Role of DNA Repair-related Gene Polymorphisms in Susceptibility to Risk of Prostate Cancer

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Abstract

Aim: We assessed the association between genetic variants of XPG, XPA, XPD, CSB, XPC and CCNH in the nucleotide excision repair (NER) pathway and risk of prostate cancer. Methods: We genotyped the XPG, XPA, XPD, CSB, XPC and CCNH polymorphisms by a 384-well plate format on the MassARRAY® platform. Multivariate logistical regression analysis was used to assess the associations between the six gene polymorphisms and risk of prostate cancer. Results: Individuals carrying the XPG rs229614 TT (OR=2.01, 95%CI=1.35-3.27) genotype and T allele (OR=1.73, 95%CI=1.37-2.57) were moderately significantly associated with a higher risk of prostate cancer. Subjects with XPD rs13181 G allele had a marginally increased risk of prostate cancer, with adjusted OR(95% CI) of 1.53 (1.04-2.37). Moreover, individuals carrying with CSB rs2228526 GG genotype (OR=2.05, 95% CI=1.23-3.52) and G allele (OR=1.56, 95% CI=1.17-2.05) were associated with a higher increased risk of prostate cancer. The combination genotype of XPG rs2296147 T and CSB rs2228526 G allele had accumulative effect on the risk of this cancer, with an OR (95% CI) of 2.23(1.37-3.59). Conclusions: Our study indicates that XPG rs2296147 and CSB rs2228526 polymorphisms are significantly associated with increased risk of prostate cancer, and that combination of XPG rs2296147 T allele and CSB rs2228526 G allele is strongly associated with an increased risk.

Keywords: XPG - CSB - DNA repair-related genes - prostate cancer - polymorphism

Introduction

Prostate cancer is the second most commonly diagnosed solid tumor in males, accounting for 10% of male cancer related death (Globocan, 2008). It is well known that the prostate is a multi-factorial disease and induced by genetic and environmental factors (Grant et al., 2004). Genomic stability and integrity are important in maintaining accurate DNA replication. DNA disruptions could induce gene rearrangements, translocations, amplifications and deletions, which could play an important role in the carcinogenesis.

DNA repair system plays a vital role in maintaining the stability of cellular functions and genomic integrity through the reversal of the damaged DNA induced by various endogenous and/or exogenous factors. There are four well known DNA repair pathways which are responsible for repairing various of DNA damage, including base excision repair (BER), nucleotide excision repair (NER), double-strand break repair (SSBR) and homologous recombination repair (HRR). Nucleotide excision repair (NER) pathway is an important mechanism that maintains genomic integrity by removing DNA bulky lesions or interstrand adducts induced by exogenous and/or endogenous factors (Neumann et al., 2005; Wu et al., 2005). The variation of DNA repair genes in the NER pathway may affect the capacity of encoded DNA repair enzymes, and subsequently enhance the risk of cancer (Goode et al., 2002; Hu et al., 2002; Hu et al., 2004).

Previous studies have examined the association between DNA repaired gene polymorphisms and risk of prostate cancer in various populations (Nock et al., 2006; Hooker et al., 2008; Berhane et al., 2012; Liao et al., 2012; Sobti et al., 2012). However, these results are inconsistent. In this case-control study, we assessed the association between genetic variants of XPG, XPA, XPD, CSB, XPC and CCNH in the NER pathway and risk of prostate cancer.

Materials and Methods

Characteristics of study subjects

The subjects were recruited from an ongoing multicenter case-control study conducted in China. This study included 279 patients with newly diagnosed and histopathologically confirmed primary prostate cancer from Shanghai Pudong New Area Zhoupu Hospital and Shanghai East Hospital Affiliated to Tongji University between January 2008 and May 2012. 241 prostate cancer cases agreed to participate with a participation...
rate of 86.38%. A total of 316 cancer-free controls who sought for health examination in the Shanghai Pudong New Area Zhoupu Hospital and Shanghai East Hospital Affiliated to Tongji University were selected during the same time period, and 264 controls agreed to participate (participation rate: 83.54%). Controls were matched with cases by age (±5 years). All patients were asked to provide 5mL of blood for genotyping, and they signed a written informed consent.

