DNA AND CELL BIOLOGY
Volume 27, Number 9, 2008

Pp. 497–503
DOI: 10.1089/dna.2007.0700

PSA and Androgen-Related Gene (*AR*, *CYP17*, and *CYP19*) Polymorphisms and the Risk of Adenocarcinoma at Prostate Biopsy

Rodrigo Mattos dos Santos,[1] Carlos Márcio Nóbrega de Jesus,[2] José Carlos Souza Trindade Filho,[2] José Carlos Souza Trindade,[2] João Lauro Viana de Camargo,[3] Cláudia Aparecida Rainho,[1] and Silvia Regina Rogatto[2,4]

The aim of the present study was to examine the impact of polymorphisms in prostate-specific antigen (*PSA*) and androgen-related genes (*AR*, *CYP17*, and *CYP19*) on prostate cancer (PCa) risk in selected high-risk patients who underwent prostate biopsy. Blood samples and prostate tissues were obtained for DNA analysis. Single-nucleotide polymorphisms in the 5′-untranslated regions (UTRs) of the *PSA* (substitution A > G at position − 158) and *CYP17* (substitution T > C at 5′-UTR) genes were detected by polymerase chain reaction (PCR)–restriction fragment length polymorphism assays. The CAG and TTTA repeats in the *AR* and *CYP19* genes, respectively, were genotyped by PCR-based GeneScan analysis. Patients with the GG genotype of the *PSA* gene had a higher risk of PCa than those with the AG or AA genotype (OR = 3.79, $p = 0.00138$). The AA genotype was associated with lower PSA levels (6.44 ± 1.64 ng/mL) compared with genotypes having at least one G allele (10.44 ± 10.06 ng/mL) ($p = 0.0687$, 95% CI − 0.3146 to 8.315, unpaired *t*-test). The multivariate analysis confirmed the association between PSA levels and *PSA* genotypes (AA vs. AG + GG; $\chi^2 = 0.0482$) and *CYP19* (short alleles homozygous vs. at least one long allele; $\chi^2 = 0.0110$) genotypes. Genetic instability at the *AR* locus leading to somatic mosaicism was detected in one PCa patient by comparing the length of *AR* CAG repeats in matched peripheral blood and prostate biopsy cores. Taken together, these findings suggest that the *PSA* genotype should be a clinically relevant biomarker to predict the PCa risk.

Introduction

Prostate cancer (PCa) is the most commonly diagnosed noncutaneous malignancy in men. Despite the substantial public health impact of PCa, little is known about its etiology. The major risk factors for the development of PCa are advanced age, familial predisposition, and ethnicity (Abate-Shen and Shen, 2000). Genetic variation or polymorphisms also have been implicated in susceptibility to cancer development, different aspects of neoplastic growth, and outcome of the disease (Loktionov, 2004). The role of steroid hormones in the etiology of PCa has been reported, and several molecules encoded by polymorphic genes (hormones, their receptors, and enzymes involved in hormone biosynthesis and metabolism) have recently been shown to be associated with PCa risk (Ntais *et al.*, 2003). In the context of hormone-sensitive diseases such as PCa, genes involved in hormone interactions are likely candidates for modulating disease susceptibility.

Prostate development and growth are dependent on androgens that act through the androgen receptor (AR), a member of the steroid hormone receptor family of molecules that regulate gene expression (Platz and Giovannucci, 2004). Variation in the CAG repeats in exon 1 of the *AR* gene has a possible direct role in PCa causation and differential treatment responses. Further, ethnic variations in CAG repeats mirror ethnic variations in PCa. Thus, it was proposed that size variation of this repeat modifies PCa risk and progression (Giovannucci, 2002). The mechanism by which short CAG alleles of *AR* increase PCa risk is related to the increased efficiency of their encoded transactivators of androgen-regulated target genes (Kazemi-Esfarjani *et al.*, 1995). AR acts as a transcription factor that regulates the prostate-specific antigen (*PSA*) gene by interactions with DNA sequences known as AREs (androgen response elements) in the *PSA* gene promoter region (Arnold *et al.*, 2007).

A single-nucleotide polymorphism (SNP) (an A > G substitution at position − 158) in the ARE-I sequence of the *PSA*

[1]Institute of Biosciences, Sao Paulo State University, UNESP, Botucatu, Sao Paulo, Brazil.
Departments of [2]Urology and [3]Pathology, Faculty of Medicine, Sao Paulo State University, UNESP, Botucatu, Sao Paulo, Brazil.
[4]AC Camargo Hospital, Sao Paulo, Sao Paulo, Brazil.

gene has been described (Rao and Cramer, 1999). Polymorphisms in the promoter region of the *PSA* gene have been associated with quantitative differences in mRNA expression in breast and prostate carcinomas (Yang *et al.*, 2000, 2001). Xue *et al.* (2001) showed that genetic variants of both *PSA* and *AR* genes modulate serum PSA levels in healthy men.

