RESEARCH ARTICLE

Association of Benign Prostate Hyperplasia with Polymorphisms in VDR, CYP17, and SRD5A2 Genes among Lebanese Men

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Abstract

Background: The aim of the study was to investigate any associations between benign prostate hyperplasia (BPH) and single nucleotide polymorphisms (SNPs) in the VDR gene (FokI, BsmI, ApaI and TaqαI loci) and the CYP17 gene (MspA1I locus), as well as TA repeat polymorphism in SRD5A2 gene among Lebanese men. Materials and Methods: DNA extracted from blood of 68 subjects with confirmed BPH and 79 age-matched controls was subjected to PCR/PCR-restriction fragment length polymorphism analysis. The odds ra=tio (OR) of having a genotype and the relative risk (RR) of developing BPH for having the genotype were calculated and the alleles were designated risk-bearing or protective. Results: Our data indicated that the A and B alleles of the VDR ApaI and BsmI SNPs were highly associated with increased risk of BPH (p=0.0168 and 0.0002, respectively). Moreover, 63% of the controls compared to 43% of the subjects with BPH were homozygous for none of the risk-bearing alleles (p=0.0123) whereas 60% of the controls and 28% of the subjects with BPH were homozygous for two or more protective alleles (p<0.0001). Conclusions: For the first time, our study demonstrated that ApaI and BsmI of the VDR gene are associated with risk of BPH among Lebanese men. Our study also indicated that overall polymorphism profile of all the genes involved in prostate physiology could be a better predictor of BPH risk.

Keywords: Benign prostate hyperplasia (BPH) - single nucleotide polymorphism (SNP) - TA repeat polymorphism

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Introduction

Benign prostate hyperplasia (BPH) is the most prevalent urological problem of the ageing men that causes morbidity but not mortality. The disease is manifested by enlargement of the prostate gland due to proliferation of non-transformed stromal-epithelial cells, resulting in moderate to severe obstruction of the urethra. The pathogenesis of BPH is vaguely understood and the underlying causes have not been fully elucidated. It is hypothesized that steroid hormone-induced cell proliferation, inflammation and inefficiency of the apoptotic cell death as well as environmental and genetic factors may contribute to the disease (Konwar et al., 2008).

The incidence rate of BPH is 2.96 per 1000 man-years among men in their forties and 34.46 per 1000 man-years among men in their seventies (Werhamme et al., 2002). Previous reports indicated that the prevalence of BPH, and the volume and cellular composition of the prostate may vary among different families and among racial and ethnic groups (Pearson et al., 2003). A study on BPH patients found that Asian men, compared to Caucasian and African American, have smaller prostate volume, and prostate in Japanese men contains more glandular tissue and less stromal component (Aoki et al., 2001).

The most useful and widely used biochemical marker for BPH is the PSA, but unfortunately PSA level increases only in later stages of clinical BPH (McConnell et al., 2006) and also in case of prostate cancer (Pca). This makes it difficult at the gray zone of PSA (between 4 and 10 ng/ml) to differentiate between BPH and Pca without prostate biopsy that is still viewed to be an invasive method (Dwivedi et al., 2011). Also, it was shown that a PSA level higher than 10 ng/ml in Middle Eastern men is more predictable of BPH and BPH with prostatitis rather than Pca (Kehinde et al., 2003; Anim et al., 2007), as it is the case among Caucasians in Europe and United States. The reason for that is still unclear, and it may be due to genetic factors and genetic-environmental interactions. More epidemiologic, molecular and genetic studies are needed to explain this discrepancy.

The genetics of BPH is still not fully understood and very few studies were previously performed worldwide in this field. The results of the published

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studies on the association between BPH risk and different genetic polymorphism are promising. Although no high penetrance genetic marker for BPH has been identified yet, certain alleles of a number of genes have been associated with the disease. Single nucleotide polymorphism (SNP) and TA repeats in several genes, including vitamin D receptor (VDR) gene (Bousema et al., 2000; Habuchi et al., 2000a; Hamasaki et al., 2002; Chaimuangraj et al., 2006), androgen receptor (AR) gene, cytochrome P-450 steroid 17α-hydroxylase/17, 20 lyase (CYP17) gene (Habuchi et al., 2000b; Madigan et al., 2003; Tigli et al., 2003), and steroid 5α reductase type II (SRD5A2) gene have been indicated in BPH development (Salam et al., 2005; Roberts et al., 2006; Konwar et al., 2008). The VDR gene encodes a vitamin D-inducible transcription factor with important roles in many physiological processes including cell proliferation (McConnell et al., 2006). In addition, the CYP17 gene encodes a lyase enzyme that regulates a rate-limiting step of androgen synthesis pathway (Kehinde et al., 2003), and the SRD5A2 gene encodes a reductase enzyme that converts testosterone to the more biologically active form of the hormone, dihydrotestosterone (Li et al., 2010).

