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Report

Breast cancer prognostic significance of a single nucleotide polymorphism in the proximal androgen response element of the prostate specific antigen gene promoter

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Key words: androgen receptor, breast cancer, mutation, polymorphism, prognosis

Summary

Prostate Specific Antigen (PSA) expression by breast epithelial cells is associated with favorable breast cancer prognosis. In preliminary studies, we found that a nucleotide variation (G → A) at position −158 in the androgen response element (ARE-1) of the PSA promoter was present in four out of 9 breast tumors examined and in a breast carcinoma cell line. We have now determined the nucleotide composition at position −158 of DNA extracted from 148 well-characterized breast tumors and compared tumor genotype with that of controls without cancer, with tumor PSA concentration and with clinicopathological variables, overall survival and disease free survival. The G → A base change at position −158 is a polymorphism. Allelotypes were similarly distributed in breast cancer patients and controls. The Mann–Whitney U Test showed a significantly higher tumor PSA concentration in tumors that presented a homozygous G as opposed to homozygous A genotype. Genotype at position −158 was not associated with clinicopathological variables in contingency table analysis. Univariate Cox regression models showed a 28% reduction in risk for death in patients with homozygous G genotype compared to those with homozygous A genotype ($P = 0.03$). However, ARE-I genotype did not significantly add to the prognostic power in the multivariate model of overall survival. In summary, the base change at position −158 is a polymorphism that may affect breast cancer prognosis, but further studies are required to confirm this possibility and to investigate the relevance of this polymorphism in terms of breast cancer susceptibility.

Abbreviations: ARE: Androgen responsive element; PSA: prostate-specific antigen; ER: estrogen receptor; PR: progesterone receptor; EDTA: ethylenediaminetetraacetic acid; DFS: disease-free survival; OS: overall survival; RR: relative risk; CI: confidence interval.

Introduction

Although it is widely recognized that the initiation and progression of breast carcinoma is influenced by the hormonal milieu of the breast epithelium, the molecular mechanisms involved remain poorly understood. Among the hormones thought to play roles are estrogens, progestins, and androgens which, acting in tandem, synergistically, or antagonistically, modulate the transcription of a variety of genes controlling cell replication, differentiation, and developmental programs [1]. One such gene which is transcriptionally regulated by steroid hormones encodes a 30–33 kDa glycoprotein, prostrate specific antigen (PSA), which is expressed predominantly in the luminal epithelial cells of the prostate but which has also been demon-

strated to display lower levels of expression in several normal and malignant tissues, including breast, ovarian, colon, liver, adrenal, and salivary epithelia *in vitro* and *in situ* [2, 3]. It is this nonprostatic expression of PSA, particularly in breast carcinomas, that has suggested cellular functions of PSA beyond its ability to liquify seminal coagulum [4] and that forms the basis of a potential clinical application of PSA in addition to its routine use as a serum marker for prostate tumor burden [5, 6]. A number of studies have shown that relatively high amounts of PSA protein in breast tumor tissues are associated with steroid hormone responsiveness, early disease stage and other clinical and pathologic features consistent with a favorable breast cancer prognosis [7, 8]. Furthermore, PSA expression, as quantified in cytosolic extracts of breast tumors, has been shown to be an independent prognostic factor insofar as breast cancer patients with elevated PSA concentrations in their tumor extracts had reduced risks of cancer relapse and death [9, 10]. PSA synthesis by non-neoplastic ductal epithelial cells of the breast and its release into nipple aspirate fluid has also been shown to be associated with lower risk for breast cancer development [11].

PSA gene expression is regulated by the binding of transcription factors to certain DNA sequences within a 5.8 kb segment upstream of the coding region. Up-regulation of PSA expression by androgens has been demonstrated in a prostate carcinoma cell line [12, 13] and breast cancer cell lines [14], and is mediated by a 15 base nucleotide sequence mediating high affinity binding of androgen receptor complexed with its cognate steroid ligand. Three such response elements have been identified, two within several hundred bases of the transcription start site (ARE-I and ARE-II from −170 to −156 and −394 to −380, respectively) [13, 15] and a third approximately 4200 bases further upstream (ARE-III) [16]. Excision and linkage of varying portions of the PSA regulatory domain to a reporter gene has shown androgen-induced transcription to be primarily driven by the distal enhancer, although the most proximal androgen responsive element ARE-I also has an important role because in its absence the distal enhancer-driven, steroid-induced, up-regulation of transcription is diminished by almost 80% [16]. Individual nucleotides comprising the ARE-1 sequence can have great functional impact on PSA gene expression. For example, alterating the −169 guanosine and the −165 cytosine to adenosine completely abolishes androgen-inducibility of reporter gene constructs [13].