**SNP selection and Genotyping**

5 ml venous blood was drawn from each cases and controls. The blood was kept in -20°C, and EDTA with 1.5~2.2 mg/ml was used for anticoagulant. DNA was isolated from peripheral blood and genotyped using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China) according to the manufacturer’s instructions.

Genomic DNA was extracted using a TIANamp blood DNA kit (Tiangen Biotech, China). Genotyping of XPG rs2296147, XPA rs1800975, XPD rs13181, CSB rs2228526, XPC rs2228001 and CCNH rs2266690 were performed in a 384-well plate format on the MassARRAY® platform. Primers and probes were designed using Sequenom® Assay Design 3.1 software (Sequenom®). The cycling programme involved preliminary denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, and annealing at 64°C for 30 s, with a final extension at 72°C for 10 min. PCR products were verified by 1.0% agarose gel electrophoresis, and the PCR products were visualized using ethidium bromide staining. Reproducibility was verified by repeat analysis of a randomly chosen subgroup of 10% of the subjects.

**Statistical analysis**

Statistical analysis was conducted using SPSS® version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Continuous variables were presented as mean ± SD and analysed using independent sample t-test. Categorical variables were presented as n of subjects (%) and analysed using χ²-test. The Hardy-Weinberg
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Table 3. Combination effect of XPG rs2296147 and CSB rs2228526 polymorphisms on prostate cancer risk

| Single nucleotide polymorphism | Cases N=241 | %   | Controls N=264 | %   | OR (95% CI) | P value | Adjusted OR (95% CI) | Adjusted P value |
|-------------------------------|-----------|-----|----------------|-----|-------------|---------|---------------------|-----------------|
| XPG rs2296147/CSB rs2228526  |           |     |                |     |             |         |                     |                 |
| CC/AA                         | 61        | 25.3| 97             | 36.7| 1.0(Ref.)   | -       | 1.0(Ref.)           | -               |
| T allele/AA                   | 29        | 12  | 27             | 10.2| 1.71(0.88-3.31)| 0.09    | 1.82(0.95-3.56)     | 0.07            |
| CC/G allele                  | 84        | 34.9| 86             | 32.6| 2.47(1.51-3.28)| <0.001 | 2.10(1.26-3.27)     | 0.02            |
| T allele/G allele            | 67        | 27.8| 54             | 20.5| 1.97(1.19-3.38)| 0.005  | 2.23(1.37-3.59)     | 0.003           |

1 Adjusted for age and family history of prostate cancer

Results

A total of 241 prostate cancer patients and 264 controls were recruited in our study. The mean ages of the cases and controls were 67.4±7.1 and 66.8±9.2 years, respectively. We did not find significant role of smoking status, drinking status and BMI in the risk of prostate cancer. However, we found prostate cancer cases were more likely to have a family history of cancer when compared with the controls (13.7% vs 1.1%, p<0.05).

Genotype distributions of the six SNPs are shown in Table 2. All genotype distributions of XPG rs2296147, XPA rs1800975, XPD rs13181, CSB rs2228526, XPC rs2228001 and CCNH rs2266690 in controls were in line with Hardy-Weinberg equilibrium (All p value>0.05).

Multivariate regression analyses showed that individuals carrying with XPG rs2296147 TT (OR=2.01, 95%CI=1.35-3.27) genotype and T allele (OR=1.73, 95%CI=1.37-2.57) were moderately significantly associated with a higher risk of prostate cancer. Subjects with XPD rs13181 G allele marginally increased the risk of prostate cancer, with adjusted OR (95%CI) of 1.53 (1.04-2.37). Moreover, individuals carrying with XPC rs2228526 GG genotype (OR=2.05, 95% CI=1.23-3.52) and G allele (OR=1.56, 95%CI=1.17-2.05) were associated with a higher increased risk of prostate cancer.

For further analysis, we identified the combination effect of XPG rs2296147 and CSB rs2228526 polymorphisms on prostate cancer risk. Subjects carrying both XPG rs2296147 CC genotype and CSB rs2228526 G allele had a moderate increased risk of prostate cancer, with a adjusted OR (95%CI) of 2.10 (1.26-3.27). Similarly, subjects carrying XPG rs2296147 T allele and CSB rs2228526 G allele was associated with an increased risk, with adjusted OR (95% CI) of 2.23 (1.37-3.59).

