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## Significance of common variants on human chromosome 8q24 in relation to the risk of prostate cancer in native Japanese men

Miao Liu<sup>1</sup>, Takayuki Kurosaki<sup>1</sup>, Motofumi Suzuki\*<sup>1</sup>, Yutaka Enomoto<sup>1</sup>, Hiroaki Nishimatsu<sup>1</sup>, Tomio Arai<sup>2</sup>, Motoji Sawabe<sup>2</sup>, Takayuki Hosoi<sup>3</sup>, Yukio Homma<sup>1</sup> and Tadaichi Kitamura<sup>1,4</sup>

Address: <sup>1</sup>Department of Urology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan, <sup>2</sup>Department of Pathology, Tokyo Metropolitan Geriatric Hospital, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan, <sup>3</sup>Department of Advanced Medicine, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka-cho, Ohbu, Aichi 474-8511, Japan and <sup>4</sup>Department of Urology, Asoka Hospital, 1-18-1 Sumiyoshi, Koutou-ku, Tokyo 135-0002, Japan

Email: Miao Liu - hndfh20012003@yahoo.co.jp; Takayuki Kurosaki - kurosan@sb3.so-net.ne.jp; Motofumi Suzuki\* - SUZUKIM-URO@h.u-tokyo.ac.jp; Yutaka Enomoto - yenomoto-ty@umin.ac.jp; Hiroaki Nishimatsu - nishimatsu-uro@h.u-tokyo.ac.jp; Tomio Arai - arai@tmig.or.jp; Motoji Sawabe - sawabe@tmig.or.jp; Takayuki Hosoi - hosoworld@cb3.so-net.ne.jp; Yukio Homma - homma-uro@umin.ac.jp; Tadaichi Kitamura - tadkitamura-ty@umin.ac.jp

\* Corresponding author

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### Abstract

**Background:** Common variants on human chromosome 8q24, rs1447295 (C/A) and rs6983267 (T/G), have been recently linked to the prevalence of prostate cancer in European and American populations. Here, we evaluated whether the single-nucleotide polymorphisms rs1447295 and rs6983267 were associated with the risk of sporadic prostate cancer as well as latent prostate cancer in a native Japanese population.

**Results:** We analyzed genomic DNA samples from 391 sporadic prostate cancer patients, 323 controls who had died from causes unrelated to cancer and 112 Japanese men who were diagnosed as having latent prostate cancer based on autopsy results. The polymorphisms were determined by allelic discrimination using a fluorescent-based TaqMan assay. The A allele of rs1447295 was significantly associated with the risk of sporadic prostate cancer ( $p = 0.04$ ; age-adjusted OR, 1.34), while the G allele of rs6983267 showed a trend towards being a high-risk allele ( $p = 0.06$ ; age-adjusted OR, 1.27). No significant difference between these two polymorphisms and the risk of latent prostate cancer was observed in the present Japanese population.

**Conclusion:** Known variants on human chromosome 8q24 may be risk factors for sporadic prostate cancer in native Japanese men.

### Background

The incidence of prostate cancer is widely known to be much lower in Asian men than in Western men [1]. Recently, genome-wide association studies have revealed a close relation between variants on human chromosome

8q24 and the risk of prostate cancer [2-13]. In 2006, Amundadottir et al. [2] first identified a region on chromosome 8q24 that was possibly linked to prostate cancer in Icelandic men. Subsequently, Freedman et al. [3] 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 [14]. In 2007, Haiman et al. [8] and Yeager et al. [9] 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 germline genetic polymorphisms among patients with latent prostate cancer diagnosed at the time of autopsy (LPCa) remain unknown. Stamey et al. first proposed the pathologic entity of clinically diagnosed latent prostate cancers, which are defined as clinically insignificant cancers [15]. 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.

## Results

The characteristics of the control, SPCa, and LPCa groups are shown in Table 1. 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. Table 2 shows the distributions of the genotypes and alleles of the rs1447295 and rs6983267 polymorphisms. The genotype distributions for each SNP were consistent with Hardy-Weinberg equilibrium (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 ( $p = 7.0 \times 10^{-3}$ ; 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 (Table 3).

