ANTICANCER RESEARCH *24*: 2431-2438 (2004)

Genetic Polymorphisms of Hormone-related Genes and Prostate Cancer Risk in the Japanese Population

TAKAHIDE FUKATSU[1,2], YOSHIFUMI HIROKAWA[1], TOMIO ARAKI[3], TAKUICHI HIOKI[4], TETSUYA MURATA[4], HIROYOSHI SUZUKI[5], TOMOHIKO ICHIKAWA[5], HIROMASA TSUKINO[6], DELAI QIU[6], TAKAHIKO KATOH[6], YOSHIKI SUGIMURA[2], RYUICHI YATANI[1], TAIZO SHIRAISHI[1] and MASATOSHI WATANABE[1,7]

[1]Second Department of Pathology and
[2]Department of Urology, Mie University School of Medicine, Tsu;
[3]Department of Urology, Matsusaka Saiseikai General Hospital, Matsusaka;
[4]Department of Urology, JA Suzuka General Hospital, Suzuka;
[5]Department of Urology, Graduate School of Medicine, Chiba University, Chiba;
[6]Department of Public Health, Miyazaki Medical College, Miyazaki;
[7]Division of Materials Science and Chemical Engineering, Graduate School of Engineering, Yokohama National University, Yokohama, Japan

Abstract. *Carcinogenesis of the prostate involves androgen influences, and associations between genetic polymorphisms of androgen receptor and metabolizing enzymes and prostate cancer risk have been reported. Roles for non-androgenic hormones are not well defined, but they also may have an impact judging from epidemiological and animal experimental αλ ζof data. The purpose of the study was to determine whether hormone-related polymorphisms are associated with prostate cancer risk. A case-control study was performed with 147 Japanese prostate cancer patients and 266 urological controls. Polymorphisms of target genes [cytochrome P450 (CYP) 1B1, Leu^{432}Val; debrisoquine hydroxylase, (CYP2D6)*★*4; aromatase (CYP19), Arg^{264}Cys; estrogen receptor (ER)α-Xx (Xba I) and Pp (Pvu II); ERβ-Rr (Rsa I); progesterone receptor (PR) Alu in intron 7] were examined by PCR-based methods. The capital and small letters signify the absence and presence of restriction sites, respectively. Odds ratios (OR) were adjusted for age using multiple logistic regression analysis with SPSS Medical Pack. Among the seven examined genetic polymorphisms, significant associations between CYP1B1 Leu^{432}Val (OR 4.80; 95% confidence interval (CI), 1.21-19.05) and Alu in intron 7 of PR (OR 4.17; 95%CI, 1.26-13.85) were found. As for combined effects, the CYP1B1 polymorphisms (Leu/Val+Val/Val) together with heterozygosity for Alu in the PR were more frequent among prostate cancer patients (1.45%) than controls (0.41%), although without significance (OR, 3.99; 95%CI, 0.36-44.8). The combination of ERα (P/p+p/p) polymorphisms with heterozygosity for Alu in the PR demonstrated an OR of 4.56 (95%CI, 1.01-20.6). This pilot study showed that CYP1B1 and PR polymorphisms, alone or in combination, might be associated with prostate cancer risk. They might, therefore, have potential as a tool for identifying high-risk individuals.*

Correspondence to: Masatoshi Watanabe, M.D., Ph.D., Second Department of Pathology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514, Japan. Tel: +81-59-232-2864, Fax: +81-59-231-5210, e-mail: mawata@doc.medic.mie-u.ac.jp

Key Words: Polymorphism, hormone-related gene, prostate cancer.

Prostate cancer is one of the most common malignancies among men in western countries, demonstrating the highest incidence rate of any neoplasm in the United States (1). Recently, the numbers of clinical cases have been increasing annually in Japan, but it is well recognized that the incidence of prostate cancer is still 2- to 3-fold higher in American Caucasians than in Asians (2,3). Reflecting the shared genetic inheritance within a group, ethnicity may be an important factor in determining risk of prostate cancer. Relative to the general population, men with a family history of prostate cancer have a 2-to 3-fold increased incidence (4) and genetic polymorphisms are hypothesized as a possible factor underlying differences among ethnic groups and familial predisposition. A relatively small number of studies have been conducted to examine the associations between prostate cancer risk and polymorphisms of genes involved in the metabolism of xenobiotics, such as cytochromes P450 (CYPs) and glutathione S-transferases (GSTs) (5-7).

Human prostate cancer is often androgen-sensitive and reacts to hormonal therapy by temporary remission, followed by relapse to an androgen-insensitive state. The available data

suggest that steroid hormones, particularly androgens, play an important role in human prostate carcinogenesis. Therefore genetic polymorphisms of androgen metabolizing enzymes and receptors might exert an influence. There has been focus on cytochrome P450c17α (CYP17), steroid 5α-reductase type II (SRD5A2) and androgen receptor genes, but the results have been equivocal (8-11). The effects of estrogens on prostate epithelium are still unclear although they have been used in the treatment of prostate cancer. However, evidence in support of a role comes from epidemiological and animal experimental studies, including an investigation of treatment of NBL rats with testosterone and estradiol (12).

