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Original Article

Association of Vitamin D Receptor (VDR) FokI Polymorphism with Type 1 Diabetes Mellitus in a Sulaimani City

Zanyar A. Hussein[a] and Gaza F. Salih[a, *]

[a]*Biology Department, College of Sciences, University of Sulaimani, Sulaimani City, Kurdistan Region, Iraq.*

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ABSTRACT

Type 1 diabetes mellitus is an autoimmune disorder characterized by pancreatic β-cell destruction and lifelong metabolic complications. Vitamin D and its receptor (VDR) play an important role in immune regulation and insulin secretion. This study aimed to investigate the association between the VDR Fok1 polymorphism (rs2228570), Vitamin D status, and T1DM in a pediatric Kurdish population from Sulaimani city, Iraq. A case-control study was conducted, including 71 participants: 40 children and adolescents with T1DM and 31 age- and sex matched healthy controls (2-18 years). Genomic DNA was extracted, and VDR Fok1 genotyping was performed using PCR and sequencing. Clinical parameter including serum vitamin D3 levels, random blood sugar, Hemoglobin A1c, and body mass index (BMI), were assessed. Allele frequency analysis revealed a significantly higher frequency of the C allele in T1DM patients than in controls (76.3% vs. 58.1%; $P < 0.05$), whereas the T allele was more prevalent in controls. The CT heterozygous genotype was significantly more frequent among controls than T1DM patients (64.5% vs. 32.5%, P=0.043). Serum vitamin D3 levels were significantly lower in T1DM patients (P<0.001), whereas HbA1c and BMI values were significantly higher compared with controls (p<0.001). In conclusion, the VDR Fok1 polymorphism, particularly the CC genotype, is associated with T1DM in the pediatric Kurdish population. These findings support a potential role of VDR genetic variation and Vitamin D status in T1DM susceptibility, warranting further studies with larger sample sizes.

1. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder that leads to elevated blood sugar levels. The disease develops when insulin production drops or when the body shows reduced response to insulin action in the periphery or through a combination of both factors (Pociot 2017). The combination of long-term high blood sugar with other metabolic issues in DM patients results in dangerous and disabling health problems that threaten their survival (Yang et al. 2024). The four primary complications of diabetes are microvascular retinopathy, nephropathy, neuropathy, and macrovascular disease, all of which collectively increase the risk of cardiovascular disease by two to four times(Garofolo et al. 2019). Type 1 diabetes mellitus causes destruction of B cell this cause stop in insulin production (Aravindhan et al. 2021). The global prevalence of type 1 diabetes shows a documented annual increase of 3%. Projections indicated that the incidence of type 1 diabetes was expected to rise by 40% compared to

* Corresponding author.
E-mail address: Gaza.salih@univsul.edu.iq (G. Salih)

1998 levels by the year 2010(Pugliese 2017). The body requires Vitamin D as an essential fat-soluble vitamin, which performs multiple vital functions, including maintaining calcium/phosphorus balance, bone health, insulin secretion, and insulin sensitivity improvement (Argano et al. 2023). It has a role in regulating innate and adaptive immunity, including suppression of pro-inflammatory cytokines and modulation of T cell activity. The human body produces 80–90% of its vitamin D by exposing the skin to ultraviolet B (UVB) rays from sunlight, while 10–20% comes from dietary sources (Alaei-Shahmiri et al. 2020). The worldwide prevalence of insufficient vitamin D levels reaches approximately 1 billion people (Holick et al. 2011).

Vitamin D does some really important things in our bodies. It works by binding to a special receptor called the Vitamin D receptor. This receptor is like a messenger that helps our body understand what to do. First, vitamin D gets converted into a different form in the liver, called 25-hydroxyvitamin D. Then, it gets sent to the kidneys where it's activated into its most powerful form, 1,25-dihydroxyvitamin D_3. This active form of vitamin D binds to the VDR, which then helps control the way certain genes work. These genes are involved in some big tasks, like helping immune system work properly, keeping pancreas healthy, and making sure pancreatic cells are working like they should. So, vitamin D plays a crucial role in keeping body running smoothly and preventing certain health problems(Carlberg and Muñoz 2022).

The VDR gene exists on chromosome 12q13.11 inside the cell nucleus where it connects with 1,25-dihydroxy vitamin D (Moradkhani et al. 2024).

This gene shows high polymorphism through 1862 known single-nucleotide polymorphisms (SNPs), which can be found in the NCBI-maintained dbSNP database(Nogales and Dediego 2019).

The VDR gene shows high polymorphism through 1862 known SNPs, which can be found in the NCBI-maintained dbSNP database. The majority of VDR SNPs exist as silent non-functional SNPs, which do not affect VDR gene or protein expression or functionality. The protein sequence can be modified through interactions between exon-based Polymorphisms and these silent SNPs. The two possible forms of exon SNPs exist as synonymous or non-synonymous variations(Moradkhani et al. 2024). The FokI polymorphism (rs2228570) located in exon 3 affects the translation initiation site and results in two protein isoforms that differ in length by three amino acids (Ruiz-Ballesteros et al. 2020). Research indicates that the shorter 'C' allele enhances VDR transcriptional activity. The BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236) polymorphisms reside in the 3' untranslated region (UTR) or intron 8 of the VDR gene and influence mRNA stability, which in turn affects the levels of VDR protein. The functional effects of these polymorphisms remain less clear than those of the FokI polymorphism (Zhai et al. 2020). The three SNPs exist in strong linkage disequilibrium because they tend to be inherited together as a unit. The Fok1 polymorphism occurs when the T/C ratio changes in DNA (Ruiz-Ballesteros et al. 2020). The mutation transforms the ATG start codon into ACG, which leads to a different start site and results in a new start codon located nine bases later. The FokI polymorphism creates a shorter VDR protein, which has 424 amino acids instead of the normal 427 amino acids found in the wild-type protein (Ruiz-Ballesteros et al. 2020). In this study we work at Sulaimani city with the child diagnosed with T1DM to detect which polymorphisms in Sulaimani dominants and detection the relationships between the Fok1 polymorphism with T1DM.

