Profiling Genetic Variation along the Androgen Biosynthesis and Metabolism Pathways Implicates Several Single Nucleotide Polymorphisms and Their Combinations as Prostate Cancer Risk Factors

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Abstract

Several candidate genes along androgen pathway have been suggested to affect prostate cancer risk but no single gene seems to be overwhelmingly important for a large fraction of the patients. In this study, we first screened for variants in candidate genes and then chose to explore the association between 18 variants and prostate cancer risk by genotyping DNA samples from unselected (n = 847) and familial (n = 121) prostate cancer patients and population controls (n = 923). We identified a novel single nucleotide polymorphism (SNP) in the CYP19A1 gene, T201M, with a mild significant association with prostate cancer [odds ratio (OR), 2.04; 95% confidence interval (95% CI), 1.03-4.03; P = 0.04]. Stratified analysis revealed that this risk was most apparent in patients with organ-confined (T1-T2) and low-grade (WHO grade 1) tumors (OR, 5.42; 95% CI, 2.33-12.6; P < 0.0001). In contrast, CYP17A1 341T>C alteration was associated with moderate to poorly differentiated (WHO grade 2-3) organ-confined disease (OR, 1.42; 95% CI, 1.09-1.83; P = 0.007). We also tested a multigenic model of prostate cancer risk by calculating the joint effect of CYP19A1 T201M with five other common SNPs. Individuals carrying both the CYP19A1 and KLF3 252A>G variant alleles had a significantly increased risk for prostate cancer (OR, 2.87; 95% CI, 1.10-7.49; P = 0.04). In conclusion, our results suggest that several SNPs may have different contributions to distinct clinical subsets as well as combinatorial effects in others illustrating that profiling and joint analysis of several genes along each pathway may be needed to understand genetic contributions to prostate cancer etiology. (Cancer Res 2006; 66(2): 743-7)

Introduction

Prostate cancer is the most common male malignancy and the second leading cause of cancer deaths in many western countries. There is large variation in the risk to prostate cancer among different racial/ethnic groups (1). Although the reason for this is mostly unknown, differences in diet and hormonal levels may be involved (2). Androgens are essential for both the normal and malignant growth and differentiation of the prostate. Castrated men never develop prostate cancer and androgen ablation is widely used as the primary treatment for extracapsular prostate cancer (3, 4). Further evidence of the role of the androgens in prostate cancer etiology comes from rat experiments wherein testosterone and dihydrotestosterone have been used to induce prostate cancer (5, 6).

Because of the importance of androgens to prostate cancer development, genes involved in the biosynthesis and metabolism of androgens have been under intensive study. Already several genes have been identified along the androgen pathway, such as SRD5A2, CYP19A1, CYP17A1, HSD3B1, and AR (7-11), of which genetic variation is suggested to be associated with an increased risk of prostate cancer. However, many of the effects observed have been rather modest. Furthermore, studies have often been done using rather small sample sets and replication of the data in independent clinical cohorts has not been possible. To establish whether the genetic variants in the androgen pathway are predictive of prostate cancer, we screened 10 genes for possible disease-associated variations and then genotyped 18 selected alterations among a large sample set, including a total of 1,891 samples from unselected and familial prostate cancer patients and controls.

Materials and Methods

Samples used in the screening of genetic variation. All samples collected and used are of Finnish origin. The initial screening by single-strand conformational polymorphism (SSCP) of genetic variation in 10 genes was done among 32 men with familial prostate cancer and 32 men with unselected prostate cancer (family history for prostate cancer unknown). Collection of the Finnish families with prostate cancer has been previously reported (12). For SSCP, we randomly picked 32 samples from families that had either at least three affected members or two affected with unselected prostate cancer (family history for prostate cancer unknown). The mean number of affected family members in these 32 families was 3.6 (range, 2-7) and the mean age at diagnosis was 64.8 years (range, 50-76 years). The samples from
the unselected prostate cancer that were used in SSCP analysis were randomly picked among cases that had been diagnosed <60 years of age. The mean age at diagnosis of the 32 men with unselected prostate cancer was 54.9 years (range, 49-58 years). The patients were diagnosed during 1990 to 1999.

