TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 626-640

doi:10.3906/sag-1501-124

Polymorphisms in androgen metabolism genes *AR*, *CYP1B1*, *CYP19*, and *SRD5A2* and prostate cancer risk and aggressiveness in Bulgarian patients

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Received: 23.01.2015 • **Accepted/Published Online:** 04.06.2015 • **Final Version:** 19.04.2016

Background/aim: The aim of our study was to elucidate the role of polymorphisms in *AR*, *CYP1B1*, *CYP19*, and *SRD5A2* genes for prostate cancer (PC) development in Bulgarian patients.

Materials and methods: We genotyped 246 PC patients and 261 controls (155 with benign prostate hyperplasia and 107 healthy population controls) using direct sequencing, PCR-RFLP, SSCP, and fragment analysis.

Results: The allele and genotype frequencies of most of the studied variants did not differ significantly between cases and controls. Increased frequencies of the C/C genotype and C allele of rs1056837 in *CYP1B1*, and genotype 7/8 of the (TTTA)n repeat polymorphism in *CYP19*, were observed in patients in comparison with controls.

The 8/9 and the 7/12 genotypes of (TTTA)n in *CYP19* showed suggestive evidence for association with decreased prostate cancer risk and the risk for aggressive disease, respectively. The haplotype analysis revealed 2 *CYP1B1* haplotypes associated with PC risk reduction.

Conclusion: Some *CYP1B1* haplotypes and genotypes of the *CYP19* (TTTA)n repeat appeared to be associated with disease risk or aggressiveness in Bulgarian PC patients. In contrast, the *SRD5A2* polymorphisms (V89L and (TA)n repeat), the CAG repeat in *AR*, and the Arg264Cys variant in *CYP19A1* are most likely not implicated in prostate carcinogenesis.

Key words: Association study, polymorphisms, prostate cancer, *AR*, *CYP1B1*, *CYP19*, *SRD5A2*

1. Introduction

1.1. Epidemiology

Benign and malignant disorders of the prostate are among the most common diseases affecting males, particularly in industrialized countries (1). Prostate cancer (PC) has been recognized as a clinical entity since antiquity, when it was first described by ancient Egyptians, while surgical procedures to remove the prostate were developed more than 100 years ago. However, the availability of highly accessible blood tests for prostate-specific antigen (PSA) has revolutionized the diagnosis of PC over the past 3 decades (2). Recent statistics demonstrate that PC is now the second leading cause of cancer death and the most commonly diagnosed malignancy in men in developed countries as a result of the increased availability of prostate-specific antigen testing (1,3–6).

PC was the second most common malignancy of all male cancers in Bulgaria in 2010 (11.2%) with 1734 new cases and it was the third leading cause for cancer deaths among males (8.1%). The expected number of new PC cases for 2013 with 95% confidence interval is 1851 (7).

1.2. Risk factors

Despite the substantial health impact of PC, the underlying etiology is still relatively poorly understood, with both genetic predisposition and environmental factors likely to be contributing to the risk of the disease (5). It is believed that the development of PC is a multiphase process and that the disease is heterogeneous (5). The only established risk factors are age, race, and family history (8). Evidence exists that the steroid hormone pathway and the genes involved in the metabolism of estrogens and androgens or having receptor function affect the risk of PC (9). Androgens are essential for the development, growth,

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and secretory activities of the prostate, whereas estrogens modulate these effects (9). A mutagenic effect of estrogen metabolites has long been hypothesized. Animal studies support this hypothesis, evidenced by an increased risk of prostatic dysplasia and carcinoma with estrogen treatment in a Noble rat model (10).

Some authors believe that high estrogen and low testosterone lead to the development of inflammation upon aging and the emergence of premalignant lesions (1), but others have shown that administration of testosterone induces prostate tumors in laboratory animals and PC regress after antiandrogen therapy (11).

The inconsistency of data from a variety of epidemiological studies might be due to the different measurements of plasma steroids, ethnic variability, and heterogeneity of the genetic background among individuals. In particular, single nucleotide polymorphisms (SNPs) of genes involved in both the metabolism and action of steroid hormones may be primarily implicated in the susceptibility to PC (12). Association between PC risk and SNPs in genes involved in sex hormone-related steroid pathways has been observed in different studies, providing further insight into the genetic basis of this disease (13,14).

The goal of the present study was to investigate the association of PC and polymorphisms in genes implicated in androgen metabolism such as *AR*, *CYP1B1*, *CYP19*, and *SRD5A2* in a cohort of Bulgarian patients and controls. All selected polymorphisms were previously examined in other populations and conflicting results have been reported due to different ethnic groups, study designs, sample sizes, environmental differences, genetic admixtures, and ascertainment bias.

2. Materials and methods

2.1. Study population (participants)

Blood samples of patients with benign prostatic hyperplasia (BPH) and PC were collected during their annual screening in the Clinic of Urology/Department of Urology, Alexandrovska University Hospital, Medical University of Sofia. For-cause biopsies were recommended for participants with abnormal digital rectal examinations and/or an increase in prostate-specific antigen. All subjects were of Bulgarian ethnicity. Each participant provided written informed consent according to protocols approved by ethical review board of the University.

We genotyped 246 patients with PC and 261 controls. The clinical characteristics of the PC cases are given in Table 1. The Gleason scoring system was used to classify tumors as low-grade (Gleason score of ≤7) or high-grade (Gleason score of >7).

The controls were matched to PC patients for age within 5-year categories. They included 155 individuals with BPH and normal PSA levels and 107 healthy individuals with unknown BPH and PSA status selected from the DNA biobank of the Molecular Medicine Center, Medical University of Sofia. The mean PSA value among controls with BPH was 1.71 ng/mL.

Table 1. Clinical characteristics of the PC cases

Parameter	N
Mean age	69.25 (SD = 8.16)
Mean PSA, ng/ml	34.56 (SD = 55.36)
PSA level at diagnosis	
< 10 ng/ml	86
10 - 30 ng/ml	91
> 30ng/ml	64
Unknown	5
Pathologic Gleason score	
≤ 6	103
7	72
≥ 8	71
Pathologic T stage	
T1	26
T2	123
T3	84
T4	13
Nodal invasion	
N0	166
N1	80
Metastasis	
M0	210
M1	36
Age at onset, yr	
≤ 64	67
> 64	172
PSA = prostate specific antigen	

2.2. DNA isolation and genotyping

DNA was isolated from blood samples using the Chemagic Magnetic Separation Station (CHENAGEN). The quality of isolated DNA was checked by agarose gel electrophoresis and the concentration was measured using a NanoDrop spectrophotometer (Thermo Scientific).