After stratification according to the Gleason score, T-stage, and serum prostate specific antigen (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  $\leq 7$ , a T-stage of T3 or T4, and a serum PSA level  $\geq 20$  ng/mL; meanwhile, the frequency of the GG genotype of rs6983267 was observed significantly higher in SPCa patients with a Gleason score  $\geq 8$ , a T-stage of T1 or T2, and a serum PSA

**Table 1: Characteristics of control, SPCa, and LPCa**

		Control (N = 323)	SPCa (N = 391)	LPCa (N = 112)	p value
Age	median	79	71	81	< 0.01
	range	49 – 100	48 – 89	66 – 98	
T-stage	T1 or T2		167	102	< 0.01
	T3 or T4		224	5	
	unknown		0	5	
Gleason score	$\leq 7$		241 (92)*	62	0.49
	$\geq 8$		150 (34)*	45	
	unknown		0	5	

\*Numbers of patients with SPCa who were undergone radical prostatectomy are described in the parenthesis.

—, not applicable.

**Table 2: Genotypic and allelic frequencies of rs1447295 and rs6983267 polymorphisms**

Genotype	Control (%) (N = 323)	SPCa (%) (N = 391)	LPCa (%) (N = 112)
<b>rs1447295</b>			
CC	218 (67.5)	217 (55.5)	71 (63.4)
CA	89 (27.5)	149 (38.1)	34 (30.4)
AA	16 (5.0)	25 (6.4)	7 (6.2)
<b>rs6983267</b>			
TT	147 (45.5)	151 (38.6)	48 (42.9)
TG	151 (46.8)	181 (46.3)	53 (47.3)
GG	25 (7.7)	59 (15.1)	11 (9.8)
<b>Allele</b>			
	Control (%) (2N = 646)	SPCa (%) (2N = 782)	LPCa (%) (2N = 224)
<b>rs1447295</b>			
C	525 (81.3)	583 (74.6)	176 (78.6)
A	121 (18.7)	199 (25.4)	48 (21.4)
HWE			
chi-square	2.91	0.01	1.09
p value	0.09	0.93	0.30
<b>rs6983267</b>			
T	445 (68.9)	483 (61.8)	149 (66.5)
G	201 (31.1)	299 (38.2)	75 (33.5)
HWE			
chi-square	2.65	0.16	0.44
p value	0.10	0.69	0.51

HWE, Hardy-Weinberg equilibrium.

level  $\geq 20$  ng/mL (Table 4). 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) (Table 5).

## Discussion

The present case-controlled study investigated the relation between variants on human chromosome 8q24 and the risk of sporadic and latent prostate cancer in native Japanese men. We confirmed that the rs1447295 and rs6983267 polymorphisms were significantly associated with the risk of sporadic prostate cancer in native Japanese men. Table 6 shows the results of allele-wise analyses for prostate cancer susceptibility in multi-ethnic studies. The frequencies of the risk alleles and the magnitudes of the effects found in our study were very similar to those previously reported for Japanese Americans [3,8]. Neither rs1447295 nor rs6983267 was associated with the risk of prostate cancer in African Americans; however, positive associations with other genetic markers on chromosome 8, such as DG8S737 [2,8], rs16901979 [7,11], rs13254738, rs6983561, broad11934905, rs7000448 [8],

and rs7008482 [11], were also reported in these subjects. Whether these markers might also be valid for native Japanese men should be confirmed in future studies. In a genome-wide association study in subjects of European ancestry [9], the ORs of the CA and AA genotypes of rs1447295 were 1.43 and 2.23, respectively. In our results, patients with the CA genotype of rs1447295 were more likely to have prostate cancer ( $p = 0.02$ ; age-adjusted OR, 1.54; 95% CI, 1.08–2.21); however, the AA genotype was not significantly associated with prostate cancer in the present study ( $p = 0.50$ ; age-adjusted OR, 1.50; 95% CI, 0.62–2.73). In addition, the CA+AA genotypes of rs1447295 were observed more frequently in the SPCa group ( $p = 0.02$ ; age-adjusted OR, 1.50; 95% CI, 1.07–2.11), similar to the results of a previous study conducted in Australian men [4]. As for the rs6983267 polymorphism, we could not find any significant association between the G allele and SPCa ( $p = 0.06$ ; age-adjusted OR, 1.27, 95% CI: 0.99–1.62), although Haiman et al. [8] reported a significant association between these two factors (OR, 1.22; 95%CI, 1.05–1.42) in a study sample of 1,450 Japanese-Americans (722 patients with SPCa and 728 controls). The disparity between the results of their report and ours might have been caused by the different sample sizes.