In the present study, we analyzed polymorphisms in the VDR, CYP17, and SRD5A2 genes among 79 healthy Lebanese men and 68 with BPH. We also included four diallelic SNPs of the VDR genes; namely, the FokI C to T transition (located at the exon 2); the BsmI A to G transition and the ApaI G to T transversion (located in the intron between exons 8 and 9); and the TaqαI T to C transition (located in exon 9). In addition, we also analyzed a single diallelic SNP of the CYP17 gene-the MspA1I T to C transition-(located in the 5'UTR of the exon one) and a diallelic TA 0 or 9 dinucleotide repeats of the SRD5A2 gene (located in the 3'UTR of the mRNA of the gene). Moreover, we studied the association of the genotype of each individual locus as well as the overall genotype profile for the important loci of the indicated genes with BPH. To the best of our knowledge, no previous studies were performed in Lebanon or any other Arab countries regarding the relationship between BPH and the polymorphism of any of the genes investigated in this study.

Materials and Methods

Subjects

The 147 subjects (68 with confirmed BPH and 79 age-matched controls) enrolled in the study were volunteer participants of prostate disorder screening campaigns organized by Dr. El Ezzi's laboratory in collaboration with several hospitals and medical centers in Lebanon. An informed consent form to participate in the prostate-specific antigen (PSA) screening, and donation of blood for DNA extraction and usage in research was obtained from each subject in accordance with the ethical standards of the 1975 Declaration of Helsinki, and following the procedures and guidelines of the Institutional Review Board (IRB) of Utah Valley University (IRB approval # 00614). Prostate health of each of the participants was evaluated by measuring the serum Total PSA (PSA-T) level (using PSA kits purchased from Immunotech, Marseille, France), followed by a digital rectal examination (DRE) if necessary. When the PSA-T level is in the gray zone (between 4 and 10 ng/ml), a free PSA test (PSA-F) was conducted to determine the F/T PSA level and to help differentiate between BPH and prostate cancer (Pca). The International Prostate Symptom Score (IPSS) value was determined and when needed, trans-rectal ultrasonography was conducted. Controls were volunteers with normal level of PSA for at least two years, normal IPSS score, and normal DRE.

Methods

DNA was extracted from blood samples using QiaAmp DNA Blood Mini Kit (Qiagen, Milan, Italy), and quantified spectrophotometrically. Appropriate DNA fragments of the three genes were amplified by polymerase chain reaction (PCR) using the primers sets shown in Table 1.

The PCR mixture contained 1x reaction buffer (Invitrogen, Grand Island, NY), 1.75 mM $MgCl_2$, 0.2 mM dNTP, 20 pico moles of the two primers, 25 ng of template DNA, and 1 unit of Platinum Taq DNA polymerase (Invitrogen) in a total volume of 25 μl. A GeneAmp 2700 thermocycler (Applied Biosystems, Carlsbad, CA) used for PCR was programed as follows: 94°C for 5 minutes

Table 1.

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'*
VDR TaqαIF	5'CAGAGCATGGACAGGGAGCAA3' and
VDR TaqαIR	5'GCAACTCCTCATGGCTGAGGTCTC3'**
CYP17F	5'CATTCGCACCTCTGGAGTC3' and
CYP17R	5'GGCTCTTGGGGTACTTG3'***
SRD5A2F	5'GCTGATGAAAACTGTCAAGCTGCTGA3' and
SRD5A2R	5'GCCAGCTGGCAGAACGCCAGGAGAC3'****

*Falleti et al., 2010; **Taylor, et al., 1996; ***Feigelson et al., 1997; and finally; Forrest et al., 2005

