Cover letter

June 16, 2015

To Editor-in-Chief

Environmental Health and Preventive Medicine

I, along with my coauthors, would like to ask you to consider the attached manuscript entitled "XPC intron11 C/A polymorphism as a risk factor for prostate cancer" for publication in Environmental Health and Preventive Medicine as an original article.

This manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review boards. All the authors have approved the manuscript and agree with submission to your esteemed journal. There are no conflicts of interest to declare.

Sincerely,

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Title page

Regular Article

Title: XPC intron11 C/A polymorphism as a risk factor for prostate cancer

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Brief running title: XPC intron11 C/A polymorphism and prostate cancer

Keyword: Cancer risk, XPC-PAT, DNA repair gene, Xeroderma pigmentosum, Prostate cancer

ABSTRACT

Objectives: DNA repair genes play an important role in protection against environmental and endogenous DNA damage, and constitute the first line of defense against cancer. Xeroderma pigmentosum complementation group C (XPC) is involved in the damage recognition step during nucleotide excision repair. The relationship between XPC intron11 C/A polymorphism and cancer risk has not been widely studied. Hence, this study evaluated the relationship between the XPC intron11 C/A polymorphism and prostate cancer risk.

Material and Methods: This hospital-based cohort consisted of 152 patients with prostate cancer and 142 male controls. The XPC intron11 C/A genotype was determined using the PCR-RFLP method. Medical, occupational, and cigarette-smoking history was obtained from each participant using questionnaires.

Results: Logistic regression analysis revealed that compared to controls, the frequencies of the A/A and C/A genotypes were significantly higher than those of the C/C genotype in cancer patients (OR = 2.03, 95% confidence interval [CI] = 1.03–3.98 and OR = 1.91, 95% CI = 1.13–3.24, respectively). We also found that the frequency of the A/A genotype was significantly higher in cancer cases than in controls among non-smokers (OR = 7.7, 95% CI = 1.38–42.88, compared to the C/C genotype).

Conclusion: We found that the XPC intron11 C/A polymorphism was associated with an increased risk of prostate cancer. Among non-smokers, the A/A genotype was significantly more prevalent in prostate cancer patients than in controls.

INTRODUCTION

Prostate cancer is the most common cancer in men in the United States [1]. However, the incidence of prostate cancer in Asia is relatively low. Risk factors for prostate cancer are diet [2], age, smoking and somatic genomic changes, including deletions, amplifications, and point mutations in tumor suppressor and DNA repair genes [3, 4], similar to those for other cancers.

DNA repair genes play an important role in protection against environmental and endogenous DNA damage, and constitute the first line of defense against cancer. The four major pathways of DNA repair are base excision repair, nucleotide excision repair (NER), double strand break repair, and mismatch repair. The xeroderma pigmentosum (XP) complementation group C (XPC) protein is involved in early damage recognition and initiation of NER by binding to HR23B to form the stable XPC-HR23B complex, which recognizes and binds to damaged DNA, leading to subsequent DNA repair [5]. There are three polymorphisms frequently detected in the XPC gene: the poly AT insertion/deletion on intron9 (PAT), the A to C substitution in exon 15 (Lys939Gln), and the C to A substitution in position 5 of intron 11 (intron11 C/A polymorphism).

Epidemiologic studies of cancer patients have shown that the PAT+/+ genotype was associated with an increased risk of squamous cell carcinoma of the head and neck [6], and lung cancer risk [7]. The A to C substitution in exon 15 that gave rise to a lysine to glutamine substitution at position 939 has been associated with relatively high risk of bladder cancer [8] and lung cancer [9], but not related to bladder cancer[10]. However, the relationship between the intron11 C/A polymorphism and cancer risk has not been widely studied; to date, the association of the intron11 C/A polymorphism with colorectal cancer, reported by Gil et al, was the only published study [11]. In the present study, we evaluated the risk of prostate cancer associated with the intron11 C/A polymorphism.

