Published in final edited form as:
Cancer Epidemiol. 2012 August ; 36(4): 347–353. doi:10.1016/j.canep.2012.04.002.

Androgen metabolism and JAK/STAT pathway genes and prostate cancer risk

Erika M. Kwon[a,b], **Sarah K. Holt**[c], **Rong Fu**[c], **Suzanne Kolb**[c], **Gabrielle Williams**[a], **Janet L. Stanford**[c,d], and **Elaine A. Ostrander**[a,*]

Erika M. Kwon: kwone@mail.nih.gov; Sarah K. Holt: skholt@fhcrc.org; Rong Fu: rfu@fhcrc.org; Suzanne Kolb: skolb@fhcrc.org; Gabrielle Williams: gabego@hotmail.com; Janet L. Stanford: jstanfor@fhcrc.org; Elaine A. Ostrander: eostrand@mail.nih.gov

[a]National Human Genome Research Institute, Cancer Genetics Branch, National Institutes of Health, Building 50, 50 South Drive, Bethesda, MD 20892, USA

[b]Program in Human Genetics and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

[c]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1124 Fairview Ave N., Seattle, WA 98103, USA

[d]Department of Epidemiology, School of Public Health, University of Washington, Seattle, WA 98195, USA

Abstract

Background—Prostate cancer (PC) is the most frequently diagnosed solid tumor in U.S. men. Genome-wide association studies (GWAS) have identified over 40 risk-associated single nucleotide polymorphisms (SNPs), including variants in androgen pathway genes (e.g., *KLK3* and *AR*). Androgens are important in PC and genes involved in this pathway are therefore candidates for conferring susceptibility to PC.

Methods—In this hypothesis-testing study, we evaluated PC risk in association with SNPs in 22 candidate genes involved in androgen metabolism or interactions with the androgen receptor *(AR)*. A total of 187 SNPs were genotyped in 1,458 cases and 1,351 age-matched controls from a population-based study. PC risk was estimated using adjusted unconditional logistic regression and multinomial regression models.

Results—Single SNP analyses showed evidence (p<0.05) for associations with 14 SNPs in 9 genes: *NKX3.1, HSD17B3, AKR1C3, SULT2A1, CYP17A1, KLK3, JAK2, NCOA4* and *STAT3*. The most significant result was observed for rs2253502 in *HSD17B3* (odds ratio, OR=0.57, 95% CI: 0.39–0.84). In addition, five SNPs in four genes (*CYP17A1, HSD17B4, NCOA4,* and *SULT2A1*) were associated with more aggressive disease (p<0.01).

Conclusions—Our results replicate previously reported associations for SNPs in *CYP17A1, HSD17B3, ARK1C3, NKX3.1, NCOA4* and *KLK3*. In addition, novel associations were observed

[*]Corresponding Author: Elaine A. Ostrander, Ph.D., National Human Genome Research Institute, NIH, 50 South Dr., Building 50, Room 5351, Bethesda, MD 20892, Phone: 301-594-5284, FAX: 301-480-0472, eostrand@mail.nih.gov.

Conflict of interest statement
The authors declare no conflict of interest.

for SNPs in *JAK2, HSD17B4,* and *SULT2A1.* These results will require replication in larger studies.

Keywords

Androgen pathway; JAK2; HSD17B3; prostate cancer; polymorphisms; genetic susceptibility

Introduction

Prostate cancer (PC) is the most commonly diagnosed solid tumor in men with more than 240,000 new diagnoses and more than 33,000 deaths predicted in 2011 [1]. The primary PC risk factors are African ancestry, older age, and a positive family history. However, despite the strong evidence that a hereditary component plays an important role in PC susceptibility from twin studies, linkage, and segregation analyses, specific genetic mutations responsible for inherited risk still remain largely uncharacterized. In recent years, however, major progress in the identification of genetic variants associated with PC has been made possible through genome-wide association studies (GWAS). GWAS studies of PC have led to discovery of over 40 germline genetic variants that are associated with PC susceptibility, explaining approximately 14% of the total genetic variation of PC [2] however, despite these newly identified SNPs large proportion of disease heritability remains unexplained.

Among the numerous biological pathways relevant to PC susceptibility and progression, there is compelling evidence that steroid hormones play a critical role due to affects on prostate cell proliferation and differentiation. The role of testosterone (T) in PC development was proposed 60 years ago by Huggins and Hodges, [3] however the historical view on the relationship between T and PC has been challenged in recent years [4]. Ross *et al.* demonstrated that the difference in PC incidence among different ethnic groups was correlated with *in vivo* activity of 5α-reductase and proposed a polygenic model that focused on a series of genes involved in androgen metabolism [5]. Several genes that play important roles in androgen metabolism, *SRD5A2, CYP17, AR,* and *HSD3B1,* have been reported previously to have SNPs associated with PC susceptibility, although few such variants have been consistently replicated. In a recent study by Lu *et al.*, a publicly available ChIP-on-chip dataset was used to identify 22,447 regions containing AR-binding sites in the genome [6] and test associations between genetic variants within AR binding sites and PC risk [7]. Interestingly, AR-binding sites can mark novel AR target genes, and the study found statistical evidence ($p<1.0\times10^{-5}$) for genetic variants identified to have strong associations with PC risk from GWAS (8p21 and 8q24) that were located within AR-binding sites. AR signaling is regulated by its co-regulatory factors and has a central role in androgen-dependent (AD) to androgen-independent (AI) progression of the disease. Thus, genes in the androgen metabolism or AR signaling pathway are important candidates for conferring genetic susceptibility to PC.

