

## Original Article

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

Zorana Z Nikolić<sup>1</sup>, Goran N Brajušković<sup>1</sup>, Dušanka Lj Savić Pavičević<sup>1</sup>, Aleksandar S Kojić<sup>1</sup>, Vinka D Vukotić<sup>2</sup>, Saša M Tomović<sup>3</sup>, Snežana J Cerović<sup>4</sup>, Vladimir Filipović<sup>5</sup>, Đuro Mišljenović<sup>5</sup>, Stanka P Romac<sup>1</sup>

<sup>1</sup>University of Belgrade, Faculty of Biology, Belgrade, Serbia; <sup>2</sup>Department of Urology, Clinical Centre "dr Dragiša Mišović", Belgrade, Serbia; <sup>3</sup>Clinical of Surgery, Clinical Centre "Zvezdara", Belgrade, Serbia; <sup>4</sup>Institute of Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Serbia; <sup>5</sup>University of Belgrade, Faculty of Mathematics, Belgrade, Serbia

Received October 3, 2012; accepted November 2, 2012; Epub November 18, 2012; Published January 1, 2012

**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 descent [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 Mišović" 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 bucal 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 bucal 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

## SNP rs3787016 and prostate cancer risk

**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  $\chi^2$  and Fisher's exact test. Association under dominant and recessive genetic models was assessed by  $\chi^2$  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-

## SNP rs3787016 and prostate cancer risk

**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	P value						Per allele OR (95% CI)
	Allelic $\chi^2$ test	Fisher's exact test	Cochran-Armitage trend test	Genotypic $\chi^2$ 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  $\geq 0.05$ ) 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 ( $P = 0.428$ )  $\chi^2$  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/

## SNP rs3787016 and prostate cancer risk

**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 $\chi^2$ test	Genotypic $\chi^2$ 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 $\chi^2$ test	Genotypic $\chi^2$ 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 $\chi^2$ test	Fisher's exact test	Cochran-Armitage trend test	Genotypic $\chi^2$ 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 vs. High-risk	0.299	0.573	0.354	0.073							
	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.

#### Acknowledgements

The research was supported by the Ministry of Education and Science of Serbia (Project no. 173016).

**Address correspondence to:** Dr. Goran Brajušković, Studentski trg 3, P.Box 52, 11000 Belgrade, Serbia. Tel: + 381 11 2639 100; Fax: + 381 11 26 39 100; E-mail: brajuskovic@bio.bg.ac.rs