MATERIALS AND METHODS

Subjects

The patient consisted of 152 patients with prostate cancer (cases), histologically diagnosed between September 1992 and June 2003 at the University of Occupational and Environmental Health (UOEH) Hospital or the University of Miyazaki Hospital, Japan. The control group consisted of 142 patients with non-cancerous diseases, randomly selected from the UOEH hospital, a hospital near the UOEH Hospital, and the University of Miyazaki Hospital between September 1996 and June 2003. Control patients were examined to rule out urothelial disease, hematuria, and cancer.

The demographic data of cases and controls are shown in Table 1. The mean ages were 71.7 and 70.2 years for cases and controls, respectively. All study subjects completed a questionnaire administered by a trained interviewer, which covered medical, occupational, and cigarette-smoking history. No exposure to carcinogens, heavy metals, or radiation was recorded in the occupational history of any participant. Cigarette-smoke exposure was calculated as pack-years (1 pack [20 cigarettes]/day X years of smoking). “never-smoker” were defined as those who did not smoke at the time of completing the questionnaire. A “light-smoker” was defined as a person who had smoked less than 35 pack-years, and a “heavy-smoker” was defined as someone who had smoked more than 35 pack-years. “Smoker” in the table 1 included “light-smoker” and “heavy-smoker”. The nature of the study was explained to all participants, and informed consent was obtained from each participant. Ethical approval for the study was obtained from the Ethical Committee of the Faculty of Medicine, University of Miyazaki.

Genotyping

Blood samples were taken from all participants, and genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction. The PCR-RFLP method, originally described by Marin et al [7], was used to identify the intron11 C/A polymorphism. The PCR primers used for amplification were as follows: forward 5'-GCCAAATGCTGACTTGCTCACCGG-3' and reverse 5'-GCCACGCGGTGTAGATTGGG-3'. Each 50 μL PCR reaction mixture contained 10 pmol of each primer, 2.0 mM MgCl2, 200 mM of each dNTP, 1 unit of Taq polymerase, and 100–300 ng of genomic DNA. The reaction mixture was preincubated for 5 min at 94ºC. The PCR conditions used were 30 cycles of 94ºC for 30s and 65ºC for 30s, followed by 72ºC for 30s. The PCR products were digested with the restriction enzyme HaeIII (New England Biolabs, Beverly, MA, USA) at 37ºC for overnight. DNA fragments were electrophoresed in 2% agarose gel and stained with ethidium bromide. The A/A genotype gave a single 128 bp band, the C/A genotype showed three bands of 24 bp, 104 bp, and 128 bp, and the C/C genotype had two bands of 24 bp and 104 bp.

Statistical analysis

Univariate analysis was initially performed to compare the distributions of age, sex, and smoking status. Differences in the distributions between cases and controls were tested using the $\chi 2$ and Mann–Whitney U tests, where appropriate. A test for Hardy-Weinberg equilibrium among the controls was conducted using observed genotype frequencies and a $\chi 2$ test. The odds ratio (OR) and 95% confidence intervals (95% CI) for prostate cancer risk were adjusted for age by multiple logistic regression analysis using the Dr SPSS Ⅱ for Windows (SPSS version11.0.1).

RESULTS

The analysis included 152 prostate cancer patients and 142 controls from the Japanese population. The characteristics of the cases and controls, such as age and smoking status, are summarized in Table 1. The prostate cancer patient group had a significantly higher number of never smokers than the control group ($P < 0.01$).

The intron11 C/A polymorphism distribution for the cases and controls is shown in Table 2. The distribution of the genotypes among the controls was consistent with the Hardy-Weinberg equilibrium ($P = 0.38$), and was similar to that in another report [7]. The frequencies of the C/A and A/A genotypes were significantly higher in cancer patients than in the control group. The adjusted ORs for prostate cancer risk associated with the C/A and A/A genotypes compared to the C/C genotype were 1.91 (95% CI, 1.13–3.24) and 2.03 (95% CI, 1.03–3.98), respectively.

