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Relationship between Vitamin D Receptor (VDR) Gene Polymorphisms and susceptibility to Type 2 Diabetes Mellitus in Moroccans population

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ABSTRACT: Vitamin D receptor (VDR) gene is recognized as candidate gene for susceptibility to Type 2 diabetes mellitus (T2DM). The goal of this study was to search the eventual association between VDR gene polymorphisms and T2DM in Moroccans patients. *Materials and Methods*: 176 patients with T2DM and 177 Healthy controls from the Moroccans population were recruited. ***Bsm*-I**(rs1544410), ***Fok*-I**(rs10735810) and ***Apa*-I**(rs7975232) SNPs of the VDR gene were determined PCR-RFLP. A Vitamin D level was determined using ELISA. *Results*: The prevalence of Vitamin D inadequancy is higher significantly in patients with T2DM than in the controls subjects. There was a strong association between fok-I polymorphisms with T2DM (OR = 0,35, 95% CI = 0.14–0.83, ***P*** = 0.018). The Fok-I polymorphism was associated significantly with increased levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides (all P values <0.05). We found significantly elevated systolic blood pressure (P = 0.042) in association with *Apa1. Conclusions*: Our study indicated that Vitamin D Deficiency was prevalent in Moroccans Patients with T2DM, and some variants of the VDR polymorphism seems to be associated with certain mechanisms that improve the lipid profile, and accordingly, reduce the risk of cardiovascular disease. However, the Fok-I f allele seems to have a protective effect against diabetes.

KEYWORDS: Diabete type 2, polymorphism, VDR, vitamin D receptor *Bsm*-I, *Fok*-I, *Apa*-I.

1 INTRODUCTION

Insulin Resistance and altered insulin secretion are the main characteristics 2 Diabetes Mellitus (T2DM). T2DM is a multifactorial disease, influenced by both genetic and environmental factors. In Morocco, the prevalence of diabetes has increased dramatically with the adoption of a new lifestyle of over nutrition and reduced physical activity [1]. T2DM is associated with a high risk of reduce in Vitamin level D [2]. This deficiency is considered now a public health because it has been associated with several risks of many pathogenesis such as diabetes, cardiovascular disease and cancer [3], [4]. The results of several studies show that vitamin D could play a major role in the onset of diabetes T2DM. Epidemiological data showed a reduction in vitamin D deficiency in a population at risk for T2DM compared with subjects not at risk [5]. Vitamin D primarily known to be involved in phospho-calcium homeostasis also regulates growth and differentiation of diverse types of cells through specific receptor [6]. Vitamin D and its metabolites could inhibit T-cell proliferation and suppress production of

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interleukin 1, interleukin 2, tumor necrosis factor-α and interferon-β [7], [8]. In the nonobese diabetic mouse model for insulin-dependent diabetes mellitus, vitamin D is necessary for normal insulin release and maintenance of glucose tolerance [9]. Vitamin D is activated after binding to it specific cytosolic-nuclear Vitamin D Receptor Vitamin D (VDR) [10], [11]. VDR is a member of Steroid-Throid Hormone Receptor family. The VDR gene as a important candidate gene for T2DM, because vitamin D exerts its effects through the VDR. Genetic alterations of the *VDR* gene may lead to defects in gene activation or changes in the protein structure of the *VDR*, both of which could affect the cellular functions of vitamin D. The gene of VDR is located on Chromosome 12q12-q14, and common polymorphisms have been identified namely *BsmI* (rs1544410), *Fok-I* (rs10735810), and *Apa-I* (rs7975232) [12]. The objective of this study was to investigate the eventual association between VDR Gene Polymorphism and T2DM in Moroccan population and evaluate its interaction with anthropometrical and biochemical parameters.

2 MATERIALS AND METHODS

2.1 STUDY POPULATION

This study concerned 353 Moroccan adult volunteers, 177 patients diagnosed as T2DM by medical corps according to International Diabetes Federation and 176 sex-matched healthy controls. The volunteers were recruited in the Pasteur Institute of Morocco in Casablanca and informed about the aim and method of the study. A questionnaire was performed including lifestyle, social demographics information's, past medical history, geographical origin, family history, the presence of hypertension, diabetes, and hyper-cholesterolemia. The subjects with co-morbidities were excluded from this study. The anthropometrics measurements age, gender, weight, height, waist and hip-circumference, body mass index and diastolic and systolic blood pressure were taken for all volunteers. All participants in this study were unrelated individuals of similar ethnicity from different geographic and ethnic backgrounds in Morocco.

2.2 ETHICS STATEMENT

All participants provided informed consent before participation in this study. The institutional committee of ethical research of Pasteur Institute of Morocco approved this study.

