Research Article

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Pharmacogenomics

Relationship between vitamin D receptor gene polymorphisms, cardiovascular risk factors and adiponectin in a healthy young population

Aim: To explore the association between *VDR* polymorphisms and several cardiovascular risk factors and adiponectin. **Materials & methods:** Three-hundred and sixty-nine healthy students were randomly selected. Five *VDR* polymorphisms were genotyped: *BsmI* rs1544410; *Cdx2* rs11568820; *ApaI* rs7975232; *TaqI* rs731236 and *FokI* rs2228570. BMI, waist circumference (WC), blood pressure, lipid/glycemic profiles and adiponectin were assessed. **Results:** In men, *BsmI*, *ApaI* and *TaqI* were associated with BMI and WC ($p < 0.05$). *FokI* was associated with triglycerides and high-density lipoprotein levels ($p = 0.0036$; $p = 0.005$) whereas *BsmI* and *Cdx2* were associated with adiponectin levels ($p = 0.026$; $p = 0.048$). Associations disappeared after BMI and WC adjustments. In women, *ApaI* was associated with systolic blood pressure ($p = 0.02$). **Conclusion:** Our study demonstrated a gender-specific difference between *VDR* SNPs and various cardiovascular risk factors and adiponectin.

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Keywords: adiponectin • cardiovascular risk factors • healthy subjects • *VDR* polymorphism • vitamin D

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Vitamin D plays a central role in a large variety of metabolic pathways. It exerts its action at a cellular level through binding of the active metabolite 1,25-dihydroxyvitamin D to the vitamin D receptor (VDR). The VDR is expressed in many different cell types such as pancreatic β cells, vascular smooth muscle cells, adipocytes, osteoblasts and chondrocytes [1,2]. It regulates more than 3% of the human genome, including genes that are crucial for glucose metabolism [3]. This finding probably explains the nonskeletal effects of vitamin D [3].

The gene encoding the VDR is located in the long arm of chromosome 12 (locus 12q12-q14) [4]. A large number of polymorphisms have been described so far in *VDR* [4,5]. Five SNPs of *VDR* named *Cdx2*, *FokI*, *BsmI*, *ApaI* and *TaqI*, have been studied and were found to be related to bone characteristics and risk of fractures, although results remain equivocal [6,7]. Beside their effects on bone metabolism, *VDR* SNPs have been associated with cardiovascular (CV) diseases [8], metabolic syndrome (MS), increased risk of Type 2 diabetes [9,10], insulin resistance [11–13], unfavorable lipid profile [11–13], increased blood pressure [14], obesity [11] and mortality [15]. However, in each of these studies, few *VDR* SNPs were analyzed in relation to CV risk factors. In addition, their relationship with adiponectin, an adipocyte specific protein with anti-atherogenic and insulin enhancer effects [16], has never been explored.

In Middle Eastern countries, more particularly in Lebanon [17,18] and Jordan [19], few studies looked at *VDR* SNPs. These studies analyzed only two or three *VDR* SNPs (*BsmI*, *TaqI* and *ApaI*) and were limited to bone density measurement.

The main objective of this study is to assess whether five different *VDR* SNPs

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(*BsmI* rs1544410; *ApaI* rs7975232 both in intron 8, *Cdx2* rs11568820; *TaqI* rs731236 in exon 9 and *FokI* rs2228570 in exon 2) are associated with 25-hydroxy-vitamin D (25[OH]D) levels, CV risk factors (mainly, body mass index [BMI], waist circumference [WC], blood pressure [BP], lipid and glycemic profiles) as well as adiponectin in a cohort of 369 young Lebanese men and women between 18 and 30 years of age.

Material & methods

Participants

Participants are students from the Saint-Joseph University Medical Sciences Campus, located in Beirut, Lebanon. This cohort has been recruited as previously published [20]. Briefly, 369 randomly selected students of both genders accepted to participate in the study. Population age ranged between 18 and 30 years. The study was approved by the University Ethical Committee (Reference number USJ 2011-13). Each participant gave a written and informed consent before enrollment. Exclusion criteria were pregnancy, use of contraceptive pills or drugs that may affect lipid profile and/or metabolic parameters. In addition, none of the students were taking vitamin D supplements.

The following anthropometric measures, performed using the same devices throughout this study, were taken by a registered nurse: height in meters (m), weight in kilograms (kg) using a manual scale and WC, taken at the umbilicus, in cm. Systolic BP (SBP) and diastolic BP (DBP) were measured in seated subjects after a rest for at least 15 min using a mercury tensiometer. BMI was calculated as weight (kg)/height (m^2).

Biological parameters

Blood was collected after a 12-h fasting period and across all seasons. In the hour following blood withdrawal, samples were centrifuged and serum was divided in several aliquots: some were stored at -80°C for later insulin, adiponectin and 25(OH)D measurements and others were sent for biochemical analysis.

Fasting insulin was measured using a commercial chemiluminescent assay (Immulite, DPC, CA, USA). The assay sensitivity for insulin was 2 mIU/ml with intra-assay CVs below 9%. Adiponectin was measured using a commercially available RIA kit (Linco Research, Inc., MO, USA). The assay sensitivity was 1 ng/ml and intraassay CV <9.5%. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA index), defined as (fasting immunoreactive insulin in mU/l × FPG in mmol/L)/22.5 [21]. The 25(OH)D was measured using the Dia Sorin radioimmunoassay (MN, USA). The assay sensitivity was 1.5 ng/ml and the CV was below 12%. Biochemical parameters (glucose, total cholesterol, triglycerides and high-density lipoprotein [HDL] cholesterol) were measured using an automated COBAS Integra 400, Roche Diagnostics. Low-density lipoprotein (LDL)-cholesterol was then calculated using the Friedwald equation.

For all biological parameters measures, at least two levels of control were added systematically to every run.