For the association study, 9 polymorphisms in 4 genes related to androgen metabolism were selected: **CAG** repeat polymorphism in exon 1 of *AR*, **rs1800440** (Asn453Ser, 4390A > G, CYP1B1*4), **rs1056837** (Asp449Asp, 4379C

> T), **rs1056836** (Leu432Val, 4326C > G CYP1B1*3), **rs1056827** (Ala119Ser, 355G > T CYP1B1*2) in *CYP1B1*, **rs700519** (Arg264Cys, R264C, 27142C > T) and **(TTTA)n** in *CYP19*, **rs523349** (Val89Leu), and **rs10529926** ((TA)n repeat polymorphism in 3'UTR) in *SRD5A2*. They were chosen according to the following criteria: 1) likely to be functional, 2) to have previously been associated with PC, and 3) to have minor allele frequency (MAF) of >0.1.

The genetic analysis was carried out with standard methods. The repeat polymorphisms in *CYP19*, *SRD5A2*, and *AR* were analyzed by fragment analysis on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems) after PCR amplification. The number of repeats was determined using molecular weight DNA standard GeneScan 500 Rox (Life Technologies). To confirm the results, some samples were sequenced.

2.2.1. *CYP1B1* SNPs

The SNPs in the *CYP1B1* gene were genotyped by polymerase chain reaction (PCR) amplification followed by direct sequencing using an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). The cycling conditions for genotyping the SNPs in exons 2 and 3 were as follows: exon 2 - 5 min at 95 °C and 35 cycles consisting of 40 s at 95 °C, 40 s at 55 °C, and 50 s at 72 °C, with a final extension of 5 min at 72 °C; exon 3 - 5 min at 95 °C and 35 cycles consisting of 30 s at 95 °C, 40 s at 56 °C, and 1 min at 72 °C, with a final extension of 7 min at 72 °C.

2.2.2. *AR* (CAG)n

PCR reactions for (CAG)n repeat polymorphism were carried out under the following conditions: 95 °C for 7 min; 35 cycles of 95 °C for 40 s, 66 °C for 40 s, and 72 °C for 1 min; and a final extension at 72 °C for 7 min. The number of repeats was determined using an ABI Prism 310 genetic analyzer running in parallel with a molecular weight DNA marker-Rox. To confirm the results, some samples were sequenced.

2.2.3. *SRD5A2* (TA)n

The (TA)n marker was genotyped using the following cycling conditions: 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 5 min.

2.2.4. *CYP19* (TTTA)n

The (TTTA)n repeat polymorphism was amplified in PCR reactions implemented as follows: after a first denaturation step of 11 min at 95 °C, reactions were submitted to 35 cycles consisting of 30 s at 94 °C, 30 s at 51 °C, and 30 s at 72 °C. A final extension of 10 min at 72 °C was performed, and then 3 µL of each PCR product was mixed with 9.25 µL of deionized formamide and 0.75 µL of a molecular marker (GeneScan 500 Rox). The fragments were then separated by capillary electrophoresis.

2.2.5. *SRD5A2* V89L

To detect the V89L polymorphism, PCR combined with restriction fragment length polymorphism (RFLP) was used. Briefly, PCR was carried out in a final volume of 20 µL containing 50 ng of genomic DNA. After a first step of 11 min at 95 °C, reactions were submitted to 35 cycles consisting of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C. A final extension of 10 min at 72 °C was then performed. After PCR amplification and testing of the products on 2% agarose gel electrophoresis, 10 µL of PCR product was digested with 5 units of RsaI in a final volume of 20 µL. Digestion was performed overnight at 37 °C. The products were then separated on 3% agarose gel stained with ethidium bromide to identify the base pair change. The genotyping was done blind to case or control status.

2.2.6. *CYP19* Arg264Cys

The *CYP19* Arg264Cys (rs700519) substitution, resulting from C-to-T polymorphism at codon 7, was detected using 2 different methods: the single-strand conformation polymorphism (SSCP) method and the RFLP method. To confirm the accuracy of the 2 methods used, we sequenced some PCR products on an ABI Prism 310 genetic analyzer (Applied Biosystems). The cycling conditions were as follows: 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were then tested by 2% agarose gel electrophoresis containing ethidium bromide. For SSCP analysis, PCR products (7 µL) were mixed with 10 µL of denaturing solution, incubated at 95 °C for 5 min, and chilled immediately on ice. Samples were electrophoresed on 15% 37.5:1 acrylamide:bis-acrylamide gel at 18 °C and 2000 V/cm for 4 h in 1X TBE buffer. After electrophoresis, gels were silver stained by Budowle method. For RFLP analysis, 10 µL of PCR products were digested with 5 units of Sfa NI in a final volume of 20 µL. Digestion was performed overnight at 37 °C. The products were then separated on 3% agarose gel containing ethidium bromide. Then fragments were visualized by UV illumination. Fragment sizes were estimated by comparison to a ladder on the same gel. The C-to-T substitution creates a recognition site for the Sfa NI restriction enzyme.

The sequences of the all primers used are shown in Table 2.

2.3. Statistics

A chi-square test was used to verify the Hardy–Weinberg equilibrium in haplotype analysis with HaploView 4.0 (Broad Institute of MIT & Harvard). The Fisher exact test was used to compare the distribution of genotypes and alleles between PC cases and controls (BPH and population controls), between PC cases and BPH controls, between PC cases and population controls, and between BPH controls and population controls. This test was performed using the online tools Vassar Stats (http://vassarstats.net/) and PLINK v.1.07 (http://pngu.mgh.harvard.edu/~purcell/

Table 2. Primers sequences used for genotyping and their annealing temperatures

Gene and studied polymorphisms	Primer sequence	Annealing temperature
AR, (CAG)n repeat polymorphism	F 5'- TCCAGAATCTGTTCCAGAGCGTGC- 3'	66°C
	R 5'- GCTGTGAAGGTTGCTGTTCCTCAT – 3'	
SRD5A2, rs523349 (V89L)	F 5' GCCACCTGGGACGTTACTTCTG-3'	60°C
	R 5'- TCCTTGGCGTTCCTCGGTGC-3'	
SRD5A2, (TA)n repeat (rs10529926)	F 5'-*Fam-GAAAACTGTCAAGCTGCTG-3'	55 °C
	R 5'-GGCAGAACGCCAGGAGAC-3'	
CYP19, rs700519 (Arg264Cys)	F 5'- CGCTAGATGTCTAAACTGAG-3'	55°C
	R 5'-CATATGTGGCATGGGAATTA-3'	
CYP19, (TTTA)n repeat polymorphism	F 5'-*Hex-TTATGAAAGGTAAGCAGGTACTTAG-3'	51°C
	R 5'-GTCGTGAGCCAAGGTCACT-3'	
CYP1B1, rs1800440 (S453N); rs1056837 (D449D) and rs1056836 (V432L)	F 5'-GCCTATTTAAGAAAAAGTGGAATTAAA-3'	56°C
	R 5'-ATTCATTTTCGCAGGCTCAT-3'	
CYP1B1, rs1056827 (A119S)	F 5'-CCCATAGTGGTGCTG AATGG-3'	55°C
	R 5'-TGTCCAGGATGAAGTTGCTG-3'	

