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Genome-Wide Association Scan for Variants Associated with Early-Onset Prostate Cancer in Romania

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Abstract

Objectives: There is evidence that prostate cancer (PCa) has a genetic predisposition, multiple genome-wide associations studies evaluating the impact of common and rare markers in the etiology of PCa and early-onset PCa. In our study we performed a genome-wide association scan for early-onset prostate cancer in a cohort of genotyped hospital patients form Romania. **Used methodology:** The study consisted of 2024 hospital patients genotyped, 990 unrelated PCa cases histopathologically confirmed and with abnormal PSA levels, and 1034 controls consisting of patients admitted for urological and surgical conditions, excluding cancer. **Conclusions:** We found genome-wide significant evidence for variants on chromosomes 2 ,12 and 22. We also evaluated the association results for the whole-genome obtaining compelling evidence regarding the importance of common genetic variants to early-onset PCa in Romania.

Keywords: prostate cancer, genome-wide associations studies, variants, susceptibility loci, single nucleotide polymorphisms, DNA

Abstract

Obiectivele studiului: Există informații ce dovedesc predispoziția genetică a cancerului de prostată (PCa). Multiple teste de asociere la nivelul întregului genom uman au evaluat impactul variantelor comune și a celor rar întâlnite în etiologia cancerului de prostată și a cancerului de prostată cu debut rapid. În studiul nostru am realizat un test de asociere la nivelul întregului genom uman pentru fenotipul cancerului de prostată cu debut rapid în care am utilizat o cohortă de pacienți genotipați din România. **Metode:** Studiul a fost realizat pe 2024 de pacienți genotipați, 990 de cazuri de PCa confirmate histopatologic și cu valori anormale ale PSA-ului seric, rezultatele fiind comparate cu un grup de 1034 de pacienți care s-au prezentat la spital pentru alte afecțiuni urologice cu excepția cancerului. **Concluzii:** Am obținut rezultate seminificative la nivelul întregului genom uman pentru variante localizate pe cromozomul 2, 12 și 22. De asemenea, am evaluat rezultatele asocierii pentru toate variantele utlizate în cadrul studiului obținând date relevante ce susțin implicarea variantelor genetice comune în cancerul de prostată cu debut rapid în România.

Cuvinte cheie: cancer de prostată, studiile de asociere genetică, variante genetice, locus asociat cu susceptibilitate, polimorfism uninucleotidic, ADN

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INTRODUCTION

Prostate cancer incidence varies more than 25-fold worldwide, largely due to the widespread practice of prostate specific antigen (PSA) testing and subsequent biopsy[1].

It is the second most frequently diagnosed cancer of men (914.000 new cases per year, accounting for 13.8% of total cancer cases) and the 5th most common cancer overall worldwide. The incidence is expected to grow to 1.7 million new cases and 499.000 deaths by 2030, mainly due to the growth and aging of the global population[2]. The *European Randomized Study of Screening for Prostate Cancer* (ERSPC)[3] showed that prostate cancer (PC) screening with prostate-specific antigen (PSA) decreased PC mortality. While screening has led to more men being diagnosed with prostate cancer than in previous years, the possibility of negative effects from over-diagnosis and treatment cannot be ignored[4]. As such, it is very important to understand the etiology of prostate cancer in relation to specific areas of the world, in hopes of avoiding over-diagnosis.

Prostate cancer is the third most common cancer diagnosed in Europe today and has emerged as the most frequent cancer amongst men in Europe, reaching an age-standardised rate of 96 per 100.000 men in 2012[5]. The incidence rate for this disease has been increasing rapidly over the past two decades in most European countries, particularly in the wealthiest countries of Northern and Western Europe[5,6]. This trend has been mainly attributed to the widespread availability of PSA tests in Northern and Western European countries. Because PSA testing has a much greater effect on incidence than on mortality, there is less variation in mortality rates worldwide (10-fold) than that observed for incidence (25-fold). The number of deaths from prostate cancer is almost the same in developed and developing regions; in 2012, the age-standardised mortality rate in Europe was 19 per 100.000 men[2,5].