In this hypothesis-testing study, we evaluated associations of common genetic variants in 15 key genes involved in androgen metabolism (*AKR1C1, AKR1C2, AKR1C3, AR, CYP11A1, CYP17A1, HSD17B3, HSD17B4, HSD17B6, HSD3B1, HSD3B2, SRD5A1, SRD5A2, SULT2A1* and *UGT2B11*) as well as seven genes that are either AR target genes (*KLK3, NKX3.1, let-7,* and *miR-222*) or co-regulators of AR activation (*JAK2, NCOA4*, and *STAT3*) with PC risk in a population-based case-control study of Caucasians and African Americans. We also tested the associations between SNPs and clinical characteristics of PC.

Materials and methods

Study Population

The study population consists of Caucasian and African American male residents of King County, WA, who were enrolled in one of two population-based case-control studies (study I and study II). The collection methodologies have been described in detail elsewhere [8, 9]. In brief, study I cases were diagnosed between January 1, 1993 and December 31, 1996 and study II cases were diagnosed between January 1, 2002 to December 31, 2005. For both studies, cases had histologically confirmed PC and were identified from the Seattle-Puget Sound Surveillance, Epidemiology, and End Results (SEER) cancer registry. Overall, 2,244 eligible patients were identified and 1,754 (78.2%) were interviewed. Of those interviewed, blood samples yielding sufficient DNA for genotyping were drawn from 1,458 (83.1%) cases. A comparison group of controls with no self-reported physician's diagnosis of PC was identified by random digit dialing. Controls were frequency matched to cases by 5-year age groups and were recruited evenly throughout each ascertainment period for cases. Overall, 1,645 (67.2%) of 2,448 identified men who met the eligibility criteria were interviewed and blood samples were drawn and DNA was prepared using standard protocols from 1,351 (81.7%) interviewed controls.

Study participants completed detailed in-person interviews conducted by trained male interviewers using standardized questionnaires. Information was collected on family structure, medical and cancer history, and social and demographic factors. Clinical information on cases (Gleason score, tumor stage, and serum prostate-specific antigen (PSA) level at diagnosis) was obtained from the cancer registry. Plasma PSA levels in controls were measured using stored samples, retrospectively. All study procedures were approved by the Institutional Review Board (IRB) of the Fred Hutchinson Cancer Research Center and the National Human Genome Research Institute and written informed consent was obtained from all study participants.

SNP selection and genotyping

A total of 187 SNPs in 22 genes were genotyped and the majority (n=150) were selected as tagSNPs to capture common haplotype blocks and to maximize coverage for each gene. TagSNP selection parameters were set to minor allele frequency ≥ 5% and a pairwise r^2 threshold of 0.8 using the phased HapMap CEU population data (version 22; http://www.hapmap.org). Tagger (www.broadinstitute.org/mpg/tagger) was used to select tagSNPs and Haploview[10] was used to visualize linkage disequilibrium (LD) blocks of targeted genomic regions (10kb upstream and 5kb downstream of genes of interest). The majority of the candidate genes (n=15) are involved in androgen metabolism, and seven genes were selected based on their potential interaction with the *AR*. Thirty-seven SNPs were selected based on functional significance and previously published associations. Genotyping was performed using the Applied Biosystems (ABI, http://www.appliedbiosystems.com) SNPlex Genotyping System and allele assignment was carried out with the use of ABI 3730xl DNA analyzer for detection and proprietary GeneMapper software to assign specific SNP alleles. Three SNPs (rs6152, rs10761581, and rs17464626) failed genotyping using the SNPlex system and were genotyped by 5'-endonuclease assay (Taqman) using the ABIPrism 7900HT sequence detection system, according to the manufacturer's instructions. In addition, the *NKX3.1* gene region was resequenced using seven primer pairs (Table S1) in 94 samples selected at random from our case-control study population to identify 11 additional SNPs that were subsequently genotyped in all samples using the SNPlex Genotyping system. In summary, 15 SNPs failed due to a low call rate (<90%) and 11 SNPs had low minor allele frequencies (MAF <0.01) in Caucasians. In the African American subset, 16 SNPs failed due to a low call rate and 12

SNPs had a low MAF of <1%. The genotyping rates were 96.1% and 95.5% for Caucasians and African Americans, respectively. The remaining SNPs had ~99% agreement between 144 blind duplicates distributed across all genotyping batches.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) [11] test for each SNP was assessed in controls separately by race using a χ^2 test. SNPs with significant departure from HWE ($p<0.05$) were excluded from further analysis (n=14 for Caucasians and n=7 for African Americans). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression [12]. Potential confounding factors (family history, PC screening history and BMI) were evaluated to test whether these covariates changed the risk estimates by ≥ 10%. All association analyses were adjusted for age and stratified by race. Additive, dominant and recessive genetic models were evaluated for all SNPs except for SNPs on the X chromosome (n=6) and SNPs for which there were no variant allele homozygotes. Polytomous regression models were used for assessing the association between SNP genotypes and cases stratified by Gleason score [2–7(3+4) vs. 7(4+3)-10], diagnostic PSA level [<20 ng/mL vs. ≥20 ng/mL], or tumor stage [local vs. regional or distant]. Statistically significant associations between individual SNPs and PC risk were evaluated using likelihood ratio-based test statistics. Multiple comparisons were adjusted for by using permutation approach where permutated data were generated by permuting SNPs within each gene while keeping other covariates and case-control status unchanged. Then recessive, dominant and additive genetic models were fit on the permutated data to obtain the ordered minimal p-values. Repeating this process 1000 times yields a null distribution of the order statistics. The permutation p-value for each SNP is the probability of observing a p-value that is less than or equal to the nominal p-value for the corresponding order statistic. SNPs were considered to be significantly associated with PC risk if both the nominal p-value and permuted p-value were less than 0.05. Nominally significant SNPs were evaluated for interaction with all other nominally significant SNPs by testing pairwise combinations. Haplotype blocks were defined by the Gabriel et al. analysis method, [13] and frequencies and measures of association for each gene were estimated using Hplus v3.1 (http://qge.fhcrc.org/hplus) [14]. Moreover, we tested the significance of androgen pathway SNPs by comparing the proportion of statistically significant genes in the pathway with a nominal 5% significance level using the method described by Gauvreau [15]. All other statistical analyses were performed using STATA v10.1 or PLINK v1.07 [16].