2. Material and Methods

2.1. Study Design

This study employed a case-control design to investigate the association between the (VDR) Fok1 polymorphism and vitamin D deficiency among patients with type 1 diabetes mellitus (T1DM) in Sulaymaniyah City, Kurdistan Region, Iraq. A total of 71 participants were enrolled between December 2024 and May 2025, including 40 T1DM patients (20

males and 20 females) and 31 healthy controls (16 males and 15 females). Participants of both sexes were included in the study. Type 1 diabetes mellitus patients were recruited from Dr. Jamal Hospital for Children, Sulaymaniyah, while molecular analyses were conducted at the Molecular Research Laboratory, University of Human Development.

The study protocol was approved according to decision number (13) at 9/12/2025 by the institutional ethics committee of the University of Sulaimani. Written informed consent was obtained from all parents or legal guardians of all participants prior to sample collection.

2.2. Inclusion and Exclusion criteria

Diagnosis of type 1 diabetes (T1DM) was established according to the World Health Organization (WHO) criteria. The case group included children and adolescents aged 2-18 years with confirmed T1DM. The control group consisted of age and sex matched individual without diabetes, defined by normal fasting blood glucose levels, no personal history of diabetes or other autoimmune disorders, and no known chronic systemic disease.

Exclusion criteria for both groups included current or recent (within the past 6 months) vitamin D supplementation, presence of chronic hepatic, renal, or endocrine disorders, acute or chronic inflammatory or infectious diseases, use of medication known to affect vitamin D metabolism, and malnutrition. Participants with incomplete clinical data were excluded.

To minimize confounding effects, information regarding disease duration, vitamin D supplementation, sun exposure, and nutritional status was collected and recorded to reduce potential confounding.

2.3. Study Protocol

A total of 10 mL of fasting venous blood was collected from each participant. The collected blood volume was justified by the need to perform multiple biochemical and molecular analyses from a single venipuncture, thereby avoiding repeated blood sampling in pediatric participants. The sample was divided in to a serum gel tube (4 ml) for measurement of random blood sugar (RBS), and serum 25-hydroxyvitamin D {25(OH) D} and two EDTA tubes for assessment of glycated hemoglobin (HbA1C) (3 ml) and genomic DNA extraction(3 ml).

Biochemical analysis of RBS and HbA1c were performed using the Roche/Hitachi Cobas C311 analyzer (Germany), while serum 25(OH)D concentration were measured using the Cobas e411 analyzer (Germany).

2.4. DNA extraction and conventional PCR

Genomic DNA was extracted from peripheral whole blood using the Addprep Genomic DNA Extraction Kit (AddBio Inc., South Korea) in accordance with the manufacturer's instructions. Briefly 200 µL of whole blood was mixed with 20 µL proteinase K and 200 µL Binding Buffer, followed by incubation at 56 °C for 10 minutes with gentle agitation. Subsequently, 200 µL of absolute ethanol was added, and the mixture was transferred to a GD spin column and centrifuged. The column was washed twice using the provided wash buffers and centrifuged to remove residual ethanol. DNA was eluted in 100 – 200 µL elution buffers and stored at -20°C until further analysis.

2.5. DNA concentration and quality control

Genomic DNA was extracted from blood samples using (Adddrop Genomic DNA extraction). Quantity and purity were assessed by NanoDrop spectrophotometry (260 – 280 nm), with results summarized in Table 1.

2.6. Conventional PCR

Primers specific for VDR Fok1 (rs2228570) polymorphic region were designed based on the reference VDR gene sequence and synthesized by Macrogen Inc. (Seoul, South Korea). Primer sequences and characteristics include forward Primer are 5'-ATGTATGAGGGCTCCGAAGG-3' with 55% of GC ratio, and reverse primer are 5' -

CTTGGGTGAGGAAGCTGTGA-3' and have same GC% as the forward primer with an expected amplicon size of 685 bp.

PCR amplification was performed in a 20 µL reaction volume containing genomic DNA, forward and reverse primers, Taq PCR Master Mix, and nuclease-free water. The amplification protocol consisted of an initial denaturation at 95 °C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 35 seconds, annealing at 62 °C for 20 seconds, and extension at 72°C for 70 seconds, with a final extension at 72 °C for 5 minutes.

To validate PCR performance and prevent contamination, negative control reactions (No template control) were included in each PCR run.

2.7. Sequencing Alignment

The obtained nucleotide sequences were subjected to alignment analysis using the National Center for Biotechnology Information (NCBI) tools. Sequence alignment was performed using the Basic Local Alignment Search Tool (BLAST) algorithm under the nucleotide-to-nucleotide (BLASTn) option. Each sample sequence derived from both Type 1 diabetes mellitus patients and healthy controls was aligned against the Human Vitamin D receptor (VDR) reference sequence retrieved from the NCBI GeneBank Database (Accession no.: NG_008731.1). This approach enables the identification of sequence variations and confirmation of the targeted polymorphic region.

