Comprehensive Assessment of Candidate Genes and Serological Markers for the Detection of Prostate Cancer

Robert K. Nam,[1] William W. Zhang,[3] John Trachtenberg,[2] Michael A. S. Jewett,[2] Marjan Emami,[1] Danny Vesprini,[3] William Chu,[3] Minnie Ho,[3] Joan Sweet,[4] Andrew Evans,[4] Ants Toi,[5] Michael Pollak,[6] and Steven A. Narod[3]

[1]Division of Urology, Sunnybrook and Women's College Health Sciences Centre, [2]University Health Network, [3]Department of Public Health Sciences, [4]Department of Pathology, [5]Department of Medical Imaging, University Health Network, University of Toronto, Toronto, Ontario, and [6]Department of Oncology, McGill University, Montreal, Quebec, Canada

Abstract

We examined whether selected polymorphisms in 11 candidate genes and serum levels of insulin-like growth factor I (IGF-I) help predict the presence of prostate cancer among patients prescreened with prostate-specific antigen (PSA) and digital rectal exam (DRE). We studied 1031 consecutive men who underwent one or more prostate biopsies because of an elevated PSA level (>4 ng/ml) or an abnormal DRE. Eleven candidate genes were examined, including the androgen receptor, *SRD5A2*, *CYP17*, *CYP3A4*, vitamin D receptor, PSA, *GST-T1*, *GST-M1*, *GST-P1*, IGF-I, and IGF binding protein 3. We also measured serum IGF-I levels before biopsy. Of the 1031 men, 483 had cancer on any biopsy (cases) and 548 men had no cancer (controls). Age, ethnicity, total PSA, and DRE result were strongly predictive of the presence of prostate cancer. The mean IGF-I level for cases (119.4 ng/ml) was lower than for controls (124.4 ng/ml, $P = 0.05$) and were not predictive for the presence of prostate cancer. We found no associations between the androgen receptor, *SRD5A2*, *CYP17*, *CYP3A4*, vitamin D receptor, *GST-M1*, *GST-P1*, and IGF binding protein 3 genotypes and prostate cancer risk. The adjusted odds ratios for having prostate cancer for patients with the *GST-T1* and IGF-I variant alleles were 1.64 (95% confidence interval, 1.1–2.4; $P = 0.01$) and 1.70 (95% confidence interval, 1.1–2.7; $P = 0.02$), respectively. Nine of 11 candidate genes were not significantly predictive for prostate cancer in a clinical setting. The *GST-T1* and IGF-I polymorphisms demonstrated modest associations with prostate cancer risk. IGF-I levels were not helpful in identifying patients with prostate cancer at the time of biopsy.

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Requests for reprints: Dr. Robert K. Nam, 2075 Bayview Avenue, MG-406, Toronto, Ontario, M4N 3M5 Canada. Phone: (416) 480-5075; Fax: (416) 480-6121; E-mail: robert.nam@utoronto.ca.

Introduction

Prostate cancer is the most common malignancy diagnosed in males and is a major public health problem (1, 2). The most frequent screening test used to help to identify patients with prostate cancer is the measurement of serological levels of serum prostate-specific antigen (PSA; Ref. 3). Two large clinical trials are under way to establish whether prostate cancer screening reduces mortality (4, 5).

The sensitivity of PSA as a screening test is high (6), but the positive predictive is relatively low for screening of the general population (7). Many benign conditions of the prostate gland, including benign prostatic hyperplasia and prostatitis, which are prevalent among older men, also elevate PSA levels (8), and consequently, many men are unnecessarily subjected to invasive prostatic biopsies. The use of variations of the PSA test, including PSA density, and the free:total PSA ratio have not eliminated this problem (9).

Association studies have examined the significance of several candidate genes based on biological pathways associated with prostate carcinogenesis. A significant number of these genes encode for proteins involved in androgen metabolism (10). Polymorphic alleles of the androgen receptor (11, 12), the vitamin D receptor (13), 5-α reductase (14, 15), *CYP17* (16), and *CYP3A4* (17) genes have been associated with the presence of prostate cancer in some studies.

The CAG repeat polymorphism of the X-linked androgen receptor gene has been well described (18). The average length of the CAG repeat in the population is reported to be 21 repeat units, with a range of 11–31 in healthy individuals (18). Men with a CAG repeat sequence of $<$19–22 units have a relative risk of 1.5–2.1 for developing prostate cancer, compared with patients with a longer CAG repeat (11, 12). We described an increased rate of prostate cancer progression for men with CAG repeats of $\leq$18 units (19).

A substitution of valine (*V*) to leucine (*L*) in codon 89 in exon 1 of the 5-α reductase enzyme gene (*SRD5A2*) has been associated with different levels of 5-α reductase-2 activity and with prostate cancer risk (14, 15). Levels of 5-α reductase-2 expression are higher in human prostate cancer cells than in benign prostatic cells (20). It is believed that polymorphic variants with the *SRD5A2* gene influence 5-α reductase-2 activity (14). A polymorphism of the *SRD5A2* gene, consisting of a valine (*V*) to leucine (*L*) substitution at codon 89 in exon 1 is associated with different levels of 5-α reductase-2 activity and with prostate cancer risk (14, 21). We reported that patients with the *V* allele have a 2.5 fold increase in risk for prostate cancer compared with men with the *L* allele (15).