Results

Selected characteristics of the study population are shown in Table 1. The mean age at diagnosis for cases was 59.9 years, and cases had a higher percentage of men with a first-degree family history of PC compared to controls. Study participants were predominantly Caucasian (91.7%), but a higher proportion of cases than controls were African American. Between cases and controls, cases had more frequent PSA test within 5-year period prior to reference date and there were no significant differences with respect to BMI. The majority of cases had localized stage tumors, PSA values less than 10.0 ng/mL, and Gleason scores less than or equal to 7 (3+4).

SNP association tests

Genotype data were available for 147 SNPs (Caucasians) and 152 SNPs (African Americans). Association results of nominally significant SNPs in candidate genes are shown in Table 2 for Caucasians and in Table S2 for African Americans.

Of the 147 SNPs analyzed in Caucasians, associations between 14 SNPs in nine genes and PC were found to be nominally significant at the 5% level. These included variants in four genes from the androgen metabolism pathway (*AKR1C3, CYP17A1, HSD17B3* and *SULT2A1)*, two (*KLK3* and *NKX3*.1) that are regulated by the AR and three (*NCOA4, JAK2* and *STAT3*) that are predicted to interact with androgen pathway genes for regulation of steroid hormone levels. Notably, three independent tagSNPs located in the *HSD17B3* gene region were associated with PC risk, with the strongest association observed for rs2253502 for TT homozygote carriers (OR=0.57; 95% CI 0.38–0.84). Permutation testing revealed only one likely significant association for SNP rs10429491 (OR=0.79, 95% CI 0.67–0.92, $p_{permuted}=0.02$), which is in the *Janus kinase 2* gene, after adjusting for multiple testing. In addition, rs1058205 in the *KLK3* gene demonstrated a borderline significant OR of 0.84 (95% CI 0.71–0.99, $p_{permuted}=0.055$). Haplotype analysis of selected tagSNPs did not improve associations compared to the single SNP analyses.

We next evaluated the association of selected SNPs with clinical variables. Due to the small number of African Americans in our dataset, we restricted these analyses to Caucasians. Stratification on Gleason score, diagnostic PSA value, or stage showed six SNPs that were associated with disease features at p <0.01 (Table 3) using an additive model. For two of those six SNPs, (rs10786712 in *CYP17A1* and rs10429491 in *JAK2*), results were also observed in the overall risk analyses. Interestingly, the *JAK2* SNP showed the strongest association. Specifically, carriers of the T allele showed a 23 % fold risk reduction in cases with lower Gleason scores (OR=0.77, 95% CI 0.64–0.91). The minor alleles of two SNPs in *HSD17B4* (rs10478424 and rs6897978) and a SNP in *SULT2A1* (rs2547238) showed associations with increased risk of PC in cases with advanced tumor stage. After accounting for multiple comparisons, none of abovementioned SNPs remained statistically significant.

In African Americans, ten SNPs were analyzed adjusted for family history since risk estimates were altered (≥10%) compared to risk estimates adjusted for age only. Nominally significant associations with overall PC risk were observed for 15 SNPs in six genes (Table S1). Associations of *HSD17B4* SNPs with PC risk were unique to African American men and six out of 15 SNPs that showed an association were located in the *SULT2A1* gene region. In *KLK3,* an intronic variant, rs174776, had the strongest association in African Americans where carriers of the TT and TC genotype had a 3.4-fold greater risk (95% CI 1.47–7.71) compared to men with CC genotype. Three SNPs, rs182420, rs174776 and rs1058205, were associated in both African Americans and Caucasians; however, the direction of the relative risk estimate for each SNP differed by race. None of the other nominally significant SNPs observed in African Americans were seen in the Caucasian population. The results presented for African Americans must be interpreted with caution due to the limited sample size.

Pathway analyses using proportion test

The analysis of single SNPs with overall PC risk in Caucasian men demonstrated that four genes in the androgen pathway contain SNPs associated with PC risk (nominal p-values < 0.05), when only 0.75 genes (15 X 0.05) are expected to be significant at the same threshold level for 16 genes analyzed. We found an excess of statistically significant associations over the proportion expected by chance in the androgen metabolizing pathway for PC risk (observed:expected = 3.8, p = 0.001).

Discussion

The role of androgen and its receptor has been shown to be critical in PC development based on data from animal studies as well as clinical trials, which further support its importance in the etiology of PC [17, 18]. Recent results from multiple GWAS of PC based on Caucasian

populations have shown that individual SNPs confer low to moderate risk for PC. An alternative approach to GWAS studies is a comprehensive evaluation of multiple genes with polymorphisms that interact in the same pathway, which has been proposed as a better way to study multifactorial diseases such as PC. For the *AR* pathway, this should include not just key genes involved in androgen metabolism and biosynthesis, but also its co-regulators [19].

