

Original Article

Relationship between vitamin D deficiency, vitamin D receptor gene variants, & the risk of coronary artery disease among South Indians: A case-control study

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Background & objectives: Vitamin D deficiency (VDD) and variations in the vitamin D receptor (*VDR*) gene are implicated in the pathogenesis of coronary artery disease (CAD). This study investigated the association between VDD, *VDR* gene variants (*Apal*, *BsmI*, *FokI*, and *TaqI*), and CAD risk among South Indians.

Methods: The case-control study was conducted in 250 CAD patients and 260 matched controls. Serum vitamin D levels were measured by ELISA. Genotyping for *VDR Apal* (A>C, rs7975232), *BsmI* (A>G, rs1544410), *FokI* (T>C, rs2228570), and *TaqI* (C>T, rs731236) was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method.

Results: VDD was significantly higher among CAD patients (90%) than in controls (63%). Individuals with vitamin D levels <20 ng/ml were 5.7 times more likely to have CAD when compared to those with vitamin D levels ≥ 20 ng/ml ($P<0.001$, OR=5.74, 95% CI=2.92-11.30). No correlation was observed between vitamin D levels and CAD risk factors, systolic and diastolic blood pressure ($r=-0.105$, $P=0.095$, $r=-0.049$, $P=0.437$), and blood glucose ($r=-0.067$, $P=0.304$). A trend for negative correlation of vitamin D levels with cholesterol ($r=-0.112$, $P=0.094$) and triglyceride levels ($r=-0.133$, $P=0.061$) was observed. The *VDR TaqI* variant showed significant association with reduced CAD risk in the overall analysis (Model II, OR=0.60, 95% CI=0.39-0.90, $P=0.016$). The *FokI* variant was associated with an increased risk of CAD in males (Model III, OR=5.9, 95% CI=2.09-16.85, $P=0.001$). However, combined analysis of VDD and *VDR* gene variants indicated that neither *FokI* 'ff' ($P=0.145$) nor *TaqI* 'tt' ($P=0.138$) genotypes significantly altered CAD risk in vitamin D-deficient subjects.

Interpretation & conclusions: The findings of this study suggested that VDD was significantly higher among the CAD patients and increases the risk of CAD by 5.7-fold. This study revealed the differing roles of *VDR* gene variants in CAD susceptibility and the influence of gender and other covariates.

Key words Coronary artery disease - genetic association - Indian population - *VDR* variants - vitamin D receptor - vitamin D deficiency

Cardiovascular diseases (CVDs) have become one of the leading causes of mortality and morbidity in

India, with coronary artery disease (CAD) accounting for 26.9 per cent of all medically certified deaths, with disorders of the circulatory system¹. Indians are particularly susceptible to premature CAD, leading to acute myocardial infarction (AMI) at an earlier age compared to the Western population. CAD affects Indians a decade earlier in their most productive years of life. The complex interactions of both biological and social determinants are considered to be responsible for this higher burden in India². Vitamin D deficiency (VDD) has been implicated as a major risk factor for CAD. A 60 per cent higher risk of heart disease was observed in patients with vitamin D levels below 15 ng/ml in the Framingham study³. Further, an increased cardiovascular risk with a hazard ratio of 1.5 in vitamin D-deficient individuals was reported in the Framingham offspring study⁴. These findings highlight the importance of considering VDD as a potential treatable cause of CAD.

VDD, as well as insufficiency, have been said to be a pandemic affecting 50 per cent of the population globally⁵. The prevalence of VDD in India is reported to range from 50-94 per cent by various studies on the Indian population over the last decade⁶, with only one study showing 34.5 per cent⁷. Vitamin D plays an essential role in the regulation of cell proliferation, differentiation, apoptosis, and modulation of immune response. It has been increasingly recognised that a deficiency in vitamin D levels increases the risk of chronic diseases like diabetes, hypertension, dyslipidaemia, cerebral or cardiovascular ailments, and chronic kidney disease. Studies have also found an association between VDD and the severity of CAD. Therefore, VDD is considered an independent risk factor for CAD.

The functioning of the active form of vitamin D is mediated through the vitamin D receptor (*VDR*), which is a ligand-dependent receptor. These receptors are found in cardiovascular cells, including cardiomyocytes, vascular smooth muscle cells, endothelial cells, immune cells, and also platelets⁸. The *VDR* gene, which encodes the *VDR* receptor, influences many regulatory genes that are of particular importance in CVD and directly modulates gene transcription. It has been suggested that *VDR* variants could be a better marker compared to traditional risk factors for earlier detection of CAD, which can aid in personalised treatment⁹.

There is a paucity of data on the association of hypovitaminosis D, *VDR* gene variants, and CAD risk

in the Indian population. Existing studies often have small sample sizes, limiting their statistical power and generalisability. Therefore, we conducted this study to investigate the relationship between VDD, *VDR* variants (*BsmI*, *ApaI*, *TaqI*, and *FokI*), and CAD risk and severity in South Indian population.

Materials & Methods

This case-control study conducted between March 2020 and December 2021 was conducted by the department of Pathology, Sri Jayadeva Institute of Cardiovascular Sciences and Research (SJICR), Bengaluru, Karnataka, India, after obtaining clearance from the Institutional Medical Ethics Committee. Written informed consent for using samples for research was obtained from all the individuals before sampling. A detailed proforma with demographic variables and clinical details was used to document data for all study participants. Total 250 CAD patients who presented at SJICR, Bengaluru, were recruited in this study. Patients above 18 yr of age, with a clinical diagnosis of CAD with echocardiographic and ECG evidence, undergoing coronary angiography (CAG) were included in the study. The study population comprised both diabetic and non-diabetic patients. Terminally ill patients, those with hepatic/renal failures/malignancy, patients reactive to HIV, HBsAg, HCV & VDRL, and those on vitamin D or calcium supplements were excluded. The control group (n=260) was recruited from healthy volunteers who were attending to the hospital patients and blood donors during the study period. Individuals with any history of CVDs and those taking calcium or vitamin D supplements were excluded.