Testosterone can be synthesized from cholesterol in several steps by a number of different enzymes. CYP17 catalyses the 17α-hydroxylation of pregnenolone and progesterone and has 17, 20-lyase activity while 5α-reductase type II (SRD5A2) converts testosterone to the metabolically more active dihydrotestosterone (11). CYP19 activity determines the local oestrogen level, catalyzing the conversion of androgens to estrogens so that specific inhibitors are used for control of breast cancers (13-15) and the prostate is also influenced by estrogen through CYP19 activity in its stroma (10,16). CYP1B1 catalyzes the conversion of 17-β-estradiol(E2) to the catechol metabolite 16-OH-E2 (13,17-19). CYP 2D6 is known to contribute to the metabolism of a large number of clinically relevant drugs and is thought to be involved in the synthesis and/or metabolism of steroid compounds in steroidogenic tissues (7). There have already been studies of relationships between CYP2D6/CYP1B1 and prostate cancer risk (7,20,21). To further investigate the possibility that genes involved in these pathways are candidates for determining prostate cancer susceptibility, we conducted a hospital-based case-control study of Japanese men focusing on polymorphisms of the CYP 1B1, 2D6, 19 and estrogen receptor (ER)α, ERβ and progesterone receptor (PR) genes.

Materials and Methods

Selection of patient and controls. Prostate cancer subjects (n=147) and urological controls (n= 266) were taken from the records of the Departments of Urology at Mie University Hospital, Chiba University Hospital, JA Suzuka Central General Hospital and Matsusaka Saiseikai Hospital, Japan, between 1991 and 2002. All were Japanese men and the prostate cancer patients were all histologically confirmed and characterized in terms of their clinical staging (Tumor-Node-Metastasis system) and grade (Japanese General Rules for Clinical and Pathological Studies on Prostate cancer in 2001). Forty-four patients (29.9%) had well-differentiated, 57 (38.8%) moderately-differentiated and 46 (31.3%) poorly-differentiated adenocarcinomas. Regarding clinical staging, 60 (40.8%) were in stage A or B, 36 (24.5%) were in stage C and 51(34.7%) were in stage D. The control group was composed of patients with BPH or other urological diseases, who were healthy and confirmed to be free of prostate cancer by no elevation of serum PSA and/or a negative biopsy. Retropubic prostatectomy was performed for 99 of controls, with pathological examination to exclude the presence of prostate cancer. The mean age of the prostate cancer patients was 71.3±8.3 years and for the controls, 70.4±7.5. All gave informed consent to participate in this molecular genetic study of prostate cancer.

DNA extraction. DNA was isolated from peripheral blood of all prostate cancer patients and 167 controls and from frozen prostate tissue in 99 controls.

Genotyping of CYP 1B1. To evaluate the CYP 1B1 Leu^{432}Val, PCR-RFLP was performed essentially as described previously (19). The primers used were as follows: 5'-CTGCCAACACCTCTGTCTTG-3' and 5'-CTGAAATCGCACTGGTGAGC-3'. The amplification was for 35 cycles with denaturation at 95˚C for 1 min, annealing at 63˚C , and extension at 72˚C for 1 min. The PCR products (271bp) were digested at 37˚C overnight using *Eco57* I. The produced fragment sizes were 105 bp and 166 bp for the Leu allele and an undigested 271 bp fragment for the Val allele. Classification was into the L/L genotype (105 and 166 bp fragments), V/L genotype (105, 166, and 271 bp fragments) and V/V genotype (271 bp fragment).

Genotyping of CYP 2D6. The genotypes of CYP2D6 (the G to A substitution at the junction of intron 3-exon 4, CYP2D6*4 allele) were analyzed using two primers: 5'-GCTTCGCCAACCACTCCG-3' and 5'-AAATCCTGCTCTTCCGAGGC-3' (20). The running conditions were for 35 cycles with a melting step at 94˚C for 30sec and annealing and elongation at 65˚C for 1min and 30sec, respectively. PCR products were digested with *BstO* 1 at 60˚C and classified into poor metabolizer (PM, homozygote mutated-type, 334 bp fragment), rapid metabolizer (RM, heterozygote wild/mutated-type, 334, 230, and 205 bp fragments) and rapid metabolizer (RM, homozygote wild-type, 230 and 105 bp fragments) type.

Genotyping of CYP 19. The primers (5'-CGCTAGATGTCTAAA CTGAG-3' and 5'-CATATGTGGC ATGGGAATTA-3') were used to amplify the coding and flanking sequences of exon 7 of CYP19 (16). Thermal cycling consisted of 35 cycles of denaturation at 94˚C for 1 min, annealing at 51˚C for 1 min and elongation at 72˚C for 1 min. The C to T substitution in exon 7, resulting in a single amino acid substitution from Arg by Cys at codon 264, creates a recognition site for *SfaN* I restriction enzyme. PCR products were digested overnight at 37˚C .

Genotyping of ERα. Detection of *Xba* I and *Pvu* II restriction sites in intron 1 was facilitated by PCR amplification of a region spanning the sites with primers: 5'-ATCCAGGGTTATGTGGCAATGAC-3' and 5'-ACCCTGGCGTCGATTATCTGA-3' (16). The amplification was for 30 cycles of denaturation at 95˚C for 60sec, annealing at 57˚C for 60sec and elongation at 72˚C for 60sec. The PCR products were digested overnight at 37˚C. The presence of the *Xba* I and *Pvu* II restriction sites is indicated by x or p, and the absence by X or P.