plink/). One-tailed and 2-tailed P-values were calculated. $P < 0.05$ was considered significant. A separate test for deviation from Hardy–Weinberg equilibrium was made with an online tool from Helmholtz Zentrum München (https://www.helmholtz-muenchen.de/) using the Pearson P-values. Unconditional logistic regression was also used for comparisons between cases and controls and for risk attribution. This analysis was performed with SPSS 20. Statistical epistasis was evaluated with PLINK v1.07.

3. Results

In total 9 polymorphic variants were genotyped in 507 samples. All variants were successfully genotyped in >90% of the samples. The distributions of the genotypes and the alleles of *CYP1B1*, *CYP19*, and *SRD5A2* SNPs in PC patients and controls (BPH and population controls) are shown in Table 3. The allele and genotype distribution of repeat polymorphisms in *SRD5A2* and *CYP19* in patients and controls are shown in Table 4 and Table 5, respectively.

All genotype frequencies in patients with PC and controls follow the Hardy–Weinberg equilibrium for all SNPs, except *SRD5A2* V89L ($P = 0.0047$ for cases and $P = 0.03$ for controls). The deviation from Hardy–Weinberg equilibrium might be due to the action of selective pressure on the population or might be influenced by the genotyping method. The frequencies among controls in other studies summarized by Li et al. also deviated from Hardy–Weinberg equilibrium (15).

Our results for the SNP V89L in *SRD5A2* are consistent with previous metaanalyses that did not find evidence for a significant main effect of this polymorphism on prostate carcinogenesis (8,15–17). The V89L site was highly polymorphic; however, we did not observe any significant differences in its allele and genotype frequencies between patients and combined controls, nor between BPH and population controls or between patients versus BPH or population controls (Table 3). No association was found with Gleason score, tumor stage, metastasis, PSA, or age of diagnosis.

At the *SRD5A2* (TA)n repeat site, the (TA)o allele was the most common in both the cases (84.94%) and the controls (85.41%), while the $(TA)_9$ allele was less frequent in the cases (13.6%) and the controls (13.81%) (Table 4). Statistical analysis did not find any association of (TA)n polymorphism alleles or genotypes with PC in Bulgarian patients. Comparisons between PC cases and the 2 groups of controls separately and between BPH and population controls were also made, but no statistically significant differences were observed.

For *CYP19A1* (TTTA)n polymorphism, 8 alleles ranging from 7 to 14 repeats followed a bimodal distribution, with a peak at 7 repeats and another at 12 repeats (Figure 1). The 7/8 genotype was more frequent in patients with PC in comparison with one of the most common genotypes, 7/7; however, this difference did not reach statistical significance. We were not able to test the

Table 3. Genotype and allele frequencies of SNP in CYP1B1, CYP19 and SRD5A2 in patients and controls.

Gene/ Polymorphism	Genotype/ Allele	Cases	Controls	OR	95%CI	P (two–tailed)
CYP1B1, rs1056836	GG	32 (13.4%)	42 (16.73%)	0.77	0.47–1.27	0.32
	GC	108 (45.2%)	118 (47.01%)	0.93	0.65–1.32	0.72
	CC	99 (41.4%)	91 (36.26%)	1.24	0.86–1.79	0.27
	G	172 (36%)	202 (40.24%)	0.83	0.64–1.08	0.19
	C	306 (64%)	300 (59.76%)	1.2	0.92–1.55	0.19
CYP1B1, rs1056837	CC	99 (41.42%)	86 (34.54%)	1.34	0.93–1.93	0.13
	CT	108(45.19%)	121 (48.59%)	0.87	0.61–1.24	0.47
	TT	32 (13.39%)	42 (16.87%)	0.76	0.46–1.25	0.31
	C	306 (64%)	293 (58.84%)	1.24	0.96–1.61	0.1
	T	172 (36%)	205 (41.16%)	0.8	0.62–1.04	0.1
CYP1B1, rs1800440	AA	145 (60.67%)	154 (61.85%)	0.95	0.66–1.37	0.85
	AG	85 (35.56%)	87 (34.94%)	1.03	0.71–1.49	0.92
	GG	9 (3.77%)	8 (3.21%)	1.18	0.45–3.1	0.8
	A	375 (78.45%)	395 (79.32%)	0.95	0.7–1.29	0.75
	G	103 (21.55%)	103 (20.68%)	1.05	0.77–1.43	0.75
CYP1B1, rs1056827	GG	104 (45.41%)	129 (50.19%)	0.83	0.58–1.18	0.32
	GT	103 (44.98%)	108 (42.02%)	1.13	0.79–1.62	0.52
	TT	22 (9.61%)	20 (7.78%)	1.26	0.67–2.37	0.52
	G	311 (67.9%)	366 (71.21%)	0.85	0.65–1.12	0.29
	T	147 (32.1%)	148 (28.79%)	1.17	0.89–1.54	0.29
CYP19, rs700519 (Arg264Cys)	CC	223 (92.53%)	242 (92.72%)	0.97	0.5–1.9	1
	CR	18 (7.47%)	18 (6.7%)	1.09	0.55–2.15	0.86
	RR	0 (0%)	1 (0.38%)	0		1
	C	464 (96.27%)	502 (96.17%)	1.03	0.54–1.96	1
	R	18 (3.73%)	20 (3.83%)	0.97	0.51–1.86	1
SRD5A2, rs523349 (Val89Leu)	VV	17 (6.94%)	24 (16.73%)	0.71	0.37–1.37	0.33
	VL	128 (52.24%)	132 (47.01%)	1.01	0.71–1.44	1
	LL	100 (40.82%)	98 (36.26%)	1.1	0.77–1.57	0.65
	V	162 (33.06%)	180 (35.43%)	0.9	0.69–1.17	0.46
	L	328 (66.94%)	328 (64.57%)	1.11	0.86–1.44	0.46

gene dosage effect due to the small number of participants with the 8/8 genotype in the study. Genotype 8/9 was more frequent in controls in comparison with patients and showed a suggestive decrease of PC risk (OR = 0.41, 95% CI = 0.17–1, P = 0.058, 2-tailed) (Table 5). Genotype 7/12 was more frequent in patients with metastasis (31.43%) in comparison with controls (17.13%) (OR = 2.22, 95% CI = 1–4.86, P = 0.062, 2-tailed) (Table 6). Similarly, this genotype was more frequent in patients with metastasis (31.43%) in comparison with patients without metastasis (17.39%) and the result was close to statistical significance (OR = 2.17, 95% CI = 0.97–4.84, P = 0.064, 2-tailed). Comparison was made between PC patients with high (>7) and low (≤7) Gleason scores. The frequency of the 7/12 genotype in patients with less differentiated tumors (26.09%) was higher in comparison with those with highly differentiated tumors (12.62%) (OR = 2.44, 95% CI = 1.12–5.39, P = 0.028 2-tailed).

