RESEARCH ARTICLE

Association of Polymorphisms in the VDR, CYP17 and SRD5A2 Genes and Prostate Cancer Among Lebanese Men

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Abstract

Aims: The goal of this study was to investigate possible associations of some single nucleotide polymorphisms (SNPs) in the VDR gene (the FokI, BsmI, ApaI and Taq**α**I loci), and the CYP17 gene (the MspA1I locus), and variable numbers of TA repeats in the SRD5A2 gene, with prostate cancer (PCa), among Lebanese men. **Materials and Methods:** Blood DNA samples of 50 subjects with confirmed PCa and 79 age-qualified controls were subjected to PCR or PCR-RFLP analyses, and the risk-bearing and protective alleles were identified. The odds ratio (OR) of having a genotype and the relative risk (RR) of developing PCa were calculated. In addition, the distribution of homozygosis in the risk-bearing and protective alleles were compared between the control and the PCa groups. **Results:** The A and B alleles of the VDR ApaI and BsmI loci and the 0 TA repeat allele of the SRD5A2 gene were found to be associated with increased risk of PCa ($p = 0.022$, 0.029 and 0.013, respectively). A higher percentage of the subjects with PCa compared to the controls was homozygous for two or more of the risk-bearing alleles (46% for PCa, 27% for controls, $p = 0.023$). In contrast, a higher percentage of the controls compared to the PCa group was homozygous in two or more of the protective alleles (71% for controls, 38% for PCa group, $p = 0.001$). **Conclusions:** To the best of our knowledge, this is the first genetic study demonstrating any association of polymorphisms of the VDR and SDR5A2 genes with an increased risk of PCa among Lebanese men. Our study also indicated that the overall polymorphism profile of all genes involved in prostrate physiology is likely to be a better indicator for PCa risk than polymorphisms in individual genes.

Keywords: prostate cancer (PCa)- single nucleotide polymorphism (SNP)- TA repeat polymorphism

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Introduction

Prostate cancer (PCa) is the second most commonly diagnosed cancer for males and the second leading cause of cancer deaths among males of all races (ACS, 2016; Seigel et al., 2011). The possible risk factors for PCa include age, diet, obesity, life style, smoking, infections and inflammation of the prostate gland, vasectomy, exposure to certain types of chemicals, and genetic predisposition (ACS, 2016). The incidence of PCa is highest among Africans, followed by Caucasians and Mongolians (Tao et al., 2015; Hsing et al., 2000). Identification and validation of specific genetic markers could be helpful in the screening and early diagnosis of prostate cancer.

Polymorphisms in several genes, including the vitamin D receptor (VDR) gene, the androgen receptor (AR) gene, the cytochrome P-450 17 alpha-hydroxylase/C(17, 20)-lyase (CYP17) gene, and the steroid 5 alpha-reductase type 2 (SRD5A2) gene, have been indicated in PCa development (Wang et al., 2016; Dianat et al., 2009, Ntais et al., 2003). The VDR gene encodes a ligand-inducible transcription factor that regulates many physiological processes, including cell growth, embryonic development and metabolic homeostasis (Gene Cards, 2016; Feldman and Malloy, 2014). The CYP17 gene encodes an enzyme that controls a rate-limiting step in androgen biosynthesis (Gene Cards, 2016; Chen et al., 2014). The SRD5A2 gene encodes an enzyme that converts testosterone to dihydrotestosterone (Gene Cards, 2016; Boer et al., 2016), the more biologically active form of the hormone. Since the prostate gland is an androgen-regulated organ, polymorphisms in CYP17 and SRD5A2 genes could be associated with PCa.

A number of previous molecular epidemiological studies reported certain polymorphisms of these genes as protective or risk-bearing factors for PCa, but other studies found no such associations (Wang et al., 2016; Norman, 2006; Ntais et al., 2003). This may be due to racial and ethnic differences (CDC, 2016; Hsing et al., 2000), and the presence of both protective and risk-bearing polymorphisms in different genes in almost every individual (Kitts et al., 2014; Latil et al., 2001). In the

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present study we examined a number of polymorphisms in the VDR gene, the CYP17 gene and the SRD5A2 gene among Lebanese men with PCa and age-qualified healthy Lebanese men. We analyzed the association of polymorphisms in each of the three genes individually as well as the overall polymorphisms in all three genes as a risk factor for PCa. Our study in relation to other similar studies indicates that the overall genotypic profile of the three involved genes rather than the genotype of an individual gene more tangibly correlates with PCa risks.

