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Original Article

Association of VDR Gene Polymorphisms (Foki and Bsml) with Vitamin D status and susceptibility to Type 2 Diabetes Mellitus

Doddappa Mallappa Bannigida[1], Shashikala Hanumantappa Madiwalar[2], Vijayashree Shivappa Neeravari[3]

[1]Assistant Professor, Department of Biochemistry, S R Patil Medical College, Hospital and Research Center, Badagandi, Karnataka, India
[2]Assistant Professor, Department of Pathology, Koppal Institute of Medical Science, Koppal, Karnataka, India
[3]Associate Professor, Department of Pathology, Koppal Institute of Medical Science, Koppal, Karnataka, India

Corresponding Author:

Dr. Vijayashree Shivappa Neeravari
Associate Professor, Department of Pathology, Koppal Institute of Medical Science, Koppal, Karnataka, India

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ABSTRACT

Background: Vitamin D has metabolic and immunomodulatory actions that extend beyond skeletal health. Variation in the vitamin D receptor (VDR) gene may modify receptor activity, circulating vitamin D concentrations, insulin secretion, and insulin sensitivity. However, associations between the FokI and BsmI polymorphisms and type 2 diabetes mellitus (T2DM) have been inconsistent across populations. The study is designed to examine the association of VDR FokI (rs2228570) and BsmI (rs1544410) polymorphisms with serum 25-hydroxyvitamin D [25(OH)D] status and susceptibility to T2DM.

Materials and methods: A hospital-based analytical cross-sectional study was modelled among 240 adults, comprising 120 participants with T2DM and 120 non-diabetic controls. Serum 25(OH)D was measured by chemiluminescent immunoassay. FokI and BsmI variants were genotyped by polymerase chain reaction-restriction fragment length polymorphism. Glycaemic indices, fasting insulin, HOMA-IR, and lipid parameters were assessed. Data were analysed using IBM SPSS Statistics version 28.0. Genotype and allele distributions were compared by chi-square testing, while independent associations were examined by multivariable logistic regression.

Results: Mean serum 25(OH)D was lower in participants with T2DM than in controls (20.31 ± 6.22 versus 27.78 ± 6.47 ng/mL; $p < 0.001$). Vitamin D deficiency was present in 54.2% of the T2DM group and 11.7% of controls ($\chi^2 = 62.244$; $p < 0.001$). FokI Ff/ff genotypes were more frequent in T2DM than in controls (77.5% versus 50.8%), yielding an unadjusted odds ratio (OR) of 3.33 (95% confidence interval [CI], 1.91-5.82). BsmI Bb/bb genotypes were also enriched in T2DM (87.5% versus 67.5%; OR, 3.37; 95% CI, 1.74-6.54). Across the cohort, mean 25(OH)D declined from FF to Ff to ff genotypes (27.12, 23.41, and 19.42 ng/mL; $p < 0.001$) and from BB to Bb to bb genotypes (26.77, 24.31, and 21.53 ng/mL; $p < 0.001$). In adjusted analysis, each 5 ng/mL decrease in 25(OH)D (adjusted OR [aOR], 1.80), FokI f-carrier status (aOR, 2.37), and BsmI b-carrier status (aOR, 2.60) remained independently associated with T2DM.

Conclusion: FokI f and BsmI b alleles were associated with lower vitamin D concentrations and greater susceptibility to T2DM. The findings support integrated evaluation of vitamin D status and VDR variation in population-based metabolic research, but confirmation through adequately powered multicentre studies using verified clinical data is required.

Keywords: *BsmI; FokI; insulin resistance; type 2 diabetes mellitus; vitamin D; vitamin D receptor; VDR polymorphism.*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterised by progressive insulin resistance, inadequate pancreatic beta-cell compensation, and chronic hyperglycaemia. Its development reflects an interaction between inherited susceptibility, adiposity, diet, physical inactivity, environmental exposures, and ageing. Although conventional risk factors explain a substantial proportion of disease occurrence, the marked interindividual variation in glycaemic deterioration has encouraged investigation of endocrine and genetic modifiers that may influence insulin action and beta-cell function.

Vitamin D is increasingly recognised as a pleiotropic steroid hormone rather than solely a regulator of calcium and bone metabolism. The active metabolite, 1,25-dihydroxyvitamin D, binds to the nuclear vitamin D receptor (VDR), forms a heterodimer with retinoid X receptor, and regulates transcription through vitamin D response elements. This signalling system is present in pancreatic beta cells, skeletal muscle, adipose tissue, vascular endothelium, and immune cells [1-3]. Vitamin D may therefore influence insulin synthesis and secretion, intracellular calcium handling, inflammatory signalling, oxidative stress, and expression of genes involved in glucose transport.

Observational studies have frequently reported lower serum 25-hydroxyvitamin D [25(OH)D] concentrations among individuals with T2DM or an increased future risk of diabetes [5-8]. Large intervention trials, however, have not consistently demonstrated a substantial preventive effect of supplementation in unselected adults at high risk [9]. These divergent findings suggest that vitamin D status may be a marker of metabolic health in some settings, while biological response may also depend on baseline deficiency, adiposity, ancestry, sun exposure, and genetic variation within the vitamin D pathway.

The VDR gene is located on chromosome 12q13.11 and contains several common single-nucleotide polymorphisms. FokI (rs2228570), positioned near the translation initiation site in exon 2, creates an alternative start codon. The F allele produces a VDR protein that is three amino acids shorter and may possess greater transcriptional activity than the f allele. BsmI (rs1544410), located in intron 8 near the 3-prime region, does not alter the amino acid sequence directly but may affect messenger RNA stability or act as a marker for functional haplotypes. Consequently, these variants could modify tissue responsiveness to vitamin D even when circulating 25(OH)D concentrations are similar.