Variants and samples used in the large-scale population study. From the detected variants, 14 changed the protein sequence (SRD5A2 A49T, SRD5A2 V89L, HSD3B1 N367T, HSD3B1 R71T, HSD17B2 A111T, HSD17B3 G289S, AKR1C3 Q5H, AKR1C3 P180S, CYP19A1 R264C, KLK3 D102N, KLK3 L132I, KLK3 I179T, CYP19A1 T201M, HSD17B3 729_735 delGAATTAC). These and two found noncoding variants (CYP17A1 −34T>C and KLK3 −252A>G), previously reported to be associated with prostate cancer risk, were selected for large-scale analysis in 1,891 Finnish men (9, 13). In addition, based on previous reports of positive association with prostate cancer, variants AR R726L and LHB I15T were included in the study (14, 15). Patients with familial prostate cancer (n = 121) used in the genotyping of 18 selected variants had two or more first- or second-degree affected family members. The youngest affected member with available genotyping of 18 selected variants had two or more first- or second-degree affected family members. The youngest affected member with available genotyping of 18 selected variants had two or more first- or second-degree affected family members. The youngest affected member with available genotyping of 18 selected variants had two or more first- or second-degree affected family members. The youngest affected member with available genotyping of 18 selected variants had two or more first- or second-degree affected family members.

Written informed consent was obtained from all living patients and their family members and research protocols were approved by the Ethical Committee of the Tampere University Hospital. All prostate cancer diagnoses were confirmed through medical records or from the Finnish Cancer Registry.

Mutation screening with SSCP analysis. SSCP analysis (16) of the entire coding sequence of the genes SRD5A2, HSD17B2, HSD17B3, HSD3B1, HSD3B2, CYP11A, CYP17A1, CYP19A1, KLK3, and AKR1C3 was done using primer sequences that were designed to include all intron-exon boundaries. All primers are available in http://www.uta.fi/imt/schleutker/indexb.html. Arrays were spotted with allele-specific oligonucleotides from forward or reverse orientations. The PCR primer pairs were grouped into multiplex PCR reactions with four, four, four, and three primer pairs per reaction for 15 variants. Specific PCR conditions can be obtained from the corresponding author. Products of two multiplex PCRs were then pooled so that in the DNA transcription step using the T7 Amplitube Kit (Epiporechete Technologies, Madison, WI), there were four, four, and seven variants in the same reaction. A 5′ Cy5-A9J 3′ blocked probe was not used in the hybridization reaction. The microscope glass slides were scanned using the confocal ScanArray 4000 (GSI Lumonics, Watertown, MA). Ten-micron resolution, 16-bit TIFF images were analyzed using the QuantArray software (GSI Lumonics).

Table 1. Clinical and pathologic findings at diagnosis of the unselected prostate cancer patients

<table>
<thead>
<tr>
<th>Clinical/pathologic category</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage</strong></td>
<td></td>
</tr>
<tr>
<td>T1 stage</td>
<td></td>
</tr>
<tr>
<td>T1c-T2 (organ confined)</td>
<td>542 (64)</td>
</tr>
<tr>
<td>T2-T4 (extracapsular)</td>
<td>305 (36)</td>
</tr>
<tr>
<td>Prostate-specific antigen value at diagnosis</td>
<td></td>
</tr>
<tr>
<td>&lt;20 ng/mL</td>
<td>625 (74)</td>
</tr>
<tr>
<td>≥20 ng/mL</td>
<td>217 (25)</td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td></td>
</tr>
<tr>
<td>WHO grade</td>
<td></td>
</tr>
<tr>
<td>I (low)</td>
<td>165 (20)</td>
</tr>
<tr>
<td>II (mid)</td>
<td>526 (64)</td>
</tr>
<tr>
<td>III (high)</td>
<td>132 (16)</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
</tr>
<tr>
<td>2-6</td>
<td>349 (45)</td>
</tr>
<tr>
<td>7-10</td>
<td>432 (55)</td>
</tr>
</tbody>
</table>

