論文の内容の要旨

論文題目：Significance of Common Variants on Human Chromosome 8q24 in Relation to the Risk of Prostate Cancer in Native Japanese Men

和訳：（染色体 8q24 上の一塩基多型と日本人前立腺癌発症リスクについて）

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Introduction

The incidence of prostate cancer is widely known to be much lower in Asian men than in Western men. Recently, genome-wide association studies have revealed a close relation between variants on human chromosome 8q24 and the risk of prostate cancer. In 2006, Amundadottir et al. first identified a region on chromosome 8q24 that was possibly linked to prostate cancer in Icelandic men. Subsequently, Freedman et al. confirmed an association between rs1447295 and the risk of prostate cancer in another ethnic cohort study. In yet another study, the prevalence of the rs1447295 polymorphism associated with prostate cancer was investigated in a population of Indian-born Asian Indians who had emigrated from India and were living in the United States. In 2007, Haiman et al. and Yeager et al. showed a strong association

between rs6983267 and the risk of prostate cancer. Their investigations included African Americans, Latino Americans, European Americans, Japanese Americans, native Hawaiians, Australians, Swedish, Icelanders, and the British, but not native Japanese or other East Asian men. Thus, we felt that it was important to replicate the study in a population of native Japanese subjects to better understand the disparities in prostate cancer risk among different ethnicities.

The characteristics of genetic polymorphisms among patients with latent prostate cancer (LPCa) diagnosed at the time of autopsy remain unknown. Stamey et al. first proposed the pathologic entity of clinically diagnosed latent prostate cancers, which are defined as clinically insignificant cancers. Strictly speaking, 'insignificant prostate cancer' is not the same as 'LPCa', and a comparative genetic analysis of clinically diagnosed sporadic prostate cancer (SPCa) and LPCa might be useful for a better understanding of the nature of this disease. From this viewpoint, we also included LPCa subjects in the present study to examine the genetic differences between SPCa and LPCa patients.

Purpose

The aim of our study was to investigate polymorphisms on human chromosome 8q24 whether they could be used as a gene marker for determining the risk of sporadic or latent cancer in native Japanese men.

Methods

Study design

A total of 391 Japanese patients with SPCa who were treated at the Department of Urology at the University of Tokyo Hospital or at our affiliated hospitals located in the Kanto area of Japan between January 1999 and August 2007 were enrolled. One hundred twenty-six cases underwent a radical prostatectomy and the remaining cases were treated with androgen-deprivation therapy. Adenocarcinoma of the prostate was pathologically confirmed in all the cases, and the Gleason score was also evaluated by pathologists working at each hospital. The clinical T stage of the patients with SPCa was evaluated based on a digital rectal examination, transrectal ultrasonography, pelvic computed tomography, and pathological findings according to the 2002 TNM staging system for cancer. The serum prostate specific antigen (PSA) levels at diagnosis were also measured. Patients with a family history of prostate cancer were carefully excluded from this study. The mean age of the SPCa patients was 70.7 ± 8.0 years (median, 71 years; range, 48 to 89 years). Genomic DNA samples were extracted from peripheral blood specimens of these patients. The study was conducted with the approval of the Ethics Committee of the University of Tokyo and the internal review board of each of the affiliated hospitals. Written informed consent was obtained from each patient prior to their enrollment in the study. We also examined 323 residence-matched Japanese men as a control group. The mean age of the patients in the control group was 79.2 ± 9.2 years (median, 79 years; range, 49 to 100 years). All the patients in the control group had died at the Tokyo Metropolitan Geriatric

Hospital and were consecutively autopsied. All the control patients were pathologically confirmed to not have suffered from any malignancy. Moreover, we examined 112 Japanese men who were diagnosed as having LPCa at the time of autopsy. The mean age of the LPCa patients was 81.9 ± 7.6 years (median, 81 years; range, 66 to 98 years). These patients had been registered in the database of Japanese single nucleotide polymorphisms for geriatric research (JG-SNP). Genomic DNA samples from the controls and LPCa cases were extracted from frozen kidney tissues. Written informed consent was obtained from the patients' family members under the Act of Postmortem Examination. This study was also reviewed and approved by the Ethics Committee of the Tokyo Metropolitan Geriatric Hospital. Moreover, this study was carried out in compliance with the Helsinki Declaration.