Materials and Methods

Study population

Men enrolled in the study were volunteers who participated in the prostate cancer campaigns launched by Dr. Asmahan A El Ezzi at the Lebanese Atomic Energy Commission in collaboration with many hospitals and medical centers in Lebanon. All subjects were Caucasian Lebanese men between 50 and 70 years of age. Informed consent to participate in the study for PSA screening, donation of blood for DNA extraction and storage, and using the DNA samples for molecular research was obtained from each of the subjects in accordance with the Declaration of Helsinki and following the guidelines of the Institutional Review Board of Lebanese University, Beirut, Lebanon. Prostate condition of the subjects was examined by measuring the serum prostate-specific antigen (PSA) level of all participants and, if consented, by digital rectal examination (DRE). Blood samples were withdrawn in non-fasting state and serum was separated and stored in freezer at -30°C till the day of PSA assay. Total PSA (PSA-T) assays were performed following the guidelines of the kits purchased from Immunotech (Marseille, France). For subjects with PSA-T levels in the gray zone (i.e. between 4-10 ng/ml), a free PSA test (PSA-F) was also conducted, and the F/T PSA ratio was determined, in order to help differentiate benign prostrate hyperplasia (BPH) and PCa. In addition, the International Prostate Symptom Score (IPSS) value was determined, and a trans-rectal prostate ultrasonography was conducted if needed. Histological analyses of prostate biopsies confirmed the diagnosis of prostate cancer. Fifty subjects with confirmed PCa and 79 controls were included in the present study. The control subjects were volunteers who had normal PSA level, normal IPSS score, and normal DRE for two consecutive years.

DNA extraction and analysis

Blood DNA was extracted using QiaAmp DNA Blood Mini Kit (Qiagen, Milan, Italy), and stored at -82° C before being transported to the United States in a temperature-controlled package for molecular analyses. DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher, Waltham, MA). The DNA samples with A260/A280 ratio between 1.8-2.0 were used for PCR-amplification of the appropriate DNA fragments for further analyses. The following primers were used: VDR Fok1F- 5'TGCAGCCTTCACAGGTCATA3' and VDR FokIR- 5'GGCCTGCTTGCTGTTCTTAC3', VDR BsmIF- 5'CAGTTCACGCAAGAGCAGAG3' and VDR BsmIR-5'ACCTGAAGGGAGACGTAGCA3'; VDR ApaIF- 5'ACGTCTGCAGTGTGTTGGAC3' and VDR ApaIR 5'TCACCGGTCAGCAGTCATAG3' (Falleti et al., 2010); VDR TaqαIF-5'CAGAGCATGGACAGGGAGCAA3' and VDR TaqαIR-5'GCAACTCCTCATGGCTGAGGTCTC3' (Taylor et al., 1996); CYP17F- 5'CATTCGCACCTCTGGAGTC3' and CYP17R- 5'GGCTCTTGGGGTACTTG3' (Feigelson et al., 1997); and SRD5A2F- 5'GCTGAT GAAAACTGTCAAGCTGCTGA3'and SRD5A2R-5'GCCAGCTGGCAGAACGCCAGGAGAC3' (Forrest et al., 2005). The reaction mixture for PCR (25 µl) contained 1x reaction buffer (Invitrogen, Grand Island, NY), 0.2 mM dNTP, 1.75 mM MgCl2, 20 pico moles of the two primers, 20 ng of template DNA and 1 unit of Platinum Taq DNA polymerase (Invitrogen). The thermocycler (GeneAmp PCR 9700, ABI, Foster City, CA) was programmed as the following: 94°C for 5 minutes (one cycle), 94°C for 45 sec, 57-64°C (depending on the primer pairs used) for 45 sec and 72°C for 60 sec (35 cycles); 72°C for 5 minutes (one cycle) and soak at 4°C.