The following variants were genotyped using allele-specific primer extension assay: LHB I15T, SRD5A2 A49T, SRD5A2 V89L, HSD3B1 N367T, HSD3B1 R71T, HSD17B2 A111T, HSD17B3 G289S, HSD17B3 729_735 delGAATTAC, AKR1C3 Q5H, AKR1C3 P180S, CYP19A1 R264C, CYP17A1 −34T>C, AR R726L, KLK3 D102N, and KLK3 L132I. Some modifications were made to the method described by Riise Stensland et al. (17). The allele-specific oligonucleotides and multiplex PCR primers are available at http://www.uta.fi/imt/schleutker/indexb.html. Sequencing was done using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit with the ABI 310 and ABI3100 sequencers (Applied Biosystems, Foster City, CA) according to the instructions of the manufacturer in 96-well format. The KLK3 −252A>G genotypes were determined by using the TaqMan SNP Genotyping Assays (Applied Biosystems). Sequencing results were confirmed by using the TaqMan Pre-Designed Assay. For KLK3 I179T genotyping, a Custom TaqMan SNP Genotyping Assay was ordered. Briefly, the DNA was amplified for I179T analysis using the following KLK3-specific primers: forward 5′-CCGTAGTCTTGGACCCCAAA-3′ and reverse 5′-CTTGGGCGGACACAGCT-3′. The KLK3 I179T genotypes were determined using the following fluorogenic allele-specific probes with a conjugated minor groove binder group: VIC-labeled 5′-CCTCTCATATTTCC-3′ for T allele and FAM-labeled 5′-CCCTCATTACCTTCC-3′ for C allele. The nucleotide sequences of the primers and probes used in the PCR were deduced from publicly available sequences deposited in the GeneBank database and were chosen and synthesized by Applied Biosystems using the Assay-by-Design service. DNA samples were genotyped by means of 5′ nuclease assay for allelic discrimination using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Known control samples previously genotyped by sequencing were run in parallel with unknown samples. After PCR, end-point fluorescence was measured and genotype calling was carried out using the allelic discrimination analysis module.

Sequencing. Sequencing was done using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit with the ABI 310 and ABI3100 sequencers (Applied Biosystems).

Minisequencing. The genotypes for CYP19 T201M alteration were determined by minisequencing. A 121-bp fragment was first amplified as follows: 100 ng of DNA, 200 mmol/L of both primers, 200 μmol/L of each deoxynucleotide triphosphate, 1.5 mmol/L MgCl2, and 1.5 units of AmpliTaqGold DNA Polymerase (Applied Biosystems) in a final volume of 50 μL. Only 95°C for 10 minutes, followed by 35 cycles of 95°C 30 seconds, 62°C for 30 seconds, and 72°C for 45 seconds, with a 5-minute extension at 72°C after the last cycle. Primers for PCR were 5′-AATCGGCAAGATGTGAGAGTG-3′ and reverse 5′-CTTCGGCGGACACAGCT-3′. The minisequencing was done as described by Syvanen (18) with detection primer 5′-CTTCGGCGGACACAGCT-3′. The results were confirmed by sequencing with ABI PRISM 310 Genetic Analyzer (Applied Biosystems) as recommended by the manufacturer. Primers used in sequencing were the same as those used in PCR.

