Single Nucleotide Polymorphisms Associated With Prostate Cancer Development in Saudi Males

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Abstract

Background: Genome-wide association studies have demonstrated that single nucleotide polymorphisms (SNPs) are important risk factors for the development of prostate cancer (PC). In this study, we investigated a group of SNPs in Saudi individuals and compared their frequency in patients and healthy normal controls.

Methods: A total of 320 individuals were included in the study: 85 with PC, 120 with benign nodular hyperplasia, and 115 healthy normal controls. DNA was extracted from paraffin-embedded formalin-fixed tissue from PC and nodular hyperplasia patients and from whole blood of healthy controls. A total of thirteen SNPs were genotyped using the TaqMan® MGB PCR assay.

Results: The rs16901979A, s629242T and rs1447295A alleles were found at significantly higher frequencies in PC patients than in controls ($p < 0.05$). The C/A genotype of the rs16901979 SNP was observed significantly more frequently in PC patients than in controls (43% vs 14%, OR = 0.2, p value = 0.0001) and more frequently in PC patients than in the benign hyperplasia group (43% vs 25%, OR = 2.3, p value = 0.03).

Conclusion: Our study highlighted several SNP genotypes associated with PC development in Saudi males. These findings have important implications for diagnosing PC and screening unaffected family members of Saudi patients.

Background

Prostate cancer (PC) is the second most common cancer worldwide and the fifth leading cause of death in males. Approximately 1.1 million men with PC were reported in 2012, while the total number of deaths for that year was 307,000 (1). PC is considered the most common type of cancer among men in many countries, and recent statistical analysis indicates that approximately 76.5% of PC patients have survival rates of 5 years postdiagnosis (2). There is no clear cause for PC to date, but obesity, age and family history are considered primary risk factors, whereas 40% of cases are attributed to inherited factors. The average age at the time of diagnosis is 70 years, and it is not common in males younger than 45 years (3). The International Agency for Research on Cancer (IARC) and the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) have considered a number of environmental factors to be possible risk factors for PC (4). It has been demonstrated that PC in the United States is more frequent in Blacks than in Whites or Hispanics (5). It has also been shown that the susceptibility of males with a family history of PC is higher than that of those with no history of PC. A male whose father has the disease has a 2.1–2.4 times greater chance of having PC. The incidence is also 2.9–3.3 times greater in men whose brothers had the disease (6,7). Available data showed a low incidence rate of PC among Saudis, which varies from 2.6 to 3.5 per 100,000 according to geographical areas (8). In 2013, a descriptive epidemiology study of the Saudi Cancer Registry ranked PC as the sixth most common cancer among Saudis (9).

Genome-wide association studies (GWAS) have been used over the last several years to measure and analyze the DNA sequence variations across the PC genome and identify possible genetic risk factors for disease associations. No single gene is responsible for PC, but some studies have demonstrated that BRCA1 and BRCA2 mutations, which are associated with ovarian and breast cancers in women, are also risk factors for PC. Men with positive BRCA2 mutations are fivefold more likely to have PC than negative individuals (10,11). A group of studies have demonstrated that single nucleotide polymorphisms (SNPs) are risk factors for PC (12–15). Carriers of the homozygous T allele of the rs10993994 SNP were found to have a higher risk for PC than those having the homozygous C allele (16). In this study, we investigated a group of SNPs in Saudi individuals with the aim of determining their possible genetic variations and comparing the outcomes between patients and healthy normal controls.

Methods

Patients and control

A total of 211 patients were initially recruited for this study, 16 of whom were later excluded because they were duplicates or were not Saudi nationals. The remaining patients were categorized as patients with PC (group 1) and patients with nodular hyperplasia (group 2). Paraffin imbedded tissue sections were obtained for each patient group. Peripheral blood in EDTA, obtained from blood bank donors, was collected from healthy normal individuals (with no history of PC) as unrelated Saudi controls (group 3). The study was approved by the institution's IRB.

