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Vitamin D receptor gene polymorphisms and vitamin D status and susceptibility to type 2 diabetes mellitus in Moroccans patients

Errouagui Abdeltif[1], Houda Benrahma[1], Hicham Charoute[1] , Hamid Barakat[1] , Mostafa kandil[2] , Hassan Rouba[1]

[1] laboratoire de génétique moléculaire et humaine, département de recherche scientifique,
Institut Pasteur du Maroc, 1 place louis pasteur, 20360 Casablanca, Maroc
[2] laboratoire des sciences anthropogenetiques et pathologiques, faculté des sciences, université chouaib doukkali, Maroc

Abstract
Introduction. Vitamin D receptor (VDR) gene is recognized as candidate gene for susceptibility to Type 2 diabetes mellitus (T2DM). The aim of this study was to investigate the association between VDR gene polymorphisms and T2DM in Moroccans patients. *Materials and Methods*. 176 clinically diagnosed T2DM patients and 177 healthy controls from the Moroccans population were recruited. ***Bsm***I(rs1544410), ***Fok***I(rs10735810) and ***Apa***I (rs7975232) single nucleotide polymorphisms (SNPS) of the VDR gene were determined using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). A Vitamin D level was determined using ELISA. *Results*. The prevalence of Vitamin D deficiency and insufficiency is significantly higher in patients with T2DM than in the control subjects. There was a strong association between fok1 polymorphisms with T2DM (OR = 0,35, 95% CI = 0.14–0.83, ***P*** = 0.018), while the VDR *BsmI* and ***Apa***I polymorphisms are not. The Fok1 polymorphism 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*. *Conclusions*. Our study indicated that vitamin D deficiency was prevalent in Moroccans patients with T2DM, and each variants in VDR polymorphism may be associated with different mechanisms that enhance the plasma lipid profil and the risk of cardiovascular disease. However, the allele *FokI* f appears to have protective effect against diabetes.

Keywords
Diabetes, genetic polymorphisms, restriction fragment length polymorphisms, vitamin D receptor

Abbreviations
T2DM, type 2 diabetes mellitus; VDR, vitamin D receptor; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index, SNPs, single nucleotide polymorphisms;

Background
Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and altered insulin secretion. 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 vitamin D deficiency [2]. This deficiency is now considered a public-health because it has been associated with greater risks of other morbidities, such as cardiovascular disease and cancer [3,4]. Several studies have shown that vitamin D deficiency might play an important role in the pathogenesis of T2DM. Epidemiological data showed a reduction in vitamin D deficiency in a London Bangladeshi 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 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 its specific cytosolic/nuclear vitamin D receptor (VDR) [10,11]. VDR is a member of the steroid/thyroid 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 VDR gene is located on chromosome 12q12–q14, and common polymorphisms have been identified namely *BsmI* (rs1544410), *FokI* (rs10735810), and *ApaI* (rs7975232) [12]. The aim of this study was to investigate the association between VDR gene polymorphisms and T2DM in Moroccans patients.

Methods
Study population
This study concerned 353 Moroccan adult volunteers, 177 patients diagnosed as T2DM by medical corps and 176 healthy controls. At enrolment in the Medical Biology Center of Pasteur Institute of Morocco in Casablanca, they completed a health and lifestyle questionnaire including social demographics characteristics, and medical history. The clinical examination consisted of questionnaire and a physical examination: age, gender, geographical origin, family history, body mass index, the presence of hypertension, diabetes, hypercholesterolemia, realized at the Pasteur Institute in Casablanca. All subjects (patients and controls groups) included in this study were from different geographic and ethnic backgrounds inMorocco.

Ethics statement
We obtained written informed consent from each subject and the research protocol was approved by the committee on research ethics of Pasteur Institute of Morocco.

Biochemicals measurements
The blood was collected in EDTA tubes. Glycemia, total cholesterol (TC), Triglycerides (TG) and High-Density Lipoprotein Cholesterol (HDL-C) levels were determined using the VITROS (5.1 FS Chemistery System). Low-density Lipoprotein Cholesterol (LDL-C) level was calculated according to the Friedwald's formula. All biochemical measurements were performed in the Biochemistry Laboratory of the Medical Biology Center in Pasteur Institute of Morocco. Serum 25-hydroxyvitamin D (25(OH)D) level was measured by ELISA. 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.

