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ORIGINAL ARTICLE

Genetic variants in RNA-induced silencing complex genes and prostate cancer

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Abstract
Purpose The purpose of this study is to evaluate the potential association between genetic variants in genes encoding the components of RNA-induced silencing complex and prostate cancer (PCa) risk. Genetic variants chosen for this study are rs3742330 in *DICER1*, rs4961280 in *AGO2*, rs784567 in *TARBP2*, rs7813 in *GEMIN4* and rs197414 in *GEMIN3*.
Methods The study involved 355 PCa patients, 360 patients with benign prostatic hyperplasia and 318 healthy controls. For individuals diagnosed with PCa, clinicopathological characteristics including serum prostate-specific antigen level at diagnosis, Gleason score (GS) and clinical stage were determined. Genotyping was performed using high-resolution melting analysis, PCR–RFLP, TaqMan SNP Genotyping Assay and real-time PCR-based genotyping assay using specific probes. Allelic and genotypic associations were evaluated by unconditional linear and logistic regression methods.
Results The study provided no evidence of association between the analyzed genetic variants and PCa risk. Nevertheless, allele A of rs784567 was found to confer the reduced risk of higher serum PSA level at diagnosis ($P = 0.046$; Difference $= -66.64$, 95 % CI -131.93 to 1.35, for log-additive model). Furthermore, rs4961280, as well as rs3742330, were shown to be associated with GS. These variants, together with rs7813, were found to be associated with the lower clinical stage of PCa. Also, rs3742330 minor allele G was found to be associated with lower PCa aggressiveness ($P = 0.036$; OR 0.14, 95 % CI 0.023–1.22, for recessive model).
Conclusions According to our data, rs3742330, rs4961280 and rs7813 qualify for potentially protective genetic variants against PCa progression. These variants were not shown to be associated with PCa risk.

Keywords Prostate cancer · rs3742330 · rs4961280 · rs784567 · rs7813 · rs197414

Introduction

Recent statistics on prostate cancer (PCa) show that this malignancy is the second most commonly diagnosed cancer among males. Furthermore, it contributes substantially to cancer-related death rates, ranking fifth on mortality scales in global male population [1]. Among the newly diagnosed, latent PCa that remains indolent during the life time represents a significant percent. According to estimations based on clinical reports from developed countries, as much as a half of patients are overdiagnosed with PCa [2], which leads to unnecessary morbidity due to application of invasive therapeutic procedures [3]. Therefore, among prevailing aims in modern scientific research on PCa is to identify genetic markers potentially significant for constructing reliable algorithms applicable for assessing the risk of disease progression to a more aggressive form [4].

Regulatory activities of microRNA molecules have been extensively studied in the last decade [5]. During this period, numerous lines of evidence have been found to support the involvement of deregulations in RNA interference

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process in carcinogenesis in various tissues, including prostatic [6–9]. These data mostly consist of the observed differences in microRNA and their targets' expression between normal and malignant cells [8, 10]. Furthermore, forced or silenced expression of numerous microRNAs was found to be correlated with presentation of various aspects of PCa malignant phenotype in in vitro systems [10].

Due to their potential effect on microRNA biogenesis and function, components of RNA-induced silencing complex (RISC) could also be involved in molecular pathogenesis of PCa. This hypothesis is augmented by the recent data indicating the aberrant expression of several components of this protein complex in malignant, compared to normal prostatic tissue [9, 11]. Also, downregulated or increased expression of several RISC proteins has been associated with cancer growth, apoptosis, as well as with the development of metastases [11, 12].

Based on these data, genetic variants potentially influencing the biogenesis and function of microRNAs qualify for candidates in case–control studies on PCa risk and progression [13–16]. Among these genetic variants are, therefore, those located in genes encoding the components of RISC: *DICER1*, *AGO2*, *TARBP2*, *GEMIN3* and *GEMIN4* [17].

To date, microRNA genetic variants have been evaluated for their potential association with PCa in both Asian and European populations [13–16]. Still, those located in RISC genes have been analyzed in a single study in Han Chinese [18]. The only component of RISC that was a subject of this study is GEMIN4. Evidence was obtained supporting the association of two *GEMIN4* variants (rs2740348 and rs7813) with PCa risk. Furthermore, three genetic variants were found to be associated with the increased stage of localized PCa [18].

Since the association between variants located within genes encoding the components of RISC and PCa risk has not been analyzed in European populations, we conducted a case–control study in Serbian population. Potentially functional genetic variants chosen for this study are rs3742330 in *DICER1*, rs4961280 in *AGO2*, rs784567 in *TARBP2*, rs7813 in *GEMIN4* and rs197414 in *GEMIN3*. Furthermore, we have evaluated the possible association of selected genetic variants with standard prognostic parameters of PCa progression, as well as with the risk of PCa progression. Genetic variants included in this study were selected based on their potential functional significance and also for their previously found association with PCa and/or other malignant diseases.