DNA extraction from whole blood and paraffin embedded tissue

Extracted DNA from archived paraffin embedded tissue has been used in the study of normal/diseased tissues utilizing various genetic techniques, including genome-wide profiling technologies, such as microarrays (17–19). A MagNa pure compact instrument (Roche Diagnostics GmbH, Roche Applied Science, 68298 Mannheim, Germany) was used for DNA extraction from whole blood, and the ReliaPrep™ FFPE gDNA Miniprep System kit (<https://worldwide.promega.com>) was utilized for DNA extraction from paraffin-embedded blocks. Both methods were performed according to the manufacturer's instructions. A Nanodrop® 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) was used to determine DNA quality and quantity, and a concentration of 20 ng/μl of DNA with a ratio of 260/280 between 1.6 and 2.0 was accepted for the assay genotyping.

SNP Genotyping

Thirteen SNPs, including rs4430796, rs1859962, rs16901979, rs6983267, rs1447295, rs1571801, rs4054823, rs1545985, rs7652331, rs629242, rs13149290, rs251177 and rs10492519, were genotyped. Applied Biosystems kits (Foster City, CA, 94404) were utilized, and the SNP assay contains sequence-specific forward and reverse primers with two TaqMan® MGB probes and their dyes (VIC™ and FAM™) used to detect alleles 1 and 2 for each SNP. The procedure was carried out according to the

manufacturer's instructions, and a Light Cycler®480 instrument (Roche Diagnostics Ltd, Ferrenstrasse, CH-6343 Rotkreus, Switzerland) was used for data collection. Multiple DNA samples were replicated to ensure the quality of the tested samples, and positive and negative samples were also included in each batch as controls.

Statistical analysis

Conformation for Hardy-Weinberg equilibrium (HWE) was analyzed in control data implementing the χ^2 distribution (degree of freedom = 1) to detect the differences between the expected and observed values in the tested SNPs, as described by Rodriguez et al.(20). The frequencies of alleles, genotypes and the genotype models for each SNP (dominant and recessive traits) were derived by an algorithm based on the direct counting method using SNPStats software (<https://www.snpsstats.net/start.htm>), and the results were expressed as percentages at 95% confidence intervals (CIs). Fisher's exact test was used to compare patients and control data. The log of the odds (logit) for the presence of each SNP was calculated to determine significant differences at p values < 0.05.

Results

A total of 320 individuals were recruited for this study; 85 were patients with PC, with ages ranging from 60 to 70 years (mean = 69.5, median = 71); 120 were patients with benign nodular hyperplasia, with ages ranging from 62 to 76 years (mean = 67.5, median = 70); and 115 were healthy normal controls, with ages ranging from 45 to 67 years (mean = 52.6, median = 60).

Tested HWE for allele frequencies on healthy control data showed a conformation to expected distribution in 11 SNPs out of 13; rs1447295 and rs1545985 SNPs were not found within the expected distribution for HWE (p values < 0.01). The comparison of the allele frequencies in the three different groups is shown in Table 1. The rs16901979A allele was found at a significantly higher frequency in PC patients than in healthy normal controls (23% vs 8%, OR = 0.3, 95% CI = 0.2,0.6; p value = 0.0002); this result indicates that individuals carrying this allele are fivefold more susceptible to developing PC than negative individuals. The rs16901979A allele was also found at a significantly greater frequency in PC patients than in benign hyperplasia patients (23% vs 14%; OR = 0.4; 95% CI = 0.3, 1.0; p value = 0.049) with 2.5-fold higher disease susceptibility. Regarding the s629242 SNP, the T allele was found at a significantly higher level in PC patients than in healthy controls (31% vs 22%; OR = 0.6, 95% CI = 0.4, 1.0; p value = 0.03). The rs1447295A allele was detected at a significantly higher level in PC patients than in benign hyperplasia patients (12% vs 5%; OR = 0.4; p value = 0.0027) and in benign hyperplasia patients than in normal healthy controls (11% vs 5%; OR = 2.1; p value = 0.045). No significant differences were demonstrated in other allele frequencies among these three different groups. The comparison of genotype analysis is shown in Table 2. In this regard, we found the C/A genotype of the rs16901979 SNP at a significantly higher frequency in PC patients than in normal healthy controls (43% vs 14%, OR = 0.2, p value = 0.0001) and in PC patients compared to the benign hyperplasia group (43% vs 25%, OR = 2.3, p value = 0.03).