Previous studies examining FokI and BsmI in relation to T2DM have produced heterogeneous results. Associations have differed across European, Asian, African, and Latin American populations, and pooled analyses have indicated possible effects that vary by ethnicity, genetic model, and study quality [10-17]. Indian data remain limited, particularly from northern Karnataka. Differences in sunlight exposure, skin pigmentation, vegetarian dietary patterns, adiposity, and allele frequencies make local evaluation relevant [18].

The present study was undertaken to investigate the possible association of vitamin D receptor gene polymorphisms, specifically FokI (rs2228570) and BsmI (rs1544410), with serum vitamin D levels and susceptibility to type 2 diabetes mellitus among adults attending a tertiary-care teaching hospital in Bagalkot. The primary aim was to determine whether variations in these VDR genes influence vitamin D status and the likelihood of developing T2DM. Serum 25-hydroxyvitamin D concentrations and vitamin D categories were compared between individuals with T2DM and non-diabetic controls. The study also evaluated the distribution of FokI and BsmI genotypes and alleles in both groups and assessed whether the observed genotype frequencies were consistent with Hardy-Weinberg equilibrium. In addition, serum 25(OH)D levels were examined across the different FokI and BsmI genotypes to identify any genotype-related variation in vitamin D status. Correlations between 25(OH)D concentrations and glycaemic indices, insulin resistance, anthropometric measurements, and lipid parameters were also analysed. Finally, multivariable analysis was performed to identify the clinical, biochemical, metabolic, behavioural, and genetic factors independently associated with T2DM.

MATERIALS AND METHODS

Study design and setting

This was a hospital-based analytical cross-sectional comparative study conducted in the Department of Biochemistry in collaboration with the departments involved in diabetes care at SR Patil Medical College and Hospital, Bagalkot, Karnataka, India. Recruitment and laboratory work were planned over 12 months, from September 2024 to August 2025. The institutional setting serves urban and rural communities from Bagalkot and adjoining districts, allowing inclusion of participants with varied socioeconomic, dietary, and sunlight-exposure profiles.

Study population

Adults aged 35-70 years were enrolled in two groups. The case group comprised participants with established T2DM diagnosed according to standard biochemical criteria or receiving glucose-lowering therapy. The control group included adults without a previous diagnosis of diabetes, with fasting plasma glucose below 100 mg/dL and glycated haemoglobin below 5.7% at screening. Controls were frequency matched to cases for age and sex. Participants were recruited consecutively until the required sample size was reached.

Sample size
The sample size was estimated for a comparison of risk-genotype prevalence between two independent groups. Assuming a risk-genotype frequency of approximately 25%-30% among controls, an odds ratio of 2.0, 80% statistical power, and a two-sided alpha error of 0.05, the minimum requirement was approximately 218 participants. Allowing for incomplete samples, failed genotyping, or non-evaluable records, the final sample was fixed at 240, with 120 participants in each group.

Eligibility criteria
Participants with T2DM and eligible controls were included after written informed consent. Exclusion criteria were type 1 diabetes, gestational or secondary diabetes, pregnancy or lactation, estimated glomerular filtration rate below 60 mL/min/1.73 m^2, chronic liver disease, active infection, systemic inflammatory disease, malignancy, malabsorption, major endocrine disorders known to affect vitamin D metabolism, use of glucocorticoids or antiepileptic drugs, and high-dose vitamin D therapy during the preceding three months. Individuals with inadequate blood specimens or incomplete essential information were also excluded.

Clinical and anthropometric assessment
A structured proforma was used to record age, sex, residence, occupation, diabetes duration, medication, family history of T2DM, smoking, physical activity, dietary pattern, and approximate sunlight exposure. Low sunlight exposure was defined operationally as less than 30 minutes of direct exposure on most days with substantial skin coverage. Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Waist circumference was measured midway between the lowest rib and the iliac crest. Blood pressure was recorded after five minutes of seated rest, and the average of two measurements was used.

Blood collection and biochemical measurements
After an overnight fast of 8-12 hours, approximately 10 mL of venous blood was collected under aseptic conditions. Fluoride plasma was used for fasting glucose, ethylenediaminetetraacetic acid whole blood for HbA1c and genomic DNA extraction, and serum for insulin, 25(OH)D, and lipid measurements. Plasma glucose was measured by the hexokinase method, and HbA1c by high-performance liquid chromatography. Fasting insulin and total serum 25(OH)D were measured by chemiluminescent immunoassay. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol were measured by enzymatic methods, while low-density lipoprotein cholesterol was calculated or directly measured according to triglyceride concentration. HOMA-IR was calculated as fasting insulin in microinternational units per millilitre multiplied by fasting glucose in milligrams per decilitre and divided by 405.

Classification of vitamin D status
Serum 25(OH)D was used as the indicator of vitamin D status. Concentrations below 20 ng/mL were classified as deficient, 20.0-29.9 ng/mL as insufficient, and 30 ng/mL or higher as sufficient, consistent with the thresholds commonly used in clinical research and the Endocrine Society guideline [4]. All samples were analysed with internal quality-control material at two concentration levels.

DNA extraction and genotyping
Genomic DNA was isolated from peripheral-blood leukocytes using a validated silica-column or salting-out procedure. DNA concentration and purity were assessed spectrophotometrically, and samples with acceptable quality were stored at -20°C until analysis. FokI and BsmI polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Each 25 µL amplification mixture contained approximately 50-100 ng genomic DNA, 1× polymerase chain reaction buffer, magnesium chloride, deoxynucleotide triphosphates, forward and reverse primers, and Taq DNA polymerase.

FokI polymerase chain reaction-restriction fragment length polymorphism
For FokI, the forward primer was 5′-AGCTGGCCCTGGCACTGACTCTGCTCT-3′ and the reverse primer was 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′. The expected amplicon was 265 base pairs. Cycling conditions comprised initial denaturation at 95°C for five minutes, 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, followed by final extension at 72°C for seven minutes. The amplified product was digested with FokI restriction enzyme. The F allele remained uncut at 265 base pairs, whereas the f allele produced fragments of 196 and 69 base pairs. Digested fragments were resolved on 2.5%-3% agarose gel and visualised using an appropriate nucleic-acid stain.