Materials and methods

The study used peripheral blood samples obtained from patients treated in the period between 2009 and 2013 at Clinical Centre "Dr Dragiša Mišović Dedinje", Belgrade, Serbia. Research was conducted with the approval of ethics committee of this medical institution. Written informed consents were obtained from participants before their inclusion in the study. Experiments are in accordance with the Helsinki Declaration of 1964.

Three hundred and fifty-five samples of peripheral blood were obtained from patients with PCa and 360 samples from patients with benign prostatic hyperplasia (BPH). The control group comprised 318 healthy volunteers who gave samples of buccal swabs. The exclusion criteria for potential controls were the presence of any self-reported diseases and family history of PCa. Controls were recruited after passing standard annual physical examination. Mean ages for PCa, BPH patients and controls were 69.91, 68.10 and 69.11 years, respectively. Diagnoses of PCa and BPH were made by using standard clinical procedure which included digital rectal examination, transrectal ultrasonography, abdominal and pelvic ultrasound, bone scintigraphy and radiography, serum prostate-specific antigen (PSA) level and biopsy of the prostate. Serum PSA levels were determined by Hybritech method of monoclonal immunoassay. Clinical stage of cancer was determined according to TNM classification system. H&E-stained slides of paraffin-embedded prostate biopsy material were used to determine histological type of cancer and Gleason score (GS).

Patients with PCa were selected into groups based on the values of standard prognostic parameters—PSA at diagnosis (PSA < 10 ng/ml; 10 ng/ml ≤ PSA ≤ 20 ng/ml; PSA > 20 ng/ml), Gleason score (GS < 7; GS = 7; GS > 7) and clinical stage (T1; T2; T3/T4). Two groups of patients were formed based on the presence of distant metastases. Based on the risk for localized cancer progression, three groups of patients were formed, according to D'Amico criteria and as recommended by European Association of Urology (EAU). Groups were defined as low-risk (PSA < 10 ng/ml, GS < 7, and clinical stage T1–T2a), intermediate-risk (PSA 10–20 ng/ml or GS = 7 or clinical stage T2b–T2c) and high-risk (PSA > 20 ng/ml or GS > 7 or stage T3/T4) [19]. Since patients with metastases were included in the study, the criteria were modified to include this subset into high-risk group. Patients were also selected into low-risk (Gleason score <7 and stage T1–T2) and high-risk (Gleason score ≥7 or stage T3/T4 or bone metastases) groups according to Medeiros et al. [20].

Genomic DNA was isolated from peripheral blood and buccal swab samples using the QIAamp® DNA Mini Kit (*QIAGEN*, Hilden, Germany) following the manufacturers' protocol.

Genotyping of rs3742330 was performed by high-resolution melting analysis (HRMA). Primers used to amplify 198-bp-long segment of DNA surrounding this genetic variant were designed by using Primer3 software [21, 22]:

5′-CAAAGTCTTCACTTCCCTGCCA-3′ and 5′-GATGTTTAACTCCTCTCCACGTGATC-3′. The amplifications were performed in 10 µl volumes containing 10–20 ng of genomic DNA, 1X MeltDoctor™ HRM Master Mix (*Applied Biosystems*, Foster City, CA, USA), 0.3 µM of both primers (*Invitrogen,* Life Technologies, Grand Island, NY, USA) and nuclease-free water (*Serva*, Westbury, NY, USA). PCR cycling included an initial denaturation and enzyme activation at 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C and 15 s at 60 °C. The heteroduplex formation step included denaturation at 95 °C for 10 s, followed by 30 s at 40 °C, while the melt curve run included hold at 60 °C for 1 min, 15 s at 95 °C with ramp rate of 0.3 % during which fluorescence was acquired and 15 s at 60 °C. The HRMA was performed by using High Resolution Melting Software version 3.0.1 (*Applied Biosystems*, Foster City, CA, USA).

Genotyping of rs4961280 was performed by custom-designed real-time PCR-based genotyping assay using specific probes (*PrimerDesign Ltd*, Southampton, UK). The assay was carried out using the standard method recommended by the manufacturer.

HRMA was used to genotype rs784567. Primers used to amplify 198-bp-long segment of DNA surrounding this genetic variant were designed by using Primer3 software [21, 22]: 5′-AGCCCTGCGGAAACAGAG-3′ and 5′-GTCGGATCCTGGCTCTTTG-3′. PCR amplification, melting curve run and the analysis of the obtained results were conducted as described for rs3742330.

Genotyping of rs7813 was performed using TaqMan® SNP Genotyping Assay (*Applied Biosystems*, Foster City, CA, USA). The assay was carried out using the standard method recommended by the manufacturer.