For the analysis of PCR-amplified DNA with various numbers of TA repeats of the SRD5A2 gene, the amplified DNA was directly resolved in 9.0% polyacrylamide gels. RFLP of various loci of the other genes was conducted by treating the PCR-amplified DNA with 2 units of an appropriate restriction endonuclease (i.e. ApaI, BsmI, FokI, or TaqαI for VDR gene and MspAI for CYP17 gene; all from New England Biolab, Beverly, MA). The reaction mixture was incubated overnight in the reaction buffer at the incubation temperature suggested by the manufacturer. The treated DNA was then resolved in a 3.0% agarose gel (for TaqαI-treated DNA) or a 6% polyacrylamide gel (for ApaI, BsmI, FokI, and MspA1I-treated DNA). The gels were stained with ethidium bromide and then documented using a digital camera (Kodak, Rochester, NY). RFLP alleles are generally codominant but for convenience, the alleles having the repeat DNA or the restriction site were considered recessive. The band pattern for different genotypes are as the following: VDR FokI: FF: 157 bp, Ff: 157, 121 and 36 bp, ff- 121 bp and 36 bp, VDR BsmI: BB-236 bp, Bb- 236 bp, 197 bp and 39 bp, bb- 197 bp and 39 bp; VDR ApaI: AA- 211 bp, Aa- 211 bp, 172 bp and 39 bp, aa-172 bp and 39 bp; VDR TaqαI: TT- 495 bp and 245 bp; Tt- 495 bp, 290 bp, 245 bp, and 205 bp, tt- 290 bp, 245 bp, and 205 bp; CYP17: A1/A1 (i.e. MM)- 459 bp, A1/A2 (i.e. Mm)- 459 bp, 335 bp and 124 bp, A2/A2 (i.e. mm)- 335 bp and 124 bp; and SRD5A2: 0/0- 98bp, 0/9- 98 bp and 116 bp, and 9/9- 116 bp. Both 0/9 and 9/9 genotypes of the SRD5A2 gene also produced three additional large-sized artifact bands that were not scored.

Statistical analyses

Whether the distribution of the genotypes and the frequency of alleles are in agreement with the Hardy-Weinberg equilibrium was tested using χ^2 statistics at the 0.05 level of significance. The null hypothesis that the population is in Hardy-Weinberg equilibrium was rejected if the test statistic was >3.84. Association between PCa and the genotypes was assessed by calculating the odds ratio (OR) and the 95% confidence interval (CI).

The OR was calculated using the formula (a/b)/(c/d) (Bland and Altman, 2000), where a/b is the ratio of certain attributes for the PCa group, and c/d is the ratio for the same attributes for the control group. Relative risk (RR) was calculated using the formula RR=[a/(a+b)]/[c/(c+d)] (Sheshkin, 2004), where a and b are the numbers of genotypes for a particular trait for the PCa group, and c and d are the numbers of genotypes for the corresponding trait for the control group. The OR, RR, 95% CI and p-values were calculated using MedCalc software (MedCalc, Mariakerke, Belgium). A value of RR >1 for a genotype is considered implicative of disease risk relative to the alternative genotype. The genotypic ratio among the controls and the subjects with PCa were compared using the two-tailed z test for the difference between two independent proportions at the 0.05 level of significance. The null hypothesis H0 was: there is no difference between the two proportions, and the alternative hypothesis H1 was: there is a difference between the two proportions. The null hypothesis was rejected and the alternative hypothesis that there is a significant difference between the two tested proportions was supported only if the p-value was ≤ 0.05. The calculations were made using Epitools software (http://epitools.ausvet.com.au/).

Results

Distribution of the genotypes and allele frequencies

This study included 50 cases of PCa (mean age 67.6 ± 7.4 years) and 79 male cohort controls (mean age 58.3 ± 10.1 years). The distribution of homozygous dominant, homozygous recessive, and heterozygote genotypes of almost all of the loci were in Hardy-Weinberg equilibrium except the TaqαI locus of the VDR gene for the control group ($\chi^2 = 7.5$; p-value = 0.006), the TA repeats of the SRD5A2 gene for the subjects with PCa ($\chi^2 = 9.3$; p-value = 0.002), and the MspA1I locus of the CYP17 gene for the control group ($\chi^2 = 8.0$; p-value = 0.005) (Table 1). In these three loci, the ratio of the homozygous dominant, homozygous recessive, and heterozygous genotypes significantly deviated from the expected ratios.