References

- [1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN. *Int J Cancer* 2010; 127: 2893-2917.
- [2] Steinberg GD, Crater BS, Beaty TH, Childs B, Walsh PC. Family history and the risk of prostate cancer. *The Prostate* 1990; 17: 337-347.
- [3] Walsh PC, Partin AW. Family history facilitates the early diagnosis of prostate carcinoma. *Cancer* 1997; 80: 1871-1874.
- [4] Grönberg H, Damber L, Damber JE. Studies of genetic factors in prostate cancer in a twin population. *J Urol* 1994; 152: 1484-1487.
- [5] Easton DF, Eeles RA. Genome-wide association studies in cancer. *Human Molecular Genetics* 2008; 17: R109-R115.
- [6] Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. *Nature Reviews* 2009; 10: 241-251.
- [7] Ng PC, Murray SS, Levy S, Venter JC. An agenda for personalized medicine. *Nature* 2009; 461: 724-726.
- [8] Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Cazier JB, Sainz J, Jakobsdottir M, Kostic J, Magnusdottir DN, Ghosh S, Agnarsson K, Birgisdottir B, Le Roux L, Olafsdottir A, Blondal T, Andresdottir M, Gretarsdottir OS, Bergthorsson JT, Gudbjartsson D, Gylfason A, Thorleifsson G, Manolescu A, Kristjansson K, Geirsson G, Isaksson H, Douglas J, Johansson JE, Bälter K, Wiklund F, Montie JE, Yu X, Suarez BK, Ober C, Cooney KA, Gronberg H, Catalona WJ, Einarsson GV, Barkardottir RB, Gulcher JR, Kong A, Thorsteinsdottir U, Stefansson K. A common variant associated with prostate cancer in European and African populations. *Nat Genet* 2006; 38: 652-658.
- [9] Freedman ML, Haiman CA, Patterson N, McDonald GJ, Tandon A, Waliszewska A, Penney K, Steen RG, Ardlie K, John EM, Oakley-Girvan I, Whittemore AS, Cooney KA, Ingles SA, Altshuler D, Henderson BE, Reich D. Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. *Proc Natl Acad Sci USA* 2006; 103: 14068-14073.
- [10] Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Xu J, Blondal T, Kostic J, Sun J, Ghosh S, Stacey SN, Mouy M, Saemundsdottir J, Backman VM, Kristjansson K, Tres A, Partin AW, Albers-Akkers MT, Godino-Ivan Marcos J, Walsh PC, Swinkels DW, Navarrete S, Isaacs SD, Aben KK, Graif T, Cashy J, Ruiz-Echarri M, Wiley KE, Suarez BK, Witjes JA, Frigge M, Ober C, Jonsson E, Einarsson GV, Mayordomo JI, Kiemeny LA, Isaacs WB, Catalona WJ, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet* 2007; 39: 631-637.
- [11] Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, Rafnar T, Gudbjartsson D, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Blondal T, Stacey SN, Helgason A, Gunnarsdottir S, Olafsdottir A, Kristinsson KT, Birgisdottir B, Ghosh S, Thorlacius S, Magnusdottir D, Stefansdottir G, Kristjansson K, Bagger Y, Wilensky RL, Reilly MP, Morris AD, Kimber CH, Adeyemo A, Chen Y, Zhou J, So WY, Tong PC, Ng MC, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Tres A, Fuertes F, Ruiz-Echarri M, Asin L, Saez B, van Boven E, Klaver S, Swinkels DW, Aben KK, Graif T, Cashy J, Suarez BK, van Vierssen Trip O, Frigge ML, Ober C, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Palmer CN, Rotimi C, Chan JC, Pedersen O, Sigurdsson G, Benediktsson R, Jonsson E, Einarsson GV, Mayordomo JI, Catalona WJ, Kiemeny LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet* 2007; 39: 977-983.
- [12] Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ, Greenway SC, Stram DO, Le Marchand L, Kolonel LN, Frasco M, Wong D, Pooler LC, Ardlie K, Oakley-Girvan I, Whittemore AS, Cooney KA, John EM, Ingles SA, Altshuler D, Henderson BE, Reich D. Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet* 2007; 39: 638-644.
- [13] Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearnhead P, Yu K, Chatterjee N, Wang Z, Welch R, Staats BJ, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cancel-Tassin G, Cussenot O, Valeri A, Andriole GL, Gelmann EP, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover R, Hunter DJ, Chanock SJ, Thomas G. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet* 2007; 39: 645-649.
- [14] Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK, Mulholland S, Leongamornlert DA, Edwards SM, Morrison J, Field HI, Southey MC, Severi G, Donovan JL, Hamdy FC, Dearnaley DP, Muir KR, Smith C, Bagnato M, Arderm-Jones AT, Hall AL, O'Brien LT, Gehr-Swain BN, Wilkinson RA, Cox A, Lewis