Sample collection and methodology: Venous blood (8 ml) was collected from the study participants in tubes containing EDTA and plain vials without an anticoagulant. The serum was separated from blood collected in plain vials and used for clinical biochemistry analysis, and a part of the serum sample was stored at -80°C for ELISA analysis. DNA was isolated from EDTA blood using the standard protocol and stored for molecular studies at -80°C.

Estimation of serum 25(OH) vitamin D [25(OH)D] levels: Serum 25(OH)D was measured by enzyme immunoassay (ELISA) using commercial kits (Cayman Chemical, MI, USA) and quality control materials provided by the manufacturer. The intra- and inter-assay coefficients of variation were 3.65 per cent and 4.8 per cent, respectively.

Table I. Demographic, clinical characteristics, and serum 25 (OH) D levels of the study participants

Parameters	Controls	Cases	P value
	n=260	n=250	
Age in yr, median (IQR)	47 (45-50)	47 (40-52)	0.062
Sex, male/ female	210/50	206/44	0.649
Smokers, n (%)	24 (9.23)	143 (57.2)	<0.001
Alcohol, n (%)	26 (10)	89 (35.6)	<0.001
Hypertension, n (%)	45 (17.30)	113 (45.2)	<0.001
Diabetes, n (%)	29 (11.15)	110 (44)	<0.001
Vitamin D status			
Serum 25 (OH)D, ng/ml, Mean \pm SE	18.12 \pm 0.62	12.05 \pm 0.44	< 0.001
\geq 30 ng/ml, n %	31 (11.92)	10 (4.00)	<0.001
20-30 ng/ml, n %	65 (24.50)	15 (6.00)	< 0.001
< 20 ng/ml, n %	164 (63.07)	225 (90.00)	< 0.001

n, the total number of subjects in each group; IQR, inter quartile range; SE, standard error; A $P < 0.05$ is considered significant

Multiplexing PCR genotyping of VDR variants - *TaqI* (*C>T*, *rs731236*), *BsmI* (*A>G*, *rs1544410*), *FokI* (*T>C*, *rs2228570*) and *ApaI* (*A>C*, *rs7975232*): Genomic DNA from human peripheral blood samples was isolated using a QiAamp DNA isolation kit (Qiagen, Germany) and was quantified using NanoDrop 2000 (Thermo Fisher Scientific, MA, USA). PCR for VDR variants (Multiplex PCR I - *TaqI* and *BsmI*; Multiplex PCR II - *FokI* and *ApaI*) was carried out with 2U of Taq DNA Polymerase (Solis Biodyne, Tartu) and supplied buffer under the following conditions: 94°C for 6 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 40 sec, followed by a 5 min final extension at 72°C. The fragments were then digested with restriction enzymes and resolved in 2.5 per cent agarose gels.

Statistical analysis: IBM SPSS statistics v.21 and GraphPad Prism v.9. (Chicago, IL, USA) were used for analysis. The sample size was calculated using OpenEpi (Open-Source Epidemiologic Statistics for Public Health, Version, available at www.OpenEpi.com). Calculations were based on a two-sided confidence level of 95 per cent, a statistical power of 80 per cent, and an equal case-to-control ratio (1:1). The input parameters were obtained by combining the prevalence of VDD and VDR polymorphisms from a previous study¹⁰. The prevalence of VDD was 52 per cent in cases and 36 per cent in controls, and the minor allele frequencies

(MAF) for VDR polymorphisms were: *FokI* (20.2% vs. 26.17%), *BsmI* (30% vs. 26.64%), *ApaI* (39.17% vs. 36.45%), and *TaqI* (56.67% vs. 59.81%). By applying a weighted prevalence approach, the estimated exposure prevalence in controls (P_0) was 36.88 per cent, and in cases (P_1) was 44.26 per cent. OpenEpi calculations estimated a minimum sample size per group of 692 (Kelsey), 691 (Fleiss), and 718 (Fleiss with continuity correction), a total of 1382–1436 participants. However, due to the feasibility of recruitment and other studies conducted with comparable sample sizes^{11,12}, the sample size of 510 participants (250 cases, 260 controls) was determined to be adequate to retain statistical power. The differences in baseline characteristics, such as gender, hypertension, diabetes, smoking, and alcohol use, between patients and healthy controls were assessed using the chi-squared test. Age distribution was compared using the Mann-Whitney U test. The correlation analysis between vitamin D levels and CAD risk factors, including blood pressure, blood glucose, cholesterol, and triglyceride levels, was performed using the Spearman rank correlation coefficient (r). Genotypic frequencies of variants were tested for Hardy Weinberg Equilibrium (HWE) by chi-squared analysis. The frequency distribution of the alleles was compared between patient and control groups using the chi-squared test. Logistic regression analysis was used to analyse the association between VDR variants and CAD risk. Covariates included age, gender, the habit of smoking or drinking, and presence of diabetes mellitus and/or hypertension. Linkage disequilibrium (LD) analysis of VDR variants was conducted using Haploview 4.2 software available at www.broadinstitute.org/haploview/haploview. $P < 0.05$ were considered statistically significant in all the analysis.

Results

Demographic and clinical characteristics of the study participants: A total of 250 CAD patients (206 males and 44 females) and 260 healthy controls (210 males and 50 females) were included in this study. The distribution of age between patients and control subjects was 47 (45-50) yr and 47 (40-52) yr ($P=0.062$), respectively. The demographic and clinical characteristics of the subjects have been presented in table I.

Serum 25 (OH)D levels in patients and controls: The mean serum 25(OH)D levels were observed to

Table II. Partial correlation analysis

Variable	Vitamin D levels (Unadjusted r)	P value (Unadjusted)	Vitamin D levels (Adjusted r)	P value (Adjusted)
Cases vs. Controls	-0.304	<0.001	-0.256	<0.001
Age	0.048	0.278	0.035	0.41
Gender	-0.031	0.485	-0.028	0.51
Hypertension	-0.129	0.003	-0.102	0.015
Diabetes	-0.119	0.007	-0.087	0.045
Smoking	-0.107	0.016	-0.092	0.035
Alcohol	-0.07	0.113	-0.065	0.15

r, Pearson correlation coefficient; A $P < 0.05$ is considered significant

be lower in patients (12.05 ± 0.4 ng/ml) compared to controls (18.12 ± 0.6 ng/ml). VDD (< 20 ng/ml) was significantly more common in cases than in controls ($P < 0.001$). Conversely, vitamin D sufficiency (≥ 30 ng/ml) was more prevalent in controls compared to patients (11.92 vs. 4%; $P < 0.001$) (Table I).