In 2008, Terada et al. [16] initially studied 507 patients with prostate cancer and 511 controls to determine the association between these common variants on chromosome 8q24 and the risk of prostate cancer in a native Japanese population. For their Japanese subjects, the A allele of rs1447295 was significantly associated with the risk of prostate cancer, while the G allele of rs6983267 was associated with the risk of prostatic enlargement rather than prostate cancer. Their findings for rs6983267 differed from the results reported by Haiman et al. regarding the risk of prostate cancer in Japanese Americans [8]. Terada et al. adopted healthy male volunteers or patients of benign disease as control, while our controls were pathologically confirmed to be the cases which have not suffered from any malignancy by autopsy. We could not read from their report whether they had surveyed of controls by a PSA test or physical examination to exclude prostate cancer subjects. We worried that they had a potential chance including prostate cancer subjects among controls and patients with benign prostatic hyperplasia. We think careful setting of controls is a critical issue that interferes with the results. During the course of our experiment, we also observed a relation between the aggressiveness of SPCa and these two SNPs. Thus, we investigated possible interactions between these two independent SNPs. Our results revealed a significant OR only in patients carrying both the CA genotype of rs1447295 and the GG genotype of rs6983267. While this result differed from that reported by Yeager et al. [9] in a European population, the magnitude of the effect in our study was higher (age-adjusted

**Table 3: Genotypic and allelic analyses of prostate cancer susceptibility**

Genotype	Control vs. SPCa			Control vs. LPCa		
	OR*	95% CI	p value	OR*	95% CI	p value
rs1447295						
CC	1.0	reference		1.0	reference	
CA	1.54	1.08 – 2.21	0.02	1.22	0.75 – 1.98	0.42
AA	1.29	0.62 – 2.73	0.50	1.45	0.53 – 3.59	0.45
CA + AA	1.50	1.07 – 2.11	0.02	1.25	0.79 – 1.97	0.33
rs6983267						
TT	1.0	reference		1.0	reference	
TG	0.95	0.67 – 1.35	0.78	1.17	0.74 – 1.86	0.50
GG	2.21	1.24 – 4.03	7.0 × 10 <sup>-3</sup>	1.40	0.62 – 3.03	0.40
TG + GG	1.12	0.80 – 1.57	0.50	1.21	0.78 – 1.88	0.41
Allele	OR*	95% CI	p value	OR*	95% CI	p value
rs1447295						
C	1.0	reference		1.0	reference	
A	1.34	1.01 – 1.79	0.04	1.23	0.84 – 1.79	0.29
rs6983267						
T	1.0	reference		1.0	reference	
G	1.27	0.99 – 1.62	0.06	1.17	0.84 – 1.62	0.35

OR\*, Age-adjusted odds ratio.

OR, 2.74; 95% CI, 1.13–7.17). The accumulation of samples in collaboration with various international centers would be indispensable for the further study of such interactions.

The functional features of these polymorphisms are still unclear. The closest characterized gene is the proto-oncogene *c-MYC*, located approximately 264 kb from rs1447295 [2,6]. Sato et al. [17] demonstrated that patients with additional increases in the *c-MYC* copy number relative to the chromosome 8 centromere showed a more rapid disease progression and earlier death from cancer. Thus, clarifying the relation between *c-MYC* amplification and the risk alleles among the polymorphic markers located in 8q24 is a crucial task.