Pang et al. [17], using genomic DNA from a prostate tumor, have reported a guanosine to adenosine (G–A) mutation at position −158 (AGAACAGCAAGTGCT to AGAACAGCAAGTACT). Interestingly, the same point mutation, established as such based on the PSA promoter sequence reported previously by other workers [18, 19], was observed in four out of 9 breast tumors and in the MCF-7 breast cancer cell line during a preliminary investigation in our laboratory [20], prompting us to undertake the present study designed to investigate the implications to breast cancer patients of the G to A base change at position −158 of the PSA gene in terms of tumor PSA protein expression, disease aggressiveness and prognosis.

Materials and methods

Breast cancer patients

Included in this study were tumor specimens from 148 women who underwent surgical treatment for primary breast carcinoma at the Department of Gynecologic 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, but other than this there was no particular bias related to sample selection. As far as could be determined, all the collected specimens were from unrelated patients. The patients represented 60% of new cases of breast cancer diagnosed and treated at the above institution during the accrual period. This study has been approved by the Ethics and Research Committees at the University of Toronto and the University of Turin.

The median age of the cancer patients was 54 years, while the range of ages was from 25 to 93 years. All patients had a histologically-confirmed diagnosis of primary breast cancer and had 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 61.5%. 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. Pathologic staging was performed according to the Postsurgical International Union Against Cancer Tumor-Node-Metastasis classification system

[21]. Of 147 patients for whom the stage was known, 43 (29.2%), 87 (59.2%), 7 (4.8%) and 10 (6.8%) had stages I, II, III and IV, respectively. Histologic grade of the tumors was determined according to criteria reported by Bloom and Richardson [22], and was known for 105 patients: six patients (5.7%) had grade I, 55 (52.4%) had grade II and 44 patients (41.9%) had grade III. Most of the tumors (70%) were of invasive ductal histologic type, whereas the remaining tumors were invasive lobular (12.6%), ductal *in-situ* (2%), medullary (2.7%), papillary (2.7%), tubular (2%), inflammatory (2.7%), tubulo–lobular (2.7%), cribriform (1.3%), and muciparous (1.3%). Post-operative treatment was known for all patients. Whereas 28% received no further treatment after tumor resection, 20% were given adjuvant chemotherapy only, 47% 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. 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 overall survival (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, 55 patients (37.6%) developed cancer relapse and 38 (26%) died.

Women with no evidence of malignant disease

Whole blood samples, collected in EDTA-containing evacuated tubes for routine hematological evaluation, were obtained from women outpatients at Mount Sinai Hospital in Toronto, Ontario, Canada. Individuals with documented evidence or suspicion of malignancy, as indicated by medical chart review, were excluded leaving 48 in the cohort. The ages of these patients ranged from 50 to 70 years; the median age was 57 years, close to that (54 years) of the breast cancer group.

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 μl of blood was used for each extraction. The breast tumor tissue, which contained more than 70% tumor cells as determined by histological examination, was pulverised into a fine powder and stored at −80°C until the extraction procedure. Briefly, following 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°C until analysis.

PCR amphlfication of the ARE-I region

The paired primer sequences flanking the ARE-I region (5′-TTTCAGGAGCATGAGGAATAA-3′ and 5′-CCCAGGAGCCCTATAAAAC-3′) were designed using Oligo 5.0 software (National Biosciences, Plymouth, MN) according to the PSA genomic DNA sequence deposited into GenBank by Schuur et al. [23] (accession # U37672) and were predicted to result in a 440 bP amplicon. PCR amplification was performed in a final volume of 25 μl, containing approximately 100–150 ng of template DNA, 10 mmol/l Tris–HCl buffer (pH 8.3), 50 mmol/l KCl, 2.5 U of AmpliTaq Polymerase (Hoffmann-La Roche, Basel, Switzerland), 250 μmol/l of deoxynucleoside triphosphates, 1.5 mmol/l $MgCl_2$, and 1 μmol/l of each primer. The thermal cycling profile consisted of an initial 5 min denaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s and extension at 72°C for 30 s. A final extension step at 72°C for 7 min completed the reaction, the success of which was verified by electrophoresing an 8 μl aliquot of the amplified PCR product on an 0.8% agarose gel containing ethidium bromide. It was thereby determined that five breast tumor specimens (out of 148) did not yield DNA of sufficient quality, even after repeated PCR amplification, for subsequent sequence analysis.

