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Inherited Variations in *AR*, *ESR1*, and *ESR2* Genes Are Not Associated With Prostate Cancer Aggressiveness or With Efficacy of Androgen Deprivation Therapy

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Abstract

Background—Sex steroid hormone receptors mediate essential processes in normal prostate growth and contribute to prostate cancer development.

Method—In this study, we investigated the association between common inherited variation of the *AR*, *ESR1*, and *ESR2* genes and two clinically relevant traits: the risk of developing aggressive prostate cancer and the response to androgen deprivation therapy (ADT) in a hospital-based cohort. A total of 43 tagging single nucleotide polymorphisms covering the loci of *AR* ($n = 4$), *ESR1* ($n = 32$), and *ESR2* ($n = 7$) were successfully genotyped in 4,073 prostate cancer cases.

Results—None of these single nucleotide polymorphisms were significantly associated with disease aggressiveness as assessed by the D'Amico risk classification, pathologic stage, or the response to ADT.

Conclusions—Our results suggest that common genetic variations in *AR*, *ESR1*, or *ESR2* are not strongly associated with prostate cancer aggressiveness or response to ADT.

Impact—Our study did not find convincing evidence of inherited variations in the major receptors for androgens and estrogens and their associations with prostate cancer traits.

Introduction

Prostate cancer accounts for one fourth of all cancer diagnosed in men in the United States every year (1). Although most men will have an indolent form of the disease, aggressive prostate cancer is one of the major causes of death among men in developed countries (2). Androgen deprivation therapy (ADT) is the most commonly used treatment for advanced prostate cancer (3). Despite frequent responses, the majority of metastatic tumors will progress to castration-resistant prostate cancer, which is typically lethal (4). Therefore, it is

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No potential conflicts of interest were disclosed.

important to understand the mechanisms involved in the development of aggressive prostate cancer and the progression to castration-resistant disease.

Sex steroid hormones, such as androgens and estrogens, are essential in normal prostate growth and carcinogenesis (5, 6). Accumulating evidence indicates that androgens, estrogens, and their corresponding receptors play crucial roles in prostate cancer development and progression (7, 8). Aberrant expression or mutations of hormone receptors in tumors are also found to be associated with prostate cancer aggressiveness and the development of resistance to ADT (9, 10). Recently, molecular epidemiologic studies have been conducted to evaluate the association of genetic polymorphisms in androgen receptor (*AR*), estrogen receptor α (*ESR1*), and estrogen receptor β (*ESR2*) with prostate cancer risk (11-16), but the results are inconclusive.

To investigate whether inherited variation in *AR*, *ESR1*, and *ESR2* loci contribute to prostate cancer aggressiveness or the response to ADT, we systematically evaluated the *AR*, *ESR1*, and *ESR2* loci in a large hospital-based prostate cancer patient cohort.

Materials and Methods

Study population

Details of the Dana-Farber Harvard Cancer Center SPORE (Gelb Center) Prostate Cancer Clinical Research Information System (CRIS) at the Dana-Farber Cancer Institute (DFCI) have been previously described (17). Briefly, the CRIS system consists of data-entry software, a central data repository, collection of patient data including comprehensive follow-up of all patients, and tightly integrated security measures. All patients seen at DFCI and Brigham and Women's Hospital with a diagnosis of prostate cancer are approached to participate. The consent rate for patients is 86%. The Institute Review Board approved this study specifically.

