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Association of Variants in Estrogen-Related Pathway Genes with Prostate Cancer Risk

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Abstract

Background—Through mediation of estrogen receptors, estradiol has been shown to have both carcinogenic and anti-carcinogenic effects on the prostate. We performed a population-based case-control study to investigate variants in estrogen-related genes *ESR1*, *ESR2*, *CYP19A1*, *CYP11A1*, and *CYP17A1* and the potential association with risk of prostate cancer.

Materials and Methods—We evaluated prostate cancer risk conferred by 73 single nucleotide polymorphisms in 1,304 incident prostate cancer cases and 1,266 age-matched controls. Analysis included stratification by clinical features and assessment of environmental modifiers.

Results—There was evidence of altered risk of developing prostate cancer for variants in *ESR1*, *CYP11A1*, and *CYP17A1*, however, only *CYP17A1* rs1056836 retained significance after adjustment for multiple comparisons. An association with risk for more aggressive prostate cancer was observed for variants in *ESR1*, *ESR2*, and *CYP19A1*, but none was significant after adjustment for multiple comparisons. There was no effect modification by obesity.

Conclusions—Germline genetic variation of these estrogen pathway genes may contribute to risk of prostate cancer. Additional studies to validate these results and examine the functional consequence of validated variants are warranted.

Keywords

Estrogen Receptor; Cytochrome P450; Aromatase; Prostate Neoplasm; Polymorphism

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There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Introduction

The relationship between estrogen and prostate cancer (PCa) has been evident since the 1940s, when diethylstilbestrol was first administered as treatment for patients with advanced disease [1]. Yet, while beneficial therapeutic effects of estrogen are being explored, studies using animal models, *in vitro* platforms, and gene expression have clearly demonstrated carcinogenic effects of the hormone [2,3]. These contradictory actions are mediated through two receptors, estrogen receptor- α (ESR α) and ESR β . The ESR α contributes to cellular proliferation, inflammation and pre-malignant pathology and has been found to be up-regulated in malignant epithelial prostate tissue [4–6]. Conversely, ESR β is down-regulated in low-grade malignant tissue and has been shown to exhibit antiproliferative and antioxidant properties [5,6]. Germline variation in genes related activation of these ESRs may be associated with PCa risk.

An additional consideration when examining potential consequences of the ESRs is bioavailability of estradiol (E₂). Intra-prostatic exposure to E₂ is rate-limited through localized aromatization of testosterone by aromatase, an enzyme found to be up-regulated in malignant prostate epithelial cells [6]. Estradiol is inactivated through catabolization via 2- or 4-hydroxylation into intermediates called catechol estrogens (CEs). 4-OH CEs, which is the predominant CEs found in prostate tissue, show an even higher binding affinity to the ESRs and subsequent induction of ESR-dependant gene expression as compared to E₂ [7]. In addition, 4-OH CEs exhibit genotoxic properties, including direct damage to DNA through the formation of dupurinating adducts and indirect damage to DNA via formation of free radicals causing DNA base oxidation [8]. Depurinating adducts have been detected at higher levels in PCa patients' urine and *CYP1B1*, the 4-hydroxylation catalyst, is over-expressed in prostate cancer cell lines and tumor tissue [9].

Genetic association studies examining estrogen pathway genes in relation to PCa risk have yielded conflicting results with limited power [10–16]. Thus, we designed a candidate gene study to test the hypothesis that common genetic variation in five main estrogen-related genes is related to PCa risk. The study focused on: *ESR1* and *ESR2*, which encode for ESR α and ESR β respectively; *CYP19A1*, which encodes for aromatase; and, *CYP1A1* and *CYP1B1*, which encode for the 2- or 4-hydroxylation enzymes CYP1A1 and CYP1B1.

Materials and Methods

Study Population

Study subjects were Caucasian men enrolled in two population-based PCa case-control studies that have been described previously [17,18]. Cases had newly diagnosed with histologically confirmed PCa during two study periods, either January 1, 1993 to December 31, 1996 (Study I, age range 40–64 years) or January 1, 2002 to December 31, 2005 (Study II, age range 35–74 years). PCa cases were identified from the Seattle-Puget Sound population-based tumor registry within the NCI Surveillance, Epidemiology and End Results program. Of the 1,548 eligible, interviewed cases we obtained peripheral blood leukocyte samples for genotyping from 1,309 men. Five subjects were excluded for lack of sufficient DNA, for a total of 1,304 cases. Controls were recruited evenly throughout case ascertainment periods using random digit telephone dialing and frequency matched by 5-year age groups. Of the 1,529 eligible, interviewed controls we obtained blood samples from 1,266 men. Detailed information about demographics, environmental exposures, and medical history was obtained from a structured in-person interview. All study participants signed an informed consent prior to data collection. The cancer registry provided further clinical information on disease stage, Gleason score, diagnostic PSA level and primary therapy.