Two genes encoding for the cytochrome *p*-450 enzymes, *CYP17* and *CYP3A4*, influence the rate of androgen metabolism. The *CYP17* enzyme is the rate-limiting step in androgen biosynthesis from 17-α hydroxylase and 17,20-lyase (16). The *A2* allele of the *CYP17* gene represents a thymine to cytosine substitution and has been associated with a 1.2- to a 2.8-fold

increase in the risk of prostate cancer (22, 23). The *CYP3A4* gene metabolizes testosterone to less active metabolites (17). A germ-line genetic variant in the 5′ regulatory region of the *CYP3A4* gene that substitutes an alanine for a glycine at codon 293 has been associated with a 1.7–9.5-fold increase in risk for prostate cancer (17, 24).

There have been many polymorphisms described in the vitamin D receptor gene (25) and several have been linked to prostate cancer risk (13). There are many hypotheses linking the role of vitamin D, its receptor, and prostate carcinogenesis, including different UV radiation and prostate cancer risk (26) and *in vitro* expression of the vitamin D receptor in prostate cancer cells (27).

Other candidate genes which have been linked to prostate cancer include the *PSA* gene and glutathione *S*-transferase (GST) enzyme genes. A *G/A* polymorphism of the androgen receptor domain of the *PSA* gene has been reported to be positively associated with prostate cancer risk (28). Xue *et al.* (28) found that this polymorphism was also associated with serum PSA levels and with prostate cancer stage at presentation. Two subsequent studies yielded conflicting results. Xu *et al.* (29) found no relationship with this polymorphism and prostate cancer risk. In contrast, another group found a positive association (odds ratio 2.9 for prostate cancer for patients with the *A*-allele) with this gene (30).

The GST group of enzymes (GSTs, type M/T/P) conjugate glutathione intermediate metabolites that can be carcinogenic (31, 32). Homozygous deletions result in absent GST enzymes, which results in poorer elimination of carcinogens, possibly increasing the risk for carcinogenesis (31). Certain deletions of the GST family of genes have been associated with increased risk of cancer of several sites known to be strongly related to carcinogens, including lung (33), colon (34), and breast (35). However, patients with one or more nondeleted *GST-T1* alleles were reported to have a 2-fold increase in prostate cancer (36).

High serum levels of insulin-like growth factor type I (IGF-I) and low serum levels of IGF binding protein type-3 (IGFBP-3) have been shown to be associated with an increased risk of prostate cancer (37, 38). Polymorphisms of the *IGF-I* and *IGFBP-3* genes have been shown to be associated with serum IGF-I and IGFBP-3 levels (39, 40), but these variants have not been examined for prostate cancer risk. A microsatellite polymorphism (variable length *CA* repeat sequence) has been identified in the promoter region of the *IGF-I* gene (41). The most common allele has *19 CA-repeats* (*CA19*) and that those with the homozygous *CA19/CA19* genotype have been found to have lower serum IGF-I levels (41). A single bp polymorphism in the promoter region of the *IGFBP-3* gene has been described and has been shown to be associated with serum IGFBP-3 levels (39). Those with the *C-allele* had lower IGFBP-3 levels (39). These polymorphisms of the *IGF-I* and *IGFBP-3* genes could be associated with prostate cancer, given the strong relationships found between their serum levels and prostate cancer risk.

The clinical significance of these biomarkers has not been established. To date, no studies have examined the use of these markers to identify patients at increased risk for having prostate cancer. Previous association studies have been based on highly selected controls who have a low inherent risk for prostate cancer, namely with a low PSA and a normal digital rectal exam (DRE) or patients with histological evidence of only benign prostatic hyperplasia. Additional case-control studies of this design are not needed. However, these biomarkers have not been tested in a true clinical setting where a patient requires a prostate biopsy because of an abnormal screening test result with PSA and DRE. It would be important to evaluate the risk for prostate cancer based on these biomarkers among unselected controls beyond the low predictive ability of current screening tests of PSA and DRE.

To establish whether these genetic markers are predictive for prostate cancer, we genotyped the androgen receptors *SRD5A2*, *CYP17*, *CYP3A4*, vitamin D receptor, *PSA*, *GST-T/M/P1*, *IGF-I*, and *IGFBP-3* polymorphisms among 1031 men who underwent a prostate biopsy because of a PSA of ≥4.0 ng/ml or an abnormal DRE. We also measured serum-free PSA, IGF-I, and IGFBP-3 levels before prostate biopsy.

Materials and Methods

Study Subjects. Patients were drawn from a consecutive sample of 1103 eligible men who were referred to the Prostate Center of the University Health Network, between June 1998 and June 2000, because of either a PSA value ≥4.0 ng/ml or because of an abnormal DRE. In Ontario, there are no formal criteria for PSA screening, but the practice is widespread. The study population was not part of a formal screening program, but it is representative of all clinical referrals to the Prostate Center. No patient had a history of prostate cancer before prostate biopsy, and no patients were included who were referred for tertiary management.