DNA sequencing

Both strands of each PCR product were sequenced on a Microgene Blaster™ automated DNA sequencer (Visible Genetics, Toronto, ON, Canada), following a protocol described in detail elsewhere [24]. The sequencing primers, 5′-TCCGCCCCTGCCCTGCTG-3′ and 5′-GCTTTGTATGAAGAATCG-3′ were labeled at the 5′-end with the fluorescent dye Cy5.5 (Visible Genetics).

Steroid hormone receptor analyses

Tumor specimens ($n = 148$) were pulverized in liquid nitrogen, homogenized in buffer, and the cytosolic fractions were obtained by ultracentrifugation and quantified for steroid hormone receptors as described elsewhere [25]. The results of the dual ligand-binding assay, in which dextran coated charcoal was used to separate bound from free ligand, were interpreted by Scatchard analysis [26]. Protein concentrations of the cytosols were determined by the Lowry method [27]. Tumors with ER and PR concentrations below or equal to 10 fmol/mg protein were considered as receptor negative, whereas tumors with receptor concentrations above such values were considered positive, as followed previously [28, 29]. Based on these cutoffs, 99 (67.3%) and 93 (63.7%) of 147 and 146 breast carcinomas were ER and PR-positive, respectively.

PSA immunoassay

Approximately 10–50 mg of the pulverized tumor tissues were combined with 500 µl of a cell lysis buffer containing 50 mmol/l Tris (pH 8.0), 150 mmol/l NaCl, 5 mmol/l EDTA, 10 g/l Nonidet NP-40 surfactant, and 1 mmol/l phenylmethylsulfonyl fluoride and incubated for 30 min on ice. Following 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 [30]. The PSA assay has a detection limit of 0.001 µg/l. PSA concentrations were adjusted for total protein content, as determined by the bicinchoninic acid method (Pierce Chemical Co, Rockford, IL), and are expressed as micrograms of PSA per gram of extracted protein.

Statistical analysis

For analysis of data, patients were subdivided into groups based on the status of different clinical or pathologic variables. Associations between ARE-I genotypes and other categorical variables were analyzed using the chi-square (χ^2) test. Estrogen receptor (ER) and progesterone receptor (PR) values were categorized into positive and negative status as described above. The cutoff value for tumor size was 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: less than 45 years, 45 to 55 years and greater than 55 years. The analysis of differences in tumor PSA concentration among the three ARE-1 allelotypes (homozygous G; heterozygous G and A; homozygous A) was carried out with the nonparametric Mann–Witney U test. Survival analyses were performed by constructing Kaplan–Meier disease free survival (DFS) and OS curves [31], where differences between curves were evaluated by the log-rank test, as well as by estimating the relative risks for relapse and death using the Cox proportional hazards regression model [32]. Only patients for whom the status of all variables was known were included in the multivariate models, which incorporated ARE-I genotypes and all other variables for which the patients were characterized. Selection of prognostic variables with the highest significant effect in DFS and OS was performed in Cox models using the stepwise regression option from SPSS software (SPSS, Richmond, CA). Only variables for which $P < 0.05$ were retained in the final model. In all statistical tests, a P value < 0.05 was considered significant.

Results

Distribution of ARE-I genotypes

Sequencing the ARE-1 region of the PSA gene promoter with the Visible Genetics Inc. methodology clearly distinguished the three allelotypes of the point mutation at position −158, homozygous G, homozygous A and heterozygous G/A. Each allelotype was present in at least 20% of specimens indicating that the base change at position −158 was a common polymorphism. Of the 48 randomly selected women without cancer, 10 (21%) were homozygous G, 22 (46%) were heterozygous (G/A), and 16 (33%) were homozygous A. Among the breast tumors, 30 (21%) had the G nucleotide at position −158 of both PSA alleles, 77 (54%) were heterozygous (G/A) and 36 (25%) were homozygous for the A genotype. The percent distribution of each genotype in the cancer and non cancer groups was remarkably similar (Figure 1), despite the fact that the cancer patients and control patients were unmatched and from different countries. No other point mutations, insertions, or deletions were revealed in the DNA sequences within, and flanking, the ARE-I region in any of the PCR products.