A self-administered food frequency questionnaire (FFQ) was completed following the interview. Only Study II FFQ collected information on dietary phytoestrogens, thus analyses with respect to diet were limited to this group. The Study II FFQ instrument ascertained frequency of consumption and portion size of approximately 120 line items two years prior to reference date (date of diagnosis for cases and an assigned date for controls that approximated the distribution of the cases' diagnosis dates). It was completed by 897 cases (89.6%) and 865 controls (91.8%) with interview data. Subjects whose daily energy intake was less than 800 kcal (eight cases, 15 controls) or greater than 5000 kcal (18 cases, 20 controls) were excluded. After Study II participants were limited to Caucasians with DNA collected, 669 cases (94.1%) and 670 controls (93.4%) had FFQ data. Daily dietary intake of genistein and daidzein were analyzed using the Nutrition Data System for Research software version 2009, developed by the Nutrition Coordination Center, University of Minnesota, Minneapolis, MN. This study was approved by Fred Hutchinson Cancer Research Center's Institutional Review Board and genotyping was approved by the Internal Review Board of the National Human Genome Research Institute.

Variant Selection and Genotyping

Single nucleotide polymorphisms (SNPs) were identified from publicly available sources, HapMap consortium or dbSNP, and were selected from upstream (5k bp), downstream (5k bp), intronic and exonic regions of each gene. A tagSNP approach using the algorithm LDselect on SeattleSNPs (www.pga.gs.washington.edu) with parameters of $r^2 \geq 0.8$ and minor allele frequency $\geq 5\%$ selected polymorphisms to capture genetic variability in each gene [19]. A total of 27 SNPs for *ESR1* (chromosome 6q25.1), 14 for *ESR2* (chromosome 14q23.1), 17 for *CYP19A1* (chromosome 15q21.1), 9 for *CYP11A1* (chromosome 15q24.1), and 8 for *CYP11B1* (chromosome 2p21) were selected. Applied Biosystems (ABI) SNPlex™ Genotyping System was used for genotyping, and proprietary GeneMapper® software was used for calling alleles (www.appliedbiosystems.com). Specific SNP alleles were determined by an ABI 3730x/DNA Analyzer, based on presence of a unique sequence assigned to each original allele-specific oligonucleotide. Quality control included genotyping of 141 blind duplicate samples, which revealed 99% agreement on genotyping calls across 68 of the 75 SNPs assayed and 96% agreement for the remaining seven SNPs. Each batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and laboratory personnel were blinded to the case-control status of samples. SNP genotype frequencies were examined for Hardy-Weinberg Equilibrium (HWE) using the χ^2 statistic and all were found to be consistent ($p > 0.05$) with HWE among Caucasian controls with the exception of rs1271572 in *ESR2* ($p < 0.001$), rs1799814 in *CYP11A1* ($p = 0.02$), and rs12900487 in *CYP19A1* ($p < 0.001$). Further review of genotyping data revealed that rs1271572 had sub-optimal separation with low intensity and rs12900487 was in the middle of two repeat elements, thus these SNPs were dropped. The third SNP, rs1799814, was of good quality, with no apparent genotyping errors, so was retained.

Statistical Analysis

Data were analyzed using unconditional logistic regression to calculate an odds ratio (OR) as an estimate of relative risk of PCa associated with SNP genotypes. All models were adjusted for age. Both dominant and co-dominant models were evaluated, except for SNPs with no individuals homozygous for the variant (less common) allele. Trend tests, which used a variable coded as number of variant alleles for each SNP, were used to assess allele dosage. All models were utilized to evaluate the SNPs potential association with PCa risk. Global tests of association, which were estimated by comparing an adjusted model with all SNPs to a null model that only included age, automatically adjusts for multiple testing based on degrees of freedom of corresponding χ^2 tests [20]. Multiple comparisons were also accounted for by using permutations to calculate exact p-values for each SNP. For each

permutation, dominant, codominant and log-additive (trend) models were fit for all SNPs and the minimum p-values kept for each SNP. P-values were ordered to approximate the null distribution of the order statistics, i.e., minimum p-value, second smallest p-value, etc. The original p-values were also ordered and permutation p-values were calculated by comparing the ordered p-values to the null distribution for the appropriate order statistic. Permutation p-values can be interpreted as the probability of observing a p-value less than or equal to what was observed for the given order statistic under the null hypothesis of no association with disease risk for any of the 73 SNPs. In permutations, the SNP vector was randomly permuted between subjects so all SNPs for a given subject were kept together, which maintained the LD structure between SNPs. A SNP was considered to be significantly associated with PCa risk if both nominal and permuted p-values were ≤ 0.05 [21]. This same methodology was applied to the stratified analysis. In addition to single SNP assessment, Haploview software version 4.1 was used to generate linkage disequilibrium (LD) estimates and define haplotype blocks for selected SNPs [22]. Haplotype risk associations were assessed within each block using HPlus version 3.1, which employs an empirical estimating equation (EE) technique [23].