CYP17 and *CYP19* genes are involved in sex hormone metabolism. The *CYP17* gene encodes the cytochrome P450c17α enzyme, which has 17alpha-hydroxylase and 17,20-lyase activities and is a key enzyme in the steroidogenic pathway. The A2 allele of *CYP17*, which has an increased rate of transcription of *CYP17* mRNA, results from a T > C substitution at nucleotide 34 upstream from the translation start site of the *CYP17* gene (Haiman *et al.*, 2001; Cussenot *et al.*, 2007). The *CYP19* gene encodes the enzyme aromatase that catalyzes the last steps of estrogen biosynthesis—three successive hydroxylations of the A ring of androgens, leading to irreversible conversion of andronestedione to estrone and testosterone to estradiol. The *CYP19* gene has a polymorphic short tandem repeat $TTTA_{7–13}$ located in intron 4 (Feigelson *et al.*, 1998). A recent study showed that the presence of the TTTA long allele of *CYP19* was an independent risk factor for death and a strong predictor of survival in PCa patients with bone metastasis at diagnosis (Tsuchiya *et al.*, 2006; Cussenot *et al.*, 2007).

The purpose of this study was to evaluate whether there is an association between well-characterized polymorphisms in genes related to androgen action (*AR*, *PSA*, *CYP17*, and *CYP19*) and PCa risk at biopsy.

Materials and Methods

Patients

A total of 96 men treated at the Department of Urology of the Clinical Hospital by the Faculty of Medicine, Sao Paulo State University, UNESP, Botucatu, Sao Paulo, Brazil, were eligible for this study because of abnormal PSA values and/or suspected PCa after digital rectal exams. The patients were accrued consecutively from March 2001 to October 2002. The inclusion criteria were no previous new histology diagnosis of PCa or prostatic lesions, suspicious digital rectum exam and/or elevated serum PSA level, and histological data of the biopsies. All patients were advised of the procedures and provided written informed consent. The study protocol was approved by the institutional ethics committee and by the Brazilian Ethics Committee on Research (CONEP). Systematic ultrasound-guided needle biopsies obtaining 3–10 cores (median of 6 cores) were done by using an 18-gauge, spring-loaded biopsy device. The primary endpoint was the histological presence of adenocarcinoma of the prostate in the biopsy specimens. For the genotyping analysis, a sample of peripheral blood was collected and matched with prostate biopsies (maximum of three additional cores/patient). The subjects were divided into two groups according to histological diagnosis of prostate adenocarcinoma ($n = 47$) or cancer-free patients ($n = 49$). Among the 47 PCa patients, 29 underwent radical suprapubic prostatectomy and 18 radiotherapy and/or hormonal therapy. After prostatectomy, histological analysis revealed organ percentage involvement raging from 5% to 85%. Other parameters investigated included extraprostate invasion (detected in 14 out of 29 patients), lymph node involvement (detected in 3 out of 30 cases), perineural invasion (observed in 15 out of 18 patients; in 11 patients, these data were not available), and bone metastasis (3 patients). The follow-up after the surgery varied between 2 and 88 months. In addition, the presurgical and the postsurgical serum PSA levels were evaluated to access biochemical recurrence. Eight out of 41 PCa patients showed biochemical recurrence.

DNA extraction

Genomic DNA was obtained from peripheral blood samples and prostate biopsies by standard SDS/proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation. *AR* genotyping was performed on matched blood and prostate DNA. The *PSA*, *CYP17*, and *CYP19* genotyping was conducted on DNA from peripheral blood samples.

PCR-based GeneScan analysis

The CAG and TTTA repeats of *AR* and *CYP19* genes, respectively, were genotyped by the size of the PCR product containing the polymorphic microsatellite. A ~ 290-bp fragment of the *AR* gene was amplified from genomic DNA with fluorescently labeled primers according to Bharaj *et al.* (1999). The fluorescent PCR products were analyzed on 5% polyacrylamide/7 M urea gels using an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, CA). Digital images of fluorescent gels were acquired using Data Collection software and analyzed using GeneScan software. The number of CAG repeats was calculated by subtracting the number of nucleotides in the invariant part of the PCR fragment from the total PCR product length and dividing the resulting number by three. Because the Brazilian population is known to be highly miscegenated, we used the cut-off data for CAG repeat lengths from Ribeiro *et al.* (2002). These authors genotyped 200 individuals from two cities of Sao Paulo State, Brazil, and found the mean CAG repeat length to be 20.65. In our study, the patients were from the same state and showed a mean of 19.8 CAG repeats. Thus, we selected the cut-off of 20 CAG repeats to dichotomize the *AR* genotypes into short or long alleles. Analysis of the TTTA repeat in intron 4 of the *CYP19* gene was performed as described by Polymeropoulos *et al.* (1991). The *CYP19* alleles were classified as short (≤164 bp) or long (≥168 bp).