To the best of our knowledge, no study to date has examined common variants on human chromosome 8 among subjects with LPCa. Our results suggested that the rs1447295 and rs6983267 polymorphisms were not associated with the risk of LPCa in native Japanese men. The frequencies of each genotype in these subjects and the controls were quite similar. We believe that such genetic research in subjects with LPCa may be useful for clarifying the genetic characteristics of clinically insignificant prostate cancer.

In Japan, the age-adjusted incidence rate of prostate cancer was determined to be 26.2 per 100,000 in 2001 [18], while that in Japanese Americans living in California was determined to be 103.7 per 100,000 (2000 to 2002) [1].

Denis et al. [19] suggested that low intakes of vitamin E, selenium, lignans and isoflavonoids affected the tumorigenesis risk of prostate cancer. Marks et al. [20] reported that Japanese Americans had more body fat, higher serum triglyceride levels, lower estradiol levels, and much lower soy-metabolite levels than native Japanese. Additionally, Bettuzzi et al. [21] and Kurahashi et al. [22] emphasized the protective effects of green tea constituents against the risk of prostate cancer. These findings suggest that we need to consider genetic and environmental factors, as well as nutrition, in our attempts to prevent prostate cancer.

This study had several limitations. First, our sample size was relatively small, and this might have influenced the robustness of the results. Further study using larger samples for both the case and control groups is necessary. Secondary, several pathologists at the affiliated hospitals had participated in the diagnosis of prostate cancer. Therefore, we cannot deny the inter-observer variability existed. Moreover, the data of Gleason score was underestimated, because two thirds of the pathological data from SPCa patients were obtained by biopsy.

Little is known about why the prevalence of prostate cancer is much lower in Asian populations than in other ethnicities. Researchers from Asia need to collaborate in addressing this issue.

**Conclusion**

The present study demonstrates that polymorphisms on 8q24 are associated with the occurrence of prostate cancer

**Table 4: Prostate cancer susceptibility stratified by Gleason score, T-stage, and serum PSA levels at diagnosis (control vs. SPCa)**

Genotype	Gleason score ≤ 7 (N = 241)			Gleason score ≥ 8 (N = 150)		
	OR*	95% CI	p value	OR*	95% CI	p value
rs1447295						
CC	1.0	reference		1.0	reference	
CA	1.62	1.08 – 2.42	0.02	1.48	0.94 – 2.34	0.09
AA	1.09	0.46 – 2.56	0.84	1.63	0.66 – 3.92	0.29
CA + AA	1.53	1.04 – 2.25	0.03	1.50	0.97 – 2.32	0.07
rs6983267						
TT	1.0	reference		1.0	reference	
TG	0.93	0.62 – 1.39	0.73	0.90	0.57 – 1.42	0.66
GG	1.84	0.96 – 3.55	0.07	2.41	1.20 – 4.86	0.01
TG + GG	1.05	0.72 – 1.55	0.79	1.10	0.72 – 1.69	0.67
Genotype	T1 or T2 (N = 167)			T3 or T4 (N = 224)		
	OR*	95% CI	p value	OR*	95% CI	p value
rs1447295						
CC	1.0	reference		1.0	reference	
CA	1.39	0.88 – 2.17	0.15	1.70	1.14 – 2.56	0.01
AA	1.34	0.53 – 3.29	0.53	1.28	0.55 – 2.95	0.57
CA + AA	1.38	0.90 – 2.11	0.14	1.64	1.11 – 2.41	0.01
rs6983267						
TT	1.0	reference		1.0	reference	
TG	0.96	0.61 – 1.51	0.87	0.91	0.61 – 1.36	0.65
GG	2.68	1.37 – 5.30	4.0 × 10 <sup>-3</sup>	1.69	0.87 – 3.31	0.12
TG + GG	1.19	0.78 – 1.83	0.42	1.02	0.69 – 1.49	0.93
Genotype	PSA < 20 ng/mL (N = 208)			PSA ≥ 20 ng/mL (N = 183)		
	OR*	95% CI	p value	OR*	95% CI	p value
rs1447295						
CC	1.0	reference		1.0	reference	
CA	1.46	0.95 – 2.23	0.08	1.71	1.12 – 2.60	0.01
AA	0.97	0.37 – 2.44	0.95	1.69	0.74 – 3.83	0.21
CA + AA	1.38	0.92 – 2.08	0.12	1.70	1.14 – 2.54	9.0 × 10 <sup>-3</sup>
rs6983267						
TT	1.0	reference		1.0	reference	
TG	0.96	0.63 – 1.47	0.86	0.92	0.60 – 1.40	0.69
GG	1.46	0.72 – 2.96	0.30	2.74	1.45 – 5.24	2.0 × 10 <sup>-3</sup>
TG + GG	1.03	0.69 – 1.55	0.88	1.16	0.78 – 1.73	0.47
Genotype	Nonaggressive (N = 99)			Aggressive <sup>a</sup> (N = 292)		
	OR*	95% CI	p value	OR*	95% CI	p value
rs1447295						
CC	1.0	reference		1.0	reference	
CA	1.69	0.98 – 2.91	0.06	1.54	1.06 – 2.25	0.02
AA	1.39	0.44 – 3.99	0.56	1.28	0.59 – 2.81	0.53
CA + AA	1.64	0.97 – 2.75	0.06	1.50	1.05 – 2.15	0.03
rs6983267						
TT	1.0	reference		1.0	reference	
TG	0.99	0.58 – 1.71	0.97	0.93	0.64 – 1.36	0.72
GG	1.45	0.58 – 3.46	0.42	2.32	1.29 – 4.26	0.01
TG + GG	1.05	0.63 – 1.78	0.85	1.12	0.79 – 1.60	0.52