Of the 1103 patients, 1031 (93.4%) consented to participate in the study. Blood samples were collected before clinical prostate examination. Plasma was separated from blood samples and was stored at −70°C. A urological history, which included the American Urological Association Symptom Score describing the severity of lower urinary tract voiding symptoms, was obtained (42). The results of DRE, performed by one evaluator (A. T.) were recorded. Systematic ultrasound-guided needle biopsies that obtain 6–12 samples were performed, using an 18-gauge spring loaded biopsy device (Bard Magnum, Murray Hill, NJ). The primary end point was the histological presence of adenocarcinoma of the prostate in the biopsy specimen. Grade was evaluated by the Gleason scoring system (43). All research was conducted with informed consent and with the approval of the hospital research ethics board.

Of the patients who did not have evidence of cancer on the initial prostate biopsy, repeat prostatic biopsies were offered because cancer can be detected in ~15–30% of patients in subsequent biopsies who have an initial negative prostate biopsy (44, 45). Of the 1031 patients, 639 patients underwent one biopsy session, of whom 347 (54.3%) had cancer. Of the remaining 392 men, 328 had two prostate biopsy sessions with 128 (39.0%) men having cancer on the second biopsy, and 63 men had three or more biopsy sessions with 8 (12.5%) having cancer on the third biopsy. In total, of the 1031 patients, 483 (46.9%) had cancer (cases) and 548 (53.1%) had no evidence of cancer (controls).

Genetic Analysis. DNA samples from each patients were extracted from peripheral blood leukocytes using standard protocols. Eleven polymorphisms were examined, including the androgen receptor, *SRD5A2*, *CYP17*, *CYP3A4*, vitamin D receptor, *PSA*, *GST-M/P/T1*, *IGF-I*, and *IGFBP-3* genes (Table 1). To ensure for quality control of the restriction digests, we randomly duplicated 25% of samples for comparison for each polymorphism. All gel readers were blinded to the primary end point and covariates.

Serological Analysis. Both free and total PSA levels were measured using commercially available kits, performed on the Immulite chemiluminescence immunoassay system (Diagnostic Products Corporation, San Diego, CA) according to the man-

Table 1 Summary of polymorphisms of the 11 candidate genes, including method of DNA analysis and upstream/downstream primers used

Gene	Polymorphism	Method	Primers		Reference
			Upstream	Downstream	
Androgen receptor	CAG repeat	Direct sequencing	GTGGAAGATTCAGCCAAGCT	TTGCTGTTCCTCATCCAGGA	19
SRD5A2	V89L	RFLP	CGCCTGGTTCCTGCAGGAGCT	GTGAAGGCGGCGTCTGTG	15
CYP17	Single nucleotide base change T/C	RFLP	CATTCGCACCTCTGGAGTC	GGCTCTTGGGGTACTTG	23
CYP3A4	NFSE[a]	CSGE	AACAGGGGTGGAAACACA	CTTTCCTGCCCTGCACAG	17
Vitamin D receptor	*BsmI* (intron 8)	RFLP	CAACCAAGACTACAAGTACGCGTCAGTGA	AACCAGCGGGAAGAGGTCAAGGG	25
PSA	Single bp change G/A	RFLP	TTGTATGAAGAATCGGGGATCGT	TCCCCCAGGAGCCCTATAAAA	28
GST-T1	Homozygous deletion	RFLP	TTCCTTACTGGTCCTCACATC	TCACCGGATCATGGCCAGCA	36
GST-M1	Homozygous deletion	RFLP	CTGCCCTACTTGATTGATGGG	CTGGATTGTAGCAGATCATGC	36
GST-P1	Single bp change I/V	RFLP	CAACTTCATCCACGTTCACC	GAAGAGCCAAGGACAGTT	32
IGF-I	19-repeat allele	VNTR	GCTAGCCAGCTGGTGTTATT	ACCACTCTGGGAGAAGGGTA	39
IGFBP-3	Single bp change A/C	RFLP	CCACGAGGTACACACGAATG	AGCCGCAGTGCTCGCATCTGG	39

[a] NFSE, nifedipine specific element; CSGE, conformation-sensitive gel electrophoresis; VNTR, variable number of tandem repeats.

ufacturer's recommendations. Plasma levels of IGF-I and IGFBP-3 were determine using assay kits based on ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX; Ref. 37).

Data Analysis. We compared the frequencies of the candidate polymorphic variants and the levels of IGF-I and IGFBP-3 between prostate cancer cases and controls. The effect of the polymorphic alleles in predicting prostate cancer was examined using univariate and multivariate logistic regression modeling, controlling for age, serum PSA level, DRE, ethnic background, and the presence of lower urinary tract voiding symptoms.

The *SRD5A2*, *CYP17*, *CYP3A4*, vitamin D receptor, *PSA*, *GST-P1*, and *IGFBP-3* genes were categorized according to 0, 1, or 2 variant alleles: (*a*) *SRD5A2*, *VV/VL/LL* (15); (*b*) *CYP17*, *A1A1/A1A2/A2A2* (23); (*c*) *CYP3A4*, *WW/WV/VV* (17); (*d*) vitamin D receptor, *bb/Bb/BB* (25); (*e*) *PSA*, *AA/AG/GG* (28); (*f*) *GST-P1*, *II/IV/VV* (32); and (*g*) *IGFBP-3*, *AA/AC/CC* (39). The CAG repeat polymorphism of the androgen receptor gene was dichotomized at ≤18 CAG repeat units and >18 units. Ours and other studies have shown this to be an important cutoff level for prostate cancer risk and progression (11, 19). The *GST-M1* and *T1* genes were dichotomized into either homozygous deletions (0) or at least one nondeleted allele (1), according to Rebbeck *et al.* (36), who showed a positive association between the nondeleted genotype of the GST-T1 gene and prostate cancer risk. The *IGF-I* gene was categorized into two *19CA* alleles, one *19CA* allele, or no *19CA* allele (40).