Table 4. Genotype and allele frequencies of SRD5A2 (TA)n repeat polymorphism in patients and controls.

Genotype/allele	Patients (n = 239)	Controls (n = 258)	OR	P (1-tailed)	P (2-tailed)
0/0	173 (72.38%)	185 (71.59%)	1.05	0.43	0.84
0/9	53 (22.18%)	67 (26.17%)	0.8	0.19	0.35
9/9	6 (2.51%)	3 (0.78%)	2.19	0.22	0.32
0/8	7 (2.93%)	4 (1.56%)	1.92	0.23	0.37
0	406 (84.94%)	440 (85.41%)	0.99	0.51	1
8	7 (1.46%)	4 (0.78%)	1.9	0.23	0.37
9	65 (13.6%)	72 (13.81%)	0.96	0.44	0.85

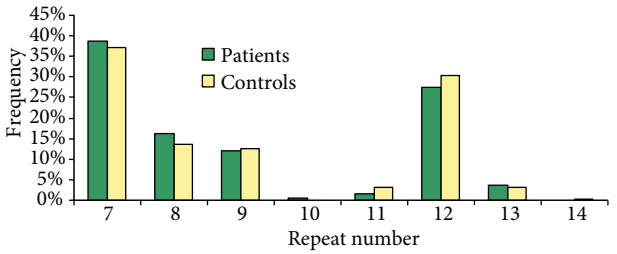

Figure 1. (TTTA)n repeat polymorphism in the *CYP19* gene in patients with PC and controls.

The *CYP19* Cys264Arg alteration showed no association with PC risk or with Gleason score, PSA, tumor stage, metastasis, or age at diagnosis in Bulgarian patients. The most plausible explanation for this result is the low allele frequency of the polymorphic variant and the small sample size. The observed allele frequency of the Arg allele was 3.73% among patients and 3.83% among controls. Homozygous carriers of the polymorphic allele were not observed among patients and only one was found among controls.

Among the polymorphic variants in *CYP1B1*, only rs1056837 showed any tendency for association with PC. The frequencies of the C allele and the C/C genotype of the rs1056837 variant were higher in patients in comparison with all controls (Table 3), and also between patients and population controls (OR = 1.46, OR = 1.46, 95% CI = 1.05–2.04, P = 0.026, 2-tailed for the C allele). The polymorphic variants in *CYP1B1* did not show any correlation with clinicopathological characteristics.

In Bulgarian patients and controls, a high linkage disequilibrium was found between the studied polymorphic variants. The 2-marker haplotype T-T (rs1056837-rs1056827) was found to be protective. It was more frequent in controls in comparison with patients (P = 0.019). The haplotypes A-T-G-T (rs1800440-rs1056837-rs1056836-rs1056827; P = 0.062) and T-G-T (rs1056837-rs1056836-rs1056827; P = 0.043) also showed association with a decreased risk of PC (Table 7). On the other hand, none of the haplotype combinations showed any association with aggressive disease.

The microsatellite (CAG)n in exon 1 of *AR* with 17 alleles found in patients and controls did not show any association with PC. The length of the repeats ranged between 14 and 29–31 with a peak at 21 repeats and another at 24 repeats (Figure 2). A repeat length of 30 was not observed. There was no significant difference between the frequencies of the short alleles (14–19 CAG repeats) among patients and controls. Correlation of the microsatellite with advanced disease was not observed.

Multigenic analysis was performed but no statistically significant additive effects or epistatic interactions were observed. Results from unconditional logistic regression are shown in Table 8. The polymorphism Cys264Arg was not included in this analysis due to its very low frequency in our samples. The polymorphisms rs1056836 and rs1056837 are in linkage disequilibrium and only one of them was included in the logistic regression analysis.

4. Discussion

Epidemiologic studies of PC risk factors, including genetic association studies, are challenging because PC is a heterogeneous disease (5). Many factors influence the genetic predisposition for prostate carcinogenesis and there are differences among populations (18).

In the present study we have explored the association of polymorphisms in genes implicated in androgen metabolism including *AR*, *CYP1B1*, *SRD5A2*, and *CYP19* with PC. This is the first study in the Bulgarian population and includes 246 PC patients and controls consisting of 155 BPH patients and 107 unaffected healthy men.

For evaluation of the results 2-tailed P-values were considered. No correction for multiple testing was performed, as we consider Bonferroni correction to

Table 5. Frequencies of the most common genotypes and frequencies of all observed alleles of CYP19 (TTTA)n repeat in patients and controls.

Genotype/allele	Patients (n = 242)	Controls (n = 251)	OR	P (2-tailed)
7/7	38 (15.70%)	48 (19.12%)	0.79	0.34
7/8	29 (11.98%)	19 (7.57%)	1.66	0.13
7/9	23 (9.50%)	13 (5.18%)	1.92	0.083
7/11	3 (1.24%)	8 (3.19%)	0.38	0.22
7/12	47 (19.42%)	43 (17.13%)	1.16	0.56
7/13	9 (3.72%)	7 (2.79%)	1.35	0.62
8/8	8 (3.31%)	7 (2.79%)	1.19	0.8
8/9	7 (2.89%)	17 (6.77%)	0.41	0.058
8/12	22 (9.09%)	16 (6.37%)	1.47	0.31
8/13	3 (1.24%)	1 (0.4%)	3.14	0.36
8/14	0 (0%)	1 (0.4%)	0	1
9/9	4 (1.65%)	3 (1.19%)	1.39	0.72
9/12	16 (6.61%)	19 (7.57%)	0.86	0.73
9/13	2 (0.83%)	5 (1.99%)	0.41	0.45
12/12	22 (9.09%)	34 (13.54%)	0.64	0.15
7	187 (38.64%)	186 (37.05%)	1.07	0.65
8	78 (16.11%)	68 (13.54%)	1.23	0.28
9	58 (11.98%)	63 (12.55%)	0.95	0.85
10	3 (0.61%)	0 (0%)	Infinity	0.12
11	7 (1.45%)	16 (3.19%)	0.45	0.09
12	133 (27.448%)	152 (30.28%)	0.87	0.36
13	18 (3.71%)	16 (3.19%)	1.17	0.73
14	0 (0%)	1 (0.2%)	0	1

be overly conservative in cases where the tests are not completely independent.