PCR assay for PSA and CYP17 genotyping

The *PSA* and *CYP17* polymorphisms were determined by a PCR-restriction fragment length polymorphism (PCR-RFLP) assay. Briefly, a 300-bp fragment of the *PSA* gene was amplified by PCR (Xue *et al.*, 2000), digested with *Nh*eI restriction enzyme (New England Biolabs, Beverly, MA), and electrophoresed on a 2% ethidium bromide–stained agarose gel. Three possible genotypes (A/A, A/G, and G/G) were distinguished depending on the presence (allele A) or absence (allele G) of the *Nh*eI restriction site. RFLP analysis of the *CYP17* gene was performed by amplification of a 421-bp region that contains the polymorphic site of the *MspA1*I restriction enzyme (Habuchi *et al.*, 2000). The PCR products were digested with *MspA1*I (New England Biolabs). When the *MspA1*I site was present, the 421-bp amplified fragment was cleaved into 130-bp and 291-bp fragments. The genotype was designated as *CYP17*A1* or *CYP17*A2* when the restriction site was absent or present, respectively.

Statistical analysis

We examined the relationship between the polymorphisms and the PCa risk at prostate biopsy. The subjects were classified as adenocarcinoma patients or cancer-free patients at prostate biopsy. The PCa-free status was confirmed after at least 54 months of follow-up. Pathological and clinical data (including clinical stage, Gleason score, PSA level, biochemical recurrence, and extraprostate invasion) were considered in PCa patient group. Analysis of data was performed using the statistical software package SAS/STAT, version 6.0 (SAS Institute, Cary, NC). Mean, median, and standard deviation (SD) were calculated for each group. For univariate data analysis, the associations of categorical data were determined using the 2×2 tables and the Fisher's exact test. The pairwise tests utilized the following dichotomous variables: age ≤ 55 versus > 55 years; serum PSA levels ≤ 4 versus > 4 ng/mL; Gleason score ≤ 6 versus ≥ 7; and clinical stage II versus III + IV, and genotypes. Multivariate logistic analysis model considered the serum PSA levels (categorized as ≤ 4 vs. > 4 ng/mL) in function of *AR* (short alleles vs. long alleles), *PSA* (*AA* vs. *AG* + *GG*), *CYP17A* (*A1*/A1** vs. *A1*A2** + *A2*A2**), and *CYP19* (short alleles homozygous vs. at least one long allele) genotypes. All statistical tests were two-sided, and $p < 0.05$ was considered significant.

Results

The mean age at biopsy of the 96 men was 66.68 years (range 46–85 years). Biopsy confirmed prostatic adenocarcinoma in 49% of the patients. Of the 49 men with no evidence of PCa, 46 had at least six biopsy cores evaluated histologically. In the group of PCa cases, 42 of 47 patients (89%) had at least six biopsies evaluated for the diagnosis.

The mean age of the PCa patients (66.87 ± 7.72 years) was not significantly different from that of the cancer-free group (66.49 ± 8.51 years). PCa patients were more likely to have higher serum PSA levels. PSA levels > 4 ng/mL were observed in 39 of 47 PCa cases (83%) and in 30 of 45 (67%) cancer-free individuals ($p = 0.0930$, Fisher's exact test). In addition, the median serum PSA level at the time of diagnosis was 10.73 ng/mL in PCa cases (SD = 10.10) and 7.44 ng/mL (SD = 6.25) in the cancer-free group (Student's *t*-test, $t = 1.823$; $p = 0.0719$). Higher PSA levels ($\geq$10 ng/mL) were significantly associated with T3 tumors (Table 1).

To determine if polymorphisms in the *AR*, *PSA*, *CYP17*, or *CYP19* genes could predict the presence of cancer, genotyping data were compared between case and control patients. The mean length of the CAG repeat of the *AR* gene in the cancer group was 19.76 ± 2.03 (range 18–25 repeats) compared with 19.85 ± 3.68 (range 11–32 repeats) in cancer-free men. There

Table 1. Clinical Characteristics and Genotyping Findings in PCa (Cases) and Cancer-Free (Controls) Patients at Prostate Biopsy

	Group		
Variables	*Cases* (n = *47*)	*Controls* (n = *49*)	p *value*[a]
Age (years) ($n = 96$)			
≤55	3	6	0.4870
>55	44	43	
Mean ± SD	66.87 ± 7.72	66.49 ± 8.51	0.8184[b]
Median	67	68	
Interval	46–84	47–85	
Serum PSA (ng/mL) ($n = 92$)			
≤4	8	15	0.0930*
4–10	19	18	1.0000
>10	20	12	0.1292
Mean ± SD	10.73 ± 10.10	7.44 ± 6.25	0.0719[c,*]
Median	8.63	6.7	
Interval	0.2–49.55	0.4–29.46	
AR genotype ($n = 92$)			
Short alleles (≤20 CAG repeats)	33	28	0.3778
Long alleles (>20 CAG repeats)	13	18	
PSA genotype ($n = 85$)			
Homozygous AA	7	19	0.0091
Heterozygous AG	19	18	0.8283
Homozygous GG	16	6	0.0138
CYP17A genotype ($n = 93$)			
Homozygous *CYP17*A1/CYP17*A1*	20	27	0.5354
Heterozygous *CYP17*A1/CYP17*A2*	22	18	0.1496
Homozygous *CYP17*A2/CYP17*A2*	1	5	0.2115
CYP19 genotype ($n = 94$)			
Short alleles homozygous	4	2	0.4021
Short/long alleles heterozygous	1	1	1.0000
Long alleles homozygous	37	49	0.4597

