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**Association of Vitamin D Receptor Gene Polymorphism (Taq1) in Sudanese Children with Type 1 Diabetes Mellitus at Gezira State, Sudan.**

Mohammed A Hamza<sup>1</sup>, Khalid E. Khalid<sup>1</sup>, Osman K Saeed<sup>2</sup> and Huda M Haroun<sup>3</sup>

1. Department of Biochemistry and nutrition, Faculty of Medicine, University of Gezira

2. Department of Medicine, Faculty of Medicine, University of Gezira.

3. Department of Pediatric, Faculty of Medicine, University of Gezira.

**Corresponding author:** Mohammed A Hamza. Department of Biochemistry and nutrition, Faculty of Medicine, University of Gezira. P.O.box:20 Wad - Medani,Sudan.Tel: +249511854279 - Fax: +249511843415 - E.mail: faggad94@yahoo.com

**Abstract:**

**Introduction:** Diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.

**Objectives:** This study aimed to determine the frequency of selected VDR gene polymorphism Taq1(rs731236) single nucleotide polymorphism in diabetic and non-diabetic children to assess its contribution to the susceptibility to type 1 diabetes (T1D).

**Methods:** One hundred children with T1D were enrolled in this study; 50 healthy non-diabetic children served as a control group for detection of VDR gene polymorphism Taq1. This polymorphism was detected by using the polymerase chain reaction (PCR) and restriction fragment polymorphism (RFLP) analysis.

**Results:** There was a significant difference between frequencies of Taq1(*t* allele) ((P= 0.05; Odd Ratio = 0.412; 95% CI 0.182 - 0.932).

**Conclusion:** VDR TaqI polymorphism was associated with T1D in Sudanese children.

**المخلص:**

يوصف مرض السكري بأنه من المسببات المرضية المتعددة التي تتميز بفرط سكر الدم المزمن واضطراب في التمثيل الغذائي ( اضطرابات من الكربوهيدرات والدهون واستقلاب البروتين) الناتج عن نقص في إفراز الأنسولين، عمل الأنسولين أو كليهما.

هدفت هذه الدراسة لتحديد تكرارات تعدد شكل الجين المستقبل لفايتمين د في مجموعتي المرضى والأصحاء ولتقييم دوره في الإصابة بالسكري عند الأطفال. اشتملت هذه الدراسة علي 100 طفل مصابين مرض السكري و50 أصحاء من نفس العمر. تضمنت هذه الدراسة تكرارات تعدد شكل الجين المستقبل لفايتمين د (Taq1) في مجموعتي المرضى والأصحاء. أظهرت هذه الدراسة ان تكرار الاليل (*t*) في الجين المستقبل لفايتمين د يختلف اختلاف ذو معني عند مجموعة الأصحاء مقارنة بالمرضى مما يعني ان لهما دور في الحماية من الإصابة بالسكري عند الأطفال.

خلصت هذه الدراسة الي ان التعدد في الشكل الجيني لمستقبل فايتمين د (Taq1) لهما علاقة بالإصابة بالسكري النوع الأول عند الأطفال السودانيين.

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### **Introduction:**

Type 1 diabetes (T1D) is an autoimmune condition in which the body's immune system has attacked and destroyed the beta cells of the pancreas. As a result, there is a shortage of insulin, and glucose cannot enter the cells. Body processes involving the storage of glucose as energy and the utilization of glucose are adversely affected. The body is essentially starved of the energy it needs for normal function <sup>(1)</sup>.

Type 1 diabetes is a multifactorial disease with strong genetic component. The major T1D susceptibility locus maps to the HLA class II genes and accounts for up to 30%–50% of genetic T1D risk. Other non-HLA T1D loci were known to produce smaller effects on disease risk compared to HLA genes. These loci include insulin gene (INS), cytotoxic T-lymphocyte associated protein 4 (CTLA4) genes and protein tyrosine phosphatase non receptor type 22 (PTPN22) gene <sup>(2)</sup>.