Risk estimates for cases stratified by Gleason score [2–7(3+4) vs. 7(4+3)–10 and stage (local vs. regional/distant) were estimated using polytomous regression. Gene-environment interactions were assessed for self-reported first-degree family history of prostate cancer (yes/no), BMI (<25 , $25\text{--}29.9$, ≥ 30 kg/m²) based on maximum adult height and weight one year prior to reference date, and dietary phytoestrogen intake (daidzein and genistein quartiles). Effect modification was evaluated by comparing dominant genetic models with and without multiplicative interaction terms using the likelihood ratio test. Gene-gene interaction was assessed through an exhaustive binary test of epistasis (all possible SNP-SNP pairs) implemented in PLINK using the dominant genetic model [24]. Intra-gene SNP-SNP interactions were included if the r^2 between the two SNPs was <0.2 . All analyses, with exception of the haplotype analyses, were done using the STATA statistical package (version 10.1, STATA Corp., College Station, TX), PLINK software (version 1.07), or R (version 2.10).

Results

Table I shows demographics of the study sample. BMI and dietary intake of genistein/daidzein were not significantly different between cases and controls. The majority of cases had localized stage tumors at diagnosis with Gleason scores of less than 7.

SNP Associations

The SNPs for which an association with PCa was observed are shown in Table II. With the exceptions of *CYP1B1* rs2855658 and rs1056836, which are in high LD ($r^2 = 0.99$), these associations did not remain significant after adjustment for multiple comparisons. For rs2855658 and rs1056836 the per-allele nominal p-value for association with PCa risk was 0.002 and 0.004, respectively, and the permuted nominal p-value was 0.02 and 0.01 respectively. Haplotype analysis did not reveal any associations between genotypes and PCa risk beyond the single SNP analysis (Table II). A comprehensive list of genotype distributions and odds ratios (95% CI) are shown in Supplement I. For SNPs shown in Table II, stratification by measures of tumor aggressiveness did not reveal any genotypes to be differentially associated with a particular Gleason score or stage stratum (Table III). Global analysis of all 73 SNPs revealed that, as a group, these SNPs are not significantly associated with PCa ($p = 0.58$). The P values for global association of the SNPs in *ESR1*, *ESR2*, *CYP19A1*, *CYP11A1* and *CYP1B1* were 0.74, 0.78, 0.89, 0.05 and 0.05, respectively. Two *ESR1* SNPs and one *ESR2* SNP were associated with higher Gleason score and 5 *CYP19A1*

SNPs were associated with advanced stage, however, no associations retained significance after adjustment for multiple comparisons (Table III).

Gene-Environment and Gene-Gene Interactions

We found significant differences in risk estimates by quartiles of dietary phytoestrogen intake (measured by daidzein and genistein) for five SNPs (*ESR1* rs3778089, *ESR2* rs1952586 and rs1887994, *CYP1A1* rs1799814, and *CYP19A1* rs2445765), however these SNPs were not associated with PCa risk overall and associations were not significant after adjustment for multiple comparisons (data not shown). There were no significant interactions observed between BMI and the genotypes associated with PCa risk overall. There were some significant associations observed by those reporting a family history of prostate cancer versus those who did not, but these observations did not retain significance after adjustment for multiple comparisons. (Supplement II). Significant pairwise interaction ($p < 0.01$) was observed between SNPs in all five genes (Supplement III). Three SNPs that were independently associated with PCa risk overall showed evidence of gene-gene interaction (rs162549, rs3020432, and rs4886605).

Discussion

In this study we confirmed some previously reported associations and found some novel associations between PCa risk and estrogen-related gene variants. These are reported with the caveat that, with the exception of *CYP1B1* rs1056836, significance was not retained after adjustment for multiple comparisons. Associations with PCa risk previously reported for *ESR1* rs926777 and *CYP1B1* rs1056836, as well as null associations for any *CYP19A1* SNPs, were substantiated by our data, however, we did not replicate reported associations with *ESR2* rs2987983. While gene-environment and gene-gene interactions reported here are exploratory in nature given our limited sample size, our results support the continued effort to account for these sources of variability in genetic association studies.

There have not been any published studies to our knowledge that have comprehensively evaluated genetic variation in *ESR1* and PCa risk; most studies have focused on two intronic SNPs, *PvuII* (rs2234693) and *XbaI* (rs9340799) with mostly null findings reported [10,11,15]. In accordance with most prior studies, we did not observe associations with these loci. We compared six *ESR1* SNPs reported to be associated with PCa risk (Table II) to data from NCI's Cancer Genetic Markers of Susceptibility (CGEMS) project, which genotyped *ESR1* as part of a genome-wide association study (GWAS) [25]. SNPs rs488133 and rs2077647 are within the 5' and intron 1 regions. Although LD information is not available, CGEMS SNPs rs532010 and rs7753153 are also in this region and were associated with more aggressive PCa. Two CGEMS SNPs (rs9340931 and rs9397463) are in LD with SNP rs926777 ($r^2 = 0.72$ and $r^2 = 0.78$, respectively, Caucasian HapMap population) and one CGEMS SNP rs2207396 is in LD with SNP rs3020432 ($r^2 = 0.75$, Caucasian HapMap population). All three of these CGEMS SNPs were associated with risk of PCa overall and with comparatively more aggressive disease.

