

Original Article

Assessment of possible association between rs3787016 and prostate cancer risk in Serbian population

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Abstract: Recent study, which included meta-analysis of two genome-wide association studies (GWAS), followed by a replication, identified the association between single nucleotide polymorphism (SNP) rs3787016 at 19p13 and prostate cancer (PCa) risk. Considering possible genetic differences between populations, we conducted the study in order to evaluate the association of this polymorphism with prostate cancer risk in Serbian population. 261 samples of peripheral blood were obtained from the patients with PCa and 257 samples from patients with benign prostatic hyperplasia (BPH). 106 volunteers who gave samples of bucal swabs comprised the control group. For individuals diagnosed with PCa clinicopathological characteristics including serum prostate-specific antigen (PSA) level at diagnosis, Gleason score (GS) and clinical stage were determined. Genotypization of rs3787016 was performed by using Taqman® SNP Genotyping Assay. The differences in allele and genotype frequencies between analyzed groups of subjects were performed by using PLINK, SPSS 17.0 for Windows and SNPStats statistical software. No significant association of rs3787016 with PCa risk was determined comparing allele and genotype frequencies among group of patients diagnosed with PCa and the control group, as well as among groups of patients with PCa and BPH. Also, no evidence of association of rs3787016 with PCa risk was shown using tests for association under dominant and recessive genetic models. SNP rs3787016 showed no significant association with standard prognostic parameters regarding PCa progression, nor with the risk of disease progression assessed according to two different risk classification systems.

Keywords: Prostate cancer, association study, single nucleotide polymorphism (SNP)

Introduction

Prostate cancer (PCa) is the second most common cancer in males with the incidence rates varying worldwide, reaching highest values primarily in developed countries. It is also the sixth leading cause of cancer death in men worldwide, with the highest mortality rate among men of African descend [1].

Due to this high mortality rate, extensive research has focused on molecular basis of PCa which still remains largely unknown. Epidemiological studies suggested that it is a multifactorial disease with genetic components involved in its etiology [2-4]. For this reason, much research effort has been focused on determining genetic variants with low penetrability associ-

ated with PCa. Recently, genome-wide association studies (GWAS) have provided a new approach in determining common genetic variants associated with human diseases, including malignancies [5]. The major contribution of this type of studies is identification of novel biomarkers which would eventually be implemented in accurate risk and disease course assessment [6, 7].

GWAS have yielded a large number of PCa risk loci, currently more than 30, located at 2p15, 3p12, 6q25, 7p15, 7q21, 8q24, 10q11, 10q26, 11q13, 12q13, 17q12, 17q24, 19q13, and Xp11 [8-21]. Studies have also shown association of several risk alleles with clinical and pathological parameters of PCa, such as Gleason score, serum prostate-specific antigen

(PSA) at diagnosis, presence of metastases, cancer aggressiveness and cancer-related mortality [22-24]. Results of association studies may vary between populations due to interpopulation genetic differences, including differences in allele frequencies and linkage disequilibrium (LD) structures [25].

The meta-analysis of two existing PCa GWAS, Johns Hopkins Hospital (JHH) and Cancer Genetic Markers of Susceptibility (CGEMS), followed by an additional replication revealed a PCa risk locus rs3787016 at 19p13 with the risk allele A (P value 7.22E-07; per allele odds ratio = 1.19; 95% confidence interval: 1.11-1.27) [26]. SNP rs3787016 localizes to the fourth intron of RNA polymerase II polypeptide E gene (*POLR2E*), which encodes a subunit of RNA polymerases I, II and III. Previous data suggested that this SNP is also located in the long non-coding RNA (lncRNA) gene AC112706.1 (Ensembl Archived Gene Stable ID: ENSG00000244958, <http://mar2010.archive.ensembl.org/index.html>) overlapping with *POLR2E* gene [26]. lncRNA gene AC112706.1 was present in Ensembl database release 57 from March 2010, while in later releases 58 and 59 it was specified as small nuclear RNA gene and finally withdrawn from release 60 (<http://www.ensembl.org/index.html>). Functional genetic variant in LD with rs3787016 is not identified and the biological mechanism underlying its effect on PCa susceptibility remains unknown [26]. The possible association of this SNP with PCa progression was not tested and the replication studies were not conducted in other populations.

