Research article

A49T, V89L and TA repeat polymorphisms of steroid 5α-reductase type II and breast cancer risk in Japanese women

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Abstract

Background: Breast cancer is hormone related, as are cancers of the endometrium, ovary, and prostate. Several studies have suggested that higher extracellular levels of androgens are associated with breast cancer risk, while biological evidence indicates that androgens are protective. The codon 49 alanine to threonine substitution (A49T), codon 89 valine to leucine substitution (V89L) and TA repeat polymorphisms of the steroid 5α-reductase type II (*SRD5A2*) gene are considered functional with respect to enzyme activity converting testosterone into dihydrotestosterone. To test the hypothesis that these three polymorphisms are associated with risk of breast cancer, a case–control study was conducted with patients of Aichi Cancer Center Hospital.

Methods: The cases were 237 patients histologically diagnosed with breast cancer, and the controls were 185 noncancer outpatients. DNA from peripheral blood was genotyped by PCR methods.

Results: The threonine allele of A49T was not found in our subjects. Compared with the *V/V* genotype of V89L, the *L/L* genotype was associated with a decreased risk (crude odds ratio [OR] = 0.61, 95% confidence interval [CI] = 0.36–1.05). This was also the case for the TA(*9/9*) genotype, with an OR of 0.58 (95% CI = 0.13–2.63) relative to TA(*0/0*). Among women with the TA(*0/0*) genotype, however, the OR for the *L/L* genotype was 0.46 (95% CI = 0.24–0.88) compared with the *V/V* genotype, and those with the *V/V* and TA(*0/0*) genotypes had the highest risk. The haplotype with the *L* and TA(*9*) repeat alleles was not found.

Conclusion: This study is the first to our knowledge focusing on Japanese women, suggesting that *SRD5A2* polymorphisms might have an association with breast cancer risk. Further large-sample studies will be required to confirm the association and to assess any interactions with environmental factors.

Keywords: breast cancer risk, Japanese women, *SRD5A2* gene polymorphisms

Introduction

Breast cancer is a hormone-related cancer, as are cancers of the endometrium, ovary, and prostate [1]. Although the molecular mechanisms involved in initiation and progression are poorly understood, there is evidence that extracellular levels of androgens are associated with the

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A49T = codon 49 alanine to threonine substitution; BMI = body mass index; bp = base pairs; CI = confidence interval; *L/L* = leucine/leucine; OR = odds ratio; PCR = polymerase chain reaction; *SRD5A2* = steroid 5α-reductase type II; V89L = codon 89 valine to leucine substitution; *V/L* = valine/leucine; *V/V* = valine/valine.

development of breast cancer [2,3]. Nested case–control studies have shown that increased circulating levels of testosterone elevate the risk of breast cancer [4–6]. Experimental studies suggest, however, that androgens exert a potent antiproliferative effect on the growth of several hormone-sensitive human breast cancer cells under both basal and estrogen-induced incubation conditions *in vitro* [7], as well as *in vivo*, using ZR-75-1 human breast cancer cells in nude mice [8]. Androgens have been used for the treatment of breast cancer [9,10].

Steroid 5α-reductase converts testosterone to the metabolically more active dihydrotestosterone, which has two isoforms; type I expressed in liver, skin and scalp by *SRD5A1* located on chromosome 5; and type II expressed in prostate by *SRD5A2* on chromosome 2. In human breast invasive ductal carcinomas, both type I (58% of 60 cases) and type II (15% of 60 cases) are reportedly expressed [11].

The *SRD5A2* gene consists of five exons and four introns, and encodes a 254 amino acid protein [12]. Several single nucleotide polymorphisms have been reported in the five exons [13]. However, only two of these polymorphisms (A49T and V89L), along with the variable number of dinucleotide TA repeat polymorphisms in the 3′ untranslated region, have been examined concerning risk and prognosis of cancers.

The V89L polymorphism that substitutes leucine for valine at codon 89 is reported to reduce almost 30% of androstanediol glucuronide, a serum marker of 5α-reductase activity, among Asians [14]. Among Caucasian men, a 10% insignificantly lower androstanediol glucuronide level was observed for individuals with the *L/L* genotype [15].