Genotypes in both *CYP1B1* and *CYP1A1* were associated with PCa risk in our dataset. A number of studies, but not all, have reported an increased risk of PCa for carriers of the rs1056836 'G' allele [11,14,15,26]. Our findings support this association; in fact this polymorphism and rs2855658, which is in strong LD with rs1056836 ($r^2 = 0.99$), were the only two polymorphisms for which the disease associations remained significant after adjustment for multiple comparisons. The SNP rs1056836 is a non-synonymous polymorphism in exon 3, which encodes the heme binding domain, and results in a G-to-C and subsequent amino acid substitutions of Valine-to-Leucine. The observation of an increased risk for cases with a higher Gleason score has also been reported by others and the

SNP has documented functional consequences on transcription, protein structure, and enzyme action [7,11,14]. The CGEMS study genotyped rs2855658 and reported a null association. We are not aware of any published studies that support our observed associations with PCa risk for *CYP19A1* rs1456432 or rs4886605, and none of the CGEMS markers are in LD with these variants.

Both *ESR2* and *CYP19A1* have been evaluated comprehensively in the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3) study which selected SNPs from resequencing data [27,28]. The BPC3 study reported an increased risk of PCa overall and with advanced stage for *ESR2* rs3020450 [27]. This SNP is in complete LD ($r^2 = 1.0$, Caucasian HapMap population) with rs2987983, a polymorphism that was also found to be associated with PCa risk in a Swedish study [29]. The CGEMS SNPs that are in LD with these SNPs (rs10137185 and rs1952586, $r^2 = 0.9$, Caucasian HapMap population) also were identified to be associated with PCa risk. We genotyped both rs2987983 and rs1952586 and observed no associations with overall PCa risk, however rs1952586 was associated with risk for higher Gleason score tumors. The BPC3 reported no significant associations with *CYP19A1*, except for rs2445762 which they found to be associated with more aggressive disease [28]. In accordance with that study we also report null associations with *CYP19A1* polymorphisms for disease risk overall, but did observe an increased risk of advanced stage disease for rs2445762.

Two theories repeatedly cited in finding the “missing heritability” of prostate cancer and other complex diseases are lack of assessment of both gene-environment and gene-gene interactions. We attempted to begin addressing these issues by modeling effect modification of dietary intake of phytoestrogens, BMI, and family history of PCa, as well as looking at inter- and intra-gene interactions for the five selected genes. Phytoestrogens are plant compounds that bind to both ERs, in some cases with a higher binding affinity to ER β , to produce both estrogenic and anti-estrogenic effects in the prostate [30]. Although we were only able to utilize part of our dataset, we included potential gene-environment interactions with phytoestrogens partly due to the dearth of published literature examining this. Our data did not definitively show an interaction between diet and genotype on risk of PCa. For our investigation of interactions with family history and BMI, we were able to compare our results to the BPC3 analyses of *ESR2* and *CYP19A1*, which reported null associations with respect to family history and an interaction between *ESR2* rs1256049 with BMI [27,28]. We did not replicate the finding with BMI, and contrary to their findings, observed several interactions between family history and *CYP19A1*. Lastly, our pairwise analysis supports evidence of interaction between genes in this pathway. While our analyses of gene-environment and gene-gene interactions are preliminary and associations do not hold up to adjustment for multiple comparisons, the results do lend credence to the idea that environmental exposures and epistasis may be important considerations when assessing PCa risk in relation to genotypic variation.

This study has several strengths as well as limitations. All candidate genes examined in this study have been resequenced as part of the BPC3 project and results made publicly available, allowing for more comprehensive gene coverage for SNP selection. In addition, results from the BPC3 analyses and GWAS data from the CGEMS project were available for comparison with our results. With our sample size we had limited power for the gene-environment, gene-gene and tumor aggressiveness subgroup analyses. In addition, since the FFQ collected retrospective information, data are subject to potential recall bias and incomplete ascertainment of total phytoestrogen intake.

Conclusions

In summary, significant associations with PCa risk for several individual SNPs in *ESR1*, *CYP11A1* and *CYP11B1* were observed, and our data support the positive association previously reported for *ESR1* rs926777 as well as the null association for *CYP11A1* SNPs. We did not confirm a previously reported association for *ESR2* rs2987983. The strongest finding from our dataset was an association with *CYP11B1* rs1056836, which remained after adjustment for multiple comparisons and has been reported in several other studies. Larger studies will be better equipped to assess genetic predictors in this pathway for specific subsets of patients with particular environmental or genetic backgrounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table I

Distributions and risk estimates for selected characteristics of population-based prostate cancer cases and controls, King County, Washington, 1993–1996 and 2002–2005.