Statistics. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated using logistic regression to estimate prostate cancer risk. Categorical variables were compared with the
Fisher’s exact test and Pearson χ² test for independence. These analyses were done with SPSS 11.0 statistical software package. The magnitude of the association between the CYPI9A1 and CYPI7A1 single nucleotide polymorphisms (SNP) and the occurrence of prostate cancer and other related outcomes was measured with the OR using polytomy logistic regression. Outcome definitions included WHO grade (2-3), Gleason score (2-10), prostate-specific antigen at diagnosis (<20 ng/mL), and T stage (T₁-T₂, T₃-T₄). Polytomy regression analyses were done with SPSS v8.0.

Bioinformatics. The effect of the T201M mutation on the structure and function of aromatase was investigated with several bioinformatic methods and tools including PHD (19, 20), PROF (19, 20), Jpred (21), SADM (22), and SIFT (23). The effect of point mutation had to be evaluated sequence based because the known structures are for the core domain of P450.

Results

Table 2 shows the genetic alterations found in the screening of 64 samples from prostate cancer patients in 10 target genes by SSCP analysis. A subset of the variants identified in the screening as well as two other variants (AR R726L, LHB I15T) previously suggested to be associated with prostate cancer (14, 15) were then selected for large-scale genotyping in 1,891 Finnish men (Supplementary Table S1). We identified an association of a previously unpublished SNP, CYPI9A1 T201M (602C>T), with unselected prostate cancer (OR, 2.04; 95% CI, 1.03-4.03; P = 0.04, Supplementary Table S1). Other variants showed no statistically significant association either with unselected or familial prostate cancer. For the rare mutations, no ORs were calculated because of a small number of carriers.

We also wanted to study whether the carrier status of the studied genotypes was associated with the clinicopathologic features (T stage, M stage, WHO grade, Gleason score, prostate-specific antigen at diagnosis, and age at diagnosis) of the unselected prostate cancer cases. KLK3 −252A>G carriers had more often a lower Gleason score (2–6) than noncarriers (P = 0.045, Pearson χ² test). LHB I15T showed a borderline association with organ-confined tumor (P = 0.074, Pearson χ² test). In contrast, carriers of the KLK3 I15T alteration were more likely to have metastases than noncarriers (P = 0.009, Pearson χ² test). To determine the nature of disease association with CYPI9A1 T201M, a polytomy logistic regression analysis was done (Table 3). Interestingly, the T201M association was only seen in patients with organ-confined disease as well as in those with a low prostate-specific antigen value at diagnosis. In contrast, individuals with severe stage classification showed no association with the CYPI9A1 T allele. We saw similar results for the histologic classifications, WHO grade, and Gleason score, in which individuals with less aggressive prostate cancer defined by a low-grade tumor (WHO grade I) were 4.5 times more likely to carry the CYPI9A1 T allele than population controls (OR, 4.5; 95% CI, 1.94-10.5; P < 0.0001). More severe cases (WHO II and III or Gleason >7) did not show an overrepresentation of the CYPI9A1 T allele.

To further refine our risk categories, we created a risk score in which an individual had severe or aggressive prostate cancer (T₃-T₄ and WHO grade II-III), moderate cancer (T₁-T₂ and WHO grade II-III), or clinically less significant cancer (Tᵢ-T₂ and WHO grade I). Individuals with clinically less significant prostate cancer were more than five times more likely to carry the CYPI9A1 T201M T allele than population-based controls (OR, 5.42; 95% CI, 2.33-12.6; P < 0.0001; Table 3). This association was still significant after the conservative Bonferroni correction (eight independent genes overall; n = 8). We used the same categories in the further analysis of CYPI7A1 −34T>C. No association was seen to clinically less significant prostate cancer (OR, 1.09; 95% CI, 0.75-1.59; P = 0.66). However, this alteration increased the risk for moderate cancer