Unfortunately, we were not able to evaluate the influence of the studied polymorphisms while taking into account other factors (diet, smoking, infections, obesity, diabetes, and others) contributing to PC risk, development, and aggressiveness. It was not possible to check this in a stratified group analysis due to lack of sufficient data on all those confounding factors. This is one of the limitations of our study and highlights the importance of collecting prospective clinical and epidemiological data for future investigations. We are well aware that some of these environmental factors and comorbid conditions may interfere with the genetic factors and may even have a greater effect on the PC susceptibility and aggressiveness than the studied polymorphisms alone.

4.1. *AR*

The growth of the prostate gland depends on circulating androgens and intracellular steroid signaling pathways. The effects of androgens are mediated through the androgen receptor (AR), a nuclear transcription factor encoded by the *AR* gene. The common polymorphism, CAG repeat encoding polyglutamine residues (Q tract), in exon 1 of this gene has been implicated as a possible risk factor.

The normal range of CAG repeats is between 8 and 35 and the mean is about 20 repeats. This microsatellite repeat is associated with differences in AR activity. The shorter

Table 6. Results from analysis for association of studied polymorphisms with metastasis.

Gene/ polymorphism	Genotype/allele	Cases with metastasis	Controls	OR	95% CI	P (2-tailed)
CYP1B1, rs1056836	GG	6 (17.65%)	42 (16.73%)	1.07	(0.42–2.73)	1
	GC	14 (41.18%)	118 (47.01%)	0.79	(0.38–1.63)	0.58
	CC	14 (41.18%)	91 (36.26%)	1.23	(0.59–2.55)	0.71
	G	26 (38.24%)	202 (40.24%)	0.92	(0.54–1.54)	0.79
	C	42 (61.76%)	300 (59.76%)	1.09	(0.64–1.83)	0.79
CYP1B1, rs1056837	CC	14 (41.18%)	86 (34.54%)	1.33	(0.64–2.76)	0.57
	CT	13 (38.23%)	121 (48.59%)	0.65	(0.31–1.36)	0.28
	TT	7 (20.59%)	42 (16.87%)	1.28	(0.52–3.13)	0.63
	C	41 (60.29%)	293 (58.84%)	1.06	(0.63–1.78)	0.9
	T	27 (39.71%)	205 (41.16%)	0.94	(0.56–1.58)	0.9
CYP1B1, rs1800440	AA	21 (67.76%)	154 (61.85%)	1	(0.47–2.1)	1
	AG	11 (32.35%)	87 (34.94%)	0.89	(0.41–1.91)	0.85
	GG	2 (5.88%)	8 (3.21%)	1.89	(0.38–9.26)	0.61
	A	53 (77.94%)	395 (79.32%)	0.92	(0.5–1.7)	0.87
	G	15 (22.06%)	103 (20.68%)	1.09	(0.59–2)	0.87
CYP1B1, rs105682	GG	14 (43.75%)	129 (50.19%)	0.77	(0.37–1.62)	0.57
	GT	14 (43.75%)	108 (42.02%)	1.07	(0.51–2.25)	1
	TT	4 (12.5%)	20 (7.78%)	1.69	(0.54–5.31)	0.49
	G	42 (65.62%)	366 (71.21%)	0.77	(0.45–1.34)	0.38
	T	22 (34.38%)	148 (28.79%)	1.3	(0.74–2.24)	0.38
CYP19, rs700519 (Arg264Cys)	CC	33 (94.29%)	242 (92.72%)	1.3	(0.29–5.82)	1
	CR	2 (5.71%)	18 (6.7%)	0.82	(0.18–3.69)	1
	RR	0 (0%)	1 (0.38%)	0	–	1
	C	68 (97.14%)	502 (96.17%)	1.35	(0.31–5.92)	1
	R	2 (2.86%)	20 (3.83%)	0.74	(0.17–3.23)	1
SRD5A2, rs523349 (Val89Leu)	VV	2 (5.71%)	24 (16.73%)	0.58	(0.13–2.57)	0.56
	VL	19 (54.29%)	132 (47.01%)	1.1	(0.54–2.23)	0.86
	LL	14 (40%)	98 (36.26%)	1.06	(0.52–2.18)	1
	V	23 (32.86%)	180 (35.43%)	0.89	(0.52–1.52)	0.69
	L	47 (67.14%)	328 (64.57%)	1.12	(0.66–1.91)	0.69
CYP19, (TTTA)n repeat *	7/7	5 (14.29%)	48 (19.12%)	0.7	(0.26–1.91)	0.64
	7/8	6 (17.14%)	19 (7.57%)	2.53	(0.93–6.84)	0.1
	7/12	11 (31.43%)	43 (17.13%)	2.22	(1–4.86)	0.062
	12/12	2 (5.71%)	34 (13.54%)	0.39	(0.09–1.69)	0.28
SRD5A2, (TA)n repeat	0/0	27 (77.14%)	185 (71.59%)	1.36	(0.59–3.12)	0.55
	0/9	7 (20%)	67 (26.17%)	0.71	(0.3–1.71)	0.54
	9/9	1 (2.86%)	3 (0.78%)	2.5	(0.25–24.72)	0.4
	0/8	0 (0%)	4 (1.56%)	0		
AR, (CAG)n repeat	Short alleles	4 (11.43%)	25 (16.56%)	0.65	(0.21–2)	0.61
	Long alleles	31 (88.57%)	126 (83.44%)	1.54	(0.5–4.7)	0.61

*Results for some genotypes are shown.

Table 7. Statistical analysis of *CYP1B1* polymorphism (1- rs1800440; 2- rs1056837; 3- rs1056836; 4- rs1056827) haplotypes' associations with the risk for PC.

Haplotype				Frequency	Case, control ratios	Chi square	P
1	2	3	4				
A	T	G	T	0.011	0.004, 0.017	3.497	0.0615
	T	G	T	0.011	0.004, 0.017	4.115	0.0425
	T		T	0.014	0.005, 0.022	5.483	0.0192

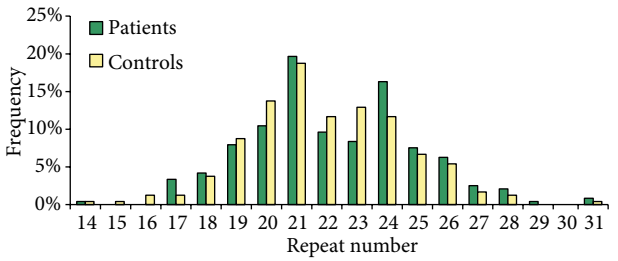

Figure 2. CAG repeat polymorphism in *AR* gene in patients with PC and controls.