2.8. Determination of Zygosity at a Specific Nucleotide

Zygosity at the polymorphic nucleotide position was determined through visual inspection of chromatin files using Chromas software (version 2.6.6). Homozygous and heterozygous genotypes were identified based on the presence of single or overlapping nucleotide peaks at the target locus. Overlapping peaks at the same nucleotide position were interpreted as heterozygous variants, while single distinct peaks indicated homozygosity.

2.9. Statistical analysis

Statistical analysis was performed using Graphpad Prism software (version 9.9). Continuous variables were expected as mean ± standard deviation (SD). Comparisons between two independent groups (Type 1 diabetes mellitus patients and healthy controls) were conducted using the independent samples t- test.

Genotype and allele frequencies were calculated by direct counting and compared between groups using the chi-square test, and the odds ratio was used. Hardly- Weinberg equilibrium (HWE) was evaluated in the control groups using the chi-square goodness-of-fit test. A P-value of 0.05 was considered statistically significant. However, p-value <0.001 and P<0.0001 were reported.

3. Results

3.1. Demographic and clinical characteristics

A total of 71 participants were included in the study, comparing 40 children with T1DM and 31 healthy controls. The mean age of T1DM group was 10.95 ±3.64 years (range: 2-18 years). No statistically significant difference in age or sex distribution was observed between the two groups ($p>0.05$).

Body mass index was evaluated using BMI – for-age-z-scores. A significant difference in MBI z-scores was observed between the T1DM and control groups ($p=0.003$).

Clinical comorbidities observed among patients with Type 1 Diabetes Mellitus (T1DM) are summarized in Table 2. Of the 40 individuals in the T1DM group, 31 (77.5%) presented with at least one associated health condition, whereas only 9 (22.5%) reported no additional comorbidities. In contrast, no chronic health conditions were identified among participants in the control group.

Table 1. NanoDrop spectrophotometry results for extracted DNA samples. All samples show pure DNA (A260/A280: 1.8-2.0) suitable for PCR and SNP genotyping. Mean ± SD values confirm high quality.

Sample ID	Concentration (ng/uL)	A260/280	A260/A280	Quality notes
Sample 1	45.2	1.85	2.10	Pure DNA, No contamination
Sample 2	52.8	1.92	2.15	Pure DNA
Sample 3	48.5	1.88	2.05	Pure DNA
Sample 4	50.1	1.90	2.12	Pure DNA
Sample 5	47.9	1.87	2.08	Pure DNA
Mean ± SD	48.9 ± 2.6	1.88 ± 0.03	2.10 ± 0.04	All suitable for downstream application

Table2. Clinical health problems observed in T1DM patients (n=40).

Health condition	Number	Percentage (%)
Eye disorder	13	32.5%
Thyroid disorder	6	15.0%
Kidney disorder	3	7.5%
Nerves disorder	3	7.5%
Skin condition	3	7.5%
Other chronic disease	3	7.5%

3.2. Vitamin D3 level

Serum Vitamin D levels were measured in both groups. Mean vitamin D levels were significantly lower in the T1DM groups (19.0±7.86 ng/mL) compared with the control group (24.61±6.50 ng/mL), with a P value of 0.019. Highlighting the need for routine monitoring and appropriate management. These data are presented in Figure 1.

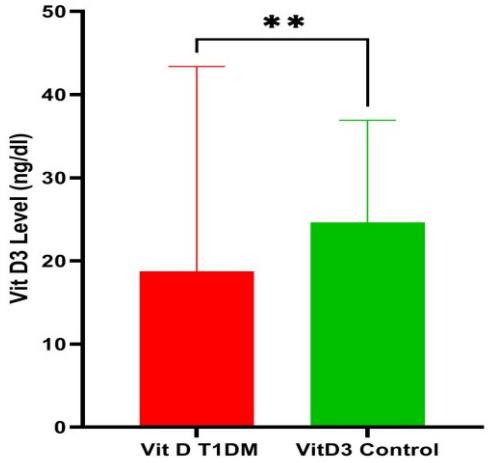

Figure 1. Show a significant difference of level Vitamin D3 in control and diabetic group.

3.3. Glycated Hemoglobin (HbA1c) Add figure for this

HbA1c levels were assessed in all participants. The T1DM group showed a mean HbA1c level of 9.67%±2.34%, whereas the control group showed a mean level of 5.24%±0.30%. The difference between groups was statistically significant ($p<0.0001$). Figure 2 explains it clearly.

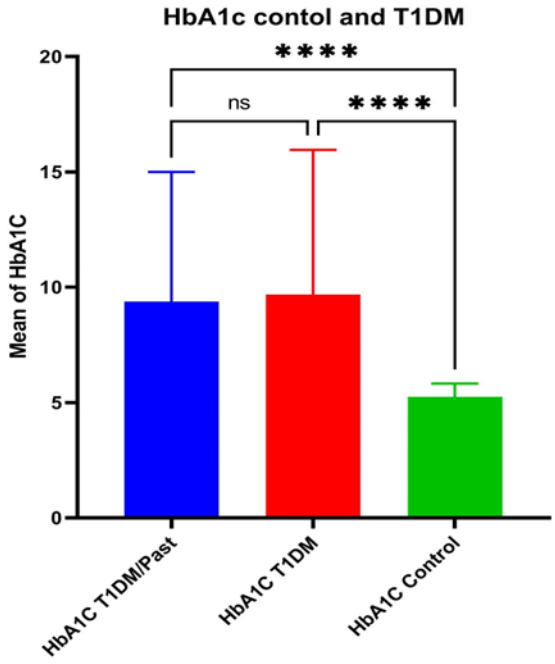

Figure 2. Comparison of mean HbA1c levels among the study groups. The bar graph illustrates the mean ± standard deviation (SD) of HbA1c (%) in patients with Type 1 Diabetes Mellitus (T1DM), individuals with a past history of T1DM, and healthy control subjects. HbA1c levels were significantly higher in both T1DM groups compared with the control group (**** $p < 0.0001$), whereas no statistically significant difference was observed between current T1DM and past T1DM groups (ns).