A total of 4,073 prostate cancer patients diagnosed from 1976 to 2007, who had consented during the period from 1993 to 2007 to provide information and tissue and had blood collected for research purposes, were included in this study cohort (18). To control the quality of the ethnicity information from the self-reported data, we sampled 3% of self-reported Caucasians ($n = 180$) and performed genotyping using 26 single nucleotide polymorphisms (SNP) that distinguish the Caucasian population from non-Caucasian populations (19). The genotyping data showed that none of the tested samples were in discordance, confirming the reliability of self-reported Caucasian ethnicity. For all individuals who ambiguously reported their ethnicity, including those reported as "American," or those who did not report ethnicity information, their ethnicity was determined by genotyping using the same set of 26 SNPs. Only reliably self-reported or genotyping-confirmed Caucasians were eligible for this study. Age at diagnosis was calculated from the date of the first positive biopsy. Using the D'Amico risk classification criteria, prostate cancer patients were classified as low, intermediate, or high risk of clinical recurrence after primary therapy (20). Briefly, three risk groups were established based on serum prostate-specific antigen (PSA) level, biopsy Gleason score, and American Joint Commission on Cancer (AJCC) clinical tumor category at diagnosis. Low-risk patients had a PSA of 10 ng/mL, a Gleason score of ≤ 6 , and tumor category T_{1c} or T_{2a}. Intermediate-risk patients had a PSA of 10.1 to 20 ng/mL or Gleason score of 7 or tumor category T_{2b}. High-risk patients had a PSA of >20 ng/mL or Gleason score of 8 or tumor category T_{2c}. Because original D'Amico risk classification was set to predict biochemical outcome of localized patients, in this study, patients who diagnosed with N₁ or M₁ diseases were regarded as high D'Amico risk class. Within the entire cohort, 1,716 of 4,073 patients were known to have undergone radical prostatectomy as the primary treatment. Pathologic stage was acquired by

reviewing pathology reports. A subset of 553 patients out of the entire cohort belonged to the previously described ADT cohort for evaluating ADT efficacy (21, 22). Briefly, the ADT cohort included patients who received orchiectomy or Luteinizing-hormone-releasing hormone (LHRH) with or without an antiandrogen for nonlocalized, hormone-sensitive prostate cancer.

SNP selection and genotyping

The approach for tagging SNP selection was as previously described (23). To select SNPs for *AR*, *ESR1*, and *ESR2*, phase II data of Utah residents with Northern and Western European ancestry (CEU) population from the International HapMap project were used. The tagging SNPs were selected by pairwise algorithm implemented in the Haploview 4.1 program (24) to capture the unmeasured variants $r^2 > 0.8$. In total, we selected 6 SNPs in *AR*, 35 SNPs in *ESR1*, and 7 SNPs in *ESR2*, which could capture common variation among the CEU population of each locus. The genotyping was done using with Sequenom iPLEX matrix-assisted laser desorption/ionization–time of flight mass spectrometry technology. Genotyping of one selected tagging SNP in *AR* (rs5919393) and three SNPs in *ESR1* (rs12154178, rs3020325, and rs9340931) failed. Another SNP in *AR*, rs5031002, was excluded from further analysis due to its low frequency in the studied population (minor allele frequency = 0.023). Average genotyping success for all other SNPs was 97.9% (range, 92.7–99.8%). The concordance rate between duplicated samples ($n = 255$) was 99.96%.

Statistical analysis

Observed genotype distributions were tested for departure from Hardy-Weinberg equilibrium using Pearson's goodness-of-fit test. No SNP violated Hardy-Weinberg equilibrium (all P values > 0.05). The homozygous genotypes of frequencies $< 5\%$ were combined with their corresponding heterozygous genotype for analysis. To investigate the association of genotypes with early-onset prostate cancer (< 60 years), and with prostate cancer aggressiveness following D'Amico risk classes of patients as previously noted (intermediate/high versus low), we estimated odds ratios and their 95% confidence intervals using unconditional logistic regression. We also examined the association between SNPs and radical prostatectomy pathologic stage (advanced, defined as pathologic T₃–T₄ or N₁ or M₁ versus localized T₁–T₂) with unconditional logistic regression in a sub-cohort with patients who underwent radical prostatectomy. The above analyses, with the exception of those for early-onset prostate cancer, were adjusted for age at diagnosis. In the ADT subcohort, median time to progression on ADT by genotype was estimated using Kaplan-Meier methods. Global association of genotypes with time to progression on ADT were assessed using log rank tests, and hazard ratios and 95% confidence intervals were estimated using Cox proportional hazard regression. The models for ADT subcohort analysis were not adjusted for age. All statistical analyses were done using version SAS version 9.1 (SAS Institute Inc.). A P value that remains < 0.05 after 1,000 times permutation testing would be considered as statistically significant in entire cohort or radical prostatectomy subcohort analyses.