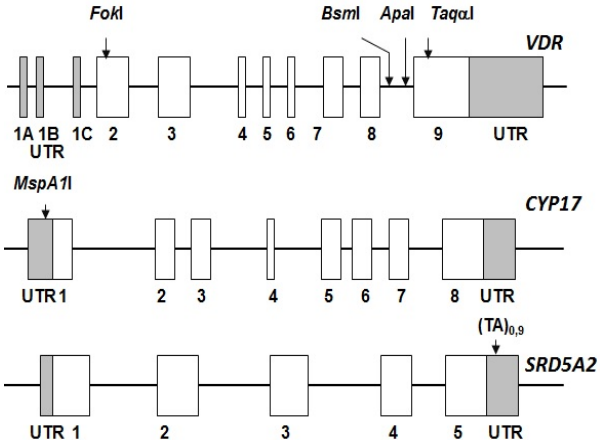

Figure 1. Schematic Diagram of the VDR Gene, CYP17 Gene, and SRD5A2 Gene, with the Exons numbered in Arabic. the untranslated region (utr) exons are shown shaded. The Relative Locations of the single Nucleotide Polymorphism (SNP) and TA Repeats are indicated by arrows. The size of the genes and the exons and introns are not drawn to scale

(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 TA repeats of the SRD5A2 gene, the amplified DNA was directly resolved in a 9.0% polyacrylamide gel. All other samples were processed for restriction fragment length polymorphism (RFLP) analysis. Briefly, the samples were treated with 2 units of restriction endonuclease for ApaI, BsmI, FokI, MspA1I or TaqαI (all from New England Biolab, Beverly, MA) overnight and then resolved in a 3.0% agarose gel for TaqαI or a 6% polyacrylamide gel for ApaI, BsmI, FokI, and MspA1I. The gels were stained with ethidium bromide and then documented using a Kodak Gel Logic 1500 scanner (Kodak, Rochester, NY). For convenience, the alleles having the restriction site or repeat DNA were considered recessive. The band patterns for different genotypes are shown in Figure 1.

Statistical analysis

The agreement of the frequencies of the dominant and recessive alleles of the genes with Hardy-Weinberg equilibrium was tested by χ^2 goodness-of-fit. The null hypothesis that the population is in Hardy-Weinberg equilibrium is rejected if the calculated χ^2 test statistic is larger than the tabulated critical value at the 0.05 level of significance or, equivalently, if the p value is smaller than 0.05. In Hardy-Weinberg (H-W) chi-square analysis, the number of degrees of freedom is equal to the number of genotypes minus the number of alleles. In our case, we have two alleles and three genotypes, therefore we have only one degree of freedom, and the tabulated chi-square critical value at the 0.05 level of significance is 3.841. A simple Excel template written by a statistician (one of the authors) was used for calculating H-W expected frequencies, the value of χ^2 test statistic, and the p value.

Association of BPH and the genotypes was evaluated by calculating the odds ratio (OR) and the 95% confidence interval (CI). The OR was calculated using the formula (a/b)/ (c/d) where a/b is the ratio of specific attributes for the BPH group and c/d is the ratio for the same attributes for the control group. Relative risk (RR) was also calculated using the formula RR=[a/(a+b)]/[c/(c+d)], where a/(a+b) is the probability of presence of certain attributes for the BPH group and c/(c+d) is the corresponding probability for the control group. The OR and RR along with 95%CI and p values were calculated using the free-on-line calculators available through MedCalc statistical software for biomedical research (MedCalc Software, Acacialaan 22, B-8400 Ostend, Belgium). A value of RR<1.0 is considered of having a protective effect of an allele or a genotype on disease risk relative to the alternative allele or genotype.

The proportions of subjects with a particular genotype among the controls and the subjects with BPH were compared with each other using the two-tailed z test for the difference between two independent proportions at 0.05 level of significance using XLSTAT software 2012. The null and alternative hypotheses were H_0: there is no significant difference between the two proportions, and H_1: there is a significant difference between the two proportions. A p value <0.05 leads to the rejection of the null hypothesis and the conclusion that there is a significant difference between the two tested proportions.

Results

Allelic distribution and allele frequencies

In the present study, the experimental group included 68 Lebanese men with confirmed BPH (mean age 65.98±9.97 years) and the control group consisted of 79 men with no known prostate pathology (mean age 58.33 ±10.14 years). The allelic distribution of all genes tested was in H-W equilibrium (Table 2) except the BPH group for the VDR FokI (p=0.0332) and the VDR BsmI loci (p=0.0138); and the control group for the VDR TaqαI (p=0.0063) and the CYP17 MspA1I loci (p=0.0047). The H-W expected frequencies of the homozygous dominant, homozygous recessive, and heterozygote genotypes significantly deviated from the observed frequencies for these four loci in the above-mentioned groups.