The allele frequency for each of the tested loci of the three genes among the controls and the subjects with PCa is shown in Table 2. There is no significant difference in the allele frequencies of the VDR TaqαI locus, the CYP17 MspA1I locus, and the SRD5A2 TA repeat locus among the controls and the subjects with PCa. The F allele of the FokI locus, the A allele of the ApaI locus, and the B allele of the BsmI locus of the VDR gene are overrepresented among the subjects with PCa compared to the controls, although the difference is not significant.

Genotype frequencies and genotypic ratios

The ratio of the homozygous recessive or dominant genotypes and heterozygous genotypes for each of the alleles among the subjects with PCa and the controls are shown in Table 3. The OR of having a particular genotype for the subjects with PCa compared to the controls and the RR of PCa for having the genotype are also shown in Table 3. In most cases, the ratio for the subjects with PCa and the controls are not significantly different except the following:

VDR BsmI: The ratio of (BB+Bb) and bb is significantly higher for the subjects with PCa compared to the controls (OR: 2.3, 95% CI: 1.1-5.1, p-value = 0.035 and the corresponding RR: 1.3, 95% CI: 1.0-1.6, p-value = 0.029).

VDR ApaI: The ratio of (AA+Aa) and aa genotypes is significantly higher among the subject with PCa compared to the controls (OR: 2.3, 95% CI: 1.1-4.7, p-value = 0.026 and the corresponding RR: 1.5, 95% CI: 1.1-2.1, p-value = 0.022).

SRD5A2 TA repeats: The ratio of 00 and 09 TA repeats is significantly higher among the subjects with PCa compared to the controls (OR: 3.1, 95% CI: 1.2-8.3, p-value =0.024 and the corresponding RR: 1.3, 95% CI 1.1-1.5, p-value = 0.013).

VDR FokI: The ratio of (FF+Ff) and ff is higher for the subjects with PCa compared to the controls but the difference is not statistically significant (OR: 1.8, 95% CI: 0.90-3.7, p-value = 0.099 and the corresponding RR: 1.3, 95% CI: 0.95-1.91, p-value =0.091).

CYP17 MspA1I: The ratio of A1A1 and (A1A2+A2A2) genotypes is higher among the subjects with PCa compared to the controls but the difference is not statistically significant ((OR: 2.0, 95% CI: 0.90-4.6, p-value = 0.095, and the corresponding RR: 1.7, 95% CI: 0.91-3.1, p-value = 0.092).

The genotypic profile

The differences in the proportions of the subjects homozygous in the potential risk-bearing alleles (A, B, F, A1 and O repeat) and the potential protective alleles (a, b, f, and A2 and 0/9 heterozygotes) are shown in Table 4. About 46% of the subjects with PCa and 27% of the controls were homozygous in two or more risk bearing alleles (p-value = 0.023), whereas 54% of the subject with PCa and 73% of the controls were homozygous in one or fewer risk bearing alleles (p-value = 0.024). In contrast, 38% of the subjects with PCa and 71% of the controls were homozygous in two or more protective alleles (p-value = 0.001); whereas, 62% of the subjects with PCa and 29% of the controls were homozygous in one or fewer protective alleles (p-value = 0.001).