2.3 BIOCHEMICALS ANALYSIS

The blood was collected in EDTA tubes and storedin a – 20 °C freezer prior to analysis. Fasting glucose plasma (FGP), Cholesterol Total (TC), triglyceride (TG) and High-Density Cholesterol (HDL-C) levels were performed using the Vitros 5.1 (F.S CHEMISTERY SYSTEM). Low-density Cholesterol (LDL-C) was determined using the Friewalds formula. was calculated according to the Friedwald's formula. 25(OH)D level was measured by enzyme linked immunosrbent assays (ELISA) (EUROIMMUM, Lubeck, Seekamp). The intra-assay variation was 2.4- 4.4% and inter assay was < 21 % Vitamin D status was classified as recommendations. A 25(OH)D level of < 20 ng/mL as deficiency, a level of 20–30 ng/mL as insufficiency, and a level of > 30 ng/mL as normal.

2.4 ISOLATION OF DNA

Genomic DNA was extracted from peripheral blood leucocytes using the salting-out method. DNA quality was determined using 1 % agarose gel electrophoresis followed by staining with ethidium bromide. Purity of DNA was determined by taking the optical density of the samples at 260 nm and 280 nm using the Nanodrop Analyzer spectrophotometer.

2.5 VDR GENOTYPING

PCR and RFLP were performed for genotyping of SNPs: *Fok-I* (rs10735810), *Bsm-I* (rs1544410), *Apa-I* (rs7975232) of VDR gene. Biometra thermal cycler and Taq Polymerase were used to perform all reactions PCR.

***Fok1* polymorphism**

Genomic DNA was amplified in a total volume of 10 µL containing approximately 50 ng of genomic DNA, 200 µMOL/L of DNTPs, 10 Pmol of each Primers, 1,5 MMOL/L of mgcl2, o,5 U Taq Polymerase and 1 µl of 10× PCR Buffer. A fragment of 270 bp including the *Fok-I* (rs10735810) polymorphism was amplified using two oligonucleotides:

Forward: 5- AGCTGGCCCTGGCACTGACTCTGGCTCT-3,

Reverse: 5- ATGGAAACACCTTGCTTCTTCTCCCTC -3.

The conditions of the PCR starts an initial denaturing at 94°C for 5 minutes, followed by 35 cycles of 94°C for 40 second, 61°C for 40 second, 72°C for 50 second, and a final extension of 72°C for 7 minutes. The PCR products were digested for 1 hour at 37°C with 3Unit of *Fok-I* restriction enzyme (*Biolabs NEW ENGLAND, R0109S*), then the PCR products of digestion were elecrophoresed on a 3% of Agarose (UltraPure Agarose, invitrogen) with bromide ethidium and visualized under UV Illumination and photographed. The wild type homozygote (FF), heterozygote (Ff) and mutant homozygote (ff) showd one band (270BP), three banbs (270, 210, 60) and tow bands (210 and 60), respectively, because the substitution create a *Fok-I* Recognition Sequence which digest the 270 bp into 210 and 60 bp.

Bsm-I Polymorphism

Genotyping for *Bsm-I* (rs1544410) was performed with the following primers:

Forward: 5- CAACCAAGACTACAAGTACCGCGTCAGTGA-3,

Reverse 5-AACCAGCGGGAAGAGGTCAAGGG -3.

A 820 bp fragment VDR gene was amplified using the PCR under reactions conditions identical to those used for the *Fok-I* polymorphism. The PCR products were digested with Bsm-I restriction enzyme (*Biolabs NEW ENGLAND, R0134S*), for one hour at 65°C. The digest fragment were seprated in a 3% of Agarose (UltraPure Agarose, invitrogen) with bromide ethidium and visualized under UV Illumination and photographed. The wild type homozygote (BB), heterozygote (Bb) and mutant homozygote (bb) showd one band (820bp), three banbs (820, 650, 170) and tow bands (650 and 170), respectively, because the substitution create a *Bsm-I* Recognition Sequence which digest the 820 bp into 650 and 170 bp.

***Apa-I* polymorphism**

Genotyping for *Apa-I (rs7975232)* was performed with the following primers:

Forward: 5- CAGAGCATGGACAGG GAGCAA-3,

Reverse 5- GCAACTCCTCATGGCTGAGGTCTC -3.