3.4. Random Blood Sugar (RBS)

Random Blood Sugar levels were measured in both T1DM and healthy controls. The mean RBS level in the T1DM group was (144.5±19.87 mg/dL), which was significantly higher than that of the control group (118.2±17.1 mg/dL). Statistical analysis showed a highly significant difference between the two groups (p<0.0001). These data are shown in Figure 3.

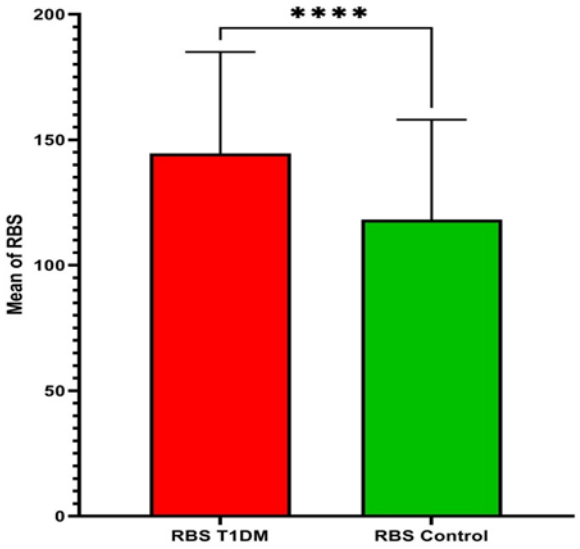

Figure 3. Comparison of Random Blood Sugar (RBS) levels between Type 1 Diabetes Mellitus (T1DM) and Control groups. Bars represent the mean of RBS value, The T1DM group shows a significantly higher mean RBS compared to the control group. (****p<0.0001).

3.5. Molecular studies

This study analyzed the distribution of the VDR Fok1 polymorphism genotypes among T1DM patients and healthy controls. For statistical analysis genotype were categorized into three groups: CC (homozygous mutant), CT (heterozygous), and other genotypes (TT, GC, and GG) due to their low frequencies.

The CC genotype was observed at a significantly higher frequency in the T1DM group compared with controls (60.0% vs. 25.81%). In contrast, the CT genotype was more prevalent among controls than T1DM patients (64.52% vs. 32.5%). The remaining genotypes were detected at low and comparable frequencies in both groups.

Chi-square analysis demonstrated a significance in genotype distribution between T1DM patients and controls (p=0.002). Furthermore, odds ratio analysis indicated that the CC genotype was significantly associated with increased risk of T1DM, while the CT genotype showed a protective association. No significant association was observed for the remaining genotypes. Genotype categorization in Table 3, while allele frequency and association analyses are presented in Table 4.

Genotype distribution of the VDR Fok1 polymorphism differed significantly between T1DM patients and controls (X2 test, p=0.043). The CC genotype was more frequent in the T1DM group (60.0%) compared with the control (25.8%). Allele frequency analysis showed a higher frequency of the C allele of in the T1DM patients (60 vs. 38) with an odds ratio of 2.31(95% CI 1.14 – 4.70, p=0.020).

3.6. BMI

In the T1DM group (n=40, mean age 10.5±4.2 years) mean BMI 19.2±3.1 kg/m2 , corresponding to a mean z-score of 0.12±1.05 (55th percentile; range: -1.8 to 1.5). Four children (10%) were obese (≥95th percentile), all adolescents. The control group (n=31, mean age 9.8±4.5years) had a mean BMI 16.8±3.6kg/m2 , z-score -0.25 ±1.12 (40th percentile), with no group differences (p=0.21, Mann-Whitney U). Updated values are in Table 5.

3.7. Gel electrophoresis

Agarose gel electrophoresis confirmed successful PCR amplification of a 685 bp fragment targeting the VDR Fok1 polymorphism region. Lane 1 contained a 100 bp DNA ladder for size reference, lane 2-11 represented patient samples, and lane 12 served as the negative control, with all sample lanes exhibiting sharp, specific bands at the expected position and no non-specific amplification or smears. Figure 4 shows gel documentation with labels.

Table 3. Genotype distribution and odds ratios of VDR Fok1 polymorphism in T1DM patients and controls.

Genotype	T1DM Group N:40	Control Group N:31	Odds Ratio (95% CI)	P- value
CC	24 (60.0%)	8 (25.81%)	1.86 – 15.28	0.002
CT	13 (32.5%)	20 (64.52%)	0.12 – 0.78	0.01
Other genotypes (TT,GC,GG)	3 (7.5%)	3 (9.67%)	0.14 – 4.18	0.75

Table 4. Allele frequency distribution and association analysis of the VDR Fok1 polymorphism in T1DM and controls. Statistical method: Odds ratio calculated using a 2*2 contingency table; 95% confidence interval computed by the Wald method.

