Role of Androgen Metabolism Genes *CYP1B1*, *PSA/KLK3*, and *CYP11α* in Prostate Cancer Risk and Aggressiveness

Mine S. Cicek,[1] Xin Liu,[2] Graham Casey,[1] and John S. Witte[2]

[1]Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic Lerner School of Medicine, Cleveland, Ohio and
[2]Department of Epidemiology and Biostatistics, University of California, San Francisco, California

Abstract

Candidate genes involved with androgen metabolism have been hypothesized to affect the risk of prostate cancer. To further investigate this, we evaluated the relationship between prostate cancer and multiple potentially functional polymorphisms in three genes involved in androgen metabolism: *CYP1B1* (two single nucleotide polymorphisms: *355G/T* and *4326C/G*), prostate-specific antigen (*PSA/KLK3* (three single nucleotide polymorphisms: *−158A/G*, *−4643G/A*, and *−5412C/T*), and *CYP11α* [(tttta)$_n$ repeat], using a moderately large (n = 918) sibling-based case-control population. When looking at all subjects combined, no association was observed between any polymorphism—or their haplotypes—and prostate cancer risk. However, among men with more aggressive prostate cancer, the *CYP1B1 355G/T* variant was positively associated with disease: carrying one or two *T* alleles gave odds ratios (OR) of 1.90 [95% confidence interval (95% CI), 1.09-3.31; P = 0.02] and 3.73 (95% CI, 1.39-10.0; P = 0.009), respectively. Similarly, carrying the *CYP1B1 355T-4326C* haplotype was positively associated with prostate cancer among men with high aggressive disease (P = 0.01). In addition, the *PSA −158G/−158G* genotype was positively associated with prostate cancer among men with less aggressive disease (OR, 2.71; 95% CI, 1.06-6.94; P = 0.04). Our findings suggest that *CYP1B1* and *PSA* variants may affect the risk of prostate cancer and tumor aggressiveness. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2173–7)

Introduction

The causes of prostate cancer are not well known, but both genetic and environmental influences likely play a role. The prostate is an androgen-dependent organ and polymorphic variants in a number of genes involved in androgen metabolism have been implicated in prostate cancer risk. For example, we previously reported that *SRD5A2*, *CYP3A4*, and *CYP3A5* variants may influence risk of developing prostate cancer or more aggressive disease (1, 2). Additional studies have suggested that variants in other genes involved in androgen metabolism or response, such as *CYP1B1*, prostate-specific antigen (*PSA/KLK3*), and *CYP11α*, may also play a role in prostate cancer risk.

CYP1B1 maps to chromosomal region 2p21-22 (3) and is involved in the activation of many procarcinogens and the hydroxylation of testosterone. Several polymorphisms in the *CYP1B1* gene have been described (4), including two common single nucleotide polymorphisms (SNP): *355G/T* (A119S) in exon 2, which is located in the substrate recognition site one (SR1S), and *4326C/G* (L432V) in exon 3, which encodes the heme-binding domain. Functional studies suggest that these nonsynonymous SNPs may alter enzymatic activity and catalytic specificity of CYP1B1 (5). Several studies have evaluated the relationship between these *CYP1B1* polymorphisms and risk of various cancers. These include colorectal (6), breast (7-11), ovarian (12), and prostate cancers (13-15). With regard to the latter, carrying the *CYP1B1 355T* allele, or the *4326G/G* genotype, has been positively associated with prostate cancer among Japanese (14) and Caucasians (13), respectively. A more recent report, however, found an inverse association between a haplotype including *355T-4326C* and sporadic prostate cancer (15).

The gene encoding PSA is regulated by the androgen receptor, which binds to androgen-responsive elements. Serum PSA level is used to screen for prostate cancer because it is often elevated in prostate cancer patients. *PSA* (also known as *KLK3*) is located on human chromosome 19q13.3-13.4, a region previously linked to advanced prostate cancer (16, 17). Both *PSA/KLK3* −158A/G promoter variant alleles (i.e., *−158A* and *−158G*) have been associated with prostate cancer risk (18, 19). In addition, this variant has been positively associated with higher PSA levels in some studies, although other studies have found inverse or null associations (19-23). The *−158A/G* variant may be in linkage disequilibrium with the potential causal variants: a recent study of men without prostate cancer found that two other promoter polymorphisms, *−4643G/A* and *−5412C/T* (but not *−158A/G*), are associated with serum PSA levels and *PSA* promoter activity (22).