A 2000 bp fragment VDR gene was amplified using the PCR under reactions conditions identical to those used for the *Fok-I* polymorphism. The PCR products were digested with *Apa-I* restriction enzyme *(Promega, REF R6361,USA)* for one hour at 65°C. The digest fragment were seprated in a 3% of Agarose (UltraPure Agarose, invitrogen) with bromide ethidium and visualized under UV Illumination and photographed. The wild type homozygote (AA), heterozygote (Aa) and mutant homozygote (aa) showd one band (2000bp), three banbs (2000, 1700, 300) and tow bands (1700 and 300), respectively, because the substitution create a *Apa-I* Recognition Sequence which digest the 2000 bp into 1700 and 300 bp.

All genetics and molecular analyses were performed in the Molecular and genetic laboratory in Pasteur institute of Morocco.

2.6 STATISTICAL ANALYSIS

The Biochemical and Anthropometrical parameters were given as means and standard deviations in the tables. The student test was applied to compare quantitative values that follow a Normal Distribution. Otherwise, the Manne-whithney test-test is used. Chi-square and logistic regression analysis were performed to evaluate the association between T2DM and genotypes and haplotypes VDR. Analysis of the regression logistic was adjusted by gender and age. The P-Value of less than o.o5 was considered statistically significantly. All analysis statistic were performed using STATA software, version 11.00. A P-Values were automatically corrected using the bonfferoni correction by multiplying with the comparisons number. For all Haplotypes frequencies, estimation and comparison, we used P-LINK SOFTWARE, VERSION 1-07. The frequencies less than 5% of all haplotypes were not considered in this analysis. Linkage Disequilibrium between each pair of VDR gene polymorphism was estimated using HAPLO-VIEW Software Version 4-2.

3 RESULTS

3.1 PATIENTS AND CONTROLS CHARACTERISTICS

Table 1. Characteristics of the studied groups.

	Controls (n=177)	**Patients (n= 176)**	**P-value**
Age (years)	56.94±11.47	57.01±11.46	0.94
BMI (kg/m2)	28.51±4.25	28.54±4.24	0.94
WC (cm)	100.79±8.83	98.44±18.12	0.84
HC (cm)	108.42±10.21	108.26±10.18	0.88
Sbp (mmgHg)	73.21±4.14	83.82±8.93	**<0.001**
Dbp (mmgHg)	127.92±6.65	146.26±15.52	**<0.001**
FGP (g/L)	0.87±0.08	1.52±0.31	**<0.001**
TC (g/L)	1.75±0.13	2.07±0.33	**<0.001**
TG (g/L)	0.95±0.36	1.37±0.75	**<0.001**
LDL-C (g/L)	1.13±0.11	1.40±0.41	**<0.001**
HDL-C (g/L)	0.45±0.07	0.43±0.08	0.08
Vitamin D level (ng/ml)	30.28±13.05	26.07±13.03	**<0.001**
Vitamin D normal (>30 ng/ml).	51% (n=90)	31% (n=55)	**<0.001**
Vitamin D insufisancy (20-29 ng/ml)	29% (n=51)	29% (n=51)	0.907
Vitamin D deficiency (<20 ng/ml)	20% (n=36)	40% (n=70)	**<0.001**
BMI: bodymass index, WC.Waist circumference, HP Hip circumference TC: Serum total cholesterol, TG: Triglycerides, FGP: Fasting plasma glucose, HDLC: High-Density Lipoprotein Cholesterol, LDL: Low-density Lipoprotein Cholesterol, Sbp systolic blood pressure, Dbp diastolic blood pressure.			

The Biochemical and Anthropometrical parameters of the study subjects are shown in Table 1. The mean age of the control group (n=177) was 56.94 years. The mean age of the T2DM group (n=176), was 57.01 years. Compared with control subjects, patients with T2DM had a lower vitamin D level (26.07±13.03 ng/ml vs. 30.28±13.05 ng/ml, p<0.01). 29% of T2DM patients were Vitamin D insufficient (20-29 ng/ml) and 40% % of the patients were Vitamin D deficient (<20 ng/ml). Among control subjects, 29% of the subjects were Vitamin D insufficient and 20% of the subjects were Vitamin D deficient. 31% of the patients with T2DM had normal vitamin D levels (> 30 ng/ml), and 51% of control subjects had normal vitamin D levels (> 30 ng/ml). The prevalence of Vitamin D deficiency is higher significantly in patients with T2DM than in the control volunteers. As expected, TG, LDL-C, TC, FGP, blood pressure and vitamin D level were significantly different between T2DM patients and controls. There was no signicantly difference in There was no significant difference BMI, WC, HP and HDL-C between both groups.

