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RESEARCH

Common Variants at 8q24 are Associated with Prostate Cancer Risk in Serbian Population

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Abstract Previous studies have shown correlation between single nucleotide polymorphisms (SNPs) at 8q24 and prostate cancer (PCa) risk. This study aimed to evaluate possible association between genotypes and alleles of 8q24 polymorphisms (rs1447295, rs4242382, rs6983267, rs7017300, and rs7837688) and PCa risk and progression. 150 patients with PCa, 150 patients with benign prostatic hyperplasia (BPH), and 100 healthy controls selected from the general population were recruited for this study. SNPs were genotyped by using PCR-RFLP analysis. There was a significant positive association between the A allele of the SNP rs4242382 and PCa risk [PCa vs. BPH comparison, P=0.014 for the best-fitting dominant model; odds ratio (OR) =1.98; 95 % confidence interval (95%CI) 1.14–3.43]. We found evidence (P=0.0064) of association between PCa risk and rs7017300 (heterozygote OR= 1.60; 95%CI 0.95–2.69) when comparing genotype distributions in PCa and BPH patients. The association between T allele rs7837688 and PCa risk was determined in PCa vs. BPH comparison with the best-fitting model of inheritance being log-additive (P=0.0033; OR=2.14, 95%CI 1.27–3.61). Odds ratio for carriers of rs6983267 TT genotype under recessive model of association with PCa was found to be 0.36 (PCa vs. control comparison, P=0.0029; 95%CI 0.19–0.71). For rs1447295, deviation from Hardy-Weinberg equilibrium was observed in BPH patients and controls. We found no association between parameters of PCa progression and five 8q24 SNPs. Locus 8q24 harbors genetic variants associated with PCa risk in Serbian population.

Keywords Prostate cancer · Association study · Polymorphism · Single nucleotide polymorphism (SNP) · 8q24

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Introduction

Prostate cancer (PCa) is one of the most common cancers among men in the majority of Western countries [1]. PCa is the most commonly diagnosed cancer in American men, accounting for more than one third of all new cancer cases [2]. The only firmly established risk factors for PCa are age, family history and ethnicity [3]. An epidemiological puzzle is the geographical distribution of PCa. Substantial differences exist in age-adjusted incidence and mortality between countries in Eastern Asia and Northwestern Europe [4]. In Serbian population, PCa shows increasing trend of newly diagnosed cases, from 662 in 1999 to 1.673 in 2009 [5, 6].

Genome-wide association analyses have identified variants in seven most important chromosomal regions associated with the risk of PCa. These variants occur in five independent regions at 8q24, in one region at 17q12 and another at 17q24.3 [7]. Five regions at 8q24 are believed to harbor genes that confer susceptibility to PCa and/or regulatory sequences affecting expression of critical genes, but these hypotheses remain to be confirmed [8]. A meta-analysis conducted by *Liu et al.* through data obtained from a large number of genome-wide association studies (GWAS), demonstrated the moderate effects of 31 single nucleotide polymorphisms (SNPs) on PCa risk [9]. The analysis also confirmed that 8q24 is the most frequently gained chromosomal region in prostate tumors [9]. In their original study, *Amundadottir* et al. identified a region on chromosome 8q24 showing suggestive linkage to PCa in Icelandic families [10]. Further analysis of this region led to the identification of two common variants (rs1447295 and DG8S737) associated with PCa risk in European and African populations [11]. Afterwards, *Zheng* et al. confirmed the association of SNPs at all five 8q24 regions with PCa risk in Swedish population [8]. Associations of 8q24 variants with aggressive PCa and/or increased tumor grade have been reported, but are yet to be confirmed. In the present population-based case–control study, we tested the association between PCa risk and SNPs rs1447295, rs4242382, rs6983267, rs7017300, and rs7837688 at 8q24 in the population of Serbia.

Materials and Methods

Subjects

Five 8q24 polymorphisms were genotyped in 150 patients with PCa (mean age: 69 years and 10 months; range: 45–96) and 150 patients with benign prostatic hyperplasia (BPH) (mean age: 68 years and 8 months; range: 33–85 years) who were treated at Clinical Centre "dr Dragiša Mišović", Belgrade, Serbia and Clinical Centre "Zvezdara", Belgrade, Serbia, in the period from 2008 to 2011.