Due to possible population differences, it is of importance to assess the association of SNP rs3787016 with PCa risk in different populations other than the one in which this association was identified (Caucasian Americans from JHH and CGEMS studies) [26]. In this study, we tested in Serbian population the reported association between rs3787016 risk allele A and PCa risk. Furthermore, we assessed the association of rs3787016 with standard prognostic parameters regarding PCa progression, as well as with the risk of cancer progression among men in the same population.

Materials and methods

The study used peripheral blood samples obtained from patients treated in the period be-

tween February 2009 and April 2012 at the Clinical Centre "Dr. Dragiša Mišević" and Clinical Centre "Zvezdara". Research was conducted with the approval of ethical committees of these medical institutions.

261 samples of peripheral blood were obtained from the patients with PCa and 257 samples from patients with benign prostatic hyperplasia (BPH). 106 volunteers who gave samples of buccal swabs comprised the control group. 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 PSA level and biopsy of the prostate. Serum PSA levels were determined by Hybritech method of monoclonal immunoassay. Pathohistological report included standard parameters for reporting PCa. Clinical stage of cancer was determined according to TNM classification system.

Patients with PCa were selected into groups based on 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 presence of metastases. Based on the risk for localized cancer progression, three groups of patients were formed, according to D'Amico criteria. Groups were defined as low-risk (PSA < 10 ng/ml, GS < 6, 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) [27]. 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 metastases) groups according to Medeiros *et al.* [28].

Genomic DNA was isolated from peripheral blood and buccal swab samples by using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturers' protocol. Genotyping of rs3787016 was performed by using Taqman® SNP Genotyping Assay (Applied Biosystems, Foster City, California, USA). Statistical analysis of SNP association was done by using PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink/> [29]), SPSS 17.0 for Windows

Table 1. Classification of patients with prostate cancer based on values of standard prognostic parameters regarding disease progression

Standard prognostic parameter	Prostate cancer patients n (%)
PSA at diagnosis	
<10 ng/ml	81 (31.3)
10-20 ng/ml	68 (26.2)
>20 ng/ml	110 (42.5)
Unknown	2
Gleason score	
<7	145 (57.5)
=7	72 (28.6)
>7	35 (13.9)
Unknown	9
Clinical stage	
T1	40 (18.2)
T2	113 (51.4)
T3/T4	67 (30.4)
Unknown	41
Metastases	
Detected	72 (32.7)
Not detected	148 (67.3)
Unknown	41

Table 2. Classification of patients with prostate cancer based on the risk for disease progression

Risk group	Prostate cancer patients n (%)
D'Amico risk criteria	
Low-risk	16 (6.4)
Intermediate-risk	87 (34.9)
High-risk	146 (58.6)
Unknown	12
Medeiros et al. risk criteria	
Low-risk	96 (40.3)
High-risk	142 (59.7)
Unknown	23

(SPSS Inc., Chicago, IL) and SNPStats software (<http://bioinfo.iconcologia.net/SNPstats> [30]). Hardy-Weinberg equilibrium was assessed by using exact test [31] implemented in PLINK software. Allelic and genotypic associations were evaluated by χ^2 and Fisher's exact test. Association under dominant and recessive genetic models was assessed by χ^2 test. Cochran-Armitage trend test was also used to assess the association under additive genetic model.

Results

We successfully genotyped SNP rs3787016 in 106 control subjects, 257 patients with BPH

and 261 patients with PCa. Clinical and pathological characteristics of the group of patients with PCa are summarized in **Table 1**. The majority of subjects had PCa with low Gleason score (GS<7). In almost one third of patients with PCa metastases were detected. Also, most of the patients were diagnosed with high-risk PCa according to both D'Amico criteria and Medeiros et al. [27, 28] (**Table 2**).

