

Association of VDR rs2228570 (FokI) and rs1544410 (BsmI) Polymorphisms with Serum Vitamin D Status in a Clinical Cohort

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Abstract: Background: Vitamin D status is influenced by environmental, nutritional, physiological, and genetic factors. The vitamin D receptor (VDR) is a key mediator of vitamin D signalling, and common VDR variants have been investigated as potential modifiers of vitamin D-related phenotypes. The present study evaluated the association of VDR rs2228570 (FokI) and rs1544410 (BsmI) polymorphisms with serum vitamin D status. **Methods:** Seventy-seven participants were classified according to the study-defined serum vitamin D categories as deficient (<20 ng/mL; n=68) or insufficient (20–30 ng/mL; n=9). Genotype and allele distributions for rs2228570 and rs1544410 were assessed, Hardy–Weinberg equilibrium (HWE) was evaluated, and inherited genetic models were examined using logistic regression with adjustment for a continuous quantitative covariate, as reported in the source analysis.

Results: Vitamin D deficiency was present in 68/77 participants (88.31%). For rs2228570, the overall genotype distribution was C/C 55 (71%), C/T 17 (22%), and T/T 5 (6%). The C/T genotype was absent in the insufficient group, in which C/C accounted for 8/9 (89%) and T/T for 1/9 (11%). The overdominant model showed a statistically significant association with vitamin D classification ($P=0.047$), whereas the codominant, dominant, recessive, and log-additive models were not statistically significant ($P>0.05$). For rs1544410, no tested genetic model was statistically significant (all $P>0.05$). **Conclusion:** In this sample, rs2228570 showed an association with vitamin D classification under an overdominant model, while rs1544410 did not show evidence of an independent association. The finding for rs2228570 should be interpreted cautiously because the insufficient group was small and contained no C/T heterozygotes, producing an unstable effect estimate. Larger, adequately powered studies incorporating environmental and clinical determinants of vitamin D status are needed.

Keywords: Vitamin D; 25-hydroxyvitamin D; vitamin D receptor; VDR; rs2228570; FokI; rs1544410; BsmI; genetic polymorphism; hypovitaminosis D

Introduction:

Vitamin D status is commonly assessed using serum 25-hydroxyvitamin D [25(OH)D], which reflects vitamin D obtained from endogenous production, food, and supplements. The U.S. National Institutes of Health Office of Dietary Supplements notes that serum 25(OH)D is the main indicator of vitamin D status and that concentrations of 20 ng/mL or higher are generally considered adequate for most people, although definitions of deficiency and optimal concentrations vary by authority and clinical context (1). The biological effects of the active vitamin D metabolite are mediated largely through the vitamin D receptor (VDR), a nuclear receptor that regulates transcription of vitamin D-responsive genes. Genetic variation within VDR has therefore attracted interest as a potential source of inter-individual differences in vitamin D-related phenotypes. Among the most extensively studied variants are rs2228570 (FokI) and rs1544410 (BsmI).

rs2228570 is a coding-region variant located near the translation initiation site and has been described as functionally relevant to VDR protein structure and activity. Previous studies of its relationship with circulating 25(OH)D have produced inconsistent results. A 2026 systematic review and meta-analysis including 45 studies and 15,256 participants found no overall significant association between rs2228570 and circulating 25(OH)D under the genetic models examined, although heterogeneity was substantial in several comparisons (2). Other individual studies have reported associations in particular populations, illustrating the importance of population, environmental, and clinical context. (3).

rs1544410 (BsmI) is another widely studied VDR polymorphism. Evidence concerning its clinical and biochemical effects is likewise mixed. Systematic reviews have found no consistent association between BsmI and several vitamin-D-related outcomes, while other studies have reported population-specific relationships (4,5).

Because vitamin D status is multifactorial and genetic effects may be modest, analysis of defined genetic models can provide useful exploratory evidence, particularly in populations for which local data are limited.

Materials and Methods:

Study design and setting:

The study included 77 subjects and analyzed VDR polymorphisms in relation to vitamin D status. The design was case control study conducted at Sudan, Gezira state, Wad Madani, Gezira Hospital for renal disease and surgery. Data was collected by using a questionnaire. Questionnaires were filled by the investigator during each time when blood samples were collected.

Participants:

Participants were classified into two vitamin D categories: deficient (<20 ng/mL) and insufficient (20–30 ng/mL). Inclusion criteria includes clinically diagnosed CKD patients age 25 – 85 years old and excluded Chronic Kidney Disease patients with other disease such as cancer.