DNA isolation & genotyping

For DNA sampling, blood was collected on EDTA tubes and white cells were isolated using a lysis solution blood lymphocyte buffer containing NH_4Cl, $KHCO_3$ and EDTA, and then frozen at -80°C.

Genomic DNA was extracted from 50 µl buffy coat samples by standard salt-precipitation methods.

RFLP-PCR genotyping

PCR were performed in a final volume of 50 µl containing approximately 100 ng of DNA, 0.25 mM dNTPs, 1.5 mM $MgCl_2$, 100 ng of each primer, 1 × PCR buffer and 0.02 U TaqDNA polymerase (Invitrogen Life Technologies, CA, USA). The amplification conditions for each PCR were 95°C for 5 min, followed by 35 cycles of: 95°C for 60 s, 55°C for 60 s and 72°C for 60 s, with a postcycling final extension of 10 min at 72°C.

Primers used for the amplification of *ApaI*, *BsmI* and *Cdx2* SNPs, were designed using Primer 3 [22] and checked for specificity using Basic Local Alignment Search Tool (BLAST) [23]. DNA sequences were obtained from UCSC or Genbank databases. Primers used for the study of *Fok*I and *TaqI* SNPs were the same as those used respectively by Harris *et al.* [24] and

Table 1. Primers sequences used in PCR.

Studied polymorphisms	Forward primer	Reverse primer	Product size
BsmI (rs1544410)	5′-cctcactgcccttagctctg-3′	5′-tgcctccaaaatcaatcagg-3′	247 bp
Cdx2 (rs11568820)	5′-ggatcccaaaaggaaaggaa-3′	5′-tgttccagatggtaaaaatagaatga-3′	396 bp
ApaI (rs7975232)	5′-ggatcctaaatgcacggaga-3′	5′-acgtctgcagtgtgttggac-3′	265 bp
TaqI (rs731236)	5′-cagagcatggacagggagcaa-3′	5′-cacttcgagcacaaggggcgttagc-3′	501 bp
FokI (rs2228570)	5′-agctggccctggcactgactctggctct-3′	5′-atggaaacaccttgcttcttctccgtc-3′	267 bp

Table 2. Clinical and biological data of the overall population.

Clinical and biological data	Total (n = 369)	Men (n = 192)	Women (n = 177)	p-value
Clinical data				
Age (years)	23.9 ± 4.0	24.1 ± 4.0	23.8 ± 4.0	0.388
BMI (kg/m^2)	23.9 ± 4.2	25.6 ± 4.1	22.0 ± 3.3	<0.001*
Waist circumference (cm)	82.7 ± 12.4	89.8 ± 11.0	74.9 ± 8.4	<0.001*
Systolic blood pressure (mmHg)	110.7 ± 13.1	117.3 ± 9.8	103.4 ± 12.3	<0.001*
Diastolic blood pressure (mmHg)	70.6 ± 9.8	74.5 ± 8.8	66.4 ± 9.1	<0.001*
Biological data				
25(OH)D (ng/ml)	31.0 ± 12.5	29.2 ± 11.3	33.0 ± 13.4	0.003*
Fasting plasma glucose (mmol/L)	4.87 ± 0.3	4.9 ± 0.28	4.8 ± 0.23	<0.001*
Insulin (mlU/ml)	9.2 ± 4.9	9.7 ± 5.2	8.5 ± 4.5	0.017*
HOMA index	2.0 ± 1.1	2.1 ± 1.2	1.8 ± 1.0	0.006*
Adiponectin (μg/ml)	11.6 ± 6.3	8.2 ± 4.1	15.3 ± 6.2	<0.001*
Triglycerides (mmol/L)	1.06 ± 0.7	1.20 ± 0.85	0.89 ± 0.55	<0.001*
HDL cholesterol (mmol/L)	1.23 ± 0.3	1.13 ± 0.27	1.34 ± 0.31	<0.001*
Total cholesterol (mmol/L)	4.57 ± 1.0	4.54 ± 0.99	4.61 ± 1.09	0.461
LDL cholesterol (mmol/L)	2.87 ± 1.0	2.88 ± 0.99	2.86 ± 1.05	0.851

*Statistically significant difference between men and women ($p < 0.05$).
HDL: High-density lipoprotein; HOMA: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein.

Riggs *et al.* [25]. All primer sequences and product sizes are listed in Table 1.

The PCR products were verified using 1% agarose gel containing SYBR® Safe (Life Technologies, CA, USA). 15 μl of each PCR were digested with 7.5 unit of each restriction enzyme (New England Biolabs, Inc., MA, USA) and incubated overnight at 25°C for *ApaI*, at 37°C for *BsmI*, *HpyCH4III* (*Cdx2*) and *FokI*, and for 3 h at 65°C for *TaqI*. 20 μl of each digested reaction mixture were then loaded and separated into 2% agarose gel.

BsmI polymorphism

The GG genotype shows only two bands of 144 and 103 bp. The AA genotype displays one fragment of 247 bp. The heterozygote displays three fragments of 247, 144 and 103 bp.

Cdx2 polymorphism (*HpyCH4III*)

The GG genotype shows three bands of 265, 80 and 51 bp. The AA genotype yields two fragments of 316 and 80 bp. The heterozygote displays four fragments of 316, 265, 80 and 51 bp.

Table 3. Genotype and allele frequencies of the studied SNPs.

