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Vitamin D Receptor Gene *ApaI* and *TaqI* Polymorphisms in Type 2 Diabetes among Saudi Population

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Abstract

BACKGROUND: Variants of the Vitamin D receptor (VDR) gene have been linked to a variety of diseases, including metabolic syndrome, cancer, bone disease, and tuberculosis. The relationship between VDR gene variants and the susceptibility of type 2 diabetes mellitus (T2DM) in different ethnic groups is yet unknown. Vitamin D and its receptor complex have a function in regulating β-cell insulin secretion as a transcription factor.

AIM: The goal of this study was to see if there is a link between VDR *Apa1* and *Taq1* polymorphisms and T2DM susceptibility in the Saudis of the Makkah environment.

MATERIALS AND METHODS: DNA was separated from peripheral blood and genotyped in 110 healthy controls and 110 unrelated people with T2DM for the VDR *ApaI* (G/T) rs7975232 and *TaqI* (A/G) rs731236 single-nucleotide polymorphisms (SNPs) using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique.

RESULTS: The distributions of the genotypes and alleles of VDR *ApaI* and *TaqI* polymorphisms were statistically indifferent across the groups investigated (p >0.05).

CONCLUSION: These findings showed that polymorphisms in the VDR *ApaI* and *TaqI* genes may not be linked to T2DM risk in Saudis.

Introduction

Diabetes mellitus (DM) is a major health condition that affects individuals worldwide, including Saudi Arabia [1]. It affects the quality of life and increases the morbidity and mortality of other illnesses [2].

Despite the reality that the etiology of T2DM is not well unknown, the pathogenesis of T2DM is suggested to be due to interactions between multiple susceptible genes and from interactions between these genes and different environmental factors [3].

However, genetic factors play a key function in different types of diabetes mellitus although inheritance is complex. Most conditions of T2DM and type 1 diabetes mellitus (T1DM) are polygenic but monogenic forms have been discovered [4].

Several genes, such as the Vitamin D receptor gene (VDR), were reported to be involved in susceptibility to T2DM in various ethnicities [5], [6], [7], [8], [9]. The VDR gene is on chromosome 12q (12-12q14) and is extraordinarily polymorphic [10]. The six VDR variants that have attracted the greatest attention include the *FokI* polymorphism, *BsmI*, *Tru9I*, *ApaI*, *TaqI,* and the poly-A polymorphism downstream of the 3' untranslated region [11].

Vitamin D binds to nuclear receptors (VDR) and affects the DNA. It has been evidenced that disrupted Vitamin D and calcium homeostasis have a role in the pathogenesis of T2DM, and it was recently shown that having a high Vitamin D level may protect against type 2 diabetes [12]. VDR is one of the steroid/thyroid hormone receptor groups [13]. Vitamin D, namely, its activated metabolite 1,25-dihydroxy Vitamin D3, is recognized to have a role in maintaining the endocrine pancreas proper functions. The action of Vitamin D is mediated by its nuclear receptor (VDR) [14].

By functioning as a transcription factor, Vitamin D and its receptor complex control beta-cell insulin synthesis. It has been shown that deficiency of Vitamin D reduces the synthesis and secretion of insulin in diabetic models of humans and animals, suggesting that it may have a role in the pathogenesis of T2DM. Supplementation with Vitamin D may help to increase insulin secretion [15], [16]. However, the

polymorphisms in the VDR genes have been shown that they may impact the VDR protein's activity [17].

FokI polymorphism has been found to play a role in VDR gene transcriptional activation [18]. The VDR is expressed in pancreatic beta-cells, and it has been found that *BsmI* polymorphism increases type 1 diabetes mellitus susceptibility [10], [19], [20]. In healthy Asians, it has been shown that VDR gene *ApaI* impacts the beta-cells insulin secretory capacity [17]. T2DM genetic background, on the other hand, is unknown. According to the published data, the VDR gene might be a new candidate gene for T2DM susceptibility [5], [6], [7], [8], [9].

In this study, we examined the connection between VDR *ApaI* and *TaqI* gene variants and the incidence of T2DM in Saudis in the Makkah environment. To the best of our knowledge, only a few studies on the genetic variants of the VDR gene in T2DM have been conducted in Saudi Arabia, necessitating more research to confirm the link between VDR polymorphism and T2DM susceptibility [21]. As a result, we set out to identify the VDR *ApaI* and *TaqI* polymorphisms in the Saudi population and determine if they have a role in the occurrence of T2DM.