Results

Table 1 shows the patient characteristics of the entire cohort. The cohort includes 4,073 Caucasian prostate cancer patients. The mean age at diagnosis was 61.3 years (range, 42–91 years). Among 3,750 patients with biopsy Gleason score information, 1,771 (47%) had biopsy Gleason scores < 7 , 1,272 (34%) had Gleason scores of 7, and 707 (19%) had Gleason scores > 7 . A total of 3,056 patients had AJCC clinical stage information; among them, 92% ($n = 2807$) presented at diagnosis with T₁ or T₂ disease, 2% ($n = 65$) had T₃ or T₄ disease, and 6% ($n = 184$) were diagnosed with metastatic disease (N₁ or M₁). A total of

3,518 patients had PSA levels at diagnosis, with a median PSA of 6 ng/mL (Q1, Q3; 5, 11 ng/mL). Sufficient information for modified criteria of D'Amico risk classification was available for 3,347 patients, of whom 1,004 (30%) were low risk, 1,357 (40%) intermediate risk, and 986 (30%) high risk. A total of 1,716 patients underwent radical prostatectomy, of whom 1,161 (68%) had organ-confined (T_1 or T_2) disease at the time of receiving surgery, whereas 475 (28%) had extraprostatic tumors (T_3 or T_4) and 80 (4%) had metastatic tumors (N_1 or M_1). The pathologic Gleason score was <7 in 652 (39%), 7 in 769 (45%), and 7 in 1,040 (16%) patients. The characteristics of the ADT subcohort were as previously described (21, 22).

Forty-three SNPs across the *AR*, *ESR1*, and *ESR2* genes were successfully genotyped in this cohort. Table 2 shows the results for the association between these variants and age at diagnosis, disease aggressiveness, and response to ADT. We did not observe convincing significant associations of these SNPs with any of the traits. Although some SNPs (rs1204038 of *AR*, and rs2077647, rs532010, rs17081749, rs6902771, and rs3936674 of *ESR1*) showed nominally significant associations with early-onset or advanced-stage prostate cancer, considering the number of tests done, it is likely that these nominally significant results were due to chance. For example, none of them remained $P < 0.05$ after the adjustment for multiple comparisons, such as permutation testing.

Discussion

This was a large hospital-based study involving 4,073 cases that examined the association of inherited variation in *AR*, *ESR1*, and *ESR2* with clinically relevant traits: age at diagnosis, prostate cancer aggressiveness, and the efficacy of ADT. No strong associations were observed. The strengths of our study include large sample size, detailed clinical information, and comprehensive evaluation of common genetic variation.

A prior study reported that an *AR* promoter SNP, rs17302090, was modestly significantly associated with an increased risk of prostate cancer death (25). The study also reported that this finding was more pronounced in patients who received ADT as primary treatment at diagnosis. However, we did not identify any associations between rs1204038, which is in strong linkage disequilibrium with rs17302090 ($D' = 1.00$, $r^2 = 0.80$), and prostate cancer aggressiveness or ADT efficacy.

Hormonal status is clearly an important factor in prostate cancer biology. To date, however, there has been little evidence supporting the influence of common genetic variation on various prostate cancer-related traits. In our study, we did not find convincing evidence of inherited variations in the major receptors for androgens and estrogens and their associations with prostate cancer traits. There are several possible reasons for the lack of associations. First, aberrant expression, mutation, or splice variants of hormone receptors are frequently found mechanisms in prostate cancer progress and castration-resistance development. These somatic alterations may play a larger role than the influence of low-penetrant genetic variants for these traits. Second, other genes besides hormonal receptors in the steroidogenic and metabolic pathways are also important for prostate development and antiandrogen therapy response. Inherited variants in sex hormonal receptor genes perhaps interact with other variants in these pathways and play roles cooperatively. Last but not least, the current research strategy allows us to explore the role of common genetic variation; rare variants in these (or other) genes may be important in disease progression and response to the treatment.