Gene		dbSNP	Alleles (B/b)†	Patients (n)	Subjects (n)					p-value§
					Genotype frequencies‡			Allelic frequencies		
					BB	Bb	bb	B	b	
VDR	*BsmI*	rs1544410	G/A	366	117 (32.0)	180 (49.2)	69 (18.9)	0.57	0.43	0.999
VDR	*Cdx2*	rs11568820	G/A	350	246 (70.3)	89 (25.4)	15 (4.3)	0.83	0.17	0.194
VDR	*ApaI*	rs7975232	T/G	369	128 (34.7)	178 (48.2)	63 (17.1)	0.59	0.41	0.998
VDR	*TaqI*	rs731236	T/C	369	134 (36.3)	180 (48.8)	55 (14.9)	0.61	0.39	0.949
VDR	*FokI*	rs2228570	F/f¶	369	209 (56.6)	146 (39.6)	14 (3.8)	0.76	0.24	0.161

†'B/b', 'B' represents the major allele and 'b' the minor allele.
‡The percentages (in brackets) are preceded by the number of patients in each group.
§The p-value represents the ones obtained with the χ^2 test for Hardy–Weinberg equilibrium.
¶AA, CC and GG genotypes are designed by FF; AT, CT or GT are designed by Ff and TT genotype is designed by ff.

Table 4. Associations of *VDR* gene variants with clinical and biological characteristics of patients.

Variables	Associations of *VDR* rs1544410 (*BsmI*) with clinical and biological characteristics of patients.							
	Men (genotypes)				Women (genotypes)			
	GG, n = 65	GA, n = 89	AA, n = 36	p-value	GG, n = 52	GA, n = 91	AA, n = 33	p-value
BMI (kg/m^2)	24.77 ± 3.71	25.57 ± 3.89	27.33 ± 4.82	0.010*	22.01 ± 3.76	22.01 ± 3.15	21.91 ± 3.33	0.987
Waist circumference (cm)	87.55 ± 10.03	89.99 ± 11.36	93.50 ± 11.49	0.034*	74.17 ± 9.81	75.42 ± 7.86	74.97 ± 7.63	0.696
Systolic blood pressure (mmHg)	11.60 ± 0.90	11.73 ± 1.01	11.94 ± 1.05	0.244	10.11 ± 1.02	10.45 ± 1.38	10.42 ± 1.12	0.264
Diastolic blood pressure (mmHg)	7.42 ± 0.88	7.49 ± 0.90	7.40 ± 0.83	0.829	6.59 ± 0.83	6.68 ± 0.99	6.62 ± 0.82	0.828
25(OH)D (ng/ml)	29.62 ± 11.31	29.82 ± 11.59	26.22 ± 10.12	0.241	35.21 ± 16.09	31.45 ± 11.95	33.91 ± 12.71	0.253
Fasting plasma glucose (mmol/L)	4.94 ± 0.31	4.92 ± 0.27	4.91 ± 0.27	0.847	4.83 ± 0.25	4.81 ± 0.24	4.80 ± 0.21	0.895
Insulin (mIU/ml)	8.66 ± 4.12	10.14 ± 5.68	10.66 ± 5.30	0.103	8.13 ± 3.53	8.92 ± 4.94	8.03 ± 4.57	0.473
HOMA index	1.91 ± 0.95	2.23 ± 1.30	2.34 ± 1.19	0.137	1.75 ± 0.76	1.92 ± 1.08	1.73 ± 1.07	0.519
Adiponectin (µg/ml)	9.17 ± 4.46	7.93 ± 4.12	6.94 ± 3.17	0.026*	15.72 ± 6.61	15.29 ± 6.03	14.77 ± 6.04	0.789
Triglycerides (mmol/L)	1.22 ± 1.15	1.19 ± 0.70	1.20 ± 0.53	0.980	0.88 ± 0.50	0.91 ± 0.61	0.88 ± 0.46	0.942
HDL-cholesterol (mmol/L)	1.20 ± 0.30	1.09 ± 0.25	1.11 ± 0.27	0.055	1.33 ± 0.33	1.38 ± 0.30	1.28 ± 0.28	0.245
Total cholesterol (mmol/L)	4.52 ± 0.95	4.53 ± 1.00	4.61 ± 1.05	0.898	4.66 ± 1.05	4.52 ± 1.10	4.77 ± 1.11	0.494
LDL-cholesterol (mmol/L)	2.83 ± 1.00	2.90 ± 1.01	2.93 ± 0.96	0.842	2.92 ± 1.03	2.72 ± 1.04	3.08 ± 1.01	0.199

Data is presented by mean ± standard deviation.
*Statistically significant difference for men ($p < 0.05$); BMI: GG <GA <AA; waist circumference: GG <GA <AA; adiponectin: AA <GA <GG.
HDL: High-density lipoprotein; HOMA: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein.

ApaI polymorphism

The TT genotype lacks an *ApaI* site and shows only one band of 265 bp. The GG genotype generates two fragments of 146 and 119 bp. The heterozygote displays three fragments of 265, 146 and 119 bp, designated as TG.

TaqI polymorphism

TaqI digestion revealed one obligatory restriction site, the homozygous TT (absence of the specific *TaqI* restriction site) yielded bands of 495 and 6 bp. The homozygous CC exhibited 294, 201, 6 bp and the heterozygous CT provided gave 495, 294, 201, 6 bp fragments.

FokI polymorphism

The AA, CC and GG genotypes (noted FF) lack a *FokI* site and shows only one band of 267 bp. The TT genotype (noted ff) yields two fragments of 195 and 72 bp. The heterozygote displays three fragments of 267, 195 and 72 bp corresponding to AT, CT or GT (all noted Ff).

DNA sequencing

Positive heterozygous and homozygous controls (defined by direct sequencing) and negative controls (water) were systematically included in experiments. In addition, genotypes of some randomly selected subjects were confirmed by Sanger sequencing. PCR products were purified using the Illustra® GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK), and both strands of the obtained products were sequenced using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) under standard conditions. The labeled products were subjected to electrophoresis on an ABI 3130 Genetic Analyzer sequencing system (Applied Biosystems). Electropherograms were analyzed using Sequence Analysis Software version 5.2 (Applied Biosystems) and compared with reference sequences using ChromasPro version 1.7.6.1 (Technelysium, Queensland, Australia).