Total serum PSA level was categorized into four groups: (*a*) ≤4.0; (*b*) 4.1–10.0; (*c*) 10.1–20.0; and (*d*) >20.0 ng/ml. Free:total-PSA was divided into quartiles of the controls grouped in descending order because low free:total PSA ratios are associated with a higher risk for prostate cancer (46). IGF-I and IGFBP-3 levels were categorized into their quartile distribution of the control group. DRE was categorized into three groups: (*a*) nonpalpable; (*b*) palpable asymmetric firmness; and (*c*) palpable nodule. Lower urinary tract symptoms were dichotomized as present or absent. Ethnicity was categorized into four groups: (*a*) Caucasian; (*b*) black; (*c*) Asian; and (*d*) other.

Results

The mean age at biopsy of the 1031 men was 65.4 years (range, 41.7–90.8 years). The mean PSA level was 12.3 ng/ml (range, 0.4–498.8 ng/ml), whereas 56.5% had a normal DRE. The majority of the patients were Caucasian (865, 83.9%); 90 (8.7%) and 59 (5.7%) were black and Asian, respectively. Twelve percent of patients had at least one relative with prostate cancer.

Of the 1031 men, 483 men (46.9%) were found to have adenocarcinoma of the prostate at biopsy (cases). Of the 548 men with no evidence of invasive cancer (controls), 292 (53.3%) had one prostate biopsy, 200 (36.5%) men had two prostate biopsies, and 56 (10.2%) had three or more prostate biopsies. Of these control patients, 39 had normal prostate tissue, 406 had inflammation/benign prostatic hyperplasia, and 103 had prostatic intraepithelial neoplasia.

The mean age of the cases (66.6 years) was higher than that of the controls (64.4 years, $P = 0.0001$). Cases were more

Table 2 Frequency distribution of established risk factors for prostate cancer among cases and controls

Subgroup	Number of cases (n = 483)	Number of controls (n = 548)	P
Age (years)			
≤50	10 (2.1%)	22 (4.0%)	
51–60	106 (21.9%)	136 (24.8%)	
61–70	195 (40.4%)	267 (48.7%)	
>70	172 (35.6%)	123 (22.5%)	0.0001
Prostate-specific antigen (ng/ml)			
≤4.0	18 (3.7%)	76 (13.9%)	
4.1–10.0	261 (54.1%)	303 (55.3%)	
10.1–20.0	143 (29.6%)	139 (25.4%)	
>20.0	61 (12.6%)	30 (5.5%)	0.0001
Digital rectal exam			
Nonpalpable	238 (49.3%)	343 (62.6%)	
Assymetry	115 (23.8%)	116 (21.2%)	
Nodule	130 (26.9%)	89 (16.2%)	0.0001
Ethnic background			
Caucasian	421 (87.2%)	444 (81.0%)	
Black	45 (9.3%)	45 (8.2%)	
Asian	12 (2.5%)	47 (8.6%)	0.0001
Other	5 (1.0%)	12 (2.2%)	
Family history of prostate cancer			
Negative	417 (86.3%)	487 (88.9%)	
Positive	66 (13.7%)	61 (11.1%)	0.22
Obstructive urinary symptoms			
Absent	112 (23.2%)	107 (19.5%)	
Present	371 (76.8%)	441 (80.5%)	0.15

Table 3 Frequency distribution of the polymorphic genotypes among cases and two control groups

Group one consists of patients with no cancer from any biopsy and group two consists of patients with no cancer from two or more prostate biopsies.

