Association of a Genetic Polymorphism of the E-cadherin Gene with Prostate Cancer in a Japanese Population

Toshiyuki Kamoto¹, Yoshiaki Isogawa¹, Yousuke Shimizu¹, Sachiko Minamiguchi², Hidefumi Kinoshita¹, Yoshiyuki Kakehi³, Kenji Mitsumori⁴, Shingo Yamamoto¹, Tomonori Habuchi¹, Tetsuro Kato⁴ and Osamu Ogawa¹

¹Department of Urology and ²Department of Clinical Pathology, Kyoto University Graduate School of Medicine, Kyoto, ³Department of Urology, Kagawa University, Takamatsu and ⁴Department of Urology, Akita Medical University, Akita, Japan

Received August 26, 2004; accepted January 8, 2005

The E-cadherin gene has been identified as having a physiological role in cellular attachment, and is hypothesized to participate in carcinogenesis. A polymorphism (an A to C substitution) in the 5′-untranslated region has a direct effect on E-cadherin gene transcriptional regulation. We explored the association between E-cadherin gene polymorphism and the risk of prostate cancer in a Japanese population. The subjects consisted of 236 patients with prostate cancer, 209 benign prostatic hyperplasia (BPH) patients and 139 male controls. A marginally significant difference was found between prostate cancer patients and male controls (P = 0.053). No significant difference was observed between prostate cancer and BPH patients. When patients with prostate cancer were divided into two groups, stage A+B and stage C+D, a significant difference was observed between progressive cancer patients (stage C+D) and male controls (odds ratio = 1.93, P = 0.016). It is possible that the presence of one A allele resulted in an increased risk of cancer progression.

Key words: E-cadherin – prostate cancer – single nucleotide polymorphism

INTRODUCTION

Prostate cancer is the most common malignancy in North American and European men, and represents a major public health challenge. Traditionally considered a disease of elderly men, a considerable proportion of cases now occur in men in their pre-retirement years. New means of identifying individuals at risk and strategies for early detection and preventive care are urgently needed.

There are some risk factors that have thus far been clearly established for prostate cancer, e.g. familial aggregation and ethnic origin (1,2). Many epidemiological studies have been conducted to evaluate the effects of environmental factors on the risk of prostate cancer in an attempt to explain the large ethnic variations in risk. However, no single environmental or lifestyle factor has consistently been associated with the risk of prostate cancer. Genetic polymorphisms of genes encoding for the androgen receptor, vitamin D receptor and cytochrome P450c17alpha enzyme have been associated with different degrees of risk for prostate cancer (3–5). E-cadherin is a 120 000 MW glycoprotein that plays a critical role in many aspects of cell adhesion, epithelial development and the establishment and maintenance of epithelial polarity (6). Loss of E-cadherin expression and the subsequent loss of homotypic cellular adhesiveness may be a critical step allowing epithelial tumor cells to invade and metastasize. E-cadherin expression is decreased or absent in poorly differentiated carcinomas of the breast, prostate and many other tumors (7,8). In prostate cancer, it is possible that the loss of E-cadherin is related to tumor aggressiveness (8).

The association between E-cadherin abnormality and tumorigenesis has been previously reported. Guilford et al. (9) reported germline mutations in the E-cadherin gene in three familial gastric cancer kindreds of New Zealand Maori. Germline mutation of the E-cadherin gene has also been implicated in the pathogenesis of sporadic colorectal cancers (10,11). There is a polymorphism (an A to C substitution) at −160 from the transcriptional start site of the E-cadherin gene promoter that might influence transcriptional efficacy (12). The present study was conducted to explore the association of this polymorphism of the E-cadherin gene with prostate cancer risk.
in Japanese men. In addition, we set up a group of men with apparent benign prostatic hyperplasia (BPH), and another group of older men without any evidence of prostate cancer or BPH.