The TA repeat polymorphism reportedly has 10 alleles with 0, 8, 9, 10, 17, 18, 19, 20, 21, and 22 repeats, among which the 0-repeat allele (designated TA(*0*)) and the 9-repeat allele (designated TA(*9*)) are common. The alleles with more than 10 repeats were found exclusively in African-Americans, the highest risk ethnic group of prostate cancer [16]. No significant difference in serum androstanediol glucuronide level was reported between the TA(*0/0*) and TA(*0/9*) genotypes among the Chinese [17].

In the A49T polymorphism that substitutes threonine for alanine at codon 49, the *T* allele was reported to be the allele with the higher enzyme activity [18].

A49T, V89L and TA repeat polymorphisms could be associated with risk of breast cancer. To our knowledge, however, there have been no studies on the potential association between *SRD5A2* gene polymorphisms and breast cancer risk except for one study of the TA repeat polymorphism, which demonstrated no significant differ-

ence in the genotype distribution between 141 cases and 70 controls [19]. However, several studies for prostate cancer risk have been reported [17,18,20–26]. Two reports have been published by the same research group in Italy concerning roles in breast cancer prognosis, one regarding the TA repeats [19] and the other regarding the V89L polymorphism [27].

The present study aims to examine the associations between three polymorphisms of *SRD5A2* and breast cancer risk for Japanese women using a prevalent case–control study conducted at Aichi Cancer Center.

Materials and methods
Study population
The cases were female breast cancer patients visited at Aichi Cancer Center Hospital [28]. Between March 1999 and April 2000, 247 breast cancer cases were interviewed, and 243 were enrolled. Two patients refused to provide a blood sample after enrollment, and two blood samples were not stored. Among the 239 blood samples, two samples of extracted DNA had concentrations too low to be genotyped. The remaining 237 samples were used in the present study. The pathology of breast tumors was examined for 204 cases at Aichi Cancer Center Hospital, and for 33 cases at other hospitals.

The controls were 187 female cancer-free outpatients, mainly presenting at the clinics for gastroenterology, breast surgery, and gynecology of Aichi Cancer Center Hospital. They were also invited during March 1999 and April 2000. One serum sample was not stored, and one had too low a DNA concentration to be genotyped. Only 185 controls were therefore available.

All participants were given a self-administrated questionnaire. Information was requested on demography, family history of breast cancer (mother and/or sisters), and food intake before the appearance of symptoms. Interviewers checked all written responses to ensure that there were no unanswered questions at the time of questionnaire collection.

Genetic analyses
PCR amplification of the A49T polymorphism was conducted by a PCR with confronting two-pair primers method [29], using the following primers: 5′-GCG GAC ACG GGT GGC GTC-3′, 5′-GAA CCA GGC GGC GCG GGT-3′, 5′-GCG GCT ACC CGC CTG CCA G-3′, and 5′-CGC CGG GAG CAG GGC AGT-3′. Aliquots of 30–100 ng genomic DNA were mixed with 25 µl reaction liquid containing 0.18 mmol/l dNTPs, 12.5 pmol each primer, 0.5 units AmpliTaq Gold, and 2.5 µl GeneAmp 10 × PCR buffer with 15 mmol/l $MgCl_2$ (Perkin-Elmer Corporation, Foster City, CA, USA). Amplification conditions were set as follows: a 10-min initial denaturation at 95°C,

followed by 30 cycles at 95°C for 1 min denaturation, 64°C for 1 min annealing and 72°C for 1 min extension, and the final extension was at 72°C for 5 min. Genotyping was 403 and 209 bp for the alanine (*A*) allele, and 430 and 257 bp for the threonine (*T*) allele.

V89L polymorphisms were genotyped by a PCR-restriction fragment length polymorphism method described by Yamada *et al.* [24].