The *CYP11α* gene encodes the cholesterol P450 side-chain cleavage enzyme that catalyzes the conversion of cholesterol to pregnenolone mediating the first rate-limiting step in testosterone synthesis (24). *CYP11α* is located on chromosome 15q23-24 and the promoter region of this gene is believed to contain multiple cyclic AMP–regulated elements that are responsible for increasing basal transcriptional activity (25). Up-regulation of this enzyme may lead to an increase in androgen production (26). A microsatellite polymorphism (tttta)$_n$ was identified in the promoter region of *CYP11α*, located 528 bp upstream from the initiation site of the translation of the gene (26). Recent studies have shown this polymorphism to be associated with increased risk of advanced prostate cancer (27), polycystic ovary syndrome (26, 28), and breast cancer (29).

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Requests for reprints: John S. Witte, Department of Epidemiology and Biostatistics, University of California, San Francisco, 500 Parnassus Avenue, MU-420 West San Francisco, CA 94143-0560. Phone: 415-476-1374; Fax: 415-476-6014. E-mail: jwitte@itsa.ucsf.edu

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Table 1. Association between *CYP1B1* variants and prostate cancer in the entire family-based study sample, stratified by disease aggressiveness

CYP1B1 variants	Entire sample			Low aggressive*			High aggressive†		
	Cases/controls	OR (95% CI)‡	*P*	Cases/controls	OR (95% CI)‡	*P*	Cases/controls	OR (95% CI)‡	*P*
Genotypes									
G355T									
GG	216 of 247	1.00		90 of 93	1.00		109 of 136	1.00	
GT	178 of 197	1.06 (0.75-1.50)	0.74	82 of 100	0.73 (0.45-1.19)	0.20	89 of 86	1.90 (1.09-3.31)	0.02
TT	43 of 35	1.68 (0.87-3.25)	0.12	14 of 14	1.00 (0.34-2.91)	0.99	27 of 18	3.73 (1.39-10.0)	0.009
C4326G									
CC	145 of 151	1.00		69 of 65	1.00		71 of 76	1.00	
GC	185 of 205	0.86 (0.56-1.30)	0.47	68 of 90	0.54 (0.28-1.05)	0.07	105 of 106	1.03 (0.57-1.89)	0.91
GG	109 of 123	0.75 (0.43-1.28)	0.29	51 of 52	0.67 (0.30-1.49)	0.32	49 of 58	0.79 (0.35-1.77)	0.57
Diplotypes									
G355T-C4326G									
G-G/G-G	89 of 101	1.00		41 of 41	1.00		40 of 48	1.00	
G-C/G-G	90 of 106	1.08 (0.61-1.89)	0.80	32 of 38	1.38 (0.54-3.54)	0.51	52 of 65	0.71 (0.33-1.51)	0.37
G-C/G-C	37 of 40	1.26 (0.59-2.65)	0.55	17 of 14	2.64 (0.78-8.89)	0.12	17 of 23	0.65 (0.23-1.82)	0.42
G-C/T-C	71 of 85	1.08 (0.59-1.97)	0.80	38 of 40	1.44 (0.59-352)	0.42	32 of 40	0.95 (0.38-2.39)	0.91
T-C/G-G	90 of 94	1.24 (0.75-2.05)	0.41	35 of 51	0.72 (0.33-1.54)	0.39	49 of 37	2.22 (1.07-4.61)	0.03
T-C/T-C	37 of 26	2.17 (1.01-4.67)	0.05	14 of 11	2.24 (0.73-6.81)	0.16	22 of 13	3.27 (1.02-10.5)	0.05
Composites§	23 of 27	0.68 (0.27-1.73)	0.42	9 of 12	0.44 (0.09-2.09)	0.30	13 of 14	1.20 (0.30-4.89)	0.79
T-C‖									
None	235 of 269	1.00		99 of 104	1.00		118 of 146	1.00	
One copy	165 of 184	1.11 (0.77-1.58)	0.58	73 of 92	0.74 (0.44-1.23)	0.24	85 of 81	1.99 (1.15-3.47)	0.01
Two copies	37 of 26	2.07 (1.03-4.16)	0.04	14 of 11	1.55 (0.53-4.58)	0.42	22 of 13	4.00 (1.36-11.8)	0.01

*Includes cases with Gleason score <7 and tumor stage $<T_{2C}$ and their brothers.
†Includes cases with Gleason score ≥ 7 and/or tumor stage $\geq T_{2C}$ and their brothers.
‡Adjusted for age.
§Includes diplotypes T-G/T-G, T-G/G-G, and T-C/T-G.
‖Presence of haplotype T-C.