In this study, we tested the hypothesis that genetic variants in androgen biosynthesis pathway genes and co-regulators of the *AR* are associated with PC risk by evaluating polymorphisms within 22 selected candidate genes. Our results confirmed previously reported associations and identified novel associations between PC risk and androgen-related genes. We observed a strong association with PC risk for a SNP in *JAK2,* rs10429491, which remained significant after correcting for multiple comparisons. We also found suggestive evidence of associations for genetic variants in *NKX3.1, KLK3, NCOA4, STAT3, HSD17B3, AKR1C3, CYP17A1* and *SULTA1* in Caucasian men. Additionally, we evaluated SNPs stratified by disease aggressiveness and showed that five SNPs were associated with comparatively more aggressive clinical phenotypes and one SNP which was associated with less aggressive disease. We also utilized genotype data from the National Cancer Institute's Cancer Genetic Markers of Susceptibility (CGEMS) project for indirect replication of 14 nominally associated SNPs with PC susceptibility [20]. Two SNPs were directly genotyped and one SNP was in LD (r^2=0.96 in the Caucasian HapMap population) with a genotyped SNP in the CGEMS dataset (http://cgems.cancer.gov/data). CGEMS data replicated our associations for SNPs in *AKR1C3, NCOA4* and *NKX3.1* and PC risk.

Surprisingly, we found a synonymous SNP, rs10429491 (H162H), located in exon 4 of the *JAK2* gene that was associated with a significant reduction in overall PC risk and the risk of less aggressive PC. To our knowledge, this is the first report of an association for a genetic variant in the *JAK2* gene with PC susceptibility. A single gain-of-function nonsynonymous mutation (rs77375493, V617F) in *JAK2* has been described in myeloproliferative neoplasia that leads to constitutive activation of *JAK2* and subsequent activation of *STAT3* [21]. However, the V617F activating mutation was found to be absent in normal and malignant prostate samples analyzed by Gu *et al.* [22]. *JAK2* encodes a cytoplasmic protein tyrosine kinase that is essential to cytokine signaling cascade in interaction with STAT3. Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway is well known to play an important role in carcinogenesis of several cell types [23]. Recently, studies demonstrating involvement of the JAK/STAT pathway in PC development have accumulated. Numerous *in vitro* functional experiments using PC cell lines have shown that STAT3 was constitutively active in PC cell lines and promotes metastatic progression of PC [24–26]. Progression of PC is often associated with transactivation of the *AR*, and STAT3 has been suggested as a mechanism to enhance the transactivation of *AR* to promote transition from androgen-dependent to androgen-independent prostate tumors [25]. We have previously reported associations of SNPs (rs12949918 and rs744166, r^2=0.98) in *STAT3* with PC risk in an analysis focused on its role in the IL6 signaling pathway, [27] and we included *STAT3* in the current analyses to evaluate its potential gene interaction with *AR* and *JAK2*.

Among genes involved in steroid hormone metabolism, germline genetic variants in *AR, SRD5A2, CYP17A1* and *CYP3A* are the most studied genes in relation to PC risk [28]. Several studies have reported conflicting results regarding the putative association of rs743572 in the 5'UTR of *CYP17A1* [29, 30]. The tagSNP rs10786712 shows evidence of a reduced risk for the variant allele with PC risk as well as advanced stage in our analyses, and is in perfect linkage disequilibrium (LD) with SNP rs743572 (r^2=1.0). The observed association most likely reflects a true causal effect for rs743572, further supporting prior evidence of an association between the *CYP17A1* variant and PC susceptibility [31].

CYP17A1 encodes a cytochrome P450 17alpha-hydroxylase, a rate-limiting enzyme that catalyzes steroid 17–20 lyase activities at key steps in the testosterone biosynthesis pathway [5] and rs743572 is predicted to increase transcription activity of CYP17A1 subsequently increasing steroid hormone production.

The *HSD17B3* gene encodes for hydroxysteroid (17-beta) dehydrogenase 3 that catalyzes the conversion of androstenedione to testosterone in the testis. There have been three published studies that examined the role of SNPs in *HSD17B3* with PC risk [29, 32, 33]. The most studied missense substitution, G289S (rs2066479), showed no association in the Cancer Prostate in Sweden (CAPS) population-based, case-control study. In contrast, Beuten *et al.* and Margiotti *et al.* reported a statistically significant increase in the risk of PC with rs2066479. None of three SNPs (rs2066485, rs2253502, and rs2257157) that showed nominal association in our data are correlated with rs2066479, thus our data demonstrates an independent association at this locus. CGEMS data show an association for a variant in *HSD17B3*; however, the CGEMS marker (rs407179) is only weakly correlated with rs2257157 (r^2=0.31). Another hydroxysteroid (17-beta) dehydrogenase, HSD17B4, catalyzes the conversion of testosterone to androstenedione. We identified two SNPs (rs10478424 and rs6897978) for which carriers of variant alleles are at a 1.5-fold increased risk of more advanced PC stage in Caucasians. To date, there are no reports of association of genetic variants in *HSD17B4* with PC risk or clinicopathologic features.

AKR1C3 and SULT2A1 have been recently identified to play critical roles in prostate carcinogenesis. AKR1C3 is a member of the aldo-keto reductase superfamily that converts DHT to 3á-diol and regulates AR occupancy and transactivation of the receptor. To date, only one suggestive association with familial PC has been reported with SNPs in the *AKR1C3* gene [34], and our finding is the first to be identified in sporadic cases. The CGEMS project directly genotyped rs4881400 and replicated our association with a similar magnitude of risk for overall PC (OR=0.78, 95% CI 0.64–0.95, p=0.016).