Genetic variant rs197414 was genotyped by using PCR–RFLP method. Primers used to amplify a segment of DNA surrounding rs197414 were designed by using Primer3 software [21, 22]: 5′-TCTTCCCAGAGCAAAGGAAA-3′ and 5′-TGGTGGTTGTTCCAAAGAAA-3′. The 15-µl PCR reaction mixture contained approximately 50 ng of genomic DNA, 0.3 µM of both primers (*Invitrogen, Life Technologies*, Grand Island, NY, USA), 200 µM of each dNTP (*Fermentas*, Hanover, MD, USA), 1.5 µl of 10X PCR buffer A (containing 15 mM MgCl2, *Kapa Biosystems*, Woburn, MA, USA), 0.04 U/µl of Taq DNA polymerase (*Kapa Biosystems*, Woburn, MA, USA) and nuclease-free water (*Serva,* Westbury, NY, USA). After initial denaturation at 97 °C for 3 min, PCR reactions were run for 35 cycles: 95 °C for 60 s, 62 °C for 60 s and 72 °C for 60 s, while final extension was at 72 °C for 10 min. The amplified fragments were separated by 1.5 % agarose gel electrophoresis and stained with ethidium bromide. Their expected length was 111 bp. Ten microliters of PCR products were digested at 37 °C overnight with 1 U of MboI enzyme (*Fermentas*, Hanover, MD, USA) per single reaction (15-µl reaction mixture). Digested products were separated by 3 % agarose gel electrophoresis. The expected lengths of fragments resulting from restriction digest were 84 and 27 bp for CC genotype, 111, 84 and 27 bp for CA genotype and 111 bp for AA genotype.

Statistical analysis of SNP association was done using SNPStats software [23, 24]. Hardy–Weinberg equilibrium was assessed using exact test implemented in SNPStats software. Allelic and genotypic associations were evaluated by unconditional linear (for serum PSA level in PCa patients) and logistic regression method with adjustment for age. Separate comparisons were done for five different genetic models: allelic (log-additive), codominant, dominant, recessive and overdominant. Odds ratio (OR) and its 95 % confidence intervals (95 % CI) were used as risk estimates. The best-fitting models were determined by using Akaike information criterion (AIC).

Results

Clinical and pathological characteristics of PCa patients are presented in Table 1. According to available data, 15.8 % of PCa patients had distant metastases at diagnosis, while the most frequently determined serum PSA scores were higher than 20 ng/ml. Also, the majority of PCa patients had GG = 6 (53.8 %) as well as clinical stage T2 of primary cancer (55 %). Not all of the PCa patients were included in the tests of association of the analyzed genetic variants with the values of standard prognostic parameters and PCa progression risk due to the lack of data on initial serum PSA, GS or clinical stage in patients' records. Also, for some patients, clinical stage was initially assessed according to different classification systems which could not be reliably converted to TNM stages. Furthermore, instead of GS, histological grades were obtained for several patients in the initial period of the collection of data.

Basic data of the genetic variants selected for the analysis in this study are presented in Table 2. Genotyping of all five genetic variants analyzed was successful for more than 99 % of subjects (Table 3). Genotype distributions, presented in Table 3, were not found to significantly deviate from HWE (Table 2).

By comparing genotype distributions among PCa patients and healthy controls, as well as among PCa and BPH patients, no evidence of association between the analyzed genetic variants and PCa was found (Table 3). Nevertheless, a statistical trend of significance was reached for association between rs784567 and the risk of developing

Table 1 Classification of patients with PCa based on the values of standard prognostic parameters of disease progression, presence of metastases and the risk of cancer progression

Standard prognostic parameter	PCa patients; *n* (%)
PSA at diagnosis	
<10 ng/ml	99 (28.2)
10–20 ng/ml	102 (29.1)
>20 ng/ml	150 (42.7)
Gleason score	
4	8 (2.3)
5	16 (4.7)
6	184 (53.8)
7	81 (23.7)
8	31 (9.1)
9	19 (5.5)
10	3 (0.9)
Clinical stage	
T1	49 (15.8)
T2	170 (55)
T3/T4	90 (29.2)
Metastases	
Distant (M+)	51 (15.8)
Regional (N+) or not detected	271 (84.2)
Risk of progression (D'Amico et al.)	
Low	22 (6.6)
Medium	115 (34.3)
High	198 (59.1)
Risk of progression (Medeiros et al.)	
Low	142 (43.1)
High	187 (56.9)

PSA prostate-specific antigen

PCa ($P = 0.055$, for dominant model). Also, rs4961280 minor allele A was found to confer the reduced risk of developing BPH ($P = 0.03$; OR 0.74, 95 % CI 0.56–0.97, for log-additive model), while the opposite direction of association was found for rs784567 minor allele A ($P = 0.013$; OR 1.52, 95 % CI 1.09–2.11, for dominant model; results not shown).

Allele A of rs784567 was found to confer the reduced risk of higher serum PSA level at diagnosis ($P = 0.046$; Difference = −66.64, 95 % CI −131.93 to 1.35, for log-additive model). Besides for log-additive genetic model, statistical significance was obtained for dominant model ($P = 0.05$; Table 4). The analysis of association between other analyzed genetic variants and the serum PSA level at diagnosis did not show statistical significance for any genetic model tested (results not shown).

