

# TA Repeat Polymorphism of the 5 $\alpha$ -Reductase Gene and Breast Cancer

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## Abstract

There is increasing evidence that androgens play a significant role in the development and progression of breast cancer. 5 $\alpha$ -Reductase (SRD5A2) is an enzyme that is expressed in androgen-dependent tissues, and it catalyzes the reduction of testosterone to its more bioactive form, dihydrotestosterone, which then transactivates a number of genes. One of these genes encodes for prostate-specific antigen (PSA), a favorable prognostic factor in breast cancer. The 3' untranslated region of the SRD5A2 gene contains either no TA repeats [(TA)<sub>0</sub>] or 9 [(TA)<sub>9</sub>] or 18 [(TA)<sub>18</sub>] repeats. Variations in the length of these dinucleotide repeats have been reported to influence the enzymatic activity of SRD5A2.

In this study, we determined the TA genotypes in DNA from 141 well-characterized breast tumors and in DNA from whole blood of 70 women without cancer. The presence of TA genotypes was then associated with tumor cytosolic PSA concentrations and with clinicopathological variables, including disease-free survival and overall survival. Three genotypes, (TA)<sub>0</sub> homozygote, (TA)<sub>0</sub>/(TA)<sub>9</sub> heterozygote, and (TA)<sub>9</sub> homozygote, were identified. No (TA)<sub>18</sub> alleles were detected in any of the two patient groups. A statistically significant association between high PSA concentrations and (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> genotypes was observed ( $P = 0.004$ ). (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> genotypes were found less frequently in patients at stage III or IV disease. TA genotypes were not associated with other clinicopathological variables by contingency table analysis. Patients with (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> repeats, when compared to those with genotypes homozygous for the (TA)<sub>0</sub> allele, showed a significant reduction in the risk for relapse ( $P = 0.043$ ). Long-term studies are needed to investigate the relevance of this polymorphism to breast cancer susceptibility.

## Introduction

The molecular mechanisms involved in the initiation and progression of breast cancer are poorly understood. Although the female breast is not generally thought to be an androgen-regulated organ, there is evidence that androgens may play a role in the development and progression of breast cancer. A number of studies have shown that increased androgen production correlates with an enhanced predisposition to contract the disease (1–4). Nearly 60% of women with breast cancer show some degree of hypertestosteronemia (5). Excessive androgen production has also been observed in subsets of women with atypical breast duct hyperplasia, a precursor of breast cancer (6). Prospective studies have also shown that increasing circulating levels of androgens precede the onset of breast cancer (4, 7). A direct role for androgens is supported by the finding of detectable ARs<sup>2</sup> in up to 90% of breast cancer specimens (8, 9) and accumulation of androgens in breast duct fluid (10), in normal and cancerous breast tissue (11, 12), and in breast apocrine cysts (13).

An enzyme expressed in androgen-dependent tissues, 5 $\alpha$ -reductase (SRD5A2) (14), may therefore play a significant role in breast cancer, considering its function in the conversion of testosterone to its more active and potent form, DHT (15). The gene encoding for this enzyme consists of five exons and four introns and in humans, it maps on chromosome 2p23, and it encodes for a 254-amino acid protein (16). DHT binds to the AR, and the DHT-AR complex so formed, transactivates a number of genes by binding to their androgen responsive elements (17). One such gene encodes for PSA, a 30-kDa glycoprotein (18). PSA is predominantly expressed in luminal epithelial cells of the prostate, and it has been established as the most useful serum marker for early detection and management of prostate cancer (19). Interestingly, PSA has also been detected in several other normal and malignant tissues, including breast, ovarian, colon, liver, adrenal, and salivary glands (20). High levels of PSA protein in breast tumor tissues are associated with steroid hormone receptor positivity, early disease stage, and other clinical and pathological features consistent with a favorable prognosis (21, 22).

Davis and Russell (23) have shown that a length polymorphism of TA dinucleotides exists in the 3' untranslated region of the SRD5A2 gene. This region of the gene consists primarily of variable numbers of TA dinucleotide repeats: (TA)<sub>0</sub>, (TA)<sub>9</sub>, and (TA)<sub>18</sub>, respectively. Although there is some minor variation in the exact number of repeats, these numbers adequately describe the three clusters of the lengths. (TA)<sub>0</sub> repeat lengths (*i.e.*, zero number of repeats) are the most common, and the (TA)<sub>18</sub> alleles (*i.e.*, 18 TA repeats) are rare, found exclusively in African-Americans (24). Increases in the lengths of these repeats have been associated with decreased intraprostatic

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<sup>2</sup> The abbreviations used are: AR, androgen receptor; DHT, dihydrotestosterone; PSA, prostate-specific antigen; DFS, disease-free survival; OS, overall survival; ER, estrogen receptor; PR, progesterone receptor; RR, relative risk; CI, confidence interval.