	Cases (%) N = 1,304	Controls (%) N = 1,266	Adjusted OR ^a (95% CI)
Age group, years			
35–49	107(8.5)	101(7.7)	
50–54	178(14.1)	189(14.5)	
55–59	343(27.1)	323(24.8)	
60–64	334(26.4)	394(30.2)	
65–69	160(12.6)	152(11.7)	
70–74	144(11.4)	145(11.1)	
First-degree relative with prostate cancer			
No	1124(88.8)	1021(78.3)	<i>Reference</i>
Yes	142(11.2)	283(21.7)	2.20(1.76–2.73)
Body mass index			
<25 kg/m ²	389(30.7)	426(32.7)	<i>Reference</i>
25–29.9 kg/m ²	618(48.8)	636(48.8)	0.94(0.79–1.12)
30 kg/m ²	259(20.5)	242(18.6)	0.85(0.68–1.07)
Daidzein ^b			
0.0 – < 0.218 mg/d	162(24.2)	158(23.7)	<i>Reference</i>
≥ 0.218 – < 0.365 mg/d	166(24.8)	148(22.2)	0.91(0.67–1.25)
≥ 0.365 – < 0.590 mg/d	172(25.7)	166(24.9)	0.99(0.73–1.34)
≥ 0.590 – 64.84 mg/d	170(25.4)	196(29.3)	1.18(0.87–1.60)
Genistein ^b			
0.0 – < 0.149 mg/d	165(24.6)	183(27.4)	<i>Reference</i>
≥ 0.149 – < 0.250 mg/d	173(25.8)	165(24.7)	0.86(0.64–1.16)
≥ 0.250 – < 0.444 mg/d	169(25.2)	136(20.4)	0.72(0.53–0.99)
≥ 0.444 – 67.84 mg/d	163(24.3)	184(27.5)	1.01(0.75–1.38)
Stage of PCa at diagnosis			
Local	1020(78.2)		
Regional	252(19.3)		
Distant	32(2.5)		
Gleason score at diagnosis			
2–6	744(57.1)		
7(3+4)	354(27.1)		
7(4+3), 8–10	202(15.5)		
Unknown	4(0.3)		
Composite aggressiveness score ^c			
Low/Moderate	871(66.8)		
High	433(33.2)		

^a Adjusted for age.

^bThese variables are limited to 669 cases and 670 controls from Study II with food frequency data.

^cClassification parameters of the composite aggressiveness score for the “High” group included diagnosis with regional/distant stage, Gleason score 7(4+3) or 8–10, *or* PSA ≥ 20 ng/mL while the “Low/Moderate” group included diagnosis with local stage, Gleason score 2–6 or 7(3+4), and diagnostic PSA <20 ng/mL.

Table II

Genotype distributions and odds ratios(95% CI) for associations between selected SNPs in *ESR1*, *CYP1B1*, and *CYP1A1* and prostate cancer risk in Caucasians

Gene	SNP	Genotype	Cases n(%) ^a (n=1,304)	Controls n(%) ^a (n=1,266)	Adjusted OR ^b (95% CI)	p ^c for trend
<i>ESR1</i>	<i>rs488133</i> ^d	CC	528(43.1)	584(48.1)	Reference	
		CT	563(45.9)	498(41.0)	1.25(1.05–1.48)	
		TT	135(11.0)	132(10.9)	1.13(0.86–1.48)	
		TT+CT			1.22(1.04–1.43)	0.06
<i>ESR1</i>	<i>rs2077647</i> ^d	AA	345(27.5)	321(25.7)	Reference	
		AG	638(50.9)	605(48.5)	0.98(0.81–1.18)	
		GG	271(21.6)	321(25.7)	0.79(0.63–0.98)	
		GG+AG			0.91(0.76–1.09)	0.04
<i>ESR1</i>	<i>rs1884051</i> ^e	AA	518(42.3)	550(45.2)	Reference	
		AG	555(45.3)	553(45.5)	1.07(0.90–1.26)	
		GG	152(12.4)	113(9.3)	1.43(1.09–1.87)	
		GG+AG			1.13(0.96–1.32)	0.02
<i>ESR1</i>	<i>rs1884054</i> ^f	AA	481(39.0)	493(40.4)	Reference	
		AC	559(45.3)	578(47.4)	0.99(0.84–1.18)	
		CC	194(15.7)	148(12.1)	1.34(1.05–1.72)	
		CC+AC			1.06(0.90–1.25)	0.07
<i>ESR1</i>	<i>rs926777</i> ^f	CC	710(55.9)	710(56.9)	ref	
		AC	470(37.0)	473(37.9)	0.99(0.84–1.17)	
		AA	91(7.2)	65(5.2)	1.40(1.00–1.95)	
		AA+AC			1.04(0.89–1.22)	0.22
<i>ESR1</i>	<i>rs3020432</i> ^g	AA	530(41.7)	551(44.4)	Reference	
		AG	582(45.8)	568(45.8)	1.07(0.90–1.26)	
		GG	159(12.5)	122(9.8)	1.36(1.04–1.77)	
		GG+AG			1.12(0.95–1.31)	0.04
<i>CYP1B1</i>	<i>rs162549</i> ^h	AA	770(61.4)	771(62.6)	Reference	
		AT	407(32.5)	410(33.3)	1.00(0.84–1.18)	