When genotype frequencies among PCa patients with GS < 7 and GS > 7 were compared, rs3742330 minor allele G was found to confer the decreased risk for high GS ($P = 0.027$; OR 0.40, 95 % CI 0.16–1.00, for log-additive model; Table 5). Furthermore, the comparison of genotype distributions among PCa patients with GS > 7 and GS = 7 showed the statistical trend for the association between this variant and GS ($P = 0.059$). Also, the same comparison showed that rs4961280 minor allele A confers the decreased risk of high GS ($P = 0.045$; OR 0.45, 95 % CI 0.20–1.00, for overdominant model). Conversely, opposite direction of association was observed in comparison of rs4961280 genotype distributions among patients with GS < 7 and GS = 7 ($P = 0.033$; OR 1.64, 95 % CI 1.04–2.58, for log-additive model).

By comparing genotype frequencies among subgroups of PCa patients with primary tumor clinical stages T1 and T2, rs4961280 and rs7813 minor alleles were found to be associated with the lower stage disease ($P = 0.0013$; OR 0.34, 95 % CI 0.18–0.66, for dominant model; and $P = 0.035$; OR 0.46, 95 % CI 0.22–0.97, for dominant model, respectively; Table 6). The results for rs4961280 were also confirmed by the comparison involving PCa patients with T3 and T1 stages ($P = 0.037$; OR 0.47, 95 % CI 0.23–0.96, for dominant model). *DICER1* genetic variant rs3742330 was also shown to be associated with the

Table 2 Genetic variants selected for the analysis

Gene	Genetic variant	Chromosomal location	Position within the gene	Major allele	Minor allele	HWE *P* value
DICER1	rs3742330	chr14:95087025	3′-UTR	A	G	0.12
TARBP2	rs784567	chr12:53500681	Promoter	G	A	0.052
AGO2	rs4961280	chr8:140637315	Promoter	C	A	0.87
GEMIN3	rs197414	chr1:111766501	Exon (p.R693S)	C	A	0.13
GEMIN4	rs7813	chr17:744946	Exon (p.R1033C)	A	G	0.13

Table 3 Association of genetic variants within *DICER1*, *AGO2*, *TARBP*, *GEMIN4* and *GEMIN3* with PCa risk

SNP	Genetic model	No of PCa patients (%)	No of controls (%)	No of BPH patients (%)	PCa versus controls			PCa versus BPH		
					OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC
rs3742330										
	Codominant									
	AA	285 (80.7)	259 (81.5)	276 (76.9)	1.00	0.91	928.7	1.00	0.49	984.2
	AG	62 (17.6)	53 (16.7)	76 (21.2)	1.09 (0.73–1.64)			0.80 (0.55–1.16)		
	GG	6 (1.7)	6 (1.9)	7 (2)	0.92 (0.29–2.92)			0.88 (0.29–2.71)		
	Dominant									
	AA	285 (80.7)	259 (81.5)	276 (76.9)	1.00	0.73	926.8	1.00	0.24	982.3
	AG + GG	68 (19.3)	59 (18.6)	83 (23.1)	1.07 (0.73–1.58)			0.80 (0.56–1.16)		
	Recessive									
	AA + AG	347 (98.3)	312 (98.1)	352 (98)	1.00	0.87	926.9	1.00	0.89	983.7
	GG	6 (1.7)	6 (1.9)	7 (2)	0.91 (0.29–2.87)			0.93 (0.30–2.83)		
	Overdominant									
	AA + GG	291 (82.4)	265 (83.3)	283 (78.8)	1.00	0.67	926.7	1.00	0.24	982.3
	AG	62 (17.6)	53 (16.7)	76 (21.2)	1.09 (0.73–1.64)			0.80 (0.55–1.16)		
	Log-additive									
	–	–	–	–	1.05 (0.74–1.47)	0.8	926.9	0.84 (0.61–1.15)	0.28	982.5
rs4961280										
	Codominant									
	CC	230 (64.8)	194 (61)	243 (67.5)	1.00	0.51	930.3	1.00	0.54	988.5
	CA	112 (31.6)	108 (34)	109 (30.3)	0.88 (0.63–1.22)			1.09 (0.79–1.50)		
	AA	13 (3.7)	16 (5)	8 (2.2)	0.68 (0.32–1.46)			1.61 (0.65–3.98)		
	Dominant									
	CC	230 (64.8)	194 (61)	243 (67.5)	1.00	0.33	928.7	1.00	0.47	987.2
	CA + AA	125 (35.2)	124 (39)	117 (32.5)	0.85 (0.62–1.17)			1.12 (0.82–1.53)		
	Recessive									
	CC + CA	342 (96.3)	302 (95)	352 (97.8)	1.00	0.38	928.9	1.00	0.32	986.8
	AA	13 (3.7)	16 (5)	8 (2.2)	0.71 (0.33–1.51)			1.56 (0.64–3.85)		
	Overdominant									
	CC + AA	243 (68.5)	210 (66)	251 (69.7)	1.00	0.53	929.3	1.00	0.7	987.6
	CA	112 (31.6)	108 (34)	109 (30.3)	0.90 (0.65–1.25)			1.06 (0.77–1.46)		
	Log-additive									
	–	–		–	0.86 (0.66–1.12)	0.26	928.4	1.14 (0.87–1.50)	0.35	986.9