Partial correlation analysis: Partial correlation analysis was done to investigate the relationship between vitamin D levels and case-controls while adjusting for confounders such as age, gender, hypertension, diabetes, smoking, and alcohol intake. The unadjusted relationship between vitamin D levels and case-control status was significant ($r = -0.304$, $P < 0.001$). After adjusting for confounders, the association was still statistically significant but slightly lower ($r = -0.256$, $P < 0.001$). Among the confounding factors, hypertension ($r = -0.102$, $P = 0.015$), diabetes ($r = -0.087$, $P = 0.045$), and smoking ($r = -0.092$, $P = 0.035$) were significantly negatively correlated with vitamin D levels (Table II).

Impact of vitamin D levels on CAD risk: We then examined the relationship between vitamin D levels and the risk of CAD by calculating the odds ratio. Our analysis revealed a strong and significant association between low vitamin D levels and an increased risk of CAD, even after adjusting for hypertension, diabetes, smoking, and alcohol. Individuals with vitamin D levels < 20 ng/ml are approximately 5.7 times more likely to have CAD compared to those with vitamin D levels ≥ 20 ng/ml ($P < 0.001$, OR=5.74).

Correlation of vitamin D levels and CAD risk factors: Vitamin D plays a crucial role in regulating blood pressure and blood glucose levels, which are significant risk factors for CAD¹³. Hence, we did a correlation analysis of vitamin D levels and CAD risk factors (blood pressure and blood glucose) for cases

and controls. However, our analysis did not reveal any significant correlation between vitamin D levels and either blood pressure or blood glucose levels in cases and controls (Fig. 1A-C for cases; Fig. 2A-C for controls).

Correlation analysis between vitamin D levels and blood lipid parameters: Vitamin D plays a significant role in lipid metabolism, influencing cholesterol synthesis and the levels of total cholesterol, LDL, and HDL. This relationship is crucial since elevated LDL cholesterol and triglycerides contribute to atherosclerosis, a major factor in CAD¹⁴. Hence, we did a correlation analysis. Plot 'D' (Fig. 1) shows a weak negative correlation between vitamin D levels and total cholesterol ($r = -0.112$, $P = 0.094$), though this relationship is not statistically significant. Plot 'E' (Fig. 1) also showed a weak negative correlation between vitamin D levels and triglycerides ($r = -0.133$, $P = 0.061$). Plot 'F' (Fig. 1) shows a near-zero correlation between vitamin D levels and HDL ($r = 0.004$, $P = 0.954$). Similarly, plot 'G' (Fig. 1) shows a near-zero correlation between vitamin D levels and LDL ($r = -0.001$, $P = 0.984$). The 'H' plot (Fig. 1) shows a weaker negative correlation between vitamin D levels and VLDL ($r = -0.044$, $P = 0.530$). The 'I' plot (Fig. 1) shows a weak negative correlation between vitamin D levels and the TC/HDL ratio ($r = -0.099$, $P = 0.164$). The correlation analysis of controls revealed no significant correlation between vitamin D levels and blood lipid parameters, including total cholesterol, triglycerides, HDL, LDL, VLDL, and the TC/HDL ratio. The correlation coefficients (r-values) for all parameters were close to zero, and the P values exceeded 0.05, indicating a lack of statistical significance (Fig. 2D-I).

Allelic frequency of VDR ApaI, FokI, TaqI, and BsmI variants: The distributions of all four VDR variants were examined for Hardy-Weinberg Equilibrium.