Table 2. Variants found by SSCP analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amino acid change</th>
<th>Nucleotide*</th>
<th>Exon/intron</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRD5A2</td>
<td>A49T</td>
<td>145G&gt;A</td>
<td>Exon 1</td>
</tr>
<tr>
<td></td>
<td>V89L</td>
<td>265G&gt;C</td>
<td>Exon 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>281+15T-C</td>
<td>Intron 1</td>
</tr>
<tr>
<td>HSD17B3</td>
<td></td>
<td>154+(30_31)insT</td>
<td>Intron 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>201+38T-C</td>
<td>Intron 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>278−67G&gt;A</td>
<td>Intron 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>672+33A&gt;G</td>
<td>Intron 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>729+375delGATAACC</td>
<td>Intron 10</td>
</tr>
<tr>
<td>CYP11A</td>
<td></td>
<td>269+49C&gt;A</td>
<td>Intron 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>939C&gt;T</td>
<td>Exon 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1167C&gt;T</td>
<td>Exon 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−34T&gt;C</td>
<td>5’-UTR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>138C&gt;T</td>
<td>Exon 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>195G&gt;T</td>
<td>Exon 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1139+19T&gt;G</td>
<td>Intron 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>451+31T&gt;C</td>
<td>Intron 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>451+(35_36)insTTT</td>
<td>Intron 3</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>V80V</td>
<td>240A&gt;G</td>
<td>Exon 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>451+31T&gt;C</td>
<td>Intron 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>602C&gt;T</td>
<td>Intron 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>743+36A&gt;T</td>
<td>Intron 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>790C&gt;T</td>
<td>Exon 6</td>
</tr>
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<td>858+26C&gt;T</td>
<td>Intron 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1512+19C&gt;T</td>
<td>3'-UTR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−158G&gt;A</td>
<td>5’-UTR</td>
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<td>−252A&gt;G</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>−205_206nsA</td>
<td>5’-UTR</td>
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<td></td>
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<td>5’-UTR</td>
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<td>536T&gt;C</td>
<td>Exon 4</td>
</tr>
<tr>
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<td>786+15C&gt;T</td>
<td>3’-UTR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>252A&gt;G</td>
<td>Exon 5</td>
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<td>90G&gt;A</td>
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<td>Exon 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>681−20C&gt;G</td>
<td>Intron 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>929+40A&gt;G</td>
<td>Intron 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>930−1T&gt;G</td>
<td>Intron 8</td>
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<tr>
<td></td>
<td></td>
<td>972+8G&gt;A</td>
<td>3’-UTR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3310&gt;A</td>
<td>Exon 2</td>
</tr>
</tbody>
</table>

*Nucleotide numbering begins at the A in the start codon except for 5’-UTR variants of the KLK3 gene in which numbering starts at the beginning of the first exon.
(OR, 1.42; 95% CI, 1.09-1.83; P = 0.007). The association was marginally significant after the conservative Bonferroni correction (n = 8).

We further tested a hypothesis of a joint effect of CYP19A1 T201M with five common polymorphism (LHB, CYP19A1, and CYP17A1) in prostate cancer risk (Supplementary Table S2). Increased risk was noted in individuals carrying all five polymorphisms (LHB, CYP19A1, CYP17A1, and CYP17A1) with an increased risk for prostate cancer among the unselected prostate cancer cases (OR, 2.04; P = 0.04). Interestingly, stratified analysis revealed a strong association with clinically less significant cancer (OR, 5.42; P < 0.0001). The observation that CYP19A1 T201M variant did not have a significant effect on prostate cancer risk in our Finnish sample set. On the other hand, after classifying the patients according to clinical data, we observed that carriers of the C allele have increased risk for moderate cancer. In contrast to Modugno et al. (8) and Suzuki et al. (29), we saw no overall association between CYP19A1 T201M and prostate cancer risk in our Finnish sample set. In the present study, the allele frequencies were 0.9% (8 of 923) and 1.3% (11 of 847), respectively, and no statistically significant association was seen.