[a]*p* value was obtained from Fisher's exact test.
[b]Unpaired *t*-test.
[c]Student's *t*-test, at 5% of significance level.
*marginally significant.

were no significant differences in the distribution of *AR* short alleles (≤20 repeats) or long alleles (>20 repeats) between the groups. No significant association was found between *AR* genotype when the group of adenocarcinoma patients was stratified according to histological grade (Gleason score ≤ 6 vs. ≥ 7) or any clinical feature (Table 2).

The length of CAG repeats in the *AR* gene was compared in matched peripheral blood and prostate biopsies. A total of 152 biopsies from 92 individuals were genotyped. Among 46 PCa patients, 4 had three biopsies, 33 had two biopsies, and 9 had one biopsy analyzed. In the group of controls, 19 individuals had two biopsies and 27 had one biopsy. Somatic mosaicism, detected as a gain of one CAG repeat in the prostate tissue compared with another biopsy (one case) or with peripheral blood (two cases), was found in individuals without cancer. In addition, one PCa patient showed a reduction from 23 repeats in peripheral blood to 18 repeats in one prostate adenocarcinoma biopsy.

A 3.79-fold increased PCa risk was determined for the GG genotype of the *PSA* gene ($p = 0.0138$, 95% CI 1.3996 to 10.9963). Further, the homozygous AA genotype was frequently observed among men who were cancer-free ($p = 0.0091$, OR 0.2526, 95% CI 0.092 to 0.6939). A marginally significant correlation was observed between serum PSA levels and *PSA* genotyping ($p = 0.0687$, 95% CI -0.3146 to 8.315, unpaired *t*-test). The AA genotype was associated with lower PSA levels (6.44 ± 1.64 ng/mL) compared with genotypes having at least one G allele (10.44 ± 10.06 ng/mL).

The Student's *t*-test was applied to simultaneously compare the *AR* and *PSA* genotyping (pairwise tests with the following dichotomous variables: *AR* short or long allele vs. *PSA* AA or AG/GG genotypes) with the serum PSA levels. When the individuals were cross-classified by *AR* and *PSA* genotypes, it was observed that the differences detected in the serum PSA levels were not influenced by the *AR* CAG repeat length.

The multivariate logistic analysis confirmed the association between PSA levels and *PSA* genotypes (AA vs. AG + GG; $\chi^2 = 0.0482$) and *CYP19* (short alleles homozygous vs. at least one long allele; $\chi^2 = 0.0110$) genotypes.

The previously reported association between the T/C substitution in the 5′-untranslated region of the *CYP17* gene and PCa risk (Haiman *et al.*, 2001; Platz and Giovannucci, 2004) was not observed in this study. However, an association between *CYP17*A1* homozygosity and clinical stage II in PCa group was detected ($p = 0.0052$, Fisher's exact test) (Table 2). Further, a high frequency of individuals with a homozygous genotype for the long TTTA repeats of the *CYP19* gene was observed in both case and control groups.

Discussion

The goals of the present study were to further characterize androgen-related gene polymorphisms and to examine the associations of these polymorphisms with serum PSA levels and other clinical and histopathological parameters in PCa patients. The choice of adequate controls is a crucial factor because many studies have used case-control designs and control groups in which the absence of cancer was not established definitively (Forrest *et al.*, 2005). In this study, we report a series of men at high risk for PCa who require a prostate biopsy. The men were classified into two groups: those with cancer and the controls. Individuals classified as controls were determined to be PCa free after histological evaluation of several biopsy cores (3–10, median of 6 cores) and after clinical follow-up (including measurement of PSA levels and ultrasonography tests) for at least 54 months.

No significant difference in CAG repeat length of the *AR* gene was observed between PCa patients and controls. In addition, there were no associations between CAG repeat length and clinical or histological parameters of the analyzed adenocarcinomas. The functional polymorphism in exon 1 is one of the better-studied *AR* gene polymorphisms, although its association with PCa remains inconclusive. This polymorphism was investigated in cohorts of several different sizes; however, recent multiethnic cohort studies failed to confirm that the common genetic variants of the *AR* gene influence the risk of PCa (Zeegers *et al.*, 2004; Freedman *et al.*, 2005).