Statistical analysis

Statistical analyses were performed using a software program (SPSS for Windows version 18.0, IL, USA). Descriptive statistics were used to describe clinical and biological data of the overall population. Chi-square (χ^2) test and Fisher Exact test were used to compare categorical variables among two or more groups. Variables were tested for normality distribution using the Kolmogorov–Smirnov test and equality of variance using the Levene test.

Student's *t*-tests were used to compare continuous variables between two groups. When the variables were not normally distributed, Mann–Whitney tests

were done. Analyses of variance followed by Tukey post-hoc tests were used to compare continuous variables between three or more groups. When the variables were not normally distributed, Kruskal–Wallis tests followed by Mann–Whitney tests were carried out. The associations between *VDR* SNPs and other variables were performed after adjustment for cofounding factors to ensure that these associations were not due to chance. A multivariate analysis was performed when needed, for BMI and WC adjustments.

Deviation from the Hardy–Weinberg equilibrium was tested using χ^2 analysis.

Data were considered statistically significant if p-values were <0.05.

Results

General characteristics of the studied population

Three hundred and sixty nine randomly selected students (192 men and 177 women) out of 390 accepted to participate in the study. The participants mean age was 23.9 ± 4 years with a mean BMI of 23.9 ± 4.2 kg/m^2. Table 2 summarizes the main clinical and biological data of the population. As shown, several studied values were significantly different between men and women.

Genotype & allele distribution

Results of genotyping and allele distribution are summarized in Table 3. The whole population as well as subpopulations (men and women) were in Hardy–Weinberg equilibrium for all SNPs (p-values for the whole population are presented in Table 3; p-values for subpopulations are presented in Supplementary Table 1). In addition, no statistical differences for genotype distribution between men and women were observed (Supplementary Table 1).

Relation between *VDR* genotypes, 25(OH)D and metabolic parameters

To explore the association between *VDR* SNPs and the different clinical and biological parameters, we performed the analyses separately for men and women taking into account the statistical differences obtained between the two groups (as shown in Table 1). Results are presented in Tables 4–8.

Association between *VDR* SNPs & 25(OH)D levels

Among all the studied SNPs, only *ApaI* was associated with 25(OH)D levels in both men and women. Students with the TT genotype for *VDR* T>G had significantly lower 25(OH)D levels than GG students (respective p-values for men and women are 0.019 and 0.024; Table 6).

Table 5. Associations of *VDR* gene variants with clinical and biological characteristics of patients.

Variables	Associations of *VDR* rs11568820 (*Cdx2*) with clinical and biological characteristics of patients							
	Men (genotypes)				Women (genotypes)			
	GG, n = 129	GA, n = 42	AA, n = 9	p-value	GG, n = 117	GA, n = 47	AA, n = 6	p-value
BMI (kg/m^2)	25.94 ± 4.44	25.10 ± 3.59	23.76 ± 1.40	0.207	21.81 ± 3.17	22.64 ± 3.92	20.65 ± 1.17	0.218
Waist circumference (cm)	90.53 ± 11.80	88.07 ± 10.36	87.78 ± 4.35	0.401	74.77 ± 7.73	75.96 ± 10.05	69.50 ± 2.43	0.195
Systolic blood pressure (mmHg)	11.81 ± 1.00	11.49 ± 0.95	11.33 ± 1.03	0.073	10.37 ± 1.30	10.21 ± 1.14	10.00 ± 0.63	0.621
Diastolic blood pressure (mmHg)	7.55 ± 0.91	7.20 ± 0.75	7.22 ± 0.83	0.059	6.65 ± 0.95	6.59 ± 0.82	6.50 ± 0.55	0.865
25(OH)D (ng/ml)	27.74 ± 10.65	29.00 ± 11.12	36.44 ± 11.99	0.065	31.99 ± 12.80	34.77 ± 15.41	34.00 ± 11.03	0.484
Fasting plasma glucose (mmol/L)	4.92 ± 0.27	4.94 ± 0.29	4.92 ± 0.28	0.888	4.81 ± 0.23	4.86 ± 0.23	4.77 ± 0.23	0.327
Insulin (mIU/ml)	10.19 ± 5.57	8.88 ± 4.04	9.01 ± 3.64	0.325	8.83 ± 4.83	8.41 ± 3.67	6.67 ± 3.14	0.480
HOMA index	2.24 ± 1.26	1.96 ± 0.90	1.99 ± 0.84	0.359	1.89 ± 1.07	1.83 ± 0.83	1.41 ± 0.65	0.493
Adiponectin (µg/ml)	7.70 ± 4.12	9.51 ± 4.22	8.58 ± 3.85	0.048*	15.59 ± 6.08	14.64 ± 6.45	19.83 ± 6.09	0.147
Triglycerides (mmol/L)	1.19 ± 0.65	1.07 ± 0.59	1.30 ± 0.52	0.498	0.89 ± 0.54	0.99 ± 0.62	0.63 ± 0.09	0.266
HDL-cholesterol (mmol/L)	1.11 ± 0.25	1.20 ± 0.30	1.16 ± 0.32	0.144	1.37 ± 0.33	1.29 ± 0.27	1.46 ± 0.15	0.232
Total cholesterol (mmol/L)	4.53 ± 1.02	4.59 ± 0.81	4.19 ± 1.40	0.550	4.51 ± 1.04	5.05 ± 1.16	4.10 ± 0.56	0.007**
LDL-cholesterol (mmol/L)	2.86 ± 1.01	3.01 ± 0.86	2.42 ± 1.34	0.044*	2.74 ± 0.97	3.30 ± 1.19	2.34 ± 0.50	0.003**

*Statistically significant difference for men ($p < 0.05$); adiponectin: GG <AA <GA; LDL-cholesterol: AA <GG <GA.
**Statistically significant difference for women ($p < 0.05$); total cholesterol: AA <GG <GA; LDL-cholesterol: AA <GG <GA.
HDL: High-density lipoprotein; HOMA: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein.