The allelic frequencies and proportions for the three genes among the controls and the subjects with BPH are shown in Table 3. There is no significant difference in

Table 2. Allelic Distribution of VDR, CYP17 and SRD5A2 Genes among the Controls (n=79) and Subjects with BPH (n=68)

Gene (locus)	Groups	Observed Freq.			Expected H-W Freq.			χ^2	p value
		WW	Ww	ww	WW	Ww	ww		
VDR (Folk)	BPH	3	37	28	6.8	29.4	31.8	4.54	0.0332*
	Control	7	27	45	5.32	30.36	43.32	0.97	0.3252
VDR (BsmI)	BPH	18	43	7	22.95	33.11	11.95	6.07	0.0138*
	Control	9	41	29	11.02	36.97	31.02	0.94	0.3324
VDR (ApaI)	BPH	9	33	26	9.56	31.88	26.56	0.08	0.771
	Control	6	27	46	4.81	29.37	44.81	0.52	0.4726
VDR (TaqαI)	BPH	7	38	23	9.94	32.12	25.94	2.28	0.131
	Control	5	48	26	10.65	36.71	31.65	7.47	0.0063*
SDR5A2 (TA0-9)	BPH	53	13	2	52.06	14.88	1.06	1.08	0.2986
	Control	53	24	2	53.48	23.04	2.48	0.14	0.7105
CYP17 (MspA1I)	BPH	21	35	12	21.8	33.4	12.8	0.16	0.6937
	Control	15	52	12	21.28	39.44	18.28	8.01	0.0047*

*Significant at 0.05 level of significance; WW, Ww and ww are equivalent to the following: for VDR gene- FokI: FF, Ff and ff; for BsmI: BB, Bb and bb; for ApaI: AA, Aa and aa; 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.

the allele frequencies of any of the loci between the two groups except that the B allele of the VDR BsmI locus is significantly more common among the subjects with BPH compared to the controls (OR=2.33, 95%CI: 1.46-3.72, p=0.0004), and the A allele of the VDR ApaI locus is also significantly more common among the subjects with BPH compared to the controls (OR=1.83, 95%CI: 1.11-3.02, p=0.0181).

Genotype frequencies and ratios

The frequencies of the homozygote recessive or dominant genotypes and heterozygote genotypes for each of the alleles among the subjects with BPH and the controls are shown in Table 4. The OR of having a particular genotype for the subjects with BPH compared to the controls and the RR of the disease for having the genotype are also shown in Table 4. The difference is generally not statistically significant except for the following instances:

VDR BsmI: The BB/bb ratio is significantly higher among the subjects with BPH compared to the controls (OR=8.29, 95%CI: 2.62-26.16, p=0.0003, and the corresponding RR=3.04, 95%CI: 1.63-5.66, p=0.0004). The (BB+Bb)/bb ratio is also significantly higher for the BPH group compared to the controls (OR=2.08, 95%CI: 1.03-4.22, p=0.0418, and the corresponding RR=1.42, 95%CI: 1.18-1.71, p=0.0002).

VDR ApaI: The (AA+Aa)/aa ratio is significantly higher for the subjects with BPH compared to the controls, (OR= 2.25, 95%CI: 1.16- 4.37, p=0.0164, and the corresponding RR=1.48, 95%CI: 1.07-2.04, p=0.0168). The ratio of AA/aa is also higher among the subjects with BPH group compared to the controls (OR=2.65, CI: 0.85-8.29, p=0.0932, and the corresponding RR=2.23, CI=0.87-5.71, p=0.0947) although the difference is not statistically significant at the 0.05 level of significance.

VDR FokI: The (FF+Ff)/ff ratio is considerably higher among the subjects with BPH compared to the controls (OR=1.89, 95%CI: 0.98-3.65, p=0.0574, and the corresponding RR=1.37, 95%CI: 0.99-1.89, p=0.0574) although the difference is barely not statistically significant at the 0.05 level of significance.