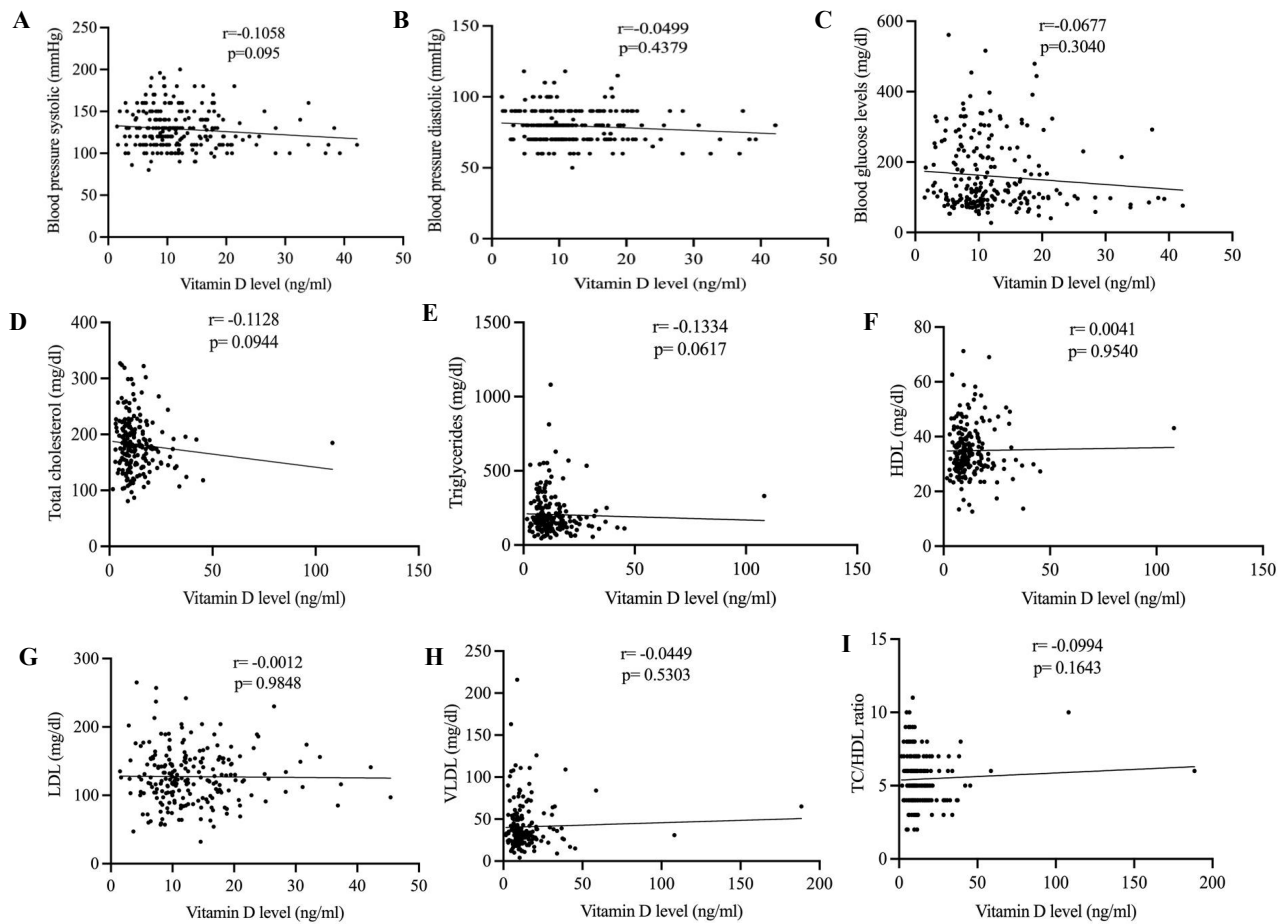


Fig. 1. The image presents three scatter plots (A, B, and C) showing the relationships between vitamin D levels (ng/ml) and CAD risk factors: Systolic blood pressure (Plot A), Diastolic blood pressure (Plot B), and Blood glucose levels (Plot C). The correlation coefficients (r) and P values for each relationship are provided. A P value <0.05 is considered significant.

No significant deviations were observed for *ApaI* ($P=0.880$), *TaqI* ($P=0.102$), and *BsmI* ($P=0.175$); however, *FokI* showed a deviation from HWE ($P=0.045$). Overall, the allelic frequency of *ApaI*, *FokI*, and *BsmI* genes was not significantly different in cases and controls. However, there was a significant difference in allelic distribution in the *TaqI* variant between cases and controls ($P=0.005$). Additionally, when the samples were analysed by gender, there was a significant difference in the distribution of the *TaqI* variant ($P=0.008$) and the *FokI* variant ($P=0.043$) in males compared to controls. The allele frequencies in the study groups have been shown in table III.

Odds ratio as estimates of risk for CAD in carriers of the VDR gene variants:

VDR *TaqI* variant: To estimate the odds ratios, we created three models with different levels of adjustments. 'Model I' was adjusted for age, ensuring the results accounted for differences in age. 'Model II'

added adjustments for smoking and alcohol use, as these lifestyle factors can affect cardiovascular risk. 'Model III' included further adjustments for hypertension and diabetes, which are common conditions linked to heart disease. Our analysis revealed that, under the dominant inheritance model (Tt + tt vs. TT), the *TaqI* variant has a potential protective association (Model I, OR=0.62, 95% CI=0.43-0.88, $P=0.008$). This association remained significant even after adjusting the odds ratio for age, smoking, and alcohol (Model II, OR=0.60, 95% CI=0.39-0.90). However, upon additional adjustment for hypertension and diabetes, the associations abrogated (Model III, OR=0.69, 95% CI=0.43-1.09, $P=0.119$). Upon gender stratification, the protective association persisted significantly in males initially (Model I, OR=0.60, 95% CI=0.37-0.99, $P=0.045$); however, abrogated after adjusting for confounders. In females, the association persisted (Model II, OR=0.42, 95% CI=0.18-0.98, $P=0.045$), even after adjusting for age, smoking, and alcohol (Supplementary table).