Genotyping of ERβ. The genotyping of ER was performed using the primers: 5'-CAGGCTTTGTGGAGCTCAG-3' and 5'-ACCTGTCCAGAACAAGATCT-3' (22). The conditions were as follows; the amplification was for 30 cycles at 95˚C for 60sec, annealing at 64.3˚C for 60sec and extension at 72˚C for 60sec. The PCR products were digested overnight at 37˚C with the *Rsa* I. In the text below, the capital R represents the absence while lower-case r indicates the presence of the restriction site.

Table I. *Distribution of allelic variants in prostate cancer patients and controls.*

	Cases (%)	Controls (%)	OR (95%CI)*	*P*-value
CYP1B1	n=136	n=255		
L/L	87 (64.0)	180 (70.6)	1	
L/V	42 (30.9)	72 (28.2)	1.19 (0.75-1.89)	0.461
V/V	7 (5.1)	3 (1.2)	4.80 (1.21-19.05)	0.026
CYP2D6	n=136	n=232		
wild/wild	133 (97.8)	231 (99.6)	1	
wild/mutated	3 (2.2)	1 (0.4)	5.52 (0.57-53.84)	0.141
CYP19	n=107	n=187		
C/C	68 (63.6)	118 (63.1)	1	
C/T	33 (30.8)	57 (30.5)	0.99 (0.58-1.67)	0.967
T/T	6 (5.6)	12 (6.4)	0.88 (0.32-2.46)	0.81
ER a	n=117	n=242		
*Xba*I	x/x	74 (63.2)	163 (67.4)	1
X/x	37 (31.6)	68 (28.1)	1.24 (0.76-2.02)	0.385
X/X	6 (5.1)	11 (4.5)	1.24 (0.44-3.47)	0.687
	n=116	n=238		
*Pvu*II	P/P	22 (19.0)	47 (19.7)	1
P/p	57 (49.1)	110 (46.2)	1.19 (0.65-2.20)	0.575
p/p	37 (31.9)	81 (34.0)	1.05 (0.55-2.01)	0.877
ERβ	n=136	n=236		
R/R	82 (60.3)	133 (56.4)	1	
R/r	43 (31.6)	91 (38.6)	0.73 (0.46-1.16)	0.182
r/r	11 (8.1)	12 (5.1)	1.45 (0.61-3.46)	0.397
PR	n=138	n=244		
A1/A1	129 (93.5)	240 (98.4)	1	
A1/A2	9 (6.5)	4 (1.6)	4.17 (1.26-13.85)	0.02

*, ORs were adjusted for age.

Table II. *Relationships between polymorphisms and clinicopathological factors.*

Factor	Genotype			OR (95%CI)	*P*-value
CYP1B1	L/L	L/V	V/V		
Stage A or B	33 (58.9)	20 (35.7)	3 (5.4)	1	
Stage C or D	53 (67.9)	21 (26.9)	4 (5.1)	0.65 (0.32-1.34)[1]	0.242
Well-diff.	27 (64.3)	13 (31.0)	2 (4.7)	1	
Moderately-diff.	37 (74.0)	10 (20.0)	3 (6.0)	0.59 (0.24-1.49)[1]	0.266
Poorly-diff.	22 (52.4)	18 (42.9)	2 (4.7)	1.73 (0.71-4.19)[1]	0.228
CYP19	C/C	C/T	T/T		
Stage A or B	25 (58.1)	14 (32.6)	4 (9.3)	1	
Stage C or D	42 (67.7)	18 (29.0)	2 (3.2)	0.60 (0.26-1.38)[2]	0.232
Well-diff.	22 (68.8)	7 (21,9)	3 (9.4)	1	
Moderately-diff.	27 (69.2)	11 (28.2)	1 (2.6)	0.97 (0.35-2.71)[2]	0.951
Poorly-diff.	18 (52.9)	14 (41.2)	2 (5.9)	1.95 (0.71-5.35)[2]	0.193
ERα-*Xba*I	x/x	x/X	X/X		
Stage A or B	30 (65.2)	12 (26.1)	4 (8.7)	1	
Stage C or D	42 (60.9)	25 (36.2)	2 (2.9)	1.22 (0.56-2.68)[3]	0.616
Well-diff.	18 (48.6)	16 (43.2)	3 (8.1)	1	
Moderately-diff.	27 (67.5)	10 (25.0)	3 (7.5)	0.46 (0.18-1.15)[3]	0.097
Poorly-diff.	27 (71.1)	11 (28.9)	0 (0.0)	0.38 (0.14-0.98)[3]	0.046
ERα-*Pvu*II	P/P	P/p	p/p		
Stage A or B	10 (21.7)	19 (41.3)	17 (37.0)	1	
Stage C or D	11 (16.2)	37 (54.4)	20 (29.4)	1.44 (0.55-3.75)[4]	0.462
Well-diff.	11 (29.7)	17 (45.9)	9 (24,3)	1	
Moderately-diff.	3 (7.7)	20 (51.3)	16 (41.0)	5.06 (1.28-20.02)[4]	0.021
Poorly-diff.	7 (18.4)	19 (50.0)	12 (31.6)	1.87 (0.63-5.53)[4]	0.257
ERβ	R/R	R/r	r/r		
Stage A or B	26 (50.0)	22 (42.3)	4 (7.7)	1	
Stage C or D	56 (68.3)	19 (23.2)	7 (8.5)	0.46 (0.22-0.94)[5]	0.033
Well-diff.	26 (65.0)	12 (30.0)	2 (5.0)	1	
Moderately-diff.	31 (60.8)	17 (33.3)	3 (5.9)	1.01 (0.42-2.44)[5]	0.978
Poorly-diff.	25 (58.1)	12 (27.9)	6 (14.0)	1.34 (0.55-3.27)[5]	0.522