Gene	SNP	Genotype	Cases n(%) ^a (n=1,304)	Controls n(%) ^a (n=1,266)	Adjusted OR ^b (95% CI)	p ^c for trend
<i>CYP1B1</i>	<i>rs2855658^h</i>	TT	77(6.1)	51(4.1)	1.53(1.06–2.20)	
		TT+AT			1.05(0.90–1.24)	0.17
		GG	367(29.1)	423(34.1)	Reference	
		AG	637(50.6)	600(48.4)	1.22(1.02–1.46)	
		AA	256(20.3)	216(17.4)	1.37(1.09–1.72)	
<i>CYP1B1</i>	<i>rs1056836^h</i>	AA+AG			1.26(1.07–1.50)	0.002
		CC	375(29.9)	435(35.2)	Reference	
		CG	624(49.7)	585(47.4)	1.24(1.04–1.48)	
		GG	257(20.5)	215(17.4)	1.39(1.11–1.75)	
		GG+CG			1.28(1.08–1.51)	0.004
<i>CYP1A1</i>	<i>rs1456432ⁱ</i>	AA	849(67.8)	886(71.9)	Reference	
		AG	373(29.8)	317(25.7)	1.23(1.03–1.47)	
		GG	30(2.4)	29(2.4)	1.08(0.64–1.82)	
		GG+AG			1.22(1.03–1.45)	0.04
		CC	863(68.1)	897(72.6)	Reference	
<i>CYP1A1</i>	<i>rs4886605^j</i>	CT	370(29.2)	311(25.2)	1.24(1.04–1.48)	
		TT	34(2.7)	28(2.3)	1.27(0.76–2.11)	
		TT+CT			1.24(1.05–1.47)	0.02

^aVariable numbers of events reflect instances of failed genotyping.

^bOdds ratios (OR) are adjusted for age and bolded if significant at **nominal** $p = 0.05$. With the exception of *CYP1B1* rs2855658 and rs1056836, these did not remain significant after adjustment for multiple comparisons.

^cAnalysis for linear trend according to the number of variant alleles. **Nominal** trend p-values ≤ 0.05 are bolded.

^dThe SNPs rs488133 and rs2077647 were contained within same haplotype block (ORTA hap vs CG hap 0.86; 95% CI 0.8–1.0).

^eThe SNP rs1884051 was independent of any haplotype block structure.

^fSNPs rs188054 and rs926777 were in same haplotype block, but no haplotype contained both variant allele. Risk estimates were retained within haplotype structure (ORCC hap vs AC hap 1.2; 95% CI 1.0–1.4; ORAA hap vs AC hap 1.7; 95% CI 1.0–2.8).

^gThe SNP rs9340954 was in a haplotype block with 7 other SNPs (rs7754762, rs7757956, rs9340954, rs3020368, rs3778082, rs3778089, and rs3020383). Haplotype analysis did not reveal association by genotypes.

^hSNPs rs162549, rs2855658, and rs1056836 were in same haplotype block (ORAC hap vs TAG hap 1.2; 95% CI 1.0–1.4).

ⁱThe SNPs rs1456432 and rs4886605 were in contained within same haplotype block (ORAC hap vs GT hap 1.2; 95% CI 1.0–1.4).

Table III

Odds ratios(95% CI) for associations between selected SNPs in *ESR1*, *ESR2*, *CYP11A1*, and *CYP19A1* and prostate cancer risk by measures of tumor aggressiveness in Caucasians

Gene	SNP	Genotype	Estimates by Gleason Score at Diagnosis						Estimates by PCa Stage at Diagnosis					
			Gleason 2-7(3+4)			Gleason 7(4+3) 10			Local			Regional/Distant		
			Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a	P for χ^2_{ab}	P for χ^2_{ab}
<i>ESR1</i>	rs488133 ^c	CC	448		79		411		117					
		CT	468	1.23(1.03–1.46)	93	1.36(0.98–1.88)	435	1.23(1.03–1.47)	128	1.31(0.99–1.73)				
		TT	117	1.16(0.88–1.53)	17	0.94(0.54–1.65)	111	1.19(0.90–1.58)	24	0.91(0.57–1.48)				
		TT+CT		1.21(1.02–1.43)		1.27(0.93–1.74)		1.22(1.03–1.45)		1.22(0.94–1.60)			0.99	
<i>ESR1</i>	rs2077647 ^c	AA	293		49		276		69					
		AG	536	0.97(0.80–1.18)	101	1.09(0.75–1.57)	490	0.94(0.77–1.15)	148	1.14(0.83–1.57)				
		GG	227	0.77(0.61–0.98)	44	0.91(0.59–1.41)	215	0.78(0.62–0.99)	56	0.80(0.55–1.18)				
		GG+AG		0.90(0.75–1.09)		1.03(0.72–1.45)		0.89(0.73–1.07)		1.02(0.76–1.39)			0.35	
<i>ESR1</i>	rs1709183	AA	564		89		525		130					
		AG	393	0.96(0.81–1.15)	88	1.36(0.99–1.87)	369	0.97(0.81–1.16)	114	1.21(0.92–1.60)				
		GG	76	0.88(0.64–1.21)	14	1.03(0.57–1.89)	66	0.82(0.59–1.15)	24	1.20(0.74–1.95)				
		GG+AG		0.95(0.80–1.12)		1.30(0.96–1.77)		0.94(0.79–1.12)		1.21(0.93–1.58)			0.07	
<i>ESR1</i>	rs1884051 ^c	AA	439		77		412		106					
		AG	464	1.05(0.88–1.25)	89	1.15(0.83–1.60)	432	1.04(0.87–1.25)	123	1.16(0.87–1.54)				
		GG	128	1.42(1.07–1.88)	24	1.52(0.92–2.51)	116	1.37(1.03–1.83)	36	1.65(1.08–2.54)				
		GG+AG		1.11(0.94–1.32)		1.22(0.89–1.66)		1.10(0.93–1.30)		1.24(0.95–1.63)			0.39	
<i>ESR1</i>	rs1884054 ^c	AA	411		68		382		99					
		AC	460	0.95(0.80–1.14)	97	1.22(0.87–1.70)	432	0.96(0.80–1.16)	127	1.10(0.82–1.46)				
		CC	167	1.35(1.05–1.75)	27	1.31(0.81–2.12)	150	1.30(1.00–1.69)	44	1.49(1.00–2.23)				
		CC+AC		1.04(0.87–1.23)		1.23(0.90–1.70)		1.03(0.87–1.23)		1.18(0.90–1.55)			0.36	
<i>ESR1</i>	rs926777 ^c	CC	603		104		562		148					
		AC	387	0.96(0.81–1.15)	82	1.19(0.87–1.63)	364	0.97(0.82–1.16)	106	1.07(0.81–1.41)				
		AA	77	1.39(0.98–1.97)	14	1.44(0.78–2.66)	68	1.31(0.91–1.87)	23	1.72(1.04–2.87)				
		AA+AC		1.02(0.86–1.20)		1.22(0.91–1.65)		1.01(0.86–1.20)		1.15(0.88–1.49)			0.37	