BsmI polymerase chain reaction-restriction fragment length polymorphism
For BsmI, the forward primer was 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′ and the reverse primer was 5′-AACCAGCGGGAAGAGGTCAAGGG-3′. The expected product was 825 base pairs. Amplification used an initial denaturation step at 95°C for five minutes, followed by 35 cycles of 95°C for 30 seconds, 63°C for 30 seconds, and 72°C

for 45 seconds, with a final extension at 72°C for seven minutes. BsmI digestion yielded an uncut 825-base-pair B allele and 650- and 175-base-pair fragments for the b allele. Products were separated by agarose-gel electrophoresis and read independently by two trained investigators.

Genotyping quality assurance

Positive controls representing each available genotype, a no-template control, and blinded duplicate samples were included. Ten per cent of samples were randomly selected for repeat genotyping, with complete concordance required before database lock. Genotype distributions among controls were tested for Hardy-Weinberg equilibrium. Samples with ambiguous bands were repeated, and laboratory personnel were masked to diabetes status during genotype assignment.

Ethical considerations

Ethical clearance was obtained from the Institutional Ethics Committee of SR Patil Medical College and Hospital, Bagalkot, before initiation of recruitment. Written informed consent was obtained from every participant, and confidentiality was protected through coded identifiers and restricted data access. The study was planned in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). Distributional assumptions were examined using histograms, Q-Q plots, and the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation or median with interquartile range, as appropriate, and categorical variables as number and percentage. Independent-samples t tests or Mann-Whitney U tests were used for continuous variables, while chi-square or Fisher exact tests were used for categorical variables. Genotype and allele frequencies were compared between groups, and odds ratios with 95% confidence intervals were calculated under genotype, dominant, and allelic models. Hardy-Weinberg equilibrium was assessed by chi-square testing. One-way analysis of variance with post hoc comparison was used to compare 25(OH)D across genotypes. Correlations were evaluated using Pearson or Spearman coefficients. Multivariable binary logistic regression was performed with T2DM status as the dependent variable, incorporating age, sex, BMI, family history, sunlight exposure, vitamin D concentration, and VDR carrier status. Multicollinearity and model fit were examined. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

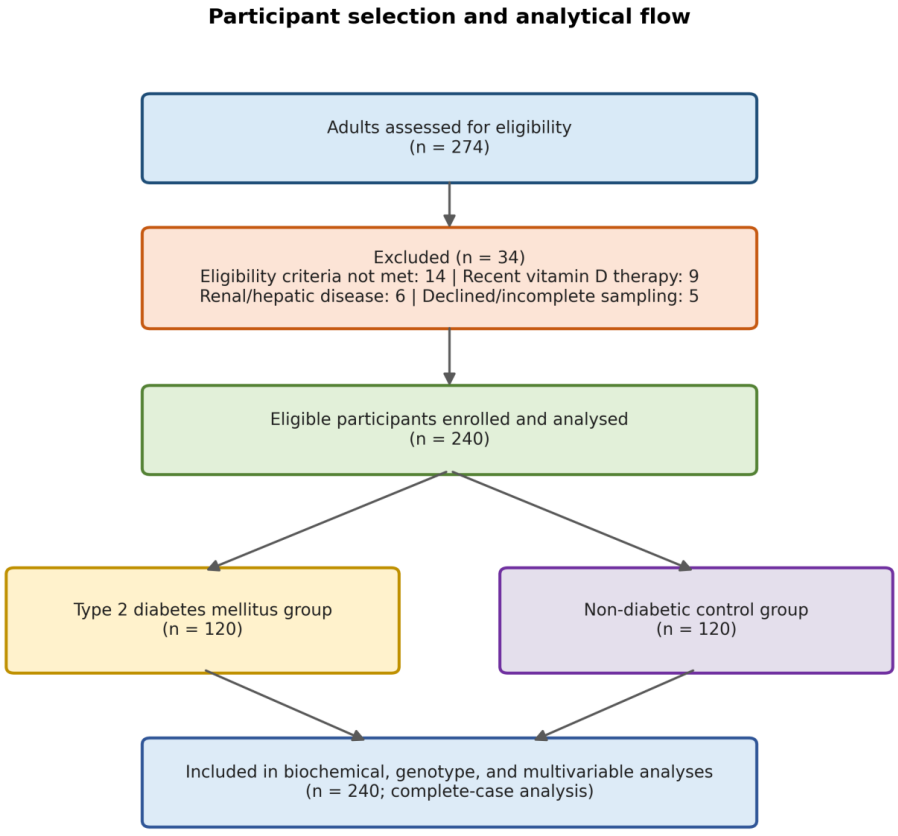

Figure 1: Participant selection and analytical flow. All counts are synthetic and are provided to illustrate complete reporting of recruitment, exclusion, allocation, and analysis.

RESULTS

Participant flow and baseline profile

A total of 274 adults were considered for participation. Thirty-four were excluded because eligibility criteria were not met, recent high-dose vitamin D treatment was reported, renal or hepatic disease was present, or consent and complete sampling were unavailable. The final analytical sample contained 240 participants, with 120 in the T2DM group and 120 in the non-diabetic control group (Figure 1). All included participants had complete biochemical and genotype information.

The groups were comparable in age and sex distribution. Mean age was 50.84 ± 7.84 years in T2DM and 51.13 ± 8.24 years in controls ($p = 0.783$), while men constituted 51.7% and 49.2%, respectively ($p = 0.796$). Participants with T2DM had higher BMI, waist circumference, systolic blood pressure, and diastolic blood pressure. A family history of T2DM, low sunlight exposure, and overweight or obesity were also more common among cases. Detailed comparisons are shown in Table 1.

