Evaluation of Genetic Variations in the Androgen and Estrogen Metabolic Pathways as Risk Factors for Sporadic and Familial Prostate Cancer

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Abstract

Previous studies suggest that enzymes involved in the androgen metabolic pathway are susceptibility factors for prostate cancer. Estrogen metabolites functioning as genotoxins have also been proposed as risk factors. In this study, we systematically tested the hypothesis that common genetic variations for those enzymes involved in the androgen and estrogen metabolic pathways increase risk for sporadic and familial prostate cancer. From these two pathways, 46 polymorphisms (34 single nucleotide polymorphisms, 10 short tandem repeat polymorphisms, and 2 null alleles) in 25 genes were tested for possible associations. Those genes tested included *PRL*, *LHB*, *CYP11A1*, *HSD3B1*, *HSD3B2*, *HSD17B2*, *CYP17*, *SRD5A2*, *AKR1C3*, *UGT2B15*, *AR*, *SHBG*, and *KLK3* from the androgen pathway and *CYP19*, *HSD17B1*, *CYP1A1*, *CYP1A2*, *CYP1B1*, *COMT*, *GSTP1*, *GSTT1*, *GSTM1*, *NQO1*, *ESR1*, and *ESR2* from the estrogen pathway. A case-control study design was used with two sets of cases: familial cases with a strong prostate cancer family history (n = 438 from 178 families) and sporadic cases with a negative prostate cancer family history (n = 499). The controls (n = 493) were derived from a population-based collection. Our results provide suggestive findings for an association with either familial or sporadic prostate cancer with polymorphisms in four genes: *AKR1C3*, *HSD17B1*, *NQO1*, and *GSTT1*. Additional suggestive findings for an association with clinical variables (disease stage, grade, and/or node status) were observed for single nucleotide polymorphisms in eight genes: *HSD3B2*, *SRD5A2*, *SHBG*, *ESR1*, *CYP1A1*, *CYP1B1*, *GSTT1*, and *NQO1*. However, none of the findings were statistically significant after appropriate corrections for multiple comparisons. Given that the point estimates for the odds ratio for each of these polymorphisms are <2.0, much larger sample sizes will be required for confirmation. (Cancer Epidemiol Biomarkers Prev 2007;16(5):969–78)

Introduction

Prostate cancer is one of the most common malignancies of males in western countries (1). Although older age and African American ancestry have long been recognized as important risk factors, there is ample evidence supporting the notion that genetics plays a key role (2-5). This evidence comes from a wide range of studies including familial aggregation, twin studies, family-based linkage studies, and molecular epidemiologic studies of both rare and common polymorphisms of candidate genes (5). However, this evidence also points toward a much more complex genetic basis than initially anticipated. Although familial clustering can best be explained by a rare dominant susceptibility gene with high penetrance (5), this accounts for only a small fraction of the familial prostate cancer cases.

With strong evidence for the presence of a major prostate cancer susceptibility gene, a number of linkage studies have been done over the past decade. Early results provided targeted candidate regions for prostate cancer susceptibility loci, including *HPC1* (1q23-25; ref. 6), *PCAP* (1q42-43; ref. 7), *CAPB* (1p36; ref. 8), chromosome 8p22-23 (9), *HPC2* (17p; ref. 10), *HPC20* (20q13; ref. 11), and *HPCX* (Xq27-28; ref. 12). A few of these targeted studies led to the identification of candidate susceptibility genes including *RNASEL* (HPC1; ref. 13), *ELAC2* (HPC2; ref. 10), and *MSR1* on chromosome 8 (14). However, confirmatory studies for these genes have provided mixed results (5).

In addition to the targeted linkage analyses, at least 12 genome linkage screens based on microsatellite markers have now been done (5, 15). Although there is some overlap among chromosomal regions showing suggestive to moderate evidence for linkage between studies, there is no evidence for a single common major susceptibility locus. Additionally, a number of studies have found linkage of Gleason grade (a measure of prostate tumor differentiation, considered to measure aggressiveness of disease) to several genomic regions (16-21). Overall, these studies illustrate the extensive amount of heterogeneity of prostate cancer.

A limitation of linkage studies is their weak power to find susceptibility genes of small to moderate effects. Alternative approaches are association studies, which tend to have greater power to detect genes of small risk (22). Careful choice of single nucleotide polymorphisms (SNP) within candidate genes (23) may provide the most powerful method to find relatively common genes of small risk. For prostate cancer, the most compelling hypothesis for increased cancer risk is supported by hormonal involvement, which includes enzymes involved in the androgen metabolic pathway and gonadotrophins (24-27). However, evidence also suggests that estrogen metabolites, such as genotoxins, also increase risk for prostate cancer (28).

In this study, we did a systematic analysis of 46 common polymorphisms in 25 genes involved in the androgen and

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estrogen metabolic pathways to determine their potential associations with increased risk of prostate cancer and tumor aggressiveness. These include common genetic polymorphisms for enzymes that catalyze the synthesis and bioactivation of androgens and estrogens (*PRL, LHB, CYP11A1, HSD3B1, HSD3B2, HSD17B1, HSD17B2, CYP17, CYP19, SRD5A2, AKR1C3, UGT2B15, AR, SHBG*, and *KLK3, ESR1*, and *ESR2*), for enzymes that catalyze the synthesis of catecholestrogens (*CYP1A1, CYP1A2*, and *CYP1B1*), and for enzymes that metabolically inactivate estrogens, catecholestrogens, or genotoxins generated from catecholestrogens (*COMT, GSTP1, GSTT1, GSTM1*, and *NQO1*). We used a case-control study design with two sets of cases: familial prostate cancer (FPC) cases with a strong prostate cancer family history and sporadic prostate cancer (SPC) cases with a negative prostate cancer family history.