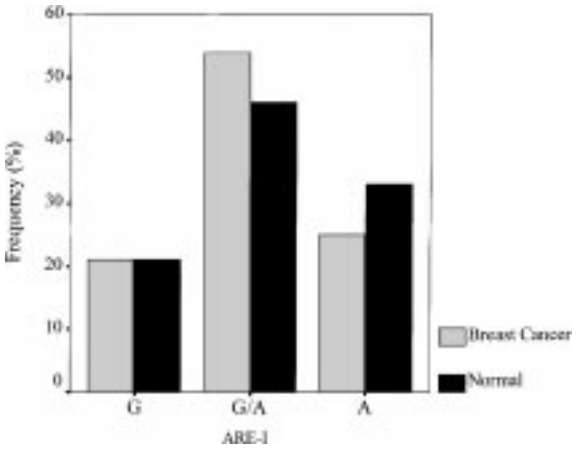

Figure 1. Distribution of ARE-I genotypes in human breast carcinomas and in leukocytes from unmatched females without malignancy. As determined by the χ^2 test, the differences were not statistically significant ($P = 0.42$).

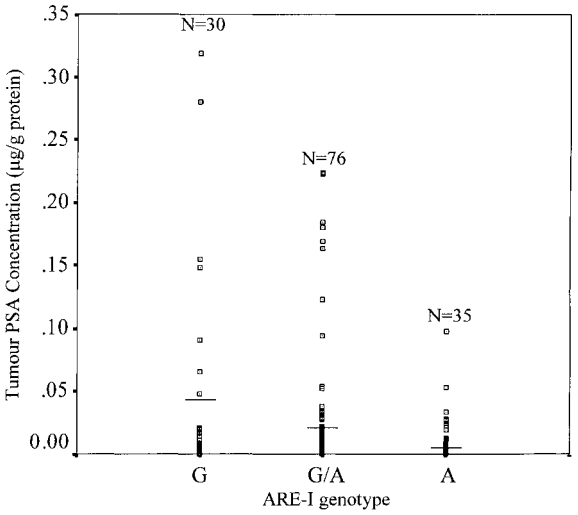

Figure 2. Relationship between breast tumour ARE-I genotype and breast tumor-extract PSA concentration. Mean PSA concentration in each allelotype is indicated by a horizontal bar. Mean values are 0.044 µg/g protein, 0.028 µg/g protein, and 0.010 µg/g protein for G/G, G/A, and A/A genotypes, respectively. The difference in PSA concentrations ($P = 0.019$) between G/G and A/A groups was statistically significant, as determined by the Mann–Whitney U test.

Relationships of ARE-I genotypes to other prognostic variables

The distribution of ARE-I genotypes – G/G, A/A, and G/A – among subgroups of patients classified by age, tumor size, nodal status, histologic grade, histologic type, disease stage, ER status, PR status, and adjuvant treatment administered was examined by χ^2 tests (Table 1). Statistically significant associations between ARE-I genotype and these other variables were not found. In the same analysis, ARE-I genotypes were shown not to differ among patients who had received different treatments postoperatively.

Tumor tissue PSA concentration among the three ARE-I genotypes showed a G/G to G/A to A/A stepwise decline in mean PSA concentration (Figure 2). The difference in PSA level between the homozygous G and homozygous A groups was determined to be significant by the Mann–Whitney U test ($P = 0.019$).

ARE-I Polymorphism and breast cancer survival

Cox regression models were developed to evaluate the effect of ARE-I genotypes on DFS and OS for breast cancer patients (Table 2). In univariate analyses in which the estimated risks for relapse or death were compared among the three groups of patients having particular ARE-I genotypes two groups at a time, the only statistically significant difference demonstrated was an approximately 28% reduction in risk for death in patients with the homozygous G genotype compared to patients who were homozygous A (RR=0.72, 95% CI=0.52–0.98; $P = 0.030$). The adverse effect of homozygous A status was further echoed by Kaplan–Meier survival plots and accompanying log-rank tests (Figure 3). In this analysis, homozygous A patients were revealed to have significantly worse OS compared to homozygous G patients and heterozygous patients combined; the analogous difference in terms of unfavourable DFS was of borderline statistical significance. There was also a significant difference in OS among all three genotypes ($P = 0.038$).