Sulfotransferase (SULT) 2A1 catalyzes dehydroepiandrosterone (DHEA) sulfation in adrenal cortex and a study by Wilborn *et al.* reported an association between genetic variants in the *SULT2A1* gene and DHEA:DHEA-S ratio in African Americans [35]. We identified nominally significant associations for six SNPs in the *SULT2A1* gene among African Americans and the strongest association was observed for rs2547238 (OR=3.17, 95% CI 1.23–8.14) located in the second intron. In Caucasians, the most significant association was observed for rs182420, which is located 1675 bases downstream of *SULT2A1,* for which carriers of the ‘GG’ genotype are at a 1.4-fold increase in PC risk. Additionally, rs2547238, located in the third intron of *SULT2A1,* and 10,285bp downstream of rs182420, was associated with increased risk for more advanced stage. To our knowledge, this is the first study to report on the positive associations of SNPs in *SULT2A1* with PC risk in African Americans and Caucasians.

Androgen-related genes include co-regulators of AR as well as genes downstream of the androgen metabolism pathway containing AR binding sites (e.g. *KLK3*). A recent GWAS identified new loci containing putative susceptibility genes at 19q13.33 (*KLK3*), 8p21 (*NKX3.1*) and 10q11 (*NCOA4*) [36]. Prostate-specific antigen (*PSA/KLK3*) was shown to positively associate with PC risk in candidate gene studies [37–39] prior to the GWAS study. The classical kallikrein gene expressed exclusively in prostate tissue is widely used in the diagnosis and monitoring of the disease. Expression of PSA is regulated by recruitment of AR and its co-activators at functional androgen-response elements (AREs) at the proximal promoter as well as an enhancer region upstream of the gene [40, 41]. Alterations (A-252G and G-158A) in the ARE1 region can lead to a change in regulation of PSA expression [37]. The most significant SNP in the GWAS (rs2735839) is located 600 bp

downstream of the *KLK3* gene when cases were compared to selected controls with low PSA levels [36, 42]. Recently, the same group identified a novel PC susceptibility coding SNP in exon 4 (rs17632542, I179T) that causes a nonsynonymous change with the potential to alter RNA splicing and affect protein stability [43]. We identified two SNPs (rs174776 and rs1058205) in the 3'UTR of *KLK3* that confer a ~25% reduced risk of PC in Caucasians. One of them, rs1058205, was also found to be associated with PC in the multi-stage study by Kote-Jarai *et al.*, demonstrating a similar risk estimate (OR=0.87, 95% CI 0.77–0.99) as we report here. Thus, we have replicated an association of rs1058201 with overall PC risk to further support the notion that common genetic variants in *KLK3* contribute to PC development.

The putative prostate tumor suppressor gene *NKX3.1* is an androgen regulated homeobox-containing transcription factor and is subject to loss of heterozygosity in human PC. Androgen stimulation has been shown to increase its expression, and He *et al.* reported that the *NKX3.1* gene plays a role in androgen-driven differentiation in prostate tissue [44]. To determine genetic and functional aspects of the 8p21 region harboring *NKX3.1* with PC risk, Akamatsu *et al.* identified a SNP in the 5'UTR through fine mapping of the initial GWAS peak. The variant rs11781886 was shown to affect transcription activity by altering binding affinity of transcriptional factor Sp1 in prostate cells [45]. We found a moderate association of two SNPs in *NKX3.1* that confers increased relative risk of PC in Caucasians. SNP rs4872175 is located 1,426 downstream and the newly identified novel variant (NKX3.1_5) is located within the 3'UTR of *NKX3.1.* The 3'UTR region has been found to contain functional AREs that are predicted to play a crucial role in the regulation of NKX3.1 expression [46], and future studies should include SNPs identified here.

An androgen receptor co-activator (ARA70/NCOA4) is located on chromosome 10q11.2 and since its initial discovery by GWAS, [36, 47] three studies have evaluated the associations between PC risk and variants at 10q11.2 [48–50]. The most significant GWAS SNP, rs10993994, is associated with reduced expression of *MSMB* and overexpression of *NCOA4* in the prostate, thus promoting anchorage-independent growth of prostate epithelial cells. In addition, there is evidence of an association between PC risk and rs1074005, which is an independent locus on 10q11. In this study we found an association between two SNPs (rs17178655 and rs7350420) and PC risk that are not correlated with the abovementioned SNPs. The CGEMS data confirmed the association of rs7350420 where carriers of the variant 'C' allele had a reduced overall risk of PC (OR=0.82, 95% CI 0.70–0.97), further supporting our findings.

Our study includes several strengths and weaknesses that should be considered when interpreting these results. One of the strength of our study is that the majority of genes examined were interrogated by tagSNPs to comprehensively analyze variation across the entire gene region. Selected candidate genes comprise key enzymes involved in the androgen metabolism pathway. Using data available from the CGEMS project, we were also able to infer significance of previously reported associations if the particular SNP is in LD with our selected tagSNP. Our population-based case-control design and the moderate sample size for evaluating common genetic variants in association with overall risk of PC in Caucasians are also strengths of the study. However, our sample size was insufficient to evaluate gene by gene interactions, which is a challenge in many studies. In addition, the analyses of African Americans were underpowered and results in this subset should be interpreted with caution.

In summary, our results support the hypothesis that androgen metabolism pathway genes and *AR* interacting genes contribute to PC susceptibility. We observed significant associations with several SNPs in steroid hormone pathway genes (*CYP17A1, HSD17B3,*

HSD17B4, AKR1C3 and *SULT2A1*) and co-regulators of *AR* (*NCOA4* and *JAK2/STAT3*). Moreover, we replicated recently identified associations at three independent loci (*KLK3, NKX3.1* and *NCOA4*) that are likely regulated by androgens within prostate tissues. Novel risk associations were observed for SNPs in *JAK2, HSD17B4,* and *SULT2A1*. Furthermore, variants in several genes (*CYP17A1, HSD17B4, JAK2, NCOA4* and *SULT2A1*) were found to be associated with PC risk when analyses stratified by clinicopathologic features. The results reported here suggest that genetic variants in androgen-related genes are independently associated with PC risk and aggressive PC, but larger studies are needed to confirm our findings considering features of aggressive PC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank all participants of this study for their time and effort. This work was supported by the Intramural Program of the National Human Genome Research Institute, by grants RO1-CA056678 and R01-CA092579 from the National Cancer Institute with additional support from the Fred Hutchinson Cancer Research Center.