Isolation of DNA
Genomic DNA was extracted from peripheral blood leucocytes using the salting-out method (8). 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.

VDR genotyping
Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed for genotyping of SNPs: *Fok*I (rs10735810), *Bsm*I (rs1544410), *Apa*I (rs7975232) of VDR gene. All PCR were performed in a Biometra thermal cycler, using Taq Polymerase (Bioline).

***Fok1* polymorphism**
PCR amplification was carried out in a total volume of 10 µL containing approximately 50 ng of genomic DNA, 200 µmol/L dNTPs, 10 pmol of each primer, 1.5 mmol/L MgCl2, 0.5 U Taq polymerase and 1 µL of 10× PCR buffer. A fragment of 270 bp including the *FokI* (rs10735810) polymorphism was amplified using two oligonucleotides:
Forward: 5′- AGCTGGCCCTGGCACTGACTCTGGCTCT-3′,
Reverse: 5′- ATGGAAACACCTTGCTTCTTCTCCCTC -3′.
The PCR conditions were an initial denaturing at 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, 61°C for 40 s, 72°C for 50 s, and a final extension of 72°C for 7 min. The PCR products were digested for one hour at 37°C with *FokI* restriction enzyme (Biolabs). Then the products of digestion were electrophoresed on a 3% agarose gel stained with ethidium bromide and visualized using ultraviolet illumination. The wild type homozygote (FF), heterozygote (Ff) and mutant homozygote (ff) showed one band (270 bp), three bands (270, 210 and 60 bp) and two bands (210 and 60 bp), respectively, because the substitution creates a Fok1 recognition sequence which digests the 270 bp into 210 and 60 fragments.

***Bsm1* 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 *FokI* polymorphism. The PCR products were digested with BsmI restriction enzyme (Biolabs) for one hour at 65°C . The digested fragments were separated in a 3% agarose gel stained with ethidium bromide and visualized using ultraviolet illumination. The wild type homozygote (BB), heterozygote (Bb) and mutant homozygote (bb) showed one band (820 bp), three bands (820, 650 and 170 bp) and two bands (650 and 170 bp), respectively, because the substitution creates a *BsmI* recognition sequence which digests the 820 bp into 650 and 170 fragments.

***ApaI* polymorphism**
Genotyping for *ApaI (rs7975232)* was performed with the following primers:
Forward: 5′- CAGAGCATGGACAGG GAGCAA-3′,
Reverse 5′- GCAACTCCTCATGGCTGAGGTCTC -3′.
A 82000 bp fragment VDR gene was amplified using the PCR under reactions conditions identical to those used for the *FokI* polymorphism. The PCR products were digested with *ApaI* restriction enzyme (Promega) for one hour at 65°C. The digested fragments were separated in a 3% agarose gel stained with ethidium bromide and visualized using ultraviolet illumination. Absence of *Apa-I* restriction site (2000bp) was assigned as a common allele A (wild-type allele) and presence of restriction site resulting in 1700 bp and 300bp fragments was assigned as infrequent allele a (mutant allele). Genotypes were assigned accordingly as homozygotes for common allele (AA) and homozygotes for infrequent allele (aa). Presence of 2000, 1700 and 300 bp fragments was assigned as heterozygotes (Aa). All molecular analyses were performed in the Human Genetic Laboratory in Pasteur Institute of Morocco.

Statistical analysis
The Clinical and biochemical parameters were expressed as means ± SD. The student's 1 test was applied for comparison of quantitative traits that follow a normal distribution. Otherwise, we used Manne-Whitney test.Chi-square test and logistic regression analysis were performed to test the association between DT2 and VDR genotypes and haplotypes. Logistic regression analysis was adjusted by age and gender. A P value of less than 0.05 was considered statistically significant. All statistical analyses were performed using STATA software, version 11.0. The P-values were corrected with the Bonferroni correction by multiplying with the number of comparisons. All haplotype frequencies estimation and comparison we used the PLINK software, version 1.07. All haplotypes with frequencies less than 5% were ignored in the analysis. Linkage disequilibrium between each pair of VDR gene polymorphisms was estimated using Haploview software, version 4.2.

Results
Characteristics of controls and patients
The clinical characteristics of the study subjects are shown in Table 1. The mean age of the control group was 56.94 years. The mean age of the T2DM group (n=176) was 57.01 years.