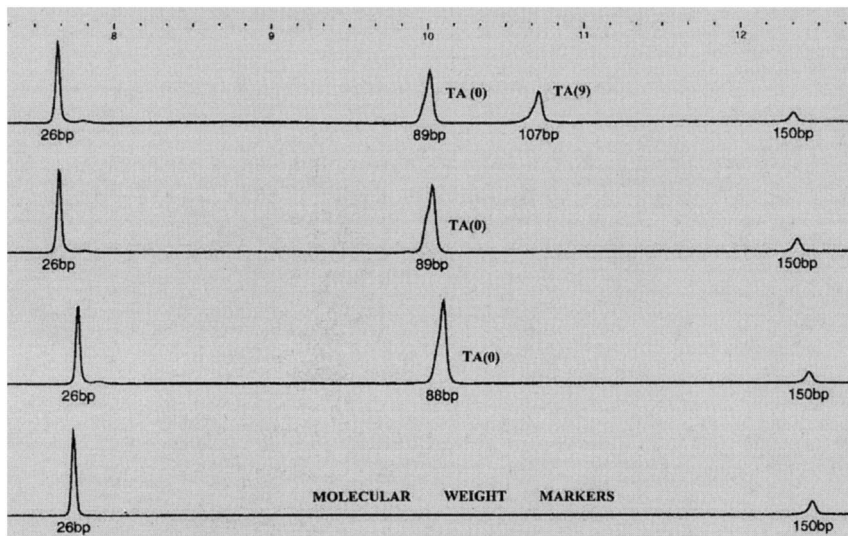


Fig. 1. Representative DNA fragment analysis of three patients and one molecular weight standard run (last lane). The various TA length alleles are shown. The first patient is heterozygous (TA)<sub>0</sub>/(TA)<sub>9</sub>. Numbers below peaks, PCR product length in bp.

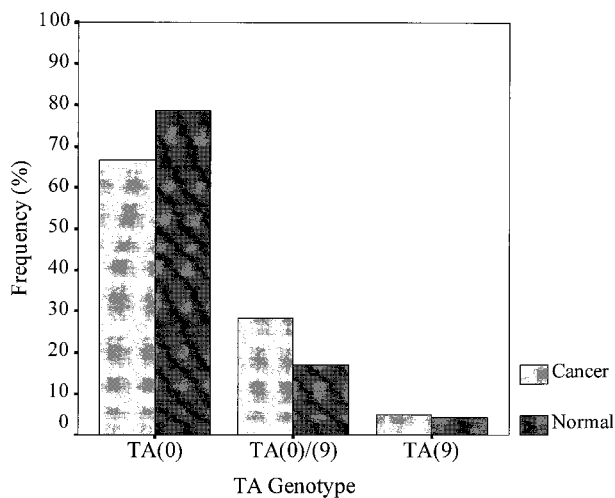


Fig. 2. Distribution of TA genotypes in DNA from breast carcinomas and in DNA from leukocytes from females without breast cancer. As determined by the  $\chi^2$  test, the differences were not statistically significant ( $P = 0.15$ ). (TA)<sub>0</sub>, no TA repeats present; (TA)<sub>9</sub>, nine TA repeats present in the 3' untranslated region of the SRD5A2 gene.

SRD5A2 activity (25), which argues for decreased risk for prostate cancer in these men. TA-rich sequences in the untranslated regions of other genes have been associated with mRNA instability (26), which in turn, affects protein synthesis.

Based on the previous observations that androgens may be implicated in breast cancer, we undertook this study to investigate the possible involvement of the TA repeats in the progression of breast cancer. We investigated the relation of the TA repeats with PSA concentration in 141 breast cancer cytosols. TA genotypes were compared with other established prognostic factors in terms of DFS and OS using univariate and multivariate analysis.

## Materials and Methods

**Samples.** Included in this study were tumor specimens from 141 breast cancer patients and whole blood samples from 70

healthy women as controls. The patients underwent surgical treatment for primary breast carcinoma at the Department of Gynecological Oncology at the University of Turin (Turin, Italy) during the period from January 1988 to December 1992. Tumor tissue had been frozen in liquid nitrogen immediately after surgery. The selection criteria for the specimens included the availability of sufficient tissue mass for extraction and assay; the eligible patients represented ~60% of new cases of breast cancer diagnosed and treated at the above institution during the accrual period. Whole blood samples from healthy women collected in EDTA-containing evacuated tubes for routine hematological evaluation were obtained from women attending outpatient clinics at the same department. Individuals with documented evidence or suspicion of malignancy, as indicated by medical chart review, were excluded, leaving 70 in the study. This study has been approved by the Institutional Review Board of the University of Turin.

The median age of the patients was 55 years, with a range from 25 to 93 years. The ages of healthy women ranged from 50 to 79 years; the median age was 59 years, close to the median of the breast cancer group (55 years). All patients had a histologically confirmed diagnosis of primary breast cancer and received no treatment before surgery. Modified radical mastectomy with axillary lymph node dissection was performed on 95% of the patients. For the patients who had axillary node dissection, the positivity rate for cancer involvement of lymph nodes was 62%. The sizes of the tumors resected during surgery ranged from 0.8 to 7.0 cm, and the mean and median sizes were 2.7 cm and 2.5 cm, respectively. Pathological staging was performed according to the Postsurgical International Union Against Cancer Tumor-Node-Metastasis classification system (27). Of 140 patients for whom the stage was known, 42 (30.0%), 80 (57.1%), 7 (5.0%), and 11 (7.9%) had stages I, II, III, and IV, respectively. Histological grade of the tumors was determined according to criteria reported by Bloom and Richardson (28) and was known for 99 patients: 6 patients (6.1%) had grade I, 55 patients (55.5%) had grade II, and 38 patients (38.4%) had grade III. Most of the tumors (70%) were of invasive ductal histological type, whereas the remaining tumors were invasive lobular (13.5%), ductal *in situ* (2.1%),