Table 6. Associations of *VDR* gene variants with clinical and biological characteristics of patients.

Variables	Associations of *VDR* rs7975232 (*ApaI*) with clinical and biological characteristics of patients							
	Men (genotypes)				Women (genotypes)			
	TT, n = 64	TG, n = 94	GG, n = 34	p-value	TT, n = 64	TG, n = 84	GG, n = 29	p-value
BMI (kg/m^2)	26.57 ± 4.58	25.59 ± 4.00	23.94 ± 2.58	0.009*	22.10 ± 3.08	21.69 ± 3.13	22.61 ± 4.40	0.422
Waist circumference (cm)	92.36 ± 12.25	89.65 ± 11.00	85.53 ± 6.77	0.013*	75.89 ± 7.67	74.18 ± 7.67	74.97 ± 11.53	0.475
Systolic blood pressure (mmHg)	11.88 ± 1.02	11.62 ± 0.97	11.74 ± 0.94	0.262	10.64 ± 1.34	10.27 ± 1.19	9.90 ± 0.97	0.020**
Diastolic blood pressure (mmHg)	7.46 ± 0.90	7.42 ± 0.89	7.51 ± 0.81	0.860	6.83 ± 0.95	6.55 ± 0.87	6.48 ± 0.87	0.105
25(OH)D (ng/ml)	26.16 ± 9.61	31.30 ± 11.92	29.03 ± 11.76	0.019*	34.02 ± 13.19	30.54 ± 11.63	38.10 ± 17.12	0.024**
Fasting plasma glucose (mmol/L)	4.94 ± 0.29	4.92 ± 0.28	4.94 ± 0.28	0.875	4.83 ± 0.24	4.80 ± 0.22	4.80 ± 0.26	0.715
Insulin (mIU/ml)	10.51 ± 5.26	9.64 ± 5.54	8.56 ± 3.47	0.198	8.91 ± 5.18	8.19 ± 4.10	8.68 ± 3.94	0.615
HOMA index	2.32 ± 1.17	2.13 ± 1.29	1.88 ± 0.77	0.215	1.92 ± 1.12	1.76 ± 0.94	1.86 ± 0.84	0.645
Adiponectin (µg/ml)	7.21 ± 3.74	8.41 ± 4.17	9.24 ± 4.42	0.046*	14.24 ± 6.33	15.88 ± 5.88	15.88 ± 6.63	0.240
Triglycerides (mmol/L)	1.08 ± 0.47	1.33 ± 1.07	1.10 ± 0.68	0.142	0.99 ± 0.71	0.81 ± 0.35	0.93 ± 0.59	0.137
HDL-cholesterol (mmol/L)	1.11 ± 0.25	1.13 ± 0.28	1.19 ± 0.29	0.362	1.34 ± 0.32	1.35 ± 0.31	1.34 ± 0.31	0.970
Total cholesterol (mmol/L)	4.61 ± 1.12	4.53 ± 0.92	4.42 ± 0.91	0.655	4.79 ± 1.16	4.44 ± 0.99	4.75 ± 1.13	0.125
LDL-cholesterol (mmol/L)	2.99 ± 1.11	2.84 ± 0.88	2.78 ± 1.07	0.506	3.00 ± 1.07	2.71 ± 0.99	2.99 ± 1.14	0.190

*Statistically significant difference for men ($p < 0.05$); BMI: GG <TG <TT; waist circumference: GG <TG <TT; 25(OH)D: TT <GG <GT; adiponectin: TT <GT <GG.
**Statistically significant difference for women ($p < 0.05$); systolic blood pressure: GG < TG <TT; 25(OH)D: TG <TT <GG.
HDL: High-density lipoprotein; HOMA: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein.

Association between *VDR* SNPs & BP

In women, higher SBP was noted with the TT genotype for *ApaI* (T>G) (Table 6). No significant differences were observed in men.

Association between *VDR* SNPs & metabolic parameters

Association with BMI & WC

In men, students carrying the AA genotype for *BsmI* (G>A), TT genotype for *ApaI* (T>G) and CC genotype for *TaqI* (T>C) had higher BMI and WC compared with those with other genotypes (for BMI, respective p-values for *BsmI*, *ApaI* and *TaqI* are 0.01, 0.009 and 0.003; for WC, respective p-values are 0.034, 0.013 and 0.009; Tables 4, 6 & 7). No significant associations were observed in women.

Association with lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol & triglycerides)

In both men and women, *VDR FokI* and *Cdx2* were associated with the lipid profile. Among men, FF carriers of the *FokI* (F>f) SNP had significantly higher triglycerides and lower HDL-cholesterol levels than Ff or ff carriers ($p = 0.036$ and 0.005 respectively; Table 8). In women, students carrying at least one f allele had significantly higher cholesterol levels (total cholesterol and LDL-cholesterol) than FF carriers ($p = 0.028$ and 0.033 respectively). On the other hand, for the *Cdx2* (G>A) SNP, male and female students with at least one G allele (GG or GA) had significantly higher LDL-cholesterol compared with the AA students (respectively $p = 0.044$ and $p = 0.003$); while for the total cholesterol, this difference was only significant in women ($p = 0.007$) (Table 5).

Association with glycemic profile (fasting plasma glucose, insulin, HOMA index)

Among the different studied SNPs, only the *VDR TaqI* (T>C) was associated with insulin levels: male students carrying the CC genotype had significantly higher insulin levels than the other groups ($p = 0.046$; Table 7). This association disappeared after adjustment for both BMI and WC ($p = 0.52$). None of the studied SNPs was related to fasting plasma glucose or HOMA index in both men and women.