References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011; 61(4): 212–36. [PubMed: 21685461]
2. Kim ST, Cheng Y, Hsu FC, Jin T, Kader AK, Zheng SL, et al. Prostate cancer risk-associated variants reported from genome-wide association studies: meta-analysis and their contribution to genetic Variation. Prostate. 2010; 70(16):1729–38. [PubMed: 20564319]
3. Huggins C, Hodges DV. Studies on prosatate cancer: effect of castration, of estrogen, and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res. 1941; 1:293–297.
4. Morgentaler A. Guilt by association: a historical perspective on Huggins, testosterone therapy, and prostate cancer. J Sex Med. 2008; 5(8):1834–40. [PubMed: 18547385]
5. Ross RK, Pike MC, Coetzee GA, Reichardt JK, Yu MC, Feigelson H, et al. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. Cancer Res. 1998; 58(20):4497–504. [PubMed: 9788589]
6. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell. 2009; 138(2):245–56. [PubMed: 19632176]
7. Lu Y, Sun J, Kader AK, Kim ST, Kim JW, Liu W, et al. Association of prostate cancer risk with snps in regions containing androgen receptor binding sites captured by ChIP-On-chip analyses. Prostate. 2011
8. Stanford JL, Wicklund KG, McKnight B, Daling JR, Brawer MK. Vasectomy and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev. 1999; 8(10):881–6. [PubMed: 10548316]
9. Agalliu I, Salinas CA, Hansten PD, Ostrander EA, Stanford JL. Statin use and risk of prostate cancer: results from a population-based epidemiologic study. Am J Epidemiol. 2008; 168(3):250–60. [PubMed: 18556686]
10. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–5. [PubMed: 15297300]
11. Hardy GH. Mendelian Proportions in a Mixed Population. Science. 1908; 28(706):49–50. [PubMed: 17779291]
12. Breslow NE, Day NE. Statistical methods in cancer research. Volume I - The analysis of case-control studies. IARC Sci Publ. 1980; (32):5–338. [PubMed: 7216345]

13. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science. 2002; 296(5576):2225–9. [PubMed: 12029063]
14. Zhao LP, Li SS, Khalid N. A method for the assessment of disease associations with single-nucleotide polymorphism haplotypes and environmental variables in case-control studies. Am J Hum Genet. 2003; 72(5):1231–50. [PubMed: 12704570]
15. Gauvreau K. Hypothesis testing: proportions. Circulation. 2006; 114(14):1545–8. [PubMed: 17015806]
16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3): 559–75. [PubMed: 17701901]
17. Pollard M, Luckert PH, Schmidt MA. Induction of prostate adenocarcinomas in Lobund Wistar rats by testosterone. Prostate. 1982; 3(6):563–8. [PubMed: 7155989]
18. Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, et al. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 2010; 362(13):1192–202. [PubMed: 20357281]
19. Hsing AW, Chu LW, Stanczyk FZ. Androgen and prostate cancer: is the hypothesis dead? Cancer Epidemiol Biomarkers Prev. 2008; 17(10):2525–30. [PubMed: 18842992]
20. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nat Genet. 2007; 39(5):645–9. [PubMed: 17401363]
21. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005; 352(17):1779–90. [PubMed: 15858187]
22. Gu L, Zhu XH, Visakorpi T, Alanen K, Mirtti T, Edmonston TB, et al. Activating mutation (V617F) in the tyrosine kinase JAK2 is absent in locally-confined or castration-resistant prostate cancer. Anal Cell Pathol (Amst). 2010; 33(2):55–9. [PubMed: 20966544]
23. Constantinescu SN, Girardot M, Pecquet C. Mining for JAK-STAT mutations in cancer. Trends Biochem Sci. 2008; 33(3):122–31. [PubMed: 18291658]
24. Barton BE, Karras JG, Murphy TF, Barton A, Huang HF. Signal transducer and activator of transcription 3 (STAT3) activation in prostate cancer: Direct STAT3 inhibition induces apoptosis in prostate cancer lines. Mol Cancer Ther. 2004; 3(1):11–20. [PubMed: 14749471]
25. De Miguel F, Lee SO, Onate SA, Gao AC. Stat3 enhances transactivation of steroid hormone receptors. Nucl Recept. 2003; 1(1):3. [PubMed: 12904256]
26. Agarwal C, Tyagi A, Kaur M, Agarwal R. Silibinin inhibits constitutive activation of Stat3, and causes caspase activation and apoptotic death of human prostate carcinoma DU145 cells. Carcinogenesis. 2007; 28(7):1463–70. [PubMed: 17341659]
27. Kwon EM, Salinas CA, Kolb S, Fu R, Feng Z, Stanford JL, et al. Genetic polymorphisms in inflammation pathway genes and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2011; 20(5):923–33. [PubMed: 21430300]
28. Mononen N, Schleutker J. Polymorphisms in genes involved in androgen pathways as risk factors for prostate cancer. J Urol. 2009; 181(4):1541–9. [PubMed: 19230916]
29. Lindström S, Zheng SL, Wiklund F, Jonsson BA, Adami HO, Bälter KA, et al. Systematic replication study of reported genetic associations in prostate cancer: Strong support for genetic variation in the androgen pathway. Prostate. 2006; 66(16):1729–43. [PubMed: 16998812]
30. Severi G V, Hayes M, Tesoriero AA, Southey MC, Hoang HN, Padilla EJ, et al. The rs743572 common variant in the promoter of CYP17A1 is not associated with prostate cancer risk or circulating hormonal levels. BJU Int. 2008; 101(4):492–6. [PubMed: 17986287]
31. Lindstrom S, Zheng SL, Wiklund F, Jonsson BA, Adami HO, Balter KA, et al. Systematic replication study of reported genetic associations in prostate cancer: Strong support for genetic variation in the androgen pathway. Prostate. 2006; 66(16):1729–43. [PubMed: 16998812]
32. Margiotti K, Kim E, Pearce CL, Spera E, Novelli G, Reichardt JK. Association of the G289S single nucleotide polymorphism in the HSD17B3 gene with prostate cancer in Italian men. Prostate. 2002; 53(1):65–8. [PubMed: 12210481]