All patients were diagnosed with PCa confirmed by histopathologic examination of specimens obtained by transrectal ultrasound (TRUS) guided biopsies, transurethral resection of the prostate and radical prostatectomy (RP). Grading was established according to Gleason score (GS) differentiation system (range 2–10) [12]. Serum prostate-specific antigen (PSA) levels were determined using Hybritech monoclonal immunoassay (*Beckman Hybritech assay*; *Beckman Coulter, Inc.,* Fullerton, CA, USA), with the cutoff value of 4.0 ng/ml [13]. Patients with BPH were given a digital rectal examination (DRE) (compatible with BPH) and serum PSA analysis. One hundred healthy volunteers with normal PSA and normal DRE who had no previous history of PCa or BPH served as the control group. The investigation was approved by the Ethics Committees of Clinical Centre "dr Dragiša Mišović" and Clinical Centre "Zvezdara", Belgrade, Serbia.

Risk of progression was determined by using two classification systems: one proposed by *D'Amico* et al [14]. and the other by *Medeiros et al* [15]. According to *D'Amico* criteria, PCa patients were divided into three groups: low risk (PSA ≤10 ng/ml, clinical stage ≤T2a, and Gleason score ≤6), medium risk (PSA from 10 ng/ml to 20 ng/ml or clinical stage T2b-c, or Gleason score 7), and high risk group (PSA ≥20 ng/ml, or clinical stage ≥T3, or Gleason score ≥8) [14]. Patients with metastases were added to high risk group. Following instructions of *Medeiros* et al., patients were stratified into two groups: high risk (Gleason score ≥7, or advanced clinical stage—T3 and T4, or presence of bone metastases), and low risk of cancer progression (low grade, early stage and absence of bone metastases) [16].

Peripheral blood samples were collected in vacutainer tubes containing Na-citrate, and maintained at 4 °C. All samples were obtained with the informed consent of the participants before their inclusion in the study.

DNA Extraction

Genomic DNA was extracted from 200 μl of peripheral blood obtained from PCa and BPH patients and buccal swabs provided by control subjects by using QIAamp DNA Blood Mini Kit (*Qiagen*, Hilden, Germany) following the manufacturers' instructions.

Amplification of the Regions Surrounding Selected SNPs

The primer sets used for amplification of the regions surrounding selected SNPs are shown in Supplementary Table S1. Each PCR was carried out in a 15 μl reaction mixture containing 0.3 μM (for rs1447295 and rs7837688) or 0.2 μM (for rs4242382, rs7017300 and rs6983267) of both primers (*Eurofins MWG Operon,* Huntsville, AL, USA), 200 μM of each dNTP (deoxyribonucleoside triphosphate, *Fermentas,* Hanover, MD, USA), 1.5 μl of 10X PCR buffer A (containing 15 mM $MgCl_2$, *Kapa Biosystems,* Woburn, MA, USA), 2 μl of genomic DNA (about 20 ng of DNA), 0.04 U/μl of Taq DNA polymerase (*Kapa Biosystems,* Woburn, MA, USA), and nuclease free water (*Serva,* Westbury, NY, USA). PCR amplification profiles are shown in Supplementary Table S2. The amplified fragments were separated by 1.5 % agarose gel electrophoresis and stained with ethidium bromide.

Genotyping

All five selected SNPs were genotyped by using PCR-RFLP analysis. 10 μl of PCR products were digested at optimal temperature overnight with 1U of specific enzyme per single

reaction (15 μl reaction mixture). Enzyme characteristics and lengths of digested PCR products are shown in Supplementary Table S3. Digested products were separated by 3 % agarose gel electrophoresis.

For each SNP, 15 random samples were chosen to be retyped by capillary gel electrophoresis in order to assess the validity of proper SNP analysis by PCR-RFLP method. Amplified fragments were purified by using QIAquick PCR Purification Kit, following manufacturers' instructions (*QIAquick PCR Purification Kit*, *Qiagen,* Hilden, Germany). Purified PCR products were sequenced with BigDyeTerm v1. 1 CycleSeq Kit (*Applied Biosystems*, Foster City, CA, USA). Afterwards, DNA sequencing reaction products were purified by using EDTA/ethanol purification method [16] and analyzed by capillary gel electrophoresis on ABI PRISM 3100 Genetic Analyzer (*Applied Biosystems,* Foster City, CA, USA).

Statistical Analysis

Descriptive statistics and exploratory analysis were used for data explanation. Deviations from Hardy-Weinberg equilibrium (HWE) were evaluated by using exact test implemented in SNPStats software (*Catalan Institute of Oncology,* Barcelona, Spain). For each SNP, multiple logistic regression models were used to assess potential association with PCa risk (dominant, recessive, codominant, overdominant and log-additive). The best-fitting models were determined by using Akaike information criterion (AIC). The odds ratio (OR) and its 95 % confidence interval (CI) were calculated as assessment measure of association between polymorphisms, genotypes and PCa risk.