Association with adiponectin

In men, both *VDR BsmI* (G>A) and *Cdx2* (G>A) were associated with adiponectin levels: students with the AA genotype for *BsmI* and GG for *Cdx2* had significantly lower adiponectin levels than other genotypes ($p = 0.026$ and 0.048 respectively; Tables 4 & 5). These associations disappeared after adjustment for both BMI and WC (respectively $p = 0.08$ and $p = 0.35$ for *BsmI* and *Cdx2*).

Discussion

This study assessed, in a Middle-Eastern cohort of young men and women aged 18–30 years, whether five *VDR* SNPs (*BsmI*, *Cdx2*, *ApaI*, *TaqI*, *FokI*) are associated with 25(OH)D levels, CV risk factors and adiponectin.

Our results showed that the *ApaI* (T>G) SNP was associated with 25(OH)D levels in both male and female students; subjects with the TT genotype showing significantly lower levels than those with the GG genotype. The relationship between *VDR* SNPs and bone biology has been largely studied in populations of different ethnic origins but studies were mainly performed on adults and in relation with bone density. The few studies that have been conducted in children and young people to investigate the influence of *VDR* SNPs on vitamin D levels have reported divergent results. Santos *et al.* [26] studied a group of Brazilian girls aged 7–18 years and found that *VDR* wild-type genotypes for *BsmI*, *ApaI* and *TaqI* were significantly associated with lower 25(OH)D levels. Other authors did not find any association [27,28]. More studies are required to elucidate the contribution of *VDR* genetic SNPs on serum 25(OH)D in healthy subjects. The exact molecular mechanism explaining the association between *VDR* polymorphisms and 25(OH)D serum levels remains unknown. Levin *et al.* [15] speculate that greater VDR activity for a given amount of 25(OH)D could provide protection in situations of low 25(OH)D substrate.

We then found an association between *ApaI* (T>G) genotype and BP, female students with TT genotype having higher levels of SBP. These results are in line with the one reported for the *BsmI VDR* SNP in Korean workers [29]. In another study, an opposite relationship was shown [14] with the same SNP in healthy men and women. Finally, a recent large genetic study failed to reproduce any association between *VDR* related SNPs and BP [30], suggesting that further research is needed to elucidate these findings. Interestingly, in our study, the *ApaI* TT genotype group, who has higher BP levels, shows also significantly lower 25(OH)D levels than the TG or GG groups, suggesting a clear link between vitamin D and BP. Our findings may suggest that, at least in women, a common genetic polymorphism may predispose to both vitamin D deficiency and high BP. The link between vitamin D and hypertension may be explained through the calcium metabolism. In fact, it has been shown that calcium supplementation [31–33] and high vitamin D intake are associated with lower BP [34]. This link may also be explained through the renin-angiotensin system since 25(OH)D and the *FokI* SNP were independently associated with lower plasma renin activity in both hyper- and normo-tensive patients [35].

Table 7. Associations of *VDR* gene variants with clinical and biological characteristics of patients.

Variables	Associations of *VDR* rs731236 (*TaqI*) with clinical and biological characteristics of patients.						
	Men (genotypes)				Women (genotypes)		
	TT, n = 75	TC, n = 88	CC, n = 29	p-value	TT, n = 59	TC, n = 92	CC, n = 26	p-value
BMI (kg/m^2)	24.70 ± 3.52	25.71 ± 3.89	27.74 ± 5.20	0.003*	22.01 ± 3.58	22.00 ± 3.13	21.92 ± 3.67	0.992
Waist circumference (cm)	87.47 ± 9.57	90.20 ± 11.33	94.76 ± 12.22	0.009*	74.44 ± 9.40	75.24 ± 7.91	74.92 ± 7.98	0.850
Systolic blood pressure (mmHg)	11.65 ± 0.86	11.71 ± 1.02	12.00 ± 1.14	0.251	10.18 ± 1.06	10.39 ± 1.35	10.58 ± 1.17	0.353
Diastolic blood pressure (mmHg)	7.41 ± 0.82	7.48 ± 0.94	7.47 ± 0.86	0.894	6.58 ± 0.82	6.64 ± 0.99	6.75 ± 0.82	0.743
25(OH)D (ng/ml)	29.41 ± 10.81	29.95 ± 12.14	26.24 ± 10.02	0.305	35.07 ± 15.48	31.52 ± 12.01	33.77 ± 13.00	0.274
Fasting plasma glucose (mmol/L)	4.94 ± 0.31	4.92 ± 0.27	4.92 ± 0.28	0.826	4.82 ± 0.24	4.81 ± 0.23	4.80 ± 0.23	0.894
Insulin (mIU/ml)	8.71 ± 4.10	10.11 ± 5.72	11.30 ± 5.45	0.046*	7.92 ± 3.47	9.05 ± 4.94	8.12 ± 4.76	0.282
HOMA index	1.93 ± 0.95	2.23 ± 1.31	2.48 ± 1.22	0.067	1.70 ± 0.75	1.94 ± 1.09	1.75 ± 1.12	0.310
Adiponectin (µg/ml)	8.89 ± 4.25	7.90 ± 4.26	7.04 ± 2.97	0.088	15.26 ± 6.64	15.37 ± 5.90	15.03 ± 6.33	0.970
Triglycerides (mmol/L)	1.24 ± 1.10	1.20 ± 0.68	1.15 ± 0.52	0.882	0.88 ± 0.49	0.91 ± 0.62	0.87 ± 0.43	0.907
HDL-cholesterol (mmol/L)	1.18 ± 0.30	1.11 ± 0.26	1.06 ± 0.19	0.081	1.34 ± 0.32	1.36 ± 0.30	1.29 ± 0.31	0.610
Total cholesterol (mmol/L)	4.47 ± 0.85	4.63 ± 1.09	4.45 ± 0.99	0.525	4.70 ± 1.06	4.52 ± 1.08	4.77 ± 1.16	0.473
LDL-cholesterol (mmol/L)	2.78 ± 0.90	2.98 ± 1.08	2.85 ± 0.93	0.440	2.95 ± 1.04	2.74 ± 1.04	3.07 ± 1.08	0.261

*Statistically significant difference for men ($p < 0.05$); BMI: TT = CT <CC; waist circumference: TT <CT <CC; insulin: TT <CT <CC.
HDL: High-density lipoprotein; HOMA: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein.