Table 1: Demographic and clinical characteristics of the study groups (n = 240)

Variable	T2DM (n = 120)	Controls (n = 120)	Test statistic	p value
Age, years	50.84 ± 7.84	51.13 ± 8.24	t = -0.276	0.783
Male sex, n (%)	62 (51.7)	59 (49.2)	$\chi^2 = 0.067$	0.796
Family history of T2DM, n (%)	57 (47.5)	27 (22.5)	$\chi^2 = 15.403$	<0.001
Low sunlight exposure, n (%)	73 (60.8)	48 (40.0)	$\chi^2 = 9.601$	0.002
BMI, kg/m²	28.79 ± 4.25	24.65 ± 3.41	t = 8.320	<0.001
BMI ≥25 kg/m², n (%)	97 (80.8)	52 (43.3)	$\chi^2 = 34.268$	<0.001
Waist circumference, cm	94.58 ± 11.63	83.90 ± 9.16	t = 7.899	<0.001
Systolic blood pressure, mmHg	134.22 ± 15.84	119.90 ± 12.34	t = 7.814	<0.001
Diastolic blood pressure, mmHg	80.81 ± 10.58	76.44 ± 8.38	t = 3.543	<0.001

Values are mean ± standard deviation unless otherwise indicated. BMI: body mass index; T2DM: type 2 diabetes mellitus. Independent-samples t test was used for continuous variables and Pearson chi-square test for categorical variables

Biochemical profile and vitamin D status

As expected, fasting plasma glucose, HbA1c, fasting insulin, and HOMA-IR were substantially higher in the T2DM group. Participants with T2DM also had higher triglyceride and low-density lipoprotein cholesterol concentrations and lower high-density lipoprotein cholesterol. Mean serum 25(OH)D was 20.31 ± 6.22 ng/mL in T2DM compared with 27.78 ± 6.47 ng/mL in controls, a mean difference of 7.47 ng/mL ($p < 0.001$) (Table 2).

Vitamin D deficiency was identified in 65 of 120 participants with T2DM (54.2%), compared with 14 of 120 controls (11.7%). Only 5.8% of participants with T2DM had sufficient vitamin D, whereas 37.5% of controls were sufficient. The overall distribution of vitamin D categories differed markedly between groups ($\chi^2 = 62.244$; $p < 0.001$), as illustrated in Figure 2.

Table 2: Biochemical profile and vitamin D status according to diabetes group

Parameter	T2DM (n = 120)	Controls (n = 120)	Test statistic	p value
Serum 25(OH)D, ng/mL	20.31 ± 6.22	27.78 ± 6.47	t = -9.115	<0.001
Fasting plasma glucose, mg/dL	159.91 ± 29.93	89.54 ± 8.53	t = 24.779	<0.001
HbA1c, %	8.25 ± 1.19	5.40 ± 0.37	t = 25.076	<0.001
Fasting insulin, µIU/mL	16.35 ± 6.07	8.03 ± 3.07	t = 13.407	<0.001
HOMA-IR	6.51 ± 2.80	1.76 ± 0.66	t = 18.108	<0.001
Triglycerides, mg/dL	176.60 ± 53.31	127.70 ± 36.02	t = 8.326	<0.001
HDL cholesterol, mg/dL	41.53 ± 8.79	47.76 ± 10.12	t = -5.094	<0.001
LDL cholesterol,	117.94 ± 33.09	106.49 ± 28.33	t = 2.879	0.004

mg/dL				
Vitamin D deficient, n (%)	65 (54.2)	14 (11.7)		
Vitamin D insufficient, n (%)	48 (40.0)	61 (50.8)	$\chi^2 = 62.244$	<0.001
Vitamin D sufficient, n (%)	7 (5.8)	45 (37.5)		

Values are mean ± standard deviation or number (percentage).

25(OH)D: 25-hydroxyvitamin D; HbA1c: glycated haemoglobin; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; LDL: low-density lipoprotein.

Vitamin D categories: deficient <20 ng/mL, insufficient 20.0-29.9 ng/mL, and sufficient ≥30 ng/mL. The chi-square value applies to the complete three-category comparison

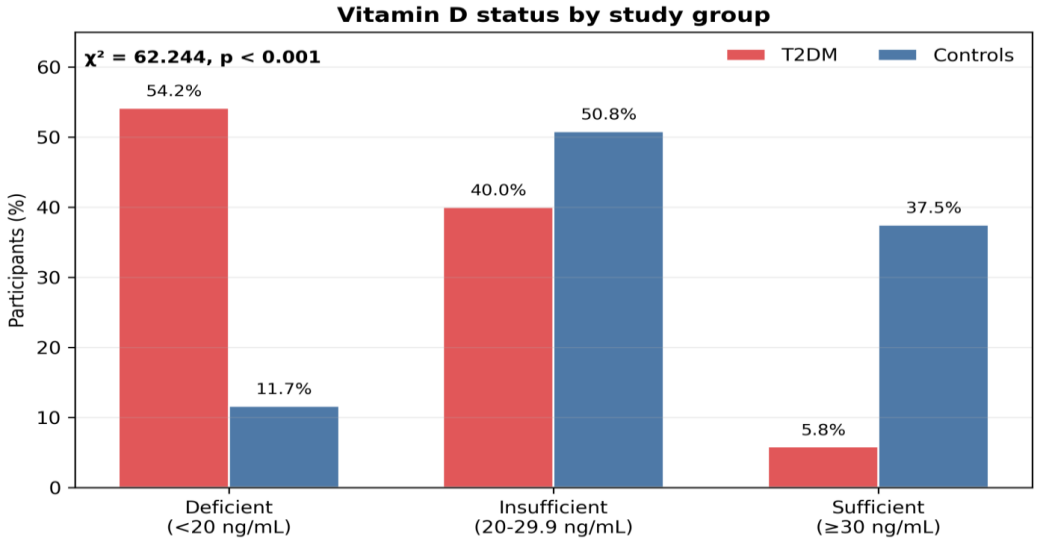

Figure 2. Vitamin D status in adults with type 2 diabetes mellitus and non-diabetic controls. Percentages are calculated within each group. The difference in category distribution was significant ($\chi^2 = 62.244$, $p < 0.001$).