The rs3787016 allele A and genotype frequencies in groups of patients with PCa and BPH, as well as in the control group, are summarized in **Table 3**. Frequencies of genotypes for SNP rs3787016 were consistent with Hardy-

Table 3. Rs3787016 allele A frequencies and genotype distributions in groups of patients with prostate cancer and benign prostatic hyperplasia and in the control group

Group	Allele A frequency	Genotype frequencies		
		GG	AG	AA
Prostate cancer	0.272	0.536	0.383	0.081
Benign prostatic hyperplasia	0.245	0.580	0.350	0.070
Controls	0.274	0.519	0.415	0.066

Table 4. Association of allele A and genotypes of rs3787016 with the prostate cancer risk

Comparison	Allelic χ^2 test	Fisher's exact test	Cochran-Armitage trend test	P value			Per allele OR (95% CI)
				Genotypic χ^2 test	Dominant model	Recessive model	
PCa vs. BPH	0.323	0.356	0.333	0.605	0.320	0.653	1.15 (0.87-1.52)
PCa vs. controls	0.966	1	0.966	0.800	0.760	0.637	0.99 (0.69-1.42)
BPH vs. controls	0.423	0.453	0.429	0.504	0.288	0.891	0.86 (0.60-1.24)

Abbreviations: OR-odds ratio; CI-confidence interval.

Weinberg equilibrium (P value ≥ 0.05) among both groups of patients with PCa and BPH, as well as among controls selected from the general population.

No significant association of rs3787016 with PCa risk was determined based on differences in rs3787016 allele frequencies and genotype distributions between the group of patients with PCa and the control group. Similar results were obtained by comparing rs3787016 allele A frequencies and genotype distributions in groups of patients with PCa and BPH. Furthermore, no significant association was observed between rs3787016 and the risk of BPH (BPH vs. controls comparison) (**Table 4**). We next performed tests for association of rs3787016 with PCa risk under alternative genetic models (dominant and recessive). These tests also showed no evidence of association of SNP rs3787016 with PCa risk. Similarly, there was no evidence of association of rs3787016 with the risk of BPH (**Table 4**).

Table 5 shows the results of tests for association of rs3787016 allele A and genotypes with standard prognostic parameters regarding progression of PCa. Since both allelic and genotypic tests yielded P values > 0.2 , no evidence to support the association of rs3787016 alleles and genotypes with standard prognostic parameters

was found. Similarly, no association between rs3787016 and the presence of metastases among patients with PCa was determined. There was also no evidence of association of rs3787016 with standard prognostic parameters, nor with the presence of metastases assuming dominant and recessive genetic models (**Table 5**).

Neither allelic ($P = 0.765$) nor genotypic ($P = 0.428$) χ^2 test revealed significant association of rs3787016 with the risk of PCa progression assessed according to D'Amico criteria [27] (**Table 6**). Rs3787016 allele A frequencies and genotype distributions in low-risk and high-risk PCa according to Medeiros *et al.* [28] are summarized in **Table 7**. Results of applied statistical tests suggested that rs3787016 is not associated with the risk of PCa progression assessed according to Medeiros *et al.* [28] (**Table 7**).

Discussion

Prostate cancer is a heterogeneous disease marked with a broad spectrum of clinical behaviors and pathological characteristics, which is likely to reflect underlying molecular heterogeneity [32]. Great research efforts have been made to identify genetic alterations, gene expression patterns and genetic variants in numerous genes associated with PCa onset and/

Table 5. Rs3787016 allele A frequencies and genotype distributions in groups of patients based on values of standard prognostic parameters. Association of rs3787016 with standard prognostic parameters regarding prostate cancer progression and the presence of metastases.

Comparison	Allele A frequency	Genotype frequencies			P value			
		GG	AG	AA	Allelic χ^2 test	Genotypic χ^2 test	Dominant model	Recessive model
PSA at diagnosis					0.960	0.262	0.506	0.328
<10 ng/ml	0.283	0.580	0.309	0.111				
vs. 10-20 ng/ml	0.279	0.485	0.471	0.044				
vs. >20 ng/ml	0.268	0.546	0.373	0.181				
Gleason score					0.481	0.807*	0.601	0.603*
<7	0.259	0.552	0.379	0.069				
vs. =7	0.285	0.528	0.375	0.097				
vs. >7	0.329	0.457	0.429	0.114				
Clinical stage					0.831	0.624*	0.873	0.389*
T1	0.300	0.525	0.350	0.125				
vs. T2	0.266	0.531	0.407	0.062				
vs. T3/T4	0.269	0.567	0.328	0.105				
Metastases					0.868	0.536	0.761	0.362
Detected	0.278	0.556	0.333	0.111				
vs. Not detected	0.270	0.534	0.392	0.074				

*contingency tables include cells with expected count less than 5. No more than 20% of the expected counts are less than 5 and all individual expected counts are 1 or greater.