3.2 GENOTYPIC MODEL DISTRIBUTIONS OF VDR POLYMORPHISMS BETWEEN DT2 SUBJECTS AND CONTROLS AND LINKAGE DISEQUILIBRIUM

The genotype frequencies of the VDR *Bsm-I*, *Fok-I* and *Apa-I* were in agreement with Hardy–Weinberg equilibrium in all groups (Table 2). In T2DM patients, the frequencies of ff genotypes of VDR *Fok*-I is significantly increased compared to control group and (OR = 0.35, 95% CI = 0.15 –0.82, *P* = 0.016). Regarding the VDR *Apa-I* and *Taq*I polymorphisms, there were no significant differences in all genetics model between cases and controls (*P* > 0.05).

Table 2. Genotypic distribution of VDR polymorphisms and statistic comparison between DT2 subjects and controls

VDR rs10735810 (Fok-I)					
Model	**Genotype**	**Controls (n= 177)**	**Patients (n= 176)**	**OR (95% CI)**	**P-value**
Codominant	FF	82 (46.33%)	87 (49.43%)	Reference	
	Ff	74 (41.81%)	80 (45.45%)	0.99(0.63-1.54)	0.958
	ff	21 (11.86%)	9 (5.11%)	**0.35(0.14-0.83)**	**0.018**
Dominant	FF	82 (46.33%)	87 (49.43%)	Reference	
	Ff+ff	95 (53.67%)	89 (50.57%)	0.84(0.55-1.28)	0.420
Recessive	FF+Ff	156 (88.14%)	167 (94.89%)	Reference	
	ff	21 (11.86%)	9 (5.11%)	**0.35(0.15-0.82)**	**0.016**
VDR rs1544410 (Bsm-I)					
Model	**Genotype**	**Controls (n= 177)**	**Patients (n= 176)**	**OR (95% C`I)**	**P-value**
Codominant	BB	18 (13.24%)	18 (13.33%)	Reference	
	Bb	57 (41.91%)	57 (42.22%)	1.00 (0.47-2.13)	1
	bb	61 (44.85%)	60 (44.44%)	0.99 (0.46-2.10)	0.979
Dominant	Bb	61 (44.85%)	60 (44.44%)	Reference	
	Bb+bb	75 (55.15%)	75 (55.56%)	0.99 (0.49-2.02)	0.989
Recessive	bb+Bb	118 (86.76%)	117 (86.67%)	Reference	
	BB	18 (13.24%)	18 (13.33%)	0.98 (0.61-1.59)	0.951
VDR rs7975232 (Apa-I)					
Model	**Genotype**	**Controls (n= 177)**	**Patients (n= 176)**	**OR (95% CI)**	**P-value**
Codominant	AA	36 (22.50%)	36 (22.64%)	Reference	
	Aa	90 (56.25%)	89 (55.97%)	0.99 (0.57-1.71)	0.965
	aa	34 (21.25%)	34 (21.38%)	1.00 (0.50-1.98)	1
Dominant	A/A	36 (22.50%)	36 (22.64%)	Reference	
	Aa+aa	124 (77.50%)	123 (77.36%)	0.99 (0.58-1.68)	0.971
Recessive	A/A+Aa	126 (78.75%)	125 (78.62%)	Reference	
	aa	34 (21.25%)	34 (21.38%)	1.01 (0.58-1.74)	0.982

Standarized Linkage-Disequilibrium coefficient D' was calculate for all pairs of polymorphism to determine the extent of Linkage Disiquilibrium (LD) among the 3 polymorphisms. Figure.1 shows that with the exception of Bsm-I and A pa-I Polymorphisms which were in strong linkage Disequilibrium (D'=49), others polymorphism were not in Linkage Disequilibrium (Figure 1).

3.3 COMPARISONS OF CLINICALS AND BIOCHEMICALS PARAMETERS BETWEEN VDR POLYMORPHISM

We compared biochemical and clinical parameters between VDR genotypes for all patients and controls combined. The Fok-I Polymorphism was associated significantly with increased levels of TC, LDL-C HDL-C and TG (all P values <0.05). We found significantly elevated systolic blood pressure (P = 0.042) in association with *Apa1* polymorphism. No Significant association was observed for *Bsm1* polymorphism (Table 3).

3.4 ANALYSIS OF VDR HAPLOTYPE

The combined effect of three variants (Fok-I, Bsm-I, Apa-I) in the VDR gene were examined. There were 7 haplotypes identified in the VDR gene in our population, with frequencies greater than 5%. No significant association was observed between the all haplotypes and DT2M.