[1]OR (V/V+V/L *vs* L/L); [2]OR (C/T+T/T *vs* C/C); [3]OR (x/X+X/X *vs* x/x); [4]OR (P/p+p/p *vs* P/P); [5]R/r+r/r *vs* R/R).

Genotyping of PR. Genotyping of PR was performed using the primers: 5'-GCCTCTAAAATGAAAGGCAGAAAGC-3' and 5'-GCGCGTATTTCTTGCTAAATGTCTG-3' (23). The amplification was for 30 cycles, each consisting of 60sec of denaturing at 94˚C, 60sec of annealing at 60˚C and 60sec of extension at 72˚C. The A1 Allele appeared as a 175 bp, and the A2 allele, the *PROGINS,* as a 481 bp fragment.

For all amplification reactions, an initial denaturation step of 5min at 94˚C or 95˚C and a final extension at 72˚C for 5min were applied. The PCR products were run on 1.6 to 2.0% agarose gels and visualized by ethidium bromide staining.

Statistical analysis. To examine the associations between genotypes and the development of prostate cancer, we calculated odds ratios OR and confidence intervals (CI). OR were adjusted for age using multiple logistic regression analysis with the SPSS Medical Pack.

Results

Data for the allelic distribution of CYP1B1, 2D6, 19, ERα, ERβ and PR genes in prostate cancer patients and controls, and their relationships to risk among Japanese males, are shown in Table I. The allele frequencies of the CYP1B1, 19, ERα (*Xba* I and *Pvu* II) and ERβ polymorphisms were compatible with the control population in Hardy-Weinberg equilibrium. The frequencies of CYP1B1 L/L, L/V and V/V alleles were 64.0, 30.9 and 5.1% in prostate cancer patients, compared with 70.6, 28.2 and 1.2% in controls. An association between CYP1B1 Val^{432} and prostate cancer risk was apparent (OR 4.80; 95%CI, 1.21-19.05; $p=0.026$). With CYP2D6, the frequencies of heterozygote wild/mutated and homozygote mutated types were

Table IIIa. *Distribution of combined allelic variants among prostate cancer patients and controls.*

Genotype	CYP1B1 L/L	CYP1B1 L/V+V/V
ERα *Xba*I		
x/x	54/112[1]	19/46
	1	0.83[2] (0.44-1.57)[3], *p*=0.562
x/X;X/X	23/56	17/20
	0.87 (0.49-1.57), *p*=0.647	1.84 (0.89-3.81), *p*=0.101
*Pvu*II P/P	9/31	11/16
	1	2.67 (0.89-7.95), *p*=0.079
P/p+p/p	67/133	25/50
	1.96 (0.85-4.50), *p*=0.1131.89	(0.75-4.73), *p*=0.176
ERβ		
R/R	51/84	28/42
	1	1.09 (0.60-1.97), *p*=0.783
R/r+r/r	33/75	18/25
	0.70 (0.41-1.21), *p*=0.702	1.13 (0.55-2.30), *p*=0.740
PR		
A1/A1	81/162	41/69
	1	1.17 (0.73-1.88), *p*=0.52
A1+A2	6/3	2/1
	4.1 (0.99-16.72), *p*=0.052	3.99 (0.36-44.8), *p*=0.26
CYP2D6		
W/W	81/164	44/60
	1	1.47 (0.91-2.36), *p*=0.113
W/M	1/1	1/0
	2.1 (0.13-34.33), *p*=0.607	-
CYP19		
C/C	48/84	20/30
	1	1.10 (0.56-2.17), *p*=0.778
C/T+T/T	21/47	17/20
	0.74 (0.39-1.39), *p*=0.349	1.48 (0.71-3.10), *p*=0.295

[1]cases/controls; [2]ORs were adjusted for age; [3]95% CI.

Table IIIb. *Distribution of combined allelic variants among prostate cancer patients and controls.*

Genotype	PR A1/A1	PR A1/A2	
ERα *Xba*I			
x/x	70/148[1]	4/3	
	1	2.88[2] (0.63-13.3)[3], *p*=0.175	
x/X+X/X	38/73	2/0	
	1.14 (0.70-1.85), *p*=0.60	-	
*Pvu*II	P/P	20/42	0/0
	1	-	
P/p+p/p	87/175	6/3	
	1.10 (0.60-2.00), *p*=0.765	4.56 (1.01-20.6), *p*=0.049	
ERβ			
R/R	76/124	3/3	
	1	1.72 (0.34-8.79), *p*=0.518	
R/r+r/r	49/97	5/1	
	0.79 (0.50-1.24), *p*=0.309	7.07 (0.80-67.7), *p*=0.079	
CYP2D6			
W/W	123/212	6/4	
	1	2.49 (0.68-9.06), *p*=0.166	
W/M	2/1	1/0	
	3.47 (0.31-38.84), *p*=0.312	-	
CYP19			
C/C	63/112	4/1	
	1	7.21 (0.79-66.0), *p*=0.080	
C/T+T/T	39/65	0/1	
	1.06 (0.64-1.76), *p*=0.824	-	

[1]cases/controls; [2]ORs were adjusted for age; [3]95% CI.