Table 3 continued

SNP	Genetic model	No of PCa patients (%)	No of controls (%)	No of BPH patients (%)	PCa versus controls			PCa versus BPH		
					OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC
rs784567										
	Codominant									
	GG	100 (28.2)	111 (34.9)	94 (26.1)	1.00			1.00		
	GA	169 (47.6)	139 (43.7)	190 (52.8)	1.36 (0.95–1.93)	0.15	927.8	0.84 (0.59–1.19)	0.33	987.5
	AA	86 (24.2)	68 (21.4)	76 (21.1)	1.43 (0.94–2.17)			1.10 (0.72–1.67)		
	Dominant									
	GG	100 (28.2)	111 (34.9)	94 (26.1)	1.00	0.055[b]	925.9	1.00	0.59	987.5
	GA + AA	255 (71.8)	207 (65.1)	266 (73.9)	1.38 (0.99–1.92)			0.91 (0.65–1.27)		
	Recessive									
	GG + GA	269 (75.8)	250 (78.6)	284 (78.9)	1.00	0.34	928.7	1.00	0.25	986.4
	AA	86 (24.2)	68 (21.4)	76 (21.1)	1.19 (0.83–1.72)			1.23 (0.86–1.75)		
	Overdominant									
	GG + AA	186 (52.4)	179 (56.3)	170 (47.2)	1.00	0.32	928.6	1.00	0.15	985.7
	GA	169 (47.6)	139 (43.7)	190 (52.8)	1.17 (0.86–1.58)			0.81 (0.60–1.08)		
	Log-additive									
	–	–	–	–	1.21 (0.98–1.49)	0.078[b]	926.5	1.04 (0.84–1.28)	0.74	987.6
rs7813										
	Codominant									
	AA	114 (32.1)	101 (31.7)	122 (33.9)	1.00			1.00		
	AG	181 (51)	169 (53)	176 (48.9)	0.96 (0.68–1.36)	0.79	933.2	1.09 (0.79–1.52)	0.86	989.4
	GG	60 (16.9)	49 (15.4)	62 (17.2)	1.12 (0.70–1.78)			1.03 (0.66–1.60)		
	Dominant									
	AA	114 (32.1)	101 (31.7)	122 (33.9)	1.00	0.99	931.7	1.00	0.65	987.5
	AG + GG	241 (67.9)	218 (68.3)	238 (66.1)	1.00 (0.72–1.38)			1.08 (0.79–1.47)		
	Recessive									
	AA + AG	295 (83.1)	270 (84.6)	298 (82.8)	1.00	0.52	931.2	1.00	0.89	987.7
	GG	60 (16.9)	49 (15.4)	62 (17.2)	1.15 (0.76–1.74)			0.97 (0.66–1.44)		
	Overdominant									
	AA + GG	174 (49)	150 (47)	184 (51.1)	1.00	0.62	931.4	1.00	0.59	987.5
	AG	181 (51)	169 (53)	176 (48.9)	0.93 (0.68–1.26)			1.08 (0.81–1.46)		
	Log-additive									
	–	–	–	–	1.04 (0.83–1.30)	0.73	931.5	1.03 (0.83–1.27)	0.81	987.7

Table 3 continued

SNP	Genetic model	No of PCa patients (%)	No of controls (%)	No of BPH patients (%)	PCa versus controls			PCa versus BPH		
					OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC
rs197414										
	Codominant									
	CC	268 (75.9)	233 (73)	264 (73.5)	1.00			1.00		
	CA	75 (21.2)	75 (23.5)	81 (22.6)	0.88 (0.61–1.27)	0.75	930.3	0.90 (0.63–1.29)	0.68	985.1
	AA	10 (2.8)	11 (3.5)	14 (3.9)	0.83 (0.34–2.00)			0.74 (0.32–1.70)		
	Dominant									
	CC	268 (75.9)	233 (73)	264 (73.5)	1.00	0.45	928.3	1.00	0.45	983.3
	CA + AA	85 (24.1)	86 (27)	95 (26.5)	0.87 (0.62–1.24)			0.88 (0.62–1.23)		
	Recessive									
	CC + CA	343 (97.2)	308 (96.5)	345 (96.1)	1.00	0.72	928.8	1.00	0.51	983.5
	AA	10 (2.8)	11 (3.5)	14 (3.9)	0.85 (0.35–2.05)			0.76 (0.33–1.73)		
	Overdominant									
	CC + AA	278 (78.8)	244 (76.5)	278 (77.4)	1.00	0.52	928.5	1.00	0.62	983.7
	CA	75 (21.2)	75 (23.5)	81 (22.6)	0.89 (0.62–1.28)			0.91 (0.64–1.31)		
	Log-additive									
	–	–	–	–	0.89 (0.66–1.20)	0.45	928.3	0.88 (0.66–1.17)	0.39	983.2