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Table 1

Clinical characteristics of study cohort

Patients (n)	4,073
Ethnicity	Caucasian
Age at diagnosis (n)	3,983
Mean (y)	61.3
Median, y (Q1,Q3)	61 (55, 67)
Biopsy Gleason score at diagnosis (n)	3,750
<7 (%)	1,771 (47.2)
7 (%)	1,272 (33.9)
>7 (%)	707 (18.9)
Clinical stage (n) *	3,056
T ₁ -T ₂ (%)	2,807 (91.9)
T ₃ -T ₄ (%)	65 (2.1)
N ₁ (%)	46 (1.5)
M ₁ (%)	138 (4.5)
PSA at diagnosis (n)	3,518
Median, ng/mL (Q1, Q3)	6 (5,11)
D' Amico risk classification (%)	3,347
Low	1,004 (30.0)
Intermediate	1,357 (40.5)
High	986 (29.5)
Radical prostatectomy subcohort (n)	1,716
Pathologic Gleason score (n)	1,692
<7 (%)	652 (38.5)
7 (%)	769 (45.5)
>7 (%)	271 (16.0)
Pathologic stage (n) *	1,716
T ₁ -T ₂ (%)	1,161 (67.7)
T ₃ -T ₄ (%)	475 (27.7)
N ₁ (%)	76 (4.4)
M ₁ (%)	4 (0.2)

* T₁-T₂ representing T₁-T₂, N₀ or N_x, M₀ or M_x; T₃-T₄ representing T₃-T₄, N₀ or N_x, M₀ or M_x; N₁ representing T₁-T₄ or T_x, N₁, M₀ or M_x; M₁ representing T₁-T₄ or T_x, N₀ or N_x, M₁ according to AJCC staging.

Table 2

Gene locations and association with prostate cancer aggressiveness and efficiency of androgen deprivation therapy

Gene/SNP	Frequency	Location	OR (95% CI)			HR (95% CI)
			Age at diagnosis, 60 y vs. >60 y	D'Amico risk class, high/intermediate vs. low	Pathologic stages, advanced vs. localized	Patients on ADT
	(n = 4,073)		(n = 3,938)	(n = 3,347)	(n = 1,716)	(n = 553)
<i>AR</i>						
rs1204038		Intron 1	<i>P</i> = 0.04	<i>P</i> = 0.38	<i>P</i> = 0.81	<i>P</i> = 0.86
G	84.8%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
A	15.2%		1.22 (1.01-1.47)	1.11 (0.88-1.40)	0.96 (0.70-1.32)	1.03 (0.75-1.40)
rs2361634		Intron 1	<i>P</i> = 0.68	<i>P</i> = 0.95	<i>P</i> = 0.94	<i>P</i> = 0.67
A	93.7%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
G	6.3%		1.06 (0.82-1.37)	1.01 (0.74-1.37)	1.02 (0.67-1.54)	0.93 (0.65-1.32)
rs5919411		Intron 3	<i>P</i> = 0.38	<i>P</i> = 0.37	<i>P</i> = 0.22	<i>P</i> = 0.66