Compared with control subjects (n=177), 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

	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
Waist circumference (cm)	100.79±8.83	98.44±18.12	0.84
Hip circumference (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-cholesterol (g/L)	1.13±0.11	1.40±0.41	**<0.001**
HDL-cholesterol (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)	
Vitamin D insufisancy (20-29 ng/ml)	29% (n=51)	29% (n=51)	
Vitamin D deficiency (<20 ng/ml)	20% (n=36)	40% (n=70)	

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 prevalences of Vitamin D deficiency is significantly higher in

Table 2. Characteristics of the studied groups.

BMI: bodymass index, TC: Serum total cholesterol, TG: Triglycerides, FGP: Fasting plasma glucose, HDL: High-Density Lipoprotein Cholesterol, LDL: Low-density Lipoprotein Cholesterol, Sbp systolic blood pressure, Dbp diastolic blood pressure.

patients with T2DM than in the control subjects. As expected, triglyceridemia (TG), LDL, total cholesterol (TC), Glycemia (Gly), blood pressure and vitamin D level were significantly different between T2DM patients and controls. There was no significant difference in body mass index (BMI), Waist circumference, Hip circumference and HDL between both groups.

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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 3). In type 2 diabetic 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).

To determine the extent of linkage disequilibrium (LD) among the three polymorphisms, standardized LD coefficient D' was calculated for all pairs of polymorphisms. Figure 1 shows that with the exception of BsmI and ApaI polymorphisms which were in strong linkage disequilibrium (D' =49), other polymorphisms were not in linkage disequilibrium (Figure 1).

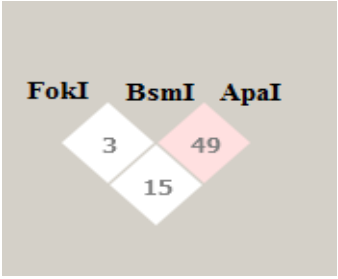
Comparisons of clinical and biochemical parameters between VDR genotypes.

We compared biological and clinical traits between VDR genotypes for all patients and controls combined. The Fok1 polymorphism 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* polymorphism. No Significant association was observed for *Bsm1* polymorphism (Table 4).

Table 3. Genotypic distribution of VDR polymorphisms and statistic comparison between DT2 subjects and controls

VDR rs10735810 (FokI)					
Model	**Genotype**	**Controls (n= 177)**	**Patients (n= 176)**	**OR (95% CI)**	**P-value**
Codominant	FF	82 (46.33%)	87 (49.43%)		
	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%)		
	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%)		
	ff	21 (11.86%)	9 (5.11%)	**0.35(0.15-0.82)**	**0.016**
VDR rs1544410 (BsmI)					
Model	**Genotype**	**Controls (n= 177)**	**Patients (n= 176)**	**OR (95% C`I)**	**P-value**
Codominant	BB	18 (13.24%)	18 (13.33%)	1.00	
	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%)	1.00	
	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%)	1.00	
	BB	18 (13.24%)	18 (13.33%)	0.98 (0.61-1.59)	0.951
VDR rs7975232 (ApaI)					
Model	**Genotype**	**Controls (n= 177)**	**Patients (n= 176)**	**OR (95% CI)**	**P-value**
Codominant	AA	36 (22.50%)	36 (22.64%)	1.00	
	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%)	1.00	
	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%)	1.00	
	aa	34 (21.25%)	34 (21.38%)	1.01 (0.58-1.74)	0.982

Figure 1 The linkage disequilibrium (LD) between the three VDR SNPs



VDR haplotype analysis

To examine the combined effect of three variants (fok1, Bsm1, Apa1) in the VDR gene. There were 7 haplotypes identified in the VDR gene in our population, with frequencies greater than 5% (Table 5). No significant association was observed between the all haplotypes and DT2M.

The frequencies all haplotypes are listed in Table 5. Analysis of the interaction between haplotypes, and clinical and biochemical showed that the H1 haplotype was associated with higer waist circonference and lower triglycerides levels (TG). The H2 haplotype was significantly associated with lower HDL cholesterol levels. No significant difference was detected between carriers and noncarriers of others haplotype (Table 6).

4. Discussion

Vitamin D is reported to be involved in a wide variety of 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 interessants genes that has been expressed in a large number of different tissues [13]. Pancreatic beta-cells is among the severals 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 such study to analyse the prevalence of vitamin D deficiency in Moroccan patients with T2DM, and the distribution of *VDR* polymorphisms in relation biochemical's and anthropometric parameters.