Gene	SNP	Genotype	Estimates by Gleason Score at Diagnosis						Estimates by pCa Stage at Diagnosis			
			Gleason 2-7(3+4)			Gleason 7(4+3) 10			Local		Regional/Distant	
			Cases N	Adjusted OR (95% CI) ^a	P for χ^2 ^b	Cases N	Adjusted OR (95% CI) ^a	P for χ^2 ^b	Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a
<i>ESR1</i>	rs3020432 ^c	AA	445			83			415		115	
		AG	490	1.07(0.90-1.27)		90	1.06(0.77-1.46)		455	1.07(0.89-1.28)	127	1.06(0.80-1.40)
		GG	133	1.35(1.02-1.78)		26	1.42(0.88-2.31)		125	1.36(1.03-1.81)	34	1.32(0.86-2.04)
		GG+AG		1.12(0.95-1.32)	0.97		1.13(0.83-1.53)	0.97		1.12(0.95-1.33)		1.11(0.85-1.45)
<i>ESR1</i>	rs2228480	GG	675			140			643		174	
		AG	349	1.10(0.92-1.31)		50	0.76(0.54-1.08)		306	1.01(0.84-1.22)	95	1.16(0.88-1.53)
		AA	31	0.82(0.51-1.31)		4	0.49(0.17-1.40)		30	0.82(0.51-1.32)	5	0.53(0.21-1.36)
		AA+AG		1.07(0.90-1.27)	0.03		0.73(0.52-1.03)	0.03		0.99(0.83-1.19)		1.09(0.83-1.44)
<i>ESR2</i>	rs1952586	AA	862			150			800		216	
		AG	194	1.05(0.85-1.31)		45	1.42(0.99-2.05)		185	1.09(0.88-1.35)	54	1.16(0.83-1.62)
		GG	7	1.03(0.37-2.86)		5	4.53(1.46-14.10)		7	1.14(0.41-3.15)	5	2.86(0.92-8.85)
		GG+AG		1.05(0.85-1.30)	0.04		1.53(1.07-2.17)	0.04		1.09(0.88-1.35)		1.23(0.89-1.69)
<i>CYP1A1</i>	rs1456432 ^c	AA	707			138			672		177	
		AG	315	1.25(1.04-1.50)		58	1.19(0.85-1.65)		284	1.19(0.98-1.43)	89	1.40(1.05-1.86)
		GG	27	1.17(0.68-1.99)		3	0.66(0.20-2.21)		23	1.05(0.60-1.83)	7	1.20(0.51-2.78)
		GG+AG		1.24(1.04-1.48)	0.63		1.14(0.82-1.58)	0.63		1.17(0.98-1.41)		1.38(1.04-1.82)
<i>CYP1A1</i>	rs4886605 ^c	CC	721			138			687		176	
		CT	313	1.25(1.04-1.51)		57	1.20(0.86-1.68)		281	1.18(0.98-1.43)	89	1.45(1.09-1.93)
		TT	30	1.33(0.79-2.25)		4	0.95(0.33-2.76)		24	1.13(0.65-1.97)	10	1.78(0.85-3.74)
		TT+CT		1.26(1.05-1.51)	0.71		1.18(0.85-1.64)	0.71		1.18(0.98-1.42)		1.48(1.12-1.95)
<i>CYP1B1</i>	rs162549 ^c	AA	652			116			610		160	
		AT	344	0.99(0.83-1.19)		61	1.00(0.72-1.39)		313	0.97(0.81-1.16)	94	1.09(0.83-1.45)
		TT	60	1.39(0.95-2.05)		17	2.33(1.30-4.19)		56	1.42(0.96-2.10)	21	1.92(1.12-3.28)
		TT+AT		1.04(0.88-1.23)	0.55		1.14(0.84-1.56)	0.55		1.02(0.86-1.21)		1.19(0.91-1.55)
<i>CYP1B1</i>	rs2855658 ^c	GG	312			54			286		81	
		AG	544	1.23(1.02-1.48)		92	1.20(0.84-1.72)		500	1.23(1.02-1.49)	137	1.19(0.88-1.61)
		AA	202	1.27(1.00-1.61)		52	1.93(1.27-2.93)		201	1.39(1.09-1.77)	55	1.31(0.89-1.91)
		AA+AG		1.24(1.04-1.48)	0.50		1.39(1.00-1.95)	0.50		1.27(1.06-1.53)		1.22(0.92-1.63)