The frequencies of all haplotypes are listed in Table 5. The interaction between haplotypes and clinical and Biochemical's parameters showed that H1 HAPLOTYPE was associated with higher waist circumference and lower triglycerides levels (TG). The H2 haplotype was associated significantly with lower HDL-C. No difference significant was detected between others Haplotypes (Table 6)

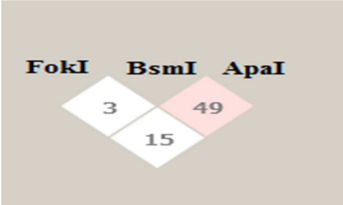

Figure 1 The Linkage Disequilibrium (LD) between the three VDR SNPs

4 DISCUSSION

Vitamin D is reported to be involved in several biological processes. Variations in this vitamin D endocrine systems can lead to several common chronic diseases, such as cardiovascular disorders, diabetes, cancer, tuberculosis, and osteoarthrosis [13], [14]. The gene of VDR is one of the important genes that have been expressed in a large number of different tissues [13]. Pancreatic beta-cells is among the several types of cells that respond to vitamin D [14]. Thanks to this, VDR genotype can play an important role in glucose homeostasis and in the mechanism of insulin release [15]. We conducted this study aiming to clarify the contribution of VDR polymorphisms in susceptibility to T2DM among Moroccan Population. To our knowledge, this is the first study to studied the prevalence of Vitamin D inadequacy in Moroccan population with T2DM, and the distribution of *VDR* polymorphisms in relation biochemical's and anthropometric parameters.

Table 3. Anthropometric and metabolic parameters according to genotypes of VDR polymorphisms

VDR rs10735810 (Fok-I)					
	FF	Ff	ff	P-value (FF vs Ff)	P-value (FF vs ff)
Age (years)	57.99±10.70	56.01±11.97	56.24±12.64	0.367	0.880
BMI (kg/m2)	28.31±4.30	28.71±4.07	28.72±4.80	0.349	0.804
WC (cm)	100.61±12.55	98.54±16.62	99.48±9.44	0.522	0.489
HC (cm)	108.26±9.79	107.92±10.26	111.36±11.96	0.622	0.293
Sbp (mmgHg)	78.37±8.60	78.92±9.09	77.46±7.96	0.777	0.573
Dbp (mmgHg)	137.20±15.41	137.00±14.69	137.21±15.44	0.778	0.759
FGP (g/L)	1.21±0.41	1.20±0.39	1.10±0.37	0.868	0.229
TC (g/L)	1.85±0.26	1.94±0.32	1.74±0.15	0.057	0.019 *
TG (g/L)	1.08±0.65	1.14±0.58	1.22±0.19	0.406	0.001 *
LDL-C (g/L)	1.21±0.24	1.31±0.36	1.08±0.26	0.020 *	0.023 *
HDL-C (g/L)	0.46±0.07	0.45±0.07	0.37±0.05	0.292	<0.001 *
Vitamin D level (ng/ml)	28.18±12.72	28.61±12.82	25.83±17.49	0.774	0.083

VDR rs1544410 (Bsm-I)					
	BB	Bb	bb	P-value (BB vs Bb)	P-value (BB vs bb)
Age (years)	61.00±10.84	57.33±10.96	56.66±12.37	0.026 *	0.112
BMI (kg/m2)	27.48±4.44	28.48±4.38	28.62±4.43	0.314	0.232
WC (cm)	98.29±18.72	100.84±13.47	98.04±16.86	0.933	0.327
HC (cm)	105.47±9.63	108.72±9.62	107.72±10.82	0.123	0.253
Sbp (mmgHg)	78.80±8.66	77.75±8.54	79.30±8.90	0.509	0.806
Dbp (mmgHg)	138.80±15.45	136.72±14.74	136.92±14.28	0.471	0.608
FGP (g/L)	1.17±0.34	1.19±0.39	1.19±0.43	0.843	0.532
TC (g/L)	1.91±0.26	1.86±0.25	1.89±0.32	0.398	0.571
TG (g/L)	1.13±0.40	1.01±0.48	1.24±0.77	0.159	0.775
LDL-C (g/L)	1.24±0.44	1.23±0.28	1.25±0.32	0.912	0.877
HDL-C (g/L)	0.44±0.08	0.46±0.08	0.43±0.07	0.437	0.956
Vitamin D level (ng/ml)	28.21±11.74	28.01±12.42	27.94±14.70	0.787	0.524