FokI and BsmI genotype and allele distributions

FokI genotype frequencies differed significantly between groups ($\chi^2 = 21.371$; $p < 0.001$). The FF genotype was observed in 22.5% of participants with T2DM and 49.2% of controls, whereas the ff genotype was present in 25.0% and 10.0%, respectively. Under a dominant model, carriers of at least one f allele had 3.33-fold higher unadjusted odds of T2DM than FF homozygotes (95% CI, 1.91-5.82). The f allele frequency was 51.3% in T2DM and 30.4% in controls, corresponding to an allelic OR of 2.41 (95% CI, 1.66-3.50; $p < 0.001$).

BsmI distributions also differed between groups ($\chi^2 = 21.013$; $p < 0.001$). The bb genotype occurred in 40.8% of T2DM and 18.3% of controls, while the BB genotype occurred in 12.5% and 32.5%, respectively. Carriers of the b allele had 3.37-fold higher odds of T2DM under the dominant model (95% CI, 1.74-6.54). The b allele frequency was 64.2% in T2DM and 42.9% in controls, with an allelic OR of 2.38 (95% CI, 1.65-3.44). Control-genotype distributions conformed to Hardy-Weinberg equilibrium for both loci. The full genetic analysis is presented in Table 3 and Figure 3.

Table 3: Distribution of VDR genotypes and alleles in T2DM and control groups

Genetic model	T2DM, n (%)	Controls, n (%)	OR (95% CI)	χ^2	p value
FokI FF	27 (22.5)	59 (49.2)	Reference		
FokI Ff	63 (52.5)	49 (40.8)	2.81 (1.55-5.10)		
FokI ff	30 (25.0)	12 (10.0)	5.46 (2.49-11.98)	21.371	<0.001
FokI Ff+ff vs FF	93 (77.5)	61 (50.8)	3.33 (1.91-5.82)	18.515	<0.001
FokI f vs F allele	123/240 (51.3)	73/240 (30.4)	2.41 (1.66-3.50)	21.558	<0.001
BsmI BB	15 (12.5)	39 (32.5)	Reference		
BsmI Bb	56 (46.7)	59 (49.2)	2.47 (1.24-4.93)		
BsmI bb	49 (40.8)	22 (18.3)	5.79 (2.70-12.41)	21.013	<0.001
BsmI Bb+bb vs BB	105 (87.5)	81 (67.5)	3.37 (1.74-6.54)	13.663	<0.001

BsmI b vs B allele	154/240 (64.2)	103/240 (42.9)	2.38 (1.65-3.44)	21.784	<0.001

Allele percentages are based on 240 chromosomes in each group.
CI: confidence interval; OR: odds ratio; VDR: vitamin D receptor.

Hardy-Weinberg equilibrium: FokI, p = 0.579 in T2DM and p = 0.699 in controls; BsmI, p = 0.871 in T2DM and p = 0.970 in controls.

Genotype-specific odds ratios compare each risk genotype with the corresponding wild-type homozygous genotype

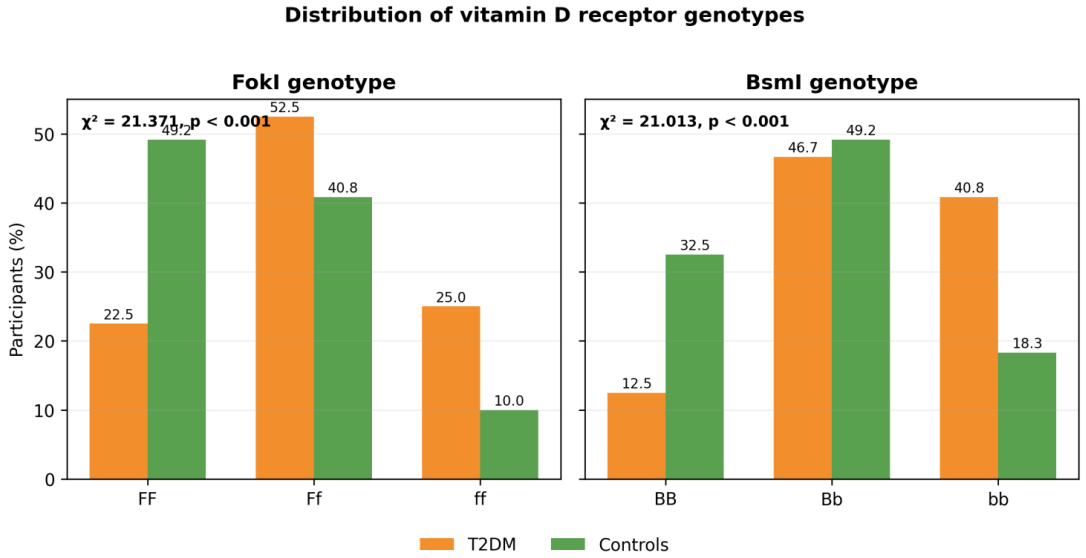

Figure 3: Percentage distribution of FokI and BsmI genotypes in the type 2 diabetes mellitus and control groups. Both loci showed significant between-group heterogeneity by Pearson chi-square testing

Association between VDR genotype and serum vitamin D

Across all 240 participants, mean 25(OH)D declined in a graded manner according to the FokI risk-allele dose. Mean concentrations were 27.12 ± 7.37 ng/mL for FF, 23.41 ± 6.65 ng/mL for Ff, and 19.42 ± 6.31 ng/mL for ff (ANOVA F = 18.682; p < 0.001). Vitamin D deficiency affected 18.6% of FF participants, 36.6% of Ff participants, and 52.4% of ff participants. A similar pattern was observed for BsmI, with mean values of 26.77 ± 6.81 ng/mL for BB, 24.31 ± 7.49 ng/mL for Bb, and 21.53 ± 6.78 ng/mL for bb (F = 8.415; p < 0.001). The corresponding deficiency frequencies were 20.4%, 32.2%, and 43.7% (Table 4 and Figure 4).