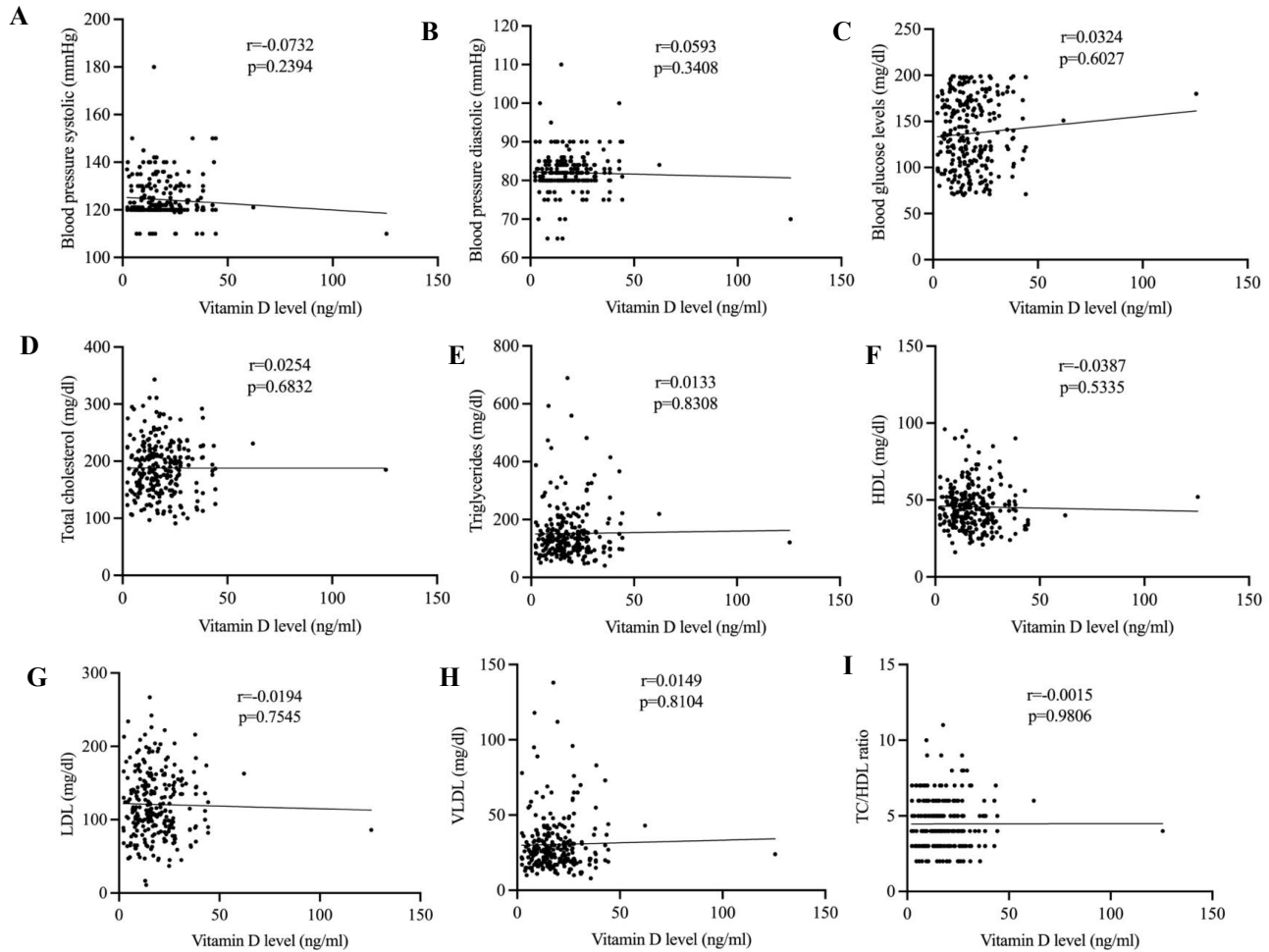


Fig. 2. The image presents six scatter plots (A to F) showing the relationship between vitamin D levels (ng/ml) and various blood lipid parameters: Total Cholesterol (Plot A), Triglycerides (Plot B), VLDL (Plot C), HDL (Plot D), LDL (Plot E), and the TC/HDL ratio (Plot F). The correlation coefficients (r) and P values for each relationship are provided. A P value <0.05 is considered significant.

VDR BsmI variant: There was no statistically significant difference observed in the distribution of alleles and genotypes of the *BsmI* gene variant between cases and controls, even when stratified according to gender (Supplementary table).

VDR ApaI variant: Logistic regression analysis did not indicate any association between the *ApaI* variant and CAD, even when stratified according to gender when analysed under various covariate adjustment models (Supplementary table).

VDR FokI variant: Overall logistic regression analysis did not reveal any significant association. However, in the recessive model of inheritance, the 'f' allele was significantly associated with CAD even after adjusting for the vascular risk factors (Model III, OR= 2.93, 95% CI=1.19-7.25, $P= 0.019$). Upon gender stratification, we found that the 'f' allele can significantly increase

the risk of CAD in males by 5.9 folds (Model III, OR= 5.9, 95% CI= 2.09-16.8, $P=0.001$) even after adjusting the OR with confounders. In women, there was no significant association of the *FokI* gene variant with the risk for CAD (Supplementary table).

Impact of VDR gene variants (TaqI and FokI) in the context of VDD: To determine whether the protective and risk effects of *TaqI* and *FokI* variants persist in the background of VDD, the combined effects of VDD and *FokI* and *TaqI* were estimated as a measure of the odds ratio. The logistic regression analysis for the *FokI* variant showed that individuals with the 'ff' genotype did not have a significantly increased risk compared to those with the 'FF' or 'Ff' genotypes (OR= 0.51, 95% CI= 0.20-1.25, $P=0.145$). Similarly, the logistic regression analysis for the *TaqI* variant indicated that individuals with the 'tt' genotype did not have a significantly increased risk compared to those with

Table III. Allelic frequency of *VDR ApaI*, *FokI*, *TaqI*, and *BsmI* variants

Genotype	Allelic frequency				P value	OR	95% CI
	Controls		Cases				
<i>VDR ApaI</i>	A	a	A	a			
Overall	306 (58.8)	214 (41.2)	294 (57.6)	226 (42.4)	>0.999	1.02	0.78-1.28
						0.99	0.77-1.28
Males	243 (57.8)	177 (42.2)	235 (53.2)	177 (46.7)	0.833	1.03	0.78-1.36
						0.96	0.73-1.27
Females	63 (63.0)	37 (37.0)	59 (66.6)	29 (33.3)	0.646	0.83	0.45-1.52
						1.19	0.65-2.18
<i>VDR FokI</i>	F	f	F	f			
Overall, n (%)	400 (76.9)	120 (23.1)	367 (73.5)	133 (26.4)	0.217	1.20	0.90-1.60
						0.82	0.62-1.10
Males, n (%)	331 (78.8)	89 (21.2)	299 (72.9)	113 (27.1)	0.043	1.40	1.02-1.93
						0.71	0.51-0.97
Females, n (%)	69 (69.0)	31 (31.0)	59 (74.7)	29 (25.3)	0.25	0.65	0.34-1.25
						1.52	0.72-2.93
<i>VDR TaqI</i>	T	t	T	t			
Overall, n (%)	349 (67.1)	171 (32.8)	375 (75.0)	125 (25.0)	0.005	0.68	0.51-0.89
						1.47	1.11-1.93
Males, n (%)	286 (68.0)	134 (32.0)	315 (76.4)	197 (23.6)	0.008	0.65	0.48-0.89
						1.52	1.12-2.06
Females, n (%)	63 (63.0)	37 (37.0)	60 (68.1)	28 (31.9)	0.539	0.79	0.43-1.45
						1.25	0.68-2.30
<i>VDR BsmI</i>	B	b	B	b			
Overall, n (%)	306 (58.8)	214 (41.2)	278 (55.6)	222 (44.5)	0.311	1.14	0.89-1.46
						0.87	0.68-1.12
Males, n (%)	249 (59.9)	171 (40.1)	224 (54.3)	188 (45.6)	0.161	1.22	0.92-1.60
						0.81	0.62-1.07
Females, n (%)	57 (57.0)	43 (43.0)	54 (61.3)	34 (38.6)	0.556	0.83	0.46-1.49
						1.19	0.66-2.14

n, Number of alleles; % percentage of allele frequency; 'A' represents the major allele frequency of the *ApaI* variant; 'a' represents the alternate allele frequency of the *ApaI* variant; 'F' represents the major allele frequency of the *FokI* variant; 'f' represents the alternate allele frequency of the *FokI* variant; 'T' represents the major allele frequency of the *TaqI* variant; 't' represents the alternate allele frequency of the *TaqI* variant; 'B' represents the major allele frequency of the *BsmI* variant; 'b' represents the alternate allele frequency of the *BsmI* variant OR, odds ratio; CI, confidence interval; A $P < 0.05$ is considered significant

the 'TT' or 'Tt' genotypes (OR=1.62, 95% CI=0.85-3.12, $P=0.138$). The results have been summarised in supplementary table.