Table 8. Associations of *VDR* gene variants with clinical and biological characteristics of patients.

Variables	Associations of *VDR* rs2228570 (*FokI*) with clinical and biological characteristics of patients.							
	Men (genotypes)				Women (genotypes)			
	FF, n = 108	Ff, n = 74	Ff, n = 10	p-value	FF, n = 101	Ff, n = 72	ff, n = 4	p-value
BMI (kg/m^2)	25.67 ± 4.12	25.38 ± 3.87	26.90 ± 5.36	0.536	21.80 ± 3.10	22.25 ± 3.70	22.08 ± 3.13	0.686
Waist circumference (cm)	89.77 ± 11.03	89.58 ± 10.58	92.20 ± 14.98	0.780	74.81 ± 7.91	75.25 ± 9.20	72.25 ± 6.24	0.768
Systolic blood pressure (mmHg)	11.75 ± 0.98	11.72 ± 0.99	11.60 ± 1.08	0.903	10.33 ± 1.27	10.41 ± 1.18	9.50 ± 1.29	0.355
Diastolic blood pressure (mmHg)	7.43 ± 0.94	7.49 ± 0.83	7.35 ± 0.58	0.835	6.65 ± 0.95	6.64 ± 0.84	6.25 ± 1.26	0.686
25(OH)D (ng/ml)	28.79 ± 11.17	29.78 ± 12.08	29.00 ± 7.63	0.844	33.44 ± 13.98	32.72 ± 12.74	28.50 ± 13.48	0.748
Fasting plasma glucose (mmol/L)	4.92 ± 0.26	4.94 ± 0.32	4.96 ± 0.34	0.828	4.82 ± 0.24	4.79 ± 0.22	4.88 ± 0.37	0.549
Insulin (mIU/ml)	9.73 ± 5.37	9.82 ± 4.94	9.25 ± 4.81	0.947	8.30 ± 4.18	8.93 ± 4.95	7.35 ± 2.92	0.574
HOMA index	2.15 ± 1.24	2.16 ± 1.12	2.03 ± 1.04	0.947	1.80 ± 0.98	1.90 ± 1.04	1.60 ± 0.68	0.718
Adiponectin (µg/ml)	8.14 ± 3.84	8.41 ± 4.52	6.43 ± 4.02	0.364	15.62 ± 6.25	14.85 ± 6.06	14.49 ± 8.00	0.699
Triglycerides (mmol/L)	1.26 ± 0.65	1.15 ± 1.11	0.97 ± 0.41	0.036*	0.90 ± 0.61	0.88 ± 0.47	0.86 ± 0.29	0.971
HDL-cholesterol (mmol/L)	1.07 ± 0.24	1.21 ± 0.30	1.18 ± 0.29	0.005*	1.36 ± 0.29	1.32 ± 0.33	1.46 ± 0.17	0.597
Total cholesterol (mmol/L)	4.55 ± 0.95	4.52 ± 1.04	4.57 ± 1.15	0.983	4.45 ± 1.06	4.84 ± 1.10	4.89 ± 1.00	0.028**
LDL-cholesterol (mmol/L)	2.93 ± 0.96	2.79 ± 1.02	2.93 ± 1.24	0.629	2.68 ± 0.99	3.10 ± 1.08	3.02 ± 1.18	0.033**

AA, CC and GG genotypes are designed by FF; AT, CT or GT are designed by Ff and TT genotype is designed by ff.
*Statistically significant difference for men ($p < 0.05$); triglycerides: ff <Ff <FF; HDL-cholesterol: FF <Ff = ff.
**Statistically significant difference for women ($p < 0.05$); total cholesterol: FF <Ff = ff; LDL-cholesterol: FF<Ff = ff.
HDL: High-density lipoprotein; HOMA: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein.

In addition, our results showed that the *BsmI* (G>A), *ApaI* (T>G) and *TaqI* (T>C) SNPs influenced BMI and WC in male students. These results suggest a strong genetic link between vitamin D and adiposity and are in agreement with two previously published studies [11,36]. Both studies – the first one, which was performed on 176 healthy Polish men [11] and the second one on 570 Saudi subjects [36] – showed that the presence of the minor allele A of *BsmI* is positively associated with obesity. A third study highlighted that healthy women with AA genotype (for *BsmI*) were characterized by higher body weight and fat mass compared with those carrying the GG genotype [37].

Importantly, we identified a relationship between *VDR* SNPs (*FokI*; F>f and *Cdx2*; G>A) and lipid profile. In men, FF carriers for the *FokI* SNP displayed significantly higher triglycerides and lower HDL-levels than Ff or ff carriers; whereas, women carrying at least one f allele had significantly higher total and LDL-cholesterol. Our results are in line with two previously reported studies. The first one showed, in 176 healthy men, that FF carriers for *FokI* SNP had significantly lower HDL-cholesterol levels compared with the other groups [11]. While the second one showed that the *FokI* variant was associated with cholesterol levels in subjects with ischemic stroke [38]. Other studies showed opposite results [13] or no associations with the *FokI* SNP [39]. As for *Cdx2* SNP, we found that both male and female students with at least one G allele had significantly higher LDL-cholesterol levels than AA students, a finding that has never been reported before. Several hypotheses could explain how the vitamin D-VDR axis affects lipid profile; vitamin D induces suppression of parathyroid hormone secretion which in turn reduces lipolysis [40]. In addition, vitamin D by increasing intestinal calcium absorption can lower hepatic triglycerides synthesis [41]. Finally, vitamin D may indirectly affect the lipid metabolism through improvement of insulin secretion and sensitivity [42].