PCa prostate cancer, *BPH* benign prostatic hyperplasia, *OR* odds ratio, *CI* confidence interval, *AIC* Akaike information criteria

[a] Adjusted for age

[b] Statistical trend of significance

Table 4 Association of rs784567 with initial serum PSA level among PCa patients

Genetic model	*n*	Response mean (s.e.)	Serum PSA level		
			Difference (95 % CI)	*P* value[a]	AIC
rs784567					
Codominant					
GG	100	186.01 (70.22)	0.00		5306.5
GA	168	95.29 (27.1)	−91.95 (−203.55 to 19.65)	0.12	
AA	84	57.3 (13.32)	**−131.09 (−262.03 to −0.14)**		
Dominant					
GG	100	186.01 (70.22)	0.00	**0.05***	5304.9
GA + AA	252	82.63 (18.62)	**−104.93 (−209.35 to −0.51)**		
Log-additive					
–	–	–	**−66.64 (−131.93 to −1.35)**	**0.046**	5304.8

CI confidence interval, *AIC* Akaike information criteria

* Statistically significant results are shown in bold

[a] Adjusted for age

decreased risk for high clinical stage, when comparing genotype distributions among PCa patients stratified into groups with T3 and T2 stages ($P = 0.022$). The exact OR could not be calculated, since there were no PCa patients with T3 stage who had GG genotype.

The genetic variants tested were not shown to be associated with the presence of bone metastases among PCa patients (results not shown).

When classifying PCa patients according to D'Amico criteria for disease aggressiveness, tests for genetic associations yielded no statistically significant results (results not shown). Conversely, when PCa patients were selected according to different criteria for progression risk assessment, rs3742330 minor allele G was found to be associated with the lower PCa aggressiveness ($P = 0.036$; OR 0.14, 95 % CI 0.023–1.22, for recessive model; Table 7). The same comparison showed statistical trend of association between rs7813 and the risk of PCa progression ($P = 0.074$, for overdominant model; results not shown).

Discussion

Dysregulation of microRNA-based regulatory mechanisms was found to be involved in the pathogenesis of PCa. These observations mainly refer to microRNAs, but similar findings were obtained for components of microRNA machinery [8, 25]. To date, evidence was found to support the association of genetic variants in microRNA genes with PCa risk and/or progression in Asians, as well as in Europeans, as found in our previous studies in Serbian population [13–16]. Nevertheless, a single case–control study on PCa analyzed the variants within microRNA machinery genes [18]. The mentioned study provided evidence of association between two genetic variants in *GEMIN4* and PCa risk and progression [18]. Still, these results needed to be replicated in other populations in order to validate the observed effects of *GEMIN4* variants on PCa risk/progression. Therefore, we decided to conduct the first case–control study on a *GEMIN4* variant and PCa in a European population.

The exonic genetic variant in *GEMIN4* chosen for this study is rs7813, for which it was previously found that genotype TT confers the increased risk of developing PCa [18]. The same genotype was also found to be associated with the higher clinical stage of PCa [18]. Our result did not support the association of this genetic variant with the risk of developing PCa. The discordance with the previous results can be explained by the differences in ethnic backgrounds, since they were obtained in a European and an Asian population. Also, the potential reasons for differences in the observed effect of rs7813 on PCa could be unmatched subject recruitment procedure. Still, our results show similarities with previously obtained regarding PCa progression, even though patient classifications differed between these two studies. Findings from both studies qualify rs7813 allele G for protective allelic variant against disease aggressiveness.

Other genetic variants selected for the present study were never previously analyzed for association with PCa. *DICER1* variant rs3742330, located in the 3′UTR, was found to be associated with both GS and clinical stage of primary PCa. Also, this genetic variant was shown to be related to disease aggressiveness. These findings, even though unique for PCa, are consistent with previously obtained suggesting the association with osteosarcoma prognosis, with minor allele G being protective [26].