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 [38,39,40]. Among the risk factors for vitamin D deficiency there extreme age, female gender, dark skin pigmentation, winter season, malnutrition, lack of sun exposure, a covered-up style of clothing and obesity [17]. 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. [18]. As a consequence, the mineral homeostasis and regulation of bone remodelling is a major biological role of vitamin D [19, 20]. Findings from several studies have shown that vitamin D deficiency may play a crucial role in the pathogenesis of T2DM; and is a multifactorial disease in which genetic and Environmental factors play an complex role and not yet clearly defined. Several genes involved in the metabolic pathway of T2DM have been regarded as candidates for the onset of the disease (21), and among these, the VDR gene is considered as a particularly good candidate for disease susceptibility (22).

Table 4. Anthropometric and metabolic parameters according to genotypes of VDR polymorphisms

VDR rs10735810 (FokI)					
	FF	**Ff**	**Ff**	**P-value (FF vs Ff)**	**P-value (FF vs ff)**
Age	57.99±10.70	56.01±11.97	56.24±12.64	0.367	0.880
BMI	28.31±4.30	28.71±4.07	28.72±4.80	0.349	0.804
Waist circumference	100.61±12.55	98.54±16.62	99.48±9.44	0.522	0.489
Hip circumference	108.26±9.79	107.92±10.26	111.36±11.96	0.622	0.293
Diastolic blood pressure	78.37±8.60	78.92±9.09	77.46±7.96	0.777	0.573
Systolic blood pressure	137.20±15.41	137.00±14.69	137.21±15.44	0.778	0.759
Fasting plasma glucose	1.21±0.41	1.20±0.39	1.10±0.37	0.868	0.229
Total cholesterol	1.85±0.26	1.94±0.32	1.74±0.15	0.057	**0.019** *
Triglycerides	1.08±0.65	1.14±0.58	1.22±0.19	0.406	**0.001** *
LDL-cholesterol	1.21±0.24	1.31±0.36	1.08±0.26	**0.020** *	**0.023** *
HDL-cholesterol	0.46±0.07	0.45±0.07	0.37±0.05	0.292	**<0.001** *
Vitamin D	28.18±12.72	28.61±12.82	25.83±17.49	0.774	0.083
VDR rs1544410 (BsmI)					
	BB	**Bb**	**bb**	**P-value (BB vs Bb)**	**P-value (BB vs bb)**
Age	61.00±10.84	57.33±10.96	56.66±12.37	**0.026** *	0.112
BMI	27.48±4.44	28.48±4.38	28.62±4.43	0.314	0.232
Waist circumference	98.29±18.72	100.84±13.47	98.04±16.86	0.933	0.327
Hip circumference	105.47±9.63	108.72±9.62	107.72±10.82	0.123	0.253
Diastolic blood pressure	78.80±8.66	77.75±8.54	79.30±8.90	0.509	0.806
Systolic blood pressure	138.80±15.45	136.72±14.74	136.92±14.28	0.471	0.608
Fasting plasma glucose	1.17±0.34	1.19±0.39	1.19±0.43	0.843	0.532
Total cholesterol	1.91±0.26	1.86±0.25	1.89±0.32	0.398	0.571
Triglycerides	1.13±0.40	1.01±0.48	1.24±0.77	0.159	0.775
LDL-cholesterol	1.24±0.44	1.23±0.28	1.25±0.32	0.912	0.877
HDL-cholesterol	0.44±0.08	0.46±0.08	0.43±0.07	0.437	0.956
Vitamin D	28.21±11.74	28.01±12.42	27.94±14.70	0.787	0.524
VDR rs7975232 (ApaI)					
	AA	**Aa**	**aa**	**P-value (AA vs Aa)**	**P-value (AA vs aa)**
Age	55.00±11.85	56.09±11.80	60.12±11.03	0.592	**0.007** *
BMI	28.44±4.56	28.46±4.42	29.45±3.47	0.677	0.140
Waist circumference	99.67±15.03	100.17±14.45	99.44±15.02	0.522	0.774
Hip circumference	109.45±10.93	108.52±9.86	107.44±10.06	0.841	0.527
Diastolic blood pressure	78.82±9.82	78.51±8.31	78.15±9.29	0.955	0.752
Systolic blood pressure	138.85±14.58	137.77±15.39	133.69±15.19	0.466	**0.042** *
Fasting plasma glucose	1.18±0.38	1.21±0.43	1.18±0.36	0.862	0.904
Total cholesterol	1.85±0.26	1.88±0.25	1.93±0.33	0.608	0.508
Triglycerides	1.06±0.41	1.12±0.50	1.15±0.97	0.477	0.360
LDL-cholesterol	1.19±0.29	1.25±0.28	1.25±0.35	0.216	0.353
HDL-cholesterol	0.43±0.08	0.44±0.08	0.46±0.08	0.435	0.085
Vitamin D	29.97±16.73	27.60±12.20	29.33±13.23	0.311	0.988

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.