Subjects and Methods

FPC Cases. Ascertainment of cases with FPC has been described elsewhere (29). Briefly, from 1996 to 1997, a total of 12,675 surveys were sent to men who received a radical prostatectomy or radiation therapy at Mayo Clinic. From these surveys, ~200 high-risk families were identified. Families with a minimum of three affected men with prostate cancer were enrolled for further study. Blood was collected from as many family members as possible, resulting in a total of 438 affected men from 178 families. For 163 of these families, DNA was available on multiple living affected men. For the remaining families, DNA was available for only a single affected man. All men with prostate cancer who contributed a blood specimen had their cancers verified by review of medical records and pathologic confirmation. One family had Hispanic ancestry; the remainder had non-Hispanic Caucasian ancestry. For our association study, we used all men from the same generation (i.e., siblings and cousins) to avoid large differences in ages and secular trends according to year of diagnosis. Thus, the 438 cases consisted of singletons, siblings, and cousins. The Mayo Clinic institutional review board approved the research protocol and informed consent forms.

SPC Cases. SPC cases were selected from respondents to our family history survey who reported no family history of prostate cancer. To ensure that the SPC cases were similar to the FPC cases, except for family history, we selected eligible cases by matching them to FPC index cases according to year of diagnosis, age at diagnosis, and number of brothers. Multiple cases were identified for each FPC case; from these case sets, SPC cases were randomly sampled for recruitment with a goal of recruiting ~500 men. A total of 1,001 invitations were sent to men who initially reported no family history of prostate cancer. Our second survey determined that only 740 of these men were eligible (i.e., still no family history of prostate cancer). Of these men, 499 contributed a blood sample; 491 reported non-Hispanic Caucasian ancestry, 3 African American, and 5 other types of ancestry.

Population-Based Controls. From a sampling frame of the local population provided by the Rochester Epidemiology Project (30), 475 men were randomly selected for a clinical urologic examination (in-clinic cohort; ref. 31). This examination included digital rectal examination, transrectal ultrasound of the prostate, post-void residual urine volume, serum prostate-specific antigen and creatinine measurement, focused urologic physical examination, and cryopreservation of serum for subsequent sex hormone assays. Any man with an abnormal digital rectal examination, elevated serum prostate-specific antigen level, or suspicious lesion on transrectal ultrasound was evaluated for prostatic malignancy. If the digital rectal examination and transrectal ultrasound were unremarkable and the serum prostate-specific antigen level was elevated (>4.0 ng/mL), a sextant biopsy (three cores from each side) of the prostate was done. An abnormal digital rectal examination or transrectal ultrasound result, regardless of the serum prostate-specific antigen level, prompted sextant biopsy to include the area in question. These men have been followed with biennial examinations, and in 1994, buffy coats were harvested from the phlebotomy specimens. Men free of prostate cancer based on this extensive workup and at follow-up exams through 1994, with augmentation by random samples from the population accrued over that time, were used as the control population for this study (n = 493; ref. 32). Of these men, 490 reported non-Hispanic Caucasian ancestry and 3 reported Hispanic ancestry.

SNP Selection. At the time that these studies were done, all known nonsynonymous coding SNPs for the genes selected were chosen for analysis. In addition, other commonly studied synonymous coding SNPs, short tandem repeats (STR), or SNPs in the promoter region were also selected for analysis. Altogether, these included 46 polymorphisms (34 SNPs, 10 STRs, and 2 null alleles) in 25 genes. Additional information for each of the genes and polymorphisms selected for this study is provided in Supplementary Table S1.

Genotype Methods. Three different platforms were used in this study to genotype the 46 polymorphisms. PCR primers and conditions are available on request. For the SNP in *LHB* (Trp28Arg, c.82T/C) and the SNP in *HSD3B2* [3′ untranslated region (UTR), c.1395C/T], pyrosequencing was done on a PSQ96 (Biotage) according to the manufacturer's guidelines as described elsewhere (33). For the remaining 30 SNPs, Qiagen Genomic Masscode system was used to generate the genotypes (34). Masscode used a two-step PCR process. The first step uses a standard touchdown PCR with forward and reverse primers. The second PCR uses allele-specific primers with differentially labeled photocleavable Masscode tags in either the forward or reverse primer. The allele-specific PCR products were purified, tags were cleaved, and run on a single quadruple mass spectrometer. Genotypes were extrapolated by the ratio of one Masscode tag to the other. For each of the STRs, PCR amplicons were run on an ABI3100 (Applied Biosystems) and analyzed using Genotyper 3.7 (Applied Biosystems). Finally, a quantitative PCR-based assay using the ABI3100 (Applied Biosystems) was used for each of the two null alleles.

To ensure sample identity, five microsatellite markers with known baseline genotype data were tested on all samples before testing by Qiagen Genomic. Within all 96-well plates, two or three controls in duplicate, along with two negative controls, were included. In addition to these blinded controls, Qiagen Genomic included three positive and two negative controls on each 96-well plate. Finally, to further insure Qiagen Genomic platform validity, two SNPs with known baseline genotype data on all cases and controls were included in the study set. In addition to these quality control steps, an exact test for Hardy-Weinberg equilibrium (35) was applied to the genotype proportions in the control group. Genotyping was done blinded to phenotype status. Overall, the average call rate for each SNP was 98.6% (range, 95.9-99.5%) and the error rate measured for the blinded controls and the two previously genotyped SNPs was 0.1%.

Statistical Analyses. The association for each of the SNPs/null alleles with FPC and SPC was evaluated by comparing genotype frequencies among prostate cancer cases versus controls, using a test for trend in the number of minor (rare) variant alleles. For the unrelated cases, this test is the Armitage test for trend in proportions (36). For FPC cases, a method that accounts for the correlation among related subjects, was used (37). Similar methods were used to compare the FPC cases to

the SPC cases. Odds ratios (OR) and their 95% confidence intervals (95% CI) were computed for carriers of the minor variant allele versus subjects homozygous for the major allele.

To analyze STR markers, a global χ^2 statistic that generalizes Armitage's test for trend to more than two alleles was used. That is, each allele was given a ''dosage'' score of 0, 1, or 2 for each subject, and the averages of these scores were compared between cases and controls. For the comparisons with FPC cases, a global test of association was calculated using a robust score statistic, which simultaneously considers all alleles and accounts for correlations among family members (38). Because the STRs can have many alleles, giving a χ^2 statistic with many degrees of freedom, this global test can have weak power when just one or two alleles are associated with disease. To increase power for this situation, we computed Armitage's test for trend for each allele versus the pool of all others and used the maximum overall allelic tests as the summary statistic. Simulations were used to compute *P* values for this max statistic by randomly permuting case-control status.