In light of these existing results, here we aimed to further clarify the relation of polymorphisms in *CYP1B1, PSA/KLK3*, and *CYP11α* with prostate cancer and tumor aggressiveness using a sibling-based case-control population.

Materials and Methods

Study Subjects. The study design and population have been described elsewhere (1). Briefly, siblings (n = 918; 439 cases and 479 controls from 413 families) were recruited from the major medical institutions in the greater Cleveland, Ohio area and from the Henry Ford Health System, Detroit, Michigan. Ninety-one percent of the subjects were Caucasian, 8% were African American, and 1% were Asian or Latino. Institutional Review Board approval was obtained from the participating institutions and all study participants gave informed consent.

Sibling sets consisted of men with prostate cancer diagnosed at age 73 or younger and at least one brother without prostate cancer. The case clinical characteristics at diagnosis (e.g., Gleason score, tumor stage) were obtained from medical records and their disease status was confirmed histologically. All controls were no more than 8 years younger than their brother's age at diagnosis. The disease status of unaffected brothers was further confirmed through testing of PSA levels whenever possible (93% of controls). If a control's PSA level was elevated (>4 ng/mL), they were referred for further evaluation by our collaborating urologists. We followed up these individuals to confirm that they remained disease-free. These criteria were selected in an attempt to minimize potential for misclassification of prostate cancer status amongst controls (30, 31). We chose to use this sibling-based study design to ensure that our control group was selected from the same genetic source population as the cases, and is thus not subject to population stratification from factors such as race (32).

Genotype Analysis. Standard venipuncture was used to collect blood samples from all study participants in tubes with EDTA as an anticoagulant. Genomic DNA was extracted from buffy coats using the QIAmp DNA Blood kit (QIAGEN, Inc., Valencia, CA). All purified DNA samples were diluted to a constant DNA concentration of 5 ng/μL in 10 mmol/L Tris, 5 mmol/L EDTA buffer (pH 8).

CYP1B1. The *CYP1B1_L432V* polymorphism (+4326C/G; rs1056836) was detected by amplifying genomic DNA. PCR products were digested with restriction enzyme and the digested products were separated on an agarose gel. Analysis of *CYP1B1_A119S* (+355G/T; rs1056827) variant was done using the Amplifluor Uniprime SNP Detection System (Chemicon Intl., Serological Corp., Norcross, GA). The method included a ''hot start'' two-step PCR reaction followed by allelic discrimination protocol using the ABI Prism 7900HT (Applied Biosystems, Foster City, CA) for an end-point read and analysis.

PSA/KLK3. The *PSA/KLK3 G−158A* (rs266882), *A−4643G* (rs925013), and *T−5412C* (rs2739448) polymorphisms were detected by amplifying genomic DNA. PCR products were digested with restriction enzyme and the digested products were separated on an agarose gel.

CYP11α. The *CYP11a* $(tttta)_n$ microsatellite polymorphism was detected by amplifying genomic DNA. The forward primer was fluorescently labeled by Beckman color green (D3). PCR product fragment lengths were analyzed on a CEQ 8000 genetic analysis system (Beckman Coulter, Fullerton, CA). The number of (tttta) repeats was confirmed by sequencing at least one sample of each PCR product length that we observed.

To ensure quality control of genotyping results, 5% of samples were randomly selected and genotyped by a second investigator and 1% was sequenced using a 377 ABI automated sequencer (all results were concordant).