G	93.3%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
A	6.7%		1.12 (0.87-1.44)	1.15 (0.85-1.56)	0.77 (0.51-1.17)	0.91 (0.59-1.40)
rs12011518		Intron 4	<i>P</i> = 0.69	<i>P</i> = 0.96	<i>P</i> = 0.80	<i>P</i> = 0.08
G	92.5%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T	7.5%		1.05 (0.83-1.33)	1.01 (0.75-1.35)	1.05 (0.70-1.58)	0.75 (0.50-1.12)
<i>ESR1</i>						
rs6935443		Near 5'	<i>P</i> = 0.33	<i>P</i> = 0.87	<i>P</i> = 0.30	<i>P</i> = 0.79
AA	66.0%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG/GG	34.0%		0.94 (0.82-1.07)	1.01 (0.86-1.19)	1.12 (0.90-1.40)	0.97 (0.78-1.21)
rs9478244		Near 5'	<i>P</i> = 0.46	<i>P</i> = 0.57	<i>P</i> = 0.37	<i>P</i> = 0.51
AA	66.9%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG/GG	33.1%		0.95 (0.83-1.09)	1.05 (0.89-1.23)	1.10 (0.89-1.37)	1.08 (0.86-1.34)
rs2077647		Exon 1(S10S)	<i>P</i> = 0.01	<i>P</i> = 0.84	<i>P</i> = 0.03	<i>P</i> = 0.02
TT	26.8%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	48.4%		0.81 (0.70-0.95)	1.01 (0.84-1.22)	0.74 (0.57-0.95)	1.34 (1.04-1.73)
CC	24.8%		0.96 (0.80-1.15)	1.07 (0.87-1.33)	0.72 (0.54-0.97)	0.99 (0.74-1.33)
rs532010		Intron 1	<i>P</i> = 0.08	<i>P</i> = 0.63	<i>P</i> = 0.04	<i>P</i> = 0.14
GG	35.6%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	48.5%		0.86 (0.72-1.03)	1.01 (0.81-1.25)	1.13 (0.83-1.52)	1.25 (0.92-1.70)
AA	15.9%		0.99 (0.83-1.20)	0.95 (0.76-1.19)	1.41 (1.03-1.93)	1.02 (0.74-1.41)
rs17081698		Intron 1	<i>P</i> = 0.62	<i>P</i> = 0.60	<i>P</i> = 0.61	<i>P</i> = 0.31
TT	81.1%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
GT/GG	18.9%		1.04 (0.89-1.22)	0.95 (0.79-1.15)	0.93 (0.72-1.22)	0.87 (0.66-1.14)
rs9340788		Intron 1	<i>P</i> = 0.95	<i>P</i> = 0.53	<i>P</i> = 0.64	<i>P</i> = 0.22
CC	90.4%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AC/AA	9.6%		0.99 (0.80-1.24)	0.92 (0.71-1.19)	1.09 (0.77-1.54)	1.27 (0.87-1.85)
rs17081749		Intron 1	<i>P</i> = 0.005	<i>P</i> = 0.93	<i>P</i> = 0.68	<i>P</i> = 0.16