In the multivariate analysis of ARE-I genotypes, the Cox models were adjusted for the concurrent effects on DFS and OS of patient age, nodal status, tumor size, and ER and PR status, all of which were used as categorical variables, except tumor size which was used as a continuous variable, as described above. Tumor grade was not included in the multivariate analysis because of the relatively large number of patients for whom this variable was unknown. Whereas established breast cancer prognostic factors – patient age, tumor size and nodal status – were thus shown to be independent factors for predicting both DFS and OS of our cohort of patients, ARE-I genotypes did not significantly add to the prognostic power in the multivariate models.

Table 1. Associations of ARE-I polymorphism with features of breast cancer

Features	Total	No. of patients (%)			*P* value[a]
		G/G	G/A	A/A	
Age (years)					
< 45	36	5 (14.7)	22 (64.7)	7 (20.6)	
45–55	38	13 (36.1)	14 (38.9)	9 (25.0)	0.091
> 55	74	12 (16.4)	41 (56.2)	20 (27.4)	
Tumor size (cm)					
< 2	43	10 (25.0)	19 (47.5)	11 (27.5)	0.621
≥2	105	19 (19.0)	56 (56.0)	25 (25.0)	
Nodal status					
Negative	55	9 (18.0)	30 (60.0)	11 (22.0)	0.348
Positive	88	20 (23.5)	40 (47.1)	25 (29.4)	
Grade[b]					
I–II	61	11 (18.0)	34 (55.7)	16 (26.2)	0.367
III	44	10 (24.4)	17 (41.5)	14 (34.1)	
Histology					
Ductal	104	23 (23.0)	48 (48.0)	29 (29.0)	0.509
Lobular	19	3 (15.8)	14 (73.7)	2 (10.5)	
Other	25	4(16.7)	15 (62.5)	5 (20.8)	
Stage[c]					
I	43	10 (23.8)	20 (47.6)	12 (28.6)	0.897
II	87	17 (20.7)	45 (54.9)	20 (24.4)	
III–IV	17	4 (16.7)	11 (61.1)	4 (22.2)	
ER status[d]					
Negative	48	10 (22.7)	20 (45.5)	14 (31.8)	0.312
Positive	99	20 (21.1)	55 (57.9)	20 (21.1)	
PR status[d]					
Negative	53	11 (21.2)	28 (53.8)	13 (25.0)	0.995
Positive	93	18 (20.9)	47 (54.7)	21 (24.4)	
Adjuvant treatment					
None	42	5 (12.2)	28 (68.3)	8 (19.5)	0.223
Tamoxifen	70	18 (26.5)	31 (45.6)	19 (27.9)	
Chemotherapy ±tamoxifen	36	7 (20.6)	18 (52.9)	9 (26.5)	

[a] χ^2 test.
[b] Bloom–Richardson grading system.
[c] TNM system.
[d] Cutoff point: 10 fmol/mg.

Discussion

Recent studies have shown that PSA expression in breast tumors is associated with favorable patient prognosis [9, 10], although the molecular basis for the differential expression levels of PSA in breast carcinoma tissues has not been established. Surgically resected breast tumors with undetectable PSA expression differ substantially in their concentration of steroid hormone receptors [9, 10], as is the case also in breast carcinoma cell lines [14], suggesting that the cellular content of PSA is not determined by steroid receptors *per se*. The identification of single nucleotide substitutions, insertions and deletions in the PSA gene promoter [14, 20] raised the possibility that variability in PSA expression might depend on the specific sequences present in *cis*-response elements. Linkage to cancer predisposition and prognosis was suggested by