Table 1 Associations of TA genotypes with features of breast cancer

Features	Total patients	No. of patients (%)		P
		(TA) <sub>0</sub>	(TA) <sub>0</sub> /(TA) <sub>9</sub> , (TA) <sub>9</sub>	
Age (yr)				
<45	35	23 (24.5)	12 (25.5)	0.65 <sup>a</sup>
45–55	36	22 (23.4)	14 (29.8)	
>55	70	49 (52.1)	21 (44.7)	
Tumor size (cm)				
<2	40	26 (28.3)	14 (30.4)	0.47 <sup>b</sup>
≥2	98	66 (71.7)	32 (69.6)	
Nodal status				
Negative	52	31 (34.8)	21 (45.6)	0.10 <sup>b</sup>
Positive	83	58 (65.2)	25 (54.4)	
Grade <sup>c</sup>				
I–II	61	44 (65.7)	17 (53.1)	0.16 <sup>b</sup>
III	38	23 (34.3)	15 (46.9)	
Histology				
Ductal	98	65 (69.2)	33 (70.2)	0.86 <sup>a</sup>
Lobular	19	12 (12.7)	7 (14.9)	
Other	24	17 (18.1)	7 (14.9)	
Stage <sup>d</sup>				
I	42	23 (24.7)	19 (40.4)	0.079 <sup>a</sup>
II	80	55 (59.2)	25 (53.2)	
III–IV	18	15 (16.1)	3 (6.4)	
ER status <sup>e</sup>				
Negative	46	31 (34.4)	15 (31.9)	0.46 <sup>b</sup>
Positive	91	59 (65.6)	32 (68.1)	
PR status <sup>e</sup>				
Negative	50	31 (34.4)	19 (41.3)	0.27 <sup>b</sup>
Positive	86	59 (65.6)	27 (58.7)	
PSA status <sup>f</sup>				
Negative	119	85 (91.4)	34 (72.3)	0.004 <sup>b</sup>
Positive	21	8 (8.6)	13 (27.7)	
Adjuvant treatment				
None	41	26 (27.7)	15 (31.9)	0.63 <sup>a</sup>
Tamoxifen	65	46 (48.9)	19 (40.4)	
Chemotherapy ± tamoxifen	35	22 (23.4)	13 (27.7)	

<sup>a</sup>  $\chi^2$  test.<sup>b</sup> Fisher's exact test.<sup>c</sup> Bloom-Richardson grading system.<sup>d</sup> TNM system.<sup>e</sup> Cutoff point: 10 fmol/mg protein.<sup>f</sup> Cutoff point: 60 ng/g protein, equal to the 75th percentile of the distribution of PSA concentrations.

medullary (2.1%), papillary (2.1%), tubular (2.1%), inflammatory (2.8%), tubulolobular (1.4%), cribriform (2.8%), Paget (0.7%), and muciparous (0.7%). Postoperative treatment was known for all patients. Whereas 29% received no further treatment after tumor resection, 25% were given adjuvant chemotherapy only, 41% were treated with endocrine therapy only, and 5% were given both chemotherapy and endocrine therapy. Disease relapse was defined as the first documented evidence of local or regional axillary recurrence or distant metastasis.

Follow-up information was available for all patients and included survival status (alive or deceased) and disease status (disease-free or recurrence/metastasis) along with the dates of the events and cause of death, if applicable. Follow-up of patients continued from time of surgery until death, loss to follow-up, or until date of analysis. The distribution of follow-up times for patients still alive at the time of analysis or lost to follow-up ranged from 22 to 120 months, with a median of 80 months; only seven and two patients had been followed for <48 and 36 months, respectively. Follow-up times for the

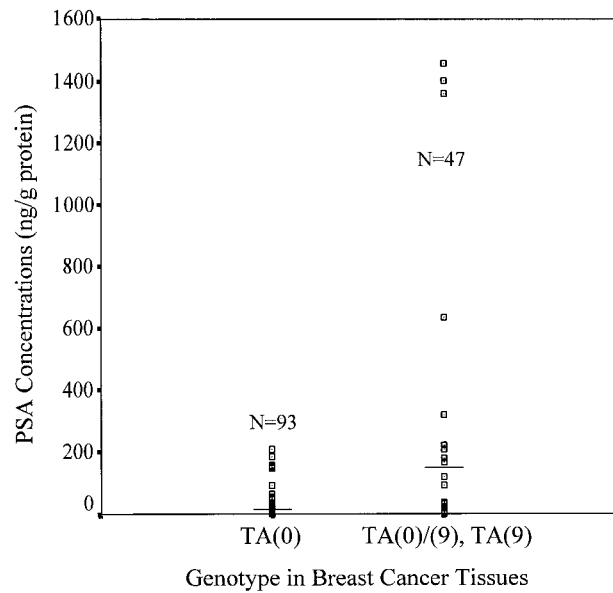


Fig. 3. Relationship between TA genotypes and breast tumor extract PSA concentrations. —, mean value. The medians were 7.1 ng/g and 10.2 ng/g for (TA)<sub>0</sub> and (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> genotypes, respectively; P = 0.041, as determined by the Mann-Whitney U test.

entire cohort, therefore, ranged from 6 to 125 months and had a median of 71 months. The relapse-free survival time in each case was the time interval between the date of surgical removal of the primary cancer and the date of the first documented evidence of relapse. The OS time was the time interval between the date of surgery and the date of death, or the date of last follow-up for those who were alive at the end of the study. During their respective follow-up periods, 50 patients (34.7%) developed cancer relapse and 35 (25%) died.

**DNA Extraction.** DNA was extracted from tissues and whole blood specimens using the Qiagen QIAmp blood and tissue DNA extraction kit (Qiagen, Chatsworth, CA). Approximately 25 mg of tissue or 200  $\mu$ l of blood were used for each extraction. The breast tumor tissue, which contained >70% tumor cells, as determined by histological examination, was pulverized into a fine powder and stored at  $-80^{\circ}\text{C}$  until the extraction procedure. Briefly, after cell lysis, the DNA was entrapped onto a silica membrane, washed and eluted into a buffer solution, quantified by absorbance measurements at 260 nm, and stored at  $4^{\circ}\text{C}$  until analysis.