In Romania, the estimated age-standardised rate of prostate cancer incidence was 37.9 per 100.000 men in 2012, while the estimated age-standardised rate for prostate cancer mortality was 16.9 per 100.000 men [5]. Due to the poor health status of Romania's population and difficulties in healthcare accessibility[7], prostate cancer might be an underdiagnosed condition. This could be attributed to the fact that prostate cancer screening is not common in Romania[8]; more than 95% of patients had an advanced stage prostate cancer at the time of diagnosis[9]. As such, causes of prostate cancer can be studied more effectively, as the Romanian population is largely unscreened and over-diagnosis is not an issue. Evaluating and comparing the causes for various racial groups, or between different geographical regions can provide useful insights into the etiology of prostate cancer.

There is evidence that prostate cancer has a genetic predisposition; more than 70 prostate cancer (PCa) susceptibility loci have been found, explaining 30% of the familial risk for this disease[10]. Each locus can have a different number of single nucleotide polymorphisms. In the 70 PCa susceptibility loci, more than 100 prostate cancer (PCa) risk-associated single nucleotide polymorphisms (SNPs) have been identified by genome wide association studies (GWAS)[11]. Genetic association studies are now at their peak, and genetic variants that affect the risk of common diseases are being published every month[12]. There is strong empirical and epidemiological evidence supporting a stronger role of genetics in early-onset prostate cancer[13].

METHODS

The study consisted of 2024 hospital patients genotyped, 990 unrelated PCa cases histopathologically confirmed and with abnormal PSA levels, and 1034 controls consisting of patients admitted for urological and surgical conditions, excluding cancer. The subjects included in this study were hospital patients admitted between 2008 and 2012 to two clinics in Bucharest (Urology Clinic "Th. Burghele" and General Surgery Clinic "St. Mary") for various medical conditions. For all individuals included in the study, blood samples were collected in order to measure biomarkers and genotyping. PSA level in plasma was measured for all subjects upon being admitted to hospital, although it was not used as an exclusion criterion. Each eligible subject gave written informed consent prior to enrolment and accepted the use of personal and clinical data, as well as biological samples for genetic research. Trained interviewers conducted face-to-face interviews, using standardised questionnaires, to collect personal data (ethnicity, marital status, education, height and weight), lifestyle data (occupation, smoking, coffee and tea consumption) and medical history (personal and family).

All subjects were of self-reported European descent. DNA was extracted from whole blood at deCODE Genetics laboratories (Reykjavik, Iceland) for genotyping. The Bioethical Committee of the Romanian College of Physicians approved the study and the study protocols were sanctioned by the National Ethical Board of the Romanian Medical Doctors Association in Romania. All subjects gave written informed consent[14]. The ave-

Table 1. Clinical characteristics of the cohort.

Decade	% cases	# cases
under 50	0.3%	3
50-60	1%	80
60-70	35.5%	320
70-80	44%	437
80-90	15%	148
Over 90	0.2%	2
T Staging	**% cases**	**# cases**
1A	2.32%	23
1B	1.11%	11
1C	15.65%	155
2A	1.71%	17
2B	1.91%	19
2C	4.04%	40
3A	43.80%	434
3B	6.06%	60
4	23.33%	231
Gleason Score	**% cases**	**# cases**
2	0.2%	2
3	0.3%	3
4	1%	10
5	3.3%	33
6	13.2%	131
7	45.1%	447
8	20.3%	201
N Staging	**% cases**	**# cases**
N0	21.5%	213
N1	3.2%	31
Nx	75.3%	656
M Staging	**% cases**	**# cases**
M0	22%	217
M1	10%	100
Mx	68%	673
PSA levels	**% cases**	**# cases**
<4	42%	852
4-9.99	18%	362
9.99-19.99	12%	262
19.99-49.99	9.5%	192
49.99-99.99	6.7%	137
>100	9.3%	187
NA	1.5%	31

rage age at diagnosis for the cases was 70 years (median, 71 years), while the range was from 46 to 89 years. The average age for controls was 60 years (median, 62 years) with a range from 19 to 87 years.

The UICC–TNM staging system was used[15]. For the T stage, the cohort was distributed as follows: 189 stage T1 cases, 76 stage T2 cases, 494 stage T3 cases and 231 stage T4 cases. The N stages were distributed as follows: 213 cases for N0, 31 N1 cases and 656 Nx cases. A similar distribution was reported for the M stage with: 217 M0 cases, 100 M1 cases and 673 Mx cases. For the Gleason score, the majority of cases were Gleason 7 (45.1%), Gleason 8 (20.3%) and lower values for Gleason 6 (13.2%) and Gleason 5 (1%). A complete description of the clinical characteristics of the cohort can be found in Table 1.