CAG repeat length correlates with higher activity of the AR and is associated with a risk of developing PC, advanced cancer, biochemical failure, and associated mortality in some studies (19). In our cohort of Bulgarian patients and controls the length of the repeats ranged between 14 and 29 to 31, with a peak at 21 repeats and another at 24 repeats (Figure 2). Our results for CAG repeat polymorphism in *AR* (Tables 6 and 8) are consistent with some recent studies that did not find any association of this microsatellite with either PC risk (20,21) or with time to progression, overall survival, Gleason score, and clinical stage at diagnosis (22). Conflicting conclusions may be due to differences in design, small sample size in some studies, differences in environment, genetic admixture, ascertainment bias (including diagnosis before or after the age of PSA testing), and differences in tract length cut off points.

4.2. *CYP1B1*

As expression of the *CYP1B1* gene is elevated in hormone-mediated cancers, it has been proposed that it may affect steroid-related cancer risk. Cytochrome P450 1B1 (*CYP1B1*) is a member of the CYP1 gene family and is one of the major enzymes involved in the hydroxylation of 17β-estradiol (E2) at the C4 position and testosterone at the C6β and C12α positions (6). No CYP1B1 protein expression was detected in normal prostate tissue, in contrast to the overexpression of CYP1B1 protein in prostate carcinoma. This suggests that CYP1B1 could biotransform anticancer agents, specifically in target cells, and play a role in drug resistance. The overexpression of CYP1B1 has also been implicated in premalignant progression but is significantly higher in PC compared with benign prostate tissue (23).

CYP1B1 is an important enzyme in the activation of both environmental (polycyclic aromatic hydrocarbons, heterocyclic and aryl amines, and nitroaromatic hydrocarbons) and endogenous (estrogen) procarcinogens to reactive metabolites that cause DNA damage (24). The importance of CYP1B1 in chemical carcinogens is well illustrated in animal models, in which metabolites of CYP1B1 have been shown to induce PC (6).

CYP1B1 is highly polymorphic and its activity and catalytic specificity are regulated by several functional nonsynonymous SNPs (3). Many studies have evaluated the relationship between these SNPs and PC.

Tanaka et al. analyzed the genetic distribution of 6 polymorphisms (both coding and noncoding) in PC patients and unaffected controls in a Japanese population (25) and observed that the frequency of polymorphism in codon 119 (rs1056827) was significantly different between patients and healthy controls. For the other polymorphisms in codons 48, 432, and 449, they did not find any association with PC. The OR of rs1056827 genotype T/T was significantly high compared to wild-type G/G. The genotype T/T variant leading to Ala-to-Ser substitution at codon 119 of the enzyme displayed the highest 4-hydroxylation activity among other variants and was up to 4-fold times higher than the wild type. Products of this carcinogenic hydroxylation activity have been shown to cause adenocarcinoma in mice as well as DNA single-strand breaks, depurination, and mutation (25). The amino acid Ala119 is located in substrate recognition site 1 (SRS1) of the enzyme and influences substrate biding (24,26). In our study we did not find any difference in genotype or allele frequencies of rs1056827 (Ala119Ser) among patients and controls as in the study of Chang et al. (24); however, they observed association with increased or decreased risk for sporadic prostate cancer of haplotype combinations in which this polymorphism was included. Similar to that, certain haplotypes showed association with prostate cancer in our study.

Table 8. Results from multivariate unconditional logistic regression for evaluation of the studied polymorphisms and PC risk.

Gene/polymorphism	Genotype	Coefficient B	P	Exp (B) (95% CI)
CYP1B1, rs1056837	CC	Reference		
	CT	0.621	0.138	1.861 (0.82–4.22)
	TT	0.275	0.414	1.317 (0.680–2.55)
CYP1B1, rs1800440	AA	Reference		
	AG	−0.137	0.581	0.872 (0.54–1.42)
	GG	−0.341	0.559	0.711 (0.23–2.23)
CYP1B1, rs1056827	GG	Reference		
	GT	−0.092	0.714	0.912 (0.56–1.49)
	TT	−0.373	0.407	0.689 (0.29–1.66)
SRD5A2, rs523349 (Val89Leu)	VV	−0.442	0.256	0.643 (0.30–1.38)
	VL	−0.041	0.960	0.960 (0.63–1.46)
	LL	Reference		
CYP19, (TTTA)n	7/8 and 8/8	0.450	0.127	1.568 (0.88–2.8)
	7/12	0.406	0.120	1.501 (0.9–2.51)
	All other genotypes	Reference		
SRD5A2, (TA)n	Genotypes containing long alleles*	−0.150	0.517	0.861 (0.55–1.35)
	All other genotypes	Reference		
AR, (CAG)n	Short alleles**	0.200	0.455	1.222 (0.72–2.1)
	Long alleles***	Reference		
Constant	−0.362		0.696	

*$(TA)_8$ and $(TA)_9$ are considered for long alleles of the (TA)n *SRD5A2* repeat polymorphism.
**Short alleles are 14–19 CAG repeats of the (CAG)n polymorphism in *AR*.
***Long alleles are 20–31 CAG repeats of the (CAG)n polymorphism in *AR*.

The frequencies of C allele (64% in patients and 58.84% in controls) and the C/C genotype (41.42% in patients and 34.54% in controls) of the rs1056837 variant (Asp449Glu) were higher in patients in comparison with controls (Table 3), but the result did not reach statistical significance. The *CYP1B1* polymorphism rs1056836 alone also has also shown association with PC analyses (6), but other studies, including ours, could not confirm this association (25). CYP1B1*3 (rs1056836) polymorphism is located near the catalytically important heme-binding domain and leads to increased expression and catalytic activity of the enzyme due to change in the tertiary or quaternary structure of the protein. The CYP1B1*3 polymorphism encodes a protein that metabolizes estrogen into 4-OHE2 more efficiently, increasing the intracellular ratio with the minor metabolite 2-OHE2. Thus, castration-resistant PC patients carrying 2 copies of CYP1B*3 had significantly shorter overall-survival after docetaxel-based therapies (3). Metaanalyses showed that L432V (rs1056836) had no evidence for association with PC in the overall population, but after subgroup analysis by ethnicity, a strong association of this polymorphism with PC risk in Asians was found (27). In agreement with these metaanalyses our results demonstrated no statistically significant association with PC risk in Bulgarian patients (Table 3), but the result should be confirmed in a larger study.