Genotypes of the TA repeat polymorphism were determined using the primers 5′-GCT GAT GAA AAC TGT CAA GCT-3′ and 5′-ACT CTA AGC AGA CAC CAC TCA G-3′, with PCR conditions the same as for the A49T polymorphism except for annealing at 54°C. Amplified DNA was 129 bp for the TA(*0*) allele and 147 bp for the TA(*9*) allele. Genotyping was confirmed for two samples of TA(*0/0*) and TA(*9/9*) by DNA sequencing.

Statistical methods
The Stata 7.0 software package (STATA Corp., College Station, TX, USA) was used to analyze the results, with the Pearson chi-square test employed to compare the distribution of characteristics between cases and controls. ORs and 95% CIs were estimated by unconditional logistic regression analysis.

Results
Characteristics of the study subjects
Our research included 237 female breast cancer cases and 185 female controls. The means and the standard deviations of age were 50.5 ± 8.5 years for cases and 52.9 ± 10.2 years for controls. The other characteristics of cases and controls are summarized in Table 1. No differences in the distributions were observed between cases and controls, except for the menopause state (women without menstruation caused by medication or surgery were included in the premenopause group if aged <50 years) and family history of breast cancer (mother and/or sisters).

Distributions of *SRD5A2* polymorphisms and crude ORs
Table 2 presents the distributions of *SRD5A2* polymorphisms. All subjects were found to have the *A/A* genotype for A49T polymorphism.

Two cases and two controls could not be genotyped for the V89L polymorphism. Frequencies of the *L/L*, *V/L* and *V/V* genotypes were 21.7, 52.3, and 26.0% for cases, and 32.8, 43.2, and 24.0% for controls, respectively. Compared with the *V/V* genotype, the *L/L* genotype demonstrated a marginally significant OR of 0.61 (95% CI = 0.36–1.05). Table 3 presents the results of the subgroup analysis. Although not significant, women with the *L/L* genotype had a reduced risk in any subgroup except those with a family history of breast cancer. There was no

Table 1

Characteristics of the study subjects

Characteristic	Cases ($n = 237$)	Controls ($n = 185$)	Chi-square	P value
Age *				
< 45 years	53	38		
≥ 45 years	184	147	0.20	0.65
Body mass index				
< 22 kg/m²	126	99		
≥ 22 kg/m²	111	86	0.005	0.94
Age at menarche				
< 14 years	113	92		
≥ 14 years	124	93	0.17	0.68
Age at first birth				
< 25 years	96	67		
≥ 25 years	112	98		
No birth	29	20	1.36	0.51
Number of births				
< 2	56	43		
≥ 2	181	142	0.009	0.93
Menopause state				
Premenopause	134	71		
Postmenopause	103	114	13.72	< 0.001
Alcohol				
< 1 day/week	184	148		
≥ 1 day/week	53	37	0.35	0.56
Smoking				
Noncurrent	211	169		
Current	26	16	0.63	0.43
Family history of breast cancer (mother and /or sisters)				
No	207	173		
Yes	30	12	4.42	0.04

* Age at diagnosis for cases and at study enrollment for controls.

difference in the OR between premenopausal women and postmenopausal women. The significance of OR was marginal among the body mass index (BMI) <22 group.

The present study found only two types of TA repeat alleles: TA(*0*) and TA(*9*). The frequencies of the TA(*0/0*), TA(*0/9*) and TA(*9/9*) genotypes were 75.9, 22.8, and 1.3% for cases, and 75.1, 22.7, and 2.2% for controls, respectively. No reduction in the OR was found for the TA(*0/9*) genotype, and women with genotype TA(*9/9*) were too few to be evaluated (Table 2). The difference in the OR was not observed between premenopausal women (OR = 0.85, 95% CI = 0.43–1.66 for TA(*0/9*) + TA(*9/9*) relative to TA(*0/0*)) and postmenopausal women (OR = 1.09, 95% CI = 0.59–2.01). Accordingly, no subgroup analysis was conducted.