CYP17 MspA1I: The A1A1/A1A2 ratio is higher

Table 3. The Allelic Frequencies and Proportions 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 (pA1A1 and A2A2) and the SRD5A2 Gene TA Repeats (0 or 9 repeats) among the Controls and Patients with BPH

Gene	Allele	Control (n=79)	BPH (n=68)	OR	95% CI	p-value
VDR	F	41 (0.23)	43 (0.32)	1	Ref	
	f	117 (0.74)	93 (0.68)	1.32	0.80-2.19	0.284
	B	59 (0.37)	79 (0.58)	1	Ref	
	b	99 (0.63)	57 (0.42)	2.33	1.46-3.72	0.0004*
	A	39 (0.25)	51 (0.38)	1	Ref	
	a	119 (0.75)	85 (0.63)	1.83	1.11-3.02	0.0181*
	T	58 (0.37)	52 (0.38)	1	Ref	
	t	100 (0.63	84 (0.62)	1.07	0.66-1.71	0.7874
CYP17	A1	82 (0.52)	77 (0.57)	1	Ref	
	A2	76 (0.48)	59 (0.43)	1.21	0.76-1.92	0.4184
SRD5A2	(TA)0	130 (0.82)	119 (0.88)	1	Ref	
	(TA)9	28 (0.18)	17 (0.13)	1.51	0.79-2.89	0.2171

*significant at 0.05 level of significance

Table 4. 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) among the Controls and Patients with BPH

Gene and Allele		Cont.	BPH	OR	95% CI	p-value	RR	95% CI	p-value
VDR (FokI)	FF *vs* Ff	7 (27)	3 (37)	0.31	0.07-1.32	0.1138	0.36	0.10-1.30	0.1200
	FF *vs* ff	7 (45)	3 (28)	0.69	0.16-2.89	0.6099	0.72	0.20-2.58	0.6125
	FF+Ff *vs* ff	34 (45)	40 (28)	1.89	0.98-3.65	0.0574	1.37	0.99-1.89	0.0574
	Ff+ff *vs* FF	62 (7)	65 (3)	2.45	0.61-9.89	0.2093	1.06	0.97-1.69	0.1986
VDR (BsmI)	BB *vs* Bb	9 (41)	18 (43)	1.91	0.77-4.73	0.1632	1.64	0.81-3.33	0.1708
	BB *vs* bb	9 (29)	18 (7)	8.29	2.62-26.16	0.0003*	3.04	1.63-5.66	0.0004*
	BB+Bb *vs* bb	50 (29)	61 (7)	2.08	1.03-4.22	0.0418*	1.42	1.18-1.71	0.0002*
	Bb+bb *vs* BB	70 (9)	50 (18)	0.36	0.15-0.86	0.0216*	0.83	0.71-0.98	0.0250*
VDR (ApaI)	AA *vs* Aa	6 (27)	9 (33)	1.23	0.39-3.88	0.7274	1.18	0.47-2.98	0.7283
	AA *vs* aa	6 (46)	9 (26)	2.65	0.85-8.29	0.0932	2.23	0.87-5.70	0.0947
	AA+Aa *vs* aa	33 (46)	42 (26)	2.25	1.16-4.37	0.0164*	1.48	1.07-2.04	0.0168*
	Aa+aa *vs* AA	73 (6)	59 (9)	0.54	0.18-1.60	0.2655	0.94	0.84-1.05	0.2717
VDR (TaqαI)	TT *vs* Tt	5 (48)	7 (38)	1.77	0.52-6.01	0.3613	1.65	0.56-4.84	0.3626
	TT *vs* tt	5 (26)	7 (23)	1.58	0.44-5.68	0.4812	1.45	0.52-4.06	0.4831
	TT+Tt *vs* tt	53 (26)	45 (23)	0.96	0.48-1.91	0.9069	0.99	0.78-1.24	0.9070
	Tt+tt *vs* TT	74 (5)	61 (7)	0.59	0.18-1.95	0.3857	0.96	0.87-1.06	0.3911
CYP17 (MspA1I	A1A1 *vs* A1A2	15 (52)	21 (35)	2.08	0.95-4.58	0.0689	1.68	0.96-2.93	0.0708
	A1A1 *vs* A2A2	15 (12)	21 (12)	1.40	0.50-3.96	0.5256	1.15	0.75-1.75	0.5308
	A1A1+A1A2 *vs* A2A2	67 (12)	56 (12)	0.84	0.35-2.01	0.688	0.97	0.84-1.12	0.6896
	A1A2+ A2A2 *vs* A1A1	64 (15)	47 (21)	0.53	0.25-1.12	0.097	0.85	0.70-1.03	0.1039
SRD5A2 (TA)n	00 *vs* 09	53 (24)	53 (13)	1.85	0.85-4.01	0.1210	1.17	0.96-1.41	0.1156
	00 *vs* 99	53 (2)	53 (2)	1.00	0.14-7.36	1.0000	1.00	0.93-1.08	1.0000
	00+09 *vs* 99	77 (2)	66 (2)	0.86	0.12-6.25	0.8792	1.00	0.94-1.05	0.8797
	99 *vs* 00+09	2 (77)	2 (66)	1.17	0.16-9.51	0.8792	1.17	0.17-8.03	0.8792
	09+99 *vs* 00	26 (53)	15 (53)	0.58	0.28-1.21	0.1456	0.67	0.39-1.16	0.1513