Materials and Methods

Subjects

The present study comprised 220 participants (110 unrelated subjects with T2DM and 110 healthy individuals) were selected from health clinics in Makkah, Saudi Arabia.

All subjects were Saudi individuals and both groups had been matched regarding the gender and age.

All participants signed a written informed consent, and the study was approved by the Ethics Committee of Umm Al Qura University, Saudi Arabia.

All subjects had their venous blood withdrawn between 9:00 and 11:00 a.m. after fasting overnight. Each sample was split in half, with one half going into sterile K_3EDTA coated tubes and the other half going into serum preparation tubes. Low-speed centrifugation was used to isolate plasma, and white cells from the buffy coat were removed for DNA separation. All specimens were maintained at –20°C until they were used.

Biochemical analyses

Fasting blood sugar was measured utilizing the glucose oxidase method, while hemoglobin A1c was measured using the HbA1c measuring kit as attested by the maker's commands (Human Diagnostics, Wiesbaden, Germany).

Genotyping

Isolation of DNA from venous blood was carried out as stated by the maker's commands using DNA extraction kit (Qiagen, Hilden, Germany). For PCR amplification, aliquots of genomic DNA were employed.

In a single amplification, primers covering the *ApaI* and *TaqI* intragenic polymorphisms were employed as described previously [10]. The primers used were 5'-CAGAGCATGGA CAGGGAGCAAG-3' and 5'-GCAACTCCTCATG GC TG AGG TCT CA-3'. After the first denaturation at 94°C for 5 min, 30 cycles of 94°C for 1 min, 68°C for 1 min, 72°C for 1 min 30 s, and a final elongation at 72°C for 7 min were performed. The restriction enzymes *TaqI* and *ApaI* were employed to digest the PCR products (740 bp). On 2% agarose, fragments were separated electrophoretically. The *ApaI* genotypes TT (740 bp), GG (530, 210 bp), and heterozygous GT were identified. The *TaqI* AA (lack of the particular *TaqI* site) gave two bands of 245 bp and 495 bp. The GG exhibits 205, 245, and 290 bp (Figure 1).

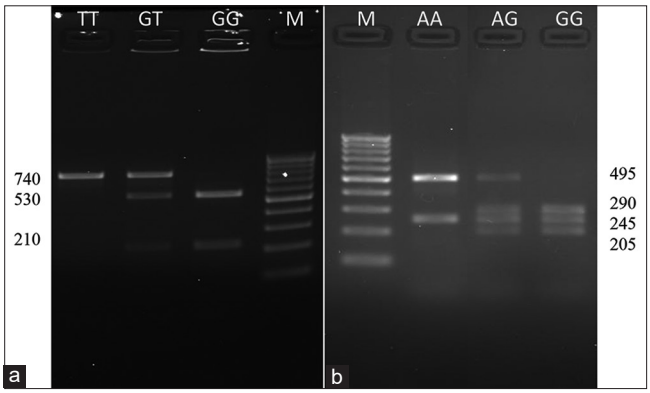

Figure 1: *Agarose electrophoresis of VDR ApaI and TaqI genotypes. The 740 bp PCR product of the VDR gene was digested with ApaI (a) and TaqI (b) restriction enzymes followed by their resolution in an agarose gel and visualization with UV light. Lane M = DNA molecular ladders (100−1000 bp). The remaining lanes correspond to the genotypes labeled at the top of each photo*

Statistical analyses

Data analyses were carried out using SPSS, version 20.0. (Chicago, IL, USA). The Student's t-test was utilized to analyze the mean values of continuous variables in patients and controls, while the χ^2 test was utilized to examine categorical data.

One-way analysis of variance (ANOVA) was used to examine the variation in different T2DM variables with the genotypes. $P < 0.05$ was considered statistically significant.

Results

Demographic and laboratory data

Table 1 shows the demographic and laboratory data of the studied groups. The mean values of age and gender were indifferent between the studied groups (p > 0.05). The BMI was higher in the diabetic group compared with the controls but not statistically significant (p > 0.05). The fasting glucose level and HbA1c were higher in diabetic subjects in comparison with the control subjects (p <0.001).