In our study, an interesting finding was the somatic mosaicism detected in four individuals by comparison of CAG repeat length between DNA obtained from matched peripheral blood and from prostate biopsy. We detected the gain of one CAG triplet in the prostate tissue from three individuals without cancer and the loss of five triplets in the prostate tumor of one patient. After a mean of 49 months of follow-up, the three cases with mosaicism and absence of cancer did not progress to adenocarcinoma. Reports investigating tissue heterogeneity of CAG repeat length in PCa are rare. Alvarado *et al.* (2005) reported a very significant shortening of CAG repeat lengths in PCa tissues, which showed a much greater degree of genetic heterogeneity than adjacent, benign prostate tissues. The authors suggested that somatic mosaicism of the AR CAG repeat may be an important genetic event in precancerous tissues that subsequently leads to cancer progression. However, we did not detect a significant association between CAG repeat length (≤20 vs. > 20 repeats) and Gleason score or clinical stage, probably due to the small sample size analyzed.

Although not conclusive, the serum PSA level remains the most important predictor of PCa. Higher PSA levels reflect an increased likelihood of a prostate tumor, more specifically of a high-grade cancer (Schatzl *et al.*, 2005). Initially, functional SNPs in the upstream regulatory region of the *PSA* gene were associated with increased promoter activity and serum PSA levels in men without prostatic disease (Cramer *et al.*, 2003). In addition, it was demonstrated that the combination of the *PSA* GG genotype and the *AR* short CAG genotype increased the risk of PCa fivefold (Xue *et al.*, 2000). In the present study, a 3.79-fold increased risk of PCa was detected for the GG genotype of the *PSA* gene. This SNP was not associated with the prognosis of PCa, although there was a tendency for an association between the G allele and higher levels of serum PSA. Moreover, all six PCa patients with serum PSA levels > 20 ng/mL (data not shown) and five patients in clinical stage III were heterozygous or homozygous for the G allele. Consistent with our findings, some other studies have shown a significant association of this SNP with PCa risk, especially when in combination with the *AR* genotype. However, in the present study, the differences in serum PSA levels were not influenced by the *AR* CAG repeat length. These data suggest that genotyping of the *PSA* gene is useful in improving the predictive value of PSA screening.

Molecular epidemiological studies have presented contradictory results concerning potential roles for *CYP17* and

Table 2. Description of Cases According to Genotyping Distribution, Clinical, and Histopathological Data in Prostate Adenocarcinoma Patients

		Genotyping[a]														
		AR (n = 46)		*PSA* (n = 42)				*CYP17* (n = 43)				*CYP19* (n = 42)				
Variables	*Number of patients (%)*	*Short*	*Long*	p *value*[b]	*AA*	*AG*	*GG*	p *value*	*A1/A1*	*A1/A2*	*A2/A2*	p *value*	*SS*	*SL*	*LL*	p *value*
Age (years) ($n = 47$)																
<55	3 (6%)	1	2	0.1884	0	1	2	1.0000	0	1	0	1.0000	0	0	1	1.0000
≥55	44 (94%)	32	11		7	18	14		20	21	1		4	1	36	
Presurgical PSA (ng/mL) ($n = 47$)																
≤4.0	8 (17%)	6	2	1.0000	2	3	1	0.2574	4	4	0	1.0000	2	0	6	0.1580
4.0–10	19 (40%)	12	6	0.7383	4	6	7	0.4133	7	10	0	0.7556	1	0	15	1.0000
>10.0	20 (43%)	15	5	0.7495	1	10	8	0.1052	9	8	1	0.7631	1	1	16	0.6227
Clinical stage ($n = 47$)																
II	32 (68%)	24	8	0.4934	5	9	13	1.0000	10	20	1	0.0052	3	1	25	1.0000
III	11 (23%)	7	4	0.7024	1	7	3	0.6536	6	2	0	0.1180	1	0	8	1.0000
IV	4 (9%)	2	1	1.0000	1	3	0	0.5322	4	0	0	0.0393	0	0	4	1.0000
Gleason score in biopsy ($n = 43$)																
≤6	16 (34%)	12	4	1.0000	2	5	7	1.0000	7	8	0	1.0000	2	0	11	0.5756
7	18 (38%)	14	4	0.5223	3	6	6	0.6858	7	8	1	1.0000	2	1	14	1.0000
≥8	13 (28%)	7	5	0.2759	2	8	3	1.0000	6	6	0	1.0000	0	0	12	0.3082
Biochemical recurrence ($n = 41$)																
Presence	8 (20%)	5	3	0.6767	1	4	3	1.0000	3	4	0	1.0000	1	1	6	0.3904
Absence	33 (80%)	23	9		5	12	11		14	15	1		1	0	28	
Laterality ($n = 47$)																
Unilateral	13 (28%)	10	3	0.7290	1	7	4	0.6514	4	9	0	0.2025	2	1	8	0.2771
Bilateral	34 (72%)	23	10		6	12	12		16	13	1		2	0	29	
Extraprostate invasion ($n = 29$)																
Presence	14 (48%)	9	5	0.4485	2	7	4	0.6447	2	8	1	0.1092	1	1	10	1.0000
Absence	15 (52%)	10	5		3	3	6		8	7	0		1	0	14	
Perineural invasion ($n = 18$)																
Presence	15 (83%)	11	4	1.0000	3	5	5	1.0000	7	7	1	1.0000	1	0	14	1.0000
Absence	3 (7%)	2	1		0	2	1		1	2	0		0	1	2	
Positive lymph involvement ($n = 30$)																
Positive	3 (10%)	1	1	1.0000	1	2	0	1.0000	1	2	0	1.0000	0	0	3	1.0000
Negative	27 (90%)	18	9		5	8	10		9	13	1		2	1	22	
Range of percentage of tumor involvement (PTI) ($n = 29$)	5–85%															