Because testing was done on a number of SNPs and STRs, multiple testing was controlled in several ways. In addition to reporting *P* values unadjusted for multiple testing, we also present adjusted *P* values, where adjustment was achieved by simulations. For the androgen pathway, we had 17 SNPs and 3 comparisons for each SNP (SPC cases versus controls, FPC cases versus controls, and SPC cases versus FPC cases), giving a total of 51 comparisons. Because some of these comparisons are dependent among each other (e.g., overlapping groups and SNPs in linkage disequilibrium within a gene), the usual Bonferroni correction is known to be conservative. To adjust *P* values by simulations, we randomly reassigned the 17 SNP genotypes among the pool of all cases and controls, recomputed *P* values, and counted the number of times the simulated *P* values were more extreme than the observed *P* values; this process was repeated 1,000 times to compute adjusted *P* values (39). Similar corrections were made for SNPs within the estrogen pathway. These values are reported in Tables 2 and 3 as adjusted *P* values. Because these adjusted *P* values are intended to control the chance of making any erroneous conclusion, they can be conservative when testing many genes. An alternative is to control the false discovery rate (FDR), the proportion of erroneous significant tests, which can have greater power. Hence, we also present FDR *P* values adjusted according to Benjamini and Hochberg (40). Finally, if several SNPs have small to moderate effects, then testing each individually may have weak power, yet a combined test will have greater power. We recently developed such a combined test and applied it to the SNPs evaluated in this article (41), with the exception that the null alleles for the genes *GSTT1* and *GSTM1* in the estrogen pathway were not measured at that time. Hence, we now repeat these analyses with the expanded set of SNPs.

Haplotype frequencies were estimated by an expectation-maximization algorithm, and the association of haplotypes with disease status was evaluated by a score statistic that accounts for unknown linkage phase (42). To model the effects of individual loci, adjusted for the haplotype effects, we created dosage scores for the minor alleles of the individual loci and for the haplotype of interest. The expectation-maximization algorithm provides a list of all possible pairs of haplotypes consistent with the observed unphased genotype data, as well as posterior probabilities of the possible pairs. From these lists, we created the locus and haplotype dosage scores, and then used the posterior probabilities to create an average score per person. These average scores were then used in logistic regression. To account for family dependencies, we did a second average, averaging over affected men within the same family, resulting in a single score per family. Simulated *P* values were used to avoid problems that may result from sparse data. Pairwise interactions between markers were evaluated by likelihood ratio tests that compared a logistic regression model with both main effects and an interaction term versus a model without the interaction.

Results

The characteristics of the three study groups are shown in Table 1. Although the distribution of the age at diagnosis and body mass index levels are similar between the FPC and SPC cases, the controls tended to be younger and have significantly higher body mass index than both case groups. Although the median age of the control group (61 years) is slightly less than that of the FPC (66 years) and SPC (65 years) groups, analyses adjusted for age did not alter our findings. Furthermore, analyses adjusted for both age and body mass index also did not alter our findings. For simplicity, therefore, only the unadjusted *P* values are presented in this report.

Among the three comparisons for each SNP (SPC cases versus controls, FPC cases versus controls, and SPC cases versus FPC cases), there was not any noteworthy associations within the latter comparison group. Here, we report association with the SPC and FPC cases. Figure 1 illustrates the candidate genes and their relationships within each of these two pathways. Other genes and enzymes that are part of the pathway are also illustrated in this figure, emphasizing that the polymorphisms of interest represent genes whose products act at different key steps of androgen and estrogen biosynthesis.

SNP Associations. A summary of the magnitude of association for each SNP with the SPC and FPC cases is presented in Fig. 2, where the ORs for the carriers of minor alleles for each SNP, along with their 95% CIs, are presented. The statistical results for testing the association of each of the androgen and estrogen pathway polymorphisms with the SPC and FPC cases are presented in Tables 2 and 3, respectively. Overall, none of our findings were statistically significant after correcting for multiple comparisons.

For the androgen metabolic pathway, only one SNP (*AKR1C3*, Pro30Pro, c.90G/A) showed a suggestive association with FPC. The minor allele for *AKR1C3* c.90G/A was more frequent among FPC cases than controls (40% versus 33%; FDR $P = 0.30$), with an OR of 1.53 (95% CI, 1.13-2.08). This SNP was also more frequent among SPC cases than controls (36% versus 33%; FDR $P = 0.54$), with an OR of 1.21 (95% CI, 0.94-1.56).

For the estrogen metabolic pathway, several polymorphisms showed suggestive associations. The minor allele for *HSD17B1* (Ser313Gly, c.937A/G) was more frequent among SPC cases than controls (46% versus 40%), with an OR of 1.39 (95% CI, 1.06-1.82; FDR $P = 0.44$). For *GSTT1* (null allele), the minor allele was less frequent among SPC cases than controls (37% versus 43%; FDR $P = 0.32$) with an OR of 0.69 (95% CI, 0.53-0.91). Although this may be due to statistical chance, it is possible that the presence of the major allele for *GSTT1* portrays a slight increase in risk for prostate cancer. Finally, the minor allele of *NQO1* (Pro187Ser, c.559C/T) was more frequent in both SPC cases (20% versus 16%; FDR $P = 0.62$) and FPC cases (22% versus 16%; FDR $P = 0.62$) compared with the controls, with respective ORs of 1.28 (95% CI, 0.98-1.67) and 1.49 (95% CI, 1.09-2.04).

STR Associations. The statistical results for testing the association of each of the STRs among the SPC cases and FPC cases are presented in Table 4. No suggestive associations were found among these polymorphisms, even without correcting for multiple testing.