OR\*, Age-adjusted odds ratio.

<sup>a</sup> Aggressive, Gleason score ≥ 8, and/or T-stage T3 or T4, and/or PSA ≥ 20 ng/mL.

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. One limitation of our study was that the number of our samples was relatively small; consequently, studies with larger populations and family-based analyses are needed in the future.

**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 [23]. The serum 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 [24]. 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) [25]. Genomic DNA samples from the controls and LPCa cases were extracted from frozen kidney tissues. The T stage of the patients with LPCa was evaluated based on pathological findings according to the 2002 TNM staging system for cancer [23]. 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 with an ABI PRISM 7000 or 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. We adjusted the concentration of the DNA solution to 100 ng/mL 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>.

**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, as just as Terada et al. reported [16]. P values less than 0.05 were considered significant.

**Abbreviations**

LPCa: latent prostate cancer diagnosed at the time of autopsy; SPCa: clinically diagnosed sporadic prostate cancer; HWE: Hardy-Weinberg equilibrium; PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval.

**Authors' contributions**

ML carried out the genotyping assay, data analysis, and drafted the manuscript. TK1 carried out the genotyping

**Table 5: Combined analysis with two polymorphisms**

		rs6983267		
		TT	TG	GG
rs1447295	OR*	1.0	0.78	1.69
	95% CI	reference	0.50 – 1.23	0.78 – 3.75
CC	OR*	1.07	1.33	2.74
	95% CI	0.58 – 1.94	0.77 – 2.29	1.13 – 7.17
CA	OR*	0.54	1.34	—
	95% CI	0.15 – 1.81	0.42 – 4.48	—
AA	95% CI	—	—	—

OR\*, Age-adjusted odds ratio.  
—, not applicable.