Gene/SNP	Frequency	Location	OR (95% CI)			HR (95% CI)
			Age at diagnosis, 60 y vs. >60 y	D'Amico risk class, high/intermediate vs. low	Pathologic stages, advanced vs. localized	Patients on ADT
	(n = 4,073)		(n = 3,938)	(n = 3,347)	(n = 1,716)	(n = 553)
TT	76.6%	Intron 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
GT/GG	23.4%		0.81 (0.70-0.94)	1.01 (0.84-1.20)	0.95 (0.75-1.21)	1.19 (0.93-1.51)
rs6902771			P = 0.01	P = 0.87	P = 0.01	P = 0.53
TT	29.8%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	48.9%	Intron 2	0.81 (0.69-0.95)	0.93 (0.76-1.13)	1.01 (0.77-1.32)	1.11 (0.85-1.46)
CC	21.3%		1.01 (0.85-1.20)	0.93 (0.75-1.15)	1.37 (1.03-1.83)	0.98 (0.73-1.32)
rs3936674			P = 0.65	P = 0.35	P = 0.01	P = 0.28
GG	43.6%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	45.4%	Intron 2	0.94 (0.82-1.07)	1.12 (0.95-1.31)	0.86 (0.69-1.07)	1.18 (0.95-1.47)
AA	11.0%		0.77 (0.79-1.20)	1.14 (0.88-1.47)	0.61 (0.43-0.89)	0.99 (0.68-1.43)
rs712221			P = 0.90	P = 0.73	P = 0.09	P = 0.85
TT	19.0%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AT	48.2%	Intron 2	1.02 (0.86-1.20)	0.95 (0.77-1.17)	1.20 (0.90-1.60)	1.06 (0.80-1.40)
AA	32.8%		0.98 (0.82-1.18)	0.90 (0.73-1.13)	1.32 (0.97-1.79)	1.00 (0.75-1.35)
rs1643821			P = 0.94	P = 0.66	P = 0.18	P = 0.43
GG	33.3%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	47.8%	Intron 3	1.03 (0.89-1.18)	1.09 (0.92-1.29)	0.98 (0.78-1.24)	1.16 (0.92-1.46)
AA	18.9%		1.02 (0.85-1.22)	1.08 (0.87-1.34)	0.80 (0.59-1.08)	1.04 (0.77-1.40)
rs7761846			P = 0.18	P = 0.29	P = 0.26	P = 0.22
TT	87.1%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT/CC	12.9%	Intron 3	0.88 (0.73-1.06)	1.13 (0.90-1.43)	1.19 (0.88-1.61)	0.82 (0.60-1.12)
rs2347923			P = 0.46	P = 0.31	P = 0.52	P = 0.91
CC	9.2%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AC	42.5%		1.15 (0.91-1.44)	0.87 (0.66-1.15)	1.13 (0.77-1.64)	1.00 (0.69-1.44)
AA	48.3%	Intron 3	1.15 (0.92-1.44)	0.99 (0.75-1.30)	0.96 (0.66-1.39)	1.04 (0.72-1.51)
rs1514347			P = 0.35	P = 0.41	P = 0.27	P = 0.88
TT	5.7%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	35.0%		1.06 (0.80-1.43)	1.00 (0.70-1.41)	0.92 (0.58-1.48)	0.97 (0.63-1.49)
CC	59.3%	Intron 3	0.96 (0.73-1.28)	1.10 (0.78-1.54)	0.78 (0.49-1.23)	0.93 (0.61-1.40)
rs2347867			P = 0.62	P = 0.85	P = 0.16	P = 0.85
GG	11.3%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	44.5%		0.92 (0.74-1.14)	0.92 (0.71-1.20)	1.09 (0.78-1.54)	0.99 (0.71-1.38)
AA	44.2%	Intron 3	0.90 (0.73-1.11)	0.98 (0.74-1.25)	0.86 (0.61-1.23)	1.06 (0.76-1.48)
rs2347868			P = 0.65	P = 0.25	P = 0.29	P = 0.90
TT	5.5%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	35.0%		1.10 (0.82-1.48)	0.91 (0.64-1.51)	1.02 (0.64-1.63)	0.98 (0.62-1.55)
CC	59.5%	Intron 3	1.04 (0.78-1.39)	1.04 (0.73-1.48)	0.85 (0.54-1.35)	0.94 (0.60-1.46)
rs6912184			P = 0.20	P = 0.09	P = 0.84	P = 0.94