Genotype	Number of cases (n = 483)	Number of Controls			P
		Control group 1 (n = 548)	P	Control group 2 (n = 256)	
SRD5A2					
VV	250 (51.8%)	257 (46.9%)		122 (47.7%)	
V/L	194 (40.2%)	238 (43.4%)		111 (36.4%)	
L/L	39 (8.1%)	53 (9.7%)	0.27	23 (9.0%)	0.57
CYP17					
A1/A1	178 (36.9%)	195 (35.6%)		89 (34.8%)	
A1/A2	228 (47.2%)	254 (46.3%)		121 (47.3%)	
A2/A2	77 (15.9%)	99 (18.1%)	0.66	46 (17.9%)	0.74
CYP3A4					
WW	411 (85.1%)	469 (85.6%)		216 (84.4%)	
WV	51 (10.6%)	55 (10.0%)		28 (10.9%)	
VV	21 (4.3%)	24 (4.4%)	0.96	12 (4.7%)	0.96
PSA					
AA	159 (32.9%)	164 (29.9%)		75 (29.3%)	
AG	205 (42.5%)	239 (43.6%)		115 (44.9%)	
GG	119 (24.6%)	145 (26.5%)	0.56	66 (25.8%)	0.60
VDR					
bb	114 (23.6%)	130 (23.7%)		57 (22.3%)	
Bb	174 (36.0%)	203 (37.0%)		93 (36.3%)	
BB	195 (40.4%)	215 (39.3%)	0.92	106 (41.4%)	0.92
GST-T1					
0	90 (18.6%)	127 (23.2%)		70 (27.3%)	
1	393 (81.4%)	421 (76.8%)	0.07	186 (72.7%)	0.006
GST-M1					
0	235 (48.6%)	266 (48.5%)		124 (48.4%)	
1	248 (51.4%)	282 (51.5%)	0.57	132 (51.6%)	0.77
GST-P1					
IL/IL	227 (47.0%)	286 (52.2%)		130 (50.8%)	
IL/V	225 (46.6%)	232 (42.3%)		111 (43.4%)	
VV	31 (6.4%)	30 (5.5%)	0.24	15 (5.8%)	0.62
IGF-I					
No 19 allele	64 (13.3%)	103 (18.8%)	0.05	53 (20.7%)	0.02
One 19 allele	230 (47.6%)	253 (46.2%)		120 (46.9%)	
Two 19 alleles	189 (39.1%)	192 (35.0%)		83 (32.4%)	
IGFBP-3					
AA	135 (28.0%)	145 (26.5%)		74 (28.9%)	
AC	233 (48.2%)	274 (50.0%)		125 (48.8%)	
CC	115 (23.8%)	129 (23.5%)	0.83	57 (22.3%)	0.89
AR					
≤18 units	421 (87.2%)	476 (86.9%)		217 (84.8%)	
>18 units	62 (12.8%)	72 (13.1%)	0.89	39 (15.2%)	0.37

likely to have had an abnormal DRE and, on average, had a higher PSA level than controls (Table 2). Asians had a lower rate of prostate cancer than Caucasians or blacks (Table 2).

To determine whether the genotypes of each candidate gene predicted the presence of cancer, we used two control groups. The first control group consisted of all patients with no evidence of cancer on any prostate biopsy. The second group consisted only of patients who had at least two prostate biopsy sessions that did not reveal any malignancy.

Two genes, the *GST-T1* and *IGF-I* polymorphisms, were found to be associated with an increased probability for prostate cancer detection. Other genes did not show any significant effects in altering the risk for prostate cancer (Table 3). Using the first control group, the adjusted odds ratio for prostate cancer for patients with the nondeleted allele of the *GST-T1* gene was 1.34 (P = 0.09), compared with patients with homozygous deletions (Table 4). Using the second control group (men with at least two negative biopsies), the odds ratio for prostate cancer based on the *GST-T1* variant allele was 1.64 [95% confidence interval (CI), 1.1–2.4; P = 0.01). Because the adjusted odds ratios for the *IGF-I* polymorphism were similar for patients with one and with two of the *19* alleles (Table 4), we combined the two groups. The adjusted odds ratios for having prostate cancer for patients with at least one *IGF-1 19-repeat* allele was 1.36 (95% CI, 0.9–2.0; P = 0.12). Using the second control group, the adjusted odds ratios for the *IGF-I* polymorphism achieved marginally significance (odds ratio = 1.70, 95% CI, 1.1–2.7; P = 0.02).

When we compared the genotypes with the established risk factors, there were no significant differences in the genotype distributions by age, PSA, DRE, histological grade, and number of needle biopsies performed. We stratified patients into the following: <65 or ≥65 years; <10 or ≥10 ng/ml PSA; normal or abnormal DRE; low, moderate, or high grade; and <8 or ≥8 needle cores. The adjusted odds ratios for prostate cancer for each of those subgroups did not significantly change from the multivariate analysis. However, there were significant differences in the distribution of the genotypes by ethnicity

Table 4 Univariate and multivariate analysis of all covariates for prostate cancer risk, including established risk factors, 11 candidate genes, and IGF-1/IGFBP-3 levels