**Table 6: Allele-wise analyses for prostate cancer risk in the multiethnic studies**

rs1447295 A allele frequency							
Author (Year)	N cases/N controls	Study population	Cases	Controls	OR	95% CI	p value
Amundadottir, et al. (2006)	1291/997	Iceland	0.17	0.11	1.72	—	1.7 × 10 <sup>-9</sup>
	1435/779	Sweden	0.16	0.13	1.29	—	4.5 × 10 <sup>-3</sup>
	458/247	European Americans	0.13	0.08	1.66	—	6.7 × 10 <sup>-3</sup>
	246/352	African Americans	0.34	0.31	1.15	—	0.29
Freedman, et al. (2006)	70/68	Native Hawaiians	0.37	0.16	3.02	1.66 – 5.50	1.5 × 10 <sup>-4</sup>
	449/465	Japanese Americans	0.24	0.17	1.48	1.18 – 1.86	3.4 × 10 <sup>-4</sup>
	640/567	Latino Americans	0.14	0.10	1.48	1.14 – 1.91	1.4 × 10 <sup>-3</sup>
	455/447	European Americans	0.13	0.10	1.35	1.01 – 1.80	0.02
Wang, et al. (2007)	989/804	African Americans	—	—	1.05	0.95 – 1.16	0.15
	491/545	Caucasians	0.12	0.10	1.16	0.85 – 1.58	0.25
Gudmundsson, et al. (2007)	435/545	Caucasians*	0.17	0.10	1.93	1.37 – 2.72	4.0 × 10 <sup>-3</sup>
	1453/3064	Iceland	0.17	0.10	1.71	1.49 – 1.95	1.6 × 10 <sup>-14</sup>
	385/892	Spain	0.10	0.07	1.44	1.07 – 1.94	1.7 × 10 <sup>-2</sup>
Haiman, et al. (2007)	367/1302	The Netherlands	0.14	0.11	1.39	1.09 – 1.78	9.0 × 10 <sup>-3</sup>
	373/372	African Americans	0.32	0.31	1.01	0.81 – 1.25	0.96
	1614/837	African Americans	—	—	—	—	—
	722/728	Japanese Americans	—	—	—	—	—
Robbins, et al. (2007)	1111/112	Native Hawaiians	—	—	—	—	—
	637/633	Latino Americans	—	—	—	—	—
	1182/942	European Americans	—	—	—	—	—
Present study (2009)	490/567	African Americans	0.34	0.31	1.40	0.70 – 1.30	0.13
	391/323	Native Japanese	0.25	0.19	1.34	1.01 – 1.79	0.04

  

rs6983267 G allele frequency							
Author (Year)	N cases/N controls	Study population	Cases	Controls	OR	95% CI	p value
Amundadottir, et al. (2006)	1291/997	Iceland	—	—	—	—	—
	1435/779	Sweden	—	—	—	—	—
	458/247	European Americans	—	—	—	—	—
	246/352	African Americans	—	—	—	—	—
Freedman, et al. (2006)	70/68	Native Hawaiians	—	—	—	—	—
	449/465	Japanese Americans	—	—	—	—	—
	640/567	Latino Americans	—	—	—	—	—
	455/447	European Americans	—	—	—	—	—
Wang, et al. (2007)	989/804	African Americans	—	—	—	—	—
	491/545	Caucasians	—	—	—	—	—
Gudmundsson, et al. (2007)	435/545	Caucasians*	—	—	—	—	—
	1453/3064	Iceland	—	—	—	—	—
	385/892	Spain	—	—	—	—	—
Haiman, et al. (2007)	367/1302	The Netherlands	—	—	—	—	—
	373/372	African Americans	—	—	—	—	—
	1614/837	African Americans	0.90†	0.84	1.43	1.17 – 1.75	—
	722/728	Japanese Americans	0.37†	0.32	1.22	1.05 – 1.42	—
Robbins, et al. (2007)	1111/112	Native Hawaiians	0.36†	0.28	1.29	0.88 – 1.89	—
	637/633	Latino Americans	0.63†	0.62	1.05	0.89 – 1.24	—
	1182/942	European Americans	0.54†	0.51	1.13	0.99 – 1.28	—
Present study (2009)	490/567	African Americans	0.08	0.11	1.40	0.90 – 2.40	0.17
	391/323	Native Japanese	0.38	0.31	1.27	0.99 – 1.62	0.06

—, not applicable.

\*, Familial prostate cancer cases.

†: We calculated the G allele frequencies of rs6983267. Genotype data were obtained from the supplementary Table 3 (reference No. 8).

assay. YE analyzed and interpreted the data. TA, MS2, and TH collected the control and latent prostate cancer subjects and interpreted the data. TK2 and MS1 conceived the study. MS1 was also involved in the statistical analysis and drafting of the manuscript. HN was involved in the manuscript's revision. TK2 and YH approved the manuscript for publication. All authors read and approved the final manuscript.