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Gene	SNP	Genotype	Estimates by Gleason Score at Diagnosis					Estimates by PCa Stage at Diagnosis				
			Gleason 2-7(3+4)			Gleason 7(4+3) 10		Local			Regional/Distant	
			Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a	<i>P</i> for χ^2 ^b	Cases N	Adjusted OR (95% CI) ^a	Cases N	Adjusted OR (95% CI) ^a	<i>P</i> for χ^2 ^b
<i>CYP17A1</i>	rs1056836 ^c	CC	320		54			289		86		
		CG	535	1.24(1.03-1.50)	88	1.21(0.85-1.74)		492	1.27(1.05-1.53)	132	1.14(0.84-1.54)	
		GG	203	1.29(1.01-1.63)	52	2.01(1.32-3.04)		201	1.42(1.12-1.82)	56	1.29(0.89-1.88)	
		GG+CG		1.25(1.05-1.50)		1.42(1.02-1.99)	0.47		1.31(1.09-1.57)		1.18(0.89-1.56)	.049
<i>CYP19A1</i>	rs12900487	CC	377		71			337		113		
		CT	424	0.97(0.80-1.17)	83	1.02(0.72-1.43)		400	1.03(0.85-1.25)	108	0.81(0.61-1.09)	
		TT	262	0.99(0.79-1.22)	43	0.88(0.59-1.33)		251	1.07(0.86-1.34)	55	0.67(0.47-0.96)	
		TT+CT		0.97(0.82-1.16)		0.97(0.71-1.32)	0.96		1.04(0.88-1.25)		0.76(0.58-0.99)	0.02
<i>CYP19A1</i>	rs727479	TT	455		87			411		135		
		GT	484	0.99(0.83-1.18)	94	1.02(0.75-1.40)		460	1.05(0.88-1.26)	118	0.80(0.61-1.06)	
		GG	132	1.07(0.82-1.39)	20	0.87(0.51-1.46)		128	1.16(0.88-1.52)	24	0.64(0.40-1.02)	
		GG+GT		1.01(0.85-1.19)		0.99(0.73-1.34)	0.92		1.07(0.91-1.27)		0.77(0.59-1.00)	0.02
<i>CYP19A1</i>	rs6493494	GG	333		57			321		71		
		AG	520	1.02(0.84-1.23)	105	1.19(0.84-1.69)		484	0.98(0.81-1.18)	142	1.31(0.96-1.79)	
		AA	206	1.03(0.81-1.30)	37	1.06(0.68-1.66)		183	0.94(0.74-1.20)	61	1.45(0.99-2.12)	
		AA+AG		1.02(0.86-1.22)		1.16(0.83-1.61)	0.46		0.97(0.81-1.16)		1.35(1.00-1.82)	0.03
<i>CYP19A1</i>	rs749292	GG	320		54			307		68		
		AG	530	1.02(0.84-1.23)	106	1.20(0.84-1.70)		493	0.98(0.81-1.19)	145	1.31(0.96-1.80)	
		AA	216	1.05(0.83-1.33)	38	1.08(0.69-1.68)		192	0.97(0.76-1.23)	63	1.46(1.00-2.14)	
		AA+AG		1.03(0.86-1.22)		1.16(0.83-1.63)	0.47		0.98(0.81-1.17)		1.36(1.01-1.83)	0.04
<i>CYP19A1</i>	rs2445762	TT	544		100			520		127		
		CT	402	1.05(0.88-1.25)	83	1.20(0.88-1.65)		365	1.00(0.84-1.20)	120	1.33(1.01-1.75)	
		CC	100	1.23(0.91-1.66)	15	1.01(0.56-1.80)		89	1.14(0.84-1.56)	27	1.43(0.90-2.28)	
		CC+CT		1.08(0.92-1.28)		1.17(0.86-1.58)	0.63		1.03(0.87-1.22)		1.35(1.04-1.75)	0.04

^aPolynomial regression used to determine odds ratios (OR). Adjusted for age and bolded if significant at *p* = 0.05.^bTest for heterogeneity, with *p*-values = 0.05 bolded.^cSNPs with an observed significant association with PCa risk (Table II).