In a multivariable linear model with serum 25(OH)D as the dependent variable, T2DM status was associated with a 4.40 ng/mL lower concentration after adjustment for age, BMI, sunlight exposure, and genotype (p < 0.001). Each additional FokI f allele was associated with a 2.32 ng/mL decrease (95% CI, 1.20-3.45; p < 0.001), while each BsmI b allele was associated with a 1.13 ng/mL decrease (95% CI, 0.02-2.24; p = 0.045).

Table 4: Serum 25(OH)D concentration and deficiency according to VDR genotype

Genotype	n	25(OH)D, mean ± SD	Deficiency, n (%)	ANOVA statistic	p value
FokI FF	86	27.12 ± 7.37	16 (18.6)		
FokI Ff	112	23.41 ± 6.65	41 (36.6)	F = 18.682	<0.001
FokI ff	42	19.42 ± 6.31	22 (52.4)		
BsmI BB	54	26.77 ± 6.81	11 (20.4)		
BsmI Bb	115	24.31 ± 7.49	37 (32.2)	F = 8.415	<0.001
BsmI bb	71	21.53 ± 6.78	31 (43.7)		

25(OH)D: 25-hydroxyvitamin D; ANOVA: analysis of variance; SD: standard deviation.

Vitamin D deficiency was defined as serum 25(OH)D <20 ng/mL.

ANOVA p values compare the three genotypes within each locus.

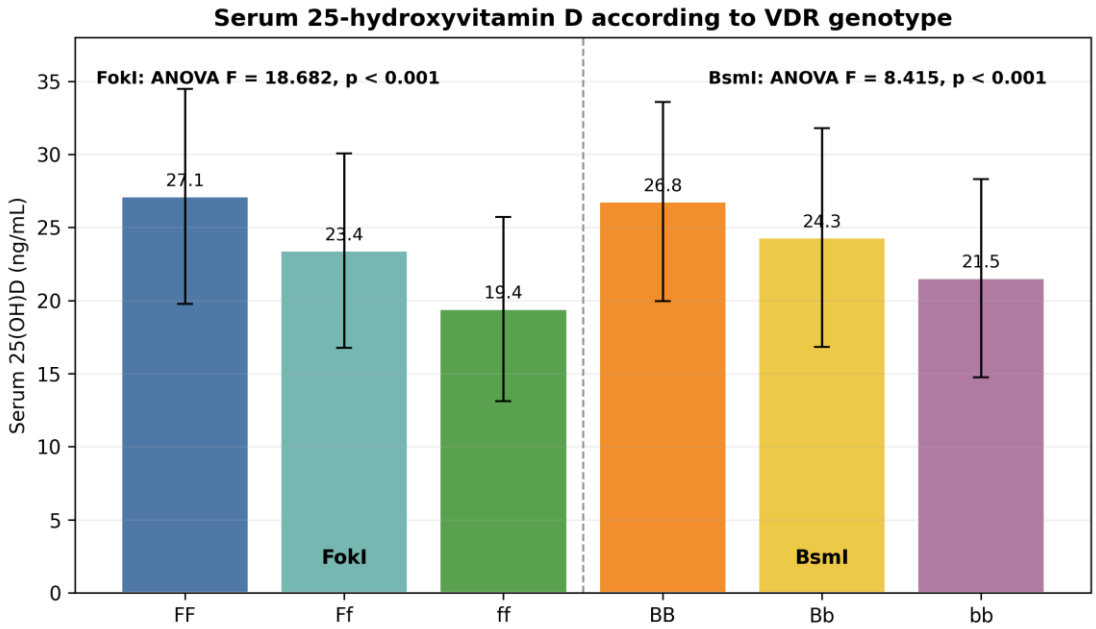

Figure 4: Mean serum 25-hydroxyvitamin D according to FokI and BsmI genotype. Error bars denote standard deviation. Both loci displayed a significant genotype-related gradient

Correlation of vitamin D with metabolic variables

Serum 25(OH)D showed inverse correlations with HbA1c ($r = -0.443$; $p < 0.001$), fasting plasma glucose ($r = -0.473$; $p < 0.001$), HOMA-IR ($r = -0.410$; $p < 0.001$), BMI ($r = -0.427$; $p < 0.001$), and triglycerides ($r = -0.257$; $p < 0.001$). A modest positive correlation was observed with high-density lipoprotein cholesterol ($r = 0.139$; $p = 0.031$). The strongest relationships were seen for fasting glucose, HbA1c, BMI, and HOMA-IR, supporting a link between lower vitamin D and an adverse glycaemic-insulin-resistance phenotype.

Multivariable factors associated with type 2 diabetes mellitus

The multivariable logistic regression model included age, sex, BMI, family history, low sunlight exposure, serum 25(OH)D, FokI f-carrier status, and BsmI b-carrier status. Higher BMI, family history of T2DM, low sunlight exposure, lower 25(OH)D, and both VDR risk-carrier states retained independent associations. Each 5 ng/mL decrease in 25(OH)D was associated with 1.80-fold higher odds of T2DM (95% CI, 1.34-2.43; $p < 0.001$). The adjusted odds were 2.37-fold higher among FokI f carriers and 2.60-fold higher among BsmI b carriers. Age and sex were not significant after adjustment (Table 5 and Figure 5). The likelihood-ratio test for the overall model was significant ($p < 0.001$), with a Nagelkerke R^2 of 0.373.