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## References

- McCracken M, Olsen M, Chen MS Jr, Jemal A, Thun M, Cokkinides V, Deapen D, Ward E: **Cancer incidence, mortality, and associated risk factors among Asian Americans of Chinese, Filipino, Vietnamese, Korean, and Japanese ethnicities.** *CA Cancer J Clin* 2007, **57**:190-205.
- Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Cazier JB, Sainz J, Jakobsdottir M, Kostic J, Magnusdottir DN, Ghosh S, Agnarsson K, Birgisdottir B, Le Roux L, Olafsdottir A, Blondal T, Andresdottir M, Gretarsdottir OS, Bergthorsson JT, Gudbjartsson D, Gylfason A, Thorleifsson G, Manolescu A, Kristjansson K, Geirsson G, Isaksson H, Douglas J, Johansson JE, Bälter K, Wiklund F, Montie JE, Yu X, Suarez BK, Ober C, Cooney KA, Gronberg H, Catalona WJ, Einarsson GV, Barkardottir RB, Gulcher JR, Kong A, Thorsteinsdottir U, Stefansson K: **A common variant associated with prostate cancer in European and African populations.** *Nat Genet* 2006, **38**:652-658.
- Freedman ML, Haiman CA, Patterson N, McDonald GJ, Tandon A, Waliszewska A, Penney K, Steen RG, Ardlie K, John EM, Oakley-Girvan I, Whittemore AS, Cooney KA, Ingles SA, Altshuler D, Henderson BE, Reich D: **Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men.** *Proc Natl Acad Sci USA* 2006, **103**:14068-14073.
- Severi G, Hayes VM, Padilla EJ, English DR, Southey MC, Sutherland RL, Hopper JL, Giles GG: **The Common Variant rs1447295 on Chromosome 8q24 and Prostate Cancer Risk: Results from an Australian Population-Based Case-Control Study.** *Cancer Epidemiol Biomarkers Prev* 2007, **16**:610-612.
- Suuriniemi M, Agalliu I, Schaid DJ, Johanneson B, McDonnell SK, Iwasaki L, Stanford JL, Ostrander EA: **Confirmation of a positive association between prostate cancer risk and a locus at chromosome 8q24.** *Cancer Epidemiol Biomarkers Prev* 2007, **16**:809-814.
- Wang L, McDonnell SK, Slusser JP, Hebring SJ, Cunningham JM, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ, Thibodeau SN: **Two common chromosome 8q24 variants are associated with increased risk for prostate cancer.** *Cancer Res* 2007, **67**:2944-2950.
- Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Xu J, Blondal T, Kostic J, Sun J, Ghosh S, Stacey SN, Mouy M, Saemundsdottir J, Backman VM, Kristjansson K, Tres A, Partin AW, Albers-Akkers MT, Godino-Ivan Marcos J, Walsh PC, Swinkels DW, Navarrete S, Isaacs SD, Aben KK, Graif T, Cashy J, Ruiz-Echarri M, Wiley KE, Suarez BK, Witjes JA, Frigge M, Ober C, Jonsson E, Einarsson GV, Mayordomo JI, Kiemeny LA, Isaacs WB, Catalona WJ, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: **Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24.** *Nat Genet* 2007, **39**:631-637.
- Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ, Greenway SC, Stram DO, Le Marchand L, Kolonel LN, Frasco M, Wong D, Pooler LC, Ardlie K, Oakley-Girvan I, Whittemore AS, Cooney KA, John EM, Ingles SA, Altshuler D, Henderson BE, Reich D: **Multiple regions within 8q24 independently affect risk for prostate cancer.** *Nat Genet* 2007, **39**:638-644.
- Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearnhead P, Yu K, Chatterjee N, Wang Z, Welch R, Staats BJ, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cancel-Tassin G, Cussenot O, Valeri A, Andriole GL, Gelmann EP, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover R, Hunter DJ, Chanock SJ, Thomas G: **Genome-wide association study of prostate cancer identifies a second risk locus at 8q24.** *Nat Genet* 2007, **39**:645-649.