SRD5A2 polymorphisms and risk factors
The associations of the polymorphism genotypes with age at menarche, age at menopause, and BMI were examined among the present controls. No associations were

Table 2

Genotype distributions of A49T, V89L and TA repeat polymorphisms

Genotype	Cases	Controls	Crude odds ratio	95% Confidence interval
A49T				
A/A	237 (100)	185 (100)	–	–
V89L				
V/V	61 (26.0)	44 (24.0)	1.00	Reference
V/L	123 (52.3)	79 (43.2)	1.12	0.70–1.81
L/L	51 (21.7)	60 (32.8)	0.61	0.36–1.05
V/L+L/L	174 (75.0)	139 (76.0)	0.90	0.58–1.41
TA repeats				
0/0	180 (75.9)	139 (75.1)	1.00	Reference
0/9	54 (22.8)	42 (22.7)	0.99	0.63–1.57
9/9	3 (1.3)	4 (2.2)	0.58	0.13–2.63
0/9 + 9/9	57 (24.1)	46 (24.9)	0.96	0.61–1.50

Percentages are shown in parentheses.

Table 3

Crude odds ratios and 95% confidence intervals for V89L polymorphism by subgroup

Subgroup	Genotype			
	V/V	*V/L*	*L/L*	*V/L + L/L*
Age at diagnosis				
< 45 years	1.00	0.71 (0.25–1.99)	0.45 (0.14–1.52)	0.62 (0.23–1.64)
≥ 45 years	1.00	1.28 (0.74–2.21)	0.70 (0.37–1.22)	1.00 (0.61–1.67)
Body mass index				
< 22 kg/m^2	1.00	1.06 (0.55–2.06)	0.48 (0.23–1.00)	0.80 (0.43–1.48)
≥ 22 kg/m^2	1.00	1.19 (0.59–2.38)	0.82 (0.37–1.79)	1.04 (0.54–1.99)
Age at menarche				
< 14 years	1.00	1.36 (0.67–2.75)	0.76 (0.35–1.66)	1.10 (0.56–2.13)
≥ 14 years	1.00	0.97 (0.50–1.88)	0.51 (0.24–1.09)	0.78 (0.42–1.43)
Age at first birth				
< 25 years	1.00	1.08 (0.49–2.38)	0.60 (0.25–1.44)	0.87 (0.42–1.82)
≥ 25 years	1.00	1.24 (0.64–2.42)	0.67 (0.31–1.42)	0.99 (0.53–1.85)
No birth	1.00	0.78 (0.18–3.36)	0.43 (0.08–2.17)	0.63 (0.16–2.46)
Number of births				
< 2	1.00	0.81 (0.27–2.38)	0.40 (0.12–1.32)	0.63 (0.23–1.74)
≥ 2	1.00	1.22 (0.71–2.08)	0.68 (0.37–1.25)	0.99 (0.60–1.63)
Menopause state				
Premenopause	1.00	1.01 (0.49–2.09)	0.67 (0.29–1.52)	0.88 (0.45–1.74)
Postmenopause	1.00	1.16 (0.60–2.23)	0.56 (0.27–1.18)	0.88 (0.48–1.62)
Alcohol				
< 1 day/week	1.00	1.19 (0.69–2.05)	0.66 (0.36–1.22)	0.97 (0.58–1.60)
≥ 1 day/week	1.00	0.94 (0.33–2.65)	0.48 (0.15–1.50)	0.72 (0.28–1.88)
Smoking				
Noncurrent	1.00	1.29 (0.78–2.13)	0.64 (0.36–1.12)	1.00 (0.63–1.59)
Current	1.00	0.16 (0.02–1.51)	0.20 (0.02–2.18)	0.17 (0.02–1.55)
Family history of breast cancer				
No	1.00	1.22 (0.73–2.03)	0.59 (0.33–1.05)	0.95 (0.59–1.52)
Yes	1.00	0.73 (0.15–3.50)	1.09 (0.19–6.20)	0.86 (0.21–3.54)

observed with mean ages at menarche and at menopause. Mean age at menarche (standard deviation) was 13.6 years (1.5 years) for the *V/V* genotype, 13.7 years (1.8 years) for the *V/L* genotype, 13.7 years (1.8 years) for the *L/L* genotype, 13.7 years (1.8 years) for the TA(*0/0*) genotype, 13.7 years (1.6 years) for the TA(*0/9*) genotype, and 13.5 years (2.4 years) for the TA(*9/9*) genotype. Among control women with natural menopause, the mean age at menopause (standard deviation) was 50.5 years (2.7 years) for the *V/V* genotype, 50.1 years (3.8 years) for the *V/L* genotype, 50.1 years (3.6 years) for the *L/L* genotype, 50.8 years (3.7 years) for the TA(*0/0*) genotype, 49.6 years (2.6 years) for the TA(*0/9*) genotype, and there were no postmenopausal controls with the TA(*9/9*) genotype.