Linkage disequilibrium analysis of VDR variants: The comparison of linkage disequilibrium (LD) patterns between cases and controls revealed a distinct difference in the genetic correlations among the four SNPs analysed (*TaqI*, *BsmI*, *ApaI*, and *FokI*) within a 34 kb genomic block. The case group demonstrated a higher LD between *ApaI* (rs7975232) and *FokI*

(rs2228570), $r^2 = 92$ and between *BsmI* (rs1544410) and *ApaI*, $r^2 = 87$. Moderate LD is also observed between *TaqI* (rs731236) and *ApaI* ($r^2 = 47$) and between *TaqI* and *BsmI* ($r^2 = 41$). The deep red colour in the LD plot for cases underlines the strong linkage among certain SNP pairs, indicating the existence of a tightly linked haplotype in affected individuals (Fig. 3A). In contrast, the control group exhibited markedly lower LD values across most SNP pairs, with the strongest LD observed between *ApaI* and *FokI* ($r^2 = 54$), which is reduced compared to the case group. The values of LD between

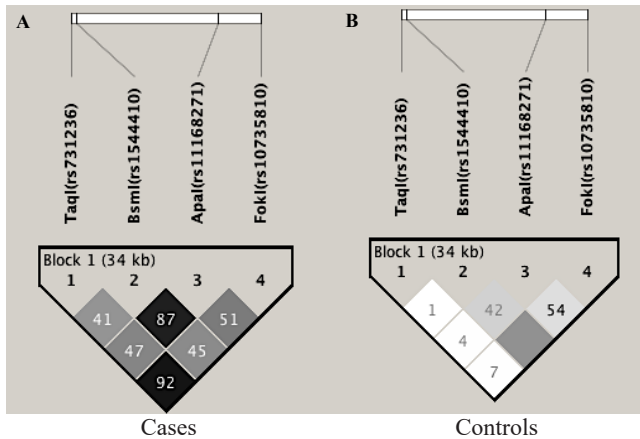


Fig. 3. Linkage disequilibrium (LD) plot: 'A' represents the LD pattern observed in cases; 'B' represents LD pattern observed in controls. The deep red colour in the LD plot underlines the strong linkage, whereas the light intensity of the LD plot reflects the weak linkage.

other pairs, such as *TaqI* and *BsmI* or *TaqI* and *ApaI*, are minimal and between $r^2=1$ to 4. The overall light intensity of the control LD plot reflects that the genetic correlations are relatively weak, which suggests a lack of closely linked haplotypes in non-affected individuals (Fig. 3B).

Discussion

This is a case-control study carried out on CAD patients in a tertiary cardiac centre in Southern India. We studied the prevalence and association between VDD and *VDR* gene variants with various risk factors for CAD and its severity.

Although both controls and cases had VDD in our study, the mean vitamin D level was significantly lower in patients. Also, 90 per cent of patients were deficient compared to 63 per cent of controls. We also found that VDD was associated with 5.7-fold increased risk of CAD. These findings suggest a strong association between VDD and CAD risk in our study group. In a meta-analysis of 44,717 participants across 65 studies from five South Asian countries, including India, Pakistan, Bangladesh, Nepal, and Sri Lanka, the prevalence of VDD was 67 per cent among Indians. The average level of vitamin D ranged from 4.7 to 32 ng/ml, with a weighted mean of 19.15 ng/ml¹⁵. The meta-analysis by Yan *et al*⁹, which included 13 studies from China, Iran, Brazil, Egypt, Poland, Croatia, and Germany, concluded that low plasma vitamin D levels are associated with CAD⁹. A study from the Northern part of India on first incident acute myocardial infarction reported a higher prevalence of

VDD and insufficiency and much lower mean values of vitamin D (98.3% of the cases had below normal values with a mean level of 6 ng/ml; 95.8% of controls had below normal values with a mean of 11.1 ng/ml). A 4.5-fold risk of myocardial infarction was also noted among individuals with severe (<10 ng/ml) VDD¹⁶. Collectively, these findings suggest that low vitamin D levels may be a significant risk factor for CAD, particularly in populations with a high prevalence of VDD. Our partial correlation analysis indicated that differences in vitamin D levels between cases and controls remain significant even after adjusting for key confounders, underscoring the potential role of vitamin D deficiency in CAD. Vitamin D plays a crucial role in regulating blood pressure and blood glucose levels, which are significant risk factors for CAD^{17,18}. Our correlation analysis suggests that there are weak negative correlations between vitamin D levels and CAD risk factors, but none of these relationships are statistically significant. In a previous study from India, higher levels of vitamin D have been associated with lower incidences of CAD⁸, but our findings do not support a strong correlation between vitamin D levels and CAD risk factors. VDD can affect lipid profiles by influencing low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, and total cholesterol levels in CAD patients^{19,20}. Additionally, VDD may impact blood sugar levels and the TC/HDL ratio²¹. Consistent with the previous findings our analysis revealed a trend for a negative correlation of vitamin D levels with cholesterol and triglyceride levels. However, other variables did not correlate. These findings suggest that maintaining adequate levels of vitamin D may have a positive impact on lipid profiles in CAD patients.

There are conflicting findings among various studies on *VDR* variants and their association with CAD^{22,23}. In the present study, the allelic frequency of the *TaqI* variant was significantly different between cases and controls. Also, a significant difference was found in the allelic distribution of the *FokI* variant in males with VDD compared to controls. Under the dominant inheritance model (Tt + tt vs TT), the *TaqI* variant suggested a potential protective association with an odds ratio of 0.6. This association remained significant even after adjusting the odds ratio for age, smoking, and alcohol. Upon gender stratification, the protective association persisted significantly in both males and females, even after adjusting for age, smoking, and alcohol. These findings suggest that the protective association of the 't' allele against CAD is

consistent across genders and remains significant after controlling for potential confounders.