Vitamin D assessment:

The serum 25-hydroxyvitamin D [25(OH)D] concentration was measured using a competitive immunoassay with a total assay duration of approximately 27 minutes. For each determination, 15 µL of serum sample was subjected to a three-step incubation procedure. During the first incubation, the sample was treated with pretreatment reagents 1 and 2 to release 25(OH)D from vitamin D-binding protein. In the second incubation, the pretreated sample was incubated with ruthenium-labelled vitamin D-binding protein, allowing formation of a complex between 25(OH)D and the ruthenium-labelled binding protein. During the third incubation, streptavidin-coated microparticles and biotin-labelled 25(OH)D were added. The labelled 25(OH)D competed with the endogenous 25(OH)D for binding sites on the ruthenium-labelled vitamin D-binding protein. The resulting complex containing biotinylated 25(OH)D was immobilized on the solid phase through the biotin–streptavidin interaction. The reaction mixture was subsequently transferred to the measuring cell, where the microparticles were magnetically captured onto the electrode surface. Unbound substances were removed using ProCell/ProCell M. Application of an electrical voltage to the electrode induced chemiluminescent emission, which was measured by a photomultiplier. The concentration of 25(OH)D was calculated from an instrument-specific calibration curve generated using two-point calibration in conjunction with a master curve encoded in the reagent barcode.

Genotyping:

The VDR gene polymorphisms “rs1544410 (BsmI)” and “rs2228570 (FokI)” were investigated. Genomic DNA was extracted using a commercial “QIAGEN DNA extraction kit (USA)”, and genotyping was performed by “PCR-Conventional” using primers designed with Primer3 and a Techne TC-312 thermal cycler. PCR amplification consisted of initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at the appropriate temperature for 30 seconds, and extension at 72°C for 30 seconds. The amplified products were subsequently digested with the corresponding restriction enzymes according to the manufacturer’s instructions for genotype determination.

Statistical analysis:

Data were analyzed and tabulated using statistical package for social sciences (IBM SPSS) and SNPStats online tool and DNA sequencing tools.

Ethical considerations:

Ethical clearance was taken from Gezira Hospital for Surgery and Kidney Diseases (Wad Medani Center) and permission was taken from ministry of health, Gezira state.

Results:

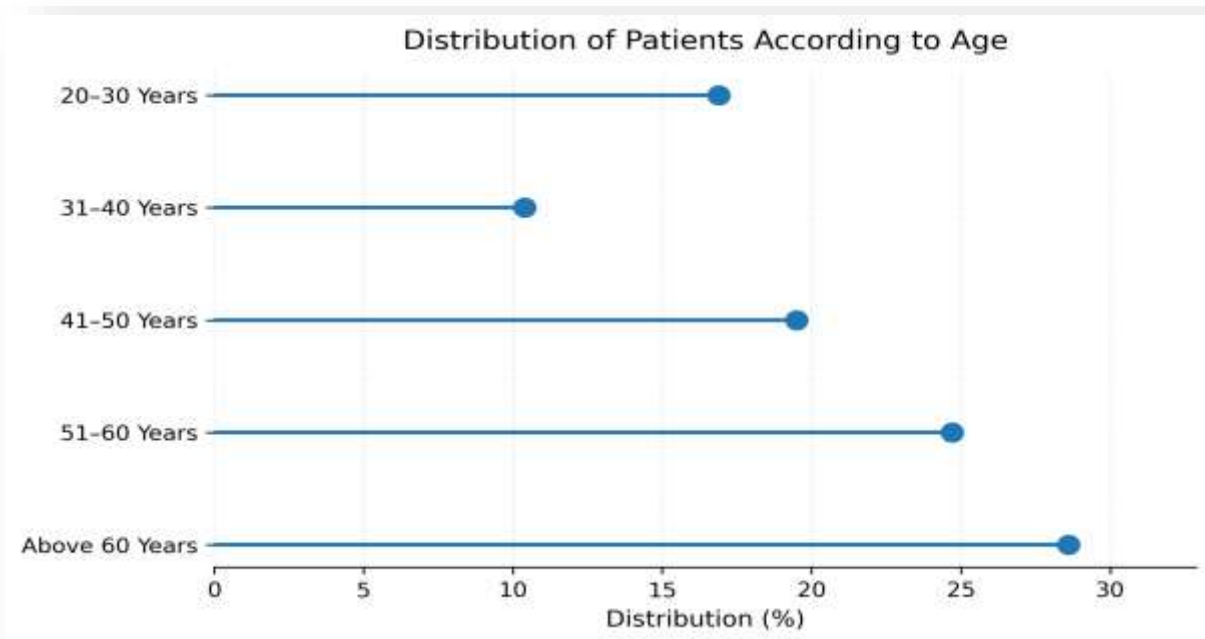


Figure 1. Distribution of patients according to age.