VDR rs7975232 (Apa-I)					
	AA	Aa	aa	P-value (AA vs Aa)	P-value (AA vs aa)
Age (years)	55.00±11.85	56.09±11.80	60.12±11.03	0.592	0.007 *
BMI (kg/m2)	28.44±4.56	28.46±4.42	29.45±3.47	0.677	0.140
WC (cm)	99.67±15.03	100.17±14.45	99.44±15.02	0.522	0.774
HC (cm)	109.45±10.93	108.52±9.86	107.44±10.06	0.841	0.527
Sbp (mmgHg)	78.82±9.82	78.51±8.31	78.15±9.29	0.955	0.752
Dbp (mmgHg)	138.85±14.58	137.77±15.39	133.69±15.19	0.466	0.042 *
FGP (g/L)	1.18±0.38	1.21±0.43	1.18±0.36	0.862	0.904
TC (g/L)	1.85±0.26	1.88±0.25	1.93±0.33	0.608	0.508
TG (g/L)	1.06±0.41	1.12±0.50	1.15±0.97	0.477	0.360
LDL-C (g/L)	1.19±0.29	1.25±0.28	1.25±0.35	0.216	0.353
HDL-C (g/L)	0.43±0.08	0.44±0.08	0.46±0.08	0.435	0.085
Vitamin D level (ng/ml)	29.97±16.73	27.60±12.20	29.33±13.23	0.311	0.988

*Data are presented as mean ± standard deviation; statistical significance is shown. *Indicates that the group is significantly different from the first genotype.*

The present study revealed that the prevalence of vitamin D deficiency was considerably higher in T2DM (40%) compared to control (20%). The prevalence of vitamin D deficiency was same in our cohort than in recent study in Caribbean subjects with T2DM who found that 42,6% [16] .

Vitamin D deficiency is a major health problem in many parts of the world, including Africa and Middle East [17], [18], [19]. Among the risk factors for vitamin D deficiency there, winter season, lack of sun exposure, dark skin pigmentation, gender female, age extreme, obesity, a covered up style of clothing and malnutrition [20]. Intestinal absorption of calcium and phosphorus and improving the reabsorption of calcium by the kidney, is stimulated by Vitamin D, which leads to higher plasma levels of these two important minerals [21]. As a consequence, the mineral homeostasis and regulation of bone remodelling is a major biological role of vitamin D [22], [23]. Findings from several studies have shown that vitamin D deficiency may play a important role in the disease of T2DM; and is a multifactorial disease in which genetic and environmental factors play an complex role and not yet clearly defined. This study demonstrated that VDR gene polymorphisms were associated with susceptibility to T2DM in the Moroccan population, which can be explained by differences in VDR *Fok-I* genotype distributions between T2DM and control subjects.

Several genes involved in metabolic processes of T2DM were considered candidates for the pathogenesis of the disease [24], and among them, the VDR gene is considered a better candidate for susceptibility to disease of T2DM [25]. The presence of a correlation between polymorphisms and T2D associated metabolic parameters, including fasting glucose, impaired glucose tolerance, Insulin secretion and Insulin resistance has been cited by many observational studies[26], [14]. VDR *Fok*-I polymorphisms and T2DM are closely correlated. In patients with T2DM, VDR ff genotype were significantly lower in patients with T2DM than in control individuals. Carrying the f allele of the *Fok*-I SNP might be protective against Vitamin D Defiviency. Recent study conclude that they can affect circulating levels of vitamin D and may also influence cardiovascular risk [27]. In agreement with our results, a recent study has demonstrated Fok-I polymorphism of the VDR gene as a possible risk factor for T2DM [28].

In addition the results of recent study in patients with T2DM suggest that higher vitamin D levels may be associated with better chronics kidney disease that this association was modified by Fok-I polymorphisms. On the contrary, there are studies founding no association between T2DM patients and controls in the allele and genotype frequencies in VDR *Fok-I* gene polymorphism [29], [30], [15]. The molecular explanation for the supposed relationship between Fok-I polymorphism and T2DM are only partly understood. The gene coding for the VDR is located on chromosome 12q12-q14. Direct effect of Vitamin D metabolism pathway and cell adiposity differentiation is a possible pathway for such an effect, as VDR is expressed in preadipocytes [31].

Table 4 Association analysis of haplotypes derived from polymorphic sites using genotype data

Haplotype	Fok-I (rs10735810)	Bsm-I (rs1544410)	Apa-I (rs7975232)	Frequency		OR(95%CI)	P-value
				Controls	Cases		
H1	F	B	A	0.07637	0.06835	1.296(0.568-2.96)	0.535
H2	F	b	A	0.1042	0.1162	0.791(0.421-1.49)	0.466
H3	F	b	A	0.3071	0.2998	1.091(0.725-1.64)	0.677
H4	F	B	A	0.07353	0.09622	0.6547(0.332-1.29)	0.216
H5	F	B	a	0.1815	0.1595	1.302(0.779-2.18)	0.312
H6	F	b	a	0.08703	0.09328	0.8981(0.456-1.77)	0.756
H7	F	b	a	0.1701	0.1666	1.059(0.631-1.78)	0.829

Table 5 Association between VDR haplotypes and clinical and biochemical parameters

