

Genetic Variation of Genes Involved in Dihydrotestosterone Metabolism and the Risk of Prostate Cancer

Sunita R. Setlur¹, Chen X. Chen², Ruhella R. Hossain², Jung Sook Ha¹, Vanessa E. Van Doren², Birgit Stenzel⁵, Eberhard Steiner⁵, Derek Oldridge², Naoki Kitabayashi², Samprit Banerjee³, Jin Yun Chen¹, Georg Schäfer⁵, Wolfgang Horninger⁵, Charles Lee¹, Mark A. Rubin², Helmut Klocker⁵, and Francesca Demichelis^{2,4}

Abstract

Purpose: Dihydrotestosterone (DHT) is an important factor in prostate cancer (PCA) genesis and disease progression. Given PCA's strong genetic component, we evaluated the possibility that variation in genes involved in DHT metabolism influence PCA risk.

Experimental Design: We investigated copy number variants (CNV) and single nucleotide polymorphisms (SNP). We explored associations between CNV of uridine diphospho-glucuronosyltransferase (UGT) genes from the 2B subclass, given their prostate specificity and/or involvement in steroid metabolism and PCA risk. We also investigated associations between SNPs in genes (*HSD3B1*, *SRD5A1/2*, and *AKR1C2*) involved in the conversion of testosterone to DHT, and in DHT metabolism and PCA risk. The population consisted of 426 men (205 controls and 221 cases) who underwent prostate-specific antigen screening as part of a PCA early detection program in Tyrol, Austria.

Results: No association between CNV in *UGT2B17* and *UGT2B28* and PCA risk was identified. Men carrying the AA genotype at SNP rs6428830 (*HSD3B1*) had an odds ratio (OR) of 2.0 [95% confidence intervals (95% CI), 1.1-4.1] compared with men with GG, and men with AG or GG versus AA in rs1691053 (*SRD5A1*) had an OR of 1.8 (95% CI, 1.04-3.13). Individuals carrying both risk alleles had an OR of 3.1 (95% CI, 1.4-6.7) when compared with men carrying neither ($P = 0.005$). Controls with the AA genotype on rs7594951 (*SRD5A2*) tended toward higher serum DHT levels ($P = 0.03$).

Conclusions: This is the first study to implicate the 5 α -reductase isoform 1 (*SRD5A1*) and PCA risk, supporting the rationale of blocking enzymatic activity of both isoforms of 5 α -reductase for PCA chemoprevention. *Cancer Epidemiol Biomarkers Prev*; 19(1); 229-39. ©2010 AACR.

Introduction

Dihydrotestosterone (DHT), the most potent male hormone, has long been considered an important factor in prostate cancer (PCA) disease progression through direct activation of the androgen receptor (1). Testosterone (T) is converted to DHT by 5 α -reductase and is then glucuronidated by members of the uridine diphospho-glucuronosyltransferase (UGT) family for excretion. The

Reduction by Dutasteride of prostate Cancer Events (REDUCE) trial (2) recently reported that dutasteride, an inhibitor of 5 α -reductase types I and II, reduced PCA risk in men at higher levels of risk of the disease (American Urological Association in Chicago, IL, April 27, 2009). This trial consisted of a total of 1,516 patients with PCA, with 659 in the dutasteride arm and 857 in the placebo arm. The REDUCE trial showed that dutasteride significantly lowered the risk of all biopsy-detectable PCA by 23% ($P < 0.0001$) over 4 years. These findings are consistent with the results of the Prostate Cancer Prevention Trial, which also showed a significant reduction in the 7-year period prevalence of biopsy-detectable PCA with finasteride, a type II 5 α -reductase-selective inhibitor (3). Thus, two large clinical studies show that decreasing production of DHT using 5 α -reductase inhibitors can effectively decrease the incidence of clinically localized PCA.

Interindividual levels of DHT, and possibly the response to inhibitors of DHT, may be influenced by germ line polymorphisms. The extent of polymorphisms has been catalogued by the HapMap consortium, which categorized single nucleotide polymorphisms (SNP) in several widely disparate populations (4). A recent discovery

Authors' Affiliations: ¹Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; ²Department of Pathology and Laboratory Medicine, ³Department of Public Health, and ⁴Institute for Computational Biomedicine, Weill Cornell Medical College, New York, New York; and ⁵Department of Urology, Innsbruck Medical University, Innsbruck, Austria

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Corresponding Author: Francesca Demichelis, Institute for Computational Biomedicine, Weill Cornell Medical College, 1305 York Avenue, Y 1307 (or Box 140), New York, NY 10065. Phone: 646-962-5616; Fax: 215-440-9354. E-mail: frd2004@med.cornell.edu

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Translational Relevance

Two recent clinical trials showed a reduction of prostate cancer diagnosis after treatment with dihydrotestosterone (DHT) inhibitors implicating the important role DHT metabolism plays in prostate cancer development. Our study evaluates the potential role of genetic germ line variation in genes along the DHT pathway on the risk of prostate cancer. Although we did not observe associations between copy number polymorphisms for *UGT2B17* or *UGT2B28* and risk of prostate cancer, we did identify significant associations between single nucleotide polymorphisms located in *HSD3B1* and *SRD5A1* with increased risk of prostate cancer. Individuals carrying both risk alleles were three times more likely to have prostate cancer compared with men carrying neither. This is the first study to implicate the 5 α -reductase isoform 1 (*SRD5A1*) in prostate cancer risk, supporting the rationale of blocking the enzymatic activity of both isoforms of 5 α -reductase for the chemoprevention of prostate cancer.

shows that common polymorphisms exist not only as SNPs (i.e., single base pair alterations) but also as larger genomic regions of DNA gain and loss called copy number variation (CNV; refs. 5, 6). Mounting evidence suggests that these two types of polymorphisms predispose individuals to risk of disease (7). PCA is a common disease that may be highly influenced by genetic variation.