The relation between VDR and glucose metabolism has been the subject of several publications. Some allelic variations in genes involved in vitamin D metabolism and VDR are associated with glucose (in)tolerance, insulin secretion and sensitivity [11–13,43]. In our study, no association was found between fasting plasma glucose or HOMA index and the *VDR* SNPs. Our results are in line with two other studies [13,44]. The absence of an obvious link with the glycemic components could be explained by the fact that our population is young and healthy.

The major new finding in our research is the association we found between two *VDR* SNPs (*BsmI* G>A and *Cdx2* G>A) and adiponectin levels in male stu-

dents. However, after adjustment for BMI and WC, these associations disappeared. To the best of our knowledge, this is the first study looking at the association between adiponectin and *VDR* SNPs in a healthy population. Such association has only been previously described in women with polycystic ovary syndrome [45,46], but not in normal healthy subjects. One hypothesis that could explain such relationship is that 1,25-dihydroxyvitamin D regulates the expression of adipokine in visceral fat, suggesting that vitamin D may upregulate the adiponectin gene [20]. Further studies are needed to clarify this association.

It is noteworthy to add that, in our study, the genetic associations between *VDR* SNPs was gender specific, some differences being noted only in the male population, whereas others were obvious in both men and women. This could be due to sex-dependent regulating factors such as estrogen. In fact, cross-talks between estrogen and vitamin D endocrine system are well known. Estrogen has been shown to upregulate VDR expression in the duodenal mucosa and concurrently, it increases the responsiveness to endogenous 1,25-dihydroxyvitamin D [47]. Another explanation could be the less favorable metabolic profile of our male subjects as compared with females.

Study strengths & limitations

We noted that when we compared our results to the literature, we faced some difficulties because of differences in the adopted genetic nomenclature and because of the lack of mention of the SNP ID references (or 'rs' ID) in most of the previously published studies. The strength of our study is that it includes the international updated SNP nomenclature, mentioning each time the ancestral and the mutated allele based on the NCBI database [48]. In addition, we performed statistical analysis after adjustment for confounding factors, to ensure that these associations were not due to chance. However, some of the negative findings may be due to other confounders such as lifestyle (dietary, smoking, alcohol intake and exercise) that have not been evaluated in the present study. Another point that deserves to be pointed out in this article, is that most of the studied SNPs are nonfunctional, which probably explains the conflicting results in the literature. Therefore, it is assumed that one or more of these SNPs might be in linkage-disequilibrium with one or more truly functional SNPs affecting vitamin D pathways.

Conclusion

In conclusion, our study is the first to look at a large panel of *VDR* SNPs in a relatively large cohort of young and healthy men and women. We identified several interesting associations between the studied *VDR* SNPs and various metabolic parameters. These associations were gender-specific, the association with BP being predominant in women, while the association with the major MS parameters such as abdominal obesity, high triglycerides, low HDL-cholesterol and adiponectin were mainly observed in men. Since the allelic frequencies in our population were similar to those described in Caucasian populations (European HapMap control population, n = 113 [48]), we assume that the observed associations in our population can be extrapolated to other populations. As far as public health is concerned, it would be interesting to follow these young subjects over time in order to assess, relying on genotype, the effects of vitamin D supplementation or lifestyle on the development of CV risk factors and subsequently on CV diseases.

Executive summary

- Numerous studies have reported significant associations between *VDR* polymorphisms and cardiovascular (CV) risk factors but none of them has combined five different polymorphisms in relation with these risk factors and adiponectin in a young healthy population.
- Our study assessed the association between *VDR* polymorphisms (*BsmI*, *Cdx2, ApaI, TaqI and FokI*) and several CV risk factors (BMI, waist circumference [WC], blood pressure, lipid and glycemic profiles) and adiponectin in a randomly selected cohort of 369 young Lebanese students.
- Our results showed that, in men, compared with other genotypes, the AA genotype for *BsmI*, TT genotype for *ApaI* and CC genotype for *TaqI* are associated with higher BMI and WC ($p < 0.05$ for all comparisons).
- In men, FF carriers of the *FokI* (F>f) have higher triglycerides and lower HDL levels ($p = 0.0036$ and $p = 0.005$) whereas the AA genotype for *BsmI* (G>A) and GG for *Cdx2* (G>A) are associated with lower adiponectin levels ($p = 0.026$ and $p = 0.048$). However, associations disappeared after BMI and WC adjustments.
- In women, the TT genotype for *ApaI* (T>G) is associated with higher systolic BP ($p = 0.02$).
- In both men and women, the presence of at least one G allele for *Cdx2* SNP is associated with higher low-density lipoprotein cholesterol ($p = 0.044$ in men and $p = 0.003$ in women).
- None of the studied SNPs is related to fasting plasma glucose or homeostasis model assessment index.
- Our study demonstrated a gender-specific difference in the relation between *VDR* SNPs and various CV risk factors and adiponectin. Further studies are needed to confirm these findings.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at:/www.futuremedicine.com/doi/full/10.2217/pgs-2016-0045

Author contributions

A Hajj participated to the data analysis and was the main author contributing to the manuscript writing. R Chedid performed the sampling of the students. E Chouery and A Megarbané performed the genetic part of the study. M-H Gannagé-Yared designed the study, performed the laboratory analysis, collected the data and participated with A Hajj in the manuscript writing. All the authors approved the final version of the manuscript

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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