**PCR Amplification.** The paired primer sequences (5'-GCT-GATGAAAAGTCAAGCTGCTGA-3' and 5'-GCCAGCT-GGCAGAACGCCAGGAGAC-3') flanking the TA repeat region in the 3' untranslated region of the SRD5A2 gene were designed based on the SRD5A2 sequence deposited in GenBank by Labrie *et al.* (Ref. 16; GenBank accession no. L03843).

The sense primer was fluorescently labeled with Cy5.5 dye as described elsewhere (29). PCR amplification of DNA was performed in a final volume of 25  $\mu$ l containing  $\sim$ 100 ng of DNA template, 10 mM "tris" (tris-hydroxymethyl-aminomethane) buffer (pH 8.3), 50 mM KCl, 2.5 units of *Taq* polymerase (Boehringer Mannheim GmbH, Mannheim, Germany), 250  $\mu$ M deoxynucleoside triphosphates, 1.5 mM  $\text{MgCl}_2$ , and 10 pmol of each of the primers. The thermal cycling profile consisted of a 30-s denaturation step at  $95^{\circ}\text{C}$

Table 2 Associations of TA genotypes with breast cancer survival

Variable	DFs		OS	
	RR <sup>a</sup> (95% CI) <sup>b</sup>	P	RR <sup>a</sup> (95% CI) <sup>b</sup>	P
Univariate analysis				
(TA) <sub>0</sub>	1.00		1.00	
(TA) <sub>0</sub> /(TA) <sub>9</sub> , (TA) <sub>9</sub>	0.50 (0.26–0.98)	0.043	0.53 (0.24–1.18)	0.12
Multivariate analysis <sup>c</sup>				
(TA) <sub>0</sub>	1.00		1.00	
(TA) <sub>0</sub> /(TA) <sub>9</sub> , (TA) <sub>9</sub>	0.58 (0.29–1.16)	0.13	0.54 (0.24–1.23)	0.14

<sup>a</sup> RR estimated from Cox proportional hazards regression model.

<sup>b</sup> CI of the estimated RR.

<sup>c</sup> Multivariate models were adjusted for lymph nodes status, tumor size, patient age, and ER and PR expression.

and a 60-s annealing step at the optimized temperature of 68°C for a total of 25–30 cycles. Each PCR was initiated with a 5-min denaturation step at 95°C and terminated with a 10-min extension at 68°C. The number of PCR cycles was adjusted (decreased or increased) if the intensities of the fragment bands were relatively high or low, respectively. The success of the PCR was verified by running an 8- $\mu$ l aliquot of the PCR product on a 0.8% agarose gel containing ethidium bromide. Fluorescently labeled standard size markers, 26 bp and 150 bp (Visible Genetics Inc., Toronto, Ontario, Canada), were used as sizing standards.

**Fragment Analysis.** Two  $\mu$ l of the PCR product were mixed with 4  $\mu$ l of a gel loading buffer (Visible Genetics) to which we also added 2  $\mu$ l each of the two molecular weight markers. This mixture was denatured at 95°C for 2 min, placed on ice, and then loaded onto the polyacrylamide gel for fragment analysis in the MicroGene Blaster (Visible Genetics Inc.) automated sequencer. More detailed description of the Visible Genetics sequencing and fragment analysis system has been recently published (29).

**Steroid Hormone Receptor Analyses.** Tumor specimens ( $n = 141$ ) were pulverized in liquid nitrogen and homogenized in buffer, and the cytosolic fractions were obtained by ultracentrifugation and quantified for steroid hormone receptors by a ligand-binding assay. The results were interpreted by Scatchard analysis (30). Protein concentrations of the cytosols were determined by the Lowry method (31). Tumors with ER and PR concentrations  $\leq 10$  fmol/mg protein were considered as receptor negative, whereas tumors with receptor concentrations above such values were considered positive, as followed previously (21). Based on these cutoffs, 91 (66.4%) and 86 (63.2%) of 137 and 136 breast carcinomas were ER- and PR-positive, respectively.

**PSA Immunoassay.** Approximately 10–50 mg of the pulverized tumor tissues were combined with 500  $\mu$ l of a cell lysis buffer containing 50 mmol/liter Tris (pH 8.0), 150 mmol/liter NaCl, 5 mmol/liter EDTA, 10 g/liter Nonidet NP-40 surfactant, and 1 mmol/liter phenylmethylsulfonyl fluoride and incubated for 30 min on ice. After centrifugation of the extracts at 15,000 g for 30 min, the supernatants were assayed, in duplicate, for total PSA concentration by an ultrasensitive time-resolved immunofluorometric method as described elsewhere (32). The PSA assay has a detection limit of 0.001 ng/ml. PSA concentrations were adjusted for total protein content, as determined by the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL), and are expressed as ng of PSA per g of extracted protein.

**Statistical Analysis.** Relationships between TA genotypes and other categorical variables were analyzed using the  $\chi^2$  test as well as the Fisher's exact test where necessary. ER and PR values were categorized into positive and negative status, as described above. Tumor size was categorized as  $< 2$  cm or  $\geq 2$  cm. Lymph node status was either positive (histological evidence of tumor extension to one or more lymph nodes) or negative. Age was categorized into three groups:  $< 45$  years, 45–55 years, and  $> 55$  years. The analysis of differences in PSA values between two groups of TA genotypes was performed with the nonparametric Mann-Whitney  $U$  test. PSA was categorized as  $< 60$  ng/g or  $\geq 60$  ng/g of total protein. Fisher's exact test was used for statistical evaluation of PSA as a categorical variable. Kaplan-Meier DFS and OS curves (33) were performed to calculate the risk for the effect of TA genotype on breast cancer survival. The differences between the curves were evaluated by the log-rank test. Cox regression analyses using the SAS statistical software (SAS Institute, Cary, NC) was used to calculate the RR and 95% CI. The multivariate models were adjusted for lymph node status, tumor size, patient age, and ER and PR expression. Only patients for whom the status of all these variables was known were included in the univariate models, as well as in the multivariate models, which incorporated TA genotypes and all other variables for which the patients were characterized.