3 and 0 in cases and 1 and 0 in controls. The overall frequency of poor metabolizers for CYP2D6 in Japanese was 0%. The heterozygote genotype was higher in cases, but not significant (OR 5.52; 95%CI, 0.57-53.84; *p*=0.141). As for CYP19, there was no significant relationship between the Arg264Cys substitution and prostate cancer risk. The frequencies of ERβ R/R, R/r and r/r alleles were 60.3, 31.6 and 8.1% in cases, compared with 56.4, 38.6 and 5.1% in controls. There was no significant association between ERβ r/r and prostate cancer risk (OR 1.45; 95%CI, 0.61-3.46; *p*=0.397). The frequencies of ERα x/x, x/X and X/X alleles were 63.2, 31.6 and 5.1% in cases, compared with 67.4, 28.1 and 4.5% in controls. The frequencies of ERα p/p, p/P and P/P alleles were 31.9, 49.1 and 19.0% in cases, compared with 34.0, 46.2 and 19.7% in controls. No association between ERα polymorphisms and prostate cancer risk was recognized. With *Alu* in intron 7 of the PR, however, a significant relationship was seen. The frequency of the A_1+A_2 heterozygote genotype was higher in cases than in controls (OR 4.17; 95%CI, 1.26-13.85; *p*=0.02).

Next, we investigated the relationships between genetic polymorphisms and clinicopathological factors including the staging or grading of the prostate cancer (Table II). As for the staging of the cancer, we classified two groups (stage A or B, stage C or D) and with the grading, divided into three groups (well-differentiated, moderately-differentiated, poorly-differentiated). ERβ (R/r+r/r *vs* R/R) together with the stage C or D group resulted in an OR of 0.46 (95%CI, 0.22-0.94; *p*=0.033). For the grading, ERα (P/p+p/p *vs* P/P) with the moderately-differentiated group resulted in an OR of 5.06 (95%CI, 1.28-20.02; *p*=0.021) and ERα (x/X+X/X *vs* x/x) with the poorly-differentiated group was 0.38 of OR (95%CI, 0.14-0.98; *p*=0.046). Contrary to our expectations, we can not find any significant association in these results.

As for the frequencies of these polymorphisms, there are no differences between patients with BPH and patients with other urological diseases (data not shown). The combined

effects of polymorphisms on prostate cancer risk were also analyzed (Table III-A and B). CYP1B1 polymorphisms (Leu/Val+Val/Val) together with heterozygosity for *Alu* in PR were more frequent among prostate cancer patients (1.36%) than controls (0.38%), although this was not significant (OR 3.99; 95%CI, 0.36-44.8; $p=0.26$), CYP1B1 (Leu/Leu) together with heterozygosity for *Alu* in PR also not being significant (OR 4.1; 95%CI, 0.99-16.72; $p=0.052$). The combination of ERα (P/p+p/p) polymorphisms with A_1+A_2 heterozygosity in the PR resulted in an OR of 4.56 (95%CI, 1.01-20.6; $p=0.049$).

Discussion

Prostatic carcinogenesis appears to be influenced by steroid hormones, especially androgens, the expression of which is regulated by genetic and environmental factors. Many studies have shown that polymorphisms and mutations in hormone-related genes may cause changes in the function of the encoded proteins (7). Cytochrome P450s (CYPs) are members of a multigene superfamily of enzymes involved in the oxidative metabolism of xenobiotics, therapeutic drugs and endogenous steroid hormones.

The hydroxylation activity of CYP1B1 is of particular importance, since activated carcinogens induce DNA single-strand breakage and mutation. Especially, CYP1B1 catalyzes the hydroxylation of 17β-oestradiol (E2) at the C4 position. Two common polymorphisms of CYP1B1 have been reported: a C to G transversion at position 1666 changes amino acid 432 from Leu to Val; a C to T transition at position 1719 is a silent mutation (17,19). Recently, Tanaka *et al.* reported frequent polymorphisms in this gene among Japanese at six different locations (intron 1, C→T; codon 48, C→G; codon 119 G→T; codon 432, C→G; codon 449, C→T; codon 453, A→G) (18). In their study, the genotype at codon 119 significantly differed between prostate cancer and controls (T/T *vs* G/G; OR 4.02; 95% CI, 1.73-9.38; $p<0.001$), but variation was not evident for the other sites including codon 432. In contrast, Tang *et al.* observed the homozygous CYP1B1 Val^{432} allele to be a risk factor (OR 3.3; 95% CI, 1.9-9.0; $p<0.03$) (19). They reported the allelic frequency distribution of the $Leu^{432}Val$ polymorphism to vary markedly among ethnic groups, with 25% Leu^{432} *versus* 75% Val^{432} in African-Americans, 57% *versus* 43% in Caucasians, and 83% *versus* 17% in Chinese. They also reported that the percentage of individuals homozygous for the CYP1B1 Val^{432} genotype was 54% in African-Americans, 18.5% in Caucasians and only 1% in Chinese. They suggested that the prevalence of the polymorphism mirrors the relative prostate cancer incidence among these three ethnic groups. Our results are in line with Tang's data, suggesting a homozygous CYP1B1 Val^{432} genotype to be a risk factor, although this is minor in frequency in the Japanese population.