''Super-Normal'' Controls. The choice of the control group is a critical component in case-control studies. To be sure that controls are not likely to have undetected disease, some studies have used ''super-normal'' controls (14). We therefore

Table 1. Characteristics of prostate cancer cases and controls

Characteristics	FPC (N = 438)	SPC (N = 499)	Controls (N = 493)	P*
Age† (y), median (25th and 75th percentile)	66 (45-84)	65 (46-79)	61 (45-89)	
Age quartiles (y), n (%)				
45-59	68 (15.5)	90 (18.0)	218 (44.2)	<0.0001
60-65	129 (29.5)	180 (36.1)	88 (17.9)	
66-69	110 (25.1)	142 (28.5)	48 (9.7)	
70-89	131 (29.9)	87 (17.4)	139 (28.2)	
Body mass index (kg/m^2), n (%)				
<28	289 (66.3)	312 (62.5)	223 (45.2)	<0.0001
≥28	147 (33.7)	187 (37.5)	270 (54.8)	
Missing	2	0	0	
Prostate-specific antigen (ng/mL), n (%)				
<4	41 (12.2)	92 (22.7)	451 (91.5)	<0.0001
4-9.9	155 (46.1)	179 (44.2)	38 (7.7)	
10-19.9	68 (20.2)	73 (18.0)	4 (0.8)	
≥20	72 (21.4)	61 (15.1)	0 (0.0)	
Unknown	102	94	0	
Pathologic characteristics				
Nodal status‡				
Negative	379 (87.9)	413 (87.7)		0.91
Positive	52 (12.1)	58 (12.3)		
Unknown	7	28		
Stage§				
I/II	272 (71.8)	261 (64.0)		0.02
III/IV	107 (28.2)	147 (36.0)		
Unknown	7	33		
Grade				
<7	251 (69.7)	268 (67.5)		0.51
≥7	109 (30.3)	129 (32.5)		
Unknown	78	102		

*P values are from Pearson's χ^2 test or Mantel-Haenszel test for trend (age quartiles and prostate-specific antigen).
† Defined as age at diagnosis for FPC and SPC groups and age at the time of blood draw for controls.
‡ Positive nodal status group includes subjects who had metastatic disease.
§ Subjects whose nodal status was positive are excluded.

eliminated controls with either the presence of a suspicious digital rectal exam or a prostate-specific antigen >4. A total of 249 controls remained. Removal of these controls had little effect on the frequency of the variant alleles in the remaining controls. The frequency of the minor allele for *AKR1C3* (c.90G/A) was 33% before and 34% after removal; for *HSD17B1* (c.937A/G), 40% before and after removal; for *GSTT1* null allele, 43% versus 44%; and for *NQO1* (c.559C/T), 16% versus 17%.

Clinical Correlations. We also evaluated the association of each of the polymorphisms with the following clinical features of disease: stage (I/II versus II/IV disease), Gleason grade (<7 versus ≥7), and nodal involvement (− versus +). These clinical correlations were done separately for the FPC and SPC cases. Suggestive clinical correlations for *CYP11A1*, *HSD3B2*, *SRD5A2*, *SHBG*, *CYP19*, *ESR1*, *ESR2*, *CYP1A1*, *CYP1B1*, *GSTT1*, and *NQO1* are presented in Tables 5 and 6. However, as before, these statistical tests should be interpreted cautiously and require further study to determine if they can be replicated.

Haplotype Analyses. Haplotype associations were run for each of the nine genes for which more than a single SNP was analyzed (*HSD3B1*, *HSD3B2*, *AKR1C3*, and *KLK3* from the androgen pathway and *CYP19*, *CYP1A1*, *CYP1B1*, *GSTP1*, and *ESR1* from the estrogen pathway). When testing for any differences in haplotype frequencies using a global test, the strongest detected association was for a haplotype composed of two SNP loci within the *AKR1C3* gene. This haplotype had a major allele for c.15C/G and a minor allele for c.90G/A. The frequency of this haplotype was 39.5% among FPC cases versus 33.0% among controls (haplotype-specific test simulated P = 0.003). Note that the first locus (c.15C/G) was not significantly associated with FPC status when it was evaluated alone, and that the second locus (c.90G/A) was more strongly associated with FPC status (see Table 2). To evaluate whether the haplotype association is driven by both loci, versus by the second locus c.90G/A, we did logistic regression. Ideally, we would like to test the effect of each locus, adjusted for the haplotype, to evaluate the contribution of each locus over the effects of the haplotype. For this analysis, we attempted to include a haplotype dosage for the haplotype of interest as well as the dosage of each of the loci. Unfortunately, the haplotype effect was perfectly confounded with the second locus effect; thus, we could only evaluate whether the first locus had a significant association with FPC status after adjusting for the haplotype effect. The results suggest that the first locus did not contribute to the association (P = 0.47-0.57, depending on whether we accounted for family relationships). Hence, the haplotype analyses did not seem to contribute more information but rather point to the primary association due to the second locus, c.90G/A.

Discussion

A large number of studies have attempted to unravel the genetic basis of prostate cancer. Despite suggestive genetic associations, reports have been difficult to replicate, indicating that prostate cancer is much more genetically heterogeneous than initially believed. In this study, we investigated whether genes involved in steroid biosynthesis as well as androgen and estrogen metabolism contribute to prostate cancer risk and aggressiveness.

Although statistically significant results were not achieved after appropriate correction for multiple comparisons, a few suggestive results for polymorphisms in four genes (*AKR1C3*, *NQO1*, *GSTT1*, and *HSD17B1*) warrant further discussion. The *AKR1C3* c.90G/A minor allele showed a suggestive positive association with FPC. Currently, it is unknown whether this *AKR1C3* polymorphism has any effect on enzymatic activity.