## Results

**Distribution of TA Genotypes.** Fragment analysis of the 3' untranslated region of the 5 $\alpha$ -reductase gene using the outlined methodology distinguishes three genotypes: (TA)<sub>0</sub> (no repeats present), (TA)<sub>0</sub>/(TA)<sub>9</sub>, and (TA)<sub>9</sub> (nine TA repeats), as shown in the examples of Fig. 1. Of the 70 women without cancer, 55 (79%) were homozygous (TA)<sub>0</sub>, 12 (17%) were heterozygous (TA)<sub>0</sub>/(TA)<sub>9</sub>, and 3 (4%) were homozygous (TA)<sub>9</sub>. Among the breast cancer patients, 94 (67%) were homozygous for (TA)<sub>0</sub>, 40 (28%) were heterozygous (TA)<sub>0</sub>/(TA)<sub>9</sub>, and 7 (5%) were homozygous for the (TA)<sub>9</sub> genotype. (TA)<sub>18</sub> repeat lengths were not detected in any of the samples in the present study. No statistically significant difference ( $\chi^2 = 3.37$ ;  $P = 0.18$ ) was found between the genotype's distributions in the cancer and non-cancer groups (Fig. 2).

**Relationships of TA Genotypes to Other Prognostic Variables.** Due to rarity of the (TA)<sub>9</sub> genotype in this population, women with the (TA)<sub>9</sub> genotype were combined with (TA)<sub>0</sub>/(TA)<sub>9</sub> for the remaining analyses. The distributions of TA genotypes [(TA)<sub>0</sub> and (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub>] between subgroups of patients differing by age, tumor size, nodal status, grade, histological type, disease stage, ER status, PR status,



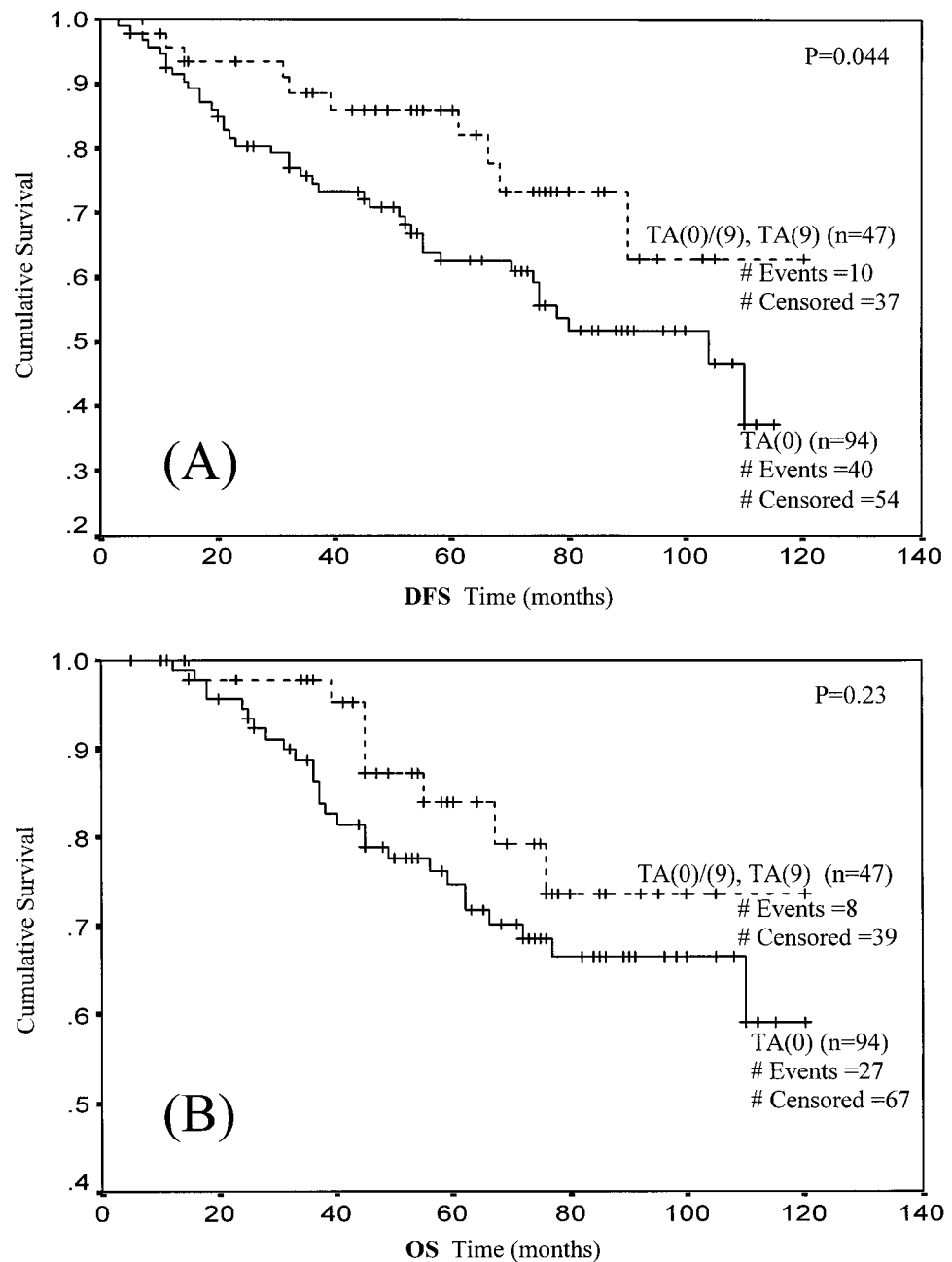


Fig. 4. DFS (A) and OS (B) curves in breast cancer patients with  $TA_0$  and  $(TA)_0/(TA)_0$  or  $(TA)_0$  genotypes, respectively. Vertical tick marks, the censored cases. Differences between genotypes were determined by log-rank tests.