In analyses of association between SNPs and PCa progression two-sided Pearson's chi-square test implemented in SPSS 17.0 (*SPSS Inc.,* Chicago, IL, USA) was used to assess *P* values for codominant, dominant and recessive inheritance models.

A 5 % level of significance was used in the analysis. Analyses of data were performed by using the computer software SPSS for Windows (Version 17.0, *SPSS Inc.,* Chicago, IL, USA) and SNPStats (*Catalan Institute of Oncology,* Barcelona, Spain).

Results

The frequencies of genotypes of five polymorphisms at 8q24 were compared between groups of 150 PCa patients, 150 BPH patients and 100 healthy control subjects. The clinical characteristics of PCa and BPH patients and histopathological characteristics of PCa patients are shown in Table 1. The mean age at diagnosis was 69 years and 10 months (range, 45–96) for PCa patients and 68 years and 8 months (range, 33–85) for BPH patients.

The distribution of rs4242382, rs7017300, rs7837688 and rs6983267 genotypes among patients in both PCa and BPH

Table 1 The clinical characteristics of patients with prostate cancer and benign prostatic hyperplasia and histopathological characteristics of patients with prostate cancer

Characteristics		PCa patients	BPH patients
Number		150	150
Mean age		69 years and 10 month (45 to 96)	68 years and 8 months (33 to 85)
Serum PSA	≤10 ng/ml	49	124
	10–20 ng/ml	39	20
	≥20 ng/ml	62	6
Tumor stage	T1	28	–
	T2	72	–
	T3 and T4	50	–
Gleason score	Gleason <7	71	–
	Gleason =7	56	–
	Gleason >7	22	–
Metastases	Absent	95	–
	Present	55	–
Risk of progression (*D'Amico* et al.)	High	14	–
	Medium	55	–
	Low	81	–
Risk of progression (*Medeiros* et al.)	High	95	–
	Low	55	–

Table 2 Association of five SNPs at 8q24 with prostate cancer risk, as assessed in comparison of genotype distributions between patients with prostate cancer and controls

SNP	Genetic model	No of PCa patients (%)	No of controls (%)	OR (95 % CI)	*P* value	AIC
rs1447295[a]	Codominant					
	CC	86 (57.3)	11 (11)	1		
	CA	61 (40.7)	82 (82)	**0.10 (0.05–0.19)**	**<0.0001**[b]	282
	AA	3 (2.0)	7 (7)	**0.05 (0.01–0.24)**		
	Dominant					
	CC	86 (57.3)	11 (11.0)	1	**<0.0001**	280.6
	CA+AA	64 (42.7)	89 (89.0)	**0.09 (0.05–0.19)**		
	Recessive					
	CC+CA	147 (98)	93 (93)	1	0.05	336.7
	AA	3 (2)	7 (7)	0.27 (0.07–1.07)		
	Overdominant					
	CC+AA	89 (59.3)	18 (18)	1	**<0.0001**	296.1
	CA	61 (40.7)	82 (82)	**0.15 (0.08–0.28)**		
	Log-additive					
	–	–	–	**0.13 (0.07–0.24)**	**<0.0001**	284
rs4242382	Codominant					
	GG	106 (70.7)	80 (80)	1		
	GA	42 (28.0)	20 (20)	1.58 (0.86–2.91)	0.11	338.2
	AA	2 (1.3)	0 (0.0)	NA (0.00–NA)		
	Dominant					
	GG	106 (70.7)	80 (80.0)	1	0.094	337.7
	GA+AA	44 (29.3)	20 (20.0)	1.66 (0.91–3.03)		
	Recessive					
	GG+GA	148 (98.7)	100 (100)	1	0.15	338.5
	AA	2 (1.3)	0 (0)	NA (0.00–NA)		
	Overdominant					
	GG+AA	108 (72)	80 (80)	1	0.15	338.4
	GA	42 (28)	20 (20)	1.56 (0.85–2.85)		
	Log-additive					
	–	–	–	1.70 (0.95–3.05)	0.068	337.2
rs7017300	Codominant					
	AA	100 (66.7)	84 (84.0)	1		
	AC	45 (30.0)	16 (16.0)	**2.36 (1.25–4.48)**	**0.0018**	329.9
	CC	5 (3.3)	0 (0.0)	NA (0.00–NA)		
	Dominant					
	AA	100 (66.7)	84 (84.0)	1	**0.0018**	330.8
	AC+CC	50 (33.3)	16 (16.0)	**2.62 (1.39–4.95)**		
	Recessive					
	AA+AC	145 (96.7)	100 (100)	1	**0.023**	335.3
	CC	5 (3.3)	0 (0)	NA (0.00–NA)		
	Overdominant					
	AA+CC	105 (70)	84 (84.0)	1	**0.01**	333.9
	AC	45 (30)	16 (16.0)	**2.25 (1.19–4.26)**		
	Log-additive					
	–	–	–	**2.63 (1.44–4.80)**	**8e-04**	329.2
rs7837688	Codominant					
	GG	101 (67.3)	82 (82.0)	1		
	GT	47 (31.3)	16 (16.0)	**2.38 (1.26–4.51)**	**0.02**	334.7
	TT	2 (1.3)	2 (2.0)	0.81 (0.11–5.89)		
	Dominant					
	GG	101 (67.3)	82 (82.0)	1	**0.009**	333.7