- Zheng SL, Sun J, Cheng Y, Li G, Hsu FC, Zhu Y, Chang BL, Liu W, Kim JW, Turner AR, Gielzak M, Yan G, Isaacs SD, Wiley KE, Sauvageot J, Chen HS, Gurganus R, Mangold LA, Trock BJ, Gronberg H, Duggan D, Carpten JD, Partin AW, Walsh PC, Xu J, Isaacs WB: **Association between two unlinked loci at 8q24 and prostate cancer risk among European Americans.** *J Natl Cancer Inst* 2007, **99**:1525-1533.
- Robbins C, Torres JB, Hooker S, Bonilla C, Hernandez W, Candreva A, Ahaghotu C, Kittles R, Carpten J: **Confirmation study of prostate cancer risk variants at 8q24 in African Americans identifies a novel risk factor.** *Genome Res* 2007, **17**:1717-1722.
- Sun J, Lange EM, Isaacs SD, Liu W, Wiley KE, Lange L, Gronberg H, Duggan D, Carpten JD, Walsh PC, Xu J, Chang BL, Isaacs WB, Zheng SL: **Chromosome 8q24 risk variants in hereditary and non-hereditary prostate cancer patients.** *Prostate* 2008, **68**:489-497.
- Menashe I, Rosenberg PS, Chen BE: **PGA: power calculator for case-control genetic association analyses.** *BMC Genet* 2008, **9**:36.
- Pemberton TJ, Mehta NU, Witonsky D, Di Rienzo A, Allayee H, Conti DV, Patel PI: **Prevalence of common disease-associated variants in Asian Indians.** *BMC Genet* 2008, **9**:13.
- Stamey TA, Freiha FS, McNeal JE, Redwine EA, Whittemore AS, Schmid HP: **Localized prostate cancer.** *Cancer* 1993, **71**:933-938.
- Terada N, Tsuchiya N, Ma Z, Shimizu Y, Kobayashi T, Nakamura E, Kamoto T, Habuchi T, Ogawa O: **Association of Genetic Polymorphisms at 8q24 With the Risk of Prostate Cancer in a Japanese Population.** *Prostate* 2008, **68**:1689-1695.
- Sato H, Minei S, Hachiya T, Yoshida T, Takimoto Y: **Fluorescence in situ hybridization analysis of c-myc amplification in stage T<sub>3</sub>N<sub>0</sub>M<sub>0</sub> prostate cancer in Japanese patients.** *Int J Urol* 2006, **13**:761-766.
- Center for Cancer Control and Information Services, National Cancer Center, Japan [<http://ganjoho.ncc.go.jp/professional/statistics/statistics.html>]
- Denis L, Morton MS, Griffiths K: **Diet and its preventive role in prostatic disease.** *Eur Urol* 1999, **35**:377-387.
- Marks LS, Kojima M, Demarzo A, Heber D, Bostwick DG, Qian J, Dorey FJ, Veltri RW, Mohler JL, Partin AW: **Prostate cancer in native Japanese and Japanese-American men: Effects of dietary differences on prostatic tissue.** *Urology* 2004, **64**:765-771.
- Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A: **Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: A preliminary report from a one-year proof-of-principle study.** *Cancer Res* 2006, **66**:1234-1240.
- Kurahashi N, Sasazuki S, Iwasaki M, Inoue M, Tsugane S, JPHC Study Group: **Green tea consumption and prostate cancer risk in Japanese men: A prospective study.** *Am J Epidemiol* 2008, **167**:71-77.
- Sabin LH, Wittekind C, Ed: **TNM classification of malignant Tumors.** 6th edition. Wiley-Liss, New York; 2002.
- Suzuki M, Kurosaki T, Arai T, Sawabe M, Hosoi T, Kitamura T: **The Val158Met polymorphism of the catechol-O-methyltransferase gene is not associated with the risk of sporadic or latent prostate cancer in Japanese men.** *Int J Urol* 2007, **14**:800-804.
- Sawabe M, Arai T, Kasahara I, Esaki Y, Nakahara K, Hosoi T, Orimo H, Takubo K, Murayama S, Tanaka N: **Development of a geriatric autopsy database and Internet-based database of Japanese single nucleotide polymorphisms for geriatric research (JG-SNP).** *Mech Ageing Dev* 2004, **125**:547-552.