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Association between Zinc transporter genes ZIP11 rs11077654 polymorphism with bladder cancer risk in Chinese Han Population

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ABSTRACT

Objective: To study the association between Zinc transporter genes ZIP11 rs11077654 polymorphism with bladder cancer risk in Chinese Han Population.
Methods: A total of 307 unrelated Chinese Han descent, including 139 bladder cancer patients as bladder cancer group and 168 healthy people as healthy control group were studied. The ZIP11 rs11077654 loci was analyzed by Polymerase Chain Reaction (PCR) and its relationship with bladder cancer was analyzed.
Results: There were three kinds of genotypes in ZIP11 gene rs11077654 loci (CC, CA and AA). Our analysis presented that the CA genotype was not associated with bladder cancer risk when the CC genotype may be served as reference (OR, 1.355; 95% CI, 0.750–2.451; $p = 0.313$). We combined CA and AA genotypes as a dominant genetic model. There was no significant association with bladder cancer risk as well in the combined group (OR, 1.333; 95% CI, 0.758–2.343; $p = 0.317$). There was no significant association observed between the CA/AA genotypes and low-grade (OR, 0.900; 95% CI, 0.411–1.973; $p = 0.793$) or high-grade bladder cancer (OR, 0.675; 95% CI, 0.349–1.308; $p = 0.243$), and nor between the CA/AA genotypes and NMIBC (OR, 0.636; 95% CI, 0.334–1.213; $p = 0.167$) or MIBC (OR, 1.100; 95% CI, 0.478–2.531; $p = 0.823$).
Conclusion: The rs11077654 of ZIP11 gene was not associated with bladder cancer in Chinese Han Population.

Introduction

Bladder cancer is one of the most frequent malignancies around the world.[1] It was estimated that 54,390 men and 18,300 women would be diagnosed with bladder cancer, and 15,580 men and women would die of this disease in the United States in 2014.[2] It is of note that the incidence of bladder cancer has been steadily increasing in China over recent years.[3] According to data in 2012 provided by the International Agency for Research on Cancer,[4] 55,486 cases and 26,820 deaths were estimated for the population in China. It has also been established that genetics have an important role in the risk of bladder cancer.[5] A recent GWAS from the Genetics of Urological Cancers Consortium identified that Zinc transporter genes ZIP11 rs11077654 played a role in bladder cancer in populations of European descent.[6]

Zinc is one of the vital minerals which contribute to maintain human health. Zinc ion transporters are significant for normal cellular functions by firmly regulating concentration of zinc ions in somatic cells. Many studies have shown proof of relationship between zinc ions imbalance, Zinc transporters dysfunction and the development of urological cancers.[7–11] ZIP11 was implicated to be a tool importing zinc for cells, and the deletion of this gene can lead to decline of cellular zinc concentrations and metallothionein levels.[6] However, the relationship between ZIP11 and the etiology of cancers is previously unknown and the mechanism of ZIP11 participating in the development of human cancers should be further investigated. Recently, Wu et al. demonstrated that no Zinc transporter variants were associated with prostate cancer risk while four variants (rs11077654, rs11077654, rs9913017, rs4969054) within Zinc transporters ZIP11 were significantly associated with bladder cancer risk.[6] Moreover, rs11077654 was significantly associated with survival of bladder cancer patients.[6]

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Therefore, Zinc transporter gene ZIP11, especially rs11077654, may play a key role in bladder cancer. At present, no research has been performed on the association between polymorphism of Zinc transporters ZIP11 rs11077654 and bladder cancer in Chinese Han Population. Due to the fact that the incidence of bladder cancer differs greatly in countries and areas,[1] it is worthwhile to validate bladder cancer risk-associated SNPs in other independent case-control series.[1] The aim of our study was to investigate the association between Zinc transporters ZIP11 rs11077654 and bladder cancer in Chinese Han Population in Northern China.

Material and methods

Human subjects

The study was performed in accordance with the principles of declaration of Helsinki and its appendices.[12] All experiments were approved by the ethical committee of Qingdao municipal hospital (Qingdao, China) and a written informed consent form was obtained from all patients prior to the initiation of the study.

We selected a total of 207 unrelated Northern Chinese Han descent, including 139 bladder cancer patients and 168 healthy controls matched for sex and age. All Subjects were collected from Qingdao municipal hospital. The diagnosis of bladder cancer patients was confirmed with transitional cell carcinoma of bladder histologically through pathological examinations.