PCA has the strongest hereditary component of common cancers, as illustrated by a study of monozygotic twins, which suggested that 42% of the incident PCA risk is genetically linked (8). More recent studies using genome-wide analyses have identified risk loci comprising SNPs on chromosome 8q24 and 17p to be associated with PCA (9-14). How such polymorphisms affect the growth of PCA or the regulation of androgens, however, is inadequately addressed in the abovementioned genome-wide studies. An alternative approach to genome-wide studies has been to focus on the role of polymorphisms involving genes regulating the steroid hormone pathway as a risk factor for developing PCA. Two recent examples studied SNPs related to 5 α -reductase type II (*SRD5A2*; ref. 15) and 3 β -hydroxysteroid dehydrogenase 1 (*HSD3B1*; ref. 16). The role of the second type of germ line polymorphism, the CNVs, has been examined by four studies that analyzed the copy number state of *UGT2B17* (UDP glucuronosyltransferase 2 family, polypeptide B17) for risk of PCA (17-20). This gene maps to chromosome 4 and plays a central role in the catabolism of T and DHT. The studies reported conflicting results regarding the association of genomic copy number alterations of this gene with PCA risk. Park et al. (20) and Karypidis et al. (18) showed that the deletion polymorphism of this gene

results in greater risk of PCA in Caucasian patients. Studies by Gallagher et al. (17) and Olsson et al. (19) did not find any association between the deletion polymorphism and risk for PCA. This candidate CNV involving a UGT locus is intriguing due to their prostate-specific role in DHT catabolism and the important role of that gene in prostate gland maintenance and growth. However, the extent to which CNV in genes involved in the metabolism of T or DHT may predispose a man to a higher risk of having PCA is still unresolved.

Using a short sequence oligonucleotide array platform (Genome-Wide Affymetrix 6.0 SNP), we were able to test genetic variation in seven genes involved in the metabolism and catabolism of T and DHT. In particular, we explored for associations between UGT genes that mediate glucuronidation and clearance of several compounds including steroid hormones, bile acids, bilirubin, xenobiotics, and drugs (21). We focused on *UGT2B17*, *UGT2B15*, *UGT2B7*, and *UGT2B28*, UGTs from the UGT2B protein subclass, because of their prostate specificity and/or involvement in steroid metabolism. Specifically, *UGT2B17* mediates the glucuronidation and subsequent clearance of DHT in the basal cells whereas *UGT2B15* mediates the clearance of T and DHT in the luminal epithelial cells. *UGT2B17*, but not *UGT2B15*, is downregulated by DHT. The mechanism of action of the UGTs in the prostate is shown in Fig. 1. Stromal cells also play a role in steroid metabolism in hormone-sensitive tissues. We also investigated the association between SNPs in genes directly related to the conversion of T in DHT, and in DHT catabolism and risk of PCA. Specifically, we investigated *HSD3B1* (1p12), the two steroid-5 α -reductase genes, *SRD5A1* (5p15.31) and *SRD5A2* (2p23.1), which catalyze the conversion of T into the more potent androgen DHT, and the aldo-keto-reductase family 1 member C2, *AKR1C2* (10p15.1).

One significant limitation to recent genetic studies in the field of PCA has been the use of cases from surgical cohorts and controls from a disparate control population. In most of these studies, controls are most frequently defined as having no reported PCA but may have no recorded prostate-specific antigen (PSA) level, and in none of the studies have the controls undergone systematic prostate needle biopsy evaluation to confirm their disease-free status. To overcome these limitations, we identified cases and controls from the same population of men screened for elevated serum PSA as part of a regional PCA early detection trial (22-24).

All men on trial underwent intense PSA screening. Abnormal results (age-adjusted PSA levels as low as 1.25 ng/mL) led to prostate needle biopsy evaluation. Controls with negative prostate needle biopsies continued to be followed with regular PSA evaluations. The current study therefore is the first to use a PSA-screened clinical trial population to explore the risk of PCA based on genetic variation of genes involved in DHT metabolism and their effect on DHT and T serum levels, both in the form of CNVs and SNPs.

Diachrome), respectively, according to the recommended procedures.

Sample DNA Preparation

Isolation of genomic DNA from blood samples was carried out in a high-throughput fashion using the QIAamp 96 DNA Blood kit (Qiagen). Ficoll-purified peripheral blood mononuclear cells were manually resuspended in 400 µL of chilled phosphate buffer saline, allowed to equilibrate to room temperature, and split into two aliquots. The aliquots were added to 20 µL of Qiagen protease in the company-provided collection microtubes to facilitate nuclear and cellular lysis. The resulting lysates were processed according to the guidelines of the manufacturer and finally resuspended in 100 µL of nuclease-free distilled water. DNA quality and quantity were evaluated by electrophoresis and NanoDrop spectrophotometry (NanoDrop; Thermo Scientific), respectively.

