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ORIGINAL ARTICLE

Association of *KLK3*, *VAMP8* and *MDM4* Genetic Variants within microRNA Binding Sites with Prostate Cancer: Evidence from Serbian Population

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Abstract

A growing number of studies have suggested that genetic variants affecting the micro-RNA- binding mechanisms (miRSNPs) constitute a promising novel class of biomarkers for prostate cancer (PCa) biology. Among the most extensively studied miRSNPs in the context of cancer is the variation rs4245739 in the *MDM4* gene, while a recent large-scale analysis revealed significant differences in genotype distributions between aggressive and non-aggressive disease for rs1058205 in *KLK3* and rs1010 in *VAMP8*. In this study, we examined a total of 1083 subjects for these three variants using Taqman® SNP Genotyping Assays. Three hundred and fifty-five samples of peripheral blood were obtained from patients with PCa and 358 samples from patients with benign prostatic hyperplasia (BPH). The control group consisted of 370 healthy volunteers. Comparisons of genotype distributions among PCa and BPH patients, as well as between PCa patients and healthy controls, yielded no evidence of association between the analyzed genetic variants and the risk of developing PCa. However, all three tested genetic variants have shown the association with the parameters of PCa progression. For *KLK3* variant rs1058205, minor allele C was found to associate with the lower serum PSA score in PCa patients (PSA > 20 ng/ml vs. PSA < 10 ng/ml comparison, Prec = 0.038; ORrec = 0.20, 95%CI 0.04–1.05). The obtained results point out the potential relevance of the tested genetic variants for the disease aggressiveness assessment.

Keywords miRSNPs · rs1058205 · rs1010 · rs4245739 · Prostate cancer

Introduction

Prostate cancer (PCa), the second most frequent malignancy in men worldwide, accounted 1.276.106 new cases and caused 358.989 deaths (6.7% of all deaths caused by cancer in men) in 2018 [1]. The well-established risk factor for PCa, apart from age and ethnicity, is family-history of the disease [2]. Rarely occurring but high-penetrant genetic variants, as well as commonly occurring low-risk variants, both contribute to genetic basis of PCa. Genome wide association studies (GWASs) have been invaluable in the discovery of these common variants associated with PCa susceptibility. In the largest PCa GWAS to date and the meta-analysis reported recently [3], 63 novel PCa susceptibility loci were identified, which raised the total number of known loci from GWAS to around 170 (GWAS Catalog) [4]. However, these commonly occurring low-risk variants can explain only about 28.4% of the familial relative risk for PCa, suggesting that additional SNPs remain to be identified [3]. Another approach to the identification of novel PCa risk loci is through candidate-gene based studies, with plausible candidates emerging from the research of the molecular pathogenesis of malignant diseases. Therefore, microRNA-based mechanisms have been recognized as a promising field of carcinogenesis research, including the case-control studies focusing on genetic variants affecting the RNA interference process [5].

MicroRNAs (miRNA) are a class of trans-acting RNAs that bind to cis-regulatory elements in their target mRNAs and negatively regulate their expression either through degrading/destabilizing the mRNA or by inhibiting their translation [6]. Target selection is critically dependent on the sequence complementarity between the miRNA nucleotides

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2–8, referred to as the miRNA seed site, and the miRNA-binding elements usually found on the 3'UTR of the mRNA. Because of the uniqueness and complexity of the miRNA-target recognition, genetic variants play an important role in the regulation of expression of miRNA targets, as well as in all the other aspects of miRNA biogenesis and function. There are two scenarios by which miRNA-related genetic variants are implicated in cancer etiology: variants creating a loss-of function or gain-of-function event [7]. The first scenario refers to the inhibition of the expression or the functional activity of a tumor-suppressive miRNAs, while the latter one presumes the opposite effects on the activity of oncogenic miRNAs. By both of these mechanisms, genetic variants related to miRNA functions may have profound effects on cancerogenesis. Direct effects of genetic variants on microRNA function are based on the alterations in pri-miRNA and pre-miRNA processing, as well as in mature RNA activities. Furthermore, genetic variants in regulatory regions may affect miRNA transcription rates, while those located in mRNAs may create or destroy a miRNA-binding site. Among the most extensively studied genetic variants are those located in the seed region or seed-complementary site, which are predicted to elicit cancer phenotype by severely affecting the target selection [8, 9].

In our previous reports, we investigated the association between genetic variants potentially affecting the transcriptional rate and/or processing of miRNA precursors and PCa risk [10–12]. The obtained results, suggesting the association between the analyzed variants and the risk of PCa onset and/or progression, encouraged us to further examine the novel candidate genetic variants with the potential effect on RNA interference, among which are variants located within microRNA binding sites. The role of this class of microRNA-related variants has been previously evaluated for the genes of biologic relevance for PCa [13, 14]. The most extensive study on this type of genetic variants, a recent large-scale analysis of 2169 microRNA single nucleotide polymorphisms (miRSNPs) and PCa risk and aggressiveness on 22,301 cases and 22,320 controls of European ancestry, revealed 22 miRSNPs associated with the risk of PCa [15]. The most significant differences in genotype distributions between aggressive and non-aggressive disease was reported for rs1058205 in *KLK3* and rs1010 in *VAMP8*. These genetic variants have also been functionally analyzed, revealing that *KLK3* variant rs1058205 creates a putative binding site for miR-3162-5p, whereas miR-370-5p was found to have a greater affinity for the *VAMP8* rs1010 A-allele. The same research group also reported *MDM4* genetic variant rs4245739 to be associated with PCa risk by creating a new miRNA-binding site for multiple miRNAs [16]. By using the reporter gene assay, it was found that miR-191-5p and miR-887 have a specific affinity for the rs4245739 C-allele, suggesting a mechanism by which the untargeted major allele A could associate with the increased risk of PCa [16].

KLK3 gene encodes the prostate specific antigene (PSA), a member of kallikrein family of serine proteases which is widely used as biomarker for PCa screening and monitoring the disease progression [17]. Therefore, variants located within this gene have been recognized as candidates for case-control and case-only studies on PCa even before the reported associations in the study by Stegeman et al. [15]. Namely, the genetic variant rs1058205, a tag SNP in the 3′-UTR of *KLK3* at the 19q13.33-locus, was previously associated with lower serum levels of PSA in African-American and Swedish men [18, 19]. Furthermore, contrasting results have been reported regarding the impact of this genetic variant on PCa susceptibility, suggesting its protective effect against PCa in at least some populations [20, 21].