SUBJECTS AND METHODS

SUBJECTS

All subjects included in the study were unrelated Japanese. A total of 584 subjects with appropriate informed consent were studied. The subjects consisted of 236 prostate cancer patients who were diagnosed from 1995 to 2001, 209 BPH patients and 139 male controls who were recruited at Tokyo University Hospital and Akita University Medical Center. All of the prostate cancer patients were diagnosed histologically with specimens obtained from transrectal needle biopsy or transurethral resection of the prostate for voiding symptoms. All of the BPH patients had various degrees of lower urinary tract symptoms and an apparent prostatic enlargement by digital rectal examination. Serum PSA levels were measured in all BPH patients, as well as in men with elevated PSA levels (\( \geq 4.0 \) ng/ml by the Tandem-R assay; Hybritech Inc., San Diego, CA) who were shown not to have prostate cancer by transurethral resectant biopsies. The male control group comprised 139 volunteers over 65 years old without any voiding symptoms. They were mainly selected from patients admitted to several community hospitals because of various non-urological diseases. They all had normal serum PSA levels (<4.0 ng/ml by the Tandem-R assay), showed no signs of prostate cancer and had no prostatic enlargement by digital rectal examination. Serum PSA levels were measured using the Tandem-R assay in most cases. When serum PSA levels were measured by kits other than the Tandem-R, the measured PSA level was adjusted to that of the Tandem-R assay using a formula published by Kuriyama et al. (13). The mean ages (\( \pm SD \)) of prostate cancer patients, BPH patients and male controls were 72.2 \( \pm \) 7.9, 69.8 \( \pm \) 8.8 and 74.7 \( \pm \) 6.9 years, respectively. There was no statistically significant difference in the mean age between these groups by unpaired two-tailed \( t \) test.

HISTOPATHOLOGICAL GRADING

Pathological grading of the prostate cancers was determined according to the General Rule for Clinical and Pathological Studies of Prostate Cancer by the Japanese Urological Association and the Japanese Society of Pathology, which is based on the WHO criteria and the Gleason pattern (14). A pathologist (S.M.) reviewed prostate cancer specimens in order to determine Gleason Grade and WHO criteria. The clinical stage was determined by review of the medical records and was classified using the Whitmore–Jewett system. In localized cancer (stages A and B), the allele distributions of A/A, C/A and C/C in cases from Akita University were 1, 7 and 34 cases, respectively, and those from Kyoto was 4, 12 and 31 cases, respectively. In invasive cancer (stages C and D), the distributions were 1, 16 and 40, and 4, 29 and 39 cases from Akita and Kyoto, respectively. There was not enough information on 18 patients with prostate cancer who did not have their clinical stage determined and thus they were excluded from the analysis.

GENOTYPING OF E-CADHERIN GENE PROMOTER POLYMORPHISMS

DNA was extracted from blood samples collected from each subject using a QIAamp Blood Kit (QIAGEN, Germany) or by the standard method with proteinase K digestion followed by phenol/chloroform extraction. There is a polymorphism (an A to C substitution) at –160 from the transcriptional start site of the E-cadherin gene promoter. The A allele created an AflIII site and the C allele created a HphiI site. The 452 bp fragment encompassing either the AflIII or HphiI polymorphic site in the promoter region of the E-cadherin gene was amplified using the primers 5'-TTCTGATCCAGGTCTTGTGGAGC-3' and 5'-GGTACCCTGACGACCAGGAC-3'. The 30 µl PCR mixture contained 1 µl of genomic DNA as a template, 10× PCR buffer (3 µl), 2.5 mM MgCl₂ (1.8 µl), 200 µM each dNTPs, 15 pmol of each primer and 0.2 U of AmpliTaq polymerase (Roche Molecular Systems). Thirty-three cycles of PCR were performed, with each cycle consisting of 94°C for 50 s, 65°C for 40 s and 72°C for 40 s. The 452 bp fragment was divided into 334 and 118 bp with AflIII digestion, or into PCR products digested for at least 2 h with either HphiI or AflIII. The digestion reactions were fractioned on a 2% agarose gel. The genotypes were designated as ‘A’ when the AflIII restriction site was present and the HphiI site was absent, and as ‘C’ when the AflIII site was absent and the HphiI site was present (Fig. 1).

STATISTICAL METHODS

Associations between disease and genotype were assessed by calculating odds ratios (ORs) and 95% confidence intervals.
Eighteen patients were not informative and were therefore excluded.

<table>
<thead>
<tr>
<th></th>
<th>E-cadherin genotype</th>
<th>Age-adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/A</td>
<td>A/C</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>11 (4.7)</td>
<td>71 (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage A.B*</td>
<td>5 (5.6)</td>
<td>19 (21.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage C,D*</td>
<td>5 (3.9)</td>
<td>45 (34.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>6 (2.9)</td>
<td>56 (26.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male control</td>
<td>5 (3.6)</td>
<td>29 (20.9)</td>
</tr>
</tbody>
</table>

*Eighteen patients were not informative and were therefore excluded. OR, odds ratio; CI, confidence interval.