Table 1

, Allele frequencies of studied SNPs in healthy control (HC), patients with prostate cancer (PC) benign hyperplasia (BH).

SNP ID	Allele A1%/A2%	PC A1%/A2%	HC A1%/A2%	BH A1%/A2%	PC vs HC OR(95% CI); p value	PC vs BH OR(95% CI); p value	BH vs HC OR(95% CI); p value
rs4430796	G/A	57/43	49/51	53/47	1.4(0.9, 2.1), 0.2	1.2(0.7, 1.8), 0.5	1.2(0.8, 1.7), 0.4
rs1859962	G/T	51/49	53/47	61/39	0.9(0.6, 1.5), 0.8	1.7(0.4, 1.1), 0.11	1.4(0.9, 2.1), 0.1
rs6983267	G/T	68/32	61/39	66/34	1.3(0.8, 2.2), 0.3	1.1(0.6, 1.8), 0.8	1.3(0.8, 1.9), 0.3
rs1447295	C/A	88/12	89/11	95/5	0.9(0.5, 1.7), 0.7	0.4(0.2, 0.9), 0.027	2.1(1.0, 4.2), 0.045
rs4054823	T/C	50/50	54/46	50/50	0.9(0.6, 1.3), 0.5	0.9(0.7, 1.5), 0.9	0.9(0.6, 1.3), 0.5
rs16901979	C/A	77/23	92/8	86/14	0.3(0.2, 0.6), 0.0002	0.4(0.3, 1.0), 0.049	0.4(0.3, 1.1), 0.07
rs251177	T/C	70/30	65/35	61/39	1.3(0.8, 2.0), 0.3	1.5 (1.0, 2.4), 0.055	0.8(0.6, 1.2), 0.4
rs7652331	C/T	76/24	79/21	81/19	0.9(0.5, 1.4), 0.6	0.7(0.5, 1.2), 0.27	1.1(0.7, 1.8), 0.6
rs1545985	A/G	79/21	76/24	79/21	1.1(0.7, 1.9), 0.6	0.9(0.6, 1.6), 0.9	1.2(0.8, 1.8), 0.5
rs1571801	G/T	88/12	85/15	84/16	1.3(0.7, 2.4), 0.4	1.4(0.8, 2.6), 0.23	0.9(0.5, 1.4), 0.06
rs629242	C/T	69/31	79/21	78/22	0.6(0.4, 1.0), 0.03	0.6(0.4, 1.0), 0.051	1.0(0.6, 1.5), 0.9
rs13149290	C/T	74/26	76/24	78/22	0.9(0.6, 1.5), 0.7	0.8(0.5, 1.3), 0.4	1.1(0.7, 1.7), 0.6
rs10492519	A/G	73/27	69/31	74/26	1.2(0.8, 2.0), 0.4	0.9(0.6, 1.6), 0.9	1.3(0.8, 1.9), 0.3
OR, odds ratio; CI, confident interval; p, p value; bold numbers mean significant association.							

Table 2

, Genotype frequencies of studied SNPs in healthy control (HC), and patients with prostate cancer (PC) and benign hyperplasia (BH).