[a]Genotyping comparisons utilized the following dichotomous variables: *AR* (short alleles ≤ 20 CAG repeats vs. long alleles > 20 CAG repeats), *PSA* (homozygous AA vs. heterozygous AG + homozygous GG), *CYP17A* (homozygous *A1*A1** vs. heterozygous *A1*A2** + homozygous *A2*A2**), and *CYP19* (S = short alleles homozygous vs. at least one L = long allele).

[b]*p* value was obtained from Fisher's exact test, 5% of significance level.

CYP19 genes in PCa (Douglas *et al.*, 2005; Forrest *et al.*, 2005; Low *et al.*, 2005; Mononen *et al.*, 2006; Tsuchiya *et al.*, 2006), probably due to the presence of several SNPs at these loci, leading to inadequate characterization of genetic variation. Recently, Onen *et al.* (2007) observed that the *CYP17*A1/CYP17*A1* genotype is more common in cases (46%) than controls (32.4%). No significant associations were found between the T/C transition of the *CYP17* gene or the TTTA repeat length of the *CYP19* gene and the risk of PCa development; however, *CYP17*A1* homozygous was more common in clinical stage II PCa patients. A recent study (Tsuchiya *et al.*, 2006) showed an association between long *CYP19* TTTA repeats and poor survival in metastatic PCa patients. In our investigation, a high frequency of this genotype was detected in both case and control groups. In three clinical stage III patients, a positive association, albeit not reaching statistical significance, was detected between *CYP19* long-allele homozygotes and metastasis presence.

Among the four polymorphisms analyzed, the substitution A > G at position –158 in the promoter region of the *PSA* gene was associated with PCa risk at biopsy. Although the etiology of PCa cannot be explained by allelic variability at a single locus, the data obtained demonstrated an association between PSA levels and *PSA* genotypes. Further studies are necessary in a larger number of samples in order to confirm if PSA genotyping could be used as an additional parameter to predict PCa risk.

References

Abate-Shen, C., and Shen, M.M. (2000). Molecular genetics of prostate cancer. Genes Dev **14,** 2410–2434.

Alvarado, C., Beitel, L.K., Sircar, K., Aprikian, A., Trifiro, M., and Gottlieb, B. (2005). Somatic mosaicism and cancer: a micro-genetic examination into the role of the androgen receptor gene in prostate cancer. Cancer Res **65,** 8514–8518.

Arnold, J.T., Liu, X., Allen, J.D., Le, H., McFann, K.K., and Blackman, M.R. (2007). Androgen receptor or estrogen receptor-beta blockade alters DHEA-, DHT-, and E(2)-induced proliferation and PSA production in human prostate cancer cells. Prostate **67,** 1152–1162.

Bharaj, B.S., Vassilikis, E.J.K., and Diamandis, E.P. (1999). Rapid and accurate determination of (CAG) repeats in the androgen receptor gene using polymerase chain reaction and automated fragment analysis. Clin Biochem **32,** 327–332.

Cramer, S.D., Chang, B.L., Rao, A., Hawkins, G.A., Zheng, S.L., Wade, W.N., Cooke, R.T., Thomas, L.N., Bleecker, E.R., Catalona, W.J., Sterling, D.A., Meyers, D.A., Ohar, J., and Xu, J. (2003). Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. J Natl Cancer Inst **95,** 1044–1053.

Cussenot, O., Azzouzi, A.R., Nicolaiew, N., Frontmont, G., Mangin, P., Cormier, L., Fournier, G., Valeri, A., Larre, S., Thibault, F., Giordanella, J.P., Pouchard, M., Zheng, Y., Hamdy, F.C., Cox, A., and Cancel-Tassin, G. (2007). Combination of polymorphisms from genes related to estrogen metabolism and risk of prostate cancers: the hidden face of estrogens. J Clin Oncol **25,** 3596–3602.

Douglas, J.A., Zuhlke, K.A., Beebe-Dimmer, J., Levin, A.M., Gruber, S.B., Wood, D.P., and Cooney, K.A. (2005). Identifying susceptibility genes for prostate cancer—a family-based association study of polymorphisms in CYP17, CYP19, CYP11A1, and LH-β. Cancer Epidemiol Biomarkers Prev **14,** 2035–2039.