The mean BMI (standard deviation) was also similar among the subgroups according to genotype except for four women with the TA(*9/9*) genotype: 22.5 (3.1), 22.2 (3.0), and 21.9 (2.9) for the V89L polymorphism, and 22.3 (3.0), 22.1 (3.1), and 19.7 (0.4) for the TA repeat polymorphism, respectively.

Relationship between V89L and TA repeat genotypes

The combined genotype frequency between TA repeat and V89L polymorphisms was also examined (Table 4). The *V/V* genotype among controls was 15.2% (21/138) for the TA(*0/0*) genotype, 46.3% (19/41) for the TA(*0/9*) genotype, and 100% (4/4) for the TA(*9/9*) genotype, while that among cases was 21.9% (39/178), 35.2% (19/54), and 100% (3/3), respectively. Fisher's exact test for 3 × 3 tables showed a significant association between the two genotype distributions both among cases and controls (*P* < 0.001).

ORs for the combination of V89L and TA repeat polymorphisms

Table 5 presents the ORs for each combination of the two polymorphisms relative to women with the *V/V* and

Table 4

Genotype distributions of V89L and TA repeat polymorphisms

TA genotype	V89L genotype (%)			Total	Fisher's exact *P* value
	V/V	*V/L*	*L/L*		
Cases					
TA(*0/0*)	39 (16.6)	88 (37.4)	51 (21.7)	178 (75.7)	
TA(*0/9*)	19 (8.1)	35 (14.9)	0 (0.0)	54 (23.0)	
TA(*9/9*)	3 (1.3)	0 (0.0)	0 (0.0)	3 (1.3)	
Total	61 (26.0)	123 (52.3)	51 (21.7)	235 (100.0)	< 0.001
Controls					
TA(*0/0*)	21 (11.5)	57 (31.2)	60 (32.8)	138 (75.4)	
TA(*0/9*)	19 (10.4)	22 (12.0)	0 (0.0)	41 (22.4)	
TA(*9/9*)	4 (2.2)	0 (0.0)	0 (0.0)	4 (2.2)	
Total	44 (24.0)	79 (43.2)	60 (32.8)	183 (100.0)	< 0.001

Percentages are shown in parentheses.

TA(*0/0*) genotypes, who had the highest risk of breast cancer. The combination of *L/L* and TA(*0/0*) had a significantly decreased risk, with an OR of 0.46 (95% CI = 0.24–0.88), and the combination of *V/V* and TA(*9/9*) showed the lowest, but insignificant, OR. The other three combinations indicated an intermediately reduced risk.

Discussion

Although one study reported that the effect of testosterone was cancelled by the adjustment of the estradiol level [30], several studies have provided epidemiological evidence that the serum level of testosterone is associated with the risk of breast cancer [2,4–6]. On the contrary, experimental data propose evidence that androgens are protective against breast cancer [7–10]. The present polymorphism study added the finding that the *SRD5A2* genotypes with a lower enzyme activity may reduce the breast cancer risk.

Table 5

Odds ratios and 95% confidence intervals for combinations of V89L and TA repeat polymorphisms

Combined genotype		Cases (*n* = 235)	Controls (*n* = 185)	Odds ratio (95% confidence interval)	
V89L	TA repeat			Crude	Adjusted*
V/V	*0/0*	39	21	1.00	1.00
V/L	*0/0*	88	57	0.83 (0.44–1.56)	0.78 (0.41–1.49)
L/L	*0/0*	51	60	0.46 (0.24–0.88)	0.45 (0.23–0.87)
V/V	*0/9*	19	19	0.54 (0.24–1.23)	0.49 (0.21–1.15)
V/L	*0/9*	35	22	0.86 (0.40–1.82)	0.91 (0.42–1.96)
V/V	*9/9*	3	4	0.40 (0.08–1.98)	0.34 (0.07–1.79)

Data presented as odds ratio (95% confidence interval). * Adjusting for family history of breast cancer and menopause state.