Table 5 Association of rs3742330 and rs4961280 with Gleason score (GS)

Genetic model	GS < 7 (%)	GS = 7 (%)	GS > 7 (%)	GS > 7 versus GS < 7			GS > 7 versus GS = 7			GS = 7 versus GS < 7		
				OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC
rs3742330												
Codominant												
AA	166 (79.8)	63 (78.8)	47 (90.4)	1.00	0.06[b]	251.7	1.00			1.00		
AG	37 (17.8)	16 (20)	5 (9.6)	0.46 (0.17–1.26)			0.40 (0.13–1.18)	0.16	175.9	1.12 (0.58–2.17)	0.75	347.5
GG	5 (2.4)	1 (1.2)	0 (0)	0.00 (0.00–NA)			0.00 (0.00–NA)			0.52 (0.06–4.51)		
Dominant												
AA	166 (79.8)	63 (78.8)	47 (90.4)	1.00	**0.045**	251.2	1.00	0.066[b]	174.3	1.00	0.88	346.1
AG + GG	42 (20.2)	17 (21.2)	5 (9.6)	0.39 (0.14–1.06)			0.38 (0.13–1.13)			1.05 (0.56–1.99)		
Log-additive	–	–	–	**0.40 (0.16–1.00)**	**0.027**	250.4	0.38 (0.13–1.11)	0.059[b]	174.1	0.98 (0.56–1.72)	0.95	346.1
rs4961280												
Codominant												
CC	140 (67.3)	44 (54.3)	37 (69.8)	1.00			1.00			1.00		
CA	63 (30.3)	33 (40.7)	12 (22.6)	0.72 (0.35–1.48)	0.29	258.2	0.45 (0.20–1.02)	0.13	178.5	1.66 (0.97–2.85)	0.1	346.1
AA	5 (2.4)	4 (4.9)	4 (7.5)	2.39 (0.57–10.07)			1.07 (0.24–4.78)			2.58 (0.66–10.05)		
Dominant												
CC	140 (67.3)	44 (54.3)	37 (69.8)	1.00	0.64	258.5	1.00	0.087[b]	177.6	1.00	**0.042**	344.5
CA + AA	68 (32.7)	37 (45.7)	16 (30.2)	0.85 (0.44–1.67)			0.53 (0.25–1.11)			**1.73 (1.02–2.92)**		
Overdominant												
CC + AA	145 (69.7)	48 (59.3)	41 (77.4)	1.00	0.28	257.6	1.00	**0.045**	176.5	1.00	0.096[b]	345.9
CA	63 (30.3)	33 (40.7)	12 (22.6)	0.68 (0.33–1.40)			**0.45 (0.20–1.00)**			1.58 (0.93–2.69)		
Log-additive												
–	–	–		1.02 (0.59–1.78)	0.94	258.7	0.69 (0.38–1.26)	0.23	179.1	**1.64 (1.04–2.58)**	**0.033**	344.1

GS Gleason score, *OR* odds ratio, *CI* confidence interval, *AIC* Akaike information criteria

* Statistically significant results are shown in bold

[a] Adjusted for age

[b] Statistical trend of significance

Table 6 Association of rs3742330, rs4961280 and rs7813 with the clinical stage of primary prostate cancer assessed according to TNM classification system

Genetic model	T1 (%)	T2 (%)	T3/T4 (%)	T2 versus T1			T3/T4 versus T1			T3/T4 versus T2		
				OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC
rs3742330												
Codominant												
AA	37 (75.5)	136 (80.5)	77 (85.6)	1.00			1.00			1.00		
AG	12 (24.5)	27 (16)	13 (14.4)	0.62 (0.28–1.34)	0.091[b]	232.9	1.42 (0.57–3.56)	0.67	185.7	0.85 (0.41–1.74)	0.067[b]	337.2
GG	0 (0)	6 (3.5)	0 (0)	NA (0.00–NA)			1.79 (0.18–18.14)			0.00 (0.00–NA)		
Recessive												
AA + AG	49 (100)	163 (96.5)	90 (100)	1.00	0.066[b]	232.3	1.00	0.65	184.3	1.00	**0.022***	335.4
GG	0 (0)	6 (3.5)	0 (0)	NA (0.00–NA)			1.67 (0.17–16.88)			0.00 (0.00–NA)		
rs4961280												
Codominant												
CC	22 (44.9)	119 (70)	57 (63.3)	1.00			1.00			1.00		
CA	25 (51)	46 (27.1)	29 (32.2)	**0.33 (0.17–0.65)**	**0.0053**	227.7	**0.44 (0.21–0.92)**	0.086[b]	181.5	1.32 (0.75–2.31)	0.52	342.1
AA	2 (4.1)	5 (2.9)	4 (4.4)	0.46 (0.08–2.57)			0.87 (0.15–5.16)			1.67 (0.43–6.46)		
Dominant												
CC	22 (44.9)	119 (70)	57 (63.3)	1.00	**0.0013**	225.8	1.00	**0.037**	180.1	1.00	0.28	340.2
CA + AA	27 (55.1)	51 (30)	33 (36.7)	**0.34 (0.18–0.66)**			**0.47 (0.23–0.96)**			1.35 (0.79–2.32)		
Overdominant												
CC + AA	24 (49)	124 (72.9)	61 (67.8)	1.00	**0.0018**	226.4	1.00	**0.027**	179.6	1.00	0.38	340.7
CA	25 (51)	46 (27.1)	29 (32.2)	**0.35 (0.18–0.67)**			**0.45 (0.22–0.92)**			1.28 (0.73–2.24)		
Log-additive												
–	–	–	–	**0.44 (0.25–0.77)**	**0.0039**	227.8	0.60 (0.33–1.09)	0.092[b]	181.6	1.31 (0.83–2.07)	0.26	340.1
rs7813												
Codominant												
AA	11 (22.4)	64 (37.6)	27 (30)	1.00			1.00			1.00		
AG	31 (63.3)	81 (47.6)	43 (47.8)	**0.44 (0.20–0.94)**	0.09[b]	233.3	0.57 (0.25–1.34)	0.22	183.5	1.26 (0.70–2.26)	0.24	340.5
GG	7 (14.3)	25 (14.7)	20 (22.2)	0.58 (0.20–1.67)			1.17 (0.38–3.58)			1.90 (0.91–4.00)		
Dominant												
AA	11 (22.4)	64 (37.6)	27 (30)	1.00	**0.035**	231.7	1.00	0.36	183.6	1.00	0.21	339.9
AG + GG	38 (77.5)	106 (62.4)	63 (70)	**0.46 (0.22–0.97)**			0.69 (0.30–1.55)			1.41 (0.82–2.45)		
Overdominant												
AA + GG	18 (36.7)	89 (52.4)	47 (52.2)	1.00	0.051[b]	232.3	1.00	0.088[b]	181.5	1.00	0.98	341.4
AG	31 (63.3)	81 (47.6)	43 (47.8)	0.52 (0.27–1.01)			0.54 (0.26–1.10)			1.01 (0.60–1.68)		