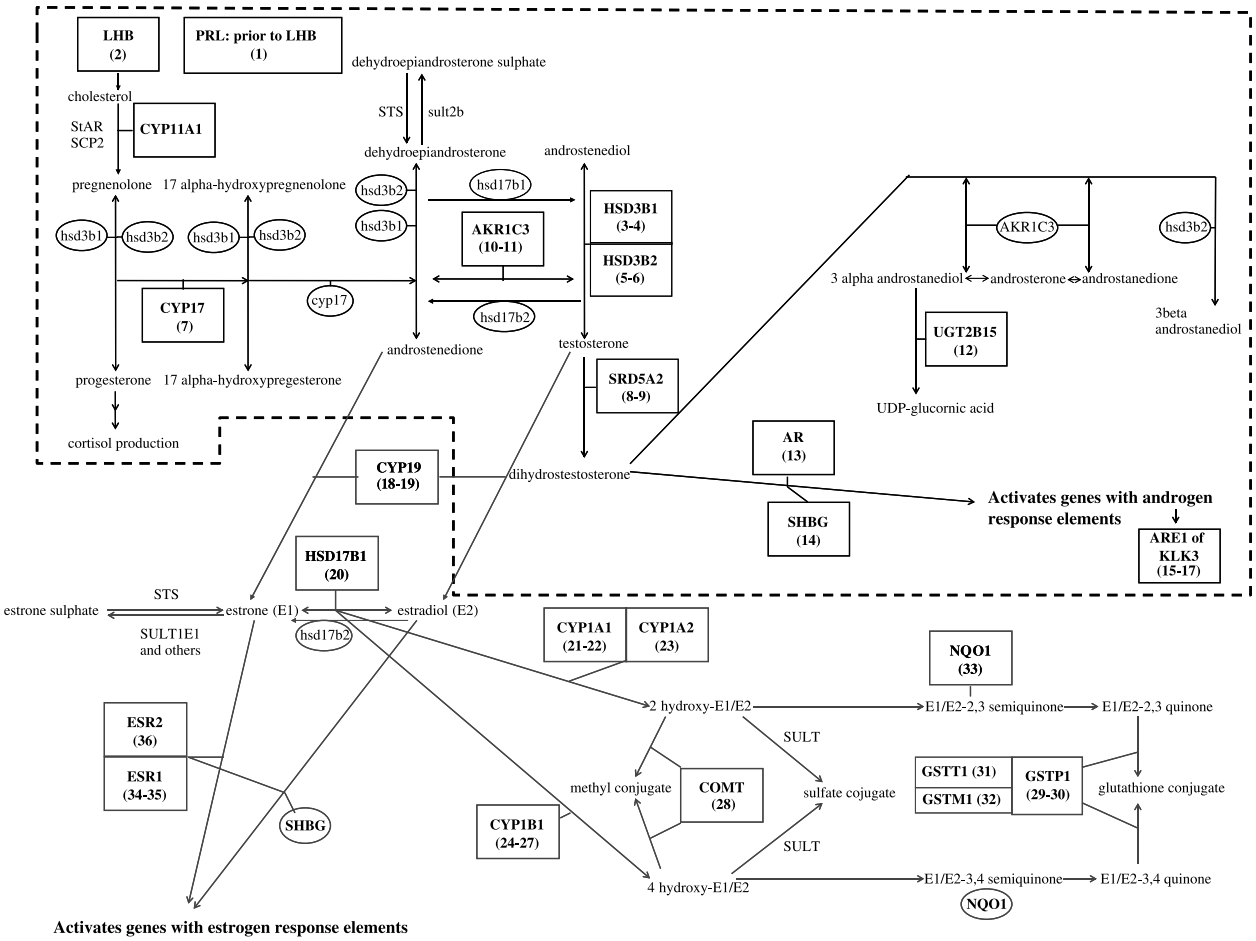

Figure 1. Candidate genes involved in androgen and estrogen metabolism. The androgen portion of the pathway is within the broken line, and the estrogen pathway is outside of the broken line, at the bottom of the figure. The genes included in the present study are indicated within boxes with their corresponding genes labeled.

Until recently, polymorphisms in the *AKR1C3* gene have not been studied for associations in prostate cancer. Mononen et al. (27) found no significant association with prostate cancer in a Finnish sample, whereas the *AKR1C3* gene was suggested to be associated with enlarged prostate in older men (43). *AKR1C3*, which is expressed highly in adenocarcinoma of the prostate, can be inhibited by nonsteroidal anti-inflammatory drugs. It is possible that the inhibition of *AKR1C3* may play a role in the chemopreventive properties of these drugs (44). The possible increased risk of cancer in both familial and sporadic cases is intriguing and warrants further investigation. It will be important to determine whether the synonymous variant in *AKR1C3* (c.90G/A) is associated with a difference in expression or enzyme activity (presumably in linkage disequilibrium with a functional variant), thereby shedding light on the role of this critical HSD.

Three genes involved in the estrogen metabolic pathway, *GSTT1*, *HSD17B1*, and *NQO1*, showed suggestive findings with increased risk of prostate cancer. Gluthathione *S*-transferase θ1 (GSTT1) enzyme activity is completely lost in men carrying the *GSTT1* null genotype (45), which may result in increasing risk of carcinogenesis (46). In a meta-analysis by Ntais et al. (26), the potential role of *GSTT1* polymorphisms was not clear. Overall, these investigators showed that the *GSTT1* polymorphism was unlikely to be the major determinant of susceptibility to prostate cancer. However, a recent large comprehensive study (n = 2088) reported a negative association between *GSTT1* and prostate cancer (47). Cumulatively, these results suggest that polymorphisms in *GSTT1* do not substantially increase the risk of prostate cancer in the population.

NQO1 and *HSD17B1* showed a marginal positive association with risk of prostate cancer, and the latter in sporadic cases only. However, the Breast and Prostate Cancer Cohort Consortium reported no association between germ line variants, including c.937A/G in *HSD17B1*, and risk of prostate cancer in a comprehensive htSNP analysis (48), suggesting that our results are false positives. Although several other polymorphisms result in reduced catalytic activity, the minor allele for *HSD17B1* (Ser313Gly, c.937A/G) was not found to affect either the catalytic or the immunologic properties of the type 1 enzyme (49). NQO1 has been implicated to have a role in carcinogenesis (50). In a comprehensive meta-analysis assessing the relationship between *NQO1 Pro187Ser* polymorphism and the risk of lung, bladder, and colon cancers by Chao et al. (51), a fairly consistent association pattern has been found across all three types of cancers, but its role in prostate cancer has not been thoroughly investigated. Steiner et al. (52) did not find any association with the *NQO1* c.559C/T SNP in sporadic cases; familial cases were not examined. This was a relatively small study, however, involving only 54 cases with prostate cancer and 100 controls. The *NQO1 Pro187Ser* substitution is reported to cause a diminished or a complete loss of enzyme activity (53). Although the biological significance is not completely understood, studies using NQO1-null mice