Since the difference in enzyme activity by the genotypes was explained in the Introduction, the potential impact on hormone concentrations should be discussed. Although hormone levels are determined by activities of several enzymes and the influence of the genotypes may differ between females and males, the V89L *L/L* genotype with low activity was found in Chinese men to be associated with a significantly higher concentration of testosterone, but not with dihydrotestosterone concentration [17]. In British men, the genotype had a significant association with a lower serum level of testosterone and free testosterone [15]. The present study suggests that the *L/L* genotype might decrease the risk for breast cancer, especially among women with BMI < 22 (Table 3). The *L/L* genotype was reported to be more frequent in Asian men (21.6%, $n = 102$) than in Caucasian men (4.1%, $n = 49$) and African-American men (3.2%, $n = 95$) [14], which may partly explain the low incidence in breast cancer among Asian women.

Of the TA repeat alleles, only TA(*0*) and TA(*9*) were observed in our subjects. The present genotype frequency for Japanese women was similar to those in Italy ($n = 70$; 79% for TA(*0/0*), 17% for TA(*0/9*), and 4% for TA(*9/9*)) [19], in the United States ($n = 802$; 75% for TA(*0/0*), 22% for TA(*0/9*), and 2% for TA(*9/9*)) [20], and in China ($n = 304$; 82% for TA(*0/0*), 17% for TA(*0/9*), and 1% for TA(*9/9*)) [17].

The present study demonstrated a tendency for risk reduction with the TA(*9/9*) genotype compared with the TA(*0/0*) genotype (Table 2). In a small-sized study with 141 cases and 70 controls in Italy, there was no significant association between the TA repeat polymorphism and risk of breast cancer [19], while the TA(*0/9*) or the TA(*9/9*) genotype demonstrates a reduction in the risk for relapse ($P = 0.043$). The combination analysis of TA repeat and V89L polymorphisms suggested that women with the TA(*0/0*) and *V/V* genotypes had the highest risk for breast cancer (Table 5). This is a plausible finding biologically, because the genotype is regarded to have the highest enzyme activity. There are no studies that examine the joint effect on prostate cancer.

The lack of alanine to threonine substitution in *SRD5A2* codon 49 in our subjects is in accordance with a previous study in Japan [24] and another in China [17], suggesting that the *T* allele may not exist among Asians. The reported frequency of the *T* allele was 1.0% of 522 alleles for African-American men and 2.3% of 400 alleles for Hispanic men [18]. In Finland, individuals with the *T* allele were 5.8% ($n = 588$) for donated blood and cancer-free autopsy samples [26]. The absence of the *T* allele among Asians may indicate that the polymorphism occurred relatively recently in comparison with V89L and TA repeat polymorphisms. For examining the effects of V89L and TA

repeat polymorphisms, our subjects had an advantage in that there was no need to consider the potential effect of A49T as a confounder or a modifier.

The present study demonstrates that the TA(*9*) allele only coexisted with the *V* allele (Table 4). The absence of the TA(*9/9*)–*L/L* genotype indicated a strong linkage disequilibrium. Another study in Italy unearthed the same result [27].

Conclusions

The present study suggests an reduced risk of breast cancer among women without the genotype combination of *SRD5A2 V/V* and TA(*0/0*). An absence of the genotypes necessitating the *L*–TA(*9*) haplotype indicated linkage disequilibrium between V89L and TA repeat polymorphisms. There appears to be no substitution of alanine to threonine in codon 49 among the Japanese. Since the metabolic pathway of steroid hormones is complicatedly regulated, activity of a single metabolic enzyme cannot solely describe the risk of breast cancer. In addition, to confirm the association observed in the present study, a systematic approach taking account of potentially relating polymorphisms is desirable in the near future.

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