CNV and SNP Genotype Evaluation

Data were generated using Affymetrix Genome-Wide Human SNP Array 6.0. Briefly, genomic DNA was processed for the Affymetrix 6.0 whole genome platform

(Affymetrix, Inc.) according to the protocols of the manufacturer. The DNA was digested with the enzymes *NspI* and *StyI* (New England Biolabs) and ligated to the respective adapters (Affymetrix) using T4 DNA ligase (New England Biolabs). The ligated DNA was amplified (Clontech), purified using magnetic beads (Agencourt), labeled, fragmented, and hybridized to the arrays. Following hybridization, the arrays were washed and stained with streptavidin-phycoerythrin (Invitrogen Corporation) and scanned. Data processing was done using Affymetrix Power Tools. SNP genotype calls were generated using the Birdseed (v2) algorithm (30). All samples included in this study passed strict data quality control measures. In addition, taking advantage of array-based genotype information, we applied an inter-sample similarity test to check for relatedness called the SNP panel identification assay (31) and tested for population stratification (32). Copy number data was evaluated by measuring the median of the log 2 values of the ratios between the normalized hybridization intensity of each target sample and a reference model built using a subset of the all-male control subjects and unrelated Caucasian HapMap female samples. For highly polymorphic loci (i.e., *UGT2B17* and *UGT2B28*), the copy number offset caused by the presence of the CNV signal in the reference model was computationally corrected.

SNP Selection

For each gene directly related to the conversion of T to DHT and in DHT catabolism, we investigated the haplo-block structure considering 10 kb flanks using Haploview (33) with default parameters. We selected one or more available SNPs for each block based on minor allele frequency as evaluated in the control group (intervals: 0.03-0.2, 0.21-0.3, and 0.31-0.5). Only SNPs with at most 3% of data missing were considered. This procedure led to the selection of 15 SNPs, specifically two for *HSD3B1* (rs6428830 and rs10754400), seven for *SRD5A1* (rs566202, rs4702379, rs248803, rs3797177, rs8192249, rs30434, and rs1691053), three for *SRD5A2* (rs4952197, rs7594951, and rs806645), and three for *AKR1C2* (rs11252866, rs11252867, and rs11816204).

Quantitative PCR for UGT2B17

The relative standard curve quantitative PCR (qPCR) method was used to investigate *UGT2B17* copy numbers. DNA from HapMap NA07022 was used for the standard curve with all assays being set up in triplicates. Thermal cycling and quantification were carried out using 7900HT Sequence Detection System from ABI with an annealing temperature of 62°C. Rho guanine nucleotide exchange factor 11 (*ARHGEF11*; forward, 5'-TCTCTGCTCCCTCACTCTCAA-3'; reverse, 5'-TGTGCCTCTTCCATC-GTTCT-3') was used as the endogenous control gene because it was copy number invariant across the samples. The *UGT2B17* primer pair (forward, 5'-CAGT-TGCCTGTAGCCACATTT-3'; reverse, 5'-TGGTGGAT-GAAGAAGACTTTTG-3') was designed to specifically

Table 1. Study cohort demographics			
	Controls (n = 205)	Cases (n = 221)	P
<hr/>			
Age (y)			
Mean	58.9	61.7	0.0001
Median	59	62.0	
Range	41-76	43-77	
PSA (ng/mL)			
≤4	126	87	<0.0001
4.1-10.0	54	99	
10.1-20.0	22	23	
>20.0	3	12	
Free PSA (%)			
Mean	17.5	16.6	0.12
Median	16.	15.1	
Range	5.1-58.6	5.2-59.6	
T (ng/mL)			
Mean	4.4	4.5	NS
Median	4.3	4.5	
Range	1.5-9.0	1.8-10.5	
DHT (ng/mL)			
Mean	0.77	0.71	NS
Median	0.58	0.54	
Range	0.16-5.8	0.2-4.1	
DHT/T			
Mean	0.19	0.17	NS
Median	0.14	0.14	
Range	0.02-1.26	0.04-1.1	
<hr/>			
Abbreviation: NS, not significant.			

Table 2. Copy number polymorphism at *UGT2B17* and *UGT2B28* in controls and cases and PCA risk association results

Gene	CN	Controls, n (%)	Cases, n (%)	Crude OR (95% CI)	P	Age-adjusted OR (95% CI)	P
<i>UGT2B17</i>	+/+	93 (45.4)	11 (50.2)	Reference		Reference	
	+/-	92 (44.9)	87 (39.4)	0.79 (0.5-1.2)	0.26	0.77 (0.51-1.16)	0.21
	-/-	20 (9.8)	23 (10.4)	0.96 (0.5-1.9)	0.91	0.88 (0.45-1.73)	0.71
<i>UGT2B28</i>	+/+	153 (74.6)	164 (74.2)	Reference	Reference		
	+/-	45 (22.0)	53 (24)	1.10 (0.70-1.73)	0.68	1.14 (0.72-1.81)	0.58
	-/-	7 (3.4)	4 (1.8)	0.53 (0.15-1.86)	0.32	0.50 (0.14-1.79)	0.29

Abbreviation: CN, copy number (-/-, homozygous deletion; +/-, hemizygous deletion; +/+, wild-type).

amplify *UGT2B17*. This primer set was checked for specificity using the BLAST, BLAT, and the Eland-based algorithms outlined below.