Covariate	Crude odds ratio	95% confidence interval	*P*	Adjusted odds ratio[a]	95% confidence interval	*P*
Established risk factors						
Age (continuous, per year)	1.04	1.0–1.1	0.0001	1.04	1.0–1.1	0.0001
PSA level (ng/ml)						
<10.0	1.00			1.00		
10.1 –20.0	1.40	1.1–1.8	0.01	1.15	0.8–1.6	0.37
>20.0	2.76	1.7–4.4	0.0001	1.78	1.1–3.0	0.03
Free:Total PSA ratio						
>0.17	1.00			1.00		
0.12 –0.17	2.14	1.4–3.4	0.001	2.40	1.5–3.8	0.0003
0.07 –0.11	3.92	2.6–6.0	0.0001	4.83	3.1–7.6	0.0001
<0.07	5.56	3.7–8.5	0.0001	6.68	4.2–10.6	0.0001
Digital rectal exam						
Normal	1.00			1.00		
Assymetry	1.43	1.1–1.9	0.02	1.65	1.2–2.3	0.004
Nodule	2.11	1.5–2.9	0.0001	2.36	1.7–3.3	0.0001
Ethnic background						
White	1.00			1.00		
Black	1.07	0.7–1.7	0.76	0.99	0.6–1.6	0.97
Asian	0.27	0.1–0.5	0.0001	0.27	0.1–0.5	0.0002
Family history						
Absent	1.00			1.00		
Present	1.26	0.9–1.8	0.22	1.33	0.9–2.0	0.17
Obstructive voiding symptoms						
Absent	1.00			1.00		
Present	0.80	0.6–1.1	0.15	0.88	0.6–1.2	0.45
Genetic risk factors						
SRD5A2						
VV	1.00			1.00		
V/L	0.84	0.6–1.1	0.18	0.86	0.6–1.3	0.28
L/L	0.76	0.5–1.1	0.22	0.97	0.6–1.6	0.89
Androgen receptor[b]						
>18 CAG repeats	1.00			1.00		
≤18 CAG repeats	0.97	0.7–1.4	0.89	1.07	0.7–1.6	0.73
CYP17						
A1/A1	1.00			1.00		
A1/A2	0.98	0.8–1.3	0.90	0.85	0.6–1.1	0.29
A2/A2	0.85	0.6–1.2	0.38	0.84	0.6–1.3	0.40
CYP3A4						
WW	1.00			1.00		
WV	1.06	0.7–1.6	0.78	1.03	0.6–1.7	0.90
VV	1.00	0.5–1.8	0.99	0.73	0.3–1.7	0.48
PSA						
AA	1.00			1.00		
AG	0.89	0.7–1.2	0.40	0.93	0.7–1.3	0.66
GG	0.85	0.6–1.2	0.32	0.86	0.6–1.2	0.41
VDR						
bb	1.00			1.00		
Bb	0.98	0.7–1.4	0.89	0.99	0.7–1.4	0.97
BB	1.03	0.8–1.4	0.84	1.09	0.8–1.5	0.63
GST-T1						
0	1.00			1.00		
1	1.32	0.9–1.8	0.07	1.34	0.9–1.9	0.09
GST-M1						
0	1.00			1.00		
1	0.94	0.8–1.2	0.59	0.91	0.7–1.2	0.42
GST-P1						
IL/IL	1.00			1.00		
IL/V	1.22	0.9–1.6	0.12	1.21	0.9–1.6	0.19
VV	1.30	0.8–2.2	0.33	1.28	0.7–2.3	0.41
IGF-I						
No 19 allele	1.00			1.00		
One 19 allele	1.46	1.0–2.1	0.04	1.30	0.9–2.2	0.08
Two 19 alleles	1.58	1.1–2.3	0.02	1.46	0.9–1.9	0.20
IGFBP-3						
AA	1.00			1.00		
AC	0.71	0.5–0.9	0.03	0.70	0.5–0.9	0.04
CC	1.00	0.7–1.3	0.98	0.90	0.6–1.2	0.52

Table 4 Continued

Covariate	Crude odds ratio	95% confidence interval	*P*	Adjusted odds ratio[a]	95% confidence interval	*P*
IGF-I levels						
<97.4	1.00			1.00		
97.4 –119.1	0.71	0.5–1.0	0.04	0.69	0.5–1.0	0.05
119.2 –150.2	0.71	0.5–1.0	0.04	0.73	0.5–1.1	0.09
>150.2	0.69	0.5–0.9	0.03	0.67	0.5–0.9	0.04
IGFBP-3 levels						
>2636.6	1.00			1.00		
2308.8 –2636.6	0.81	0.6–1.2	0.25	0.79	0.5–1.2	0.24
1967.0 –2308.7	1.14	0.8–1.6	0.46	1.02	0.7–1.5	0.91
<1967.0	1.29	0.9–1.8	0.14	1.27	0.9–1.2	0.21

[a] Each genetic marker assumed to be independent. Thus, each genetic marker adjusted for age, PSA, free:total prostate-specific antigen (PSA) ratio, digital rectal exam (DRE), ethnicity, family history and obstructive voiding symptoms. IGF-1 and IGFBP-3 considered together in a multivariate model adjusting for age, PSA, free:total PSA ratio, DRE, ethnicity, family history and obstructive voiding symptoms.
[b] CAG repeat units also considered as continuous variable. Adjusted odds ratio = 1.01 for prostate cancer per CAG repeat unit increase (95% confidence interval, 0.97–1.05; $P = 0.57$).

(Table 5). To control for possible confounding by ethnicity, we determined the probability for prostate cancer by each genotype within each ethnic group. No significant differences were found.

Finally, we examined whether serum IGF-I and IGFBP-3 levels predicted the presence of prostate cancer and whether the levels of these proteins correlated with their genotypes. The mean IGF-I levels were slightly lower for cases (119.4 ng/ml) than for controls (124.4 ng/ml, $P = 0.05$). The mean IGFBP-3 levels were also lower among the cases (2227.7 ng/ml) than the controls (2306.3 ng/ml, $P = 0.02$). The adjusted odds ratio for detecting prostate cancer for patients with the highest quartile of IGF-I levels was 0.7 (95% CI, 0.5–0.9), compared with patients with lowest quartile level (Table 5). The adjusted odds ratio for detecting prostate cancer for patients with the lowest quartile of IGFBP-3 levels was 1.3 (95% CI, 0.9–1.2), compared with patients with highest quartile level (Table 5). We found no correlation between serum IGF-I levels and the *IGF-I* polymorphism ($P = 0.69$). However, there was a strong correlation between serum IGFBP-3 levels and the *IGFBP-3* polymorphism. The mean IGFBP-3 levels for the *AA*, *AC*, and *CC* genotypes were 2361.2, 2277.9, and 2174.3 ng/ml, respectively ($P = 0.0002$). IGF-I and IGFBP-3 levels were positively correlated with each other ($r = 0.64$, 0.0001) and were negatively correlated with age ($r = -0.25$ for IGF-I, $P = 0.0001$; $r = -0.26$ for IGFBP-3, $P = 0.0001$). Neither level correlated with PSA level at biopsy ($r = -0.03$ for IGF-I, $P = 0.35$; $r = -0.05$ for IGFBP-3, $P = 0.09$).