Feigelson, H.S., Ross, R.K., Yu, M.C., Coetzee, G.A., Reichardt, J.K., and Henderson, B.E. (1998). Sex steroid hormones and genetic susceptibility to breast and prostate cancer. Drug Metab Rev **30,** 421–434.

Forrest, M.S., Edwards, S.M., Houlston, R., Kote-Jarai, Z., Key, T., Allen, N., Knowles, M.A., Turner, F., Ardern-Jones, A., Murkin, A., Williams, S., Oram, R., Bishop, D.T., Eeles, R.A., and CR-UK/BPG UK prostate cancer study collaborators. (2005). Association between hormonal genetic polymorphisms and early-onset prostate cancer. Prostate Cancer Prostatic Dis **8,** 95–102.

Freedman, M.L., Perace, C.L., Penney, K.L., Hirschhorn, J.N., Kolonel, L.N., Henderson, B.E., and Altshuler, D. (2005). Systematic evaluation of genetic variation at the androgen receptor locus and risk of prostate cancer in a multiethnic cohort study. Am J Hum Genet **76,** 82–90.

Giovannucci, E. (2002). Is the androgen receptor CAG repeats length significant for prostate cancer? Cancer Epidemiol Biomarkers Prev **11,** 985–986.

Habuchi, T., Liqing, Z., Suzuki, T., Sasaki, R., Tsuchiya, N., Tachiki, H., Shimoda, N., Satoh, S., Sato, K., Kakehi, Y., Kamoto, T., Ogawa, O., and Kato, T. (2000). Increased risk of prostate cancer and benign prostate hyperplasia associated with a CYP17 gene polymorphism with a gene dosage effect. Cancer Res **60,** 5710–5713.

Haiman, C.A., Stampfer, M.J., Giovannucci, E., Ma, J., Decalo, N.E., Kantoff, P.W., and Hunter, D.J. (2001). The relationship between a polymorphism in *CYP17* with plasma hormone levels and prostate cancer. Cancer Epidemiol Biomarkers Prev **10,** 743–748.

Kazemi-Esfarjani, P., Trifiro, M.A., and Pinsky, L. (1995). Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenic relevance for the $(CAG)_n$-expanded neuropathies. Hum Mol Genet **4,** 523–527.

Loktionov, A. (2004). Common gene polymorphisms, cancer progression and prognosis. Cancer Lett **208,** 1–33.

Low, Y.L., Taylor, J.I., Grace, P.B., Dowsett, M., Folkerd, E., Doody, D., Dunning, A.M., Scollen, S., Mulligan, A.A., Luben, R.N., Khaw, R.T., Day, N.E., Wareham, N.J., and Bingham, S.A. (2005). Polymorphisms in the CYP19 gene may affect the positive correlations between serum and urine phytoestrogen metabolites and plasma androgen concentrations in men. J Nutr **135,** 2680–2686.

Mononen, N., Seppälä, E.J., Duggal, P., Autio, V., Ikonen, T., Ellonen, P., Saharinen, J., Saarela, J., Vihinen, M., Tammela, T.L., Kallioniemi, O., Bailey-Wilson, J.E., and Schleutker, J. (2006). Profiling genetic variation along the androgen biosynthesis and metabolism pathways implicates several single nucleotide polymorphisms and their combinations as prostate cancer risk factors. Cancer Res **66,** 743–747.

Ntais, C., Polycarpou, A., and Tsatsoulis, A. (2003). Molecular epidemiology of prostate cancer: androgens and polymorphisms in androgen-related genes. Eur J Endocrinol **149,** 469–477.

Onen, I.H., Ekmekci, A., Eroglu, M., Polat, F., and Biri, H. (2007). The association of 5-alpha-reductase II (SRD5A2) and 17 hydroxylase (CYP17) gene polymorphisms with prostate cancer patients in the Turkish population. DNA Cell Biol **26,** 100–107.

Platz, E.A., and Giovannucci, E. (2004). The epidemiology of sex steroid hormones and their signaling and metabolic pathways in the etiology of prostate cancer. J Steroid Biochem Mol Biol **92,** 237–253.

Polymeropoulos, M.H., Xiao, H., Rath, D.S., and Merril, C.R. (1991). Tetranucleotide repeat polymorphism at the human

aromatase cytochrome P450 gene (CYP19). Nucleic Acids Res **19,** 195.

Rao, A., and Cramer, S.D. (1999). Identification of a polymorphism in the ARE1 region of the PSA promoter. Proc Am Assoc Cancer Res **40,** 65.

Ribeiro, M.L., Santos, A., Carvalho-Salles, A.B., and Hackel, C. (2002). Allelic frequencies of six polymorphic markers for risk of prostate cancer. Braz J Med Biol Res **35,** 205–213.

Schatzl, G., Marberger, M., Remzi, M., Grösser, P., Unterlechner, J., Haidinger, G., Zidek, T., Preyer, M., Micksche, M., and Gsur, A. (2005). Polymorphism in the ARE-I region of prostate-specific antigen gene associated with low serum testosterone level and high-grade prostate cancer. Urology **65,** 1141–1145.