PSA status, and adjuvant treatment administered were examined by  $\chi^2$  and Fisher's exact test where necessary (Table 1). The differences in TA genotype frequencies by stage or nodal status were not found to be statistically significant ( $P = 0.079$  and  $P = 0.10$ , respectively). Nevertheless, the  $(TA)_0$  genotype was found more frequently in patients with stage III-IV. Of 18 patients with stage III-IV disease, 15 were homozygous for  $(TA)_0$  genotype, whereas only 3 were heterozygous  $(TA)_0/(TA)_0$  and none had the  $(TA)_0$  genotype. Differences in tumor cytosol PSA concentrations between the TA genotypes were found to be statistically significant by the Mann-Whitney  $U$  test ( $P = 0.041$ ; Fig. 3). When PSA values were classified into two categories using a cutoff point of 60 ng/g protein, equal to the 75th percentile of the

distribution of PSA concentrations, a strong association ( $P = 0.004$ ) between PSA status and TA genotypes was observed (Table 1). Statistically significant associations between TA genotype and patient age, tumor size, grade, histological type, and steroid hormone receptors were not found. In the same analysis, TA genotypes were shown not to differ between patients who received different postoperative treatment modalities.

**Relationship of TA Polymorphism to Patient Survival.** Univariate Cox regression models were developed to evaluate the effect of TA genotypes on DFS and OS for breast cancer patients (Table 2). These regression models demonstrated a reduction in risk for relapse in patients with the  $(TA)_0/(TA)_0$  or  $(TA)_0$  genotype compared to those with the  $(TA)_0$  genotype

(RR, 0.5; 95% CI, 0.26–0.98). The Kaplan-Meier survival curves (Fig. 4) also show that (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> patients had better disease-free rates than (TA)<sub>0</sub> patients. The survival plots also demonstrate the tendency of (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> patients to have better OS than (TA)<sub>0</sub> patients, but the difference was not statistically significant. In the multivariate analysis of TA genotypes, the Cox regression models were adjusted for age, nodal status, tumor size, and ER and PR status, all of which were used as categorical variables. Tumor grade was not included in the multivariate analysis because of the relatively large number of patients for which this variable was unknown. Whereas patient age, tumor size, and nodal status were shown to be independent factors for predicting both DFS and OS, TA genotypes did not significantly add to the prognostic power in the multivariate models.

### Discussion

This study was undertaken to investigate whether TA dinucleotide repeats in the 3' untranslated region of the SRD5A2 gene have prognostic value in primary breast carcinoma. We found a positive relationship between (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> genotype and PSA levels (a favorable prognostic marker). Longer TA repeats appear to be favorable prognostic indicators in breast cancer patients. Presently, only two studies have addressed the relationship between TA alleles in prostate tumor tissue and risk of developing prostate cancer (24, 25, 34). This is the first report indicating that TA polymorphism in the 3' untranslated region of the SRD5A2 gene has prognostic value in breast cancer.

The role of the SRD5A2 enzyme in prostate cancer is evident from biological and epidemiological studies. Androgens are known to play an important role in prostate cancer development and progression, and SRD5A2 may be involved through increased production of DHT (35). However, very little is known about the physiological involvement of this enzyme in breast cancer, which is also a hormonally dependent cancer. Besides PSA, two other proteins expressed in breast tumor tissues, pepsinogen C and pS2, have also been shown to be favorable prognostic indicators (36–40). These proteins are also regulated by the steroid hormone receptor system. The increased levels of PSA observed in (TA)<sub>0</sub>/(TA)<sub>9</sub> or (TA)<sub>9</sub> genotypes (Fig. 3) may be due to increased activity of the 5- $\alpha$ -reductase enzyme in breast tissues, which results in increased DHT production. It is possible that certain enzyme variants encoded by the SRD5A2 gene, including those with variable TA repeats, may have increased enzyme activity, which facilitates higher DHT production and PSA expression (24). This explanation is based on prostate cancer studies showing that longer TA repeat lengths may decrease the activity of this enzyme, lowering the DHT production and thereby decreasing PSA production (24). In our study, longer repeats appear to associate better with hyperandrogenism if we accept PSA expression to be a marker of this condition. Elevated PSA in breast cytosols is a favorable prognostic factor for breast cancer patients (21, 22). It is evident from this study that longer TA repeats are associated with breast tumors that have higher PSA content (Table 1 and Fig. 3). Based upon these observations, we speculate that the longer repeats in the case of breast tumors up-regulate the SRD5A2 activity, in contrast to the published data for prostate cancer.

The genotype distribution shows a 12% decrease in the (TA)<sub>0</sub> repeats and an 11% increase in the (TA)<sub>0</sub>/(TA)<sub>9</sub> repeats in the breast cancer group over the leukocyte controls. Al-

though the samples in this study were not matched, the occurrence of somatic mutations cannot be overlooked. Recently, Akalu *et al.* (41) have published interesting findings from matched samples of constitutional (germ-line) DNA from peripheral blood lymphocytes and microdissected pure DNA from prostate tumors. These authors report somatic mutations and microsatellite instability (both expansions and contractions) in 57% of their samples at a polymorphic TA(n) dinucleotide repeat marker in the 3' untranslated region of the SRD5A2 gene. Loss of heterozygosity was also prevalent in cancer tissues with this marker. It is believed that such molecular mechanisms can lead to cytogenetic abnormalities, which in turn can alter the 5 $\alpha$  reductase activity. We cannot rule out if similar events take place in breast cancer tissue and thereby, up-regulate the activity of the reductase enzyme. Our ongoing studies with matched samples may, therefore, provide some explanations to this effect.