Discussion

We studied associations between selected polymorphisms of 11 candidate prostate cancer genes and the probability for prostate cancer among an unselected population of men prescreened with PSA and DRE. Among these genes, only the *GST-T1* and *IGF-I* polymorphisms were associated with a higher risk for having prostate cancer (adjusted odds ratios 1.64 and 1.70 for the variant alleles, respectively).

There have been numerous studies examining the association between specific polymorphisms of candidate genes and prostate cancer. However, these studies have mainly been case-control or nested-case control study designs with selected controls (11–17, 22–24, 26, 28–32, 36). In our study, our controls were derived from a group prescreened with PSA and a DRE. For these patients, there could be a clear clinical application in predicting prostate cancer by these markers. Ours is the first study to apply these polymorphisms to predict the presence of prostate cancer in a screening setting. Given the high frequency of the variant alleles of each of the polymorphisms, it is possible that these could be used as adjuncts to current prostate cancer screening tests. We chose 11 polymorphisms from selected candidate genes most frequently reported to be associated with increased prostate cancer risk. Marginal positive findings were found for the *GST-T1* and *IGF-I* polymorphisms.

This was found with the second control group who underwent at least two or more negative prostate biopsies. This was done to reduce misclassification of cases and controls. It has been well established that patients who undergo one prostate biopsy may have a 15–30% chance of having cancer after a second prostate biopsy is performed (44, 45, 47–50). After having two negative prostate biopsies, the chances of having a malignancy is <5% (44, 45). However, 360 patients who had an initial negative biopsy did not undergo a repeat exam. The reasons for this included patient refusal, referring physician's refusal, and loss to follow-up. After excluding these patients, the odds ratio estimates did not significantly change from the initial control group. Further study will be required in these genes to establish whether they will be of clinical importance but may be important in determining which patients will require a repeat biopsy after a negative biopsy.

On the other hand, despite using a control group who had repeat biopsies that demonstrated no evidence of cancer, the potential for misclassifying patients as controls is an important limitation to recognize in this study. The ideal control group would be men with normal PSA and DRE results who have no histological evidence of cancer, which is beyond the scope of this study and would require a large prospective study with long follow-up to assess for prostate cancer development. However, in our study, our objective was to determine whether these genetic and serological factors would be an important adjunct to enhance the ability to detect prostate cancer in a cross-sectional, clinical setting. Past association studies with more rigid selection of controls have examined their relationship with prostate cancer. This study has shown that regardless of the association with prostate cancer, these factors may not be clinically important in detecting prostate cancer. Finding clinically significant results for these types of markers is possible. We have recently shown that a polymorphism of the human kallikrein-2 gene was an important predictor for prostate cancer in a similar study

Table 5 Frequency distribution of genotypes by ethnic background

Covariate	Ethnic background			*P*
	Caucasian	Black	Asian	
SRD5A2				
VV	433 (50.1%)	54 (60.0%)	13 (22.0%)	
V/L	371 (42.9%)	29 (32.2%)	25 (42.4%)	0.0001
L/L	61 (7.0%)	7 (7.8%)	21 (35.6%)	
Androgen receptor				
>18 CAG repeats	768 (88.8%)	58 (64.4%)	55 (93.2%)	0.0001
≤18 CAG repeats	97 (11.2%)	32 (35.6%)	4 (6.8%)	
CYP17				
A1/A1	316 (36.5%)	32 (35.6%)	18 (30.5%)	
A1/A2	412 (47.6%)	38 (42.2%)	26 (44.1%)	0.22
A2/A2	137 (15.9%)	20 (22.2%)	15 (25.4%)	
CYP3A4				
WW	792 (91.5%)	14 (15.6%)	57 (96.6%)	
WV	67 (7.8%)	37 (41.1%)	2 (3.4%)	0.0001
VV	6 (0.7%)	39 (43.3%)	0 (0%)	
PSA				
AA	279 (32.3%)	32 (35.6%)	9 (15.3%)	
AG	375 (43.3%)	44 (48.9%)	18 (30.5%)	0.0001
GG	211 (24.4%)	14 (15.5%)	32 (54.2%)	
VDR				
bb	221 (25.5%)	12 (13.3%)	5 (8.5%)	
Bb	317 (36.7%)	42 (46.7%)	11 (18.6%)	0.0001
BB	327 (37.8%)	36 (40.0%)	43 (72.9%)	
GST-T1				
0	164 (19.0%)	30 (33.3%)	18 (30.5%)	0.001
1	701 (81.0%)	60 (66.7%)	41 (69.5%)	
GST-M1				
0	432 (49.9%)	25 (27.8%)	36 (61.0%)	0.0001
1	433 (50.1%)	65 (72.2%)	23 (39.0%)	
GST-P1				
IL/IL	439 (50.7%)	29 (32.2%)	35 (59.3%)	
IL/V	375 (43.4%)	52 (57.8%)	24 (40.7%)	0.002
VV	51 (5.9%)	9 (10.0%)	0 (0%)	
IGF-I				
No 19 allele	106 (12.3%)	43 (47.8%)	16 (27.1%)	0.0001
One 19 allele	407 (47.0%)	38 (42.2%)	32 (54.2%)	
Two 19 alleles	352 (40.7%)	9 (10.0%)	11 (18.7%)	
IGFBP-3				
AA	251 (29.0%)	15 (16.7%)	9 (15.3%)	
AC	433 (50.1%)	44 (48.9%)	24 (40.7%)	0.0001
CC	181 (20.9%)	31 (34.4%)	26 (44.0%)	