Statistical Analysis

χ^2 test and Wilcoxon test were applied for categorical and continuous variables, respectively, to assess the statistical significance of differences observed in the control and case groups. Two-sided $P \leq 0.05$ values were considered statistically significant. For each polymorphic location considered in the study, we applied binary logistic regression to evaluate the risk for PCA. Each regression was also considered with age as covariate (age adjustment). We report odds ratios (OR) and 95% confidence intervals (CI). Logistic regression P values are unadjusted. With respect to the potential increased risk for PCA in the presence of *UGT2B17* deletion previously reported by other authors, we estimated that we were powered ($\beta = 80\%$; $\alpha = 0.05$) to detect an OR of 1.9 or above and 1.6 or above for homozygous deletion and any deletion, respectively (proportions equal to 0.13 and 0.40 for deletions were considered, respectively). Analysis was done using R 2.9.0 (ref. 34; accessed April 23, 2008).

Primer Analysis of Published Studies

The specificity of the primers we designed for this study and those designed by other groups (17-20) was evaluated by implementing an Eland-like⁶ alignment algorithm based on the short-read alignment indexing strategy for finding perfect and 1- to 2-bp mismatch hits of short sequences. Briefly, every n -mer sequence was broken into four nonoverlapping tiles. If a pair of tiles was found to have a perfect match with a paired 6-mer tile index of the genome, an ungapped extension was done to determine if the subalignment generated a hit of interest. By design, the algorithm detects perfect match,

single base pair mismatch, and two-base pair mismatch hits with perfect sensitivity and specificity. The hits that generated amplicons of ≤ 2 kb were reported.

Results

Association of Age, PSA, Free PSA, DHT and T Levels with Cases and Controls

The cases and controls included in the study were selected to be age-matched. The cases were, on average, 2 years older than the controls ($P = 0.001$; Table 1). Initial PSA levels ranged between 0.4 and 29.2 ng/mL and between 1.25 and 469 ng/mL in controls and cases, respectively ($P < 0.0001$). No association was observed for serum-free PSA, DHT, T, and DHT over T ratios with respect to end point (case/control). We evaluated the behavior of PSA, free PSA, and DHT/T with respect to age in the control group and observed a moderate increase of PSA and free PSA with age ($P < 0.001$ for linear trends) and no trend for DHT/T ($P = 0.1$). We also observed that individuals with high DHT levels tend to have low PSA and vice versa. We evaluated individual ethnicity by population stratification analysis (32), which revealed that all individuals reflect Caucasian ancestry.

Incidence of Germ Line CNVs and SNP Genotypes in a Study Cohort

A total of 426 cases and controls were used to evaluate the germ line copy number frequencies for the genes of interest. Table 2 shows the breakdown of the copy number analysis results of the two genes exhibiting CNVs, *UGT2B17* and *UGT2B28*. Due to poor coverage, we were not able to assess *UGT2B15* copy number state in our cohort. The observed frequencies of the homozygous and hemizygous deletion in *UGT2B17* (9.8% and 44.9%, respectively) and *UGT2B28* (3.4% and 22.0%, respectively) in controls are consistent with previous reports (35). qPCR confirmed the copy number of *UGT2B17* determined by the Affymetrix SNP array platform (Fig. 2). The PCR amplicons were sequenced to confirm the

⁶ A.J. Cox, unpublished data.