	H1 FBA			H2 fbA			H3 FbA		
	Carriers	Noncarriers	P-value	Carriers	Noncarriers	P-value	carriers	Noncarriers	P-value
Age (years)	64.88±9.9	56.08±11.43	0.0003 *	57.79±13.51	56.57±11.21	0.1648	56.64±11.21	56.94±12.25	0.5627
BMI (kg/m2)	29.86±4.18	28.47±4.26	0.0798	29.19±4.06	28.46±4.30	0.2601	28.52±4.15	28.67±4.51	0.8600
WC (cm)	104.04±6.31	99.40±15.06	0.0476 *	97.95±17.94	100.07±13.98	0.5023	99.31±15.22	100.63±13.40	0.4153
HC (cm)	105.52±11.28	108.78±10.09	0.0975	106.90±10.25	108.79±10.19	0.2692	108.58±9.72	108.38±11.11	0.4744
Sbp (mmgHg)	78.04±8.90	78.60±8.74	0.6831	79.09±9.74	78.47±8.59	0.9729	78.62±8.45	78.43±9.32	0.5344
Dbp (mmgHg)	136.40±15.85	137.33±15.15	0.8239	135.63±15.06	137.51±15.21	0.3760	136.60±15.06	138.51±15.39	0.3219
FGP (g/L)	1.20±0.34	1.19±0.40	0.3871	1.14±0.37	1.20±0.40	0.3567	1.20±0.41	1.17±0.37	0.8845
TC (g/L)	1.90±0.30	1.88±0.28	0.8987	1.88±0.37	1.88±0.26	0.2024	1.90±0.29	1.84±0.26	0.0860
TG (g/L)	0.89±0.41	1.14±0.61	0.0342 *	1.19±0.57	1.11±0.61	0.2456	1.15±0.69	1.06±0.39	0.7966
LDL-C (g/L)	1.31±0.43	1.23±0.29	0.9752	1.24±0.33	1.24±0.30	0.5546	1.26±0.30	1.20±0.31	0.1801
HDL-C (g/L)	0.48±0.07	0.44±0.08	0.0538	0.41±0.07	0.45±0.08	0.0285 *	0.45±0.07	0.43±0.08	0.0567
Vit D (ng/ml)	28.26±11.69	28.29±13.50	0.8039	28.47±13.72	28.25±13.33	0.9845	27.92±-12.54	29.03±14.95	0.5096
	H4 fBa			H5 FBa			H6 fba		
	Carriers	Noncarriers	P-value	Carriers	Noncarriers	P-value	carriers	Noncarriers	P-value
Age (years)	55.95±11.90	56.92±11.48	0.7771	56.75±11.06	56.73±11.82	0.1759	53.45±11.69	57.78±11.33	0.0128 *
BMI (kg/m2)	27.95±3.98	28.70±4.32	0.1844	28.07±4.47	28.83±4.14	0.1954	28.76±4.41	28.51±4.23	0.5565
WC (cm)	96.82±19.76	100.45±13.07	0.3135	101.20±13.34	98.98±15.23	0.1572	101.06±8.89	99.40±15.84	0.6711
HC (cm)	106.58±8.72	108.96±10.49	0.1685	109.48±9.70	108.01±10.45	0.2349	110.92±11.26	107.82±9.80	0.0742
Sbp (mmgHg)	78.20±8.10	78.63±8.90	0.7397	78.91±9.43	78.35±8.35	0.7060	78.95±8.99	78.43±8.68	0.6691
Dbp (mmgHg)	137.37±15.28	137.23±15.18	0.9721	138.06±15.08	136.81±15.25	0.3914	138.46±15.09	136.88±15.22	0.3131
FGP (g/L)	1.18±0.39	1.20±0.40	0.7452	1.20±0.38	1.19±0.41	0.4541	1.20±0.40	1.19±0.40	0.9115
TC (g/L)	1.88±0.23	1.88±0.29	0.8699	1.87±0.24	1.88±0.29	0.9250	1.88±0.26	1.88±0.28	0.7175
TG (g/L)	1.18±0.47	1.11±0.63	0.1708	1.03±0.42	1.17±0.66	0.0766	1.12±0.53	1.12±0.63	0.6208
LDL-C (g/L)	1.23±0.35	1.24±-0.30	0.8904	1.22±0.23	1.24±0.34	0.9015	1.25±0.29	1.23±0.31	0.5710
HDL-C (g/L)	0.43±0.09	0.45±0.07	0.2108	0.45±0.08	0.44±0.08	0.4259	0.43±0.07	0.45±0.08	0.0511
Vit D (ng/ml)	27.61±13.28	28.43±13.40	0.5709	28.59±11.24	28.12±14.38	0.1819	27.49±13.77	28.53±13.25	0.3519