Tsuchiya, N., Wang, L., Suzuki, H., Segawa, T., Fukuda, H., Narita, S., Shimbo, M., Kamoto, T., Mitsumori, K., Ichikawa, T., Ogawa, O., Nakamura, A., and Habuchi, T. (2006). Impact of IGF-I and CYP19 gene polymorphisms on the survival of patients with metastatic prostate cancer. J Clin Oncol **24,** 1982–1989.

Xue, W., Coetzee, G.A., Ross, R.K., Irvine, R., Kolonel, L., Henderson, B.E., and Ingles, S.A. (2001). Genetic determinants of serum prostate-specific antigen levels in healthy men from a multiethnic cohort. Cancer Epidemiol Biomarkers Prev **10,** 575–579.

Xue, W., Irvine, R.A., Yu, M.C., Ross, R.K., Coetzee, G.A., and Ingles, S.A. (2000). Susceptibility to prostate cancer: interaction between genotypes at the androgen receptor and prostate-specific antigen loci. Cancer Res **60,** 839–841.

Yang, Q.F., Sakurai, T., Shan L., Yu, Z., Yoshimura, G., Suzuma, T., Umemura, T., Nakamura, M., Nakamura, Y., Mori, I., and Kakudo, K. (2000). Novel polymorphisms of prostate-specific antigen (PSA) gene associated with PSA mRNA expression in breast cancer. Hum Genet **45,** 363–366.

Yang, Q., Shan, L., Segawa, N., Nakamura, M., Nakamura, Y., Mori, I., Sakurai, T., and Kakudo, K. (2001). Novel polymorphisms in prostate specific antigen gene and its association with prostate cancer. Anticancer Res **21,** 197–200.

Zeegers, M.P., Kienemey, L.A.L.M., Nieder, A.M., and Ostrer, H. (2004). How strong is the association between CAG and GGN repeat length polymorphisms in the androgen receptor gene and prostate cancer risk? Cancer Epidemiol Biomarkers Prev **13,** 1765–1771.

Address reprint requests to:
Silvia Regina Rogatto, Ph.D.
NeoGene Laboratory
Department of Urology
Faculty of Medicine
UNESP
Botucatu, SP 18618-000
Brazil

E-mail: rogatto@fmb.unesp.br; silvia.rogatto@hcancer.org.br

Received for publication November 1, 2007; received in revised form March 31, 2008; accepted April 1, 2008.

This article has been cited by:

1. Emanuela Taioli, Vestra Sears, Alexis Watson, Rafael E. Flores-Obando, Maria D. Jackson, Flora A. Ukoli, Ilce M. de Syllos Cólus, Pedro Fernandez, Norma McFarlane-Anderson, Elaine A. Ostrander, Iara S. Rodrigues, Janet L. Stanford, Jack A. Taylor, Marshall Tulloch-Reid, Camille C.R. Ragin. 2013. Polymorphisms in CYP17 and CYP3A4 and prostate cancer in men of African descent. *The Prostate* **73**:6, 668-676. [CrossRef]
2. Johanna Schleutker. 2012. Polymorphisms in androgen signaling pathway predisposing to prostate cancer. *Molecular and Cellular Endocrinology* **360**:1-2, 25-37. [CrossRef]
3. Abha Soni, Anju Bansal, Ashwani Kumar Mishra, Jyotsna Batra, Laishram Chandreshwor Singh, Anurupa Chakraborty, Dhirendra Singh Yadav, Nayan K. Mohanty, Sunita Saxena. 2012. Association of Androgen Receptor, Prostate-Specific Antigen, and CYP19 Gene Polymorphisms with Prostate Carcinoma and Benign Prostatic Hyperplasia in a North Indian Population. *Genetic Testing and Molecular Biomarkers* **16**:8, 835-840. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Monika Kmeťová Sivoňová, Dušan Dobrota, Róbert Dušenka, Iveta Waczulíková, Peter Slezák, Tatiana Matáková, Silvia Mahmoodová, Dušan Mištuna, Ján Kliment. 2012. Effect of CYP17 and PSA gene polymorphisms on prostate cancer risk and circulating PSA levels in the Slovak population. *Molecular Biology Reports* **39**:8, 7871-7880. [CrossRef]
5. Hellen Kuasne, Iara Sant'Ana Rodrigues, Paulo Emílio Fuganti, Roberta Losi-Guembarovski, Kazuhiro Ito, Marina O. Kishima, Marco Aurélio de Freitas Rodrigues, Silvia Regina Rogatto, Rodrigo Mattos dos Santos, Ilce Mara de Syllos Cólus. 2010. Polymorphisms in the AR and PSA Genes as Markers of Susceptibility and Aggressiveness in Prostate Cancer. *Cancer Investigation* **28**:9, 917-924. [CrossRef]