Table 2 (continued)

SNP	Genetic model	No of PCa patients (%)	No of controls (%)	OR (95% CI)	*P* value	AIC
	GT+TT	49 (32.7)	18 (18.0)	**2.21 (1.20–4.08)**		
	Recessive					
	GG+GT	148 (98.7)	98 (98)	1	0.68	340.3
	TT	2 (1.3)	2 (2)	0.66 (0.09–4.78)		
	Overdominant					
	GG+TT	103 (68.7)	84 (84.0)	1	**0.0052**	332.7
	GT	47 (31.3)	16 (16.0)	**2.40 (1.27–4.53)**		
	Log-additive					
	–	–	–	**1.89 (1.07–3.33)**	**0.022**	335.3
rs6983267	Codominant					
	GG	53 (35.3)	25 (25.0)	1		
	GT	80 (53.3)	49 (49.0)	0.77 (0.43–1.39)	**0.0081**	332.9
	TT	17 (11.3)	26 (26.0)	**0.31 (0.14–0.67)**		
	Dominant					
	GG	53 (35.3)	25 (25.0)	1	0.082	337.5
	GT+TT	97 (64.7)	75 (75)	0.61 (0.35–1.07)		
	Recessive					
	GG+GT	133 (88.7)	74 (74.0)	1	**0.0029**	331.6
	TT	17 (11.3)	26 (26.0)	**0.36 (0.19–0.71)**		
	Overdominant					
	GG+TT	70 (46.7)	51 (51)	1	0.5	340.1
	GT	80 (53.3)	49 (49)	1.19 (0.72–1.97)		
	Log-additive					
	–	–	–	**0.58 (0.39–0.85)**	**0.0043**	332.4

[a] Significant deviation from HWE

[b] Statistically significant results are shown in bold

groups, as well as among controls were compatible with Hardy-Weinberg equilibrium. For rs1447295, deviation from Hardy-Weinberg equilibrium was observed in groups of BPH patients and controls. For this reason, results regarding association of rs1447295 with PCa risk were not considered reliable, even though statistical significance was reached, and A allele was shown to confer reduced PCa susceptibility.

Table 2 shows genotype frequencies of 5 SNPs at 8q24 in PCa patients and controls, as well as the results of tests for association with PCa risk. Genotype distributions in PCa and BPH patients, together with the results of association tests regarding PCa risk are summarized in Table 3. Data concerning association of 8q24 SNPs with BPH risk are shown in Table 4.

When considering possible association between rs4242382 and PCa risk, the comparison of genotype frequencies in PCa patients and controls yielded no statistical significance for any genetic model tested. Nevertheless, the best-fitting log-additive model of association between A allele and PCa risk showed statistical trend ($0.05<P<0.1$) of significance (P=0.068; OR=1.70, 95%CI 0.95–3.05). When comparing genotype distributions in PCa and BPH patients, our results showed statistically significant association of rs4242382 with PCa risk (P=0.047, for codominant model; GA heterozygote OR=1.97, 95%CI 1.12–3.44; AA homozygote OR=2.34, 95%CI 0.21–26.17). AIC score suggested dominant model as the best-fitting one (P=0.014). Carriers of A allele were found to have 1.98 fold increased risk of PCa (95%CI 1.14–3.43) compared to non-carriers. No evidence of association between rs4242382 and BPH risk were obtained in our analysis (P=0.31, for the best-fitting recessive model).