Discussion

The study of cancer as a public health problem of Lebanon gained significant attention after a national cancer registry was reestablished in 2005 as an institution within the Ministry of Public Health, and two reports, Cancer 2003 and Cancer 2004, were published in 2006 and 2008, respectively. In 2004, the population of Lebanon was 3,946,342 and 7,197 cases of cancer were diagnosed in that year. About 50% of the cases were men and PCa was the third-most common cancer (behind lung cancer and bladder cancer) among Lebanese men, with an age-standardized incidence rate of 27.6 cases/100,000 (Shamseddine and Musallam, 2004). The incidence rate for prostate cancer increased to 39.2 cases/100,000 by

Table 1. Allelic Distribution of VDR, CYP17 and SRD5A2 Genes among the Controls (n=79) and the Subjects with PCa (n=50)

Genes	loci/groups	Observed ratio DD: Dd: dd	Expected ratio DD': Dd': dd'	χ^2	*p* values R or NR
VDR					
	FokI/PCa	7.0: 22.0: 21.0	6.5: 23.0: 20.5	0.1	0.752
	FokI/Cont.	7.0: 27.0: 45:0	5.0: 30.4: 43.3	0.97	0.325
	BsmI/PCa	10.0: 28.0: 12.0	11.5: 24.9:13.5	0.74	0.39
	BsmI/Cont.	9.0: 41.0: 29.0	11.0: 36.9: 31.0	0.94	0.332
	ApaI/PCa	4.0: 27.0:19.0	6.1: 22.8: 21.1	1.7	0.192
	ApaI/Cont.	6.0: 27.0: 46.0	4.8: 29.4: 44.8	0.52	0.471
	TaqαI/PCa	6.0: 31.0: 13.0	9.2: 24.5: 16.2	3.5	0.061
	TaqαI/Cont.	5.0: 48.0: 26.0	10.6: 36.7: 31.6	7.5	0.006*
SRD5A2					
	TA 0:9/PCa	41.0: 6.0: 3.0	38.7: 10.6: 0.7	9.3	0.002*
	TA 0:9/Cont.	53.0: 24.9: 2.0	53.5: 23.0: 2.5	0.14	0.708
CYP17					
	A1:A2/PCA	16.0:25.0: 9.0	16.2: 24.5: 9.2	0.02	0.888
	A1:A2/Cont.	15.0: 52.0: 12.0	21.3: 39.4:18.3	8	0.005

*- significant at the 0.05 level of significance. DD, Dd and dd are equivalent to; for VDR gene- FokI: FF, Ff and ff; for BsaI: BB, Bb and bb; for ApaI, AA; Aa and aa; and for TaqαI, TT; Tt and tt; for SDR5A2 TA repeats- 0/0, 0/9 and 9/9; and for CYP17 gene- MspA1I: A1/A1, A1/A2 and A2/A2, respectively. DD, Dd and dd are the observed numbers, DD', Dd' and dd' are the corresponding expected numbers; The corresponding p-values rounded to three decimals are also reported

2008, making prostate cancer the most prevalent cancer diagnosed in Lebanon, and the trend indicates further increase of the same (Shamseddine et al., 2014). The current study was a part of the pioneering prostate cancer campaign in Lebanon for molecular research on prostatic diseases.

In the present study, we examined four diallelic polymorphisms of VDR gene, of them the FokI C/T transition is located in the exon 2, the BsmI A/G transition and the ApaI G/T transversion are located in the intron between exons 8 and 9; and the Taq**α**I T/C transition is located in exon 9 of the VDR gene. The FokI and Taq**α**I polymorphisms change one codon each without altering the amino acid sequence of the VDR polypeptide. Although none of the above mutations alters the primary structure of the VDR polypeptide, some of the mutations have been found associated with varying plasma levels of the VDR ligand (Morrison et al., 1994). Our results indicate that the BsmI B allele and the ApaI A allele of the VDR gene are associated with an increased risk for PCa. The data also indicate that the FokI F allele is possibly associated with increased risk of PCa but the TaqαI T or t allele of the VDR gene has no association with PCa risk. A study on the Pakistani population indicated that the ApaI A allele is protective and the FokI and TaqaI polymorphisms have no appreciable association with PCa (Yousaf et al., 2014). Earlier, Maistro and colleagues (2004) found no association of ApaI and TaqαI polymorphisms and the risk for PCa in a Brazilian population. In another population study, Cheteri et al., (2004) found no association between

Table 2. The Allelic Frequencies of the VDR FokI (F and f), BsmI (B and b), ApaI (A and a) and TaqαI (T and t), the CYP 17 Gene MspA1I (A1 and A2) and the SRD5A2 Gene TA Repeats (0 and 9 Repeats) among the Controls and Patients with PCa