*significant at 0.05 level of significance

Table 5. The Difference in the Proportions of Homozygotes in Potential Risk-Bearing Alleles (A, B, F and A1) and Potential Protective Alleles) (a, b, f and A2) among the BPH and Control Subjects

Attributes	Number of homozygotes	Controls (n=79)	BPH (n=68)	p-value
Risk-bearing alleles	0	50 (0.633)	29 (0.426)	0.0123*
	1	22 (0.278)	28 (0.412)	0.0889
	0-1	72 (0.911)	57 (0.838)	0.1777
	2	6 (0.076)	10 (0.147)	0.1675
	3	1 (0.013)	1 (0.015)	0.9126
	4	0 (0.000)	0 (0.000)	N/A
	2-4	7 (0.089)	11 (0.162)	0.1777
Protective alleles	0	7 (0.089)	24 (0.353)	<0.0001*
	1	25 (0.316)	25 (0.368)	0.5135
	0-1	32 (0.405)	49 (0.721)	0.0001*
	2	34 (0.430)	13 (0.191)	0.0019*
	3	12 (0.152)	5 (0.074)	0.1387
	4	1 (0.13)	1 (0.015)	0.9126
	2-4	47 (0.595)	19 (0.280)	0.0001*

*significant at 0.05 level of significance

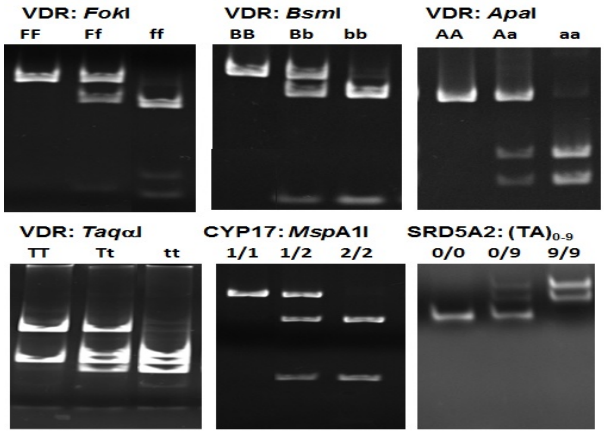

Figure 2. Polyacrylamide gels Showing the Band Patterns of the PCR Amplified DNA fragments (for SRD5A2 gene) and Restriction Endonuclease-digested PCR Amplified DNA Fragments (VDR and CYP17 genes) for the Homozygous Dominant, Homozygous Recessive and Heterozygous Genotypes

among the subjects with BPH compared to the controls (OR=2.08, 95%CI: 0.95-4.58, p=0.0689, and the corresponding RR= 1.68, 95%CI: 0.96-2.93, p=0.0708) although the difference is not statistically significant at the 0.05 level of significance.

Genetic profile

The differences in the proportions of homozygotes in the potential risk-bearing alleles (A, B, F, and A1) and the potential protective alleles (a, b, f, and A2) among the subjects with BPH and the controls are shown in Table 5. About 42.6% of the subjects with BPH compared to 63.3% of the controls are homozygous in none of the risk bearing alleles (p=0.0123). In terms of protective alleles, about 72.1% of the subjects with BPH and 40.5% of the controls were homozygous in one or fewer protective alleles (p=0.0001), whereas about 28% of the subjects with BPH and 59.5% of the controls are homozygous in two or more protective alleles (p=0.0001).

Discussion

In this study we examined the association between polymorphisms in six different markers of three different genes and BPH risk among Lebanese men. Our data showed that the ApaI A allele and BsmI B allele of the VDR gene were associated with increased risk of BPH, and the association was statistically significant. In addition, the calculated RR values showed that the FokI F allele of the VDR gene and MspA1I allele of the CYP17 gene were likely to be associated with increased risk of BPH among Lebanese men although the association was not strong enough to show statistical significance at the preselected 0.05 level of significance. We found no significant association between VDR TaqαI SNP and SDR5A2 0 or 9 repeats and BPH risk.