Another genetic variant showed to be strongly associated with aggressive PCa by Stegeman et al. [15], rs1010 located in *VAMP8*, has not been previously analyzed in other cancers or validated in subsequent replication studies. The functional significance of VAMP8 in the molecular basis of PCa remains relatively poorly understood. Still, this protein was found to be expressed in prostatic glandular epithelium [22], while it was also determined that it plays a complex role in glucose metabolism and energy expenditure which makes it a potential candidate for carcinogenesis research [23].

As for the *MDM4*, this oncogene negatively regulates p53 and several other tumor suppressor genes in PCa and in the range of malignant tumors. Therefore, the genetic variant rs4245739 in *MDM4* has been associated with the risk of various human cancers, including ovarian, breast and small cell lung cancer, as well as esophageal squamous cell carcinoma (ESCC) [24–27]. The meta-analysis by Xu et al. [28] indicated that the rs4245739 A > C genetic variant tend to reduce the overall cancer risk, with the more prominent association in Asian populations. Conversely, Gansmo et al. [29] reported rs4245739 genetic variant to be associated with the reduced risk of breast cancer but not to be associated with either lung, colon cancer or PCa.

Considering the functional significance of the miRSNPs as potential diagnostic and prognostic biomarkers of PCa, as well as the previous contrasting findings on the effects of rs1058205 and rs4245739 on PCa in different ethnic populations, the aim of the present study is to analyze their impact on PCa susceptibility and aggressiveness in Serbian population. Since the number of case-control studies on this issue is relatively limited, we consider that performing the association study in another population of European origin would contribute to the better understanding of the effect of these genetic variants on PCa risk and progression. Furthermore, since the effect of rs1010 located in *VAMP8* on PCa risk and aggressiveness was shown in a single study, additional case-control studies are needed in order to provide further data on this issue, validate the obtained results and to elucidate the effect of this genetic variant [15]. Therefore, rs1010 was also chosen

for the analysis in the present study, focusing on the effects of genetic variants located in microRNA-binding sites on prostate carcinogenesis.

Material and Methods

This study used DNA samples obtained from the collections of the Center for Human Molecular Genetics. The collection consisted of patients treated in the period between 2008 and 2013 at Clinical Centre "Dr Dragiša Mišović Dedinje", Belgrade, Serbia and Clinical Centre "Zvezdara", Belgrade, Serbia. Research was conducted with the approval of ethics committees of these medical institutions (18–5309/29 and 01–1907/17). Written informed consent was obtained from all participants included in this study. Experiments were conducted in accordance with the Helsinki Declaration of 1975.

In this study we examined a total of 1083 subjects. Three hundred and fifty-five samples of peripheral blood were obtained from patients with PCa and 358 samples from patients with benign prostatic hyperplasia (BPH). The control group consisted of 370 healthy volunteers who gave samples of either buccal swabs or peripheral blood. The exclusion criteria for potential controls were the presence of any self-reported diseases and family history of PCa. After passing standard clinical examination, which includes measurement of prostate-specific antigene (PSA), digital rectal examination (DRE), transrectal ultrasonography (TRUS), bone scintiography and radiography and prostate biopsy patients were separated into 2 groups as BPH or PCa patients. TNM classification system was used to determine clinical stage of tumor, while hematoxylin and eosin-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 $\leq PSA \leq 20$ 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. According to criteria recommended by European Association of Urology (EAU), PCa patients were divided into three groups. PCa patients with $PSA < 10$ ng/ml, $GS <$ 7, and clinical stage T1-T2a comprised low-risk group, while intermediate risk-group consisted of PCa patients with PSA 10–20 ng/ml or $GS = 7$ or clinical stage T2b-T2c. High-risk group of PCa patients was defined by $PSA > 20$ ng/ml or $GS > 7$ or clinical stage T3/T4. Patients with the presence of distant metastasis were automatically classified into high-risk group [30].

Genotyping of rs1010, rs1058205 and rs4245739 was performed by using Taqman® SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA). Statistical analysis of SNPs associations was performed by SNPStats software [31]. Hardy–Weinberg equilibrium was assessed by the exact test implemented in SNPStats software. Allelic and genotypic associations were evaluated by unconditional 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

The available clinical and pathological data on PCa patients are shown in Table 1. According to the patient classification, most of the men diagnosed with PCa had initial serum PSA score higher than 20 ng/ml (42.9%), Gleason score 6 (53.8%) or 7 (24%), as well as T2 clinical stage of primary PCa (55%). Distant metastases were detected at diagnosis in about 16% of PCa patients included in the study.

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

Standard prognostic parameter	PCa patients; n (%)
PSA at diagnosis	
< 10 ng/ml	100 (28.4)
10–20 ng/ml	101 (28.7)
> 20 ng/ml	151 (42.9)
Gleason score	
4	7 (2)
5	16 (4.7)
6	184 (53.8)
7	82 (24)
8	31 (9.1)
9	19 (5.5)
10	3 (0.9)
TNM stage	
T1	49 (15.9)
T2	170 (55)
T3/T4	90 (29.1)
Metastases	
Distant (M+)	51 (15.8)
Regional (N+) or not detected	271 (84.2)
Risk of progression (EAU 2014)	
Low	22 (6.6)
Medium	115 (34.3)
High	198 (59.1)

Abbreviations: *PSA* prostate-specific antigen

Genotyping was successful in more than 98% of samples for all three genetic variants tested. The acquired genotyping data are presented in Table 2, suggesting the lack of deviations from HWE in the control group ($P = 0.09$, $P = 0.52$ and $P = 0.8$, for rs1058205, rs1010 and rs4245739, respectively). For all genetic variants included in this study, C allele was found to be minor allele in Serbian population. Comparisons of genotype distributions among PCa and BPH patients, as well as between PCa patients and healthy controls, yielded no evidence of association between the analyzed genetic variants and the risk of developing PCa (Table 2).