The involvement of vitamin D in the etiology of both type 1 and type 2 diabetes has been suggested. Vitamin D compounds are known to suppress T-cell activation by binding to the vitamin D receptor (VDR). Polymorphisms of the VDR gene are likely to be related to T-cell mediated autoimmune disease <sup>(3)</sup>.

Vitamin D is a fat-soluble vitamin that plays an important role in bone metabolism and seems to have some anti-inflammatory and immune-modulating properties <sup>(4)</sup>. Most of these biological actions of vitamin D are considered to be exerted through the nuclear vitamin D receptor (VDR)-mediated control of target genes <sup>(5)</sup>.

The VDR gene, located on chromosome 12q13.11 encodes a polypeptide that binds 1, 25-dihydroxy-calciferol and interacts with target nuclei to produce a variety of effects <sup>(6)</sup>. Several major polymorphic sites have been described within the VDR gene. FokI in exon 2, Bsm I and Apa I both in intron 10 and Taq I in exon 11 are the four common single nucleotide polymorphisms (SNPs) for the VDR gene <sup>(7)</sup>.

Taq I polymorphism (rs731236) is a Restriction Fragment Length Polymorphism (RFLP) in exon 11 of the VDR gene. Taq I is a thymine to cytosine polymorphism (thymine: T allele and cytosine t allele). SNP lies on the forward strand of the VDR gene on position 65058 <sup>(8)</sup>.

**Study Objective:** to test the association VDR (TaqI) gene polymorphism to the susceptibility to T1 DM in Sudanese children.

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### **Methods:**

This cross-sectional study was carried out in Wad Medani Paediatric Hospital. One hundred Sudanese children with type 1 diabetes mellitus were enrolled in this study (diabetic group) with age up to 16 years, and 50 healthy children with no history of DM, or other autoimmune diseases serving as age-matched (control group),

Exclusion Criteria Age >16 years.

A questionnaire was designed to obtain data including: demographic data (age, sex) clinical information (duration of clinical symptoms, date of diagnosis, age at onset of disease, duration of the illness, family history and laboratory investigations).

Two ml of blood sample was collected from the diabetic patients and from the control in a tube containing the anti-coagulant (EDTA). Detection of VDR Polymorphism (Taq1) by genotyping methods polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP)

Genomic DNA was prepared using the QIAamp DNA Blood Mini Kit (Qiagen, USA).

### **DNA extraction:**

Genomic DNA was prepared using the QIAamp DNA Blood Mini Kit, 2 ml of EDTA blood was washed three times using Tris Base and Ethylene Diamine Tetra Acetic Acid (EDTA) 20:5 buffer (TE 20:5), centrifuged and the cells pellet was added to 1.5 ml microcentrifuge tube containing 20µl of Proteinase K enzyme and vortexed for 15 seconds, then 200 µl of lyses buffer (AL buffer) was added and the tube was vortexed for 15 seconds and incubated in water bath at 55 °C for 10 minutes after that 200 ml of cold absolute ethanol was added and vortexed for 15 seconds and transferred to the QIAamp spun column and centrifuged at 8000 round per minute (rpm) for one minute, then the column was placed in a new 2 ml collection tube. The tube containing the filtrate was discarded and washed with 500 µl of AW1 buffer and centrifuged at 8000 rpm for one min, the column was placed in a new 2 ml collection tube and the tube containing the filtrate was discarded and washed with 500 µl AW2 buffer and centrifuged at 8000 rpm for 3 minutes, then the column was placed in a new 1.8 ml microcentrifuge tube and the tube containing the filtrate was discarded, after that 100 µl of elution buffer (AE buffer) was added and the tube was incubated at room temperature for 5 minutes followed by centrifugation at 8000 rpm for one minute and preserved at -20 °C till used.

