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VDR Gene Polymorphisms in Kurdish Population and Its Relation to T1DM in Erbil-Iraq

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ABSTRACT

This research assessed the relationship among type 1 diabetes VDR gene polymorphisms (*Apa*I and *Taq*I) in the Kurdish population in Erbil-Iraq. Forty individuals with type 1 diabetes and thirty healthy people were recruited from the Kurdish population in Erbil, Iraq. Genomic DNA was taken from blood, being genotyped for SNP (single nucleotide polymorphisms). The distribution of VDR polymorphisms in two restriction fragment length polymorphism sites, *Taq*I and *Apa*I, was investigated in patients and controlled by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) utilizing *Apa*I and *Taq*I restriction enzymes. Using SPSS software (V15.0), the genotype dispersal and allelic incidences in patients and controls were compared. VDR polymorphism genotype dispersal and allele incidences vary dramatically among patients and controls. The results confirmed that the genotype GT in SNP *Apa*I was a risk factor among type 1 diabetes mellitus patients' combination that imparted the strongest susceptibility to T1DM (P=0.00023). Still, the SNP *Taq*I showed no relevance between cases and controls (P=0.35). Our findings indicate that VDR gene polymorphisms in the combination of genotypes are related to an increased risk of T1DM in the Kurdish community and warrant further investigation as a possible genetic risk marker for T1DM. More research is needed to corroborate this finding, particularly the VDR gene, which was studied for the first time in the Kurdish population.

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Introduction

T1DM is a kind of diabetes that most commonly affects children and adolescents with age below 30. Earlier research has revealed that the distribution of T1DM varies by population; the Scandinavian and Pacific Rim regions have the greatest and lowest incidence rates, respectively (1). The vitamin D receptor (VDR) gene, on chromosome 12q (12–12q14), is highly polymorphic (2,3). This gene has eight protein-coding exons (2–9) and also six untranslated exons (1a–1f), being alternatively spliced. 4 main sole nucleotide polymorphisms (SNPs) found at the three ends of the VDR gene, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I, have been widely studied (3,4).

Vitamin D receptor connections, in turn, could have a deleterious impact on patient diabetes and result in frequent DNA damage9. Although some studies have suggested that vitamin D intake has a favorable effect on DNA damage levels4, no study has found a link between VDR polymorphisms and the risk of DNA damage. The majority of VDR gene polymorphisms, including *Bsm*I, *Apa*I, and *Taq*I restriction fragment length polymorphisms (RFLPs), are found in the 3′ untranslated region (3′ UTR) in the VDR gene (5). 3′UTR of genes is vital in gene expression regulation, mainly through mRNA regulation (6).

The gene of vitamin D receptor (VDR) is found on chromosome 12 (12q13.11), has 11 exons, and spans 63495 base pairs (bp). SNPs (single nucleotide polymorphisms) in 8 exons (2–9). The three polymorphisms, *Apa*I, *Taq*I, and BsmI, are situated in the VDR gene's 3′ untranslated region (3'UTR) (7). The 3'UTR is believed to play a role in gene expression regulation, probably via controlling mRNA stability (8,9). Certain allelic variations of VDR polymorphisms have been linked to T1DM (10).

Thus, it is critical to comprehend the significance of genes in Iraqi communities, as these people may have a hereditary predisposition to T1DM. The current study sought to assess the polymorphism locations in the VDR genes *Taq*I and *Apa*I linked with type 1 diabetes in a group of Iraqi Kurds. This is the first study to show that our group's polymorphism locations in the VDR genes *Taq*I and *Apa*I are linked to type 1 diabetes.

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Materials and methods

A total of 40 type-1 diabetic patients (15 females and 25 males) mean age of 15.2 years and 30 healthy individuals with no disease were studied in this research. (12 females and 18 males) mean age ∓SD. 25.2 ± 5.39, and the family history in type 1 was (50% positive & 50% negative). The sample enrolled in this study was obtained from Layla Qassim Diabetic center in Erbil City. The Biology Department approved the study, and then it was performed in the same department, the College of Education, the University of Salahuddin. Patients were diagnosed according to WHO criteria, and all participants were informed about the study.

About (3-5) ml samples of venous blood were obtained by sterile syringe in sterile EDTA tubes. The DNA was taken from samples of blood by Wizard genomic DNA purification kit (Promega-USA) and saved until use at -20 °C.

To amplify genomic DNA, the PCR method with primers for every SNPs polymerase chain reaction (PCR) was used; further, to analyze the VDR gene, the PCR-RFLP technique was used (11,12). The primers were used to amplify A 745-bp fragment; Forward: 5′-CAGAGCATGGACAGGGAGCAAG-3′ and Reverse:5′-ACTCCTCATGGCTGAGGTCTCA-3′ (11). The PCR conditions program was done at 95°C for 5 min (initial denaturation), 35cycles at 94°C for 25 s, at 64°C for 30 s primer nailing, for 45 s at 72°C, and 5 min at 72°C (final extension). VDR gene's PCR products (5 µl) were processed in a 20-µl reaction volume with 1.5 U of *Apa*I at 37°C *for 4 h* and *Taq*I restriction endonucleases (Promega-USA) at 65°C. After visualizing under UV light, the restriction enzyme digestion products were examined on 2% agarose gel electrophoresis and staining with Ethidium bromide. There was no restriction enzyme cleavage site in the appearance of the T-allele, and a 745 bp product was produced. The cleavage products 529- and 215-bp were found in subjects harboring the 'G-allele.' The existence of 495bp and 245bp cleavage products was related to allele 'T,' while the existence of 290, 245, and 210-bp fragments were related to allele 'C' (11,12). The *Apa*I product G allele formed 528 & 217 bp fragments; T allele, 745 bp (Fig. 1), and *Taq*I T allele formed 494 & 251 bp fragments; C allele, 293, 251, and 201 bp (Fig.2).