When analyzing the potential association of rs1010 with the initial PSA score among PCa patients, the obtained results were found to be statistically insignificant. However, the association of minor allele C of rs1058205 with the lower PSA score was determined by comparing genotype distributions between PCa patients with PSA > 20 ng/ml and PSA < 10 ng/ml ($P_{rec} = 0.038$; $OR_{rec} = 0.20$, 95%CI 0.04–1.05) (Table 3). In contrast with these results, minor allele C of rs4245739 was found to associate with higher initial serum PSA scores in PSA 10–20 ng/ml vs PSA < 10 ng/ml comparison, with the lowest AIC found for both dominant and log-additive model ($P = 0.026$ for both models). At the same time, statistical trend of significance was found for association of rs4245739 with serum PSA score under log-additive and dominant genetic models when genotype distributions among patients with PSA > 20 ng/ml and PSA < 10 ng/ml were compared ($P_{log\text{-}additive} = 0.052$, $OR_{log\text{-}additive} = 1.54$, 95%CI 0.99–2.39; $P_{dom} = 0.078$; $OR_{dom} = 1.61$, 95%CI 0.94–2.75) (Table 3).

By comparing genotype frequencies among PCa patients with GS = 7 and GS < 7, rs1010 minor allele C was shown to be associated with higher GS, with statistical significance being reached for recessive and log-additive genetic models ($P_{rec} = 0.036$ and $P_{log\text{-}additive} = 0.024$). Similarly, comparisons of rs4245739 genotype distributions among PCa patients with GS > 7 and patients within both lower GS score categories demonstrated the association of minor allele C with higher GS. The statistical significance was found for multiple genetic models tested, while the lowest AIC in both comparisons was shown for dominant model (Table 4).

The comparisons of rs1058205 genotype frequencies among PCa patients with T2 and T1 clinical stages, as well as with T3/4 and T1 stages, demonstrated the protective effect of minor allele C against primary PCa progression to higher TNM stage. In both tests, statistical significance of association was shown for multiple genetic models, while the lowest AIC score suggested the over-dominant being the best-fitting one (Table 5). When analyzing the association of rs1010 with TNM clinical stage of primary PCa, statistically significant results were obtained for multiple genetic models in the comparison of genotype distributions among patients with T3/4 and T2 stages. Nevertheless, the opposite direction of the effect of heterozygous and CC homozygous genotype was determined, while the recessive model was found to be the best-fitting one, according to AIC score ($P_{rec} = 0.017$; $OR_{rec} = 2.08$, 95%CI 1.14–3.81). At the same time, C allele of rs4245739 associated with higher TNM clinical stage of primary PCa under recessive genetic model, as determined in T3/4 vs. T1 comparison ($P_{rec} = 0.033$; $OR_{rec} = 6.28$, 95%CI 0.77–50.85). Statistical significance was also reached for the association under codominant model, with the slightly higher AIC score ($P_{codom} = 0.044$) (Table 5).

Contrary to these results, the genetic variants tested in this study were not found to be associated with the presence of distant PCa metastases (results not shown). Also, tests of association with the risk of PCa progression yielded no statistical significance. Nevertheless, statistical trend was obtained for the association of rs4245739 minor allele C with higher PCa aggressiveness, as determined in both high-risk vs. low-risk, as well as in intermediate-risk vs. low-risk disease comparisons. The lowest AIC in these tests was determined for log-additive genetic model (Table 6).

Discussion

Single nucleotide variants (SNVs) are the most common source of variation within the human genome, with approximately 10 million identified so far, occurring every several hundred base pairs (every 100–300 nucleotides) [32]. Taking into account these results from the genomic sequencing project, researchers in the area of cancer genetics have focused on this type of genetic variants in their pursuit for the sources of heritability of malignant diseases. The vast majority of cancer-associated loci originated from genome-wide approach in the case-control study design, which was enabled by the technological improvements allowing the high-throughput SNV analyses. Even though the association of functional SNVs in gene coding regions with cancer is well known, it accounts for only a very small proportion of SNVs identified by GWAS. Namely, estimations are that 93% of functional SNPs in the GWAS catalogue are in the non-coding regions, having significant effects on gene expression by disrupting transcription regulatory sites or by affecting posttranscriptional events, including the binding of miRNAs [33].

MiRNAs are small non-coding RNAs (21-23 nt long) that negatively regulate protein expression, either by inhibiting the translation of the subsequent mRNA, or by inducing the transcript destabilization. Since regulation by miRNAs is dependent on base-pair complementarity, any slight change in the miRNA-binding site in the 3`-UTR of a mRNA can have profound downstream effects [34]. Not only that the genetic variant, even a small one as a SNV, could significantly reduce the binding affinity, but could also completely destroy the

Table 2 Association of genetic variants within genes *KLK3*, *VAMP8* and *MDM4* with PCa risk