Table 2. Associations of ARE-I polymorphism with breast cancer survival

Variable	Disease-free survival			Overall survival		
	Univariate *P* value	Multivariate *P* value[a]	RR (95% CI)[b]	Univariate *P* value	Multivariate *P* value[a]	RR (95% CI)[b]
ARE-I genotypes						
G/A vs G	0.74			0.34		
A vs G	0.36			0.030		
A vs G/A	0.43			0.12		
Patient age						
45–55 yrs vs <45 yrs	0.53			0.13	0.013	1.81 (1.13–2.92)
>55 yrs vs <45 yrs	<0.001	<0.001	0.48 (0.31-0.74)	0.019	0.0056	0.45 (0.25-0.79)
Tumor size						
>2 cm vs <2 cm	0.0027	0.013	1.34(1.06–1.69)	0.043	0.025	1.38 (1.04–1.83)
Grade[c]						
III vs I–II	0.17			0.25		
Nodal status						
Positive vs negative	0.0033	0.022	2.34 (1.13–4.87)	0.011	0.021	2.54 (1.08–5.89)
ER[d]						
Positive vs negative	0.095			0.038		
PR[d]						
Positive vs negative	0.47			0.51		

[a] *P* values in multivariate analyses are from the final models in which only variables with $P < 0.05$ were retained.
[b] Relative risk (RR) and 95% confidence interval (CI) are presented only for the retained variables which were significant in the multivariate analysis.
[c] Grade was not included in multivariate analysis because of the large number of missing values.
[d] ER and PR positivity based on 10 fmol/mg cutoff points.

the occurrence of these apparent mutations in prostate and breast carcinoma cell lines [14, 20] and in breast tumors [20].

In this study, we confirmed the presence of nucleotide variability (G or A) at position −158 in a much larger number of breast tumor tissues and further observed its occurrence in leukocyte DNA from women without clinically evident malignancy. Frequencies of G/G, G/A and A/A alleles did not differ significantly between tumor tissues and nonmalignant leukocytes which inferred that the position −158 variability detected in the tumor specimens represented constitutive germline polymorphisms, and not somatic mutations, but direct testing against patient -matched leukocyte DNA would be required to confirm this. PSA protein expression was modestly affected by the nucleotide composition at position −158, being significantly higher in tumors with the homozygous G compared to the homozygous A allelotype. However, the effect of nucleotide composition at position −158 on androgen-mediated PSA (gene) expression was less pronounced than that which has been reported to result from mutations at other positions within the ARE-1 [13]. No such mutations were detected throughout the ARE-1 (or flanking regions) by the direct sequencing techniques employed in this study. This removed additional local base changes as an explanation for the relatively wide variation in PSA concentration observed among tumor specimens, even within each of the G/G, G/A and A/A groups. However, distant (ie.non local) nucleotide changes within the other two androgen response elements in the 5′ regulatory region of the PSA gene, particularly the potent far-upstream ARE-III enhancer, are not ruled out by our data and may have contributed to the variability in PSA concentration. We have shown in previous work that sequence alterations are much more prevalent throughout the 5′ flanking region of the PSA gene than within the coding region [20, 33].

Although the ARE-I genotype showed no association with conventional clinical or pathological breast cancer prognostic factors, analysis of OS revealed