33. Beuten J, Gelfond JA, Franke JL, Weldon KS, Crandall AC, Johnson-Pais TL, et al. Single and multigenic analysis of the association between variants in 12 steroid hormone metabolism genes and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18(6):1869–80. [PubMed: 19505920]
34. Cunningham JM, Hebbring SJ, McDonnell SK, Cicek MS, Christensen GB, Wang L, et al. Evaluation of genetic variations in the androgen and estrogen metabolic pathways as risk factors for sporadic and familial prostate cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16(5):969–78. [PubMed: 17507624]
35. Wilborn TW, Lang NP, Smith M, Meleth S, Falany CN. Association of SULT2A1 allelic variants with plasma adrenal androgens and prostate cancer in African American men. J Steroid Biochem Mol Biol. 2006; 99(4–5):209–14. [PubMed: 16617014]
36. Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK, et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet. 2008; 40(3):316–21. [PubMed: 18264097]
37. Cicek MS, Liu X, Casey G, Witte JS. Role of androgen metabolism genes CYP1B1, PSA/KLK3, and CYP11alpha in prostate cancer risk and aggressiveness. Cancer Epidemiol Biomarkers Prev. 2005; 14(9):2173–7. [PubMed: 16172228]
38. Mononen N, Seppälä EH, Duggal P, Autio V, Ikonen T, Ellonen P, et al. Profiling genetic variation along the androgen biosynthesis and metabolism pathways implicates several single nucleotide polymorphisms and their combinations as prostate cancer risk factors. Cancer Res. 2006; 66(2): 743–7. [PubMed: 16424004]
39. Pal P, Xi H, Sun G, Kaushal R, Meeks JJ, Thaxton CS, et al. Tagging SNPs in the kallikrein genes 3 and 2 on 19q13 and their associations with prostate cancer in men of European origin. Hum Genet. 2007; 122(3–4):251–9. [PubMed: 17593395]
40. Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol. 1991; 5(12):1921–30. [PubMed: 1724287]
41. Wang Q, Carroll JS, Brown M. Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. Mol Cell. 2005; 19(5):631–42. [PubMed: 16137620]
42. Ahn J, Berndt SI, Wacholder S, Kraft P, Kibel AS, Yeager M, et al. Variation in KLK genes, prostate-specific antigen and risk of prostate cancer. Nat Genet. 2008; 40(9):1032–4. author reply 1035–6. [PubMed: 19165914]
43. Kote-Jarai Z, Amin Al Olama A, Leongamornlert D, Tymrakiewicz M, Saunders E, Guy M, et al. Identification of a novel prostate cancer susceptibility variant in the KLK3 gene transcript. Hum Genet. 2011; 129(6):687–94. [PubMed: 21465221]
44. He WW, Sciavolino PJ, Wing J, Augustus M, Hudson P, Meissner PS, et al. A novel human prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer. Genomics. 1997; 43(1):69–77. [PubMed: 9226374]
45. Akamatsu S, Takata R, Ashikawa K, Hosono N, Kamatani N, Fujioka T, et al. A functional variant in NKX3.1 associated with prostate cancer susceptibility down-regulates NKX3.1 expression. Hum Mol Genet. 2010; 19(21):4265–72. [PubMed: 20716579]
46. Thomas MA, Preece DM, Bentel JM. Androgen regulation of the prostatic tumour suppressor NKX3.1 is mediated by its 3' untranslated region. Biochem J. 2010; 425(3):575–83. [PubMed: 19886863]
47. Kote-Jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, et al. Multiple novel prostate cancer predisposition loci confirmed by an international study: the PRACTICAL Consortium. Cancer Epidemiol Biomarkers Prev. 2008; 17(8):2052–61. [PubMed: 18708398]
48. Wang Y, Ray AM, Johnson EK, Zuhlke KA, Cooney KA, Lange EM. Evidence for an association between prostate cancer and chromosome 8q24 and 10q11 genetic variants in African American men: the Flint Men's Health Study. Prostate. 2011; 71(3):225–31. [PubMed: 20717903]
49. Pomerantz MM, Shrestha Y, Flavin RJ, Regan MM, Penney KL, Mucci LA, et al. Analysis of the 10q11 cancer risk locus implicates MSMB and NCOA4 in human prostate tumorigenesis. PLoS Genet. 2010; 6(11):e1001204. [PubMed: 21085629]

50. Chang BL, Cramer SD, Wiklund F, Isaacs SD, Stevens VL, Sun J, et al. Fine mapping association study and functional analysis implicate a SNP in MSMB at 10q11 as a causal variant for prostate cancer risk. Hum Mol Genet. 2009; 18(7):1368–75. [PubMed: 19153072]