Table 6 continued

Genetic model	T1 (%)	T2 (%)	T3/T4 (%)	T2 versus T1			T3/T4 versus T1			T3/T4 versus T2		
				OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC	OR (95 % CI)[a]	*P* value[a]	AIC
Log-additive												
–	–	–	–	0.70 (0.43–1.13)	0.14	234	1.01 (0.60–1.70)	0.96	184.4	1.36 (0.95–1.97)	0.096[b]	338.6

OR odds ratio, *CI* confidence interval, *AIC* Akaike information criteria

* Statistically significant results are shown in bold

[a] Adjusted for age

[b] Statistical trend of significance

Table 7 Association of rs3742330 with the risk of PCa progression assessed according to Medeiros et al.

Genetic model	Low	High	Risk of PCa progression		
			OR (95 % CI)[a]	*P* value[a]	AIC
rs3742330					
Codominant					
AA	111 (78.2)	155 (83.8)	1.00		447.6
AG	26 (18.3)	29 (15.7)	0.78 (0.43–1.39)	0.077[b]	
GG	5 (3.5)	1 (0.5)	0.13 (0.02–1.17)		
Recessive					
AA + AG	137 (96.5)	184 (99.5)	1.00	**0.036***	446.3
GG	5 (3.5)	1 (0.5)	0.14 (0.02–1.22)		
Log-additive					
–	–	–	0.63 (0.39–1.04)	0.068[b]	447.3

OR odds ratio, *CI* confidence interval, *AIC* Akaike information criteria

* Statistically significant results are shown in bold

[a] Adjusted for age

[b] Statistical trend of significance

Similarly, minor allele of rs784567 located in *TARBP2* promoter was found to be associated with the lower serum PSA level. Still, these results are in contrast to those obtained for renal cell carcinoma outcome [27].

Genetic variant rs4961280 located in *AGO2* promoter showed opposing direction of association with GS when different groups of PCa patients were compared. This can be explained by the relatively small number of patients within some GS-based groups. More significant are results regarding the association with the clinical stage of PCa, suggesting the protective effect of minor allele A. The observed results are concordant with the potential biological function of rs4961280 as a repressive promoter variant, since AGO2 was found to be upregulated in PCa [28].

Our study provided no evidence of association between the analyzed genetic variants in RISC genes and PCa susceptibility. Nevertheless, the protective effect of rs3742330, rs4961280 and rs7813 on PCa progression risk was shown. The most significant results obtained in multiple comparisons are those implying the association of rs3742330 located in DICER1 with both GG and clinical stage of PCa, as well as with cancer aggressiveness. Still, in order to make further conclusions about the association between the analyzed genetic variants and the risk of PCa progression, additional studies in European and non-European populations are required.

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Compliance with ethical standards

Authors' contribution Protocol development, data collection, data analysis and manuscript writing were done by Z Nikolić. Protocol development, data analysis and manuscript editing were carried out by D Savić Pavićević. Data collection and data analysis were performed by N Vučić, S Cerović and V Vukotić. Protocol development, data collection, data analysis and manuscript writing/editing were done by G Brajušković.

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments.

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