SNP	Genetic model	No of PCa patients (%)	No of controls (%)	No of BPH patients (%)	PCa vs controls			PCa vs BPH		
					OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC
rs1058205										
Codominant										
	TT	249 (70.3)	262 (70.8)	265 (74.2)	1.00	0.6	1008	1.00	0.61	982.6
	CT	95 (26.8)	93 (25.1)	81 (22.7)	1.07 (0.76–1.49)			1.19 (0.84–1.68)		
	CC	10 (2.8)	15 (4)	11 (3.1)	0.69 (0.30–1.57)			1.00 (0.42–2.42)		
Dominant										
	TT	249 (70.3)	262 (70.8)	265 (74.2)	1.00	0.92	1007	1.00	0.36	980.8
	CT + CC	105 (29.7)	108 (29.2)	92 (25.8)	1.02 (0.74–1.40)			1.17 (0.84–1.63)		
Recessive										
	TT + CT	344 (97.2)	355 (96)	346 (96.9)	1.00	0.35	1006.2	1.00	0.93	981.6
	CC	10 (2.8)	15 (4)	11 (3.1)	0.68 (0.30–1.54)			0.96 (0.40–2.31)		
Overdominant										
	TT + CC	259 (73.2)	277 (74.9)	276 (77.3)	1.00	0.63	1006.8	1.00	0.32	980.6
	CT	95 (26.8)	93 (25.1)	81 (22.7)	1.09 (0.78–1.52)			1.19 (0.84–1.68)		
Log-additive										
	–	–		–	0.97 (0.74–1.27)	0.81	1007	1.12 (0.84–1.49)	0.44	981
rs1010										
Codominant										
	TT	124 (34.9)	119 (32.7)	118 (33.1)	1.00	0.34	1000.3	1.00	0.14	979.8
	CT	161 (45.4)	184 (50.5)	184 (51.7)	0.84 (0.61–1.17)			0.83 (0.60–1.16)		
	CC	70 (19.7)	61 (16.8)	54 (15.2)	1.11 (0.72–1.70)			1.25 (0.81–1.95)		
Dominant										
	TT	124 (34.9)	119 (32.7)	118 (33.1)	1.00	0.54	1000.1	1.00	0.64	981.5
	CT + CC	231 (65.1)	245 (67.3)	238 (66.8)	0.91 (0.67–1.24)			0.93 (0.68–1.27)		
Recessive										
	TT + CT	285 (80.3)	303 (83.2)	302 (84.8)	1.00	0.30	999.4	1.00	0.095‡	979
	CC	70 (19.7)	61 (16.8)	54 (15.2)	1.22 (0.84–1.79)			1.40 (0.94–2.07)		
Overdominant										
	TT + CC	194 (54.6)	180 (49.5)	172 (48.3)	1.00	0.17	998.6	1.00	0.088‡	978.8
	CT	161 (45.4)	184 (50.5)	184 (51.7)	0.81 (0.61–1.09)			0.77 (0.57–1.04)		
Log-additive										
	–	–	–	–	1.02 (0.83–1.25)	0.87	1000.4	1.07 (0.86–1.32)	0.55	981.4
rs4245739										
Codominant										
	AA	198 (56.2)	182 (51)	204 (57.3)	1.00			1.00		
	AC	131 (37.2)	144 (40.3)	122 (34.3)	0.84 (0.62–1.15)	0.32	987.2	1.10 (0.80–1.51)	0.53	978.1
	CC	23 (6.5)	31 (8.7)	30 (8.4)	0.69 (0.39–1.22)			0.79 (0.44–1.41)		
Dominant										
	AA	198 (56.2)	182 (51)	204 (57.3)	1.00	0.18	985.6	1.00	0.79	977.3
	AC + CC	154 (43.8)	175 (49)	152 (42.7)	0.82 (0.61–1.10)			1.04 (0.77–1.40)		
Recessive										
	AA+AC	329 (93.5)	326 (91.3)	326 (91.6)	1.00	0.28	986.3	1.00	0.34	976.5
	CC	23 (6.5)	31 (8.7)	30 (8.4)	0.74 (0.42–1.29)			0.76 (0.43–1.34)		
Overdominant										
	AA+CC	221 (62.8)	213 (59.7)	234 (65.7)	1.00	0.43	986.8	1.00	0.43	976.7
	AC	131 (37.2)	144 (40.3)	122 (34.3)	0.89 (0.65–1.20)			1.13 (0.83–1.54)		
Log-additive										
	–	–	–	–	0.84 (0.66–1.06)	0.13	985.2	0.98 (0.77–1.24)	0.85	977.3

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

† adjusted for age

‡ statistical trend of significance

Table 3 Association of rs1058205 and rs4245739 with the initial serum PSA scores

Genetic model	PSA < 10 ng/ml (%)	10 ng/ml ≤ PSA < 20 ng/ml (%)	PSA > 20 ng/ml (%)	PSA > 20 ng/ml vs PSA < 10 ng/ml			PSA > 20 ng/ml vs 10 ng/ml ≤ PSA < 20 ng/ml			10 ng/ml ≤ PSA < 20 ng/ml vs PSA < 10 ng/ml		
				OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC
rs1058205												
Codominant												
TT	68 (68)	68 (67.3)	111 (74)	1.00			1.00			1.00		
CT	26 (26)	31 (30.7)	37 (24.7)	0.79 (0.43–1.45)	0.088‡	329.4	0.70 (0.39–1.24)	0.43	340.3	1.17 (0.63–2.18)	0.28	282.3
CC	6 (6)	2 (2)	2 (1.3)	0.19 (0.04–0.99)			0.60 (0.08–4.39)			0.32 (0.06–1.66)		
Dominant												
TT	68 (68)	68 (67.3)	111 (74)	1.00	0.18	330.5	1.00	0.2	338.4	1.00	0.98	282.9
CT + CC	32 (32)	33 (32.7)	39 (26)	0.68 (0.38–1.20)			0.69 (0.40–1.21)			1.01 (0.56–1.83)		
Recessive												
TT + CT	94 (94)	99 (98)	148 (98.7)	1.00	**0.038***	327.9	1.00	0.68	339.8	1.00	0.13	280.6
CC	6 (6)	2 (2)	2 (1.3)	**0.20 (0.04–1.05)**			0.66 (0.09–4.82)			0.31 (0.06–1.57)		
Overdominant												
TT + CC	74 (74)	70 (69.3)	113 (75.3)	1.00	0.6	331.9	1.00	0.23	338.6	1.00	0.5	282.5
CT	26 (26)	31 (30.7)	37 (24.7)	0.85 (0.47–1.55)			0.71 (0.40–1.25)			1.24 (0.67–2.30)		
Log-additive												
–	–	–		0.64 (0.39–1.04)	0.069‡	328.9	0.71 (0.43–1.19)	0.2	338.4	0.88 (0.53–1.45)	0.62	282.7
rs4245739												
Codominant												
AA	65 (65)	50 (50)	82 (55)	1.00			1.00			1.00		
AC	31 (31)	43 (43)	55 (36.9)	1.50 (0.85–2.62)	0.15	330.1	0.79 (0.46–1.35)	0.67	339.4	1.84 (1.01–3.34)	0.076‡	278
CC	4 (4)	7 (7)	12 (8.1)	2.52 (0.76–8.40)			1.01 (0.37–2.74)			2.45 (0.67–8.96)		
Dominant												
AA	65 (65)	50 (50)	82 (55)	1.00	0.078‡	328.8	1.00	0.45	337.6	1.00	**0.026***	276.2
AC + CC	35 (35)	50 (50)	67 (45)	1.61 (0.94–2.75)			0.82 (0.49–1.37)			**1.91 (1.08–3.38)**		
Recessive												
AA + AC	96 (96)	93 (93)	137 (92)	1.00	0.18	330.1	1.00	0.83	338.2	1.00	0.3	280.1
CC	4 (4)	7 (7)	12 (8.1)	2.17 (0.66–7.11)			1.11 (0.42–2.95)			1.93 (0.54–6.87)		
Overdominant												
AA + CC	69 (69)	57 (57)	94 (63.1)	1.00	0.25	330.6	1.00	0.37	337.4	1.00	0.073‡	277.9
AC	31 (31)	43 (43)	55 (36.9)	1.38 (0.79–2.39)			0.79 (0.47–1.33)			1.70 (0.95–3.05)		
Log-additive												
–	–	–		1.54 (0.99–2.39)	0.052‡	328.1	0.90 (0.60–1.35)	0.61	338	**1.71 (1.06–2.77)**	**0.026***	276.2