Statistically significant difference in rs7017300 genotype frequencies was found between PCa patients and controls (P=0.0018, for codominant model). Minor allele C was shown to confer increased risk of PCa (heterozygote OR=2.36, 95%CI 1.25–4.48; homozygote OR not available due to lack of subjects with CC genotype in the control group). For PCa vs. controls comparison, log-additive model was selected as the best-fitting model of association between rs7017300 and PCa risk based on AIC score (P=8e-04; OR=2.63, 95%CI 1.44–4.80). When comparing genotype distributions in PCa and BPH patients, association of C allele with PCa risk was confirmed (P=0.0064, for codominant model). According to AIC score, the best-fitting model was found to be codominant (heterozygote OR=1.60, 95%CI 0.95–2.69; homozygote OR not available due to lack of subjects with CC genotype in the group of

Table 3 Association of five SNPs at 8q24 with prostate cancer risk, as assessed in comparison of genotype distributions between patients with prostate cancer and benign prostatic hyperplasia

SNP	Genetic model	No of PCa patients (%)	No of BPH patients (%)	OR (95 % CI)	*P* value	AIC
rs1447295[a]	Codominant					
	CC	86 (57.3)	96 (64.0)	1		
	CA	61 (40.7)	53 (35.3)	1.28 (0.80–2.05)	0.34	419.7
	AA	3 (2.0)	1 (0.7)	3.35 (0.34–32.80)		
	Dominant					
	CC	86 (57.3)	96 (64.0)	1	0.24	418.5
	CA+AA	64 (42.7)	54 (36.0)	1.32 (0.83–2.11)		
	Recessive					
	CC+CA	147 (98)	149 (99.3)	1	0.3	418.8
	AA	3 (2)	1 (0.7)	3.04 (0.31–29.57)		
	Overdominant					
	CC+AA	89 (59.3)	97 (64.7)	1	0.34	419
	CA	61 (40.7)	53 (35.3)	1.25 (0.79–2.00)		
	Log-additive					
	–	–	–	1.35 (0.87–2.10)	0.18	418.1
rs4242382	Codominant					
	GG	106 (70.7)	124 (82.7)	1		
	GA	42 (28.0)	25 (16.7)	**1.97 (1.12–3.44)**	**0.047**[b]	415.8
	AA	2 (1.3)	1 (0.7)	2.34 (0.21–26.17)		
	Dominant					
	GG	106 (70.7)	124 (82.7)	1	**0.014**	413.8
	GA+AA	44 (29.3)	26 (17.3)	**1.98 (1.14-3.43)**		
	Recessive					
	GG+GA	148 (98.7)	149 (99.3)	1	0.56	419.5
	AA	2 (1.3)	1 (0.7)	2.01 (0.18–22.45)		
	Overdominant					
	GG+AA	108 (72)	125 (83.3)	1	**0.018**	414.3
	GA	42 (28)	25 (16.7)	**1.94 (1.11–3.40)**		
	Log-additive					
	–	–	–	**1.89 (1.12–3.19)**	**0.014**	413.9
rs7017300	Codominant					
	AA	100 (66.7)	117 (78.0)	1		
	AC	45 (30.0)	33 (22.0)	1.60 (0.95–2.69)	**0.0064**	411.8
	CC	5 (3.3)	0 (0.0)	NA (0.00–NA)		
	Dominant					
	AA	100 (66.7)	117 (78.0)	1	**0.028**	415
	AC+CC	50 (33.3)	33 (22.0)	**1.77 (1.06–2.96)**		
	Recessive					
	AA+AC	145 (96.7)	150 (0)	1	**0.0081**	412.9
	CC	5 (3.3)	0 (0)	NA (0.00–NA)		
	Overdominant					
	AA+CC	105 (70)	117 (78.0)	1	0.11	417.4
	AC	45 (30)	33 (22.0)	1.52 (0.90–2.56)		
	Log-additive					
	–	–	–	**1.87 (1.16–3.02)**	**0.0091**	413.1
SNP	Genetic model	No of PCa patients (%)	No of controls (%)	OR (95 % CI)	*P* value	AIC
rs7837688	Codominant					
	GG	101 (67.3)	122 (81.3)	1		
	GT	47 (31.3)	28 (18.7)	**2.03 (1.19–3.47)**	**0.0082**	412.3
	TT	2 (1.3)	0 (0.0)	NA (0.00–NA)		
	Dominant					

Table 3 (continued)