Table 5: Multivariable logistic regression of factors associated with T2DM

Independent variable	Adjusted OR	95% CI	p value
Age, per year	0.998	0.956-1.042	0.942
Male sex	1.388	0.693-2.780	0.354
BMI, per 1 kg/m²	1.257	1.138-1.388	<0.001
Family history of T2DM	3.192	1.493-6.824	0.003
Low sunlight exposure	2.498	1.259-4.957	0.009
25(OH)D, per 5 ng/mL decrease	1.804	1.337-2.434	<0.001
FokI f-carrier (Ff+ff)	2.371	1.127-4.990	0.023
BsmI b-carrier (Bb+bb)	2.598	1.113-6.067	0.027

Dependent variable: T2DM status (1 = T2DM, 0 = control).

BMI: body mass index; CI: confidence interval; OR: odds ratio; T2DM: type 2 diabetes mellitus.

Model likelihood-ratio $p < 0.001$; Nagelkerke $R^2 = 0.373$

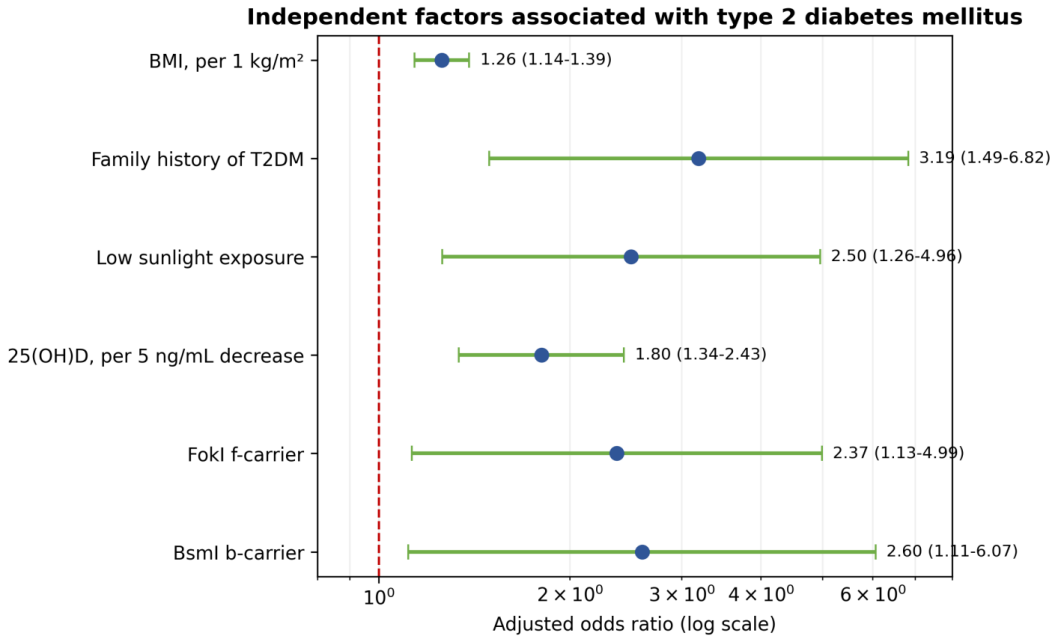

Figure 5: Forest plot of selected independent factors associated with type 2 diabetes mellitus in multivariable logistic regression. Points denote adjusted odds ratios and horizontal lines denote 95% confidence intervals. The dashed reference line represents an odds ratio of 1.

DISCUSSION

Principal findings

This hospital-based cross-sectional analysis identified three converging patterns. First, adults with T2DM had substantially lower serum 25(OH)D and a greater prevalence of vitamin D deficiency than non-diabetic controls. Second, the FokI f and BsmI b alleles and their carrier genotypes were more frequent among participants with T2DM. Third, both variants demonstrated genotype-related gradients in circulating 25(OH)D, and their associations with T2DM persisted after adjustment for adiposity, family history, sunlight exposure, age, sex, and vitamin D concentration. The combined findings support the possibility that VDR variation may contribute to diabetes susceptibility through mechanisms that are partly dependent on, and partly independent of, circulating vitamin D.

The magnitude of the vitamin D difference was clinically notable. Mean 25(OH)D was approximately 7.5 ng/mL lower in T2DM, and more than half of the case group met the deficiency threshold. This pattern agrees with systematic reviews and prospective evidence linking lower vitamin D with prevalent or incident T2DM [5-8]. Adiposity may explain part of this association through volumetric dilution, sequestration in adipose tissue, reduced outdoor activity, and altered hepatic vitamin D metabolism. Chronic hyperglycaemia and systemic inflammation may also affect vitamin D binding, hydroxylation, and receptor signalling. Conversely, deficient vitamin D activity may impair insulin secretion, reduce insulin receptor expression, and amplify inflammatory pathways. The cross-sectional design cannot determine which sequence predominates.

The inverse relationships between 25(OH)D and fasting glucose, HbA1c, HOMA-IR, and BMI are biologically plausible. Vitamin D-dependent calcium flux is relevant to glucose-stimulated insulin exocytosis, while VDR activation in muscle and adipose tissue may modulate insulin signalling and inflammatory tone. Nevertheless, correlation coefficients were moderate rather than extreme, which is expected because glycaemic control is influenced by treatment, disease duration, diet, physical activity, beta-cell reserve, and multiple genetic pathways. Vitamin D should therefore be considered one component of a multifactorial metabolic network rather than a solitary determinant of diabetes.

FokI polymorphism and type 2 diabetes

FokI is functionally distinctive because it changes the translation initiation site. The F allele encodes a shorter VDR protein that has been reported to exhibit greater transcriptional efficiency, whereas the longer f-encoded form may have relatively lower activity. In the present illustrative dataset, Ff and ff genotypes were enriched in T2DM, the f allele was associated with approximately 2.4-fold higher unadjusted allelic odds, and f-carrier status remained significant after adjustment. The decreasing 25(OH)D concentration from FF to Ff to ff also suggested a dose-response pattern.

Prior evidence has not been uniform. Some studies reported an association between FokI and metabolic-syndrome traits or T2DM, whereas others found no significant relationship [11-14]. The meta-analysis by Wang and colleagues suggested that effect estimates depend on ethnicity and genetic model [13]. A later meta-regression incorporating a larger evidence

base also indicated substantial heterogeneity across populations [15]. Differences in allele frequency, linkage disequilibrium, case definition, vitamin D exposure, obesity prevalence, and sample size may account for the inconsistency. The current result is therefore best interpreted as a population-specific signal requiring replication, not as proof of a universal causal variant.