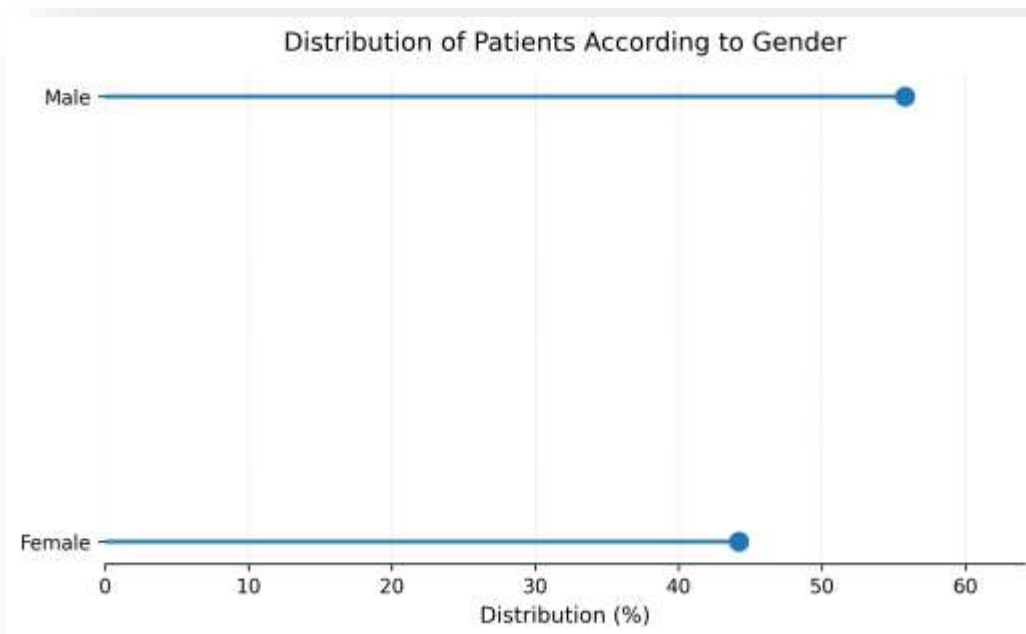


Figure 2. Distribution of patients according to gender.