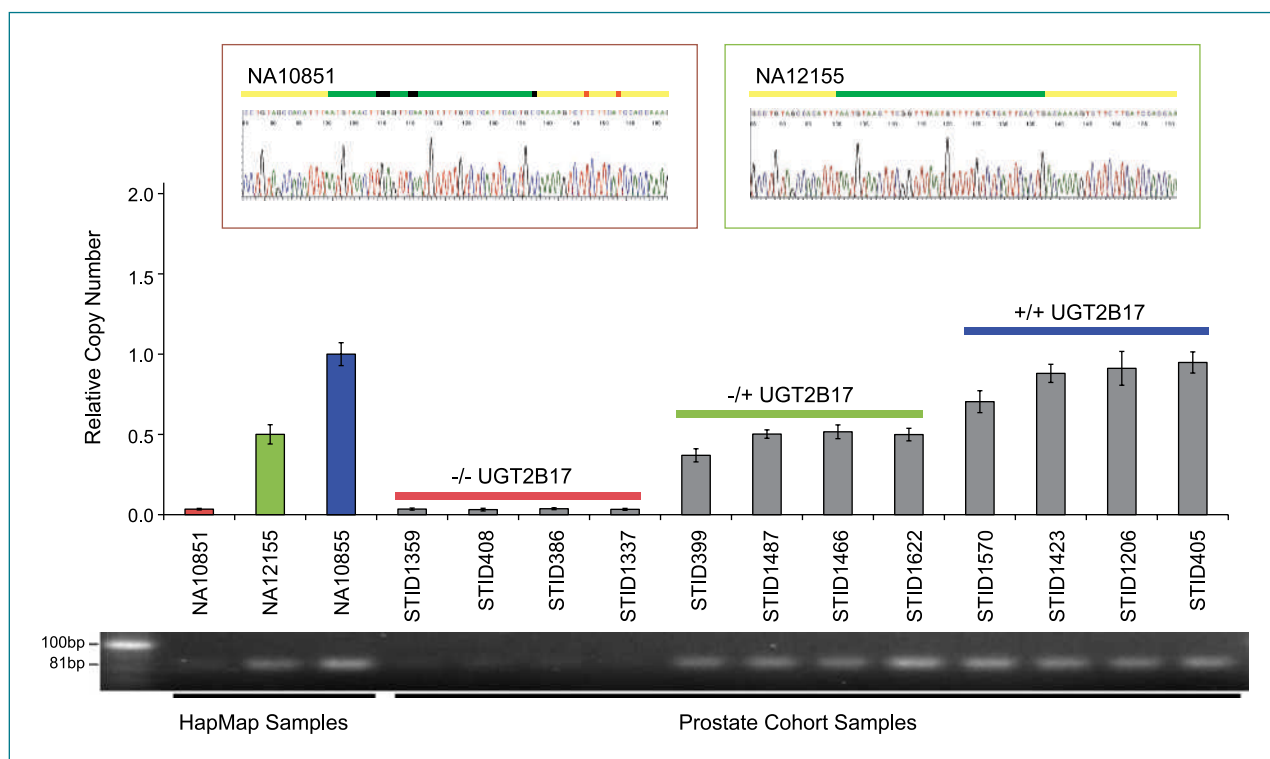


Figure 2. Confirmation of the copy number state of *UGT2B17* by qPCR. Three HapMap samples and 12 samples from the Tyrol PSA Screening Cohort were selected based on known copy number states (HapMap, far left) and array-based copy number data. Array-based copy number calls are color-coded: *UGT2B17*-/- (red), *UGT2B17*+/- (green), and *UGT2B17*+/+ (blue). The qPCR results agree with the array-based copy number calls. The PCR amplicons were sequenced to confirm the specificity of the amplified products (top, insets). The samples that had either one or two copies of the gene showed the specific product (top right, inset). A nonspecific product was observed in the samples that were homozygous for deletion (-/-; top left, inset). Sequencing identified this as *UGT2B15*, which shares 95% nucleotide homology with *UGT2B17* (Fig. 2, left, inset). We did not detect any copy number alterations in the *UGT2B7* gene, or in *SRD5A1*, *SRD5A2*, *HSD3B1*, and *AKR1C2*. *HSD3B1* was reported by two independent studies as copy number polymorphic in <1% of Caucasian individuals (36, 37).

specificity of the products amplified. The samples that had either one or two copies of the gene showed the specific product (Fig. 2, right, inset). A nonspecific product was observed in the samples that were homozygous for deletion. Sequencing identified this as *UGT2B15*, which shares 95% nucleotide homology with *UGT2B17* (Fig. 2, left, inset). We did not detect any copy number alterations in the *UGT2B7* gene, or in *SRD5A1*, *SRD5A2*, *HSD3B1*, and *AKR1C2*. *HSD3B1* was reported by two independent studies as copy number polymorphic in <1% of Caucasian individuals (36, 37).

We identified 15 SNPs on *HSD3B1* (2), *SRD5A1* (7), *SRD5A2* (3), and *AKR1C2* (3) based on linkage disequilibrium, minor allele frequency, and platform design constraints. Supplementary Table S1 summarizes the characteristics of the selected SNPs in terms of location, minor allele frequency in Caucasian HapMap individuals, study controls and cases, and Hardy-Weinberg equilibrium. It also reports *P* values for allele and genotype frequency differences based on Fisher exact test. Supplementary Fig. S1 depicts allele scatter plots for each SNP and shows good cluster separation for low genotype frequencies.

Clinical Parameters and Genetic Polymorphism

To assess the influence of genetic variants on PSA, DHT, and T serum levels, we considered individuals from the study control group. We observed higher levels of DHT and DHT/T ratio in the presence of minor allele homozygous genotype for one intronic SNP on *SRD5A2*, rs7594951 (nonparametric test, *P* = 0.028 and 0.046 for DHT and DHT over T, respectively). The minor allele frequency for rs7594951 in controls was 0.14.

Association of Polymorphisms and PCA Risk

The individuals with germ line deletion polymorphisms in *UGT2B17* and *UGT2B28* were examined for conferring risk to PCA. We did not find any association between the copy number states of either gene and risk of PCA (Table 2), nor any association between copy number and serum levels of DHT. Figure 3A and B shows the copy number counts in cases and controls in the light of DHT over T levels for cases and controls. No association between copy number state and risk for PCA was detected even when considering gene-gene interaction (data not shown) nor in combination with SNPs.