SNP ID	Genotype	PC %	HC %	BH %	PC vs HC OR(95%CI); P	BH vs PC OR(95%CI); P	BH vs HC OR(95%CI); P
rs4430796	GG	33	22	29			
	GA	48	54	48	1.6(0.8,3.37); 0.2	0.9(0.4, 1.8); 0.7	0.9(0.4, 1.8); 0.7
	AA	19	24	23	1.9 (0.8, 4.6); 0.2	0.7(0.3, 1.8); 0.5	0.7(0.3, 1.8); 0.7
rs1859962	GG	26	29	41			
	GT	49	47	39	0.9(0.4, 1.9); 0.7	1.9(0.9, 4.4); 0.1	2.0(0.9, 4.4); 0.1
	TT	25	24	20	0.9(0.4, 2.2); 0.8	1.9(0.7, 4.9); 0.2	2.0(0.7, 4.9); 0.2
rs6983267	GG	47	37	44			
	GT	42	49	44	1.5(0.7, 3.1); 0.3	0.9(0.4, 2.0); 0.8	0.9(0.4, 2.0); 0.8
	TT	11	15	12	1.7 (0.5, 5.1); 0.4	0.9(0.3, 3.1); 0.9	0.9(0.3, 3.1)0.9
rs1447295	CC	82	84	92			
	CA	13	11	5	0.8(0.3, 2.1); 0.4	2.6(0.9, 7.8); 0.08	2.6(0.9, 7.8); 0.08
	AA	6	5	3	0.9(0.2, 3.2); 0.8	2.3(0.5; 10.8); 0.3	2.3(0.5, 10.8); 0.3
rs4054823	CC	25	24	31			
	TC	50	44	39	0.9(0.5, 1.9); 0.8	1.5(0.8, 3.1); 0.2	1.5(0.8, 3.1); 0.2
	TT	25	32	30	1.3(0.6, 2.9); 0.5	1.1(0.5, 2.3); 0.9	0.9(0.5, 2.3); 0.9
rs16901979	CC	57	85	74			
	CA	43	14	25	0.2(0.1, 0.5); 0.0001	2.3(1.1, 4.9); 0.03	2.3(1.1, 4.8); 0.03
	AA	2	1	1			
rs251177	TT	47	41	35			

OR, odds ratio; CI, confident interval; p, p value; bold numbers mean significant association.

SNP ID	Genotype	PC %	HC %	BH %	PC vs HC OR(95%CI); P	BH vs PC OR(95%CI); P	BH vs HC OR(95%CI); P
	TC	47	47	51	1.1(0.6, 2.1); 0.7	0.6(0.4, 1.3); 0.2	0.7(0.4, 1.3); 0.2
	CC	6	12	14	2.1(0.7, 6.2); 0.2	0.3(0.1, 1.0); 0.6	0.3(0.1, 1.0); 0.06
rs7652331	CC	58	62	67			
	TC	37	33	28	0.8(0.4, 1.5); 0.5	1.5(0.8, 2.9); 0.2	1.5(0.8, 2.9); 0.2
	TT	5	5	5	0.6(0.2, 2.1); 0.4	1.2(0.3, 4.4); 0.8	1.2(0.3, 4.4); 0.8
rs1545985	AA	57	53	59			
	AG	43	47	41	1.2(0.7, 2.2); 0.6	1.1(0.6, 2.0); 0.9	1.1(0.6, 2.0); 0.9
	GG	0	0	0			
rs1571801	GG	78	72	73			
	GT	21	26	22	1.3(0.7, 2.6); 0.4	0.9(0.4, 1.8); 0.8	0.9(0.4, 1.8); 0.8
	TT	1	2	5			
rs629242	CC	46	61	57			
	TC	46	35	41	0.6(0.3, 1.1); 0.06	1.4(0.8, 2.6); 0.3	1.4(0.8, 2.6); 0.3
	TT	8	4	2	0.4(0.1, 1.3); 0.12	1.4(0.8, 2.6); 0.3	0.8(0.46, 1.4); 0.4
rs13149290	CC	54	56	58			
	TC	39	39	39	0.9(0.5, 1.8); 0.9	1.1(0.6, 1.9); 0.9	1.1(0.6, 1.9); 0.9
	TT	7	5	3	0.7(0.2, 2.6); 0.6	2.6(0.6, 11.8); 0.2	2.7(0.6, 11.8); 0.2
rs10492519	AA	56	51	54			
	AG	34	35	40	1.1(0.6, 2.1); 0.7	0.8(0.4, 1.5); 0.5	0.8(0.4, 1.5); 0.5
	GG	1	13	6	1.5(0.6, 4.1); 0.4	1.5(0.5, 4.6); 0.5	1.5(0.5, 4.6); 0.5
OR, odds ratio; CI, confident interval; p, p value; bold numbers mean significant association.							