Discussion

Numerous studies have indicate prostate cancer is a multi-factorial disease, and both genetic and environmental factors play an important role in the development of prostate cancer (Chen et al., 2013; Farrell et al., 2013; Priyadarshini et al., 2013). Previous studies demonstrated that DNA adducts are formed in the prostate tissue as a result of exposure to oxidative stress and environmental toxins (Ryblicki et al., 2006; Tang et al., 2007), and DNA repair mechanisms play an important role in removal of oxidative DNA compounds or DNA adducts from damaged genomic DNA sites. However, polymorphisms in DNA repaired genes play a critical role in altering the function of repairing DNA damages and removal of oxidative DNA compounds or DNA adducts. Since several studies have indicated that some DNA repaired gene polymorphisms are associated with prostate cancer in various populations (Agalliu et al., 2012; Berhane et al., 2012; Mittal et al., 2012). In this case-control study in a Chinese population, we found that XPG rs2296147 and CSB rs2228526 were strongly associated with prostate cancer risk, both individually and in combination.

Thus, the XPG rs2296147 and CSB rs2228526 genes may influence the diagnosis and treatment of gastric cancer. Our finding is consistent with those of several other studies in Indian population, American population and African Americans (Hyytinen et al., 1999; Hooker et al., 2008; Berhane et al., 2012). However, the significance of XPG rs2296147 polymorphisms in the development of prostate cancer in Chinese population is unclear. Berhane et al. reported that polymorphism XPG rs2296147 contributes to cancer risk susceptibility and can affect the development of prostate cancer in an Indian population, with a adjusted OR of 2.53 (Berhane et al., 2012). Hyytinen et al. (1999) reported that the polymorphism XPG Asp1104His plays an important role in the development of prostate cancer, while Hooker et al. conducted a population-based study in United State reported no significant correlation between XPG polymorphisms and risk of prostate tumors (Hooker et al., 2008).

Since these studies were conducted in different populations, it is difficult to make direct comparisons between them. It can be presumed that the discrepancies may be due to differences in variant frequencies between races, and thus the XPG gene polymorphisms can play different roles in the development of prostate cancer between populations.

Previous studies indicated that polymorphism of CSB rs2228526 was associated with susceptibility and prognosis of various cancer, such as skin cancer, bladder cancer and bone malignant tumor as well as glioblastoma (Chang et al., 2009; Grunda et al., 2010; Wheless et al., 2012; Sun et al., 2013). Another experimental study indicated that chronic exposure to arsenic causes DNA damage and increased cell survival that may ultimately
result in neoplastic transformation of human prostate epithelial cells (Singh et al., 2011). Only a recent study conducted in African-American men reported that the CSB polymorphism have no effect on the risk of prostate cancer (Hooker et al., 2008). The inconsistency of these studies may be explained by differences in genetic origin, population background, source of controls, and sample size, or by chance. Further studies are greatly warranted to confirm their association.

Our study showed the combination of XPG rs2296147 T allele and CSB rs2228526 G allele e were associated with a higher risk of prostate cancer, which indicated that the XPG rs2296147 and CSB rs2228526 polymorphisms had accumulative effect on the risk of this cancer.

Our study has several limitations that should be carefully considered when interpreting the results. Firstly, we only examined only a small number of SNPs in DNA repair genes with respect to risk of prostate cancer, and did not explore the interaction between SNPs and environmental factors. Another limitation is the small number of Chinese men in our study that limited the statistical power to examine associations in this group.

In conclusion, our study indicates that XPG rs2296147 and CSB rs2228526 polymorphisms are significantly associated with increased risk of prostate cancer, and that combination of XPG rs2296147 T allele and CSB rs2228526 G allele was strongly associated with an increased risk. Our findings may be helpful in identifying individuals at increased risk for developing prostate cancer. Future large population-based studies are needed to identify the exact mechanism underlying the involvement of the DNA repaired genes in the development of prostate cancer.

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