The presence of the 'ff' genotype of *FokI* conferred a 5.9-fold risk of CAD in our cohort of males. In contrast, a previous study on 40 South Indian CAD subjects did not show any significant association of CAD with the *FokI* variant²⁴. This discrepancy in the results may be due to differences in sample size, genetic heterogeneity within the population, or variations in environmental factors that were not accounted for in either study. Hence, it is important to consider other genetic variations that may contribute to the development of CAD. Also, a case-control study from China did not find any significant association of *FokI* (rs2228570) and *BsmI* (rs1544410) with CAD²⁵. A recent meta-analysis⁹ evaluated thirteen studies using trial sequential analysis (TSA) for the association of four common *VDR* polymorphisms for CAD susceptibility. In their analysis, on stratification by race, the White people were found to have an increased CAD risk in *BsmI* ('b' allele), *FokI* ('f' allele), and *TaqI* ('t' allele) polymorphisms and a protective association of 'a' allele of *Apal*; Asians exhibited only 60 per cent risk in 'ff' genotype of *FokI* and no association with *TaqI* and *BsmI* polymorphisms⁹. The present study correlates with these findings except for *TaqI* polymorphism, which has shown a protective association in our study. A meta-analysis of 158 studies has reported an increased risk of CAD in patients with *TaqI* polymorphism and also suggested a possible protective role of *FokI* polymorphism²⁶. Their observations are in total contrast to our study findings. Overall, our findings suggest that the *VDR* *TaqI* variant may play a role in reducing the risk of CAD, regardless of gender. However, the *VDR* *FokI* variant conferred a risk of CAD in our cohort of males. These findings indicate that genetic variations in the *VDR* gene may have differing effects on CAD risk based on gender.

Overall, the analysis of *VDR* gene variants revealed distinct associations with CAD under specific conditions. The *TaqI* variant demonstrated a potential protective effect under the dominant inheritance model, with the association remaining significant even after adjusting for age, smoking, and alcohol. Gender stratification showed that the protective effect persisted in both males and females, even after accounting for these covariates. In contrast, the *BsmI* variant showed no significant association with CAD, irrespective of gender or covariate adjustments. Similarly, the *Apal* variant did not exhibit any association with CAD across various models of analysis, including gender

stratification. On the other hand, the *FokI* variant displayed a significant association in the recessive model, where the 'f' allele notably increased the risk of CAD, particularly in males, with a marked 5.9-fold higher risk even after adjusting for vascular risk factors. This comparison highlights the differing roles of *VDR* gene variants in CAD susceptibility and the influence of gender and other covariates on these associations.

Then, we were interested in knowing whether *TaqI* and *FokI* variants maintain their protective and risk effects under the context of VDD. A measure of the odds ratio was calculated to assess the combined effects of VDD and *VDR* gene variants *FokI* and *TaqI*. We found that neither *FokI* 'ff' nor the *TaqI* 'tt' genotype significantly alters the risk in the context of VDD entirely. The lack of significance suggests that multiple factors likely contribute to the outcome. Firstly, there may be a threshold effect for vitamin D levels below which the impact of *VDR* gene variants becomes negligible. Secondly, if VDD is severe enough, the relative contribution of the *VDR* variants might be overshadowed by the overall deficiency. Biological systems often have compensatory mechanisms that can mitigate the effects of genetic variations. For example, individuals with 'ff' or 'tt' genotypes might have other compensatory pathways that help maintain some level of *VDR* activity or vitamin D metabolism.

The comparison of LD patterns between cases and controls revealed a distinct difference in the genetic correlations among the four SNPs analysed (*TaqI*, *BsmI*, *Apal*, and *FokI*). These differences in LD patterns suggest that certain SNPs, particularly *Apal* and *FokI*, may be associated with the cases. In contrast, the weaker LD in controls implies a more random distribution of alleles. The observed patterns warrant further investigation to explore the functional roles of these SNPs and their contribution to disease susceptibility.

Although our study focused on *VDR* variants, we recognise the importance of other genes involved in vitamin D metabolism, such as *CYP27B1*, which encodes the enzyme responsible for converting 25-hydroxyvitamin D into its active form, 1,25-dihydroxyvitamin D (calcitriol)²⁷. Variants in *CYP27B1* can lead to reduced activity of this enzyme, impairing the activation of vitamin D even when precursor levels are normal²⁸. Loss-of-function mutations in *CYP27B1* have been associated with conditions such as vitamin D-dependent rickets

type 1 (VDDR1), characterised by bone deformities and growth retardation due to insufficient calcitriol levels^{29,30}. Including the analysis of *CYP27B1* variants in future studies could provide a more comprehensive understanding of vitamin D metabolism and its role in CAD, particularly when *VDR* function is normal but calcitriol synthesis may be disrupted.

Some limitations of this study were that it did not comprehensively account for environmental and dietary factors, such as diet, sun exposure, and lifestyle, which can significantly influence vitamin D levels and CAD risk. Furthermore, the role of other genetic factors in conjunction with *VDR* variants, which might provide a more comprehensive understanding of CAD development and progression were not explored.

In conclusion, while our study provides valuable insights into the association between *VDR* gene variants and CAD risk, more research is necessary to fully comprehend the underlying mechanisms and potential treatment options.