Allele	Control	T1DM	OR	95% CI	P- value
C	38	60	2.31	1.14 – 4.70	0.020
T	20	17	Reference	_____	_____
G	4	3	_____	_____	_____

Table 5. Age – sex-BMI Z score and Percentile.

Group	n	Mean age Years	Mean BMI (kg/m²)	Mean Z score	Mean percentile	% obese (≥95th)
T1DM	40	10.5±4.2	19.2±3.1	0.12±1.05	55th	10%
Control	31	9.8±4.5	16.8±3.6	0.25±1.12	40th	3%

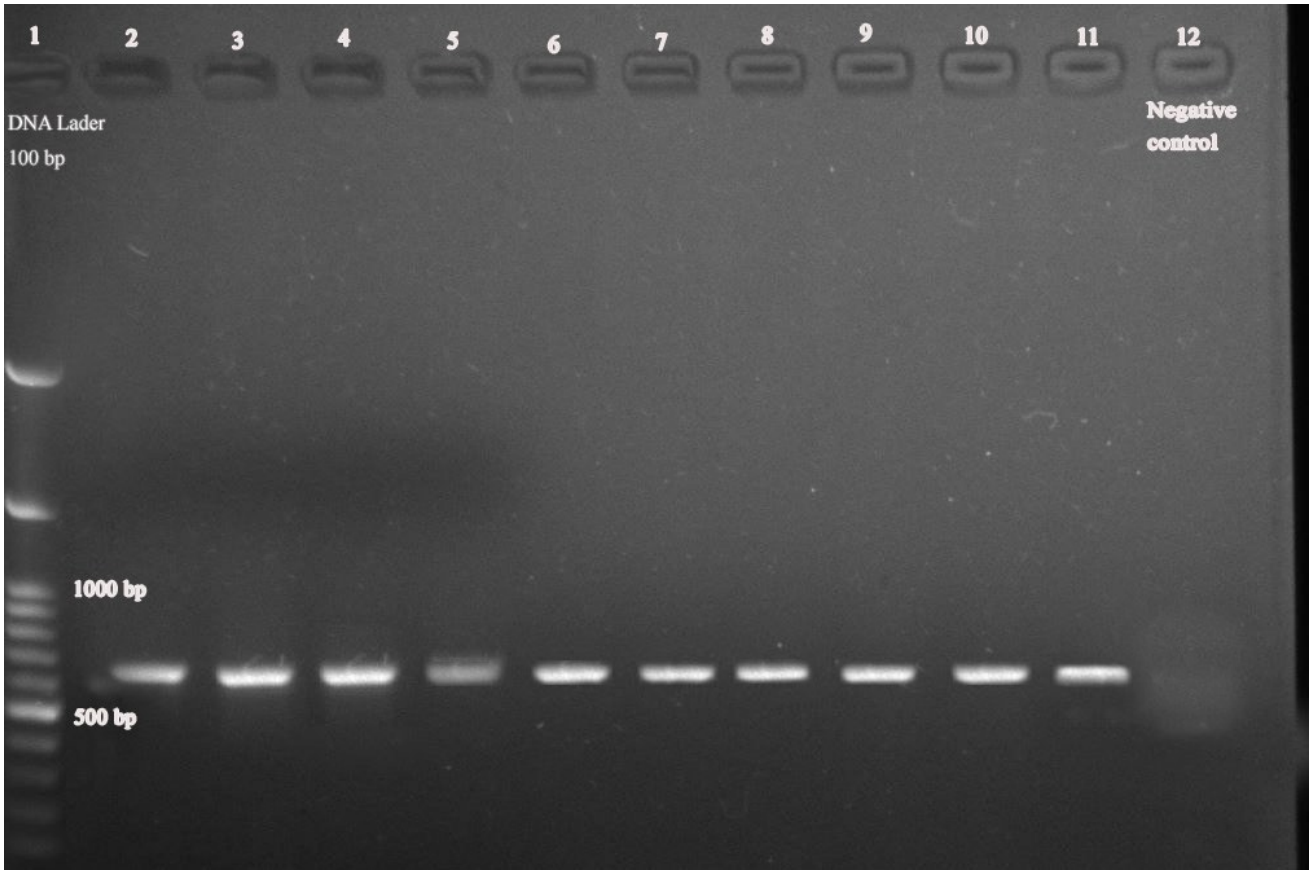

Figure 4. Agarose gel electrophoresis of VDR Fok1 PCR products. Lane 1:100 bp ladder, Lane 2-11: T1DM patient samples; negative control. All lanes show a distinct 685 bp band indicating specific amplification.

Sequence alignment provides clear data to detect a specific type of nucleotide at a specific point. Figure 5 show alignment sample with the subject sequence it shows that the targeted sequence site was (cytosine) instead of (thymine). As a result, this polymorphism (rs2228570) changes the start codon from ATG (Met; F allele) to ACG (Thr f allele), producing a shorter VDR protein. (424 vs. 427 amino acids). Sequence alignment for all samples was done.