## SNP rs3787016 and prostate cancer risk

- S, Brown PM, Jhavar SG, Tymrakiewicz M, Lophatananon A, Bryant SL; UK Genetic Prostate Cancer Study Collaborators; British Association of Urological Surgeons' Section of Oncology; UK ProtecT Study Collaborators, Horwich A, Huddart RA, Khoo VS, Parker CC, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Fisher C, Jamieson C, Cooper CS, English DR, Hopper JL, Neal DE, Easton DF. Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet* 2008; 40: 316-321.
- [15] Gudmundsson J, Sulem P, Rafnar T, Bergthorsson JT, Manolescu A, Gudbjartsson D, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Blondal T, Jakobsdottir M, Stacey SN, Kostic J, Kristinsson KT, Birgisdottir B, Ghosh S, Magnusdottir DN, Thorlacius S, Thorleifsson G, Zheng SL, Sun J, Chang BL, Elmore JB, Breyer JP, McReynolds KM, Bradley KM, Yaspan BL, Wiklund F, Stattin P, Lindström S, Adami HO, McDonnell SK, Schaid DJ, Cunningham JM, Wang L, Cerhan JR, St Sauver JL, Isaacs SD, Wiley KE, Partin AW, Walsh PC, Polo S, Ruiz-Echarri M, Navarrete S, Fuertes F, Saez B, Godino J, Weijerman PC, Swinkels DW, Aben KK, Witjes JA, Suarez BK, Helfand BT, Frigge ML, Kristjansson K, Ober C, Jonsson E, Einarsson GV, Xu J, Gronberg H, Smith JR, Thibodeau SN, Isaacs WB, Catalona WJ, Mayordomo JI, Kiemenev LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet* 2008; 40: 281-283.
- [16] Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, Yu K, Chatterjee N, Welch R, Hutchinson A, Crenshaw A, Cancel-Tassin G, Staats BJ, Wang Z, Gonzalez-Bosquet J, Fang J, Deng X, Berndt SI, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cussenot O, Valeri A, Andriole GL, Crawford ED, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover R, Hayes RB, Hunter DJ, Chanock SJ. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet* 2008; 40: 310-315.
- [17] Al Olama AA, Kote-Jarai Z, Giles GG, Guy M, Morrison J, Severi G, Leongamornlert DA, Tymrakiewicz M, Jhavar S, Saunders E, Hopper JL, Southey MC, Muir KR, English DR, Dearnaley DP, Arden-Jones AT, Hall AL, O'Brien LT, Wilkinson RA, Sawyer E, Lophatananon A; UK Genetic Prostate Cancer Study Collaborators/British Association of Urological Surgeons' Section of Oncology; UK Prostate testing for cancer and Treatment study (ProtecT Study) Collaborators, Horwich A, Huddart RA, Khoo VS, Parker CC, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Cooper C, Donovan JL, Hamdy FC, Neal DE, Eeles RA, Easton DF. Multiple loci on 8q24 associated with prostate cancer susceptibility. *Nat Genet* 2009; 41: 1058-1060.
- [18] Eeles RA, Kote-Jarai Z, Al Olama AA, Giles GG, Guy M, Severi G, Muir K, Hopper JL, Henderson BE, Haiman CA, Schleutker J, Hamdy FC, Neal DE, Donovan JL, Stanford JL, Ostrander EA, Ingles SA, John EM, Thibodeau SN, Schaid D, Park JY, Spurdle A, Clements J, Dickinson JL, Maier C, Vogel W, Dörk T, Rebbeck TR, Cooney KA, Cannon-Albright L, Chappuis PO, Hutter P, Zeegers M, Kaneva R, Zhang HW, Lu YJ, Foulkes WD, English DR, Leongamornlert DA, Tymrakiewicz M, Morrison J, Arden-Jones AT, Hall AL, O'Brien LT, Wilkinson RA, Saunders EJ, Page EC, Sawyer EJ, Edwards SM, Dearnaley DP, Horwich A, Huddart RA, Khoo VS, Parker CC, Van As N, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Cooper CS, Southey MC, Lophatananon A, Liu JF, Kolonel LN, Le Marchand L, Wahlfors T, Tammela TL, Auvinen A, Lewis SJ, Cox A, FitzGerald LM, Koopmeiners JS, Karyadi DM, Kwon EM, Stern MC, Corral R, Joshi AD, Shahabi A, McDonnell SK, Sellers TA, Pow-Sang J, Chambers S, Aitken J, Gardiner RA, Batra J, Kedda MA, Lose F, Polanowski A, Patterson B, Serth J, Meyer A, Luedeke M, Stefflova K, Ray AM, Lange EM, Farnham J, Khan H, Slavov C, Mitkova A, Cao G; UK Genetic Prostate Cancer Study Collaborators/British Association of Urological Surgeons' Section of Oncology; UK ProtecT Study Collaborators; PRACTICAL Consortium, Easton DF. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. *Nat Genet* 2009; 41: 1116-1121.
- [19] Gudmundsson J, Sulem P, Gudbjartsson DF, Blondal T, Gylfason A, Agnarsson BA, Benediktsdottir KR, Magnusdottir DN, Orlygsdottir G, Jakobsdottir M, Stacey SN, Sigurdsson A, Wahlfors T, Tammela T, Breyer JP, McReynolds KM, Bradley KM, Saez B, Godino J, Navarrete S, Fuertes F, Murillo L, Polo E, Aben KK, van Oort IM, Suarez BK, Helfand BT, Kan D, Zanon C, Frigge ML, Kristjansson K, Gulcher JR, Einarsson GV, Jonsson E, Catalona WJ, Mayordomo JI, Kiemenev LA, Smith JR, Schleutker J, Barkardottir RB, Kong A, Thorsteinsdottir U, Rafnar T, Stefansson K. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet* 2009; 41: 1122-1126.
- [20] Yeager M, Chatterjee N, Ciampa J, Jacobs KB, Gonzalez-Bosquet J, Hayes RB, Kraft P, Wacholder S, Orr N, Berndt S, Yu K, Hutchinson A, Wang Z, Amundadottir L, Feigelson HS, Thun MJ, Diver WR, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Cancel-Tassin G, Cussenot O, Valeri A, Andriole GL, Crawford ED, Haiman