The control healthy subjects without previous history of cancer were confirmed by medical history, general examinations and laboratory examinations at the same hospital during the same period of time. The exclusion criteria for the control subjects were significant mental impairment or blood transfusion in the past months; controls were also excluded if they had symptoms suggestive of bladder cancer, such as hematuria.[1]

Patient characteristics and clinical assessment

Demographic data were collected by in-person interviews. Tumor staging for all patients with bladder cancer was assessed with the 2002 International Union against Cancer (UICC) tumor-nodes-metastasis classification and tumor grading referenced the World Health Organization (WHO)/International Society of Urological Pathology (ISUP) 2004 grading of urothelial papilloma. Tumor grading refers to either high grade or low grade. Meanwhile, tumor stage was classified into NMIBC (pTa–pT1 stage) and MIBC (pT2–pT4 stage) in all patients.

Genetic DNA extraction and genotyping

The genomic DNA purification kit (BioTeke, Biotechnology, Beijing, China) was used for isolating DNA from peripheral venous blood in accordance with the instructions of the manufacturer and stored at −80 °C until use. Genotyping for ZIP11 (rs11077654) was performed by polymerase chain reaction (PCR) using the following primers: forward, 5′-ACGTTGGATGCTGGGT TATGTTTCATTCTCC-3′ and reverse, 5′-ACGTTGGATGCTTTTGCAA TAGGAGAAGAC-3′. The PCR amplification (ABI veriti-384 PCR, United States) procedure consisted of initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 3 min. The PCR products were run on 2% agarose gel electrophoresis and all cases were confined to a single band of the expected size with a 99-base pair product in size. The ZIP11 genotypes were detected by direct DNA sequencing using MassARRAY Analyzer 4.0 mass spectrometer (Sequenom, Inc, United States) and the results were analyzed with TYPER4.0 software (Sequenom, Inc, United States). The genotyping call rate was more than 95% and the completion rate was above 99%. Genotyping was carried out in a blinded fashion.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was performed for the distribution of genotypic frequencies in the control group using the χ^2 test. Genotype and alleles were assayed by chi-square test and DNA distributions between bladder cancer patients and controls were evaluated by Pearson's χ^2 test or Fisher's exact test where appropriate. The characteristics of the subjects were shown as mean ± SD. Differences in characteristics between cases and controls were estimated using student's t test, paired samples test or χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to show the relative risk degree. Statistical analyses were performed using SPSS 17.0 software (SPSS Inc. Chicago, IL, USA). $p < 0.05$ was defined statistically significant.

Results

The characteristics of patients and controls

The clinical manifestations, demographic characteristics, TNM staging and grading are shown in Table 1. No significant difference was found in age ($p = 0.406$) between patients and controls. There was no significant difference in sex distribution (x^2 test, $p = 0.953$).

Association between ZIP11 rs11077654 genotypes and bladder cancer susceptibility

The genotypes distribution of ZIP11 rs11077654 was in accordance with the Hardy–Weinberg equilibrium between patient and control groups ($P_{patient} = 0.136$, $P_{control} = 0.332$). To ensure the accuracy of our genotyping, we randomly repeated DNA sequencing in 100 subjects for reverse sequencing. The success rate of duplicated genotyping was more than 100%. The allele and genotype frequencies of rs11077654 for the patients and controls are shown in Table 2.

Our analysis presented that the CA genotype was not associated with bladder cancer risk when the CC genotype was served as reference (OR, 1.355; 95% CI, 0.750–2.451; $p = 0.313$). Additionally, considering the association of rs11077654 C allele with bladder cancer and the relatively small number of CA genotype, compared with CC genotype, we combined CA and AA genotypes as a dominant genetic model. There was no significant association with

Table 1
The characteristics of patients and controls.

Variables	Patients ($n = 139$) n (%)	Controls ($n = 168$) n (%)	p value
Age (years)			
Mean ± SD	67.5 ± 10.9	66.9 ± 11.2	0.406
Sex			
Female	31 (22.3)	37 (22.0)	0.953
Male	108 (77.7)	131 (78.0)	
Tumor grade			
Low grade	48 (34.53)	–	–
High grade	91 (65.47)	–	–
Tumor stage			
NMIBC	102 (73.38)	–	–
MIBC	37 (26.62)	–	–

Low grade contains papillary urothelial neoplasm of low malignant potential (PUNLMP) and low grade urothelial carcinoma; NMIBC: non-muscle-invasive bladder cancer; MIBC: muscle invasive bladder cancer.