Table 1: Demographic and laboratory data for control subjects and patients with type 2 diabetes mellitus

Characteristics	Control group	Patient group	p
Subjects (*n*)	110	110	> 0.05
Age (years)	43 ± 5.2	44 ± 5.4	> 0.05
Gender (male/female)	72/38	68/42	> 0.05
BMI (kg/m^2)	23.76 ± 1.12	24.17 ± 1.13	> 0.05
FBS	84 ± 7.4	168 ± 15.3	< 0.001
HbA1c	4.11 ± 0.91	7.32 ± 1.64	< 0.001

Data are shown as mean ± SD. FBS: Fasting blood sugar, HbA1c: Hemoglobin A1c, BMI: Body mass index, SD: Standard deviation.

Genotype and allele frequencies of VDR ApaI and TaqI

Both T2DM and control groups have genotype distributions of *ApaI* and *TaqI* polymorphisms that were in Hardy-Weinberg equilibrium. The genotype and allele distribution of both SNPs in T2DM and control groups are shown in Table 2. For *ApaI* polymorphism, the TT, GT, and GG genotypes were 44.55%, 35.45%, and 20%, respectively, in the controls and were 34.55%, 47.27%, and 18.18% in the T2DM group, respectively. The percentage of the T allele was 62.27% and 58.18% while the G allele was 37.73% and 41.82% in the control and T2DM groups, respectively. The frequencies of genotypes and alleles in both groups were statistically indifferent (p > 0.05).

Table 2: Genotype distribution and allele frequencies of Vitamin D receptor *ApaI* and *TaqI* polymorphisms in subjects with type 2 diabetes mellitus and control group

VDR polymorphism	Control (n = 110)	T2D (n = 110)	χ^{2a}	p	OR	95% CI
ApaI genotypes						
TT	49 (44.55)	38 (34.55)			1	
GT	39 (35.45)	52 (47.27)	3.226	0.099	1.719	0.950–3.111
GG	22 (20)	20 (18.18)	0.178	0.709	1.172	0.56–2.455
Alleles						
T	137 (62.27)	128 (58.18)			1	
G	83 (37.73)	92 (41.82)	2.301	0.168	1.522	0.884–2.622
TaqI genotypes						
AA	46 (41.82)	51 (46.36)	0.953	0.375	1.336	0.746–2.392
AG	47 (42.73)	39 (35.46)			1	
GG	17 (15.45)	20 (18.18)	0.785	0.434	0.705	0.325–1.529
Alleles						
A	139 (63.18)	141 (64.09)	0.461	0.587	1.203	0.706–2.049
G	81 (36.82)	79 (35.91)			1	

[a]Chi-square test. OR: Odds ratio, CI: Confidence interval, T2D: Type 2 diabetes, VDR: Vitamin D receptor.

For *TaqI* variants, the genotypes and allele frequencies in the control group and T2DM group are presented in Table 2. The AA, AG, and GG genotypes were 41.82%, 42.73%, and 15.45%, respectively, in the controls and 46.36%, 35.46%, and 18.18% in subjects with T2DM, respectively. The percentage of the A allele was 63.18% and 64.09% while the G allele was 36.82% and 35.91% in the control and T2DM groups, respectively. The genotype and allele frequencies of *TaqI* were statistically indifferent between both studied groups (p > 0.05).

These results indicate that the VDR *ApaI* and *TaqI* polymorphisms may not be associated with T2DM risk in studied Saudi subjects.

FBS and HbA1c in different genotypes of T2DM subjects

The level of the FBS in the *ApaI* TT, GT, and GG genotypes was 169.65 ± 14.28, 166.19 ± 14.47, and 169.75 ± 15.25, respectively, while the HbA1c ratio in these genotypes was 7.23 ± 1.38, 7.25 ± 1.7, and 7.69 ± 1.99, respectively. There was no significant difference in the FBS and HbA1c ratio between different genotypes of the VDR *ApaI* polymorphism (p = 0.455 and 0.555, respectively). The FBS concentration in the *TaqI* AA, AG, and GG genotypes was 169.07 ± 14.59, 165.15 ± 12.7, and 171 ± 17.25, respectively, while the HbA1c ratio in these genotypes was 7.53 ± 1.83, 7.19 ± 1.62, and 7.04 ± 1.11, respectively. There was no significant difference in the FBS level and HbA1c ratio between different genotypes of VDR *TaqI* polymorphism (p = 0.27 and 0.445, respectively) (Table 3).