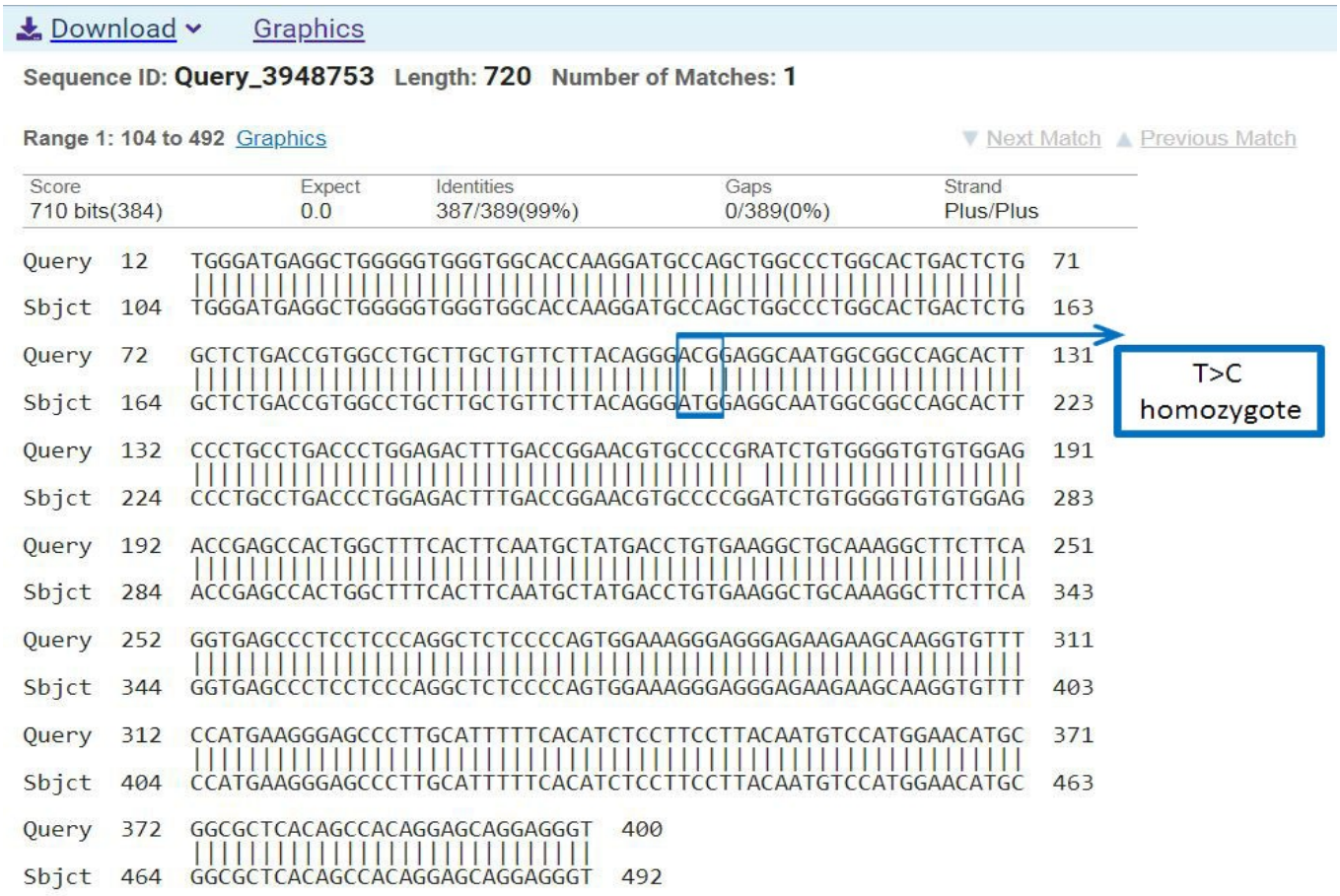

Figure 5. NCBI BLASTn alignment of the sample sequence (query) against the reference VDR Fok1 sequence (subject). The alignment reveals a Cytosine (C) at the polymorphic site (rs2228570) instead of thymine (T), corresponding to the F allele of the Fok1 polymorphism.

3.8. Zygosity Profiling for Fok1 polymorphism (rs2228570)

Determination of heterozygote or homozygote by using Chromas Graph is clearly shown by the explanation of peaks. Figure 6, show homozygote of a specific sequence; just a single peak appears it mean a single nucleotide for this specific location. In the displayed sequence, the target nucleotide of interest is position 108, which is cytosine (C). Chromas displays sequence peaks with specific colours for each base: green for A, red for T, blue for C, and black for G, enabling the identification and assessment of nucleotide composition at each position of the sequence.

Detecting the number of peaks at a specific location indicate homo or a heterozygote of a sample. Figure 7, show the heterozygote. This Chromas chromatogram shows the DNA sequence around position 238, where there is a notable double peak represented by the letter "Y," which indicates the presence of both cytosine (C, blue peak) and thymine (T, red peak) at this position. This double peak pattern signifies a heterozygous genotype, meaning

the sample contains two different nucleotides at the same locus, reflecting genetic variation. Chromas visualize such heterozygous sites by displaying two overlapping peaks of similar height and intensity for the involved nucleotides. This visual confirmation is important for identifying (SNPs) and verifying genotype calls, with homozygous sites showing a single, sharp peak and heterozygous sites showing distinct dual peaks at the same position.