design (51). Nevertheless, it is important to acknowledge that this bias of misclassifying controls would tend to dilute case-control differences that could miss modest effects.

For a large number of the polymorphisms, there were significant differences in the allelic frequencies between ethnicities. However, when restricted to Caucasian subjects, no significant associations were found. Among these, the *SRD5A2* polymorphisms have been known to highly be related to ethnic group as also confirmed in our data (52, 53). However, the V89L polymorphism after adjusting for ethnicity did not show any positive association despite our earlier positive findings (15). In our earlier study, we examined 320 men, compared with the larger sample of 1031 men in this study. It is possible that a type-1 ($\propto$) error occurred in the first study. Another polymorphism, the A49T substitution of the *SRD5A2* gene, has been highly associated with prostate cancer risk among blacks (53). We did not examine this among our cohort given the small proportion of black patients in our group and the low frequency (1.0%) of the A49T mutation (53). The lack of any meaningful findings of these polymorphisms and prostate cancer risk makes these ethnic relationships difficult to make any reasonable conclusions. There could be significant associations among other ethnic groups which we could not detect.

Serum IGF-I levels were not higher in cases than in controls. In fact, IGF-I levels were lower for cases than for controls. No previous studies have examined the relationship between the *IGF-I*, *IGFBP-3* polymorphisms, and prostate cancer risk. In contrast, a number of studies have examined the role of serum IGF-I and IGFBP-3 levels and prostate cancer risk (37, 38, 54–57). It has been well established that IGF-I has mitogenic and antiapoptotic influences on rat prostate epithelial cells suggesting that IGFs may promote prostate carcinogenesis (58). Male mutant mice with no IGF-I activity showed lowered sex drive and are infertile and a significant reduction in the mass of prostate gland and in testosterone levels (59).

Chan *et al.* (37) first demonstrated the ability of serum IGF-1 levels to predict the probability of a future diagnosis of prostate cancer using the cohort from the Physicians' Health Study. Men in the highest quartile of IGF-I levels had a relative risk of 4.3 for developing prostate cancer when compared with men in the lowest quartile. A follow-up study recently further showed that patients with IGF-I levels and low IGFBP-3 levels may be at increased risk for developing advanced stage prostate cancer (54). Because their original report, many other case-control and cohort studies have examined the relationship between IGF-I and prostate cancer showing both positive and negative associations, although all prospective studies have shown a positive association for the future development of prostate cancer (38, 55–57, 60).

Among our patients, both IGF-I and IGFBP-3 levels were significantly lower among the cases compared with the controls. It is possible that IGF-I levels may be altered in the presence of prostate cancer in our study where levels were measured immediately before prostate biopsy. This is an important limitation of case-control studies. Chan *et al.* (37) in the Physicians' Health Study had blood samples obtained ~8 years before the diagnosis of prostate cancer. This is consistent with the argument that a high IGF-I serum level is a risk factor for a future development of prostate cancer, rather than a tumor marker at diagnosis. In our study, IGF-I levels could have already been negatively affected by the presence of prostate cancer, given the case-control nature of the study.

We found a modest correlation with the *IGF-I* polymorphism and prostate cancer risk (odds ratio, 1.70). There was no correlation observed between the IGF-I levels and the *IGF-I* polymorphism. Allen *et al.* (40) also did not find a correlation between serum IGF-I levels and the *CA* repeat polymorphism among healthy middle-aged men. In contrast, we found a strong correlation with the *IGFBP-3* polymorphism and serum IGFBP-3 levels but not with prostate cancer risk. Jernstrom *et al.* (39) reported a strong positive association with IGFBP-3 levels and its polymorphism among premenopausal females, which were in the same direction as our study.

In summary, none of the selected polymorphisms examined from the 11 candidate genes was identified to be a strong predictor of prostate cancer at biopsy and high serum IGF-I levels also was not predictive. Modest significant findings were found for the *GST-T1* and *IGF-I* polymorphisms and further study will be required to examine their clinical significance for patients who require a prostate biopsy. Although strong correlations were found between the *IGFBP-3* polymorphism and serum IGFBP-3 levels, their clinical significance among men at risk for prostate cancer is still unclear. Future studies will be required to evaluate other polymorphic variants of the same and new genes implicated to be associated with prostate cancer.

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