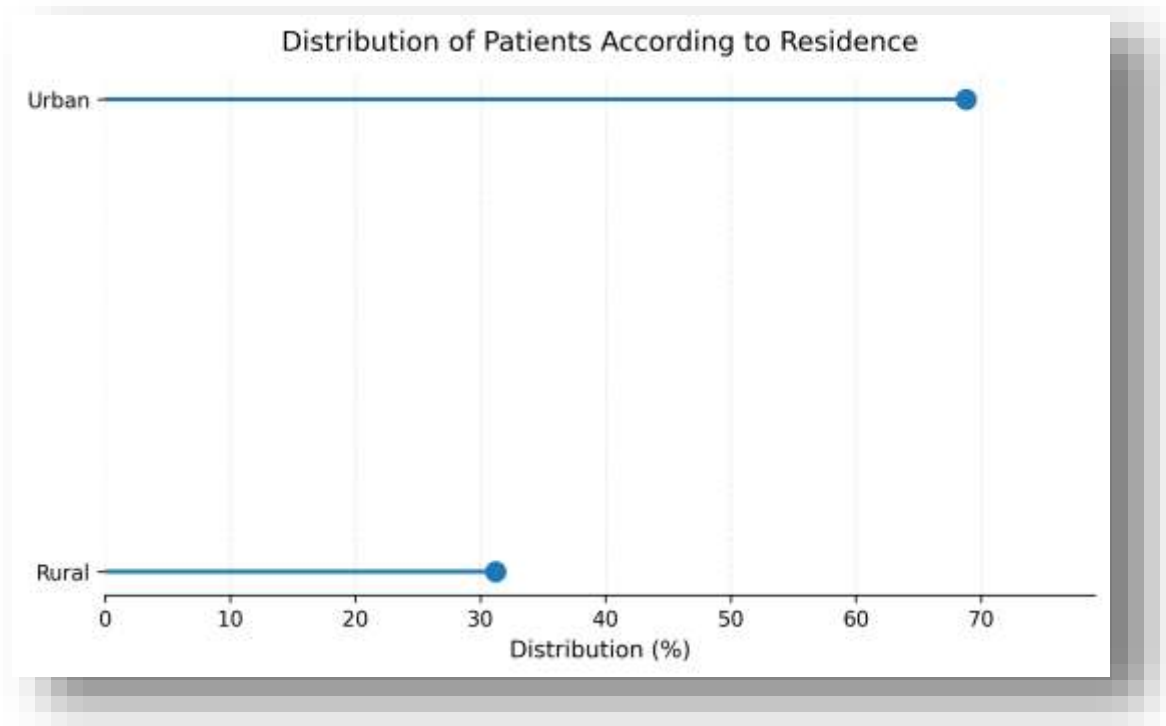


Figure 3. Distribution of patients according to residence.

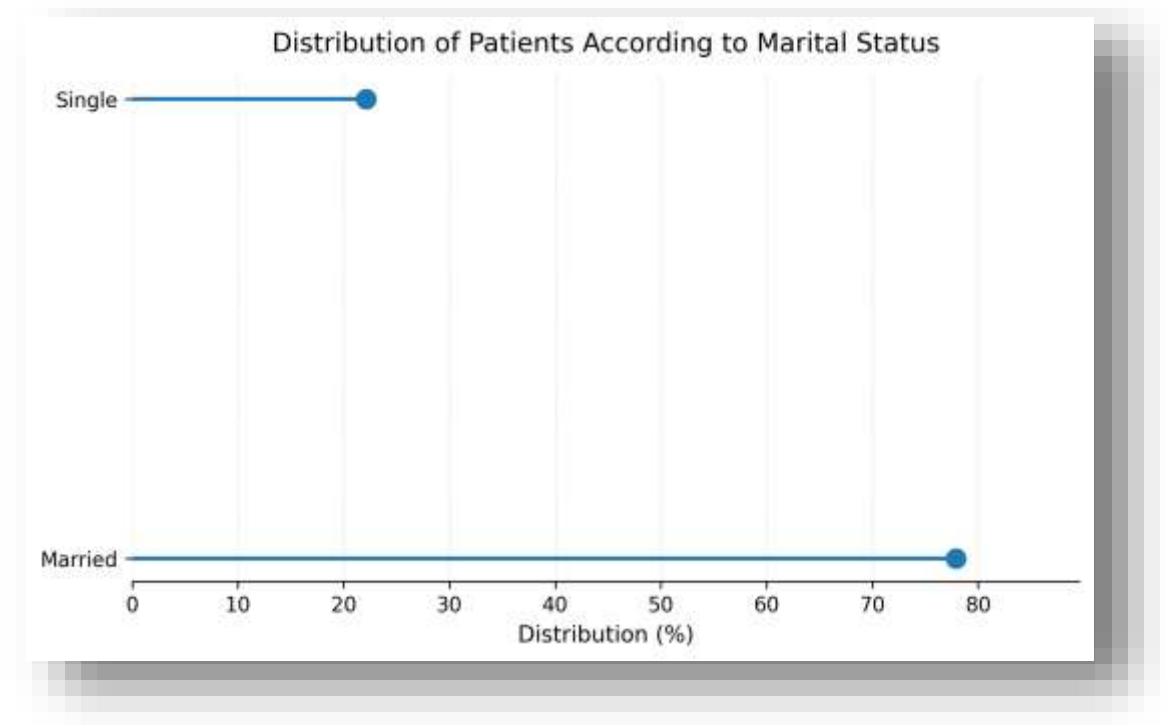


Figure 4. Distribution of patients according to marital status.

