Genomic DNA was extracted using proteinase K digestion following phenol and chloroform extraction. SNPs in CYP1A1 MspI site, SULT1A1 His213Arg, and EPHX1 His139Arg were performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Wang et al., 2002, 2003). Multiplex PCR with some modifications for GSTM1 and GSTT1 null genotype was performed according to the Lin et al. method (Lin et al., 1998). All PCR products were obtained in a total volume of 30 μL, consisting of an 80 ng sample DNA, 10× PCR buffer, 2.5 mM dNTP, 2 μM of each primer and 2 U Taq polymerase. Detailed sequences of individual primers, annealing temperature, restricted enzyme, and fragment sizes of CYP1A1, SULT1A1, EPHX1, GSTT1 and GSTM1 are presented in Table 1. Genotypes were analyzed by electrophoresis on 2% and 3% agarose gels for GSTT1 and GSTM1 genotypes as well as for CYP1A1, SULT1A1, and EPHX1 genotypes, respectively. For quality control, a random 5% of the samples were repeated with a concordance of 100%.

Statistical analysis. Hardy–Weinberg equilibrium was fitted by the goodness of χ^2 test. The SNPs of CYP1A1, SULT1A1, and EPHX1 were divided into three classes, wild-type homozygotes (WW), variant heterozygotes (WV) and variant homozygotes (VV). Other SNPs of GSTT1 and GSTM1 were divided into two classes, null and non-null genotypes. Cigarette smoking status included never, former and current. Former smokers and current smokers were defined as those who had quit cigarette smoking and those who were still smoking at the time of the recruitment, respectively. All urinary arsenic profiles were normalized by urinary creatinine (μg/g creatinine or mg/g creatinine). Urinary total arsenic (μg/g creatinine) was defined as the sum of As^{3+}, As^{5+}, MMA^{5+} and DMA^{5+}. The relative proportion of each arsenic species (InAs%, MMA% and DMA%) was calculated by dividing the concentration of each species by the total arsenic concentration. Multivariate logistic regression models were used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) to determine the association between genotypes of CYP1A1, SULT1A1, EPHX1, GSTT1 and GSTM1 and the risk of UC after adjustment for age, sex, and educational level. Finally, we used the additive model (synergy index) to evaluate the combined effects of cigarette smoking status and various urinary arsenic profiles on UC risk (Hosmer and Lemeshow, 1992). All analyses were conducted using Statistical Analysis Software (SAS) statistical package (SAS, version 8.0, Cary, NC, USA).

Results

The distributions of sociodemographic characteristics and cigarette smoking status, as well as urinary arsenic profiles are shown in Table 2. The mean age of all subjects at recruitment was 62.6 years. Most subjects (70%) were male. Healthy controls had higher educational levels than UC patients. On average, half of the UC patients were never smokers. The ORs for UC were 1.21 (95% CI, 0.68–2.17) and 2.52 (95% CI, 1.56–4.06) in subjects who were former smokers and current smokers compared with those who were non-smokers, respectively.