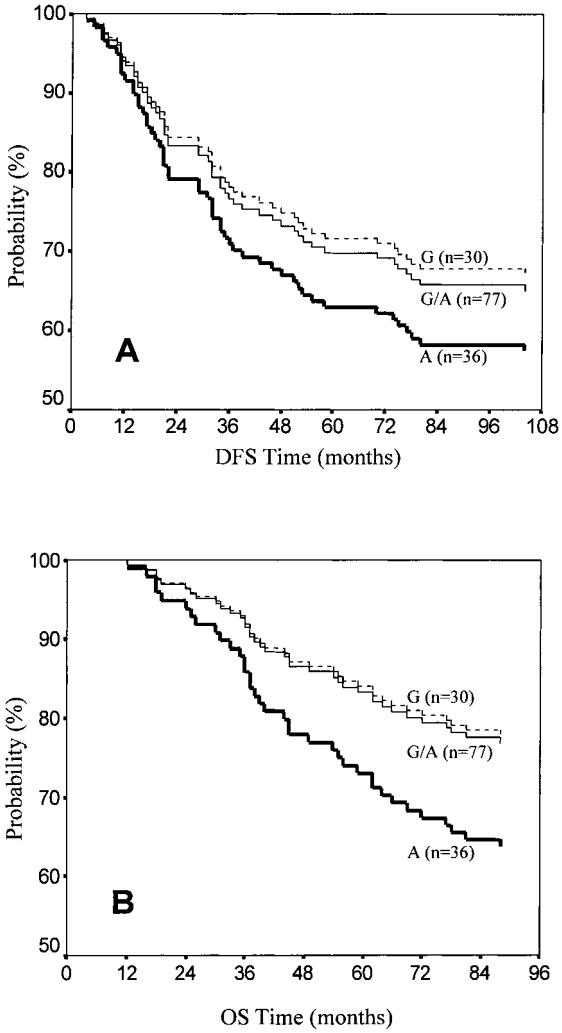

Figure 3. Kaplan–Meier plots of DFS (A) and OS (B) of 143 patients with G/G, G/A and A/A ARE-I genotypes. Differences among the three genotypes for DFS ($P = 0.23$) and OS ($P = 0.038$) were determined by log-rank tests, which were also applied to comparisons of genotype A/A versus genotypes G/G and G/A combined ($P = 0.058$ for DFS and $P = 0.028$ for OS).

patients with homozygous A at position −158 had worse overall prognosis than patients with homozygous G at the same position. Differences in relapse rates (DFS) between the same groups of patients followed a comparable trend, albeit of borderline statistical significance in Kaplan–Meier analysis. Interestingly, patients who were heterozygous were shown to have relapse and death rates similar to patients who were homozygous G, suggesting that a single copy of the G-containing allele was protective from either clinical endpoint. How this protection links with PSA expression is obscure at present given that the protective effect (28% improvement in OS) seems disproportionately high relative to the modest increase in tumour PSA protein concentration found in homozygous G specimens and given that the protection holds even for the heterozygous group where a statistically significant increases in PSA concentration relative to the A homozygotes could not be demonstrated. Whatever the link, the protective effect of ARE-1 allelotype did not survive multivariate analysis, suggesting additional associations with conventional prognostic clinicopathological variables.

In conclusion, we have employed a rapid automated DNA sequencer to ascertain the status of a polymorphic nucleotide within the proximal ARE of the PSA gene in women with breast cancer and in others without the disease, and have presented evidence that this polymorphism may be associated with postoperative breast cancer prognosis.

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References

1. Helzlsouer KJ, Couzi R: Hormones and breast cancer [Review]. Cancer 76(10): 2059–2063, 1995
2. Levesque MA, Yu H, D'Costa M, Diamandis EP: Prostate specific antigen expression by various tumors. J Clin Lab Anal 9: 123–128, 1995
3. Smith MR, Biggar S, Hussain M: Prostate specific antigen mRNA is expressed in non prostatic cells; implications for detection of micrometastases. Cancer Res 55: 2640–2644, 1995
4. Lee C, Keefer M, Zhao ZW, Krose R, Berg L, Liu XX, Sensibar J: Demonstration of the role of prostate-specific antigen in semen liquefaction by two-dimensional electrophoresis. J Androl 10: 432–438, 1989
5. Catalona WJ, Smith DS, Ratcliff TL, Dodds KM, Copelan DE, Yuan JJ, Petios JA, Andriole GL: Measurement of prostate specific antigen in serum as a screening test for prostate cancer. N Eng J Med 324: 1156–1161, 1991
6. Oesterling JE: Prostate specific antigen, a critical assessment of the most useful marker for adenocarcinoma of the prostate [Review]. J Urol 145: 907–923, 1991
7. Yu H, Diamandis EP, Levesque M, Giai M, Roagna R, Ponzone R, Sismondi P, Monne M, Croce CM: Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. Breast Cancer Res Treat 40: 171–178, 1996