Gene(allele)	Control	PCa	OR	95% CI	p-values
VDR(F)	41(0.26)	36(0.36)	1.0	ref	
VDR(f)	117(0.74)	64(0.64)	1.8	1.1-3.2	0.092
VDR(B)	59(0.37)	48(0.48)	1.0	ref	
VDR(b)	99(0.63)	52(0.52)	1.5	0.93-2.6	0.091
VDR(A)	39(0.25)	35(0.35)	1.0	ref	
VDR(a)	119(0.75)	65(0.65)	1.6	0.95-2.8	0.075
VDR(T)	58(0.37)	43(0.43)	1.0	ref	
VDR(t)	100(0.63)	57(0.57)	1.3	0.78-2.2	0.313
CYP17(A1)	82(0.52)	57(0.57)	1.0	ref	
CYP17(A2)	76(0.48)	43(0.43)	1.2	0.74-2.0	0.423
SRD5A2(0)	130(0.82)	88(0.88)	1.0	ref	
SRD5A2(9)	28(0.18)	12(0.12)	1.6	0.76-3.3	0.218

Table 3. The Genotypic Frequencies of the VDR FokI (FF, Ff and ff), BsmI (BB, Bb and bb), ApaI (AA, Aa and aa) and TaqαI (TT, Tt and tt), the CYP 17 gene MspA1I (A1A1, A1A2 and A2A2) and the SRD5A2 Gene TA Repeats (00, 09 and 99 Repeats) among the Controls and Patients with PCa

Locus (alleles)	Control	PCa	OR	95%CI	p-value	RR	95%CI	p-values
VDR FokI								
FF/Ff	7/27	7/22	1.2	0.4-4.0	0.735	1.2	0.5-2.9	0.735
FF/ff	7/45	7/21	2.1	0.7-6.9	0.201	1.8	0.8-4.8	0.197
(FF+Ff)/ff	34/45	29/21	1.8	0.9-3.7	0.099	1.3	0.9-1.9	0.091
(Ff+ff)/FF	62/7	43/7	0.7	0.2-2.1	0.521	1.0	0.8-1.1	0.531
VDR BsmI								
BB/Bb	9/41	10/28	1.6	0.6-4.5	0.350	1.4	0.7-3.2	0.349
BB/bb	9/29	10/12	2.6	0.9-8.3	0.085	1.9	0.9-3.9	0.081
(BB+Bb)/bb	50/29	48/12	2.3	1.0-5.1	0.035*	1.3	1.0-1.6	0.029*
(Bb+bb)/BB	70/9	40/10	0.5	0.2-1.4	0.184	0.9	0.8-1.1	0.209
VDR ApaI								
AA/Aa	6/27	4/27	0.7	0.2-2.6	0.563	0.7	0.2-2.3	0.564
AA/aa	6/46	4/19	1.6	0.4-6.4	0.495	1.5	0.5-4.8	0.490
(AA+Aa)/aa	33/46	31/19	2.3	1.1-4.7	0.026*	1.5	1.1-2.1	0.022*
(Aa+aa)AA	73/6	46/4	1.0	0.3-3.5	0.933	1.0	0.9-1.1	0.933
VDR Taqαl								
TT/Tt	5/48	6/31	1.9	0.5-6.6	0.339	1.7	0.6-5.2	0.338
TT/tt	5/26	6/13	2.4	0.6-9.4	0.207	1.9	0.7-5.5	0.206
(TT+Tt)/tt	53/26	37/13	1.4	0.6-3.1	0.405	1.1	0.9-1.4	0.394
(Tt+tt)/TT	74/5	44/6	0.5	0.1-1.7	0.268	0.9	0.8-1.1	0.297
CYP17 MspA1I								
A1A1/A1A2	15/52	16/25	2.2	0.9-5.2	0.066	1.7	0.9-3.1	0.063
A1A1/A2A2	15/12	16/9	1.4	0.5-4.3	0.535	1.2	0.7-1.8	0.545
(A1A1+A1A2)/ A2A2	67/12	41/9	0.8	0.3-2.1	0.673	1.0	0.8-1.1	0.679
A1A1/ (A1A2+A2A2)	15/64	16/34	2.0	0.9-4.6	0.095	1.7	0.9-3.1	0.092
SRD5A2 TA repeat								
00/09	53/24	41/6	3.1	1.2-8.3	0.024*	1.3	1.1-1.5	0.013*
00/99	53/2	41/3	0.5	0.1-3.2	0.479	1.0	0.9-1.1	0.488
(00+09)/99	77/2	47/3	0.4	0.7-2.5	0.334	1.0	0.9-1.0	0.366
00/(09+99)	53/26	41/9	2.2	0.9-5.3	0.067	1.2	0.9-1.5	0.078