Statistical Analysis. A χ^2 goodness of fit test was used to assess deviation from Hardy-Weinberg equilibrium for genotype frequencies of each variant. For the three SNPs in *PSA/KLK3* and two SNPs in *CYP1B1*, linkage disequilibrium was calculated using HAPLOVIEW (http://www.broad.mit.edu/personal/jcbarret/haploview/index.php) in Caucasian or

African American controls (randomly selecting one control from each family). Next, we reconstructed diplotypes (i.e., joint haplotypes) for both *PSA* and *CYP1B1* with the program PHASE (33), initially ignoring relations among siblings. We then estimated diplotypes incorporating relations for families with >2 siblings in the study, using MERLIN (MERLIN v1.0-α, http://www.sph.umich.edu/csg/abecasis/Merlin). The assigned diplotypes were identical using these two approaches.

Conditional logistic regression (using family as the matching variable, and a robust variance estimator that incorporates familial correlations) was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) for the relation between genotypes/diplotypes and prostate cancer. The rare diplotypes (observed in <4% of the entire sample) were collapsed into one group. We also did analyses by grouping diplotypes according to the number of haplotype of interest. The regression model was adjusted for potential confounding by age. To investigate the potential effect of genotypes/diplotypes on disease aggressiveness, we stratified the analyses by the cases clinical characteristics at diagnosis (n = 860). A total of 58 individuals (cases and controls) did not have a complete clinical data and, therefore, were not included in the analyses stratified by disease aggressiveness. Following Rebbeck et al. (34), aggressiveness was defined as ''low'' if the Gleason score of a case was <7 and the tumor category was $<T_{2C}$, and ''high'' if the Gleason score was ≥7 or the tumor category was $\geq T_{2C}$. The tumor category reflects the tumor-node-metastasis system. All P values are from two-sided tests, and all analyses were undertaken with SAS software (version 8.2, SAS Institute, Cary, NC).

Results and Discussion

The present study evaluated the potential association between prostate cancer and polymorphisms in three genes involved in androgen metabolism: *CYP1B1, PSA/KLK3*, and *CYP11α*. The mean ages of cases and controls were 61 and 63 years, respectively, as expected based on our recruitment of slightly older controls. Also as expected, cases had much higher PSA levels in comparison with the controls (mean PSA: 14.8 versus 2.2). About half of our cases were diagnosed with more advanced prostate cancer: 191 (45%) had Gleason scores ≥7, and 55 (13%) have tumor stage $\geq T_{2C}$ (Supplementary Table S1). The variants in *PSA* and *CYP1B1* were in Hardy-Weinberg equilibrium and had strong linkage disequilibrium within genes ($D' > 0.8$).

Table 1 provides ORs for the association between variants in *CYP1B1* and prostate cancer. Among all study subjects, there was no association between the two variants (*G355T* and *C4326G*) and prostate cancer, although there was a weak positive association for the T-C/T-C diplotypes (OR, 2.17; 95% CI, 1.01-4.67; P = 0.05). This result was strengthened among men with more aggressive disease: in comparison with men homozygous for the G-G haplotype (i.e., for SNPs *G355* and *4326G*), men carrying the T-C haplotype had statistically significantly increased risk, except for the diplotype G-C/T-C (Table 2). When we grouped diplotypes according to the number of T-C haplotypes, carrying one and two copies of this haplotype was associated with aggressive prostate cancer, with ORs of 1.99 (95% CI, 1.15-3.47; P = 0.01) and 4.00 (95% CI, 1.36-11.8; P = 0.01). This effect seemed to be primarily due to the G355T SNP. Specifically, in comparison with the *GG* genotype, carrying one or two *T* alleles gave ORs for prostate cancer of 1.90 (95% CI, 1.09-3.31; P = 0.02) and 3.73 (95% CI, 1.30-10.0; P = 0.009), respectively (P for trend = 0.004). This finding is in agreement with studies of prostate (14) and breast and lung cancers (35). Other studies, however, reported an inverse association between prostate cancer and a haplotype including *355T-4326C* (15), and a

Table 2. Relation between variants in *PSA/KLK3* and prostate cancer in the entire family-based study sample, stratified by disease aggressiveness