### **Genotyping of VDR gene TaqI polymorphism**

PCR amplification of the region containing the polymorphism were performed using the forward primer in intron 8 (5' GGGACGCTGAGGGATGGCAGAGC-3) and the reverse primer in exon 9 (5 GGAAAGGGGTTAGGTTGGACAGGA-3).

The PCR conditions were as follows: initial denaturation step at 94°C for 10 min, and 30 cycles using the following temperature profile: denaturation at 94°C for 1 min, , annealing at 62°C for 1 min, extension at 72 °C for 1 min, and final elongation for 5 min. The PCR products were bands of 716 (T alleles).

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**Digestion of Taq I polymorphism with TaqI (ER0671 Restriction) Enzymes:**

PCR Product was digested with TaqI # (ER0671 3000u) Lot: 00095040.

5' ...TCGA...3

3'... AGCT...5

Concentration: 10u/ µL

Source: *Thermus aquaticus* YT-1

Supplied with: 2 x 1 ml of 10X Buffer TaqI

1 ml of 10X Buffer Tango

10 mM Tris – HCL (pH 8.0), 5 mM MgCl<sub>2</sub>, 100 mM NaCl, 0.1mg/ml BSA.

PCR reaction mixture 10 µL (0.5 µg of DNA)

Nuclease free water 18 µL

10XBuffer B 2 µL

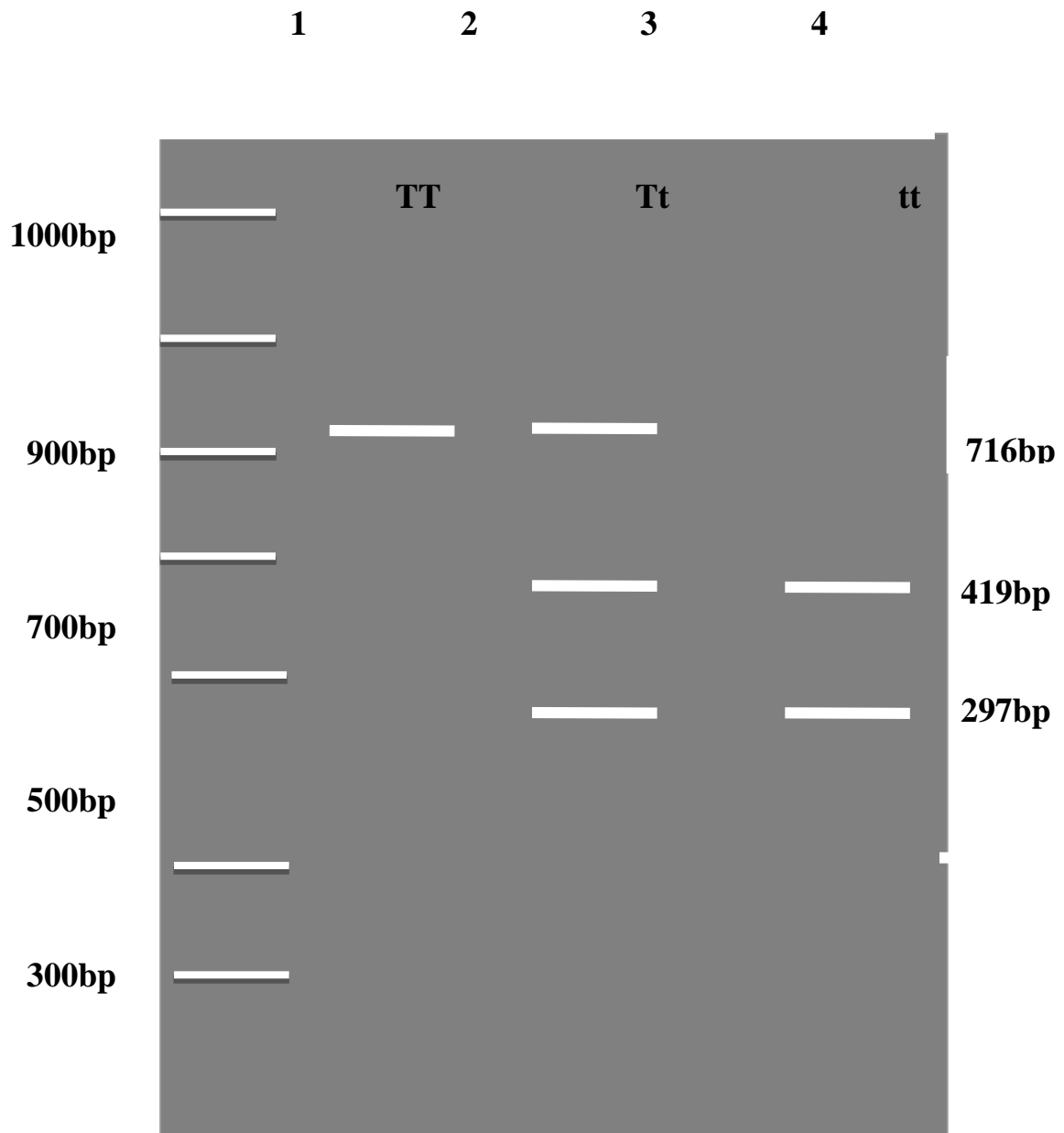
TaqI 2 µL

The tubes contents were mixed gently and spun down for a few seconds. Incubated at 65 °C for 16 hours.

**Interpretation of TaqI digestion reaction electrophoresis profiles:**

Complete cleavage into the 419 bp and 297 bp fragments is characteristic profile of the homozygous mutant (tt) and incomplete cleavage into 419 bp, 297 bp and 716 bp is of the heterozygous (Tt). While absolute absence of digestion is characteristic of the homozygous wild type (TT) (Figure 1). Digested DNA was loaded on a 2 % agarose gel (SeaKem<sup>®</sup> GTG<sup>®</sup> agarose, FMC BioProducts) stained with 5ul of ethidium bromide before electrophoresed at 150 V for 30 minute. Then it was visualised by UV light gel documentation system as described earlier by <sup>(9)</sup>.