Statistical methods

The Romanian chip-typed data was filtered using multiple criteria. A Hardy-Weinberg equilibrium threshold of 10e-6 significance was used for filtering. In addition, markers with a minor allele frequency lower than 0.01 were excluded from the study. Relatedness criteria were assessed by detecting extended haplotypes shared identical by descent and SNPs with extreme heterozygosity computing observed and expected autosomal homozygous genotype counts for each sample. All of the above-mentioned filtering was carried out using Plink! v1.07[16]. After genotyping, 716.503 SNPs were available for each individual included in the study. Two association tests were calculated with the SNPTEST software using a single binary variable for prostate cancer; all reported P values are based on a two-sided test[17].

RESULTS

In an attempt to identify susceptibility loci for early-onset PCa, 104 PCa cases diagnosed before 60 years old and 1021 controls were tested. A total of 716503 SNPs with a successful genotyping rate of >0.99% in each sample were included in the association test.
Results across the genome are graphically illustrated in Figure 1, while the top findings are presented in Table 2.

The top two results were for uncommon (minor allele frequency estimated to be 2% in case sample and 9% in control sample) chromosomes, namely 22 SNP, RS9632053 and RS42929. For reasons described in the Discussion section, we believe that the result for this SNP should be considered with caution. Two novel SNPs (RS11059458, P = 4,69x10-8; RS7576486, P=4,92x10-8) also reached genome-wide significance. Results were similar when restricting the follow-up case sample to cases diagnosed prior to the age of 60 years.

A similar test was completed for 863 cases diagnosed after the age of 60 years and 1021 controls form the same cohort. Results across the genome are graphically illustrated in Figure 2, while the top findings are presented in Table 3.

In our second test, we observe lower p-values compared with the early-onset results due to the larger sample size.

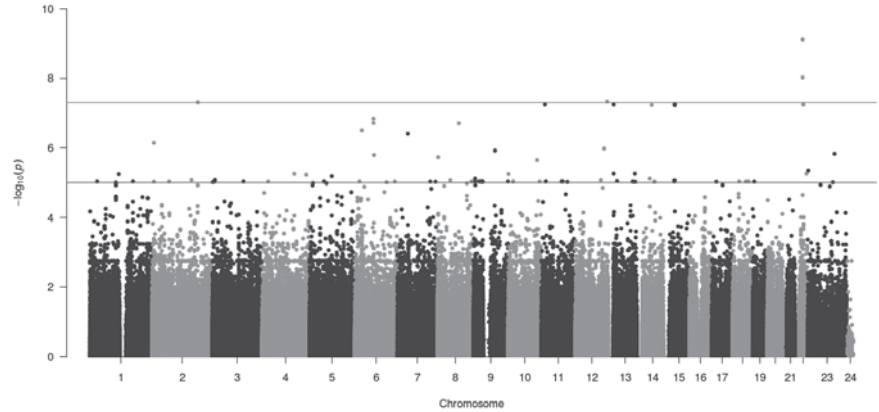

Figure 1. For the plot, the –log10 P-values (y axis) of sequence variants are shown according to their chromosomal position (x axis). The red line indicates the threshold for genome-wide statistical significance, which takes into account the effects of multiple testing (P = 0.05/28.3 million = 1.8×10^{-9}).

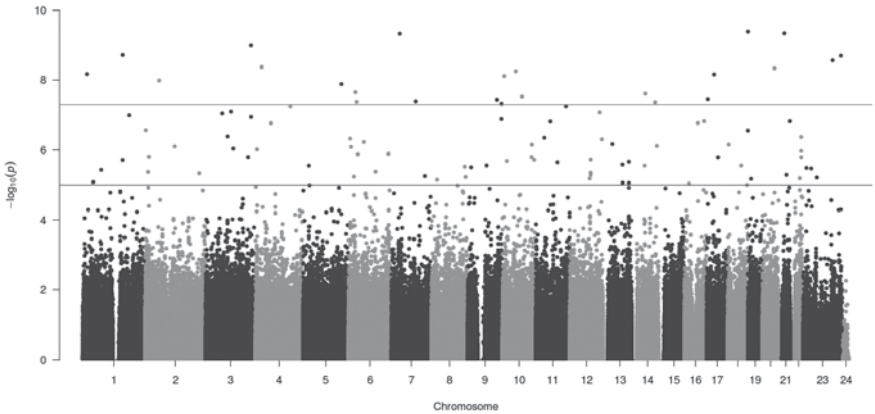

Figure 2. For the plot, the –log10 P-values (y axis) of sequence variants are shown according to their chromosomal position (x axis). The red line indicates the threshold for genome-wide statistical significance, which takes into account the effects of multiple testing (P = 0.05/28.3 million = 1.8×10^{-9}).