Lebanon is a small country with an estimated 2013 population of 4.1 million with males constituting about half of the population (CIA, 2013). We found no published reports on the occurrence or the prevalence of BPH or any other genetic or epidemiological studies on BPH in Lebanon. During our prostate disease screening campaign, we found BPH was quite common among ageing Lebanese men (data not shown). It is worth mentioning that very few studies were published earlier in the Middle East concerning the relationship between BPH risk and the polymorphism of CYP17 or SDR5A2 gene (Tigli et al., 2003; Gunes et al., 2007; Izmirli et al., 2011). However, no studies were found related to the VDR gene and its involvement in BPH risk. To the best of our knowledge, this is the first genetic epidemiological report on BPH among Lebanese men, and the first study about VDR gene and BPH risk among Middle Eastern men.

Worldwide, the studies concerning these genes involvement and the risk of BPH were also very limited in the number of published articles and the number of samples studied. We only found 4 studies related to the link between BPH risk and the BsmI of the VDR gene. Our results concerning the association of BPH with the B allele of BsmI in the VDR gene among Lebanese men were consistent with that among Japanese men (Habuchi et al., 2000a). A similar positive link was also found in USA (Mullan et al., 2006) and India (Manchanda et al., 2010), but no association was found of VDR BsmI among a small group of Thai men (Chaimuangraj et al., 2006). As for the Apa1 A allele of the VDR gene, our results showed significant association with BPH risk, but were not consistent with that of the two previous published studies on this locus conducted in Japan (Habuchi et al., 2000a) and in Thailand (Chaimuangraj et al., 2006).

The association between BPH and the TaqαI locus in the VDR gene was investigated in seven publications we are aware of. Our study showed lack of association between BPH and T or t allele of this locus. Compared to these studies, our findings were consistent with four of these results from Japan (Habuchi et al., 2000a), Thailand (Chaimuangraj et al., 2006), Netherlands (Bousema et al., 2000), and Austria (Schatzl et al., 2001), but were in contradiction with the other three studies that found association in Japan (Hamasaki et al., 2002), India (Manchanda et al., 2010), and USA (Mullan et al., 2006).

It is interesting to note that the two studies from Japan (Habuchi et al., 2000a; Hamasaki et al., 2002) revealed contradictory results. Other investigators have observed the TT genotype as a risk factor for subjects with BPH of developing PCa (Tayeb et al., 2004; Manchanda et al., 2010; Guo et al. 2012). A recent Korean study involving 47 SNPs of VDR gene indicated some VDR gene polymorphisms in Korean men are associated with prostate cancer risk and also significantly related to prostate cancer-related risk factors such as PSA level, tumor stage, and Gleason score (Oh et al., 2014).

As for the VDR FokI allele, our results did not show significant association with BPH risk. We found only two published studies on this allele; both were conducted in India. The first (Manchanda et al., 2010) did not establish link, confirming our findings, but the second (Ruan et al., 2011) found positive association. However, the second study was performed on 79 BPH cases complicated with histological prostatitis, and no one could say for sure if the conflicting outcomes of these two studies were due to the presence of prostatitis in the BPH cases studied, although it is difficult, in general, to rule out the presence of prostatitis in BPH patients.

CYP17 gene is a key enzyme in testosterone biosynthesis. We found association between BPH and this gene but the result was not statistically significant at the 0.05 level of significance. We are aware of six studies that investigated earlier this relationship, and similar to the VDR gene, these results were contradictory of each other. In particular, four of these studies from Japan (Habuchi et al., 2000b), Turkey (Gunes et al., 2007), Austria (Schatzl et al., 2001) and France (Azzouzi et al., 2002) found association with BPH. However the other two studies from China (Madigan et al., 2003) and Turkey (Tigli et al., 2003) did not show any association. Once again, the results were not only contradictory between different countries but were also within the same country as in the case of Turkey. Two previous studies found that the A2 allele of the CYP17 gene is associated with increased risk of PCa among Indian men (Sobti et al., 2009) and Iranian men (Karimpur-Zahmatkesh et al., 2013). Other studies also indicated that the same group of genetic polymorphisms in VDR genes and CYP17 gene is associated with PCa (Habuchi et al., 2000a, Habuchi et al., 2000b; Hamasaki et al., 2002).