SNP	Genetic model	No of PCa patients (%)	No of BPH patients (%)	OR (95% CI)	*P* value	AIC
	GG	101 (67.3)	122 (81.3)	1	**0.0053**	412.1
	GT+TT	49 (32.7)	28 (18.7)	**2.11 (1.24–3.61)**		
	Recessive					
	GG+GT	148 (98.7)	150 (0)	1	0.095	417.1
	TT	2 (1.3)	0 (0)	NA (0.00–NA)		
	Overdominant					
	GG+TT	103 (68.7)	122 (81.3)	1	**0.011**	413.4
	GT	47 (31.3)	28 (18.7)	**1.99 (1.16–3.40)**		
	Log-additive					
	–	–	–	**2.14 (1.27–3.61)**	**0.0033**	411.3
rs6983267	Codominant					
	GG	53 (35.3)	44 (29.3)	1		
	GT	80 (53.3)	80 (53.3)	0.83 (0.50–1.38)	0.25	419.2
	TT	17 (11.3)	26 (17.3)	0.54 (0.26–1.13)		
	Dominant					
	GG	53 (35.3)	44 (29.3)	1	0.27	418.7
	GT+TT	97 (64.7)	106 (70.7)	0.76 (0.47–1.23)		
	Recessive					
	GG+GT	133 (88.7)	124 (82.7)	1	0.14	417.7
	TT	17 (11.3)	26 (17.3)	0.61 (0.32–1.18)		
	Overdominant					
	GG+TT	70 (46.7)	70 (46.7)	1	1	419.9
	GT	80 (53.3)	80 (53.3)	1.00 (0.64–1.57)		
	Log-additive					
	–	–	–	0.76 (0.54–1.07)	0.11	417.4

[a] Significant deviation from HWE

[b] Statistically significant results are shown in bold

BPH patients). No significant association was found between rs7017300 and BPH risk.

Genotype frequencies of SNP rs7837688 were found to differ significantly between PCa patients and controls (P=0.02, for codominant model; GT heterozygote OR=2.38, 95%CI 1.26–4.51; TT homozygote OR=0.81, 95%CI 0.11–5.89). The best-fitting model for association between rs7837688 and PCa risk was found to be overdominant (P=0.0052). Men with GT genotype were shown to have 2.40 fold increased risk of PCa compared to men with homozygous genotypes (95%CI 1.27–4.53). The association between this SNP and PCa risk was confirmed by comparing genotype distributions among PCa and BPH patients (P=0.0082, for codominant model; heterozygote OR=2.03, 95%CI 1.19–3.47; homozygote OR not available due to lack of subjects with TT genotype in the group of BPH patients). The best-fitting model was shown to be log-additive (P=0.0033; OR=2.14, 95%CI 1.27–3.61). The comparison of genotype distributions among BPH patients and controls yielded no evidence of association of rs7837688 with BPH. Nevertheless, statistical trend of significance was determined for association of rs7837688 with BPH under recessive model, which is the best-fitting model according to AIC (P=0.055).

The comparison of rs6983267 genotype distribution among PCa patients and controls showed a 0.77 fold decrease in PCa risk for men with GT genotype compared to men homozygous for the G allele (P=0.0081, 95%CI 0.43–1.39). As for the men with TT genotype, the observed decrease in PCa risk compared to carriers of GG genotype was 0.31 fold (95%CI 0.14–0.67). Odds ratio for carriers of TT genotype under the best-fitting recessive model of association was found to be 0.36 (P=0.0029; 95%CI 0.19–0.71). Observed association of rs6983267 was not confirmed by comparing genotype frequencies in PCa and BPH patients. Comparison of genotype distributions among BPH patients and controls yielded no evidence of association between rs6983267 and BPH risk (P=0.098, for the best-fitting recessive model; OR=0.60, 95%CI 0.32–1.10).

When analyzing whether these 5 SNPs are in association with PCa progression, no correlation has been found (Supplementary Tables S4–S6). Genotype distributions among groups of patients have led to forming a great proportion of contingency tables with more than 20 % of cells

Table 4 Association of five SNPs at 8q24 with the risk of benign prostatic hyperplasia, as assessed in comparison of genotype distributions between patients with benign prostatic hyperplasia and controls