KLK3/PSA	Entire sample			Low aggressive*			High aggressive†		
	Cases/controls	OR (95% CI)‡	*P*	Cases/controls	OR (95% CI)‡	*P*	Cases/controls	OR (95% CI)‡	*P*
Genotypes									
A−158G									
AA	124 of 124	1.00		57 of 71	1.00		61 of 52	1.00	
GA	196 of 236	0.81 (0.52-1.26)	0.34	84 of 97	1.40 (0.66-2.99)	0.38	99 of 122	0.62 (0.34-1.14)	0.12
GG	119 of 119	0.95 (0.55-1.64)	0.85	47 of 39	2.71 (1.06-6.94)	0.04	65 of 66	0.60 (0.28-1.30)	0.19
A−4643G									
AA	266 of 281	1.00		124 of 134	1.00		132 of 134	1.00	
AG	141 of 163	0.87 (0.56-1.34)	0.52	50 of 60	0.89 (0.44-1.79)	0.74	76 of 89	0.84 (0.46-1.52)	0.56
GG	32 of 35	1.03 (0.47-2.27)	0.94	14 of 13	2.11 (0.59-7.57)	0.25	17 of 17	0.91 (0.34-2.44)	0.84
T−5412C									
TT	259 of 273	1.00		121 of 133	1.00		127 of 126	1.00	
TC	160 of 184	0.87 (0.56-1.36)	0.55	59 of 67	1.08 (0.53-2.17)	0.84	87 of 102	0.78 (0.43-1.43)	0.42
CC	20 of 22	0.99 (0.44-2.22)	0.98	8 of 7	2.01 (0.51-7.89)	0.32	11 of 12	0.80 (0.28-2.26)	0.67
Diplotypes									
A−158G-A−4643G-T−5412C									
AAT/AAT	117 of 111	1.00		55 of 65	1.00		57 of 45	1.00	
AAT/GAT	102 of 124	0.72 (0.43-1.21)	0.22	48 of 61	1.56 (0.63-3.89)	0.34	51 of 65	0.50 (0.25-0.99)	0.05
AAT/GGC	83 of 96	0.74 (0.42-1.30)	0.30	31 of 39	1.06 (0.42-2.62)	0.91	44 of 49	0.60 (0.27-1.29)	0.19
GAT/GAT	36 of 30	0.93 (0.44-1.99)	0.86	17 of 12	2.42 (0.70-8.41)	0.16	18 of 14	0.65 (0.22-1.93)	0.44
GAT/GGC	52 of 57	0.72 (0.38-1.37)	0.32	18 of 16	2.27 (0.77-6.67)	0.14	29 of 36	0.41 (0.17-0.96)	0.04
GGC/GGC	18 of 20	0.83 (0.33-2.05)	0.68	8 of 6	3.48 (0.88-13.7)	0.07	9 of 11	0.46 (0.14-1.49)	0.20
Composites§	31 of 41	0.56 (0.26-1.26)	0.15	11 of 18	0.43 (0.06-3.25)	0.68	17 of 20	0.48 (0.17-1.34)	0.16
GGC‖									
None	270 of 290	1.00		125 of 139	1.00		133 of 137	1.00	
One copy	151 of 169	0.96 (0.61-1.49)	0.84	55 of 62	1.13 (0.57-2.24)	0.73	83 of 92	0.90 (0.49-1.65)	0.73
Two copies	18 of 20	1.11 (0.47-2.59)	0.81	8 of 6	2.90 (0.75-11.3)	0.12	9 of 11	0.80 (0.26-2.44)	0.70

*Includes cases with Gleason score <7 and tumor stage $<T_{2C}$ and their brothers.
†Includes cases with Gleason score ≥7 and/or tumor stage $\geq T_{2C}$ and their brothers.
‡Adjusted for age.
§Composites include AAT/AAC, AAT/GGT, AAC/AGT, AAC/GAT, AGT/AGT, GAT/GAC, GAT/GGT, GGC/AAC, GGC/AGT, GGC/GAC, GGC/GGT.
‖Presence of haplotype GGC.