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

† adjusted for age

‡ statistical trend of significance

* statistically significant results are shown in bold

Table 4 Association of rs1010 and rs4245739 with Gleason score

Genetic model	GS < 7 (%)	GS = 7 (%)	GS > 7 (%)	GS > 7 vs GS < 7			GS > 7 vs GS = 7			GS = 7 vs GS < 7		
				OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC
rs1010												
Codominant												
TT	77 (37.2)	22 (26.8)	18 (34)	1.00			1.00			1.00		
CT	93 (44.9)	36 (43.9)	26 (49.1)	1.14 (0.57–2.27)	0.93	259.9	0.86 (0.38–1.94)	0.28	181	1.34 (0.73–2.48)	0.071‡	347.2
CC	37 (17.9)	24 (29.3)	9 (17)	1.03 (0.41–2.55)			0.46 (0.17–1.26)			2.26 (1.12–4.55)		
Dominant												
TT	77 (37.2)	22 (26.8)	18 (34)	1.00	0.76	258	1.00	0.36	180.7	1.00	0.095‡	347.7
CT + CC	130 (62.8)	60 (73.2)	35 (66)	1.11 (0.58–2.12)			0.70 (0.33–1.50)			1.61 (0.91–2.82)		
Recessive												
TT + CT	170 (82.1)	58 (70.7)	44 (83)	1.00	0.91	258.1	1.00	0.12	179.1	1.00	**0.036***	346.1
CC	37 (17.9)	24 (29.3)	9 (17)	0.95 (0.42–2.16)			0.51 (0.21–1.21)			**1.91 (1.05–3.45)**		
Overdominant												
TT + CC	114 (55.1)	46 (56.1)	27 (50.9)	1.00	0.7	257.9	1.00	0.63	181.3	1.00	0.85	350.4
CT	93 (44.9)	36 (43.9)	26 (49.1)	1.13 (0.61–2.09)			1.19 (0.59–2.41)			0.95 (0.57–1.59)		
Log-additive												
–	–	–		1.03 (0.67–1.60)	0.88	258.1	0.69 (0.43–1.13)	0.14	179.4	**1.50 (1.05–2.13)**	**0.024***	345.4
rs4245739												
Codominant												
AA	125 (60.7)	44 (54.3)	19 (36.5)	1.00			1.00			1.00		
AC	68 (33)	31 (38.3)	29 (55.8)	**3.15 (1.61–6.17)**	**0.0028***	246.8	2.29 (1.07–4.87)	0.091‡	177.5	1.31 (0.76–2.26)	0.6	348
CC	13 (6.3)	6 (7.4)	4 (7.7)	1.96 (0.57–6.80)			1.83 (0.44–7.54)			1.31 (0.47–3.65)		
Dominant												
AA	125 (60.7)	44 (54.3)	19 (36.5)	1.00	**8e-04***	245.4	1.00	**0.03***	175.6	1.00	0.31	346
AC + CC	82 (39.3)	37 (45.7)	33 (63.5)	**2.94 (1.54–5.61)**			**2.22 (1.07–4.62)**			1.31 (0.78–2.20)		
Recessive												
AA+AC	193 (93.7)	75 (92.6)	48 (92.3)	1.00	0.82	256.5	1.00	0.81	180.2	1.00	0.75	347
CC	13 (6.3)	6 (7.4)	4 (7.7)	1.15 (0.35–3.75)			1.18 (0.31–4.55)			1.18 (0.43–3.22)		
Overdominant												
AA+CC	138 (67)	50 (61.7)	23 (44.2)	1.00	**0.0011***	245.8	1.00	**0.043***	176.1	1.00	0.38	346.3
AC	68 (33)	31 (38.3)	29 (55.8)	**2.88 (1.52–5.48)**			**2.09 (1.02–4.30)**			1.27 (0.74–2.17)		
Log-additive												
–	–	–		**1.93 (1.20–3.13)**	**0.0071***	249.3	1.71 (0.96–3.06)	0.066‡	176.9	1.21 (0.81–1.83)	0.35	346.2

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

† adjusted for age

‡ statistical trend of significance

* statistically significant results are shown in bold

microRNA-binding site or create a new one [34]. Furthermore, since the first large-scale analysis focused on the potential cancer associated SNVs in miRNA-binding sites by Yu et al. [35], many genetic variants of this type were found to associate with various human malignancies.

Among the most extensively studied miRSNPs in the context of cancer is the variation rs4245739 in the 3'UTR of the *MDM4* gene [33]. At the same time, sequence alteration in the *KLK3* gene has been recognised as a candidate for genetic association studies regarding PCa, due the functional significance of PSA expressed from *KLK3* gene. Namely, besides being the serum biomarker of PCa, PSA is involved in the proteolytic breakdown of the extracellular matrix in PCa tumorigenesis, which contributes to tumour invasion and metastasis [17]. Both of these genetic variants were among the major hits of a recent large-scale study on genetic variants located within microRNA-binding sites potentially associated with PCa. The mentioned study, performed by Stegeman et al. [15], also identified a novel PCa-susceptibility locus within the *VAMP8* gene. More importantly, all three genetic variants have been functionally characterized, providing potential mechanism of action and the evidence that miRSNPs could play significant roles in PCa development and progression [15, 16]. Having all this in mind, as well as the importance

Table 5 Association of rs1058205, rs1010 and rs4245739 with the clinical stage of localized PCa