When assessing the association between SNPs and risk for PCA (Table 3), we observed that men with a minor allele homozygous genotype in rs6428830 (*HSD3B1*) had an age-adjusted OR of 2.0 (95% CI, 1.1-4.1) as compared with men with GG. The rs6428830 SNP is 2.5 kb apart from the locus previously reported by Park et al. (ref. 16; rs1047303) as associated with increased risk of PCA in Caucasian men with a family history of PCA. Individuals with AG or GG versus AA in rs1691053 (*SRD5A1*) have an OR of 1.8 (95% CI, 1.04-3.13). Figure 3C and D shows genotype counts in cases and controls in the light of DHT over T levels for cases and controls. Interestingly, rs1691053 risk allele shows an OR of 2.2 (95% CI, 1.2-4.0) for early onset risk. We then evaluated the effect of carrying both risk alleles at rs6428830 and at rs1691053, and estimated an OR of 3.1 (95% CI, 1.4-6.7) when compared with carrying neither of these risk alleles ($P = 0.005$).

Discussion

Our study examined the association of polymorphisms in *UGT2B* genes (*UGT2B7*, *UGT2B17*, and *UGT2B28*) and in genes involved in the metabolism of T and DHT with the risk of PCA. We used a well-defined cohort for the

study in which the cases and controls were intensively screened for PSA and the controls had a negative prostate biopsy and a mean follow-up without cancer diagnosis for 7.6 years. We were unable to detect any association between the deletion polymorphism of *UGT2B* genes examined and the risk of PCA. We also investigated if polymorphisms in these genes would result in differential levels of serum hormones.

The association between *UGT2B17* deletion and PCA risk was previously investigated leading to conflicting results (17-20). One primary difference between our study and the previous studies is the use of whole genome array platform for the assessment of copy number states and independent verification by qPCR on a subset of samples (three HapMap samples sequenced at the *UGT2B17* locus were included as a gold standard; ref. 38), whereas the other studies used qPCR as the only platform for genotyping. Because *UGT2B17* shares 95% sequence homology with the *UGT2B15* gene, designing specific primers for PCR is necessary for accuracy. Of interest, the genomic region encompassing *UGT2B17* and *UGT2B15* (4q13.2) is a complex genomic region whose reference sequence was recently reinterpreted (38). Hence, we tested the specificity of our primers and the primers used in other studies by applying an alignment

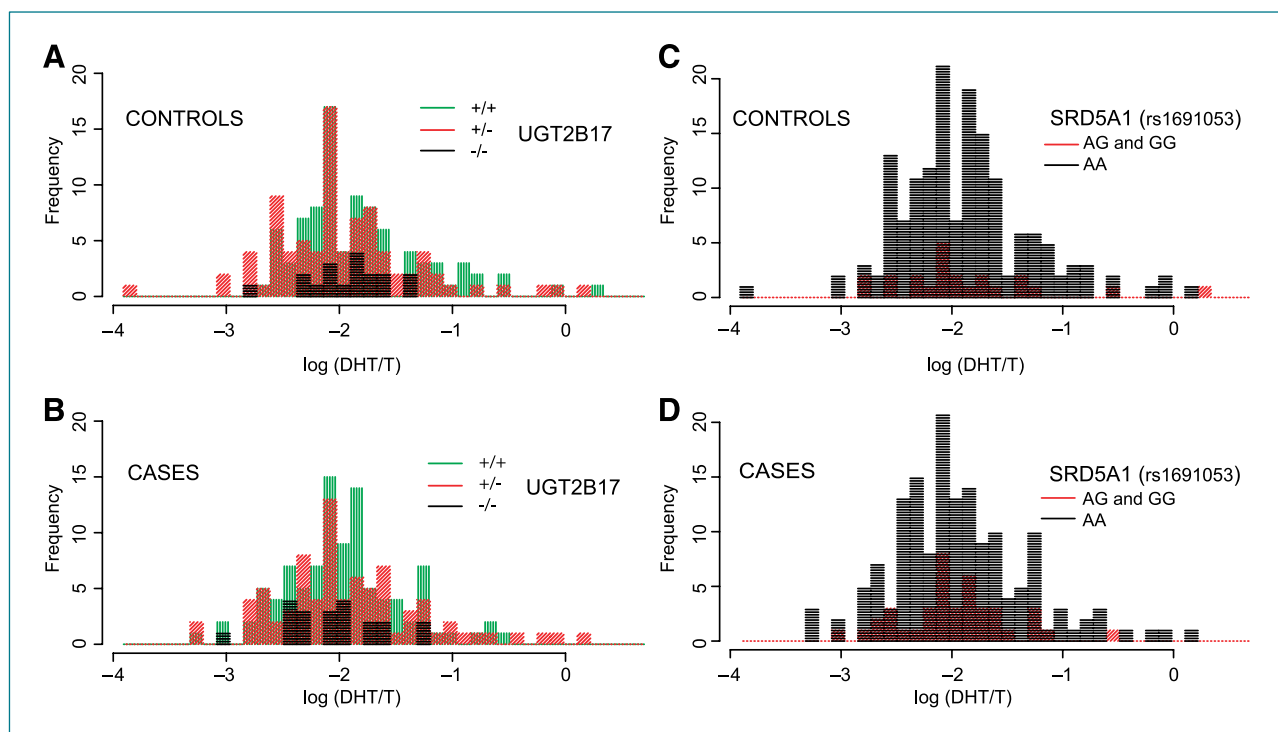


Figure 3. Relationship between serum hormone levels in cases and controls and genotypes. The frequencies of the ratios of DHT and T serum levels for controls (A and C) and cases (B and D) with respect to *UGT2B17* copy number states (A and B) and to rs1691053 (*SRD5A1*) genotype. No association between *UGT2B17* copy number state and PCA risk was detected, and no evidence of DHT/T level differentiation based on *UGT2B17* copy number state classes was evaluated, whereas the presence of G nucleotide at rs1691053 in *SRD5A1* was associated with increased risk of PCA (OR, 1.8; 95% CI, 1.04-3.13), with slightly higher effect for early onset (OR, 2.2; 95% CI, 1.2-4.0). No association between genotype and serum hormone levels was appreciated.