SNP	Genetic model	No of BPH patients (%)	No of controls (%)	OR (95 % CI)	*P* value	AIC
rs1447295[a]	Codominant					
	CC	96 (64.0)	11 (11)	1		
	CA	53 (35.3)	82 (82)	**0.07 (0.04–0.15)**	**<0.0001**[b]	263.8
	AA	1 (0.7)	7 (7)	**0.02 (0.00–0.15)**		
	Dominant					
	CC	96 (64.0)	11 (11.0)	1	**<0.0001**	264.5
	CA+AA	54 (36.0)	89 (89.0)	**0.07 (0.03–0.14)**		
	Recessive					
	CC+CA	149 (99.3)	93 (93)	1	**0.0045**	332.4
	AA	1 (0.7)	7 (7)	**0.09 (0.01–0.74)**		
	Overdominant					
	CC+AA	97 (64.7)	18 (18)	1	**<0.0001**	284.7
	CA	53 (35.3)	82 (82)	**0.12 (0.07–0.22)**		
	Log-additive					
	–	–	–	**0.08 (0.04–0.16)**	**<0.0001**	262.5
rs4242382	Codominant					
	GG	124 (82.7)	80 (80)	1		
	GA	25 (16.7)	20 (20)	0.81 (0.42–1.55)	0.49	341.1
	AA	1 (0.7)	0 (0.0)	NA (0.00–NA)		
	Dominant					
	GG	124 (82.7)	80 (80.0)	1	0.6	340.2
	GA+AA	26 (17.3)	20 (20.0)	0.84 (0.44–1.60)		
	Recessive					
	GG+GA	149 (99.3)	100 (100)	1	0.31	339.5
	AA	1 (0.7)	0 (0)	NA (0.00–NA)		
	Overdominant					
	GG+AA	125 (83.3)	80 (80)	1	0.5	340.1
	GA	25 (16.7)	20 (20)	0.80 (0.42–1.53)		
	Log-additive					
	–	–	–	0.88 (0.47–1.65)	0.7	340.4
rs7017300	Codominant					
	AA	117 (78.0)	84 (84.0)	1		
	AC	33 (22.0)	16 (16.0)	1.48 (0.77–2.86)	0.24	–
	CC	0 (0.0)	0 (0.0)	–		
	Dominant					
	AA	117 (78.0)	84 (84.0)	–	–	–
	AC+CC	33 (22.0)	16 (16.0)	–		
	Recessive					
	AA+AC	150 (0)	100 (100)	–	–	–
	CC	0 (0)	0 (0)	–		
	Overdominant					
	AA+CC	117 (78.0)	84 (84.0)	–	–	–
	AC	33 (22.0)	16 (16.0)	–		
	Log-additive					
	–	–	–	–	–	–
SNP	Genetic model	No of controls (%)	No of controls (%)	OR (95 % CI)	*P* value	AIC
rs7837688	Codominant					
	GG	122 (81.3)	82 (82.0)	1		
	GT	28 (18.7)	16 (16.0)	1.18 (0.60–2.31)	0.14	338.6
	TT	0 (0.0)	2 (2.0)	0.00 (0.00–NA)		

Table 4 (continued)

SNP	Genetic model	No of BPH patients (%)	No of controls (%)	OR (95% CI)	*P* value	AIC
	Dominant					
	GG	122 (81.3)	82 (82.0)	1	0.89	340.5
	GT+TT	28 (18.7)	18 (18.0)	1.05 (0.54–2.01)		
	Recessive					
	GG+GT	150 (0)	98 (98)	1	0.055	336.8
	TT	0 (0)	2 (2)	0.00 (0.00–NA)		
	Overdominant					
	GG+TT	122 (81.3)	84 (84.0)	1	0.59	340.2
	GT	28 (18.7)	16 (16.0)	1.20 (0.61–2.36)		
	Log-additive					
	–	–	–	0.93 (0.50–1.70)	0.8	340.4
rs6983267	Codominant					
	GG	44 (29.3)	25 (25.0)	1		
	GT	80 (53.3)	49 (49.0)	0.93 (0.51–1.70)	0.25	339.8
	TT	26 (17.3)	26 (26.0)	0.57 (0.27–1.18)		
	Dominant					
	GG	44 (29.3)	25 (25.0)	1	0.45	339.9
	GT+TT	106 (70.7)	75 (75)	0.80 (0.45–1.42)		
	Recessive					
	GG+GT	124 (82.7)	74 (74.0)	1	0.1	337.8
	TT	26 (17.3)	26 (26.0)	0.60 (0.32–1.10)		
	Overdominant					
	GG+TT	70 (46.7)	51 (51)	1	0.5	340.1
	GT	80 (53.3)	49 (49)	1.19 (0.72–1.97)		
	Log-additive					
	–	–	–	0.76 (0.53–1.10)	0.15	338.4

[a] Significant deviation from HWE

[b] Statistically significant results are shown in bold

having expected counts less than 5 (codominant and recessive model).