Table 3: Comparison between Vitamin D receptor *ApaI* and *TaqI* genotypes with respect to fasting blood sugar and hemoglobin A1c in type 2 diabetes mellitus group

SNPs	VDR polymorphism	n	FBS concentration	F*	p	HbA1c (%)	F*	p
ApaI	TT	38	169.65 ± 14.28	0.792	0.455	7.23 ± 1.38	0.592	0.555
	GT	52	166.19 ± 14.47			7.25 ± 1.7		
	GG	20	169.75 ± 15.25			7.69 ± 1.99		
TaqI	AA	51	169.07 ± 14.59	1.324	0.27	7.53 ± 1.83	0.816	0.445
	AG	39	165.15 ± 12.7			7.19 ± 1.62		
	GG	20	171 ± 17.25			7.04 ± 1.11		

Data are shown as mean ± SD. *p values were calculated using ANOVA test. SD: Standard deviation, ANOVA: Analysis of variance, VDR: Vitamin D receptor, FBS: Fasting blood sugar, HbA1c: Hemoglobin A1c, SD: Standard deviation, SNPs: Single nucleotide polymorphisms.

Discussion

Diabetes mellitus is becoming a major health problem worldwide, particularly in Saudi Arabia. As stated by the World Health Organization (WHO), diabetes is a big problem in Saudi Arabia, ranking second worst in the Middle East and seventh worst in the world [22]. Both inherited and environmental variables are thought to have a role in the illness etiology [5]. Several potential genes – such as Vitamin D receptor – that are likely to cause T2DM susceptibility in diverse populations have been examined by various research groups.

However, to date, only few studies in Saudi Arabia have looked into the link between VDR gene polymorphism and susceptibility to T2DM.

In our study, we investigated the association between VDR *ApaI* and *Taq1* gene variants and T2DM

in a sample of Saudi persons suffering from T2DM who were matched with the control participants for gender and age.

The results showed that the BMI is higher – but not statistically significant – in T2DM subjects compared with the controls while the fasting blood sugar and HbA1c were significantly higher in the diabetic group compared with the controls. There was no significant change in the genotype and allele distributions of both *ApaI* and *TaqI* variants of the VDR gene between the studied groups. We also examined the association between the biochemical parameters in the form of FBS concentration and HbA1c ratio between the different genotypes of both *ApaI* and *TaqI* SNPS and found no statistical difference between the different genotypes of both SNPS.

The previous reports addressing the relationship between the VDR polymorphisms and the susceptibility to T2DM in different ethnicities showed differing results.

VDR gene (*BsmI, TaqI, FokI,* and *ApaI*) SNPs were investigated in T2DM in several ethnic populations and the results showed no association between these four SNPs and the susceptibility of T2DM (Polish population [23], French Caucasian population [24], and Turkish population [25]). These observations agree with and corroborate our findings that the VDR gene polymorphisms did not affect diabetes risk.

Interestingly, various researches investigating the association of VDR variants with diabetes in other ethnicities came up with opposing conclusions. In Kashmiri population, Malik *et al.* studied VDR *TaqI* and *BsmI* polymorphisms and discovered that the *BsmI* G allele is related to T2DM risk [26]. Similarly, Safar *et al*. in the United Arab Emirates investigated three VDR SNPs and their connection with T2DM, finding that the *TaqI* polymorphism is not linked to T2DM risk in the Emirati population [27]. Aldaghri *et al.* investigated the polymorphism of four SNPs in the VDR gene (*ApaI, FokI, TaqI, and BsmI*) in the Saudi population of Riyadh region and found a relationship between *BsmI* T allele and C/T genotype and T2DM [21]. These findings contrast with our observations in the Makkah district, which might be explained, among other things, by changes in the participants' genetic backgrounds or by unexplained environmental variables such as daily exposure to sunshine and temperature fluctuations.

Conclusion

Our findings from the VDR gene polymorphisms in the Makkah area imply that the VDR *ApaI* and *TaqI* SNPs may not have a role in T2DM risk among Saudis.

However, the present study has some limitations due to the small number of participants. More research is needed to evaluate VDR serological levels and associated metabolites, as well as related genetic analyses, in a larger T2DM cohort with clinical data. These studies will be crucial in determining the involvement of VDR in the pathogenesis of T2DM in a specific geographic and ethnic location.

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