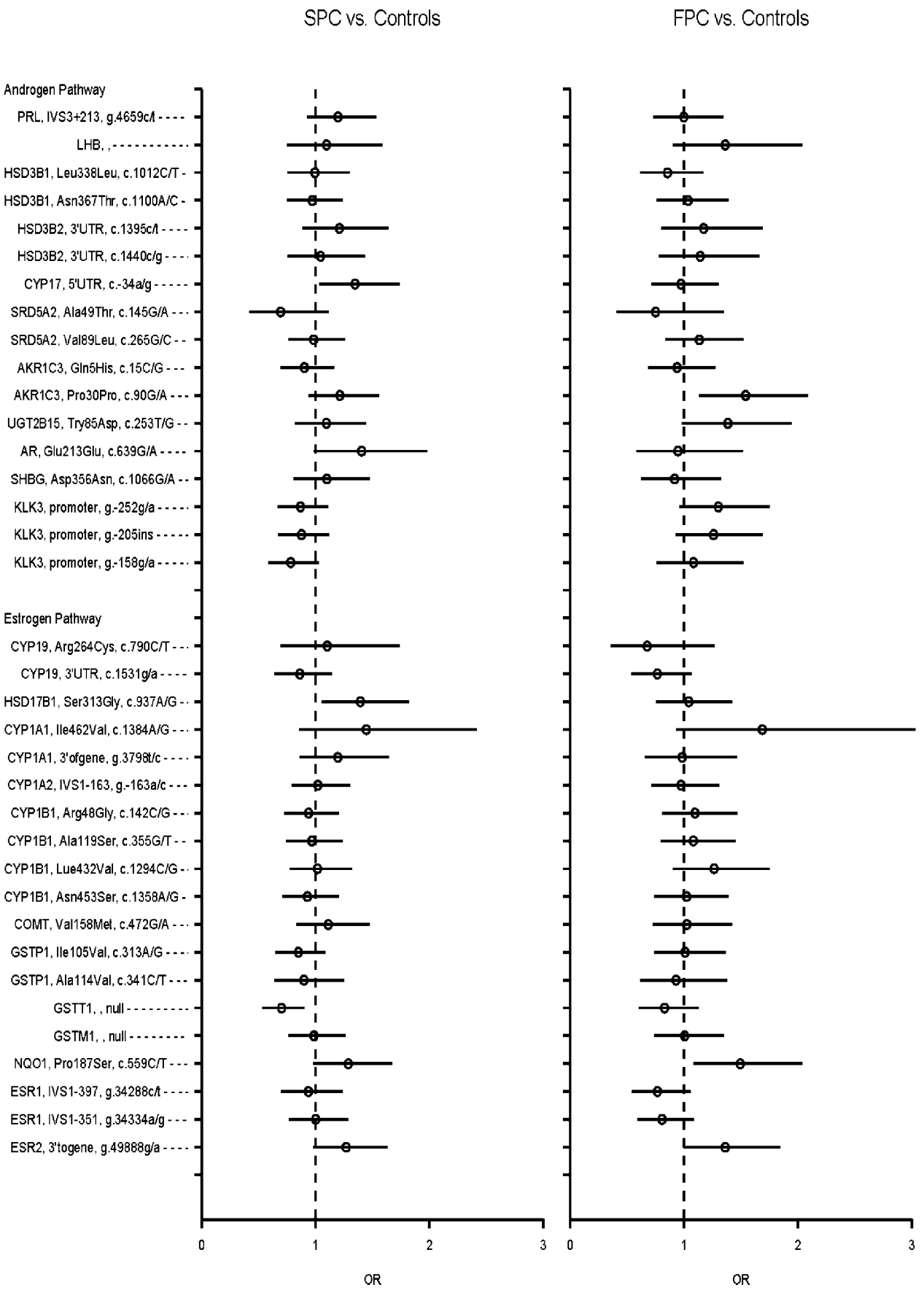

Figure 2. Results of the association study for the SPC cases versus controls and FPC cases versus controls. The left column lists each of the genes and the SNPs studied, and the remaining columns present ORs and their 95% CIs for the comparison of carriers of the minor allele versus subjects homozygous for the major allele.

Table 2. Association of androgen pathway SNPs with SPC, FPC, and controls

Gene	SNP polymorphisms	SNP ID	SPC vs controls			FPC vs controls		
			*P**	Adjusted *P* †	FDR *P*	*P**	Adjusted *P* †	FDR *P*
PRL	IVS3+213, g.4659C/T	rs7739889	0.40	1.00	0.79	0.52	1.00	0.86
LHB	Trp28Arg, c.82T/C	rs1800447	0.68	1.00	0.96	0.71	1.00	0.96
HSD3B1	Leu338Leu, c.1012C/T	rs6203	0.75	1.00	0.98	0.45	1.00	0.83
	Asn367Thr, c.1100A/C	rs1047303	0.99	1.00	0.99	0.83	1.00	0.99
HSD3B2	3′UTR, c.1395C/T	rs1819698	0.11	0.99	0.53	0.31	1.00	0.70
	3′UTR, c.1440C/G	rs1361530	0.59	1.00	0.86	0.33	1.00	0.70
CYP17	5′UTR, c.-34A/G	rs743572	0.13	0.99	0.54	0.11	0.98	0.53
SRD5A2	Ala49Thr, c.145G/A	rs9282858	0.10	0.98	0.53	0.52	1.00	0.86
	Val89Lue, c.265G/C	rs523349	0.86	1.00	0.99	0.50	1.00	0.86
AKR1C3	Gln5His, c.15C/G	rs12529	0.18	1.00	0.60	0.28	1.00	0.70
	Pro30Pro, c.90G/A	rs7741	0.14	1.00	0.54	0.01	0.41	0.30
UGT2B15	Try85Asp, c.253T/G	rs1902023	0.19	1.00	0.60	0.32	1.00	0.70
AR	Glu213Glu, c.639G/A	rs6152	0.05	0.86	0.53	0.81	1.00	0.99
SHBG	Asp356Asn, c.1066G/A	rs6259	0.57	1.00	0.86	0.90	1.00	0.99
KLK3	promoter, g.-252G/A	rs4802754	0.32	1.00	0.70	0.27	1.00	0.70
	promoter, g.-205ins	rs11575894	0.40	1.00	0.79	0.28	1.00	0.70
	promoter, g.-158G/A	rs266882	0.05	0.85	0.53	0.71	1.00	0.96

*P values unadjusted.
† Adjusted P values for multiple comparisons (see Materials and Methods).

are under way to understand the role of NQO1 deficiency in carcinogenesis (54).