Table 3. Association between *CYP11α* variants and prostate cancer, in the entire family-based study sample, stratified by disease aggressiveness

CYP11α	Entire sample			Low aggressive*			High aggressive†		
	Cases/controls	OR (95% CI)‡	*P*	Cases/controls	OR (95% CI)‡	*P*	Cases/controls	OR (95% CI)‡	*P*
$(tttta)_n$§									
None	85 of 90	1.00		40 of 42	1.00		38 of 43	1.00	
One copy	199 of 218	0.86 (0.53-1.38)	0.52	87 of 92	0.72 (0.33-1.59)	0.42	100 of 107	1.11 (0.59-2.11)	0.75
Two copies	153 of 170	0.84 (0.47-1.48)	0.54	60 of 73	0.59 (0.24-1.45)	0.25	86 of 89	1.21 (0.55-2.67)	0.64

*Includes cases with Gleason score <7 and tumor stage $<T_{2C}$ and their brothers.
†Includes cases with Gleason score ≥7 and/or tumor stage $\geq T_{2C}$ and their brothers.
‡Adjusted for age.
§Presence of 4 (tttta) repeat allele.

positive association with the *C4326G* SNP alone (13). These conflicting results might be explained by the very small sample size included in the latter study (n = 50) and our observation that not all haplotypes carrying the *355T* allele led to an increased risk. Whereas functional studies support the direct role of these variants on disease risk (5), there may be a causal variant residing on or near the corresponding haplotype. No noteworthy associations were observed among men with low aggressive prostate cancer (Table 2).

When looking at *KLK3/PSA*, no associations were observed for the three SNPs (*A−158G*, *A−4653G*, and *T−5412C*), or their haplotypes, among the entire sample (Table 3). However, we observed a statistically significant association between the *A−158G* variant and prostate cancer among men with nonaggressive disease. In comparison with men homozygous for the *−158A* allele, carrying the *GG* genotype gave an OR of 2.71 (95% CI, 1.06-6.94; P = 0.04). And there was a slight trend across genotypes, as each additional copy of the 158G variant seemed to increase the respective ORs (P for trend = 0.03). Similar, albeit weaker, results were observed for the *PSA* haplotypes: men with diplotypes carrying two copies of the −158G variant exhibited increased risk of disease (though not statistically significant; Table 2). In contrast, within the higher aggressiveness stratum, all nonreferent diplotypes (i.e., not AAT/AAT) were weakly inversely associated with disease ($P \geq 0.04$). Analyses for the number of GGC haplotypes did not materially change the above results (Table 3). This is in general agreement with two previous studies (18, 36); however, another study observed a positive association between carrying two copies of the −158A variant and disease (19). Of course, the *−158A/G* SNP may be in linkage disequilibrium with the causal variants in the *PSA/KLK3* promoter region. However, we found no associations for two other promoter polymorphisms (*G−4643G* and *C−5412T*). In addition, three-locus (*A−158G*, *G−4643A*, and *C−5412T*) haplotype analyses did not improve the results observed for the single *A−158G* variant. To this end, functional studies suggest that the proliferation, migration, and metastasis of prostate cancer cells may be due to the enzymatic activity of *PSA/KLK3* (37). Our findings suggest that the −158G/−158G may be involved in disease risk via this enzymatic pathway reflecting the effect of other functional variants.

Finally, we observed no associations between the *CYP11α* (tttta) repeat and prostate cancer risk or tumor aggressiveness (Table 3). Here we recoded genotypes for *CYP11α* as three groups: noncarriers of 4 (tttta) repeat allele (none), subjects carrying one 4 (tttta) repeat allele (one copy), and homozygotes for 4 (tttta) repeat alleles (two copies). Using other codings for the genotypes did not alter our null results (not shown). Our observation of no overall association between prostate cancer and the *CYP11A* $(tttta)_n$ variant is consistent with a previous study (27). Nevertheless, Kumazawa et al. (27) did observe that the absence of the $(tttta)_n$ variant was associated with an increased risk of stage D disease (i.e., $T_{1\text{-}4}N_1M_{0\text{-}1}$ or $T_{1\text{-}4}N_{0\text{-}1}M_1$ according to the Whitmore-Jewett system; ref. 38). Our inability to replicate their results may in part reflect the small number of men in our study with such high-stage prostate cancer.

By using a family-based design, the current study is not susceptible to confounding bias due to population stratification (39). Nevertheless, studying brothers with and without prostate cancer can lead to overmatching and subsequent reduced power for detecting genetic effects (32).

In summary, we found evidence that polymorphisms in *CYP1* and *PSA/KLK3* may affect the risk of prostate cancer among men with more and less advanced disease, respectively. Moreover, these genes may contain other functional variants that affect the aggressiveness of prostate cancer.

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