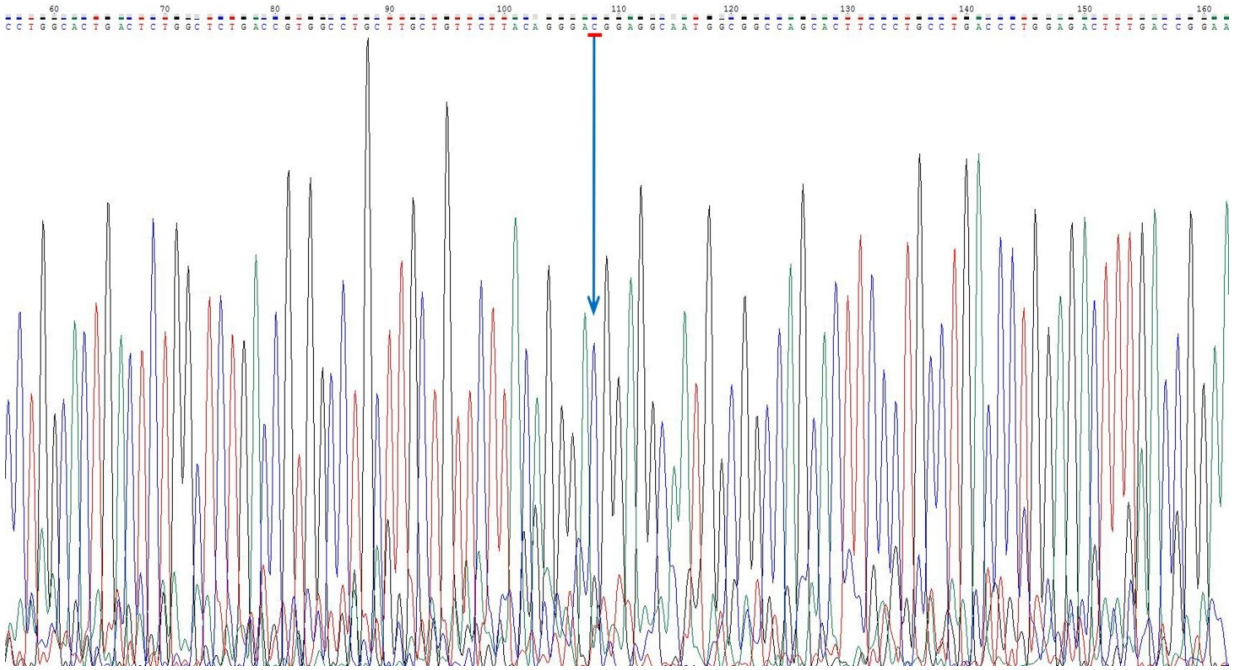

Figure 6. Chromas chromatogram of homozygous CC (Fok1 F allele) sample at position 108 (rs2228570). A single Blue peak (C) confirms homozygosity.

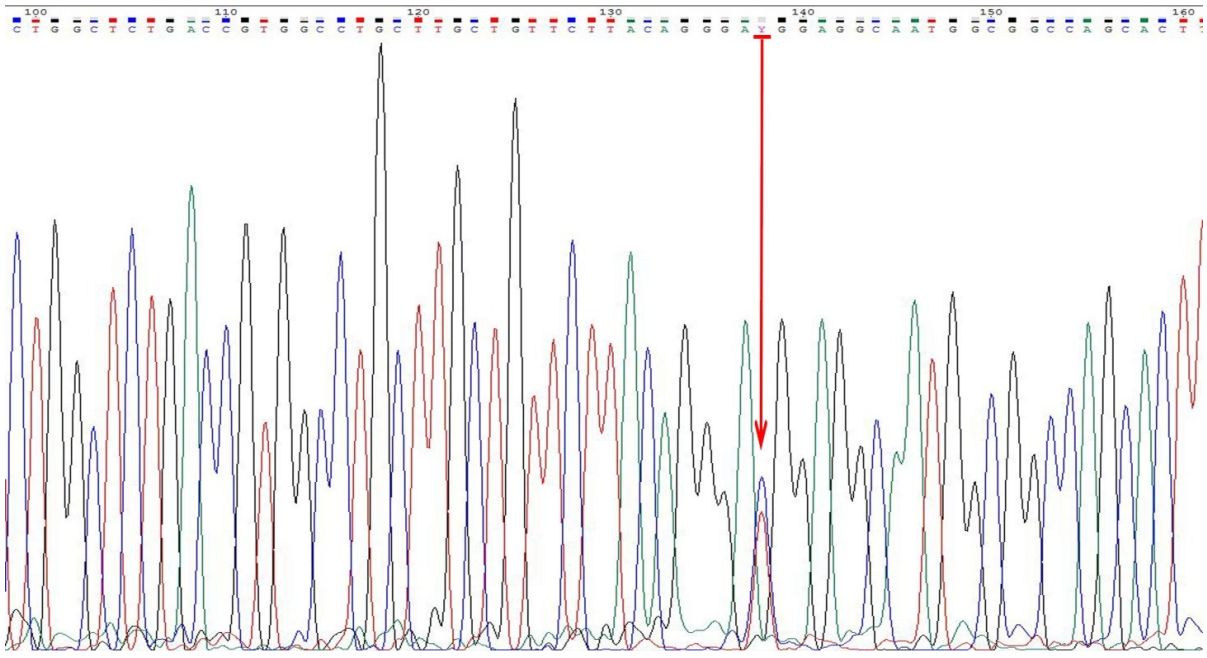

Figure 7. Chromas chromatograph of a heterozygous CT (Fok1 F/f allele) sample at position 238 (rs2228570). Overlapping Blue (C) and red (T) peaks of equal height indicate heterozygosity.

4. Discussion

The present study examined the association between Vitamin D receptor (VDR) Fok1 polymorphism (rs2228570) and Type 1 Diabetes Mellitus (T1DM) in a pediatric cohort, alongside key clinical and anthropometric parameters.