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**Figure (1).The expected bands pattern of PCR-RFLP genotyping of Taq1 polymorphism**

Lane1:100-bp DNA marker, Lane2:wild-type homozygote (TT),Lane3:heterozygote (Tt) and lane 4:mutant homozygote (tt).

**Statistical analysis:**

A chi-squared test ( $\chi^2$  test) was used to evaluate the associations between different genotypes of Bsm1, Taq1, and Apa1 variants and the disease (T1DM vs. controls). Odd

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ratios (ORs) and 95% confidence intervals (CIs) were calculated for each allele and genotype using logistic regression. The differences were considered significant if the *P*-value less than 0.05.

**Results:**

**Gender, age and BMI**

The characteristics of the study subjects are shown in Table 1. The male to female ratio in diabetic group was 53/47.while in non diabetic group was 28/22.

The family history of diabetes mellitus (FHDM) classified as negative or positive.

The negative to positive ratio of FHDM in control group was 36/14, while in the diabetic group was 34/66.

The mean ( $\pm$ SD) age for diabetic subjects was  $11.48 \pm 3.39$ ; and the mean age at onset of disease was  $7.68 \pm 3.59$  years, and for the control is depicted in Table 1.

The mean ( $\pm$ SD) BMI in the diabetic group was significantly low ( $P < 0.001$ ) as compared with the control group ( $16.22 \pm 2.37$  vs.  $19.10 \pm 4.61$  respectively) as presented in table 1.