*, significant at the 0.05 level of significance

the risk of PCa, and FokI and BsmI polymorphisms of the VDR gene. Taylor and colleagues (1996) observed the tt homozygosis to be associated with a reduced risk of PCa. Another study on a Taiwanese population revealed no association of ApaI and TaqαI polymorphisms with the risk of PCa, but the study found the B allele of the BsmI polymorphism as a risk factor for PCa (Huang et al., 2004), and a nearly identical result was observed previously in a Japanese population (Habuchi et al., 2000). In all, a consensus on the association of the VDR alleles with PCa risk, severity or prognosis of PCa has not emerged; although it is apparent that the association, if there is any, is affected by the race or ethnicity (reviewed in Dianat et al., 2009).

For the CYP17 gene, we studied a single diallelic polymorphism, the MspA1I T/C transition in the 5'UTR of the exon 1 (the absence of the MspA1I site is considered the A1 allele and the presence thereof is considered the A2 allele). This transition creates a binding site for the transcription factor Sp-1 although it is uncertain if SP1 actually interacts with the binding site (Nedelcheva et al., 1999). Our result indicates that the A1 allele is possibly associated with increased risk of PCa, which supports two previous studies (Habuchi et al., 2000b, Wadelius et al., 1999). However, other groups have found an association of the A2 allele with increased risk for PCa (for example, Song et al., 2016; Stanford et al., 2002; Gsur et al., 2000), yet another study failed to established any clear relationship between one or the other allele with the risk of PCa (Sivonova et al., 2012). A meta-analysis of over 25 different studies (Cai et al., 2012) indicated it unlikely that the MspA1I polymorphisms of the CYP17 gene affect the risk of PCa. Of the several polymorphisms of the SRD5A2 gene reported (Peters et al., 2010), we genotyped the diallelic TA dinucleotide repeats (0 or 9 repeats) present in the 3'UTR of the gene. Our results indicate that the (TA)0 allele is possibly associated with an increased risk of PCa and the heterozygote (i.e. 0/9)

Table 4. The Difference in the Proportions of Homozygotes in Potential Risk-Bearing (i.e. A, B, F, A1 and 0) Alleles and Potential Protective (i.e. a, b, f, A2 and 09) Alleles among the Control Subjects and the Subjects with PCa

Attributes	Control(n=79)	PCa (n=50)	p-values
Number of homozygotes in the potential risk-bearing alleles			
0	13(0.16)	5(0.10)	0.302
1	45(0.57)	22(0.44)	0.151
0-1	58(0.73)	27(0.54)	0.024*
2	17(0.22)	15(0.30)	0.277
3	4(0.05)	6(0.12)	0.151
4	0(0.00)	2(0.04)	0.073
2-4	21(0.27)	23(0.46)	0.023*
Number of homozygotes in the potential protective alleles			
0	3(0.4)	9(0.18)	0.006*
1	20(0.25)	22(044)	0.027*
0-1	23(0.29)	31(0.62)	0.001*
2	37(0.29)	12(0.24)	0.009*
3	16(0.20)	6(0.12)	0.225
4	2(0.03)	1(0.02)	0.846
5	1(0.01)	0(0.00)	0.425
2-5	56(0.71)	19(0.38)	0.001*

*, significant at the 0.05 level of significance

may be protective for PCa. The (TA)9 allele is rare in the Lebanese population and thus our result on this locus is not statistically rigorous. Some previous studies (Rajender et al., 2009; Neslund-Dudas et al., 2007) indicated that the (TA)9 allele is associated with increased risk of PCa. Other studies (Kachakova et al., 2016; Salam et al., 2005) found no relationship between the longer TA repeat and prostate cancer risk. An earlier meta-analysis indicated that the TA repeat polymorphism might have a modest effect on prostate cancer susceptibility (Ntais et al., 2003).