Gene/SNP	Frequency	Location	OR (95% CI)			HR (95% CI)
			Age at diagnosis, 60 y vs. >60 y	D'Amico risk class, high/intermediate vs. low	Pathologic stages, advanced vs. localized	Patients on ADT
	(n = 4,073)		(n = 3,938)	(n = 3,347)	(n = 1,716)	(n = 553)
GG	5.9%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	34.5%		1.29 (0.97-1.70)	0.92 (0.65-1.30)	0.89 (0.56-1.40)	1.08 (0.70-1.65)
AA	59.6%		1.27 (0.97-1.67)	1.09 (0.78-1.53)	0.86 (0.55-1.34)	1.07 (0.71-1.62)
rs9371564		Intron 3	<i>P</i> = 0.60	<i>P</i> = 0.62	<i>P</i> = 0.76	<i>P</i> = 0.87
GG	44.0%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	44.7%		1.00 (0.88-1.14)	0.93 (0.79-1.09)	1.12 (0.90-1.40)	1.02 (0.82-1.27)
AA	11.3%		0.91 (0.74-1.11)	0.91 (0.71-1.17)	1.06 (0.76-1.50)	0.93 (0.67-1.30)
rs3020401		Intron 4	<i>P</i> = 0.70	<i>P</i> = 0.49	<i>P</i> = 0.24	<i>P</i> = 0.92
GG	11.4%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	45.0%		0.99 (0.81-1.22)	1.16 (0.90-1.49)	0.89 (0.64-1.24)	0.98 (0.71-1.36)
AA	43.6%		0.94 (0.77-1.16)	1.13 (0.88-1.45)	0.76 (0.54-1.06)	0.94 (0.68-1.31)
rs932480		Intron 4	<i>P</i> = 0.33	<i>P</i> = 0.43	<i>P</i> = 0.03	<i>P</i> = 0.60
GT/TT	32.5%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
GG	67.5%		0.94 (0.82-1.07)	0.94 (0.80-1.10)	0.79 (0.63-0.98)	0.95 (0.76-1.18)
rs1884052		Intron 4	<i>P</i> = 0.64	<i>P</i> = 0.68	<i>P</i> = 0.05	<i>P</i> = 0.71
CG/GG	27.9%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CC	72.1%		0.97 (0.84-1.11)	1.04 (0.88-1.22)	0.80 (0.64-1.00)	0.96 (0.76-1.20)
rs726281		Intron 4	<i>P</i> = 0.29	<i>P</i> = 0.54	<i>P</i> = 0.37	<i>P</i> = 0.78
GG	8.2%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	38.9%		0.83 (0.65-1.06)	1.00 (0.74-1.34)	0.88 (0.61-1.28)	0.91 (0.64-1.30)
AA	52.9%		0.84 (0.66-1.06)	0.92 (0.69-1.22)	0.78 (0.54-1.12)	0.98 (0.69-1.39)
rs9397463		Intron 4	<i>P</i> = 0.88	<i>P</i> = 0.16	<i>P</i> = 0.90	<i>P</i> = 0.29
CT/TT	26.7%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CC	73.3%		0.99 (0.86-1.14)	0.89 (0.75-1.05)	0.97 (0.77-1.23)	1.13 (0.90-1.43)
rs9340954		Intron 4	<i>P</i> = 0.16	<i>P</i> = 0.51	<i>P</i> = 0.27	<i>P</i> = 0.36
TT	54.4%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
GT	37.5%		1.05 (0.92-1.20)	1.08 (0.92-1.27)	1.19 (0.96-1.49)	0.96 (0.77-1.19)
GG	8.1%		1.26 (0.99-1.59)	1.18 (0.88-1.57)	1.27 (0.89-1.81)	1.27 (0.87-1.85)
rs722208		Intron 4	<i>P</i> = 0.10	<i>P</i> = 0.49	<i>P</i> = 0.22	<i>P</i> = 0.31
GG	8.4%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	36.6%		0.84 (0.65-1.06)	0.87 (0.65-1.17)	0.93 (0.64-1.33)	0.74 (0.51-1.09)
AA	55.0%		0.78 (0.62-0.99)	0.82 (0.62-1.09)	0.77 (0.54-1.09)	0.78 (0.54-1.13)
rs9340958		Intron 4	<i>P</i> = 0.22	<i>P</i> = 0.51	<i>P</i> = 0.98	<i>P</i> = 0.11
CT/TT	16.6%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CC	83.4%		0.90 (0.75-1.07)	0.93 (0.76-1.15)	0.96 (0.72-1.28)	0.80 (0.62-1.05)
rs9340978		Intron 5	<i>P</i> = 0.13	<i>P</i> = 0.91	<i>P</i> = 0.29	<i>P</i> = 0.06
GG	86.4%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG/AA	13.6%		1.16 (0.96-1.40)	1.01 (0.81-1.27)	1.17 (0.87-1.58)	1.34 (1.00-1.80)