CYP1B1*4 polymorphisms (rs1800440, N453S) are not associated with catalytic changes like CYP1B1*3 but have been associated with decrease in protein expression due to an increase in the CYP1B1 proteolytic degradation rate (3). We were not able to find any association of this polymorphic variant with PC, similar to several other studies (Table 3) (9).

Our results were consistent with the previous observations for association of some haplotype combinations, including rs1056836, with increased or decreased risk for sporadic PC. The study of Beuten et al. showed that a common haplotype C – G – C –C – G – A of rs2567206, rs2551188, rs2617266, rs10012, rs1056836, and rs1800440 is inversely associated with PC risk in Hispanic Caucasians and with aggressive disease status in non-Hispanic Caucasian cases. They also found that a second major haplotype T – A – T – G – C – A was positively associated with high-grade disease in non-Hispanic Caucasians (6). Association of T-C/T-C diplotypes of rs1056827 and rs1056836 with aggressiveness has been found by Cicek et al. (26).

In the present study we did not observe an association between the haplotype combinations of studied polymorphic variants and disease aggressiveness, but we found statistically significant association of the haplotype T – G – T (rs1056837-rs1056836-rs1056827) (P = 0.043) and T – T (rs1056837-rs1056827) (P = 0.019) with decreased PC risk in Bulgarian patients (Table 7).

4.3. *CYP19*

This cytochrome P450 enzyme is present in the gonads and in the extragonadal tissue, including the fibromuscular stroma of prostate and adipose tissue (28,29). *CYP19* encodes the enzyme aromatase that catalyzes the irreversible conversion of androstenedione to estrone and of testosterone to estradiol (13,30). These reactions are the last steps of estrogen biosynthesis (5). Lack of normal function could affect testosterone concentrations, in turn influencing the risk of PC (9). Aromatase mRNA and protein have both been detected in BPH and PC tissue (29). In light of these data, normal and benign prostates clearly have the capacity to locally metabolize androgens to estrogens via aromatase. The hormone balance and the ratio of androgens to estrogens play a pivotal role in prostate disease, particularly during later life (1). Significantly, the intraprostatic hormone levels do not always mirror systemic levels and, ultimately, the local hormone levels are those that are important for the maintenance of the prostatic health, as well as the development and progression of prostate disease (1,10).

Aromatase is expressed locally within the prostate and is aberrantly expressed in PC. Specifically, the induction of expression and altered promoter utilization with malignancy imply a shift in the local hormone balance and T:E (androgen:estrogen) ratio (1). Moreover, circulating estrogens can compete with androgens for binding to sex hormone-binding globulin, and it is generally assumed that sex hormone-binding globulin synthesis is regulated by and is a reflection of androgen/estrogen balance (14). This balance is critical for prostate health, and, consequently, any alteration in aromatase expression has the potential to shift this balance and exert profound effects via ERα, ERβ, and/or nonreceptor mediated effects (1). Therefore, it has been suggested that genetic variations in the aromatase gene *CYP19* alter an individual's risk of PC.

Several studies have investigated the role of different *CYP19* polymorphisms in PC. The most extensively studied polymorphisms are rs700519 (Arg264Cys, 27142C > T) in exon 7 and tetranucleotide repeat (TTTA)n in intron 4 with 7 alleles ranging in length from 7 to 13. The (TTTA)n repeat polymorphism is not close to any intronic splice sites, and therefore it is unlikely that it directly affects aromatase activity (10). It has been reported that the long allele of this repeat polymorphism is associated with early-onset PC (13) in one study, with PC risk in familial cases in another (31), and with poor survival in PC patients with bone metastasis at diagnosis in a third (32). In concordance, in our study we found that the long allele in genotype 7/12 increases the risk for development of metastasis (Table 6), but it was not possible to test allele dosage effect due to the rare 12/12 genotype and the small number of patients with metastasis.

Tang et al. found that the 8-repeat allele is more common in cases than in controls and the 7/8 genotype is significantly associated with an increased risk of PC, regardless of Gleason score, similar to our results. They also observed differences in serum estrone levels depending on individuals' genotypes. The 8/8 genotype had the highest levels of serum estradiol and estrone and the lowest level of serum testosterone (10). In our study there was no way to test the gene-dosage effect due to the low numbers of participants with the 8/8 genotype (Table 5).

There were studies that could not confirm the association of (TTTA)n polymorphism with PC (33). Furthermore, no association between pathological grade or stage, patient's age at onset, or preoperative PSA and repeat polymorphism was found by Cunningham et al. (34) and Latil et al. (31).

Cussenot et al. found an additive effect when they combined 2 risk alleles, rs1056836 in *CYP1B1* and the long allele of (TTTA)n in CYP19 (13), -but in our study we did not observe such a cumulative effect.

The polymorphism Arg264Cys is located in exon 7, and the C-to-T substitution in nucleotide 826 leads to an Arg to Cys change at codon 264 (12). It shows a tendency toward increased risk, especially with high-grade carcinoma (14). Modugno et al. evaluated the effect of the *AR* CAG repeat length and the aromatase genotype of rs700519. They found that short AR and C/T genotypes of aromatase have a 3-fold increase in risk for PC in a Japanese population (14). Using a "super control" group composed of male Sicilian centenarians, Balistreri et al. observed a significant difference in genotype distribution of Arg264Cys between

PC patients and controls (12). However, other large studies have failed to confirm the association of rs700519 with PC in different populations (18).

Travis et al. made a comprehensive study of the genetic variation at *CYP19* locus in relation to PC risk and to circulating steroid hormone concentrations in men from the Breast and Prostate Cancer Cohort Consortium. They analyzed 21 SNPs, including rs700519. Their results suggested that, although germline variations in *CYP19A1* characterized by the haplotype-tagging SNPs produce measurable differences in sex hormone concentrations in men, they do not substantially influence the risk of PC (28). In our study, rs700519 also did not show any association with PC (Table 3).

4.4. *SRD5A2*

Steroid 5-α-reductase type 2 (SRD5A2) is a critical enzyme in androgen metabolism (8,15). The *SRD5A2* gene encodes a membrane-bound enzyme, SRD5A2, which catalyzes the irreversible conversion of testosterone into a more potent androgen, dihydrotestosterone (DHT) (8). DHT binds to AR, and the DHT-AR complex stimulates the transcription of several genes with androgen responsive elements (5). The AR is normally associated with heat shock proteins in an inactive state. Androgen binding induces dissociation from heat shock proteins, hyperphosphorylation, conformational changes, and dimerization of the receptor. The binding affinity of DHT to the prostatic AR is 5 times higher than that of testosterone (29).