- CA, Henderson B, Kolonel L, Le Marchand L, Siddiq A, Riboli E, Key TJ, Kaaks R, Isaacs W, Isaacs S, Wiley KE, Gronberg H, Wiklund F, Stattin P, Xu J, Zheng SL, Sun J, Vatten LJ, Hveem K, Kumle M, Tucker M, Gerhard DS, Hoover RN, Fraumeni JF Jr, Hunter DJ, Thomas G, Chanock SJ. Identification of a new prostate cancer susceptibility locus on chromosome 8q24. *Nat Genet* 2009; 41: 1055-1057.
- [21] Takata R, Akamatsu S, Kubo M, Takahashi A, Hosono N, Kawaguchi T, Tsunoda T, Inazawa J, Kamatani N, Ogawa O, Fujioka T, Nakamura Y, Nakagawa H. Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. *Nat Genet* 2010; 42: 751-754.
- [22] Zheng SL, Sun J, Cheng Y, Li G, Hsu FC, Zhu Y, Chang BL, Liu W, Kim JW, Turner AR, Gielzak M, Yan G, Isaacs SD, Wiley KE, Sauvageot J, Chen HS, Gurganus R, Mangold LA, Trock BJ, Gronberg H, Duggan D, Carpten JD, Partin AW, Walsh PC, Xu J, Isaacs WB. Association Between Two Unlinked Loci at 8q24 and Prostate Cancer Risk Among European Americans. *J Natl Cancer Inst* 2007; 99: 1525-1533.
- [23] Gallagher DJ, Vijai J, Cronin AM, Bhatia J, Vickers AJ, Gaudet MM, Fine S, Reuter V, Scher HI, Halldén C, Dutra-Clarke A, Klein RJ, Scardino PT, Eastham JA, Lilja H, Kirchoff T, Offit K. Susceptibility loci associated with prostate cancer progression and mortality. *Clin Cancer Res* 2010; 16: 2819-2832.
- [24] Pomerantz MM, Werner L, Xie W, Regan MM, Lee GS, Sun T, Evan C, Petrozziello G, Nakabayashi M, Oh WK, Kantoff PW, Freedman ML. Association of Prostate Cancer Risk Loci with Disease Aggressiveness and Prostate Cancer-Specific Mortality. *Cancer Prev Res* 2011; 4: 719-728.
- [25] Neale BM, Sham PC. The Future of Association Studies: Gene-Based Analysis and Replication. *Am J Hum Genet* 2004; 75: 353-362.
- [26] Jin G, Sun J, Isaacs SD, Wiley KE, Kim ST, Chu LW, Zhang Z, Zhao H, Zheng SL, Isaacs WB, Xu J. Human polymorphisms at long non-coding RNAs (lncRNAs) and association with prostate cancer risk. *Carcinogenesis* 2011; 32: 1655-1659.
- [27] D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, Tomaszewski JE, Renshaw AA, Kaplan I, Beard CJ, Wein A. Biochemical Outcome After Radical Prostatectomy, External Beam Radiation Therapy, or Interstitial Radiation Therapy for Clinically Localized Prostate Cancer. *JAMA* 1998; 280: 969-974.
- [28] Medeiros R, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, Lopes C. Endothelial nitric oxide synthase gene polymorphisms and genetic susceptibility to prostate cancer. *European Journal of Cancer Prevention* 2002; 11: 343-350.
- [29] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a toolset for whole-genome association and population-based linkage analysis. *Am J Hum Genet* 2007; 81: 559-575.