Table 6. Association of rs3787016 with the risk of PCa progression assessed according to D'Amico criteria.

Comparison	Allele A frequency	Genotype frequencies			P value			
		GG	AG	AA	Allelic χ^2 test	Genotypic χ^2 test	Dominant model	Recessive model
D'Amico risk criteria					0.765	0.428*	0.494	0.495*
Low-risk	0.219	0.687	0.188	0.125				
vs. Intermediate-risk	0.264	0.529	0.414	0.057				
vs. High-risk	0.277	0.541	0.363	0.096				

*contingency tables include cells with expected count less than 5. No more than 20% of the expected counts are less than 5 and all individual expected counts are 1 or greater.

or progression. Along with the identification of somatic mutations and epigenetic perturbations involved in prostate carcinogenesis, a large number of loci associated with PCa risk has been determined, mainly through GWAS [33]. First identified SNPs associated with PCa risk were located within the 8q24 chromosomal region [8-10]. Afterwards, a large number of other PCa risk loci were identified, many of which were replicated in different populations [11-21, 34-36].

Observed variations in results of replication studies were attributed to differences in ancestral backgrounds [35, 36]. For this reason, it is of importance to study previously reported risk loci in multiple populations to confirm association with disease risk. Since some of the PCa susceptibility loci also showed association with clinicopathological characteristics of PCa [22-24], it is important to evaluate the possible association of novel PCa risk loci with disease progression.

Table 7. Association of rs3787016 with the risk of PCa progression assessed according to Medeiros *et al.*

Comparison	Allele A frequency	Genotype frequencies			P value					Per allele OR (95%CI)	
		GG	AG	AA	Allelic χ^2 test	Fisher's exact test	Cochran-Armitage trend test	Genotypic χ^2 test	Dominant model		Recessive model
Medeiros <i>et al.</i> risk criteria					0.240	0.254	0.251	0.512	0.269	0.493	1.281 (0.85-1.94)
Low-risk	0.299	0.573	0.354	0.073							
vs. High-risk	0.250	0.500	0.401	0.099							

Abbreviations: OR-odds ratio; CI-confidence interval.

Jin *et al.* conducted a meta-analysis of two GWAS and a replication study through which they identified a novel PCa risk locus rs3787016 at 19p13 [26]. This chromosomal region was previously associated through linkage studies with familial type of PCa in several populations with various ancestral backgrounds, but its functional implication remained unknown [37, 38]. Since rs3787016 locates to an intron of *POLR2E* [26], functional genetic variants underlying reported association with PCa could also be located in the coding or non-coding regions of this gene, causing its abnormal structure and/or expression pattern with possible involvement in carcinogenesis. Jin *et al.* also suggested possible link of PCa susceptibility with the lncRNA gene overlapping with *POLR2E*, which was not functionally characterized and was later withdrawn from Ensembl database ([26] <http://www.ensembl.org/index.html>).

In contrast to results from the study conducted by Jin *et al.* [26], our findings showed lack of association between rs3787016 and PCa risk. Since the previous study included a far larger number of subjects (1906 PCa cases from JHH GWAS and 3084 controls, 1176 PCa cases from CGEMS study and 1157 controls, as well as 1114 cases and 822 controls comprising the replication population) compared to ours, difference in sample size could possibly be one of the reasons underlying the observed discordance in results of these two studies. Lack of evidence of association between the SNP rs3787016 and the risk of PCa could also reflect genetic differences between Serbian population and the populations of subjects included in the study by Jin *et al.* [26].

Our results also show lack of evidence for the association of rs3787016 with standard prognostic parameters regarding PCa progression, as well as with the risk of progression assessed according to D'Amico *et al.* and Medeiros *et al.* [27, 28]. Since there were no previous studies considering possible association between rs3787016 and PCa progression, lack of evidence of this association in Serbian population couldn't be compared with the data from other populations.

In order to evaluate the possible effect of sample size on results of our study, further analysis with the larger number of subjects should be performed. Increasing the sample size could lead to differences in rs3787016 allele and genotype frequencies between analyzed groups of subjects reaching statistical significance. A significant increase in sample size is required to determine if genetic variation between populations of diverse ancestral background underlie the observed results of the tests for possible association of rs3787016 with PCa risk in Serbian population. In order to confirm results suggesting that rs3787016 is not associated with PCa progression further analyses are required following the increase in sample size.

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