**Table (1): Characteristics of diabetics and control groups.**

Characteristics		Diabetics (N=100)	Control (N=50)
Gender	Male	53	28
	Female	47	22
Age (years)		$11.48 \pm 3.39$	$8.50 \pm 4.23$
Age at onset of disease		$7.68 \pm 3.59$	-
Duration (years)		$3.82 \pm 2.82$	-
Family history of diabetes	Negative	34	36
	Positive	66	14
BMI (kg/m <sup>2</sup> )		$16.22 \pm 2.37$	$19.10 \pm 4.61$ ( <b>P &lt; 0.001</b> )

**Genetic analysis**

**Taq1 genotype and allele frequency in diabetic and control groups**

There was a significant difference between genotypes frequencies of Taq1 in the two groups (*P*-value = 0.012) (Figure 2 and Table 2).

The allele's frequencies in control group were 0.49 for *t* and 0.51 for *T* and the allele frequencies in diabetic group 0.46 for *t* and 0.54 for *T*. There was a significant difference

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between allele frequencies of *t* ( $P= 0.05$ ; Odd Ratio = 0.412; 95% CI 0.182 - 0.932) (Table 3).

**Table (2): Genotype frequency of Taq1 in diabetic patients and controls**

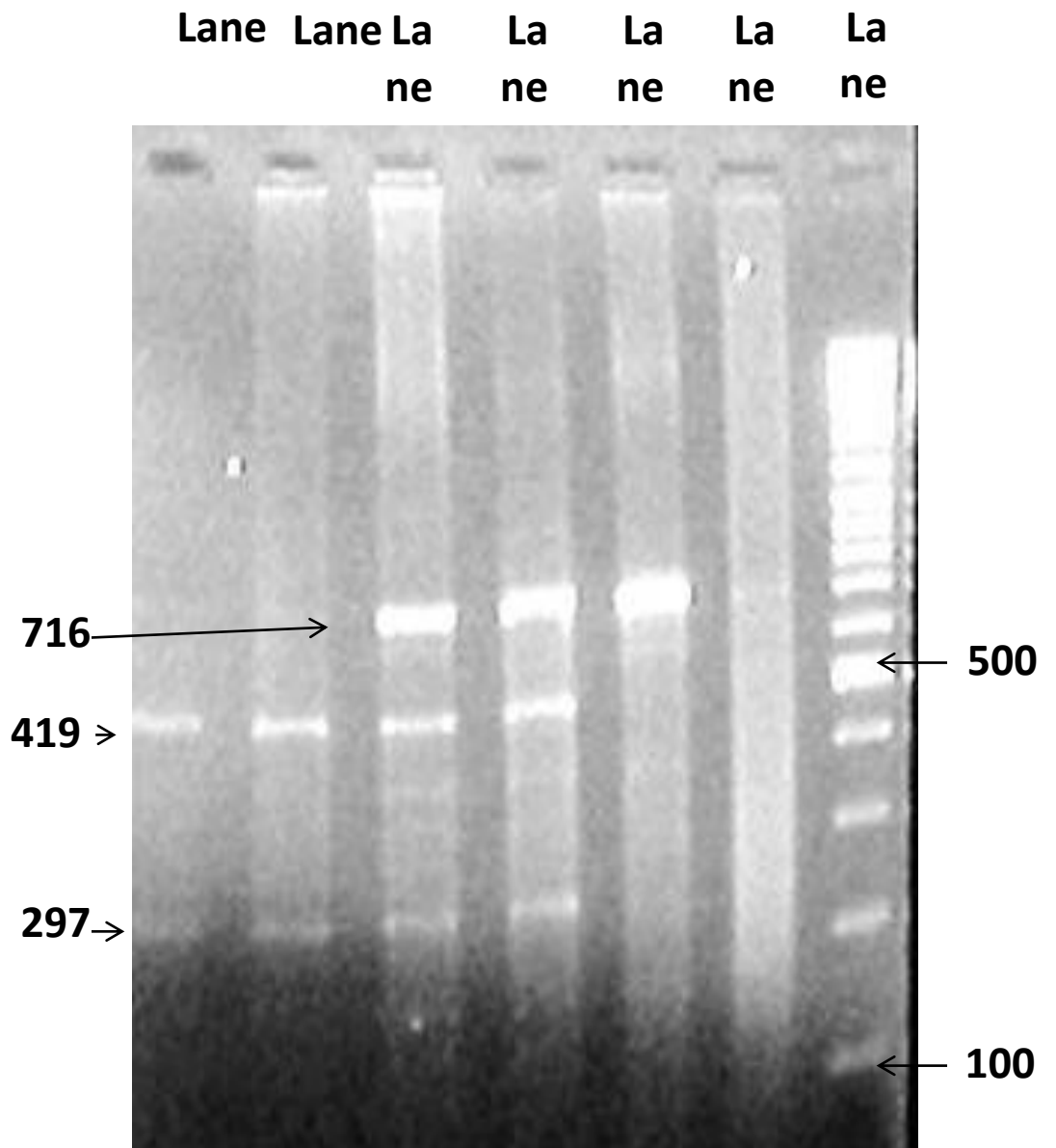
Taq1 Group	Genotypes		
	TT	Tt	tt
Diabetic patients	22(22%)	63(63%)	15(15%)
Control	16(32%)	19 (38%)	15(30%)
<i>P-value</i>		<b>0.012</b>	
$\chi^2$		<b>8.877</b>	