DISCUSSION

Given the small proportion of PCa cases diagnosed in this age range, most genetic studies for PCa have concentrated on men diagnosed with the disease later in life, despite evidence that early age at diagnosis is an indicator of increased genetic susceptibility. The relative risk of developing.

Table 2. Summary of top GWAS results for early-onset

RS ID	Chromosome	Position	Tested Allele	P-value	OR
rs9621053	22	30635146	T	7.70E-010	5.779
rs42929	22	30560432	T	9.41E-009	4.318
rs11059458	12	128052616	T	4.69E-008	39.94
rs7576486	2	183710710	A	4.92E-008	39.82
rs16886053	6	75053443	A	1.43E-007	17.04
rs12664835	6	75055012	T	1.89E-007	16.74
rs986633	8	86623223	A	1.94E-007	3.541
rs2215220	6	28521097	T	3.13E-007	4.681
rs846277	7	42021903	T	3.89E-007	2.44
rs10490219	2	8260765	T	7.22E-007	2.991
rs1366031	12	115611338	G	1.04E-006	20.16
rs7036724	9	86221371	C	1.19E-006	19.96
rs1859169	23	107319661	G	1.46E-006	6.339
rs16888388	6	77015323	A	1.58E-006	12.52
rs2258265	8	3941805	T	1.82E-006	2.107
rs953920	10	116187631	C	2.20E-006	2.998

Table 3. Summary of top GWAS results for late-onset

RS ID	Chromosome	Position	Tested Allele	P-value	OR
rs8110536	19	756985	3	4.14E-010	0.5523
rs2827540	21	22474931	3	4.64E-010	0.5848
rs2598392	7	33581287	2	4.75E-010	0.5869
rs9866700	3	180563658	2	1.01E-009	0.6147
rs12090877	1	160406270	4	1.91E-009	0.5206
rs4828787	23	150996411	4	1.99E-009	2.008
rs1890385	23	117864540	1	2.72E-009	2.011
rs16876269	4	24868715	4	4.22E-009	0.6279
rs4810663	20	47711762	4	4.60E-009	0.5851
rs2135720	10	53995731	4	5.57E-009	0.6064
rs12067141	1	18033086	1	6.71E-009	0.6092
rs7211084	17	29049811	4	6.88E-009	0.6023
rs6602234	10	7275164	2	7.63E-009	0.5289
rs7563088	2	56737504	2	1.03E-008	0.6159
rs2974554	5	152677932	4	1.30E-008	0.5622
rs4711143	6	27205620	4	2.23E-008	0.4328

PCa for a man whose father was diagnosed with PCa at age 60 or older was estimated to be 1.5. The relative risk for developing PCa increased to 2.5 if the father was diagnosed prior to 60 years of age. Similarly, if one brother was diagnosed with PCa at age 60 or older, then the relative risk of a man developing PCa was estimated to be 2, whereas this changed to 3 if the brother was diagnosed with PCa prior to age 60[18].

We describe a GWA study for early-onset PCa based entirely on cases diagnosed prior to age 60. A total of four single novel SNPs, namely chromosome 2, 12 and 2 markers on chromosome 22 reached genome-wide significance. Arguably, the most interesting result among these is the novel locus on chromosome 22, RS9632053 and RS42929. Indeed, similar results had previously been reported by the International Consortium for Prostate Cancer Genetics. The 22q12.3 region is the most consistently identified and smallest linkage region for PRCA[19].

In conclusion, we describe results from the first stage of a Genome Wide Association study for early-onset PCa. Our results provide proof that in the Romanian population the early onset of PCa shows a strong genetic component. Indeed, this is indicated by strong evidence at a number of previously established PCa loci and a lack of evidence for spurious results, although possible exceptions include chromosome 22 RS9632053 and RS42929. Overall, our results provide compelling evidence regarding the importance of common genetic variants to early-onset Pca in Romania.

Conflict of interests: none declared.
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