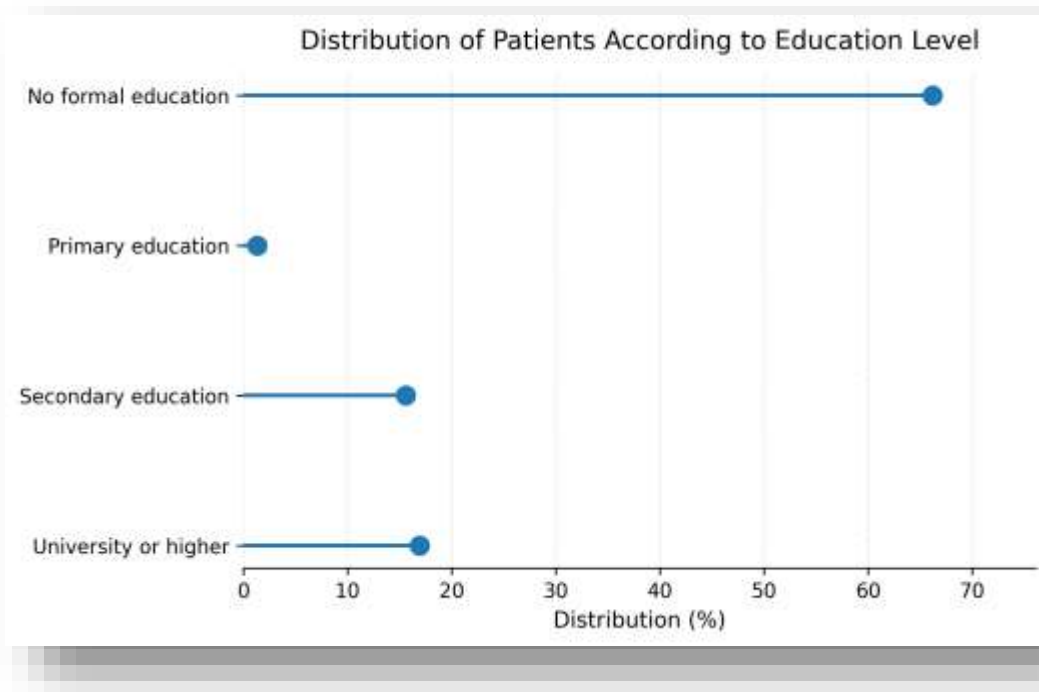


Figure 5. Distribution of patients according to education level.

Among the 77 participants, 68 (88.31%) were classified as vitamin D deficient and 9 (11.69%) as insufficient. For rs2228570, the observed genotypes were C/C 55 (71%), C/T 17 (22%), and T/T 5 (6%). The corresponding allele counts were C=127 (82%) and T=27 (18%). For rs1544410, the observed genotypes were G/G 44 (57%), G/A 30 (39%), and A/A 3 (4%), corresponding to G=118 (77%) and A=36 (23%).

Table 2: Vitamin D status and genotype distributions:

Variant/measure	Total (n=77)	Deficient (n=68)	Insufficient (n=9)	HWE P-value reported
Vitamin D status	68 (88.31%) deficient; 9 (11.69%) insufficient	68 (100%)	9 (100%)	—
rs2228570 C allele	127 (82%)	111 (82%)	16 (89%)	0.046 overall; subgroup values 0.21 and 0.059
rs2228570 T allele	27 (18%)	25 (18%)	2 (11%)	—
rs2228570 C/C	55 (71%)	47 (69%)	8 (89%)	—
rs2228570 C/T	17 (22%)	17 (25%)	0 (0%)	—
rs2228570 T/T	5 (6%)	4 (6%)	1 (11%)	—
rs1544410 G allele	118 (77%)	107 (79%)	11 (61%)	0.75 overall; subgroup values 0.27 and 0.49
rs1544410 A allele	36 (23%)	29 (21%)	7 (39%)	—
rs1544410 G/G	44 (57%)	40 (59%)	4 (44%)	—
rs1544410 G/A	30 (39%)	27 (40%)	3 (33%)	—

Variant/measure	Total (n=77)	Deficient (n=68)	Insufficient (n=9)	HWE P-value reported
rs1544410 A/A	3 (4%)	1 (1%)	2 (22%)	—

Table 3: Genotype comparison and associated analysis:

Variant	Model	Genotype comparison	OR (95% CI)	P-value
rs2228570	Codominant	C/C vs C/T vs T/T	Ref 1.00; 0.00 (0.00–NA); 1.72 (0.16–19.01)	0.13
rs2228570	Dominant	C/C vs C/T–T/T	0.34 (0.04–3.14)	0.30
rs2228570	Recessive	C/C–C/T vs T/T	2.53 (0.23–27.96)	0.48
rs2228570	Overdominant	C/C–T/T vs C/T	0.00 (0.00–NA)	0.047
rs2228570	Log-additive	Not reported in source table	—	0.68
rs1544410	Codominant	G/G vs G/A vs A/A	Ref 1.00; 0.94 (0.16–5.38); 8.13 (0.19–349.71)	0.50
rs1544410	Dominant	G/G vs G/A–A/A	1.20 (0.23–6.31)	0.83
rs1544410	Recessive	G/G–G/A vs A/A	8.36 (0.21–331.01)	0.24
rs1544410	Overdominant	G/G–A/A vs G/A	0.74 (0.13–4.05)	0.72
rs1544410	Log-additive	Not reported in source table	—	0.52

The rs2228570 overdominant model was statistically significant at $P=0.047$. This result is driven by the absence of C/T heterozygotes among the nine participants in the insufficient category. The remaining rs2228570 models were not statistically significant. None of the rs1544410 models reached $P<0.05$.