**Table (3): allele frequency of Taq1 in diabetic patients and controls**

Taq1 Group	Genotypes		Allele	
	TT + Tt	tt	T	t
Diabetic patients	85(85%)	15(15%)	0.54	0.46
Control	35(70%)	15(30%)	0.51	0.49
<i>P-value</i>	<b>= 0.05</b>			
$\chi^2$	<b>= 4.688</b>			

(Odd Ratio = 0.412; 95% CI 0.182 - 0.932).

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**Figure (2): Detection of Taq1 polymorphism using PCR-RFLP method**

Lane 1 and 2: mutant homozygote (tt), Lane 3 and 4: heterozygote (Tt), Lane 5: wild-type homozygote (TT), and Lane 7: 100-bp DNA marker.

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### **Discussion:**

Vitamin D has important biological functions, such as modulating immunity system, influencing insulin secretion and improving insulin resistance<sup>(10)</sup>, thereby could be involved in the etiology of DM and more likely to be influenced by VDR gene polymorphisms. A significant difference between genotypes frequencies of TaqI in the two groups. TT genotype and T allele was more frequent in T1DM patients compared with healthy controls. These results are similar to those reported by<sup>(11)</sup>.

This finding in line with two previous studies from Germany and France which showed an association between the TT genotype and diabetes<sup>(12,13)</sup>.

In this study, comparing patients to controls revealed that T alleles was more prevalent in patients than in controls on expense of t alleles which were observed to be higher in control group. This result may propose the possibility that t alleles have a protective role against T1D. This result showed disagreement with other studies on Egyptians conducted on healthy individuals<sup>(14-16)</sup>. Studies on Iranians<sup>(17)</sup> and Syrians<sup>(18)</sup>, reported that T allele is predominant in control group. The apparent discrepancies between our results and other studies could be due to ethnic differences and sample heterogeneity related to the distribution of VDR polymorphisms in these populations, as well as to interactions with other genetic or environmental factors involved in the pathogenesis of type 1 diabetes.

The most frequent allele of Taq I polymorphism among T1D patients was T allele. This finding in line with Other results on Italian<sup>(19)</sup> populations, the T allele was predominant

### **Conclusions:**

VDR TaqI polymorphism is associated with type 1 diabetes in Sudanese children, and the carrier of mutant homozygote tt were protected from type 1 diabetes than wild-type homozygote TT and heterozygote Tt.

Sudanese children who have TaqI polymorphisms and carrier for tt are more protected from diabetes than carriers of other genotypes.

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