Investigators from USA (Salam et al., 2005; Roberts et al., 2006) and France (Azzouzi et al., 2002) studied the association between BPH and the TA repeats in the SRD5A2 gene. The results of these three studies showed lack of association, confirming the results of our study, while one of the studies (Azzouzi et al., 2002) not only didn't show association but also established protective effect of the SRD5A2 gene against BPH risk. Two other studies conducted respectively in Austria (Schatzl et al., 2001) and Turkey (Izmirli et al., 2011) investigated this relationship and both found increased risk of BPH associated with the TA repeats of this gene, in contrary to our study and the previous three studies. One study revealed that other polymorphisms in SRD5A2 are associated with increased risk of PCa and BPH (Roberts et al., 2006). Another study found that TA(9) repeat is a risk factor of PCa but not BPH (Rajender et al., 2009).

As presented above, most of the studies on genetic polymorphisms of the BPH revealed contradictory results and still far from being conclusive to establish a set of markers that could be used in early diagnosis of BPH. This might be due to many factors that include but are not limited to the racial and ethnic differences (Platz et al., 2000), a possible polygenic basis for the disease, variability in penetrance, study plans, and environmental effects on BPH development (Konwar et al., 2008). If multiple genes were involved in BPH, and if some of the genes were polymorphic, a subject might have some protective and some risk-bearing alleles of the genes (Azzouzi et al., 2002); and this adds another layer of complication on the phenotypic outcomes. Therefore, the overall genotypic profile with regards to a group of genes associated with a disease could be a more accurate indicator of the disease risk. We investigated that possibility for the genes studied here. Our study showed that homozygosis in risk-bearing alleles was significantly less prevalent among the control subjects compared to the subjects with BPH. For example, compared to the 43% of the subjects with BPH, 63% of the control subjects were homozygous in none of the six risk-bearing alleles ($p=0.0123$). In addition, about 16% of the subjects with BPH compared to about 9% of the controls were homozygous in two or more risk-bearing alleles, although the difference was not significant ($p=0.1777$). In contrast, about 60% of the control subjects were homozygous in two or more of the protective alleles compared to 28% of the subjects with BPH ($p=0.0001$). Therefore, our data indicated that the overall SNP/TA profile of the genes associated with a disease could be a more useful marker for genetic diseases such as BPH.

It is accurate to say that the genetics and epidemiology of the BPH disease are not investigated broadly enough so far and more studies are needed. This may be due to the fact that the disease is not a life threatening one. However, the impact of this disease on patients and societies is great, especially in this decade as the number of ageing men is increasing worldwide. Since BPH is a treatable disease, it may be interesting to find a genetic biomarker to screen for it and to detect it early, especially in men at high risk. This can reduce the number of unnecessary biopsies done on patients to differentiate between the BPH and Pca cases, especially for those who are in the gray zone (PSA level between 4 and 10 ng/ml). Better yet is to find an effective therapy for this disease.

The strength of our study can be contributed to many factors that include the following: (1) both control and BPH groups have been selected very carefully. The control group was selected from men with normal PSA. However, we lowered the cut-off point for detecting normal PSA level from 4.0 ng/ml-which is usually used by physicians and researchers alike-to 2.5 ng/ml, because we know there is a 20% false-negative result in men with PSA < 4.0 ng/ml who may have Pca, (2) while the control group was selected from men with PSA level less than or equal to 2.5 ng/ml, many of them underwent DRE testing that showed normal prostate. They were also followed up for a minimum of 3 years with PSA screening at least once a year, and all of them had PSA level that was always in

the normal cut-off level that we selected, (3) all Pca cases were selected from men with borderline or abnormal PSA values, who had abnormal DRE test, and their cancer was confirmed by biopsy, and (4) this is the first study of its type that was done in Lebanon and the Middle East area dealing with six different markers of three different genes in one study.

It is worth mentioning that BPH and PCa are distinct in terms of histopathology and localization and, in general, BPH is not considered a risk factor for PCa (Chang et al., 2012). However, both BPH and PCa are cytoproliferative diseases and the same hormones and inflammatory processes play crucial roles in the development of both BPH and PCa (Orsted and Bojesen, 2013). Since BPH is an easily detectable disease, and PCa is an insidious disease and a silent killer, establishing specific attributes of BPH as predictive signs of PCa will have a significant clinical relevance.

Results from gene polymorphism studies are promising and since SNP is extremely common and many diseases have a polygenic basis, focused studies such as the present one can be useful in identifying the candidate genes and the important SNPs and TA repeats of the genes involved. And this may provide new genetic biomarkers the BPH. Higher number of samples should be investigated and more studies in the future need to be undertaken on diverse population and different ethnic groups.

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