Table 1

Selected characteristics of population-based prostate cancer cases and controls in King County, WA

Characteristic	Cases		Controls		OR[a]	95% CI	
	n=1,458	%	n=1,351	%			
Age at reference date							
35–49	118	8.1	126	9.3			
50–54	215	14.7	209	15.5			
55–59	357	24.5	358	26.5			
60–64	433	29.7	348	25.8			
65–69	177	12.1	164	12.1			
70–74	158	10.8	146	10.8			
Race							
Caucasian	1,309	89.8	1,266	93.7	1.00	Reference	
African American	149	10.2	85	6.3	1.74	1.32	2.30
First-degree family history of PC							
No	1,145	78.5	1,199	88.8	1.00	Reference	
Yes	313	21.5	152	11.3	2.19	1.77	2.71
Body mass index (kg/m^2)							
Normal (<25)	467	32.0	411	30.4	1.00	Reference	
Overweight (25–29.9)	707	48.5	650	48.1	0.95	0.80	1.13
Obese (≥30)	284	19.5	290	21.5	0.83	0.67	1.02
Prostate cancer screening history[b]							
None	157	10.8	182	13.5	1.00	Reference	
Digital rectal examination only	258	17.7	519	38.4	0.57	0.44	0.74
PSA test	1,043	71.5	650	48.1	1.94	1.52	2.47
PSA value (ng/mL)[c]							
0–3.9	189	12.9	1,253	92.8			
4.0–9.9	814	55.8	80	5.9			
10.0–19.9	208	14.2	16	1.2			
≥20.0	138	9.47	2	0.1			
Missing	109	7.47	0				

Characteristic	Cases		Controls		OR[a]	95% CI
	n=1,458	%	n=1,351	%		
Gleason score						
2–4	72	5.0				
5–6	741	51.0				
7 (3+4)	408	28.1				
7 (4+3)	91	6.3				
8–10	140	9.6				
Missing	6					
Stage of disease						
Local	1,141	78.3				
Regional	280	19.2				
Distant	37	2.5				

[a]OR adjusted for age.

[b]Prostate cancer screening history in five years prior to the reference date.

[c]Plasma prostate-specific antigen (PSA) level at diagnosis (cases) or at interview (controls).

Cancer Epidemiol. Author manuscript; available in PMC 2013 August 01.

Table 2

Prostate cancer risk estimates for candidate SNPs in androgen-related genes among Caucasian men

Gene	SNP	Allele[a]	Chr	BP	Genetic Model	MAF		OR[b] (95% CI)	*p-value*[c]
						Controls (n=1,266)	Cases (n=1,309)		
CYP17A1	rs10786712	C/T	10	104586386	Additive	0.407	0.378	0.88 (0.78–0.99)	0.03
HSD17B3	rs2066485	A/G	9	98043085	Additive	0.172	0.147	0.84 (0.72–0.97)	0.02
HSD17B3	rs2253502	T/C	9	98048264	Recessive	0.240	0.215	0.57 (0.39–0.84)	0.004
HSD17B3	rs2257157	T/C	9	98058263	Dominant	0.432	0.462	1.23 (1.03–1.47)	0.03
AKR1C3	rs4881400	T/G	10	5134037	Recessive	0.236	0.238	0.67 (0.47–0.96)	0.03
SULT2A1	rs182420	A/G	19	53064007	Recessive	0.230	0.252	1.41 (1.02–1.94)	0.04
NKX3.1	rs4872175	G/A	8	23590747	Additive	0.435	0.468	1.14 (1.02–1.27)	0.02
NKX3.1	NKX3.1_5	C/T	8	23593330	Additive	0.008	0.013	1.82 (1.02–3.25)	0.04
KLK3	rs174776	C/T	19	56051664	Additive	0.147	0.122	0.81 (0.69–0.95)	0.01
KLK3	rs1058205	T/C	19	56055210	Dominant	0.196	0.174	0.84 (0.71–0.99)	0.04
NCOA4	rs17178655	G/A	10	51231805	Additive	0.222	0.248	1.16 (1.01–1.32)	0.03
NCOA4	rs7350420	T/C	10	51264468	Additive	0.345	0.309	0.85 (0.75–0.95)	0.007
JAK2	rs10429491	C/T	9	5040706	Dominant	0.315	0.284	0.79 (0.67–0.92)	0.002
STAT3	rs744166	T/C	17	37767727	Dominant	0.425	0.404	0.83 (0.70–0.98)	0.03

[a] Major/minor alleles, based on frequencies in controls.

[b] OR adjusted for age.

[c] Nominal *P* values based on likelihood ratio-based test.

Table 3

Prostate cancer risk estimates for candidate SNPs in androgen-related genes among Caucasian men stratified by feature of disease aggressiveness

Gene	SNP	Allele	Chr.	BP	Variable	OR (95% CI)[a]	*p-value*[b]
CYP17A1[c]	rs10786712	C/T	10	104586386	Stage	0.67 (0.51–0.88)	0.004
HSD17B4	rs10478424	A/T	5	118816919	Stage	1.35 (1.08 – 1.69)	0.009
HSD17B4	rs6897978	A/G	5	118817237	Stage	1.37 (1.09 – 1.72)	0.007
JAK2[c]	rs10429491	C/T	9	5040706	Gleason	0.77 (0.64 – 0.91)	0.003
NCOA4	rs10761581	T/G	10	51238384	PSA	0.59 (0.40 – 0.87)	0.007
SULT2A1	rs2547238	C/G	19	53074292	Stage	1.43 (1.09 – 1.86)	0.009

[a] Polytomous regression was used to generate OR, adjusted for age.

[b] *p-value* < 0.01

[c] SNPs with nominal association with PC risk from Table 2.