In humans, two 5α-reductase isoenzymes have been identified. The type 2 enzyme (encoded by the *SRD5A2* gene) is localized primarily in androgen target tissue, including genital skin and the prostate gland. Type 2 enzyme is involved in prostate development and growth.

DHT is mainly responsible for prostate growth, and it has been demonstrated that tissue DHT level is a useful marker in predicting the clinical response of PC to antiandrogen therapy. The levels of DHT and resulting androgen action vary among different individuals depending on the activity of 5α-reductase (5).

It has been shown that young Japanese men have lower 5α-reductase activity than young Caucasian-American and African-American men. DHT to testosterone ratio was highest in African-Americans, intermediate in Caucasian-American, and lowest in Asian-Americans, corresponding to the respective risk of developing PC in these groups (15).

Certain *SRD5A2* polymorphisms may encode 5α-reductase enzyme variants with different activities, probably due to altered mRNA stability (29). A number of mutations/polymorphisms have been identified in the *SRD5A2* gene; however, A49T, V89L, and (TA)n repeat polymorphisms are the most frequent. No clear consensus has been reached on the association between them and PC risk (15).

Markidakis et al. reported for the first time the missense substitution in the *SRD5A2* gene, which replaces valine at codon 89 with leucine. The V89L substitution results in an almost 30% reduction of reductase activity both in vitro and in vivo (35). It has been reported that the leucine allele reduces almost 30% of androstanediol glucuronide, a serum marker of 5α-reductase activity, among Asian men. Among Caucasian men, a 10% insignificantly lower androstanediol glucuronide level has been observed in individuals with the L/L genotype (12).

This polymorphism has different distributions in various ethnic groups and populations. It has low frequency in the African-American population and it is most common in Chinese and Japanese populations, who have the lowest risk for PC among the different racial groups (8).

Several studies supported the association of V89L and PC risk. In support of the finding that the distribution of V89L genotypes parallels the patterns of PC incidence in high- and low-risk populations, some authors have found that the Val allele increases the risk for PC in different populations (36). Conversely, other authors claimed that the Leu allele is responsible for increased PC risk (37).

Despite the studies that find a correlation of V89L with PC, the metaanalysis of Li et al. (8), including 24 case-control studies, and of Li et al. (15), including 31 studies, found that PC was not associated with this variant. The last metaanalysis including all 45 eligible studies of PC since January 1995 and combining a total of 15,562 cases and 15,385 controls also reported a lack of significant associations between V89L polymorphism and PC under all genetic models (38). Thus, our results for V89L polymorphism support the findings from the metaanalyses (Tables 3 and 8).

Another polymorphism in *SRD5A2* that has been extensively studied is the (TA)n dinucleotide repeat (rs10529926) on the 3' UTR region. Originally, 3 alleles were identified: $(TA)_0$, $(TA)_9$, and $(TA)_{18}$. The most common allele, accounting for 96% of the chromosomes, was $(TA)_0$. The observation that the rare $(TA)_{18}$ allele was limited to African-Americans, who have a higher rate of PC than Asian-American or Caucasian men, suggested that a longer allele might be associated with increased enzyme activity (39). The repeat was thought to cause instability of mRNA transcripts with UA-rich 3' UTRs but no association of the (TA)n variation with serum 3α-androstanediol glucuronide level was observed (5). Longer alleles were associated with a modest, insignificant decrease in PC risk, although some studies have failed to confirm this association. No association with PC was found for this polymorphism in a British population, in Caucasians and Asians, and only a modest association was found in a South Indian population (15). Our results show

that (TA)n repeat polymorphism is not associated with PC risk in a Bulgarian population (Tables 4 and 8), but a larger sample size needs be studied in order to confirm this conclusion (Table 4).

The metaanalysis of Li et al., including 31 association studies, concluded that there was a significant association between PC and the (TA)n polymorphism, with a long TA repeat decreasing the PC risk as compared with a short TA repeat (15).

Consequently, the biochemical effects of *SRD5A2* polymorphisms on enzymatic activities or androgen levels remain controversial, but, on the other hand, androgen deprivation therapy reduces the risk of PC recurrence and death, and chemoprevention with finasteride reduces PC incidence by approximately 25% (8). Another metaanalysis showed that (TA)n repeat polymorphism and A49T have some effect on PC risk but the probably additive effects of different loci have to be considered to evaluate the risk. In our study we did not observe any additive effects between the studied polymorphisms, probably due to small sample size.

Briefly, we have evaluated the role of polymorphisms in the androgen metabolism genes *AR*, *CYP1B1*, *CYP19*, and *SRD5A2* for PC development in Bulgarian patients. The allele and genotype frequencies of most of the studied variants did not significantly differ between cases and controls. An increased frequency in PC cases in comparison with controls was observed for the C/C genotype and C allele of rs1056837 in *CYP1B1*, and genotype 7/8 of the (TTTA)n repeat polymorphism in *CYP19A1*. Some tendency for association with decreased PC risk and the risk for developing metastasis was shown for the 8/9 genotype and the 7/12 genotype of the same polymorphism, respectively.

For the polymorphisms in *CYP1B1* we performed a haplotype analysis in order to check their cumulative effects. The *CYP1B* haplotypes T – T (rs1056837-rs1056827; P = 0.019) and T – G – T (rs1056837-rs1056836-rs1056827; P = 0.0425) showed statistically significant association with PC risk reduction.

In conclusion, the polymorphisms in the *CYP1B1* gene are probably associated with PC risk in Bulgarian patients since we obtained statistically significant results for some haplotype combinations. Some genotypes of the (TTTA)n repeat polymorphism in *CYP19* also showed association with PC susceptibility or aggressiveness.

Our results suggested that the polymorphisms V89L and (TA)n repeat in *SRD5A2*, as well as the CAG repeat in *AR* and the Arg264Cys variant in *CYP19A1*, most likely were not associated with PC in this Bulgarian population. The polymorphic variant Arg264Cys in *CYP19* was too rare to make meaningful conclusions for its impact on PC risk.

Even though the present data are consistent with some previous results from other populations and metaanalyses, further studies with larger sample sizes are needed to elucidate the contribution of the androgen metabolism-related polymorphisms to the risk of PC in Bulgarian men.

Acknowledgements

We are grateful to all patients and healthy volunteers for their participation in the study. This work was supported by the following grant funded by the National Science Fund: "Investigation of genetic variants associated with increased risk and searching for diagnostic and prognostic expression markers in PC" project (contract № D002 - 119/2009; and infrastructure grant DUNK01-2/2009).

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