In a large meta-analysis of 10 studies by Ntais et al. (28) including samples from subjects of European, Asian, and African descent, no overall association between CYP19A1 T201M and prostate cancer risk was observed. However, in a subgroup of African descent, the C allele increased the prostate cancer risk, unlike in subjects of European and Asian descent. In this study, we saw no overall association between CYP19A1 T201M and prostate cancer risk in our Finnish sample set. On the other hand, after classifying the patients according to clinical data, we observed that carriers of the C allele have increased risk for moderate cancer. In contrast to Modugno et al. (8) and Suzuki et al. (29), we saw no association between the CYP19A1 R264C alteration and prostate cancer risk. However, the T allele of another alteration, T201M, in prostate cancer families, the possible effect may be masked by some other stronger genetic component.

The results from previous studies of prostate cancer predisposition have often been conflicting. The discrepancy between the studies may be due to sampling bias (e.g., small number of cases, often from highly selected hospital-based series), the choice of controls (benign prostate hyperplasia or non-prostate cancer controls, blood donors, etc.), or population stratification. However, the Finnish population is genetically very homogeneous (30) and, therefore, ideal for unbiased allele association studies. Our study was based on a large sample of unselected prostate cancer cases from the whole Pirkanmaa Hospital District, which represents

### Table 3. Association of the CYP19A1 T201M variant with prostate cancer

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>n (CT and TT)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 923)</td>
<td>13</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>T1-T2 (n = 542)</td>
<td>20</td>
<td>2.68 (1.32-5.43)</td>
<td>0.006</td>
</tr>
<tr>
<td>T1-T4 (n = 305)</td>
<td>4</td>
<td>0.93 (0.30-2.87)</td>
<td>0.90</td>
</tr>
<tr>
<td>Prostate-specific antigen at diagnosis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 923)</td>
<td>13</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&lt;20 ng/ml (n = 625)</td>
<td>19</td>
<td>2.19 (1.07-4.47)</td>
<td>0.031</td>
</tr>
<tr>
<td>≥20 ng/ml (n = 217)</td>
<td>5</td>
<td>1.65 (0.58-4.68)</td>
<td>0.88</td>
</tr>
<tr>
<td>WHO grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 923)</td>
<td>13</td>
<td>1.00</td>
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</tr>
<tr>
<td>I (n = 165)</td>
<td>10</td>
<td>4.51 (1.94-10.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>II (n = 526)</td>
<td>11</td>
<td>1.49 (0.66-3.36)</td>
<td>0.33</td>
</tr>
<tr>
<td>III (n = 132)</td>
<td>2</td>
<td>1.08 (0.24-4.82)</td>
<td>0.92</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 923)</td>
<td>13</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2-7 (n = 349)</td>
<td>12</td>
<td>2.49 (1.12-5.51)</td>
<td>0.024</td>
</tr>
<tr>
<td>7-10 (n = 432)</td>
<td>9</td>
<td>1.49 (0.63-3.51)</td>
<td>0.36</td>
</tr>
<tr>
<td>Combined WHO and T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 923)</td>
<td>13</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>WHO I and T1-T2 (n = 139)</td>
<td>10</td>
<td>5.42 (2.33-12.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WHO II-III and T1-T2 (n = 403)</td>
<td>10</td>
<td>1.78 (0.77-4.10)</td>
<td>0.17</td>
</tr>
<tr>
<td>WHO II-III and T1-T4 (n = 305)</td>
<td>4</td>
<td>0.93 (0.30-2.87)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

NOTE: The unselected prostate cancer cases were classified according to T stage, prostate-specific antigen value at diagnosis, WHO grade, and Gleason score.

### Discussion

Cancer growth depends on the ratio of cells proliferating to those dying. In prostate gland, androgens are the main regulator of this ratio by both stimulating proliferation and inhibiting apoptosis. Here, we carried out a comprehensive evaluation of the role of selected androgen pathway candidate genes in prostate cancer risk and in the clinical characteristics of the disease in large study cohorts involving almost 2,000 samples from patients and controls. First, prostate cancer patients were screened for genetic variation to identify those alterations that may be enriched in the prostate cancer population. Then 13 missense variants, one deletion resulting in a truncated protein and two 5'-untranslated region (UTR) variants, were selected for further analyses. In addition, the AR R726L and LHB I15T variants were included in the study based on information on disease association from the previous literature (14, 15).