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References

- Vijayalakshmi IB, Nemani L, Kher M, Kumar A. The gamut of coronary artery disease in Indian women. *Indian J Cardiovasc Dis Women* 2023; 8 : 43-51.
- Sharma M, Ganguly NK. Premature coronary artery disease in Indians and its associated risk factors. *Vasc Health Risk Manag* 2005; 1 : 217-25.
- Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. *Am J Med Sci* 2009; 338 : 40-4.
- Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, *et al*. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008; 117 : 503-11.
- Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br J Nutr* 2005; 94 : 483-92.
- Aparna P, Muthathal S, Nongkynrih B, Gupta SK. Vitamin D deficiency in India. *J Family Med Prim Care* 2018; 7 : 324-30.
- Chowdhury R, Taneja S, Bhandari N, Sinha B, Upadhyay RP, Bhan MK, *et al*. Vitamin-D deficiency predicts infections in young North Indian children: A secondary data analysis. *PLoS One* 2017; 12 : e0170509.
- Akhtar T, Aggarwal R, Jain SK. Serum Vitamin D level in patients with coronary artery disease and association with sun exposure: Experience from a tertiary care, teaching hospital in India. *Adv Med* 2019; 2019 : 6823417.
- Yan X, Wei Y, Wang D, Zhao J, Zhu K, Liu Y, *et al*. Four common vitamin D receptor polymorphisms and coronary artery disease susceptibility: A trial sequential analysis. *PLoS One* 2022; 17 : e0275368.
- Thirunavukkarasu R, Chitra A, Asirvatham A, Jayalakshmi M. Association of Vitamin D deficiency and Vitamin D receptor gene polymorphisms with type 1 diabetes risk: A south Indian familial study. *J Clin Res Pediatr Endocrinol* 2024; 16 : 21-30.
- Raljević D, Peršić V, Markova-Car E, Cindrić L, Miškulin R, Žuvić M, *et al*. Study of vitamin D receptor gene polymorphisms in a cohort of myocardial infarction patients with coronary artery disease. *BMC Cardiovasc Disord* 2021; 21 : 188.
- Shaik MV, Kuragayala S, Madhuri S, Shaik M, Babulal S, Gangapatnam S. Association of Vitamin D receptor genetic variant Fok1, Bsm1, Apa1, and Taq1 polymorphism and Vitamin D deficiency with increased incidence of coronary artery disease. *Biomed Biotechnol Res J* 2023; 7 : 303.
- Aggarwal R, Akhthar T, Jain SK. Coronary artery disease and its association with Vitamin D deficiency. *J Midlife Health* 2016; 7 : 56-60.
- Surdu AM, Pinzariu O, Ciobanu DM, Negru AG, Căinap SS, Lazea C, *et al*. Vitamin D and its role in the lipid metabolism and the development of atherosclerosis. *Biomedicines* 2021; 9 : 172.
- Siddiquee MH, Bhattacharjee B, Siddiqi UR, Meshbahur Rahman M. High prevalence of vitamin D deficiency among the South Asian adults: a systematic review and meta-analysis. *BMC Public Health* 2021; 21 : 1823.
- Roy A, Lakshmy R, Tarik M, Tandon N, Reddy KS, Prabhakaran D. Independent association of severe vitamin D deficiency as a risk of acute myocardial infarction in Indians. *Indian Heart J* 2015; 67 : 27-32.
- Papandreou D, Hamid ZT. The role of Vitamin D in diabetes and cardiovascular disease: An updated review of the literature. *Dis Markers* 2015; 2015 : 580474.
- Jeong HY, Park KM, Lee MJ, Yang DH, Kim SH, Lee SY. Vitamin D and hypertension. *Electrolyte Blood Press* 2017; 15 : 1-11.
- Wang Y, Si S, Liu J, Wang Z, Jia H, Feng K, *et al*. The associations of serum lipids with Vitamin D status. *PLoS One* 2016; 11 : e0165157.
- Huang X, Yang Y, Jiang Y, Zhou Z, Zhang J. Association between vitamin D deficiency and lipid profiles in overweight and obese adults: A systematic review and meta-analysis. *BMC Public Health* 2023; 23.
- Wang L, Yan N, Zhang M, Pan R, Dang Y, Niu Y. The association between blood glucose levels and lipids or lipid ratios in type 2 diabetes patients: A cross-sectional study. *Front Endocrinol (Lausanne)* 2022; 13 : 969080.

22. Akhlaghi B, Firouzabadi N, Foroughinia F, Nikparvar M, Dehghani P. Impact of vitamin D receptor gene polymorphisms (TaqI and bsmI) on the incidence and severity of coronary artery disease: A report from southern Iran. *BMC Cardiovasc Disord* 2023; 23.
23. Tabaei S, Motallebnezhad M, Tabaei SS. Vitamin D receptor (VDR) gene polymorphisms and risk of coronary artery disease (CAD): Systematic review and meta-analysis. *Biochem Genet* 2021; 59 : 813-36.
24. Sowjanya B, Selvi V, Suneel B, Balakrishna D, Kumar D, Krishna B, *et al*. Vitamin D receptor fok I gene polymorphism in angiographically proven coronary artery disease subjects: Case - control study. *Br J Med Med Res* 2015; 8 : 612-7.
25. Pan XM, Li DR, Yang L, Wang EY, Chen TY, Liu YJ, *et al*. No association between vitamin D receptor polymorphisms and coronary artery disease in a Chinese population. *DNA Cell Biol* 2009; 28 : 521-5.
26. Lu S, Guo S, Hu F, Guo Y, Yan L, Ma W, *et al*. The associations between the polymorphisms of Vitamin D receptor and coronary artery disease: A systematic review and meta-analysis. *Medicine (Baltimore)* 2016; 95 : e3467.
27. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *J Lipid Res* 2014; 55 : 13-31.
28. Khammissa RAG, Fourie J, Motswaledi MH, Ballyram R, Lemmer J, Feller L. The biological activities of Vitamin D and its receptor in relation to calcium and bone homeostasis, cancer, immune and cardiovascular systems, skin biology, and oral health. *Biomed Res Int* 2018; 2018 : 9276380.
29. Demir K, Kattan WE, Zou M, Durmaz E, Bin Essa H, Nalbantoğlu Ö, *et al*. Novel CYP27B1 gene mutations in patients with vitamin d-dependent rickets type 1A. *PLoS One* 2015; 10 : e0131376.
30. Zalewski A, Ma NS, Legeza B, Renthal N, Flück CE, Pandey AV. Vitamin d-dependent rickets type 1 caused by mutations in CYP27B1 affecting protein interactions with adrenodoxin. *J Clin Endocrinol Metab* 2016; 101 : 3409-18.

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