Discussion

The identification of prostate cancer susceptibility loci has proven to be challenging. Over the years, GWA studies have shown that the frequencies of risk alleles vary considerably across ethnic groups and that association of some SNPs with PCa is significant only in populations with specific ancestral background. These results are supportive to the hypothesis that the effect of genetic factors on PCa risk may be heterogeneous among different ethnic populations. The variations in results of studies on gene polymorphisms in PCa may be explained partially by ethnic differences, as well as with limited sample size, and other risk or protective factors [17].

First reported GWAS of PCa examined possible association of markers at chromosome 8q24 with the risk of PCa in Icelandic population [10]. Further analyses found a larger number of PCa risk markers in this region, of which many were replicated in multiple populations [3, 7–9, 17–42].

In this study, we examined the possible association of allelic variants of 5 SNPs at 8q24 with PCa risk in Serbian population for the first time. Additionally, we evaluated the possible linkage of these SNPs with standard prognostic parameters of PCa progression, as well as with the risk of disease progression assessed according to two classification systems.

Due to observed deviation from HWE, the statistically significant difference in rs1447295 genotype frequencies in PCa and BPH patients compared to controls should not be considered valid. Nevertheless, these findings are in concordance with the results of previous studies that characterized SNP rs1447295 as significantly associated with PCa risk [20]. Our data showing that A allele confers decreased risk of PCa are in contrast to the results of other GWA studies, which identified CA and AA genotypes as associated with PCa susceptibility [9, 19–21, 23–26, 36, 38, 39]. Studies conducted in European and several other populations

showed similar results [1, 3, 8, 10, 11, 18, 27–30]. However, study involving Dutch population did not replicate the previously observed association of rs1447295 allele A (P=0.10) and PCa risk [31]. Lack of evidence for this association was also observed in African American population [32, 33], as well as in European Americans [34]. Obtained results require a confirmation in further analyses that would include an increase in sample size in the population of Serbia in order to validate whether they are influenced by the interpopulational genetic differences.

We have detected the existence of statistically significant differences in rs4242382 genotype frequency between PCa and BPH patients. Results suggesting that A allele confers increased risk of PCa are consistent with findings obtained in studies conducted in other populations [8, 9, 18, 25, 36, 37, 39].

Evidence of association between rs7017300 and PCa risk were found by comparing genotype frequencies in PCa patients with those in both BPH and controls. C allele was identified as risk allelic variant, which is in consistence with other findings obtained in studies involving men of European ancestry [8, 18, 39, 40]. Furthermore, *Xu* et al. showed that rs7017300 C allele is associated with the increased risk of PCa in African Americans (P=0.03; allelic OR=1.2; 95%CI 1.00–1.5) [40].

When considering association between rs7837688 and PCa risk, we found that there is statistically significant difference in the genotype frequencies between PCa patients and controls, as well as between PCa and BPH patients. Our results regarding elevated PCa risk associated with T allele are consistent with finding of *Salinas* et al. who observed that allele T confers susceptibility to PCa in Caucasians (P=0.0006, for codominant model; heterozygote OR=1.37, 95%CI 1.13–1.67, homozygote OR=2.14, 95%CI 1.12–4.10), but not in African Americans (P=0.18, for dominant model OR=1.69, 95%CI 0.77–3.73) [22].

Several studies have suggested association between rs6983267 and PCa risk [8, 9, 23, 24, 26, 28, 35, 38–42]. Our results showing decreased risk of PCa associated with GT and TT genotypes, which is demonstrated by comparing genotype frequencies in PCa patients and controls, were consistent with previously published data. However, some studies did not replicate the previously observed association of rs6983267 allele G with PCa susceptibility [18, 29, 32–34, 36, 37].

We found that the values of parameters of PCa progression were independent of five PCa-susceptibility SNPs in Serbian population. Similarly, no association of these SNPs and PCa aggressiveness was previously determined in Swedish (for all five SNPs), African-American (for rs1447295), and European American (for rs4242382 and rs6983267) populations [8, 19, 37]. Conversely, association between rs1447295 and PCa aggressiveness was reported among European American and Polish populations [11, 30], as well as in Japanese population [3], in which association between rs1447295 and high Gleason score was shown [1, 27]. When interpreting our results, it is of great importance to consider possible errors and misleadingness associated with violations of Cochran's rule about small expected cell counts [43].

Conclusion

Five SNPs included in our study appear to be associated with the risk of prostate cancer in general, rather than with the more or less aggressive form of disease.

Conflict of Interest The Authors declare that there is no conflict of interest.

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