The TA repeats are located in the 3' noncoding region of the SRD5A2 gene, and this polymorphism may not directly affect the function of the protein. Similar TA-rich regions and other dinucleotide repeats in the 3' untranslated region of other genes have been associated with mRNA instability (26). Hence, the variation in the lengths of TA dinucleotides may be related to RNA instability, resulting in altered amounts of enzyme produced. This change may be small, but because this polymorphism is heritable, the effects will be present throughout the individual's life, and the cumulative outcome could be significant. It will be interesting to determine in parallel the TA repeat allele genotype and the SRD5A2 activity in breast cancer tissues to address more precisely the biological implications of this polymorphism.

In conclusion, our data support the view that longer TA repeats in the 3' untranslated region of the 5 $\alpha$ -reductase gene are associated with a more favorable outcome of breast cancer patients. Further studies are necessary to establish the value of this polymorphism, in combination with others markers, for prognosis and to examine if this genetic polymorphism is a risk factor for developing breast cancer.

### References

- Grattarola, R. Androgens in breast cancer. Atypical endometrial hyperplasia and breast cancer in married pre-menopausal women. *Am. J. Obstet. Gynecol.*, *116*: 423–8, 1973.
- Grattarola, R., Secreto, G., and Recchione, C. Androgens in breast cancer. Breast cancer recurrences years after mastectomy and increased androgenic activity. *Am. J. Obstet. Gynecol.*, *121*: 169–72, 1975.
- Key, T. J., and Pike, M. C. The role of estrogens and progestogens in the epidemiology and prevention of breast cancer. *Eur. J. Cancer Clin. Oncol.*, *24*: 29–43, 1988.
- Canley, J. A., Lucas, L. F., Kuller, L. H., Stone, K., Browner, W., and Cummings, S. R. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. *Ann. Intern. Med.*, *130*: 270–277, 1999.
- Secreto, G., Fariselli, G., Bandieramonte, G., Recchione, C., Dati, V., and Di Pietro, S. Androgen excretion in women with a family history of breast cancer or with epithelial hyperplasia or cancer of the breast. *Eur. J. Cancer Clin. Oncol.*, *19*: 5–10, 1983.
- Stoll, B. A., and Secreto, G. New hormone-related markers of high risk to breast cancer. *Ann. Oncol.*, *3*: 435–438, 1992.
- Berrino, F., Muti, P., Micheli, A., Bolelli, G., Krogh, V., Sciajno, R., Pisani, P., Panico, S., and Secreto, G. Serum sex hormone levels after menopause and subsequent breast cancer. *J. Natl. Cancer Inst.*, *88*: 291–296, 1996.
- Bryan, R. M., Mercer, R. J., and Bennet, R. Androgen receptors in breast cancer. *Cancer (Phila.)*, *54*: 2436–2440, 1984.
- Soreide, J. A., Lea, O. A., Varhaug, J. E., Skarstein, A., and Kvinnsland, S. Androgen receptors in operable breast cancer: relation to other steroid hormone receptors, correlation to prognostic factors and predictive value for the effect of adjuvant tamoxifen treatment. *Eur. J. Surg. Oncol.*, *18*: 112–118, 1992.