Genetic model	T1 (%)	T2 (%)	T3/T4(%)	T2 vs T1			T3/T4 vs T1			T3/T4 vs T2		
				OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC
rs1058205												
Codominant												
TT	25 (51)	128 (75.7)	64 (71.1)	1.00			1.00			1.00		
TC	23 (46.-9)	37 (21.9)	22 (24.4)	**0.33 (0.17–0.66)**	**0.0069***	227.6	**0.36 (0.17–0.78)**	**0.022***	178.8	1.19 (0.65–2.19)	0.57	341.4
CC	1 (2)	4 (2.4)	4 (4.4)	0.83 (0.09–7.73)			1.67 (0.18–15.83)			2.00 (0.48–8.28)		
Dominant												
TT	25 (51)	128 (75.7)	64 (71.1)	1.00	**0.0024***	226.3	1.00	**0.018***	178.9	1.00	0.42	339.9
TC + CC	24 (49)	41 (24.3)	26 (28.9)	**0.35 (0.18–0.69)**			**0.42 (0.20–0.87)**			1.27 (0.71–2.26)		
Recessive												
TT + TC	48 (98)	165 (97.6)	86 (95.6)	1.00	0.86	235.5	1.00	0.41	183.8	1.00	0.37	339.8
CC	1 (2)	4 (2.4)	4 (4.4)	1.22 (0.13–11.25)			2.40 (0.26–22.22)			1.92 (0.47–7.86)		
Overdominant												
TT + CC	26 (53.-1)	132 (78.1)	68 (75.6)	1.00	**0.0016***	225.6	1.00	**0.0064***	177	1.00	0.64	340.3
TC	23 (46.-9)	37 (21.9)	22 (24.4)	**0.33 (0.17–0.66)**			**0.36 (0.17–0.75)**			1.15 (0.63–2.12)		
Log-additive												
–	–	–		**0.45 (0.25–0.81)**	**0.0086***	228.7	0.57 (0.31–1.07)	0.08‡	181.4	1.28 (0.79–2.08)	0.32	339.6
rs1010												
Codominant												
TT	16 (32.-6)	58 (34.1)	31 (34.4)	1.00			1.00			1.00		
CT	24 (49)	83 (48.8)	32 (35.6)	1.00 (0.49–2.06)	0.97	238	0.71 (0.31–1.58)	0.23	183.5	**0.72 (0.40–1.31)**	**0.033***	336.6
CC	9 (18.4)	29 (17.1)	27 (30)	0.91 (0.36–2.32)			1.56 (0.59–4.13)			1.74 (0.88–3.45)		
Dominant												
TT	16 (32.-6)	58 (34.1)	31 (34.4)	1.00	0.95	236.1	1.00	0.87	184.4	1.00	0.96	341.4
CT + CC	33 (67.-3)	112 (65.9)	59 (65.6)	0.98 (0.49–1.93)			0.94 (0.45–1.98)			0.99 (0.58–1.69)		
Recessive												
TT + CT	40 (81.-6)	141 (82.9)	63 (70)	1.00	0.82	236	1.00	0.13	182.2	1.00	**0.017***	335.8
CC	9 (18.4)	29 (17.1)	27 (30)	0.91 (0.40–2.09)			1.90 (0.80–4.47)			**2.08 (1.14–3.81)**		
Overdominant												
TT + CC	25 (51)	87 (51.2)	58 (64.4)	1.00	0.91	236.1	1.00	0.14	182.3	1.00	**0.039***	337.2
CT	24 (49)	83 (48.8)	32 (35.6)	1.04 (0.55–1.97)			0.59 (0.29–1.20)			**0.58 (0.34–0.98)**		
Log-additive												
–	–	–		0.96 (0.61–1.52)	0.86	236	1.19 (0.75–1.88)	0.46	183.9	1.26 (0.89–1.79)	0.19	339.7
rs4245739												
Codominant												
AA	25 (51)	99 (58.9)	51 (56.7)	1.00			1.00			1.00		
AC	23 (46.-9)	58 (34.5)	29 (32.2)	0.61 (0.32–1.19)	0.14	233.3	**0.62 (0.30–1.28)**	**0.044***	180.2	0.97 (0.55–1.70)	0.45	340.1
CC	1 (2)	11 (6.5)	10 (11.1)	2.78 (0.34–22.72)			5.12 (0.62–42.48)			1.77 (0.70–4.43)		
Dominant												
AA	25 (51)	99 (58.9)	51 (56.7)	1.00	0.28	234.1	1.00	0.53	184.1	1.00	0.73	339.6
AC + CC	24 (49)	69 (41.1)	39 (43.3)	0.70 (0.37–1.34)			0.80 (0.40–1.62)			1.10 (0.65–1.84)		
Recessive												
AA+AC	48 (98)	157 (93.5)	80 (88.9)	1.00	0.18	233.4	1.00	**0.033***	179.9	1.00	0.21	338.1
CC	1 (2)	11 (6.5)	10 (11.1)									

Table 5 (continued)

Genetic model	T1 (%)	T2 (%)	T3/T4(%)	T2 vs T1			T3/T4 vs T1			T3/T4 vs T2		
				OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC
				3.41 (0.43–27.27)			**6.28 (0.77–50.85)**			1.78 (0.73–4.38)		
Overdominant												
AA+CC	26 (53.-1)	110 (65.5)	61 (67.8)	1.00	0.096‡	232.5	1.00	0.086‡	181.5	1.00	0.71	339.6
AC	23 (46.-9)	58 (34.5)	29 (32.2)	0.57 (0.30–1.10)			0.53 (0.26–1.09)			0.90 (0.52–1.55)		
Log-additive												
–	–	–		0.89 (0.53–1.51)	0.68	235.1	1.10 (0.63–1.91)	0.74	184.3	1.18 (0.79–1.75)	0.42	339.1

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

† adjusted for age

‡ statistical trend of significance

* statistically significant results are shown in bold

of miRSNPs for cancer aetiology, we questioned the effect of rs1058205, rs1010, and rs4245739 on the risk for PCa development and progression in Serbian population.

Since Stegeman et al. [15] identified rs1058205 as one of the 22 variants associated with the PCa risk, as well as one of the variants with the most significant effect on PCa aggressiveness among the tested miRSNPs, they also conducted the functional analysis. This genetic variant is located within the region encoding the 3`-UTR of KLK3 mRNA and was predicted to be functional, potentially creating an aberrant miRNA-binding site for miR-3162-5p, miR-219-1-3p and miR-4278. Therefore, reporter vector assay was used to test