- [30] Solé X, Guinó E, Valls J, Iñiesta R, Moreno V. SNPstats: a web tool for the analysis of association studies. *Bioinformatics* 2006; 22: 1928-1929.
- [31] Wigginton JE, Cutler DJ, Abecasis GR. A Note on Exact Tests of Hardy-Weinberg Equilibrium. *Am J Hum Genet* 2005; 76: 887-893.
- [32] Mackinnon AC, Yan BC, Joseph LJ, Al-Ahmadie HA. Molecular biology underlying the clinical heterogeneity of prostate cancer: an update. *Arch Pathol Lab Med* 2009; 133: 1033-1040.
- [33] Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* 2010; 24: 1967-2000.
- [34] Kote-Jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, Schaid D, Thibodeau S, Dörk T, Neal D, Donovan J, Hamdy F, Cox A, Maier C, Vogel W, Guy M, Muir K, Lophatananon A, Kedda MA, Spurdle A, Steginga S, John EM, Giles G, Hopper J, Chappuis PO, Hutter P, Foulkes WD, Hamel N, Salinas CA, Koopmeiners JS, Karyadi DM, Johanneson B, Wahlfors T, Tammela TL, Stern MC, Corral R, McDonnell SK, Schürmann P, Meyer A, Kuefer R, Leongamornlert DA, Tymrakiewicz M, Liu JF, O'Mara T, Gardiner RA, Aitken J, Joshi AD, Severi G, English DR, Southey M, Edwards SM, Al Olama AA; PRACTICAL Consortium, Eeles RA. Multiple novel prostate cancer predisposition loci confirmed by an international study: The PRACTICAL Consortium. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 2052-2061.
- [35] Waters KM, Le Marchand L, Kolonel LN, Monroe KR, Stram DO, Henderson BE, Haiman CA. Generalizability of Associations from Prostate Cancer Genome-Wide Association Studies in Multiple Populations. *Cancer Epidemiol Biomarkers Prev* 2009; 18: 1290-1294.
- [36] Hooker S, Hernandez W, Chen H, Robbins C, Torres JB, Ahaghotu C, Carpten J, Kittles RA. Replication of Prostate Cancer Risk Loci on 8q24, 11q13, 17q12, 19q33, and Xp11 in African Americans. *The Prostate* 2010; 70: 270-275.
- [37] Wiklund F, Gillanders EM, Albertus JA, Bergh A, Damber JE, Emanuelsson M, Freas-Lutz DL, Gildea DE, Göransson I, Jones MS, Jonsson BA, Lindmark F, Markey CJ, Riedesel EL, Stenman E, Trent JM, Grönberg H. Genome-wide scan of Swedish families with hereditary prostate cancer: Suggestive evidence of linkage at 5q11.2 and 19p13.3. *The Prostate* 2003; 57: 290-297.

## SNP rs3787016 and prostate cancer risk

- [38] Hsieh CL, Oakley-Girvan I, Balise RR, Halpern J, Gallagher RP, Wu AH, Kolonel LN, O'Brien LE, Lin IG, Van Den Berg DJ, Teh CZ, West DW, Whittemore AS. A Genome Screen of Families with Multiple Cases of Prostate Cancer: Evidence of Genetic Heterogeneity. *Am J Hum Genet.* 2001; 69: 148-158.