The E-cadherin gene polymorphism was further analyzed to clarify the influence of the A allele by dividing it into two groups, i.e. the C/C genotype or the C/A+A/A genotypes. Because there was an extremely small frequency of A/A, we divided these groups according to the presence of the A allele. To adjust for age, multivariate logistic regression analysis was performed for each polymorphism alone. A marginally significant difference was found in prostate cancer patients and male controls ($P = 0.053$). No significant differences were observed between the prostate cancer and BPH patients. When we divided patients with prostate cancer into two groups, stage A+B and stage C+D, a significant difference was observed between progressive cancer (stage C+D) and male controls ($P = 0.016$). It is possible that having one A allele resulted in an increased risk of cancer progression (Table 1). We examined the distribution of E-cadherin promoter genotypes in prostate cancer patients by Gleason score (GS). The frequency of the genotype C/C was 75% in those with a GS $<6$, 56.6% with a GS = 7 and 59.1% with a GS $>7$. There was no statistically significant difference among these GS groupings. Indeed, in the prostate cancer patients, the frequency of at least one A allele being present was higher in a GS $>9$ compared with a GS $<6$ (48.9% versus 25%; $P = 0.038$).

**DISCUSSION**

The E-cadherin gene has been identified as having a physiological role in cellular attachment, and several investigators have hypothesized that this gene participates in carcinogenesis (9,10,15). Abnormalities in the expression and cellular localization of E-cadherin are frequently associated with high tumor grading, infiltrative growth, and lymph node metastasis in a variety of human malignancies (7,8,16,17). For patients with prostate cancer, it is now well documented that decreased expression of E-cadherin is associated with a poor prognosis (8). The biological functional relationship between the loss of E-cadherin expression and the acquisition of invasive behavior has been described in several reports. Moreover, the restoration of an epithelial phenotype and a concomitant reduction in invasiveness after DNA-mediated transfection of E-cadherin has been reported by several investigators (18,19). These results suggest that E-cadherin gene abnormalities participate in several human malignancies, including carcinoma of the prostate. At present, it is not clear that the single nucleotide polymorphism (SNP) of this gene determines susceptibility to developing carcinomas as a common human disease.

Recently, the association of genetic polymorphism of the E-cadherin gene with prostate cancer has been reported (20–22). Verhage et al. (20) reported that the A-allele frequencies among 82 low grade prostate cancer cases consisted of 52 sporadic cases, and 25 familial cases and 188 controls were 39.0% and 24.7%, respectively. Thus, in their study population, A-allele carriers had an increased risk of prostate cancer, which was statistically significant (OR = 3.6; 95% CI 2.0–6.4). In our present study, the frequencies of the A allele were 34.7% in prostate cancer, 29.7% in BPH and 24.5% in male controls. Although our age-adjusted data did not support an association of the A allele with prostate cancer, we cannot exclude the possibility of a relationship between prostate cancer and this polymorphism.

On the other hand, however, we did find a significant association between the E-cadherin genotype and disease status in prostate cancer patients. Considering the function of E-cadherin intracellular attachment, it seems natural that the SNP of the E-cadherin gene promoter correlates with tumor invasiveness rather than tumorigenesis. In this study, there was a statistically significant difference between high stage prostate cancer (stage C+D) and male controls. It is possible that the presence of one A allele resulted in an increased risk of prostate cancer progression. Tsukino et al. (22) have recently reported that this E-cadherin polymorphism is not related to the incidence and progression of prostate cancer in the Japanese population. These previous results differ from our study. One of the reasons for this discrepancy might be a
difference in the setting of the control group. Tsukino et al. set the control as the age-matched general population. This may have included a number of people who had latent prostate cancer. On the other hand, in our study we set up two control groups. One group was a normal population without BPH with a serum PSA value of <4 ng/ml, while the other was those with BPH. In addition, the male volunteer controls were examined by both PSA test and digital rectal examination to confirm the absence of detectable prostate cancer.

Factors that affect susceptibility to prostate cancer include genetic factors, diet and environmental factors. There are many reports about the differences in prevalence of prostate cancer. Because there is so much variance in the prevalence of prostate cancer between races, especially Asians including Japanese and Caucasians, further studies on different racial groups with different prevalences of clinical prostate cancer are required to establish the association of $E$-cadherin gene polymorphism.

This genetic polymorphism seems to be correlated with a risk of developing prostate cancer. The polymorphism of the $E$-cadherin gene can then not only identify those at high risk for prostate cancer, but may become a marker to determine the clinical significance of the disease.

References