Table 6 Association of rs4245739 with the risk of cancer progression

Genetic model	low-risk (%)	intermediate-risk (%)	high-risk (%)	intermediate vs low-risk			high vs low-risk			high vs intermediate-risk		
				OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC	OR (95% CI) †	*P* value †	AIC
rs4245739												
Codominant												
AA	16 (72.7)	63 (55.3)	109 (55.6)	1.00			1.00			1.00		
AC	6 (27.3)	44 (38.6)	71 (36.2)	1.87 (0.68–5.17)	0.13	124.3	1.79 (0.67–4.83)	0.091‡	144.5	0.95 (0.58–1.55)	0.79	408.9
CC	0 (0)	7 (6.1)	16 (8.2)	NA (0.00-NA)			NA (0.00-NA)			1.33 (0.51–3.44)		
Dominant												
AA	16 (72.7)	63 (55.3)	109 (55.6)	1.00	0.12	124	1.00	0.11	144.7	1.00	0.99	407.3
AC + CC	6 (27.3)	51 (44.7)	87 (44.4)	2.16 (0.79–5.95)			2.18 (0.81–5.81)			1.00 (0.63–1.60)		
Recessive												
AA+AC	22 (100)	107 (93.9)	180 (91.8)	1.00	0.11	123.8	1.00	0.066‡	143.9	1.00	0.51	406.9
CC	0 (0)	7 (6.1)	16 (8.2)	NA (0.00-NA)			NA (0.00-NA)			1.36 (0.54–3.44)		
Overdominant												
AA+CC	16 (72.7)	70 (61.4)	125 (63.8)	1.00	0.31	125.3	1.00	0.35	146.4	1.00	0.73	407.2
AC	6 (27.3)	44 (38.6)	71 (36.2)	1.67 (0.61–4.61)			1.58 (0.59–4.23)			0.92 (0.57–1.49)		
Log-additive												
–	–	–	–	2.21 (0.87–5.62)	0.073‡	123.1	2.22 (0.91–5.40)	0.055‡	143.6	1.05 (0.73–1.53)	0.78	407.3

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

† adjusted for age

‡ statistical trend of significance

the results of in silico analysis, revealing that the miR-3162-5p has specific affinity for the rs1058205 T-allele. Protein, as well as mRNA levels of *KLK3*, were also found to be decreased in the presence of over-expressed miR-3162-5p in cells homozygous for T allele.

Our results are in contrast to those obtained by Stegeman et al. [15], since we obtained no evidence of association between rs1058205 and PCa susceptibility. This observation also contrasts the reported association between this genetic variant and PCa risk in the meta-analysis by Ding et al. [36]. Still, in the present study, C allele was associated with the lower serum PSA score among patients with PCa, which is consistent with the results obtained by Penney et al. [21] in their Caucasian American subjects. Still, their association of rs1058205 with the serum PSA score was determined in control subjects, similarly as in the study by Stegeman et al. [15], as well as in the study conducted by Savblom et al. [19] in Swedish population. Furthermore, Bensen et al. [18] showed association between rs1058205 and serum PSA level in their African-American PCa patients. Given the relationship between *KLK3* rs1058205 and serum PSA score, as well as the importance of this standard prognostic and diagnostic parameter of PCa, it should be noted that rs1058205 may have implications for PSA-based diagnostics and management protocols, potentially requiring genotype-dependent adjustments of PSA ranges [15]. The detected correlations potentially reflect the effect of rs1058205 on the regulation of PSA expression by regulatory factors other than miR-3162-5p, since this microRNA requires T allele for inhibitory action, while C allele was found to associate with the reduced serum PSA score in previous and the present studies.

Similarly with the finding concerning serum PSA score, we found that the rs1058205 minor allele C associates with lower clinical stage of primary tumour, while for the other tested associations statistical significance was not reached. Stegeman et al. [15] did not perform the test of association between genetic variants and TNM stages of primary PCa, while they observed for the *KLK3* rs1058205 allele-C a strong association with the nonaggressive disease. Our results seem to contradict these previous ones, but the criteria for aggressive PCa differed in the present study and the one conducted by Stegeman et al. [15]. Also, Chen, Xin [20] reported the TT genotype of rs1058205 to be associated with moderate to high-risk PCa in Chinese men. Still, they compared genotype frequencies in their control group and in the groups of PCa patients classified according to the risk of disease progression, while we made comparisons in a case-only manner. Also, they compared just TC and TT genotype counts, excluding the individuals with CC genotype and, therefore, not performing the allelic association estimates. Another previous study, conducted by Bensen et al. [18], suggested that rs1058205 is associated with the PCa aggressiveness, but the statistically significant results were obtained in their group of African-American patients, while such association was not determined for European-Americans. In their analysis, they used a similar disease aggressiveness classification system as we did in the present study. In contrast to our results, they found no associations between rs1058205 and TNM stage, while this genetic variant was shown to associate with Gleason score in European-American group of PCa patients [18].

The other most significant association with PCa susceptibility and aggressiveness in the study by Stegeman et al. [15] was found for rs1010 in *VAMP8* gene. VAMP8 belongs to the family of soluble N-ethylmaleimide-sensitive factor-attachment protein receptors (SNAREs), essential proteins for fusion of cellular membranes. This integral membrane protein is involved in granule secretion, vesicle trafficking, endocytosis and phagocytosis [37], while its direct function in carcinogenesis is not yet known. Potentially, VAMP8 could attribute to the Warburg effect, an important feature of malignant transformation, including the one that occurs in prostatic glandular epithelium [23]. To date, genetic variants in VAMP8 have not been investigated for their relation with human cancer, except for the study by Stegeman et al. [15]. Also, this genetic variant is in strong LD with a previous PCa susceptibility GWAS hit [38].

The functional characterization of rs1010 showed that the minor allele C of this genetic variant lowers the affinity of the miR-370-5p for its binding site, as predicted in silico and confirmed through reporter assay. Also, rs1010 showed a statistical trend of significance when genotype correlation with transcript expression was evaluated [15]. Therefore, the mechanism underlying the potential involvement of rs1010 in the genetic basis of PCa was proposed to rely on the action of microRNA miR-370-5p, previously found to be overexpressed in PCa tumours [15]. However, in our study, we did not find any association between rs1010 and the risk of developing PCa, which could reflect the potential differences in ethnic backgrounds between the study groups included in the present study and the previous one.

At the same time, rs1010 minor allele C was not found to associate with the higher Gleason score in the present study. Furthermore, when comparing genotype distributions among patients stratified into groups with T3/4 and T2 TNM categories, the same direction of association with clinical stage of primary PCa was determined. We could not compare these results with the data from other studies, since Stegeman et al. [15] did not examine the effect of rs1010 on the values of standard prognostic parameters of PCa, other than serum PSA score. Both of the studies did not show the relation of rs1010 with the serum PSA levels, while for the association of this genetic variant with PCa aggressiveness discordant results were obtained. That is, we did not show the association of rs1010 with the risk of PCa progression, but the association with Gleason score and the clinical stage of disease could still suggest that the minor C allele has contributive effect on the disease aggressiveness.