Table 3 shows the distribution of the three genotypes according to smoking status. Thirty-five pack-years was the mean median smoking exposure among the smoking participants in this study. Among never-smokers, the A/A genotype was significantly more frequent in cancer patients than in the controls; the OR was 7.70(95% CI, 1.38–42.88) compared to the C/C genotype.

DISCUSSION

This is the first reported study of the intron11 C/A polymorphism in a Japanese population. In this study, we evaluated the association of the intron11 C/A polymorphism with risk of prostate cancer. The genotypic distribution of cases and controls is shown in Table 2. The adjusted ORs for prostate cancer associated with the C/A genotype and A/A genotype compared to C/C genotype were 1.912 (95% CI 1.13–3.24) and 2.03 (95% CI 1.03–3.98), respectively. Similar to the findings of Gil et al in colorectal cancer [11], our results show that the C/A and A/A genotypes are associated with an increased risk of prostate cancer compared to the C/C genotype.

XP is a rare recessive disorder associated with a high rate of sunlight-induced skin cancer [12]. XPC is one of seven xeroderma pigmentosum (XP) complementation groups with three common polymorphisms. Several published reports have previously described associations of cancer risk with these XPC polymorphisms [6, 7, 13-18]. Epidemiologic studies of cancer patients have shown an association between the PAT+/+ genotype and a 1.85-fold increase in the occurrence of squamous cell carcinoma of the head and neck [6] and a 1.6-fold increase in the occurrence of lung cancer [7]. However, the relationship between the three XPC polymorphisms and several types of cancer remains controversial [6, 7, 10, 19, 20].

Only one study evaluating the relationship between the intron11 C/A polymorphism and colorectal cancer has been reported[11], therefore, the evaluation of the intron11 C/A polymorphism as a risk factor for cancer has not been established. This is only the second report of a relationship between the intron11 C/A polymorphism and cancer risk. The XPC-PAT polymorphism had linkage disequilibrium with XPC exon 15 Lys939Gln polymorphism and intron 11 C/A polymorphism [21]. But the exon 15 Lys939Gln polymorphism didn’t change XPC function in vitro [21]. The intron11 C/A polymorphism is a splice accepter site polymorphism, and is related to an increased frequency of exon 12

skipping[7]. The abnormally spliced XPC mRNA iso-form has diminished DNA repair activity and may thereby contribute to cancer susceptibility [22]. The homogenous A/A variant is associated with about 50% reduction of DNA capacity [22]. Our result was supported with these finding.

Some studies indicated that the polymorphism of XPC was risk for cancer, but some studies didn't indicate same result. The results of studies concerned XPC polymorphisms was inconsistency. Linkage disequilibrium was reported to the reason for this discrepancy [23]. That article indicated that the discrepancy was that the XPC polymorphism evaluated exist in variable degrees of linkage disequilibrium with other that were not evaluated in their investigations [23]. There was no report concerned to linkage disequilibrium in Japanese. Therefore, further study concerned linkage disequilibrium of XPC was needed to evaluation the relation between XPC polymorphism and cancer risk.

In general, the distribution of a polymorphism could be changed depending on race. However, there were no reports about the intron 11 C/A polymorphism among Japanese; this report was first article evaluated the intron 11 C/A polymorphism. Our distribution of intron 11 C/A polymorphism was the same to other reports [7, 11]. The distribution of the intron 11 C/A polymorphism of Japanese couldn't be so different to other results.