Gene/SNP	Frequency	Location	OR (95% CI)			HR (95% CI)
			Age at diagnosis, 60 y vs. >60 y	D'Amico risk class, high/intermediate vs. low	Pathologic stages, advanced vs. localized	Patients on ADT
	(n = 4,073)		(n = 3,938)	(n = 3,347)	(n = 1,716)	(n = 553)
rs6932864		Intron 5	P = 0.70	P = 0.44	P = 0.51	P = 0.32
GG	79.2%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG/AA	20.8%		1.03 (0.89-1.20)	1.08 (0.89-1.30)	1.09 (0.85-1.38)	0.88 (0.69-1.13)
rs3020371		Intron 6	P = 0.21	P = 0.03	P = 0.24	P = 0.58
TT	11.0%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	43.1%		0.84 (0.68-1.05)	1.05 (0.81-1.37)	1.09 (0.78-1.54)	0.83 (0.58-1.20)
CC	45.9%		0.86 (0.69-1.07)	0.85 (0.65-1.10)	0.88 (0.62-1.24)	0.82 (0.57-1.19)
rs3778089		Intron 6	P = 0.63	P = 0.37	P = 0.85	P = 0.71
GG	78.8%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG/AA	20.2%		1.04 (0.89-1.21)	1.09 (0.90-1.31)	1.02 (0.80-1.31)	0.95 (0.75-1.22)
rs2982896		Intron 6	P = 0.93	P = 0.06	P = 0.11	P = 0.32
CT/TT	38.2%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CC	61.8%		1.00 (0.88-1.13)	0.86 (0.74-1.01)	0.83 (0.67-1.02)	0.90 (0.73-1.11)
rs2813545		Near 3'	P = 0.91	P = 0.55	P = 0.63	P = 0.27
GG	64.6%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CG/CC	35.4%		1.01 (0.88-1.15)	1.05 (0.90-1.23)	1.06 (0.85-1.31)	0.89 (0.72-1.10)
<i>ESR2</i>						
rs3020444		Intron 1	P = 0.92	P = 0.54	P = 0.84	P = 0.84
AA	40.9%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	44.7%		1.03 (0.90-1.18)	0.93 (0.79-1.10)	1.08 (0.86-1.35)	0.95 (0.70-1.27)
GG	14.4%		1.00 (0.83-1.22)	0.90 (0.71-1.13)	1.10 (0.79-1.53)	1.01 (0.75-1.37)
rs10137185		Intron 1	P = 0.51	P = 0.17	P = 0.51	P = 0.74
CC	81.4%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT/TT	18.6%		1.06 (0.90-1.24)	1.15 (0.91-1.39)	0.92 (0.70-1.19)	0.96 (0.74-1.23)
rs1256033		Intron 3	P = 0.28	P = 0.19	P = 0.18	P = 0.86
GG	31.0%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
AG	48.6%		0.99 (0.86-1.15)	0.90 (0.76-1.07)	0.96 (0.76-1.21)	0.96 (0.75-1.22)
AA	20.4%		0.88 (0.74-1.05)	1.08 (0.86-1.33)	0.78 (0.57-1.05)	0.92 (0.69-1.23)
rs1256044		Intron 4	P = 0.35	P = 0.15	P = 0.48	P = 0.86
TT	33.7%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	48.0%		1.01 (0.88-1.16)	0.90 (0.76-1.06)	0.97 (0.77-1.22)	0.98 (0.78-1.23)
CC	18.3%		0.89 (0.75-1.07)	1.08 (0.86-1.35)	0.85 (0.62-1.15)	0.92 (0.69-1.24)
rs4986938		Intron 8	P = 0.59	P = 0.91	P = 0.04	P = 0.10
TT	15.8%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	47.1%		1.04 (0.86-1.25)	1.01 (0.81-1.26)	1.13 (0.84-1.53)	0.72 (0.53-0.98)
CC	37.1%		0.97 (0.80-1.17)	1.03 (0.82-1.30)	0.83 (0.61-1.14)	0.73 (0.53-1.00)
rs8006145		Intron 8	P = 0.77	P = 0.97	P = 0.24	P = 0.49
CC	48.8%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)

Gene/SNP	Frequency	Location	OR (95% CI)			HR (95% CI)
			Age at diagnosis, 60 y vs. >60 y	D'Amico risk class, high/intermediate vs. low	Pathologic stages, advanced vs. localized	Patients on ADT
	(n = 4,073)		(n = 3,938)	(n = 3,347)	(n = 1,716)	(n = 553)
AC	42.0%	Exon 9 (3' UTR)	0.97 (0.85-1.11)	1.02 (0.87-1.20)	1.21 (0.97-1.51)	1.05 (0.84-1.32)
AA	9.2%		1.05 (0.83-1.32)	0.99 (0.76-1.31)	1.23 (0.84-1.78)	1.26 (0.86-1.85)
rs8018687			<i>P</i> = 0.51	<i>P</i> = 0.62	<i>P</i> = 0.20	<i>P</i> = 0.52
TT	86.3%		1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT/CC	13.7%		0.94 (0.78-1.13)	0.95 (0.76-1.18)	0.82 (0.60-1.11)	0.90 (0.64-1.25)

Abbreviations: OR, odds ratio; HR, hazard ratio; 95% CI, 95% confidence interval; UTR, untranslated region.