Discussion:

This study examined two common VDR polymorphisms in relation to vitamin D classification in 77 participants. The principal finding was an association between rs2228570 and vitamin D classification under the overdominant model, whereas no statistically significant association was observed for rs1544410 under any tested model.

An evaluation of our cohort of 77 participants revealed a universal prevalence of sub-optimal vitamin D status, comprising an 88.31% deficiency and an 11.69% insufficiency rate (6). This extreme skew demonstrates a severe background burden of hypovitaminosis D, matching widespread public health reports that document systemic nutritional inadequacies within modern populations. Because every participant fell below optimal thresholds, the baseline risk environment remains heavily homogenous, making it critical to examine genetic modulators that may influence how individuals segregate across these clinical categories.

Association analyses identified a statistically significant relationship under the rs2228570 overdominant model ($P=0.047$) (7). Association analyses identified a statistically significant relationship under the rs2228570 overdominant model ($P=0.047$), driven by the absence of C/T heterozygotes in the insufficient category compared to a 25% prevalence in the deficient group. Biochemically, the rs2228570 alteration creates a translation start site polymorphism that modifies VDR protein structure, where the shorter variant exhibits higher transcriptional activity and the alternate allele yields weaker functional binding capacity for the active hormone. For further details on the genetics of vitamin D metabolism, visit (8). While this unique overdominant heterozygous distribution implies that carrying both alleles may affect boundary transitions between clinical categories, all other tested genetic models including codominant ($P=0.13$), dominant ($P=0.30$), and recessive ($P=0.48$) failed to yield significant results, indicating a limited overall predictive capacity within this specific cohort size.

Conversely, the rs1544410 polymorphism (*BsmI*) exhibited general genetic stability, strictly conforming to Hardy-Weinberg Equilibrium ($P=0.75$) without any independent, direct correlation to baseline serum 25(OH)D levels (9). No significant associations with vitamin D classification were observed across the codominant, dominant, or log-additive inheritance models for rs1544410. The extreme nominal odds ratio observed under the recessive model reflects statistical instability driven by a low minor allele frequency rather than a direct alteration of the amino acid sequence (10).

Several limitations restrict the broad generalizability of these findings. First, the small sample size (n=77) compromised our statistical power, yielding highly inflated confidence intervals for rare variant arrangements like the rs1544410 A/A genotype (11). Second, the complete absence of a healthy, vitamin D-sufficient control group creates a phenotypic restriction, potentially masking subtle genetic effects that would typically distinguish sufficient states from deficient ones. Furthermore, vitamin D status is highly polygenic and heavily mediated by non-genetic environmental factors like sun exposure, seasonal variations, and dietary behaviors (12). To fully map these pathways, future multi-center trials employing larger, phenotypically balanced cohorts with adequate control groups are strictly necessary.

Strengths and Limitations:

Strengths include evaluation of two established VDR polymorphisms, presentation of allele and genotype distributions, and assessment across several commonly used genetic models. The study also reports effect estimates and confidence intervals rather than relying only on P values.

The main limitations are the small total sample size and particularly small insufficient group (n=9), which creates sparse genotype cells and unstable regression estimates. The absence of C/T heterozygotes in the insufficient group is especially important when interpreting the significant overdominant result. The source manuscript also does not provide enough information about participant selection, assay methodology, genotyping quality control, clinical/environmental covariates, missing data, or ethical approval. Finally, the use of a dichotomized vitamin D outcome loses information that would be retained by analyzing measured 25(OH)D as a continuous variable, provided the raw values are available.

Conclusion:

In this sample of 77 participants, VDR rs2228570 (FokI) showed a statistically significant association with vitamin D classification under the overdominant genetic model ($P=0.047$), while rs1544410 (BsmI) showed no significant association under the tested models. Because the significant rs2228570 result was based on a small insufficient group with no observed C/T heterozygotes, the finding should be considered exploratory and requires confirmation in larger studies. Future research should incorporate measured serum 25(OH)D values, relevant environmental and clinical determinants, robust genotyping quality control, and sufficiently powered samples.

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