8. Diamandis EP, Yu H, Sutherland DJ: Detection of prostate-specific antigen immunoreactivity in breast tumors. Breast Cancer Res Treat 32: 301–310, 1994
9. Yu H, Giai M, Diamandis EP, Katsaros D, Sutherland DJA, Levesque MA, Roagna R, Ponzone R, Sismondi P: Prostate specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 55: 2104–2110, 1995
10. Yu H, Levesque MA, Clark GM, Diamandis EP: Prognostic value of prostate-specific antigen for women with breast cancer: a large United States cohort study. Clin Cancer Res 4: 1489–1497, 1998
11. Sauter ER, Daly M, Lenahan K, Ehya H, Engstrom PF, Bonney G, Ross EA, Yu H, Diamandis E: Prostate specific antigen levels in nipple aspirate fluid correlate with breast cancer. Cancer Epidemiol Biomarkers Prev 5: 967–970, 1996
12. Young CYF, Montgomery BT, Andrews PE, Qui SD, Bilhartz DL, Tindall DJ: Hormonal regulation of prostate specific antigen messenger RNA in human prostate adenocarcinoma cell line LNCaP. Cancer Res 51: 3748–3752, 1991
13. Riegman PHJ, Vlietstra RJ, van der Korput JAGM, Brinkman AO, Trapman J: The promoter of the prostate specific antigen contains a functional androgen responsive element. Mol Endocrinol 5: 1921–1930, 1991
14. Zarghami N, Grass L, Diamandis EP. Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. Br J Cancer 75: 579–588, 1997
15. Cleutjens KBJM, van Eekelen CCEM, van der Korput HAGM, Brinkmann AO, Trapman J: Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 271: 6379–6388, 1996
16. Cleutjens KBJM, van der Korput HAGM, van Eekelen CCCM, van Riooji HCJ, Faber PW, Trapman J: An androgen responsive element in a far upstream enhancer region is essential for high androgen-regulated activity of the prostate specific antigen promoters. Mol Endocrinol 11: 148–1461, 1997
17. Pang S, Taneja S, Dardashti K, Cohan P, Kaboo R, Sokoloff M, Tso CL, DeKernion JB, Belldegrun AS: Prostate tissue specificity of prostate-specific antigen promoter isolated from a patient with prostate cancer. Human Gene Therapy 6: 1417–1426, 1995
18. Lundwall A: Characterization of the gene for prostate-specific antigen, a human glandular kallikrein. Biochem Biophys Res Commun 161: 1151–1159, 1989
19. Klobeck HG, Combriato G, Schultz P, Arbusow V, Fittler F: Genomic sequence of human prostate specific antigen (PSA). Nucleic Acids Res 17: 3981, 1987
20. Majumdar S, Diamandis EP: The promoter of the enhancer region of the KLK 3 (prostate specific antigen) gene is frequently mutated in breast tumors and breast carcinoma cell lines. Br J Cancer 79: 1594–1602, 1999
21. Spiessl B, Beahrs OH, Hermanek P, Hutter RVP, Scheibe O, Sobin LH, Wagner G: Illustrated guide to the TNM/pTNM classification of malignant tumors. TNM atlas, 3rd edn Springer-Verlag, Berlin, New York, 1989, p. 343
22. Bloom HJG, Richardson WW: Histological grading and prognosis in breast cancer. Br J Cancer 11: 359–377, 1957
23. Schuur ER, Henderson GA, Kmetec LA, Miller JD, Lamparski HG, Henderson DR: Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 271: 7043–7051, 1996
24. Bharaj BS, Angelopoulou K, Diamandis EP: Rapid sequencing of the p53 gene with a new automated sequencer. Clin Chem 44: 1397–1403, 1998
25. Dressler LG, Seamer LC, Owens MA, Clark GM, McGuire WL: DNA flow cytometry and prognostic factors in 1131 frozen breast cancer specimens. Cancer 61: 420–427,1988
26. Scatchard G: The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51: 660–672, 1949
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with folin–phenol reagent. J Biol Chem 193: 265–275, 1951
28. Alexiera-Figusch J, van Putten WLS, Blankestein MA, Blonk-Van Der wijst J, Klijn JGM: The prognostic value and relationships of patient characteristics, estrogen and progestin receptors and site of relapse in primary breast cancer. Cancer 61: 758–768, 1988
29. Reiher A, Kolb R, Reiner G, Jakesz R, Schemper M, Spona J: Prognostic significance of steroid hormone receptor and histopathological characterization of human breast cancer. J Cancer Res Clin Oncol 113: 285–290,1987
30. Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP: 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
31. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53: 457–481, 1958
32. Cox DR: Regression models and life tables. R Stat Soc B 34: 187–202, 1972
33. Tsuyuki D, Grass L, Diamandis EP: Frequent detection of mutations in the 5′flanking region of the prostate-specific antigen gene in female breast cancer. Eur J Cancer 33: 1853–1856, 1997

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