Table 3. Significant results from regression analysis of SNP genotypes and PCA risk

SNP	Genotype	Controls, n (%)	Cases, n (%)	OR (95% CI)		Age-adjusted OR (95% CI)	
rs6428830 (HSD3B1)	AA	15 (7.4)	26 (11.8)	2.0 (0.9-3.93)	0.058	2.0 (1.1-4.1)	0.047
	AG	84 (41.4)	103 (46.6)	1.4 (0.93-2.07)	0.11	1.4 (0.93-2.11)	0.10
	GG	104 (51.2)	92 (41.6)	Reference		Reference	
	AA and AG	99 (48.8)	129 (58.4)	1.47 (1.00-2.16)	0.048	1.50 (1.02-2.21)	0.042
	GG	104 (51.2)	92 (41.6)	Reference		Reference	
	AA	15 (7.4)	26 (11.8)	1.67 (0.86-3.25)	0.13	1.73 (0.88-3.40)	0.11
rs1691053 (SRD5A1)	AG and GG	188 (92.6)	195 (88.2)	Reference		Reference	
	AA	178 (88.1)	176 (80.7)	Reference		Reference	
	AG	22 (10.9)	37 (17.0)	1.70 (0.97-3.00)	0.066	1.74 (0.98-3.10)	0.059
	GG	2 (1.0)	5 (2.3)	2.53 (0.48-3.21)	0.27	2.44 (0.46-13.03)	0.30
	AA and AG	200 (99.0)	213 (97.7)				
	GG	2 (1.0)	5 (2.3)	2.35 (0.45-2.24)	0.31	2.26 (0.43-12.03)	0.34
	AA	178 (88.1)	176 (80.7)	Reference		Reference	
	AG and GG	24 (11.9)	42 (19.3)	1.77 (1.03-3.05)	0.039	1.80 (1.04-3.13)	0.036

algorithm, which is highly sensitive and specific for homology searches of short sequences. We found that most studies were capable of recognizing additional products in the *UGT2B15* gene (results are summarized in Supplementary Table S2). For instance, one group first showed associations between *UGT2B17* and PCA risk (18), but then was unable to confirm this result in a follow-up study (19). They used primers which were capable of detecting *UGT2B15* as shown in the current study. Our primer did yield a product in *UGT2B15*, although this nonspecific weak amplification occurred only in the complete absence of *UGT2B17* and in the later cycles of the qPCR reaction. One consideration to be made is that the highly homologous gene *UGT2B15* has been shown to play a role in conferring PCA risk (39-41). A SNP, D85Y, reduces the enzymatic activity of *UGT2B15*, resulting in downregulation of its catabolic activity on DHT, thereby leading to DHT accumulation in the prostate. Because *UGT2B15* is expressed in the luminal epithelial cells, decreased activity of the gene product could lead to increased intraepithelial DHT levels. One might then predict that genes modulated by androgens would be more active in individuals with higher levels of DHT due to a lack of *UGT2B15* enzymatic activity. As a consequence over time, unchecked androgenic stimulation could contribute to PCA development. Future work will focus on determining the *in situ* activity of these genes to confirm these observations along different states of PCA development.

As is evident from Supplementary Table S2, only the primers used by Park et al. (20) seem to be specific. This group reported a positive association between deletion polymorphism of *UGT2B17* and the risk of PCA (16, 20) after adjustment for cigarette smoking (20) or age

and family history (16). The study by Park et al. (16) also showed that the N367 SNP in *HSD3B1* increased the risk of PCA when examined in combination with the deletion polymorphism of *UGT2B17*.

Given the influence of both these genes on androgen catabolism, it becomes important to examine their combined effects. Hence, it is important to examine the role of deletion polymorphism in *UGT2B15* in PCA. The *UGT2B15* gene region is not a segmental duplication as currently annotated in the latest genome build (hg19). Evidence for this comes from a recent study by Xue et al. (38), which showed by sequencing that *UGT2B15* is present as a single-copy gene. We were unable to use the genomewide platform to carry out this analysis due to the limitation of a single probe covering that region. However, we think it is important to look further into the role of this gene because it plays a central role in DHT catabolism in the prostate luminal epithelial cells. The effect of deletion polymorphisms in *UGT2B15* in combination with *UGT2B17* deletions will also be important to examine in future studies.

Altogether, conflicting results between the present study and Park's study leave room for investigation in an independent cohort as to whether the deletion of *UGT2B17* is associated with PCA. One potential source of discordant results might be the difference in the two study cohorts, which were sampled with different criteria from the population. The Park et al. study included individuals from prostatectomy series detected through PSA elevation, in which our study cohort is from a PSA screening trial confined to a specific European cohort. These cohort differences can lead to different accrual of PCA stage. Of interest, RNA expression data for *UGT2B17* and *UGT2B15* show consistent high expression in hormone-refractory PCA versus primary PCA

in two independent studies (42, 43), suggesting that enrichment for homozygous deletions in aggressive PCA is unlikely.