Among the three genetic variants chosen for the analysis in the present study, rs4245739 has been the most extensively studied to date, due to the functional significance of MDM4 for malignant transformation process. The MDM4 protein plays a major role in P53 tumour suppressor pathway through negatively regulating its function [39]. Maintaining the correct levels of P53 is pivotal to a cell, as P53 is a crucial protein for maintenance of genomic stability and control of the cell growth and apoptosis. Furthermore, MDM4 interacts with p21, a cyclin-dependent kinase inhibitor whose deregulation is associated with the higher proliferation rate in PCa. By binding to the transcription factor E2F1, MDM4 represses its transactivation and induces the changes in the regulation of cell cycle and apoptosis. Also, MDM4 inhibits the transactivation of Smad3 and Smad4, components of TGF-beta signalling, by which it further exhibits the promoting activity on tumour growth [39, 40].

The genetic variant rs4245739 locates at the 3'UTR of *MDM4* and is found to create the illegitimate miRNA-binding site. By using the reporter gene assays, Stegeman et al. [16] have shown that miR-191-5p and miR-887 have specific affinity for rs4245739 C-allele, presenting a mechanism by which the untargeted A-allele may be associated with the increased risk of PCa. Previously, Wynendaele et al. [41] have obtained the similar results in their experiments involving ovarian cancer cells, also demonstrating the allele-specific effects on the *MDM4* mRNA targeting by miR-191-5p. Therefore, this genetic variant, identified as a PCa susceptibility locus in GWA study [42], has been annotated as microRNA-binding site variant, but other functional consequences of this A > C substitution cannot be ruled out.

Besides GWAS on PCa, various other case-control studies have also associated rs4245739 with the susceptibility to specific types of cancer, such as ovarian, breast cancer, ESCC, SCLC and non-Hodgkin lymphoma [25–27, 41, 43]. Still, different studies have found this variant to have weak or almost no effect on cancer risk in their case-control comparisons [24, 29, 44–46]. Inconsistences in the results of these studies investigating the association between rs4245739 and cancer risk are found regarding not just the statistical significance of the tested association, but also regarding the susceptibility allele. For example, some studies, including those on PCa and also meta-analyses on the association with cancer risk in general, have reported the minor allele C of rs4245739 to be associated with the decreased cancer risk [16, 28, 47]. On the contrary, a study by Garcia-Closas et al. [26] reported the same allelic variant to be associated with the increased breast cancer risk, which is consistent with the previous data from the other breast cancer GWASs [48, 49]. Still, Gansmo et al. [29] have shown the reduced risk of breast cancer to be associated with rs4245739 allele C in their Norwegian case-control study, which matches the results Liu et al. [50] have obtained in Chinese population. Other reports regarding the association of rs4245739 with susceptibility to the specific type of cancer and the disease outcomes have shown the opposite effects of the same allelic variant. For instance, Wynendaele et al. [41] reported A-allele of rs4245739 in patients with ovarian cancer not expressing the estrogen receptor to be associated with increased risk of recurrence and increased risk of tumour-related deaths. Contrary to these findings, Gansmo et al. [24] showed C-allele of rs4245739 to be associated with increased risk of serious ovarian cancer.

Even though rs4245739 is a widely studied genetic variant in terms of many cancers, its role in PCa development and progression remains relatively poorly investigated, with only several studies aiming to elucidate its relation to PCa. In the present study, we found no evidence of association between rs4245739 and the risk of PCa, which is in contrast with the findings of Stegeman et al. [16], as well as with the results of iCOGS GWAS [42]. Still, our results match the ones by Gansmo et al. [29], who found the association of rs4245739 with PCa risk to be statistically insignificant. Furthermore, in our study, minor allele C was found to associate with higher PSA, higher GS, as well as with higher clinical stage of the tumour. In line with these findings, we also observed a statistical trend for the association of rs4245739 C-allele and higher PCa aggressiveness. Since Stegeman et al. [16] and iCOGS GWAS [42] also evaluated the association of this genetic variant with serum PSA score, as well as with disease aggressiveness, we can conclude that our results significantly differ from theirs. Still, the criteria for the evaluation of disease aggressiveness in our and the previous studies were discordant. On the other hand, Gansmo et al. [29] did not provide any data on the potential association of rs4245739 with the values of standard prognostic parameters of PCa, or with the risk of PCa progression. For these reasons, our results on potential association of genetic variant rs4245739 with Gleason score and clinical stages of primary PCa could not be compared with any previously obtained results from other populations.

According to the results of the present study, all three tested genetic variants have shown the association with the parameters of PCa progression. Still, discordances with the previous results were detected, which mainly refer to the lack of association with PCa susceptibility. Among the reasons for such disparity are potential differences in the genetic background of the tested populations, as well as in the environmental factors affecting the PCa development and progression. As an illustration of the ethnic differences, according to genetic variant databases, the distributions of the alleles of these three genetic variants are quite different between populations of European, Asian and African descent. This could affect the power of the specific studies and have consequence on the risk estimates. Also, the results could be affected by the study design and the participant recruitment criteria. As for the associations between the tested genetic variants and the PCa aggressiveness parameters, the differences in subgrouping criteria could have

attributed to the discordances in the obtained results, together with the potential stage-specific effects of these genetic variants. Still, the main limitation of our study is its sample size, even though more than 1000 participants were included. This could have resulted in the lack of ability to validate the previously detected associations with PCa susceptibility. Furthermore, the number of PCa patients in several subgroups was small, which suggests that the obtained results should be interpreted with caution. Still, the same direction of association of the tested genetic variants with different parameters of PCa progression point out their relevance for the disease aggressiveness assessment. However, future studies with larger sample sizes in populations of different origin are needed to better clarify the potential association of genetic variants rs1058205, rs1010 and rs4245739 with PCa.

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Authors' Contributions N.K. performed genetic analysis and wrote the manuscript. Z.D. performed the statistical analysis and reviewed the manuscript. S.M. supported genetic analysis. D.S.P. and GB reviewed the manuscript.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with Ethical Standards

Conflict of Interests The authors declare that there is no conflict of interests.

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