10. Hill, P., Garbaczewski, L., and Wynder, E. L. Testosterone in breast fluid. *Lancet*, *1*: 761, 1983.
11. Vermeulen, A., Deshyperre, J. P., Paridaeus, R., Leclercq, G., Roy, F., and Heuson J. C. Aromatase 17- $\beta$ -hydroxysteroid dehydrogenase and intratissular concentrations in cancerous and normal glandular breast tissue in post menopausal women. *Eur. J. Cancer Clin. Oncol.*, *22*: 515–525, 1986.
12. Secreto, G., Venturelli, E., Bucci, A., Piromalli, D., Fariselli, G., and Galante, E. Intratumor amount of sex steroids in elderly breast cancer patients. An approach to the biological characterisation of mammary tumours in elderly. *J. Steroid Biochem. Mol. Biol.*, *5*: 557–561, 1996.
13. Silver, Z. R. I., Wiley, E. L., Thigpen, A. E., Guileyardo, J. M., McConnell, J. D., and Russell, D. W. Cell specific expression of steroid 5 $\alpha$  reductase 2. *J. Urol.*, *152*: 438–442, 1992.
14. Wilson, J. D., Griffin, J. E., and Russell, D. W. Steroid 5 $\alpha$  reductase deficiency. *Endocrinol. Rev.*, *14*: 577–593, 1993.
15. Anderssen, S., Berman, D. M., Jenkins, E. P., and Russell, D. W. Deletion of steroid 5 $\alpha$  reductase 2 gene in male pseudohermaphroditism. *Nature (Lond.)*, *354*: 59–61, 1991.
16. Labrie, F., Sugimoto, Y., Luu-The, V., Simard, J., Lachance, Y., Bachvarov, D., LeBlanc, G., Durocher, F., and Paquet, N. Structure of human type II 5 $\alpha$  reductase gene. *Endocrinology*, *131*: 1571–1573, 1992.
17. Beato, M., and Sanchez-Pacheco, A. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr. Rev.*, *17*: 587–609, 1996.
18. Diamandis, E. P. Prostate specific antigen-its usefulness in clinical medicine. *Trends Endocrinol. Metab.*, *9*: 310–316, 1998.
19. Oesterling, J. E. Prostate specific antigen, a critical assessment of most useful tumor marker for adenocarcinoma of the prostate. *J. Urol.*, *145*: 907–923, 1991.
20. Diamandis, E. P., and Yu, H. Non prostatic sources of prostatic-specific antigen. In: J. E. Oesterling (ed.), *The Urologic Clinics of North America: Prostate-specific Antigen: the Best Prostatic Tumor Marker*, Vol. 24, pp.275–282. Philadelphia: W. B. Saunders Co., 1997.
21. Yu, H., Giai, M., Diamandis, E. P., Katsaros, D., Sutherland, D. J. A., and Levesque, M. A. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. *Cancer Res.*, *55*: 2104–2110, 1995.
22. Yu, H., Levesque, M. A., Clark, G. M., and Diamandis, E. P. Prognostic value of prostate specific antigen for women with breast cancer: a large United States cohort study. *Clin. Cancer Res.*, *4*: 1489–1497, 1998.
23. Davis, D. L., and Russell, D. W. Unusual length polymorphism in human steroid 5 $\alpha$ -reductase type 2 gene (*SRD5A2*). *Hum. Mol. Genet.*, *2*: 820, 1993.
24. Reichardt, J. K. V., Makridakis, N., Henderson, B. E., Yu, M. C., Pike, M. C., and Ross, R. K. Genetic variability of the human *SRD5A2* gene: implications for prostate cancer risk. *Cancer Res.*, *55*: 3973–3975, 1995.
25. Kantoff, P. W., Febbo, P. G., Giovannucci, E., Krithivas, K., Dahl, D. M., Chang, G., Hennekens, C. H., Brown, M., and Stampfer, M. J. A polymorphism of the 5 $\alpha$  reductase gene and its association with prostate cancer: a case control analysis. *Cancer Epidemiol. Biomark. Prev.*, *6*: 189–192, 1997.
26. Zubiaga, A. M., Belasco, J. G., and Greenberg, M. E. The nanomer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol. Cell Biol.*, *15*: 2219–2230, 1995.
27. Spiessl, B., Beahrs, O. H., Hermanek, P., Hutter, R. V. P., Scheibe, O., Sobin, L. H., and Wagner, G. Illustrated guide to the TNM/pTNM classification of malignant tumors. *TNM Atlas*, Ed. 3, p. 343. Berlin: Springer-Verlag, 1989.
28. Bloom, H. J. G., and Richardson, W. W. Histological grading and prognosis in breast cancer. *Br. J. Cancer*, *11*: 359–377, 1957.
29. Bharaj, B. S., Angelopoulou, K., and Diamandis, E. P. Rapid sequencing of the *p53* gene with a new automated DNA sequencer. *Clin. Chem.*, *44*: 1397–1403, 1998.
30. Scatchard, G. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci.*, *51*: 660–672, 1949.
31. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with folin-phenol reagent. *J. Biol. Chem.*, *193*: 265–275, 1951.
32. Ferguson, R. A., Yu, H., Kalyvas, M., Zammit, S., and Diamandis, E. P. Ultrasensitive detection of prostate specific antigen by a new time-resolved immunofluorometric assay and the Immulite immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. *Clin. Chem.*, *42*: 675–684, 1996.
33. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.*, *53*: 457–481, 1958.
34. Makridakis, N., Ross, R. K., Pike, M. C., Chang, L., Stanczyk, F. Z., Kolonel, L. N., Shi, C. Y., Yu, M. C., Henderson, B. E., and Reichardt, J. K. A prevalent missense substitution that modulates activity of prostatic steroid 5 $\alpha$ -reductase. *Cancer Res.*, *57*: 1020–1022, 1997.
35. Silver, R. I., Wiley, E. L., Davies, D. L., Thigpen, A. E., Russell, D. W., and McConnell, J. D. Expression and regulation of steroid 5 $\alpha$  reductase 2 in prostate disease. *J. Urol.*, *152*: 433–437, 1994.
36. Foekens, J. A., van Putten, W. L. J., and Portegen, H. Prognostic value of pS2 and cathepsin D in 710 human primary breast tumors: multivariate analysis. *J. Clin. Oncol.*, *11*: 899–908, 1993.
37. Scorilas, A., Yotis, I., Stravolemos, K., Gouriotis, D., Keramopoulos, A., Ampela, K., Talieri, M., and Trangas, T. c-erbB2 overexpression may be used as an independent prognostic factor for breast cancer patients. *Anticancer Res.*, *15*: 1543–1548, 1995.
38. Ardavanis, A., Gerakini, F., Amanatidou, A., Scorilas, A., Pateras, C., Garoufali, A., Pissakas, G., Stravolemos, K., Apostolikas, N., and Yiotis, I. Relationships between cathepsin D, pS2 protein and hormonal receptors in breast cancer cytosols 58 Inconsistency with their established prognostic significance. *Anticancer Res.*, *17*: 3665–3670, 1997.
39. Scorilas, A., Diamandis, E. P., Levesque, M. A., Diamandi, A., Khosravi, M. J., Giai, M., Ponzzone, R., Roagna, R., Sisoni, P., and López-Otin, C. Immunoenzymatically determined pepsinogen C concentration in breast tumor cytosols: an independent favorable prognostic factor in node-positive patients. *Clin. Can. Res.*, *5*: 1778–1785, 1999.
40. Lopez-Otin, C., and Diamandis, E. P. Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. *Endocr. Rev.*, *19*: 365–396, 1998.
41. Akalu, A., Elmajian, D. A., Highshaw, R. A., Nichols, P. W., Reichardt, J. K. V. Somatic mutations at the *SRD5A2* locus encoding prostate steroid 5 $\alpha$  reductase during prostate cancer progression. *J. Urol.*, *161*: 1355–1358, 1999.