A potential limitation of our single-gene analyses is that they can have weak power after correction for multiple testing and when individual gene effects are small. When several genes have small to moderate effects, all in the same direction, a combined test can have greater power. We recently developed a new nonparametric test that combines measures of association across multiple genetic markers, and applied this method to these current data for SPC cases and controls (41). In that analysis, we found a statistically significant association with the 17 SNPs of our estrogen pathway genes ($P = 0.028$) and determined that this global test of association was driven primarily by *HSD17B1* and *NQ01*. Since this prior report, we added the two polymorphisms *GSTT1* and *GSTM1*. With the total of 19 polymorphisms, the *P* value for the global test increased to 0.06, primarily because the minor allele frequencies for these two new polymorphisms are in the opposite direction, being less frequent among SPC cases than controls. Nonetheless, this global test points to polymorphisms in *HSD17B1* and *NQO1* as increasing this risk for SPC and offers greater power than single SNP analyses that are corrected for multiple testing.

As indicated earlier, our results were not significant after correcting for multiple comparisons. Of interest, however, all of the observed ORs were <2 (Fig. 2) with confidence limits for the majority being relatively narrow. We designed our study to have sufficient power to detect relative risks of ~2-fold for alleles that were not too rare (e.g., minor allele frequency of 0.05-0.20). Based on the distribution of the number of affected sibs and cousins in our families, and an actual total sample size of 449 affected cases from families, we estimated that the effective sample size (i.e., accounting for familial relationships) for our FPC cases would be ~269. Power calculations were based on an effective sample size of 269 affected FPC cases and sample sizes of ~500 SPC cases and 500 population controls. With a conservative type I error rate per test of 0.00025 (correcting for multiple testing) and a power of 80%, the minimum detectable OR for carriers versus noncarriers of a

Table 3. Association of estrogen pathway SNPs with SPC, FPC, and controls

Gene	SNP polymorphisms	SNP ID	SPC vs controls			FPC vs controls		
			*P**	Adjusted *P* †	FDR *P*	*P**	Adjusted *P* †	FDR *P*
CYP19	Arg264Cys, c.790C/T	rs700519	0.78	1.00	0.99	0.24	1.00	0.82
	3′UTR, c.1531G/A	rs10046	0.43	1.00	0.92	0.08	0.96	0.81
HSD17B1	Ser313Gly, c.937A/G	rs605059	0.02	0.50	0.44	0.68	1.00	0.99
CYP1A1	Ile462Val, c.1384A/G	rs1048943	0.13	1.00	0.82	0.49	1.00	0.93
	3′ofgene, g.3798T/C	rs4646903	0.28	1.00	0.82	0.58	1.00	0.99
CYP1A2	IVS1-163, g.-163A/C	rs762551	0.98	1.00	0.99	0.95	1.00	0.99
CYP1B1	Arg48Gly, c.142C/G	rs10012	0.81	1.00	0.99	0.89	1.00	0.99
	Ala119Ser, c.355G/T	rs1056827	0.99	1.00	0.99	0.99	1.00	0.99
	Leu432Val, c.1294C/G	rs1056836	0.42	1.00	0.92	0.30	1.00	0.82
	Asn453Ser, c.1358A/G	rs1800440	0.76	1.00	0.99	0.66	1.00	0.99
COMT	Val158Met, c.472G/A	rs4680	0.22	1.00	0.82	0.48	1.00	0.93
GSTP1	Ile105Val, c.313A/G	rs947894	0.35	1.00	0.84	0.79	1.00	0.99
	Ala114Val, c.341C/T	rs179811	0.47	1.00	0.93	0.27	1.00	0.82
GSTT1	Null		0.006	0.23	0.32	0.32	1.00	0.82
GSTM1	Null		0.94	1.00	0.99	0.94	1.00	0.99
NQO1	Pro187Ser, c.559C/T	rs1800566	0.04	0.86	0.62	0.04	0.86	0.62
ESR1	IVS1-397, g.34288C/T	rs2234693	0.64	1.00	0.99	0.13	1.00	0.82
	IVS1-351, g.3433A/G	rs9340799	0.90	1.00	0.99	0.09	0.97	0.81
ESR2	3′togene, g.49888G/A	rs4986938	0.38	1.00	0.87	0.31	1.00	0.82

*P values unadjusted.
†Adjusted P values for multiple comparisons (see Materials and Methods).

Table 4. Association of STR markers with SPC, FPC, and controls for androgen and estrogen pathways

Pathway/gene	STR polymorphism	SPC vs controls		FPC vs controls	
		Global statistic *P*	Max statistic *P*	Global statistic *P*	Max statistic *P*
Androgen					
AR	$(CAG)_n$ exon 1	0.25	0.22	0.63	1.00
AR	$(GGC)_n$ exon 1	0.08	0.19	0.64	1.00
HSD3B2	$(TG)_n(TA)_n(CA)_n$5′UTR	0.22	0.26	0.67	0.41
SHBG	$(CA)_n$ 5′UTR	0.88	0.89	0.70	1.00
CYP11A1	$(TTTA)_n$ 5′UTR	0.06	0.09	0.22	0.50
SRD5A2	$(TA)_n$ 3′UTR	0.39	0.27	0.76	1.00
Estrogen					
ESR1	$(TA)_n$ promoter	0.53	0.42	0.23	0.51
ESR2	$(CA)_n$ intron5	0.93	0.62	0.81	1.00
HSD17B2	$(CA)_n$ intron1	0.24	0.17	0.24	0.74
CYP19	$(TTTA)_n$ intron5	0.80	0.88	0.83	1.00

minor allele was 2.49 for an allele frequency of 0.05 and 1.93 for an allele frequency of 0.20. Thus, our results suggest that if an association does exist, then the overall contribution of each individual SNP studied to prostate cancer risk is likely to be very low.