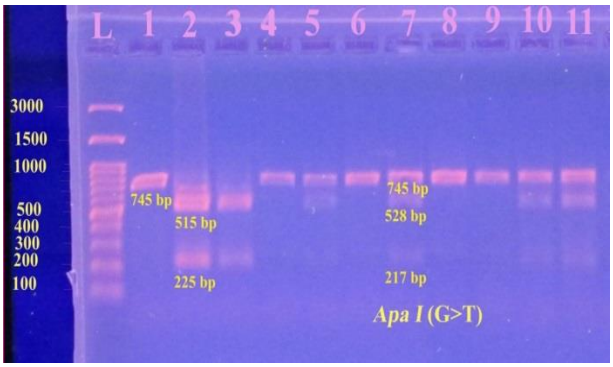

Fig. 1 The products of cleavage PCR-RFLP were analyzed on 2% agarose gel, and once stained using Ethidium bromide, they were visualized under UV light.
The polymorphism of VDR gene *ApaI*; the genomic DNA's PCR amplification was performed; then, amplification products were cleaved using restriction enzyme *ApaI.* Lane L, 100bp DNA Ladder; lane 1, 4, 6, 8, & 9 uncleaved PCR product; lanes 2 and 3 product from homozygous GG genotype; lanes 5, 7, 10, and 11 product from homozygous GT genotype; lane 1, 4, 6, 8 & 9 product from homozygous TT genotype.

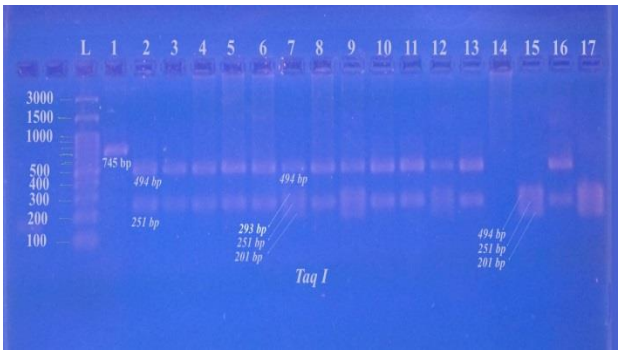

Fig. 2 VDR gene *Taq*I polymorphism detection. The genomic DNA's PCR amplification was performed; then, the amplification products were cleaved using restriction enzyme *Taq*I. Lane L, DNA Ladder; lane 1, uncleaved PCR product with 745 bp; lane 2, 3, 4, 5, 6, 8, 10, 11, 13, & 16 *Taq*I cleavage pattern from homozygous TT genotype; lanes 7 and 9 *Taq*I cleaved PCR product using heterozygous TC genotype; lanes 15 and 17 *Taq*I cleaved PCR product from CC genotype, lane 6, no sample. On 2% agarose gel, products were analyzed, and once stained using ethidium bromide, they were visualized under UV light. The number on the right side shows the product size (bp)

Statistical analysis

The Graphpad prism V 6 and Statistical Package for Social Science SPSS V (24) computer software analyzed data. The relationship significance of T1DM with Controls data was evaluated by the Chi-square ($\chi 2$) test. Genotype and allelic odds ratio, as well as a confidence interval of 95%, were measured for

determining the relationship between VDR gene *Apa*I and *Taq*I gene polymorphisms and T1DM. A *p-value* below 5% ($p<0.05$) was regarded as significant statistically.

Results and discussion

The technique applied to define the genotype of VDR gene *Taq*I polymorphism indicated significant differences in *Taq*I in both groups. The TC heterozygous genotype 57.5% was more common in the T1DM group compared to the control group (20%). The TT genotype of *Taq*I was more frequent in the control group as compared with the T1DM group (60% vs7.5%) repeatedly. Moreover, the CC allele was more frequent 35% in the T1DM group, while the TT allele was less (20%) frequent in the control group. The results confirmed a significant difference of the T1DM group with the control group in Case *ApaI* genotypes of VDR, P-value =0.00023. A similar drift was demonstrated in the case of 'C' allele was increased frequency in the T1DM group63.7% and decreased in the healthy control group was 30%. No significant difference was seen in the incidences of the genotypes or alleles between T1DM cases and controls, except for the G allele, being more widespread in T1DM patients than controls (Table 1 and 2). Even though *Apa*I polymorphism was unreliable in the present cohort and numerous other populations, the G allele was the minor among Iraqi Kurds.