Genotyping assay

Genotyping of the rs1447295 and rs6983267 polymorphisms was conducted using a TaqMan assay according to the manufacturer's instructions. We adjusted the concentration of the DNA solution to 100 ng/μL before using it. The sequences of the specific primers and TaqMan probes and the conditions for quantitative real-time polymerase chain reaction were obtained from the website of the National Cancer Institute (http://snp500cancer.nci.nih.gov/snp.cfm). All volume of the standard PCR reaction mix was 25 μL reactions containing 25 ng of genomic DNA, 12.5 μ L of 2× Taqman universal PCR master mix, 0.3 μ L of 20×Taqman SNP Genotyping Assays mix. Isolated DNA was incubated with two flanking primers (forward and reverse) for amplification of the sequence of interest and two TaqMan probes for detecting

specific alleles containing a fluorescent reporter dye (VIC and FAM) at the 5' end of each allele specific probe and non-fluorescent quencher at the 3' end of the probe. The real-time PCR analysis was performed using TaqMan Universal Master Mix and purified in 96-well optical plate using ABI PRISM 7000 or 7300 sequence detection system. PCR amplification conditions were: 2 min at 50℃, and 95℃ for 10min, followed by 49 cycles at 92℃ for 30 sec, 60℃ for 1 min. Quantitative real-time PCR was done using ABI Prism 7000 or 7300 Sequence Detection Systems.

Statistical analysis

All the statistical analyses were conducted using JMP software, version 7.0 (SAS, Cary, NC, USA). The chi-square test was used to examine Hardy-Weinberg equilibrium (HWE) and to compare the distribution of the genotypes and alleles among the control, SPCa and LPCa patients. To estimate the odds ratios (ORs) and 95% confidence intervals (CIs), logistic regression analyses were performed using age as a covariate to statistically adjust for its potential confounding effects. Additionally, we estimated ORs and 95%CIs after stratification according to the Gleason score, T-stage, and serum PSA levels at diagnosis. P values less than 0.05 were considered significant.

Results

The age distributions of the control, SPCa, and LPCa groups were significantly different (p <0.01). The T-stage distributions in the SPCa and LPCa groups were also significantly different (p <0.01). The Gleason score distributions in the SPCa and

LPCa groups were not significantly different (p = 0.49). Genotyping assays were successfully performed in all the subjects. The genotype distributions for each SNP were consistent with HWE.

Compared with the major allele homozygous genotype as a reference, both the CA genotype of rs1447295 (p = 0.02; age-adjusted OR, 1.54; 95% CI, 1.08 - 2.21) and the GG genotype of rs6983267 ($p = 7.0\times10^{-3}$; age-adjusted OR, 2.21; 95% CI, 1.24 - 4.03) were significantly associated with the risk of SPCa. Moreover, the CA + AA genotypes of rs1447295 was also significantly associated with the risk of SPCa (p = 0.02; age-adjusted OR, 1.50; 95% CI, 1.07 - 2.11). In allele-wise analyses, the A allele of rs1447295 was significantly associated with the risk of prostate cancer (p = 0.04; age-adjusted OR, 1.34; 95% CI, 1.01 - 1.79), while the G allele of rs6983267 showed a tendency towards an increase in the risk of prostate cancer (p = 0.06; age-adjusted OR, 1.27; 95% CI, 0.99 - 1.62). One hundred and twelve patients among 1,179 autopsied men were diagnosed as having LPCa (9.5%). No significant differences were observed between the control and the LPCa patients.

After stratification according to the Gleason score, T-stage, and PSA levels at the time of diagnosis, the frequencies of the CA and CA+AA genotypes of rs1447295 were observed significantly higher in SPCa patients with a Gleason score ≤7, a T-stage of T3 or T4, and a serum PSA level ≥20 ng/mL; meanwhile, the frequency of the GG genotype of rs6983267 was observed significantly higher in SPCa patients with a Gleason score ≥8, a T-stage of T1 or T2, and a serum PSA level ≥20 ng/mL. We also investigated a combined model examining the joint effect of both rs1447295

and rs6983267 on the prostate cancer risk. Individuals with SPCa who carried both the CA (rs1447295) and the GG (rs6983267) genotypes had a significantly higher risk of prostate cancer (p = 0.03; age-adjusted OR, 2.74; 95% CI, 1.13 - 7.17).

Conclusions

The present study demonstrates that polymorphisms on 8q24 are associated with the occurrence of prostate cancer in the native Japanese population. Our results confirm that these polymorphisms are closely related to the risk of SPCa; however, we did not observe an association between these polymorphisms and the risk of LPCa.