The absence of a significant age difference between T1DM patients and controls suggests that age-related developmental factors are unlikely to confound the observed genetic associations. This aligns with previous pediatric studies indicating that VDR polymorphisms exert their potential influence independently of age during childhood and adolescence, supporting the validity of age-matched genetic comparisons in autoimmune disease research (Khalid, 2016)

The higher BMI observed among children with T1DM reflects a growing shift in the phenotypic presentation of the disease. Traditionally associated with lean body mass, T1DM is now increasingly reported alongside overweight and obesity in the pediatric population (Fellinger et al. 2019). This trend has been particularly noted in Middle Eastern and Mediterranean regions, where rapid nutritional transition, reduced physical activity, and urbanization contribute to increased adiposity in children(Pappa et al., 2022; S Aljabri, 2018). In children with T1DM, insulin therapy may further promote weight gain through enhanced anabolic activity and improved caloric utilization. However, BMI in the paediatric population in an indirect measure of adiposity and is influenced by age and sex; therefore, these findings should be interpreted as coexistence rather than a pathophysiological consequence of T1DM (Zheng et al. 2022). Importantly, excess adiposity may exacerbate insulin resistance and complicate glycemic management, highlighting its clinical relevance in pediatric diabetes care (Boucher-Berry, Parton, and Alemzadeh 2016).

Elevated HbA1c levels in T1DM patients reflect persistent hyperglycemia and remain a central indicator of long-term glycemic control. Poor glycemic control is a well-recognized risk factor for the development of diabetes –related complications, particularly in pediatric patients with longer disease duration. In addition to disease duration, glycemic control in children and adolescents is influenced by multiple factors, including insulin regimen and adherence, dietary patterns, physical activity, frequency of blood glucose monitoring, and psychological aspects such as diabetes distress and family support (Costa et al. 2025). The observed HbA1c levels are consistent with international pediatric diabetes cohorts and underscore the ongoing challenge of achieving optimal metabolic control during childhood and adolescence (Boteva et al. 2020; Mojun et al., 2025). These findings reinforce established clinical knowledge rather than introducing novel mechanistic interpretations.

The presence of multiple diabetes-related complications among T1DM patients underscores the systemic and progressive nature of the disease. Microvascular complications, including ocular, renal, and neurological involvement, are known to emerge with prolonged hyperglycemia and immune-mediated tissue damage. Additionally, the relatively high frequency of thyroid disorders supports the autoimmune clustering characteristic of T1DM, as autoimmune thyroid disease frequently coexists due to shared genetic and immunological pathway (Viswanathan 2015). These observations emphasize the need for integrated, multisystem monitoring in pediatric T1DM rather than implying direct causation from genetic variation alone.

With respect to genetic findings, differences in the distribution of Fok1 genotype between T1DM patients and controls suggest a possible association between VDR polymorphism and disease susceptibility. The Fok1 polymorphism alters the translational start site of the VDR gene, resulting in a receptor with modified transcriptional activity, which may influence immune regulation. Vitamin D signalling plays a critical role in modulating T-cell response and autoimmune vulnerability (Carlberg and Muñoz, 2022; Christakos et al., 2015).

However, previous studies investigating the association between Fok1 polymorphism and T1DM have produced inconsistent results. Notably, the magnitudes of the association observed in the present study appear stronger than those reported in several investigations. While earlier studies reported modest effect sizes for the CC genotype, with odds ratios generally ranging from approximately 1.5 to 3.0 (Zemunik et al. 2005; S. B. Sahin et al. 2012). The present study demonstrated a higher effect size (OR=5.33). This difference may reflect population–specific genetic background, environmental variation in vitamin D exposure, differences in sample size, or methodological heterogeneity among studies. In this context, the present findings contribute to the existing body of evidence suggesting population-specific genetic susceptibility rather than a universal genetic determinant.

Importantly, this study does not support causal inference. As an association study, it can only indicate a correlation between the VDR Fok1 polymorphism and T1DM risk, without establishing a direct mechanistic role. The absence of functional analysis limits interpretation of how this polymorphism may influence VDR activity, immune signalling, or pancreatic B-cell autoimmunity.

Several limitations must be acknowledged. The most sample size reduces statistical power and may limit generalizability. The lack of functional assays prevents direct evaluation of the biological impact of the Fok1 variant. Additionally, serum 25-hydroxyvitamin D levels were not integrated into genotype-phenotype interaction analysis, which could provide further insight into gene- environment interactions. Future studies incorporating larger, ethnically diverse cohorts, functional experiments, and longitudinal follow-up are warranted to clarify the immunogenetic role of VDR polymorphism in T1DM.

In summary, this study supports a potential association between VDR Fok1 polymorphism and T1DM susceptibility in children, within a boarder framework of metabolic and autoimmune factors. These findings highlight the complexity of T1DM pathogenesis and reinforce the need for cautious interpretation of genetic associations in pediatric autoimmune diseases.

5. Conclusions

Based on his study, the CC genotype of the CDR gene polymorphism was significantly associated with Type 1 Diabetes Mellitus (T1DM) in Kurdish population of Sulaimani city. T1DM participants carrying the CC genotype exhibited higher HbA1c and BMI values and lower 25-hydroxyvitamin D levels compared to controls. These results indicate a potential association between VDR genetic variation and metabolic parameters in T1DM. However, no conclusions regarding causality, disease management, or prevention can be drawn from the present data. Further studies with a larger sample size and functional analyses are required to clarify the role of VDR polymorphisms in T1DM susceptibility.

Conflict of interest

The authors declare that there is no conflict of interests.

CRediT authorship contribution statement

Fund

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