This study compared the genotype frequency of patients and controls to see if there was a connection between two VDR gene variants and susceptibility to T1DM in Iraqi Kurds. A statistically significant relationship was identified among VDR gene *Taq*I polymorphism and T1DM in Kurds (Table 1).

Contrary to the positive relationships with the *Taq*I C-allele found in Kurdistan, Korea, & Kuwait, the findings in Germany revealed the prevalence of the TT genotype of the VDR gene *Taq*I polymorphism to be higher in T1DM patients as compared to in controls (11).

In Caucasians, the 3 VDR gene polymorphisms, *Apa*I and *Taq*I, are in substantial linkage disequilibrium, but there is no significant association disequilibrium with the *Fok*I polymorphism (13). The data from our research in Iraqi Kurds are strikingly similar to those in Caucasians since the relationship with T1DM was observed exclusively in the situation of *Taq*I polymorphism. The data from our study in Kuwaiti Arabs are also remarkably similar in *Taq*I polymorphism (14). A meta-analysis of Chinese adult subjects found that having the VDR gene BsmI polymorphism increased the incidence of T1DM in Africans, South Americans, Asians, and Turks (15).

It has been proposed that environmental factors, genetic susceptibility, lifestyle, and other factors cause variability in the link of the VDR gene polymorphisms with T1DM in different populations. This high variability and rapidly increased incidence of T1DM in various world populations could be owing to differences in ethnic background and nationality. These 4 VDR gene polymorphisms were examined for their connection with T1DM in a variety of populations, such as J*Apa*n, India, Kuwait, Iran, Turkish, and Finland, with mixed results (16,17). A South Indian analysis revealed that the b-allele of the VDR gene, BsmI polymorphism, was linked to T1DM (18). Nevertheless, no link was found between VDR gene polymorphisms and T1DM in Finnish cases (19). However, no significant link was found between three VDR gene polymorphisms (BsmI, *Apa*I, *Taq*I) and T1DM in Chilean cases (19). Sudanese data revealed a link between two VDR gene polymorphisms (BsmI, *Taq*I) and T1DM (20). In a Pakistani investigation, the VDR gene polymorphisms FokI and *Apa*I were indicated to be related to T1DM, whereas the *Taq*I polymorphism was not (21). An association of VDR gene polymorphisms with T1DM was shown in the Chinese Hans (22), Taiwan (23), Saudi Arabia (24), Korea (25), and Spain (26). Recent reviews and meta-analyses have explored the link between hereditary distinction in vitamin D genes and diabetes (27-29). Given the disparities in findings between population/ethnic groups, we investigated the incidence of two VDR gene variants in Iraqi Kurds in Erbil and their association with T1DM patients.

Conclusion

According to this research, the VDR gene *Taq*I polymorphism is connected with susceptibility to T1DM in Iraqi Kurds in the Kurdistan area. *Apa*I polymorphisms in the VDR gene did not demonstrate a favorable correlation with T1DM.

Table 1. Genotype/allele frequency of *VDR Taq*I (T>C) gene polymorphism in cases with T1DM and healthy control group

Genotypes/Allele	Frequency of T1DM Patients (N= 40) and (%)	Frequency of Healthy Controls (N= 30) and (%)	OR	(95% CI)	*P-Value*
g.60058 T>C					
TT (Wild type)	16 (40%)	10 (33.3 %)	1.371	0.4546 to 4.138	0.5745
TC (Heterozygous)	10 (25%)	8 (26.6 %)	1.071	0.3201 to 3.586	0.9109
CC (Mutant)	14 (35%)	12 (40 %)	1.238	0.4657 to 3.292	0.6683
T – Allele	42 (52.5%)	28 (46.66 %)	1.263	0.6458 to 2.471	0.4945
C – Allele	38 (47.5%)	32 (53.33 %)	0.7917	0.4048 to 1.548	0.4945

T1DM: Type 1 diabetes, CI: Confidence Interval; OR: Odds Ratio

Table 2 Allele/genotype frequency of *VDR ApaI* (G>T) gene polymorphism in cases with T1DM and healthy control group

Genotypes/Allele	Frequency of T1DM Patients (N= 40) and (%)	Frequency of Healthy Controls (N= 30) and (%)	OR	(95% CI)	*P-Value*
g.59979 G>T					
GG (Wild type)	3 (7.5%)	18 (60 %)	0.07143	0.0151 to 0.337	0.0003
GT (Heterozygous)	23 (57.5%)	6 (20.0 %)	1.643	0.4422 to 6.104	0.4563
TT (Mutant)	14 (35%)	6 (20.0 %)	0.4643	0.1536 to 1.403	0.1692
G – Allele	29 (36.25 %)	42 (70.0 %)	0.2437	0.1191 to 0.4988	< 0.0001
T – Allele	51 (63.73 %)	18 (30.0 %)	4.103	2.005 to 8.398	< 0.0001

T1DM: Type 1 diabetes mellitus, CI: Confidence Interval; OR: Odds Ratio

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None

Conflict interest

The authors declare no conflict of interest.

Author's contribution

Galawezh O. Othman did all the works alone.

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