It is evident that the outcomes of the studies involving a single polymorphism of a gene of interest or a group of polymorphisms of one gene have not provided a dependable model in predicting the risk of PCa, age of onset of the disease or prognosis of the disease after therapeutic interventions (Chen et al. 2015; Dianat et al., 2009; Ntais et al., 2003). This may be due to racial and ethnic differences (Dianat et al., 2009) and due to the presence of risk-bearing and protective alleles of different genes in almost every person (Kitts et al., 2014; Latil et al., 2001). It is likely that the overall genotype profile of a subject regarding all the critical genes involved in PCa can be a more reliable predictor of PCa, at least for specific racial and ethnic groups. To this end, we investigated if homozygosis in the risk-bearing alleles (A, B, F, and A1) or the protective alleles (a, b, f, A2) and the frequency of 00 and 09 alleles are unevenly distributed among the subjects with PCa and the controls. Our data indicates that a higher proportion of the subjects with PCa are homozygous for more of the risk-bearing alleles and fewer of the protective alleles. The result indicates that a subject homozygous in several of the risk-baring alleles or few or none of the protective alleles of the genes may have a higher risk for developing PCa.

We investigated only six alleles of three different genes that are potentially associated in PCa. Polymorphisms in many additional genes such as the androgen receptor gene (Kachakova et al., 2016; Huang et al., 2003), p53, p21 and p73 genes (Simonova et al., 2015; Mittal et al., 2011), and BCL-2 gene (Lin et al., 2016) could be associated with cancers. The overall genotype profile regarding all of the genes associated with PCa could be a more reliable indicator of PCa risk of an individual. A system biology approach has been suggested to investigate the association of genetic polymorphisms and PCa and BPH (Prakash et al., 2002; Luo et al., 2001). The approach is now known as the genome-wide association studies (GWAS) of SNP and SSLP and disease risks (Kar et al. 2016; Patnala et al., 2013). However, since SNPs are extremely common, studies like the present one involved in identification of important SNPs relevant to disease risks of different ethnic groups will remain useful.

Benign prostate hyperplasia (BPH) is another very common Lower Urinary Tract Syndrome (LUTS) of the ageing man. BPH and PCa share many signs and symptoms (Sausville and Naslund, 2010) but the signs of BPH are more overt and they become evident earlier in the disease progression. Thus, establishing BPH as the causal factor for PCa could improve early diagnosis and intervention (Orsted and Bojesen, 2013). In a previous study, we investigated the polymorphisms in the VDR gene, CYP17 gene and SRD5A2 gene in 68 Lebanese men with confirmed BPH and observed the ApaI A allele, BsmI B allele and FokI F allele of the VDR gene and the MspA1I A1 allele of the CYP17 gene to be associated with BPH (El Ezzi et al. 2014). Moreover, we also observed that a higher percentage of the controls were homozygous for 2 or more protective alleles compared to the BPH group (60% for controls, 28% for BPH group, p-value <0.01), as we observed in the present study. These results support and extend several previous studies showing that some common genetic polymorphisms in some of the genes

are associated with BPH as well as PCa (Hamasaki et al., 2002; Habuchi et al., 2000; Habuchi et al., 2000b). Although BPH and PCa are histopathologically distinct diseases and BPH is not considered a risk factor for PCa (Chang et al., 2012; De Nunzio et al., 2011), both BPH and PC are cytoproliferative diseases with similar hormonal and inflammatory risk factors (Miah and Catto, 2014; Orsted and Bojesen, 2013). It will be a great breakthrough if certain attributes of BPH can be established as a predictive sign of PCa.

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Statement of Conflict of Interest

There is no conflict of interest to be reported

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