Our results indicate that most of these alterations are not significantly associated with prostate cancer. We found no disease association for the LHB I15T, HSD3B1 N367T, SRD5A2 V89L, and HSD17B3 G289S variants which have been previously reported to increase prostate cancer risk (10, 15, 25, 26). In addition, we confirmed our earlier report in an extended cohort that the SRD5A2 variant A49T does not increase prostate cancer risk (27).

We have previously reported an increased risk among the AR R726L carriers (14). In the previous study, the allele frequency of the R726L mutation was 0.3% (3 of 900) in controls and 1.9% (8 of 418) among the unselected cases. In the present study, the allele frequencies were 0.9% (8 of 923) and 1.3% (11 of 847), respectively, and no statistically significant association was seen.

In Table 3, the association of the CYP19A1 T201M variant with prostate cancer is shown. The OR for the T201M variant is 2.04 (P = 0.04), indicating a significant increase in prostate cancer risk. The table also shows the association with other prostate-specific antigens and WHO grade.

The effect of the T201M mutation on the structure and function of aromatase was investigated with numerous bioinformatic methods and tools. Based on sequence database searches, position 201 is not highly conserved in aromatases and in P450 family. The residue most common in this position in the aromatase family is arginine. T201 is predicted to be close to the COOH-terminal end of a long α-helix by several methods including PHD, PROF, and Jpred. The mutation was predicted not to increase disorder or aggregation tendency of the protein. Predictions of surface accessibility of T201 are somewhat contradictory. It is possible that the residue is on the buried surface of the helix. Program SIFT (sorting intolerant from tolerant) uses multiple sequence information to predict whether an amino acid substitution affects protein function. According to SIFT prediction, T201M mutation is not tolerated. SIFT predictions have been shown to be very accurate in detecting deleterious mutations (24).
unbiased sampling of the entire population. The same is true for the control samples, which were collected from central, west-
southern, and eastern areas in Finland. Obviously, a small fraction of
population controls will get prostate cancer later in life. According to the statistics by the Finnish Cancer Registry, the
cumulative crude probability of a prostate cancer diagnosis up to
84 years of age is 8.5% based on current incidence rates.

Despite the small percentage of patients with CYP19A1 T201M
mutation, we were able to carry a preliminary gene–gene interaction
study of this alteration due to the large overall study
cohorts. A significant association to prostate cancer was seen when
the patient carried both CYP19A1 T201M and KLK3 252A>G
variants.

Due to the extensive use of prostate-specific antigen screening,
the most typical prostate cancers today are small organ–confined
cancers. Thus far, no diagnostic biomarkers indicating disease
aggressiveness and progression have been discovered. The CYP19A1
gene codes for a cytochrome P450 enzyme complex,
called aromatase, engaged in the biosynthesis of estrogens from
androgens in the testis. The T201M mutation of this protein could,
through altered enzyme function, affect testosterone levels in
the body. Based on the bioinformatic analyses, we can assume that
T201 is in structurally important α-helix, most likely at least partly
buried to the core of the protein. Substitution by methionine affects
the packing of the helix and, consequently, the local and
global fold of the enzyme. The effect may also rise from losing
stabilizing polar interaction(s) formed by the hydroxyl group of
threonine. Apparently, the change is not completely altering the
function of the protein, which could lead to modified activity and
phenotype. A higher activity of the variant enzyme could result in
lower levels of androgens in carriers as androgens are more
efficiently converted into estrogens.

Our findings suggest that the rare CYP19A1 T201M variant may
be associated with less aggressive prostate cancer and may serve as
a marker for individuals with clinically less significant disease. The
biochemical significance of the T201M alteration in aromatase
function warrants further studies. In addition, a multigenic model of
prostate cancer susceptibility is supported.

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