There have been a number of high-profile large-scale genetic studies exploring for associations between SNPs and associations with PCA. A series of independent studies have confirmed the original observations made in 2006 by Amundadottir et al. (14) and Freedman et al. (13) that SNPs located in three regions of 8q24 are associated with an increased risk of PCA. Amundadottir et al. observed a link between 8q24 (region 1) and PCA using linkage analysis followed by fine-mapping in an Icelandic family and case-control data (14). Freedman et al. detected significant associations between chromosome 8q24 markers (region 2) common in West African ancestry and risk of PCA, by analyzing up to 1,600 African American men with PCAs, using an admixture scan approach (13). One specific locus, rs1447295 (chr8:128554220, hg17), narrowed down by the deCODE study (14), has been further investigated and validated by two epidemiologic studies, a nested case control using the Cohort Consortium (44) and a case control study (45) on a Caucasian population. In April 2007, Gudmundsson et al. (46) and Haiman et al. (47) confirmed previous results and expanded their studies. Haiman et al. reported on a new region (region 3) spanning 128.47 to 128.54 Mb and on five new variants, each independently predicting risk for PCA.

More recently, a study by Zheng et al. received widespread public attention when they proposed a clinical test for the detection of PCA by combining three SNPs at 8q24 and one each at 17q12 and 17q24.3 (48). Overall, they evaluated 16 SNPs from five chromosomal regions in a Swedish population (2,893 subjects with PCA and 1,781 control subjects). Together, five SNPs and family history accounted for 46% of the PCA cases. In men with five or more of these factors, the OR for PCA was 9.46 ($P = 1.29\text{E-}8$), as compared with men without any of the factors. PSA did not add any additional power to their model. Although these findings are potentially interesting, they were not validated in a prospectively collected PSA-screened population such as the Tyrol Cohort or on another data set. Most of these studies used controls that contained up to 25% occult PCA—as suggested by the Tyrol Cohort and the Prostate Cancer Prevention Trial—possibly altering the findings.

None of the PCA SNP studies have presented a solid hypothesis as to how any of these SNPs might affect disease development or progression. In the current study, we posited that SNPs in genes involved in DHT metabolism might play a role in PCA development or progression. Several recent studies have attempted to evaluate SNPs in these pathways using standard assays to evaluate SNPs. These studies did not come to any overall conclusive results regarding SNP in the DHT metabolism pathway and association with PCA.

The current study discovered that two SNPs in the DHT pathway involving *HSD3B1* and *SRD5A1* were associated with an increased risk of PCA. Particularly interesting was that men carrying both risk alleles have a 3.1 greater likelihood for PCA compared with men carrying neither of these risk alleles ($P = 0.005$). This is in line with the current understanding of genetic variation, which would argue that cumulative effects of genes along pathways are more important than the alteration of single genes (7). Importantly, rs6428830 located in intron 3 of *HSD3B1* is in strong linkage disequilibrium with neighboring SNPs including 10 nonsynonymous SNPs, suggesting a potential functional role in the conversion of DHEA into T. *SRD5A1* only harbors synonymous SNPs and its haplostructure seems more complex; rs1691053 resides in the 3'-end of the gene and is in linkage disequilibrium with few coding SNPs. Supplementary Fig. S2 shows the haplostructure of the two genes. We also observed that the *SRD5A2* genotype is associated with DHT serum levels. Based on the current study results, one can speculate that *SRD5A2* is not the crucial isoform determining DHT levels in the prostate or that higher DHT does not contribute to PCA development but to progression of tumor cells or precancerous tumor lesion to clinical cancer. As indicated by several publications, in a tumor cell, the type I to type II ratio seems to increase as compared with benign glands. Future independent validation studies will be required to verify these findings.

Understanding how genetic variation can affect the risk of disease is ultimately critical for diagnosis and the development of prevention strategies. With regards to DHT metabolism, SNPs can significantly influence the enzymatic activity of *SRD5A1* and *SRD5A2*. For example, an A49T missense substitution in the *SRD5A2* gene results in increased enzymatic activity *in vitro* (49). This effect seems to be an inherent gain-of-function encoded by the amino acid substitution because the steady-state concentrations of the normal and mutant enzymes were identical.

One important limitation in this analysis is that DHT levels measured in the serum are probably most reflective of DHT produced by the entire body, only a portion of which can be attributed to the prostate gland. Therefore, future efforts will focus on determining the associations between genetic variants related to DHT metabolism and the local concentrations of DHT within the prostate gland.

In summary, using a pathway approach to evaluating genetic variation involved in androgen metabolism, we observed that two SNPs accounted for an increased risk of PCA. Intriguingly, this is the first study to implicate a SNP in the 5 α -reductase isoform 1 (*SRD5A1*) with risk of PCA, supporting the rationale of blocking enzymatic activity of both isoforms of 5 α -reductase for the chemoprevention of PCA. Future studies should evaluate the pharmacogenetic effects of these SNPs given the potential broad use of dutasteride in PCA chemoprevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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