Discussion

SNPs may have a functional role in causing amino acid changes, mRNA transcript instability and transcription factor binding affinity variations (21–23). In this study, we characterized 13 different SNPs utilizing TaqMan Real Time PCR assays. The rs16901979 SNP showed significant differences in allele and genotype frequencies. The rs16901979A allele was found at a higher frequency in PC patients than in normal healthy controls, and individuals with this allele were 3.3 times more susceptible to PC. However, no significant difference was observed between the nodular hyperplasia patients and normal healthy controls. Accordingly, this SNP may be considered a risk genetic factor for PC disease but not for nodular hyperplasia development. A comparison of the genotype model for rs16901979 (C/C vs A/C) has shown that individuals with the A/C genotype are at 5 times higher risk for PC development than those with the C/C genotype but not for nodular hyperplasia. Additionally, we observed a minor presence of the A/A genotype among the three groups. The presence of this genetic component may indicate its lethal effect for carrier individuals among the Saudi population. The rs16901979 SNP was found to be associated with PC cancer in a case control study by Robbins et al. that included 490 prostate cancer patients and 567 healthy controls of African American individuals (24). A similar association was found in a Taiwanese study by Chen et al. (25). In a multicenter study of the Swedish population that included 2893 patients with PC and 1781 control subjects, rs16901979 was also found to be associated with PC (12). The Rs16901979 SNP is present within the non-protein-coding region of the CASC8 gene, located at 8q24 of the human genome map. GWAS and several case–control studies have demonstrated the association of specific variants in the CASC8 gene with prostate cancer(26), and it has been suggested that 8q24 regions can independently influence the risk for prostate cancer development and advanced disease status in particular (27).

Additionally, the rs629242T allele was found at a significantly higher frequency among PC patients than among healthy normal controls. In general, rs629242T is observed less frequently than rs629242C, which is classified as the mutated allele for the rs629242 SNP (23) and is considered a risk factor for PC occurrence with a 1.6 odds ratio. A study investigating the role of rs629242 in PC found that the rs629242T allele infers a 29% increased risk for disease progression in the African American population (28). The rs629242 SNP is present within the KIAA1211 gene located at the 4q12 region. This gene encodes an actin cytoskeletal regulator that plays a role in maintaining the integrity of epithelial cells and suppressing tumorigenesis (29).

The rs1447295A allele was found at a significantly lower rate in benign nodular hyperplasia patients than in normal healthy controls or PC groups but with no difference between the healthy controls and PC. This result is in keeping with findings obtained by Robbins et al., who concluded that there is no association between the rs1447295 SNP and PC and among the African American population (24,30). However, the rs1447295 SNP was demonstrated to be associated with PC and related clinical covariables in northern Chinese men(31) and in a meta-analysis study performed by Cheg et al.(27). These contradictions may be observed because the interaction between genetic susceptibility and environmental factors plays an important role in disease development (24,30). Based on our findings, we suggest that this rs1447295

SNP may be used as a genetic marker to differentiate between benign and malignant tumors in Saudi males with prostatic disease.

Conclusions

Our study has highlighted the association of several SNP genotypes with PC development in Saudi males. In addition to the potential utility of these results in PC diagnosis, these findings may be useful in screening unaffected family members of patients.

Abbreviations

PC

Prostate Cancer

IARC

International Agency for Research on Cancer

WCRF/AICR

World Cancer Research Fund/American Institute for Cancer Research

GWAS

Genome-wide association studies

SNPs

Single Nucleotide Polymorphisms

HWE

Hardy-Weinberg equilibrium

Declarations

Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics declarations

This study was approved by Institutional Review Board at King Fahad Medical City, Riyadh (IRB Log No. 15-175). Patients and Controls consent were exempt by IRB committee because we used leftover samples after clinical tests were performed.

Consent for publication

Not applicable

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Authors' contributions:

AD was responsible for design, acquisition, analysis and interpretation of the data, drafting the manuscript and revising the manuscript. AM was responsible design, oversee the project and review all drafts of the manuscripts. AF Ahmed was responsible for acquisition of the data and review all drafts of the manuscript. All authors have read and approved the manuscript.

Conflicts of Interest:

None

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