It is now suggested that the underlying mechanism of the relationship Vitamin D deficiency and chronic diseases is the presence of VDR in several tissues and cells, including pancreatic beta cells [32]. Furthermore, an association between polymorphisms of the VDR and body weight and insulin secretion has also been reported [33], [5]. In the recent meta-analysis, Li et al. (2013) examined the association among four well-characterized VDR polymorphisms with T2D, and showed that allele f and variant homozygote ff of Fok-I were significantly associated with T2D. This meta-analysis conclude that the Fok-I polymorphism polymorphism of VDR gene could be a risk factor for T2DM especially in asian population [34]. The Fok-I polymorphism can be detected by the presence or absence of a Fok-I restriction site within the ATG transcriptional start site of the VDR gene. The gene is transcribed into normal length when the restriction site (f allele) is present and into shortened length when the restriction site (f allele) is absent. The longer VDR protein appears to possess decreased transcriptional activity, leading to lower activation of target cells [35] [36]. Another study showed that Ff/ff subjects are more insulin

resistant and obese than FF subjects [37]. VDR *Apa-I* and *Bsm-I* polymorphisms were not correlated with T2DM in Moroccan population. The distribution of VDR Apa-I and Bsm-I genotype showed no statistical difference between the control and T2DM patient. In agree with our results a recent meta-analysis did not find any association of the other three polymorphisms (Bsm1, Apa1 and Taq1) with an increased T2DM risk in overall and subgroup analysis [34]. In contrast, a studies in Chinese population found that VDR Apa-I gene polymorphism was associated with T2DM [38], [39]. Previous studies (genome-wide association and candidate gene polymorphism) have focused on the association between VDR gene and development of T2DM, but findings have often been inconsistent among different populations worldwide. Generally the discrepancies between studies may be due to false positive finding, replication study lacks power, heterogeneity between studies and heterogeneity across studies. Some studies have reported interactions between VDR polymorphisms and vitamin D in diseases such as Type 1 diabetes mellitus [40], tuberculosis [41] and prostate cancer[42] . Although to our knowledge, this is the first time an interaction between VDR polymorphisms and vitamin D levels has been reported in T2DM patients in Moroccan population.

The findings of this study confirm the relationship between biological and clinical traits and VDR genotypes for all patients and controls combined. The Fok1 FF variant was significantly associated with increased levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides (all P values <0.05). We found significantly elevated systolic blood pressure (P = 0.042) in association with *Apa1* AA variant. No Significant association was observed for *Bsm1* polymorphism. In addition, the VDR H1 haplotypes is significantly associated with increase in triglyceride level and Waist circumference. In addition, the H2 Haplotype was significantly associated with lower HDL-C level. These results suggest that each variant in VDR haplotypes may be associated with different biological pathway that improve plasma lipid levels and accordingly reduce the risk of cardiovascular disease. In agreement with our study, the results of a study conducted in Caribbean patients revealed that vitamin D deficiency was high in our T2DM patients, and was associated with the *VDR* gene *Fok*-I and *Apa*-I polymorphisms and cardiovascular risk profile, and that measurements of vitamin D may help to detect T2DM patients with cardiovascular risk, and *VDR* polymorphisms may explain why vitamin D deficiency is often observed in some patients with T2DM. Another study demonstrated that the Bsm-I VDR polymorphism influenced BMI, while the Fok-I VDR polymorphism was associated with lower serum HDL-C level [43]. In addition, a study realized in postmenopausal women showed that the Bsm-I polymorphism in the VDR gene had no association with susceptibility to obesity and insulin resistance, while it was related to a higher LDL-C level [44].

There are a few limitations of our study. Firstly, our sample numbers considered relatively small. Secondly, lack of replication studies of the association of VDR gene polymorphisms and T2DM in Moroccan population. Consequently, further studies including larger sample numbers and replication of significant findings are necessary to clarify the role of the VDR gene polymorphism in T2DM.

5 CONCLUSION

In conclusion, it is evident that vitamin D deficiency has prevailed in Moroccan population with T2DM. Alterations in vitamin D action could be may affect Insulin-Sensitivity, B-Cell action or both. Moreover our study documents a correlation between VDR Fok-I gene polymorphisms and susceptibility to T2DM in the Moroccan population. The possible action of vitamin D in the pathogenesis of T2DM is far from being completely understood. Additionally, further knowledge on this issue may identify new candidate targets in the treatment and prevention of the disease. Therefore, further investigations on this issue are warranted.

CONFLICT OF INTEREST

The all authors declare that they have no conflict of interest.

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