The presence of a correlation between VDR polymorphisms and T2DM associated metabolic parameters, including fasting glucose, glucose intolerance, insulin sensitivity, insulin secretion, and calcitriol levels, has been reported by observational studies [23,24]. 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 deficiency: an earlier study found that they can affect circulating levels of vitamin D and may also influence cardiovascular risk [25].

In agreement with our results, a recent study has demonstrated FokI polymorphism of the VDR gene as a possible risk factor for T2D [26]. 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 FokI were significantly associated with T2D. The conclusions of this meta-analysis that the FokI polymorphism of the VDR gene could be a risk factor for T2DM especially in an Asian population[34]. 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 and 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 [27,28,29]. The molecular explanation for the supposed relationship between FokI polymorphism and type 2 diabetes are only partly understood. The FokI polymorphism can be detected by the presence or absence of a FokI 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 *ApaI* and *BsmI* polymorphisms were not correlated with T2DM in Moroccan population. The distribution of VDR ApaI and BsmI 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 ApaI gene polymorphism was associated with T2DM [41,42]. 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 [43], tuberculosis [44] and prostate cancer [45]. 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 associated with lowe HDL cholesterol level. These results suggest that each variants in VDR haplotypes may be associated with different mechanisms that enhance the plasma lipid levels and 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 T2D 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 might explain why vitamin D deficiency is so frequently seen in some T2D patients [16]. Another study demonstrated that the BsmI VDR polymorphism influenced BMI, while the FokI VDR polymorphism was associated with lower serum HDL-C level [46]. In addition, a study realized in postmenoposal women showed that the BsmI 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 [47].

The gene encoding for VDR is located on the 12q12-q14. A direct effect of vitamin D on adipocyte differentiation and metabolism is a possible mechanism for such an effect, as VDR is expressed in preadipocytes [30]. It has also been suggested that the underlying mechanism of the relationship between vitamin D deficiency and chronic disease is the presence of VDR in several tissues and cells, including pancreatic beta cells and adipocytes [31]. In addition, an association between *VDR* polymorphisms and body weight and insulin secretion has also been reported [32,33].

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.

In conclusion, it is evident that vitamin D deficiency has prevailed in Moroccan population with T2DM. Alterations in vitamin D action may affect insulin sensitivity, b-cell function or both. Moreover our study documents a correlation between VDR *Fok*I gene polymorphisms and susceptibility to T2DM in the Moroccan population. The possible role 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 authors declare that they have no conflict of interest.

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AUTHORS

First Author – Errouagui abbdeltif, phd, Institut Pasteur du Maroc, abdellatif.errouagui,@pasteur.ma .

Second Author – houda benrahma, docteur, , Institut Pasteur du Maroc, ben.houda@hotmail.com.

Third Author – hicham charoute, PHD, , Institut Pasteur du Maroc, hcharoute@gmail.com

Correspondence Author – Errouagui Abdeltif , email address, abdellatif.errouagui@pasteur.ma, alternate email address, abdellatif.errouagui@gmail.com, contact number.

Table 5 Association analysis of haplotypes derived from polymorphic sites using genotype data

Haplotype	FokI (rs10735810)	BsmI (rs1544410)	ApaI (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 6 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	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	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
Waist circumference	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
Hip circumference	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
Diastolic blood pressure	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
Systolic blood pressure	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
Fasting plasma glucose	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
Total cholesterol	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
Triglycerides	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-cholesterol	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-cholesterol	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
Vitamin D	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	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	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
Waist circumference	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
Hip circumference	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
Diastolic blood pressure	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
Systolic blood pressure	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
Fasting plasma glucose	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
Total cholesterol	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
Triglycerides	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-cholesterol	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-cholesterol	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
Vitamin D	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