CYP2D6 located on chromosome 22q13.1 has been well studied since a lack of its activity is the basis for adverse events occurring during therapy with some drugs (7,20,21). About 5-10% of Caucasians have inactivating mutations in both alleles and have been designated poor metabolizers (PM). The most frequent of the inactivating mutations is the splice site G1934A transition (CYP2D6*4 or B allele) that causes a truncated protein. Two previous reports have suggested a possible association between this allele and an increased risk of prostate cancer in Caucasians and the Danish (20,21). Especially, an association between CYP2D6 PM and prostate cancer was detected among smoking Danes. In the present study, the heterozygote genotype was more frequent in cases, but this was not significant (OR 5.52; 95%CI, 0.57-53.84; $p=0.141$). Chida *et al.* reported that the CYP2D6*4 allele was very rare in Japanese, and the population frequency of the CYP2D6 PM was estimated to be 0.29% (24). This might partly explain racial differences in the incidences of prostate cancer.

CYP19 (Aromatase) catalyzes the conversion of C19 androgens to C18 estrogens. Polymorphisms in introns 4, 5, 6 and 7, the 5' regulatory area and 3'UTR have been described (10,14-16,25) and a significant association of one CYP19 polymorphism (tetranucleotide (TTTA) simple tandem repeat in intron 4 has been demonstrated with breast cancer risk in Caucasian women (15). In a Scandinavian case-control study, an association between the C to T substitution in the untranslated region of exon 10 and breast cancer risk was evident (26). With reference to prostate cancer, an association between the 171 and 187 bp alleles of CYP19 and prostate cancer risk in White French has been described (10). In Modugno's study, the frequencies of the C to T substitution in exon 7 of CYP19 were 94% for C/C and 6% for C/T in controls, whereas in cases they were 63.1% for C/C, 30.5% for C/T and T/T for 6.4% (16). A C to T substitution in exon 7 showed an increased risk of borderline significance in prostate cancer among Caucasian patients (OR 2.50; 95% CI, 0.99-6.28) and combined effects of short androgen receptor CAG repeats (OR 1.77; 95%CI, 1.00-3.14) (16). Our results from the present study are not in line with Modugno's findings, possibly due to ethnicity.

The ERα is located on chromosome 6p25.1. Two SNPs have been identified in the first intron of this gene: a T/C polymorphism recognized by the restriction endonuclease *Pvu* II, and an A/G by *Xba* I. Reports on associations between these polymorphisms and bone mineral density have been reported, but are inconsistent (27). The *Xba* I X allele has been associated with an increased risk of breast cancer in Norway while the *Pvu* II polymorphism was not (28). ERα polymorphisms may be linked with endometrial and breast cancer (29-31). ERβ is located on chromosome

14q23-24 and polymorphic dinucleotide CA repeat in the noncoding 3'-portion of the gene has been identified and suggested to be associated with bone mineral density in women (32). Recently, Arko *et al.* described the presence of a silent mutation in codon 328 with nucleotide GTG to GTA, which is recognized by *Rsa* I (22). In the previous study, in the rat prostate, the up-regulation of PR mRNA expression mediated *via* ERβ action has been reported to be included in the growth of testosterone plus estradiol-17β-induced dysplasia (33) and the expression of ERβ has been reported in human prostate cancer (34). No reports about relationships between ERβ polymorphisms and prostate cancer risk have appeared, and examination of the *Rsa* I polymorphism in the present study did not demonstrate any significant impact.

The human PR gene is located on chromosome 11q22-23, and its product belongs to the steroid-thyroid-retinoic acid receptor superfamily of transcription factors (35). Linkage disequilibrium has been reported to exist between three polymorphisms of the PR: an intronic *Alu* insertion, an exon 4 amino acid substitution codon 660 T variant, and an exon 5 synonymous codon 700 T variant (35-37): this complex is designated PROGINS. Although *Alu* insertion occurs within an intron of the PR, it is possible that it alters the expression of the PR locus. Associations between this polymorphism and risk of cancers in the breast and ovary have been reported (35-37). A reduced risk of breast cancer in German women by the age of 50 years who carry at least one A2 allele (PROGINS) has been found, while an opposite increased risk of ovarian cancer was evident (37). Tong *et al.* suggested that discrepancies might be due to different A2 frequencies at the population level (37). Previous data on PR expression are equivocal in the prostate case. Latil *et al.* reported a positive association between ERα, ERβ and PR expression and growth in human prostate cancer on Real-Time Quantitative RT-PCR, suggesting that these genes may modulate the response to hormone withdrawal (34) In the present study, the frequency of PR A1 + A2 allele was higher in cases than in controls (OR 4.17; 95% CI, 1.26-13.85; p=0.02), although the Japanese population overall appears to have a very low A2 frequency. To our knowledge, this is the first case-control study suggesting an association between our PR polymorphism and prostate cancer risk.