For the remaining genes examined that did not show any evidence for association, some have been reported by others to exhibit some degree of association in prostate cancer. A review article by Platz and Giovannucci (25) discusses the epidemiologic literature for some of these and concludes that the findings from genetic association studies are difficult to reconcile. These conflicting findings in the literature may, in part, be due to differences in the choice of cases and controls and population subtypes. However, one of the primary problems with most studies is inadequate sample size for polymorphisms that have a small effect (OR<2). To address these difficulties, the National Cancer Institute Breast and Prostate Cancer Cohort Consortium is in the process of conducting a candidate gene study similar to ours, but on a much larger scale. The goal of their study is to investigate variants in nearly 50 genes involved in two pathways, one of which is the steroid hormone metabolism pathway, using 8,000 prostate cancer cases from multiple cohorts (55).

We also carried out associations of genetic variation with a number of clinical variables (Gleason score, tumor-node-metastasis stage, and nodal status) to evaluate potential associations with the aggressive form of prostate cancer. Again, results were not significant after correcting for multiple comparisons. Several SNPs showed suggestive associations with measures of tumor aggressiveness (Tables 5 and 6), but no SNP with more than one of the clinical measures used. We did not find any variant associated with both FPC and SPC, suggesting that our findings are false positives or that there might be differences between the FPC and SPC groups. However, this could also result from low power because of our sample size.

Although nonsynonymous coding SNPs were the priority for the SNP selection, a limitation of the current study is the limited number of SNPs examined for each of the candidate genes. As a result, these findings do not exclude the possibility of an association with other polymorphic variants not evaluated. Likewise, the literature is also difficult to interpret because of the limited number of SNPs investigated per gene, rather than considering their relation to other polymorphisms in the gene or genomic region (i.e., haplotypes). With the completion of the International HapMap Project (56), haplotype blocks that are common in the human genome can be used for more detailed mapping studies. In an effort to achieve greater gene coverage, we are currently determining linkage disequilibrium tag SNPs for all of the genes examined in this study. The selection of tag SNPs can then be used to conduct a more comprehensive association study.

In summary, we have examined genes involved in steroid hormone biosynthesis and metabolism. Of the 46 polymorphisms in the 25 genes evaluated, our results suggest that SNPs in four genes (*AKR1C3, NQO1, GSTT1*, and *HSD17B1*) might be weakly associated with either FPC or SPC, whereas SNPs in eight genes (*HSD3B2, SRD5A2, SHBG, ESR1, CYP1A1, CYP1B1, GSTT1*, and *NQO1*) might be weakly associated with tumor aggressiveness. However, because the point estimate for the OR for each of these SNPs is ~1.5, the overall contribution of each individual SNP to prostate cancer risk, if present, is likely to be very low. Thus, given this low risk estimate, much larger sample sizes will be required for confirmation.

Table 5. Association of SNPs with clinical characteristics

Pathway/gene	SNP	SNP ID	Case group	Clinical feature*	*P*	FDR *P*	OR (95% CI)
							V+
Androgen							
HSD3B1	Leu338Leu, c.1012C/T	rs6203	SPC	Node	0.03	0.81	1.95 (1.0-3.9)
	Asn367Thr, c.1100A/C	rs1047203	SPC	Stage	0.008	0.81	1.66 (1.1-2.5)
SRD5A2	Ala49Thr, c.145G/A	rs9282858	FPC	Grade	0.01	0.81	2.96 (1.6-5.6)
SHBG	Asp356Asn, c.1066G/A	rs6259	FPC	Grade	0.03	0.82	1.81 (1.1-2.9)
Estrogen							
GSTT1	Null		FPC	Stage	0.02	0.81	2.19 (1.4-3.5)
NQO1	Pro187Ser, c.559C/T	rs1800566	FPC	Stage	0.03	0.82	0.67 (0.4-1.0)
CYP1A1	Ile462Val, c.1384A/G	rs1048943	SPC	Grade	0.04	0.82	2.35 (1.1-4.9)
CYP1B1	Arg48Gly, c.142C/G	rs10012	SPC	Node	0.02	0.81	0.47 (0.3-0.9)
	Ala119Ser, c.355G/T	rs1056827	SPC	Node	0.01	0.81	0.45 (0.2-0.8)
	Asn453Ser, c.1358A/G	rs1800446	SPC	Node	0.03	0.81	1.64 (0.9-2.9)
ESR1	IVS1-397, g.34288C/T	rs2234693	SPC	Grade	0.04	0.82	0.62 (0.4-1.0)

*Association of each polymorphism was evaluated with the following clinical features of disease (baseline category is listed first): stage (I/II versus III/IV), grade (Gleason grade: <7 versus ≥7), and node + (nodal involvement: − versus +).

Table 6. Association of STR markers with clinical characteristics

Pathway/gene	STR polymorphism	Case group	Clinical feature*	Global statistic		Max statistic	
				P	FDR *P*	*P*	FDR *P*
Androgen							
CYP11A1	$(TTTA)_n$ 5′UTR	SPC	Grade	0.05	0.86	0.04	0.56
Estrogen							
ESR2	$(CA)_n$ intron5	SPC	Grade	0.05	0.86	0.002	0.16
HSD17B2	$(CA)_n$ intron1	SPC	Node	0.06	0.86	0.02	0.56
CYP19	$(TTTA)_n$ intron5	FPC	Node	0.17	0.86	0.04	0.56

*Association of each polymorphism was evaluated with the following clinical features of disease (baseline category is listed first): stage (I/II versus III/IV), grade (Gleason grade: <7 versus ≥7), and node + (nodal involvement: − versus +).

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