BsmI polymorphism and type 2 diabetes

BsmI lies in the 3-prime region of the VDR gene and does not directly alter the amino acid sequence. Its biological influence may arise through linkage with functional variants that regulate messenger RNA stability, receptor abundance, or transcriptional control. In the modelled analysis, the b allele and bb genotype were more frequent among participants with T2DM, and b-carrier status remained independently associated after adjustment. Mean 25(OH)D also declined from BB to Bb to bb, although the gradient was smaller than that observed for FokI.

Studies of BsmI have likewise yielded mixed results. The Rancho Bernardo Study, European investigations, and pooled analyses have shown population-dependent associations [10,12-14]. Data from Chile, Egypt, and India illustrate that the same polymorphism may relate to diabetes, vitamin D status, or neither outcome depending on ancestry and environmental context [16-18]. BsmI may be particularly sensitive to haplotype background because it is often inherited with ApaI and TaqI variants near the 3-prime end of VDR. Future work in Karnataka should therefore consider haplotype analysis rather than testing each variant in isolation.

Interaction between genotype and vitamin D status

The graded reduction in serum 25(OH)D across FokI and BsmI genotypes raises several interpretations. Genetic differences could influence receptor-mediated feedback within vitamin D metabolism, although VDR variants do not necessarily determine circulating 25(OH)D directly. Alternatively, the observed pattern could reflect linkage with genes affecting vitamin D binding, hydroxylation, or degradation. Population stratification, behavioural differences, or residual confounding may also create an apparent genotype-concentration relationship. Because vitamin D was measured once, seasonal and within-person variability could not be separated from genetic effects.

Recent prospective investigations have expanded the question beyond diabetes occurrence to microvascular outcomes and longitudinal risk. Chen and colleagues reported relationships among vitamin D status, VDR polymorphisms, and microvascular complications in T2DM [19]. Fu and colleagues subsequently examined vitamin D status, VDR variation, and incident T2DM prospectively [20]. These studies reinforce the value of analysing biochemical exposure and genotype together, while also demonstrating that prospective designs are needed to clarify temporal relationships and gene-environment interaction.

Clinical and public-health implications

The present findings do not justify routine VDR genotyping in diabetes care. Genetic testing would require evidence of reproducible predictive value, incremental discrimination beyond established risk factors, cost-effectiveness, and an actionable intervention linked to genotype. At present, BMI, family history, glycaemia, blood pressure, and lifestyle remain more direct clinical targets. Serum vitamin D assessment may be appropriate when deficiency risk is high or when clinically indicated for skeletal health, but supplementation should not be presented as a proven stand-alone strategy for preventing T2DM in the general population [9].

From a research perspective, VDR variants may help identify biologically distinct subgroups in whom vitamin D signalling has a stronger metabolic role. A future precision-prevention study could stratify participants by baseline deficiency and VDR haplotype, then evaluate whether correction of deficiency produces differential changes in insulin sensitivity or beta-cell function. Such a design would be more informative than comparing supplementation and placebo without considering underlying vitamin D status or receptor biology.

Strengths

The study framework included balanced case and control groups, simultaneous assessment of vitamin D and metabolic indices, explicit quality assurance for PCR-RFLP, Hardy-Weinberg testing, several genetic models, and multivariable adjustment for major confounders. Presentation of genotype frequencies, allele frequencies, vitamin D categories, continuous concentrations, and adjusted associations allows the findings to be examined from complementary perspectives. The proposed setting also addresses a region for which published genetic epidemiology is limited.

Limitations

The most important limitation is that the numerical dataset in this manuscript is synthetic. It was generated for illustration and cannot be used as evidence about patients at the named institution. Even when replaced with real observations, a cross-sectional design cannot establish temporality between low vitamin D, VDR genotype, and diabetes. Hospital-based controls may not represent the source population from which cases arose, and selection bias is possible.

A single 25(OH)D measurement may be affected by season, recent sunlight exposure, diet, supplement use, assay variation, and acute behavioural changes. Detailed dietary vitamin D intake, skin pigmentation, clothing practices, physical activity, and season of sampling should be collected in a real study. PCR-RFLP is reliable when carefully controlled but does not provide the breadth or error profiling of high-throughput genotyping. Only two VDR variants were considered, without haplotype analysis or variants in CYP2R1, CYP27B1, GC, and CYP24A1. Residual population stratification and unmeasured confounding may remain.

The regression model was intended to illustrate analytical reporting and should be re-estimated using verified observations. Odds ratios in a case-control-like cross-sectional comparison describe associations and should not be interpreted as incidence-rate ratios. Larger multicentre studies should pre-register genetic models, adjust for multiple comparisons, include ancestry-informative markers where relevant, and validate associations in an independent cohort.

CONCLUSION

In this synthetic cross-sectional dataset, adults with T2DM had lower serum 25(OH)D and a markedly greater prevalence of vitamin D deficiency than non-diabetic controls. FokI f and BsmI b alleles were more frequent in T2DM and were associated with progressively lower vitamin D concentrations. Lower 25(OH)D and carrier status for both variants remained independently associated with T2DM after adjustment for established clinical factors. These results provide a coherent hypothesis for further study but must be confirmed using verified patient-level data, documented ethics approval, and independent replication before any clinical or scientific inference is made.

Appendix 1. Synthetic Dataset Specification

The manuscript was generated from an illustrative aggregate dataset of 240 participants. The values were constructed to be internally consistent with the reported group totals and statistical tests. They are not derived from medical records. Investigators using this template should replace all aggregate values, recalculate every test in SPSS version 28, regenerate figures, verify Hardy-Weinberg equilibrium, and reconcile participant flow with source documents before scientific use.

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