It is generally assumed that a multiple-gene model of susceptibility based on a hormone biosynthesis can help us understand the underlying etiology of hormone-dependent tumors, leading to the hypothesis that combined genetic variations can alter an individual's risk of prostate cancer (16,38). As for combined effects of polymorphisms, the CYP1B1 polymorphism (Leu/Val+Val/Val) together with heterozygosity for *Alu* in the PR was more frequent among prostate cancer patients than controls, but not significantly, contrary to our prediction. To our expectations, no relationships between the sole polymorphism of the ERα (*Pvu* II) and prostate cancer risk were recognized, but an increase was found for the combination of ERα (P/p+p/p) with heterozygosity for *Alu* of the PR. This result is not expected from the rodent results and previous reports (33,34), but may suggest the possibility of a feeble relationship considering that PR is an estrogen-regulated gene.

In conclusion, the present investigation showed that the CYP1B1 V/V genotype and the A2 allele of the PR or combined effects of polymorphisms are associated with prostate cancer, suggesting that assessment of genes for hormone-metabolizing enzyme and receptors may offer an approach for identifying individuals at high prostate cancer risk, especially in specific ethnic groups.

Acknowledgements

This study was supported in part by a Grant-in-Aid for the 2nd Term Comprehensive 10-years Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan, and by a Grant-in-Aid for Scientific Research on Priority Areas of Cancer from the Ministry of Education, Culture, Sport, Science and Technology of Japan.

References

1 Landis SH, Murray T, Bolden S and Wing PA: Cancer Statistics, 1999. CA Cancer J Clin *49*: 8-31, 1999.

2 Shimizu H, Ross RK and Bernstein L: Possible underestimation of the incidence rate of prostate cancer in Japan. Jpn J Cancer Res *82*: 483-5, 1991.

3 Ruijter E, Kaa CVE, Miller G, Ruiter D, Debruyne F and Schalken J: Molecular genetics and epidemiology of prostate carcinoma. Endocrine Reviews *20*: 22-45, 1999.

4 Carter BS, Bova GS, Beaty TH, Steinberg GD, Childs B, Isaacs WB and Walsh PC: Hereditary prostate cancer: Epidemiologic and clinical features. J Urol *150*: 797-802, 1993.

5 Murata M, Watanabe M, Yamanaka M, Kubota Y, Ito H, Nagao M, Katoh T, Kamataki T, Kawamura J, Yatani R and Shiraishi T: Genetic polymorphisms in cytochrome P450 (CYP) 1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. Cancer Lett *165*: 171-177, 2001.

6 Hayes RB: Gene-environment interrelations in prostate cancer. Epidemiol Rev *23*: 163-167, 2001.

7 Chen C: Risk of prostate cancer in relation to polymorphisms of metabolic genes. Epidemiol Rev *23*: 30-35, 2001.

8 Habuchi T, Liqing Z, Suzuki T, Sasaki R, Tsuchiya N, Tachiki H *et al*: Increased risk of prostate cancer and benign prostatic hyperplasia associated with a CYP17 gene polymorphism with a gene dosage effect. Cancer Res *60*: 5710-5713, 2000.

9 Yamada Y, Watanabe M, Murata M, Yamanaka M, Kubota Y, Itou H, Katou T, Kawamura J, Yatani R and Shiraishi T: Impact of genetic polymorphisms of 17-hydroxylase cytochrome P-450 (CYP17) and steroid 5α-reductase type II (SRD5A2) genes on prostate-cancer risk among the Japanese population. Int J Cancer *92*: 683-686, 2001.