We also evaluated the association between smoking status and intron11 C/A polymorphism genotype with regard to the risk of prostate cancer. We have shown that the prevalence of the A/A genotype in non-smokers is significantly higher in cancer patients than in controls (adjusted OR = 7.70, 95%CI ＝1.38–42.88). Amos et al [24], Khoury et al [25], and Wang et al [26] have also reported that genetic variation associated with cancer risk might be smaller when carcinogen exposure is greater. Jin et al [27] also indicated that the high risk associated with the Pro/Pro genotype of p53 codon 72 polymorphism was associated with lighter smoking. Wang et al. also reported that the same p53 polymorphism was slightly

over-represented in lung cancer patients who were non-smokers [26]. There was other report with same result[28]. An explanation for this might be that smoking also alters the level by triggering and up-regulating DNA repair enzymes [29]. Shen et al showed that either inadequate response to DNA damage or inaccurate repair of DNA may have contributed to the risk of lung cancer development in non- or light smokers [30].

This was only the second study describing the relationship between the XPC intron11 C/A polymorphism and cancer risk. And our result was first article concerned to prostate cancer. However, in this article there are some limitations. First of all, we collected sample randomly to delete bias. But our sample was small, therefore there could be bias in the sample. Second limitation was more never-smokers in cases than controls. Though smoking could be confounding factor, the reason that cases contained more nonsmokers was that there were many unknown person concerned to smoking status in cases and controls. We evaluated odds ratio by using multiple logistic regression analysis. We thought that the effect of the difference of nonsmokers to the result would be small. The third limitation was that control was hospital control. Hospital control might had some diseases, and the effect of their disease to occurrence of prostate cancer couldn't remove completely. But we excluded persons with urothelial disease, hematuria, and any cancer. More over linkage disequilibrium was needed to evaluate. For these limitations, further evaluation would be needed to confirm the significance of the intron11 C/A polymorphism as a risk factor for prostate cancer.

This is the first study reporting that the allele of the intron11 C/A polymorphism of the XPC gene may be a risk factor for prostate cancer in the Japanese population. The prevalence of the A/A genotype in non-smokers was significantly higher in cancer patients than in the controls, and therefore, the A/A genotype may represent a specific cancer risk factor for non-smokers.

Conflict of interest

We acknowledge that we have no conflict of interest.

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Tables

Table1 Characteristics of prostate cancer cases and health controls.

	Cases	Controls
n	152	142
Age		
Mean±SD	71.68±8.97	70.19±10.86
Range	35-93	32-92
Smoking Status		
Non-Smoker	48	23
Smoker(light and heavy)	86	96*
Unknown	18	23

Two sided $\chi 2$ test and Mann-Whitney where appropriate

*:P<0.01 Two-sided $\chi 2$ test

Table 2 XPC Intron 11 C/A genotype frequency and distribution

Genotype	Cases(n=152)	Controls(n=142)	Ajusted OR† (95% CI)	P‡value
C/C	39(25.7%)	57(40.1%)	1.00(Reference)	
C/A	81(53.3%)	62(43.7%)	1.91(1.13-3.24)	0.02
A/A	32(21.1%)	23(16.2%)	2.03(1.03-3.98)	0.04

†: Ajusted by age

‡: Two-sided χ^2 test

Table 3 XPC Intron 11 C/A genotype frequency and distribution divided into smoking status

Smoking status	Cases(%)				Controls(%)				Ajusted OR† (95% CI)		P‡ value
	n	C/C	C/A	A/A	n	C/C	C/A	A/A	C/C	A/A	
Non-Smoker	48	11(22.9)	22(45.8)	15(31.3)	23	11(47.8)	10(43.5)	2(8.7)	1.00(reference)	7.70(1.38-42.88)	0.02
Light-Smoker(＜35packs year)	40	14(35.0)	20(50.0)	6(15.0)	43	15(34.9)	21(48.8)	7(16.3)	1.00(reference)	0.86(0.24-3.31)	0.86
Heavy-Smoker(≧35packs year)	46	10(21.7)	29(63.0)	7(15.2)	53	21(39.6)	23(43.4)	9(17.0)	1.00(reference)	1.26(0.35-4.61)	0.44

†: Adjusted by age

‡: Two-sided χ^2 test