10 Latil AG, Azzouzi R, Cancel GS, Guillaume EC, Cochan-Priollet B, Berthon PL and Cussenot O: Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. Cancer *92*: 1130-1137, 2001.
11 Simard J, Dumont M, Soucy P and Labrie F: Perspective: prostate cancer susceptibility genes. Endocrinology *143*: 2029-40, 2002.
12 Bosland MC: The role of steroid hormones in prostate carcinogenesis. J Natl Cancer Inst *27*: 39-66, 2000.
13 Kristensen VN and Borresen-Dale AL: Molecular epidemiology of breast cancer: genetic variation in steroid hormone metabolism. Mutation Res *462*: 323-333, 2000.
14 Miyoshi Y, Iwao K, Ikeda N, Egawa C and Nogughi S: Breast cancer risk associated with polymorphism in CYP19 in Japanese women. Int J Cancer *89*: 325-328, 2000.
15 Baxter SW, Choong DYH, Eccles DM and Campbell IG: Polymorphic variation in CYP19 and risk of breast cancer. Carcinogenesis *22*: 347-349, 2001.
16 Modugno F, Weissfeld JL, Trump DL, Zmuda JM, Shea P, Cauley JA and Ferrell RE: Allelic variants of aromatase and the androgen and estrogen receptors: toward a multigenetic model of prostate cancer risk. Clin Cancer Res *7*: 3092-3096, 2001.
17 Bailey LR, Roodi N, Dupont MD and Parl EF: Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. Cancer Res *58*: 5038-5041, 1998.
18 Tanaka Y, Sasaki M, Kaneuchi M, Shiina H, Igawa M and Dahiya R: Polymorphisms of the CYP1B1 gene have higher risk for prostate cancer. Biochem Biophy Res Com *296*: 820-826, 2000.
19 Tang YM, Green BL, Chen G, Thompson PA, Lang NP, Shinde A, Lin D, Tan W, Lyn-Cook BD, Hammons GJ and Kadlubar FF: Human CYP1B1 Leu432Val gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. Phamacogenetics *10*: 761-766, 2000.
20 Febbo PG, Kantoff PW, Giovannucci E, Brown M, Chang G, Hennekeus CH and Stampfer M: Debrisoquine hydroxylase (CYP2D6) and prostate cancer. Cancer Epidem Biomar Prev *12*: 1075-1078, 1998.
21 Wadelius M, Autrup JL, Stubbins MJ, Andersson SO, Johansson JE, Wadelius C, Wolf CR, Autrup H and Rane A: Polymorphisms in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association with prostate cancer. Pharmacogenetics *9*: 333-340, 1999.
22 Arko B, Prezelj J, Komel R, Kocijancic A and Marc J: No major effect of estrogen receptor beta gene Rsa I polymorphism on bone mineral density and response to alendronate therapy in postmenopausal osteoporosis. J Steroid Biochem Mol Biol *81*: 147-152, 2002.
23 Wang-Gohrke S, Chang-Claude J,Becher H, Kieback DG and Runnebaum IB: Progesterone receptor gene polymorphism is associated with decreased risk for breast cancer by age 50. Cancer Res *60*: 2348-2350, 2000.
24 Chida M, Yokoi T, Kosaka Y, Chiba K, Nakamura H, Ishizaki T *et al*: Genetic polymorphism of CYP2D6 in the Japanese population. Pharmacogenetics *9*: 601-605, 1999.
25 Probst-Hensch NM, Ingles SA, Diep AT, Haile RW, Stanczyk FZ, Kolonel LN and Henderson BE: Aromatase and breast cancer susceptibility. Endocrinol Relat Cancer *6*: 165-173, 1999.
26 Kristensen VN, Harnda N, Haraldsen E, Yoshimura N, Lonning PE, Erikstein B, Karesen R, Kristensen T and Borresen-Dale AL: Genetic variants of CYP 19 (aromatase) and breast cancer risk. Oncogene *19*: 1329-1333, 2000.
27 Yamada Y, Ando F, Niino N, Ohta S and Shimokata H: Association of polymorphisms of the estrogen receptor β gene with bone mineral density of the femoral neck in the elderly Japanese women. J Mol Med *80*: 452-460, 2002.
28 Andersen TI, Heimdal KR, Skrede M, Tveit K, Berg K and Borresen AL: Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum Genet *94*: 665-670, 1994.
29 Weiderpass E, Persson I, Melhus H, Wedren S, Kindmark A and Baron JA: Estrogen receptor gene polymorphism and endothelial cancer risk. Carcinogenesis *21*: 623-627, 2000.
30 Kang HJ, Kim SW, Kim HJ, Ahn SJ, Bae JY, Park SK, Kang D, Hirvonen A, Choe KJ and Noh DY: Polymorphisms in the estrogen receptor-alpha gene and breast cancer risk. Cancer Lett *178*: 175-180, 2002.
31 Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi N and Dahiya R: Polymorphisms of estrogen receptor β gene in endometrial cancer. Biochem Biophy Res Com *297*: 558-564, 2002.
32 Ogawa S, Hosoi T, Shiraki M, Orimo H, Emi M, Muramatsu M, Ouchi Y and Inoue S: Association of estrogen receptor β gene polymorphism with bone mineral density. Biochem Biophy Res Com *269*: 537-541, 2000.
33 Lau KM, Leav I and Ho SM: Rat estrogen receptor α and β, and progesterone receptor mRNA expression in various prostatic lobes and microdissected normal and dysplasia epithelial tissues of the Noble rats. Endocrinology *139*: 424-427, 1998.
34 Latil A, Bieche I, Vidaud D, Lidereau R, Berthon P, Cussenot O and Vidaud M: Evaluation of androgen, estrogen (ERα and ERβ), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. Cancer Res *61*: 1919-1926, 2001.
35 Leodolter S and Zeillinger R: Human progesterone receptor gene polymorphism PROGINS and risk for breast cancer in Austrian women. Breast Cancer Res Treat *72*: 131-137, 2002.
36 Spurdle AB, Hopper JL, Chen X, McCredie MR, Giles GG, Venter DJ, Southey MC and Chenevix-Trench G: The progesterone receptor exon 4 Val660Leu G/T polymorphism and risk of breast cancer in Australian women. Cancer Epidem Biomar Prev *11*: 439-443, 2002.
37 Tong D, Fabjani G, Heinze G, Obermair A, Leodorter S and Zeillinger R: Analysis of the human progesterone receptor gene polymorphism progins in Austrian ovarian carcinoma patients. Int J Cancer *95*: 394-397, 2001.
38 Henderson BE and Feigelson HS: Hormonal carcinogenesis. Carcinogenesis *21*: 427-433, 2000.

Received February 19, 2004
Accepted May 24, 2004