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Original Research Article

# Emerging Genetic Associations between ACE2 and Vitamin D Receptor Variants in Type 2 Diabetes Mellitus

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**Conflict of interest: Nil**

**Abstract:**

**Background:** Diabetes mellitus type 2 (T2DM) is an example of a multifactorial disease affected by multiple genetic and environmental factors. Studies have shown that receptor-based pathways like the Vitamin D receptor (VDR) and angiotensin-converting enzyme 2 (ACE2) pathways have significant involvement in glucose metabolism, inflammation, and insulin resistance. Variations in these receptors may be responsible for diabetes predisposition.

**Objective:** This study was conducted to elucidate the relationship between the polymorphisms of VDR gene (BsmI, FokI, and TaqI) and ACE2 gene (II, DD, and I/D) and their involvement in the development of T2DM**.**

**Methods:** The study population consisted of 365 subjects, among which there were 185 T2DM patients and 180 normal control subjects. The serum concentration of 25-hydroxy vitamin D was quantified using enzyme-linked immunosorbent assay. The genomic DNA was isolated from blood samples, and the polymorphisms in the ACE2 gene were determined using polymerase chain reaction analysis.

**Results:** The incidence of VDR polymorphisms was found to be much higher among T2DM patients compared to the control group, with BsmI (77 against 17), FokI (60 against 0), and TaqI (51 against 1) polymorphisms showing a significant increase. In contrast, there is a clear predominance of the DD genotype in the diabetic population (133 subjects), whereas the II genotype is more common among the control group (169 subjects). The I/D heterozygote genotype was present only rarely in both populations. Overall, there is evidence of a strong correlation between the presence of VDR polymorphisms and the ACE2 DD genotype.

**Conclusion:** The study results show that there is a strong relationship between the polymorphisms of genes VDR and ACE2 and susceptibility to T2DM. The presence of both the polymorphic forms of VDR genes and ACE2 genotype DD underscores the importance of the relationship between the VDR–ACE2–RAAS axis in the development of diabetes.

**Keywords:** Type 2 Diabetes Mellitus, Vitamin D Receptor, ACE2, Gene Polymorphism, RAAS, Insulin Resistance.

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## Introduction

Type 2 Diabetes Mellitus (T2DM) is a global health problem that involves complex gene-environment interactions. It is defined by persistent hyperglycemia secondary to both insulin resistance and deficiency in insulin secretion (WHO, 2024). Recent developments in molecular biology have focused on the importance of receptor-mediated mechanisms in metabolism regulation. These include the Vitamin D Receptor (VDR) and ACE2 receptors, which are involved in glucose balance regulation, inflammation, and cardiovascular regulation. Vitamin D is a pleotropic hormone that modulates insulin release and sensitivity through VDR activation. Similarly, ACE2 controls the

RAAS through converting Angiotensin II to Angiotensin-(1–7). As such, it has anti-inflammatory effects and increases insulin sensitivity (Gheblawi et al., 2020). Therefore, the interaction between VDR and ACE2 receptors implies a new molecular pathway involved in T2DM pathology. Type 2 Diabetes Mellitus (T2DM) is a multifactorial metabolic disorder marked by persistent hyperglycemia due to impaired insulin secretion and peripheral insulin resistance. Growing research indicates that receptor-mediated molecular pathways play a crucial role in T2DM pathogenesis. In particular, the VDR signaling pathway and RAAS, especially through Angiotensin-Converting Enzyme 2 (ACE2), have been identified as significant modulators of metabolic homeostasis.

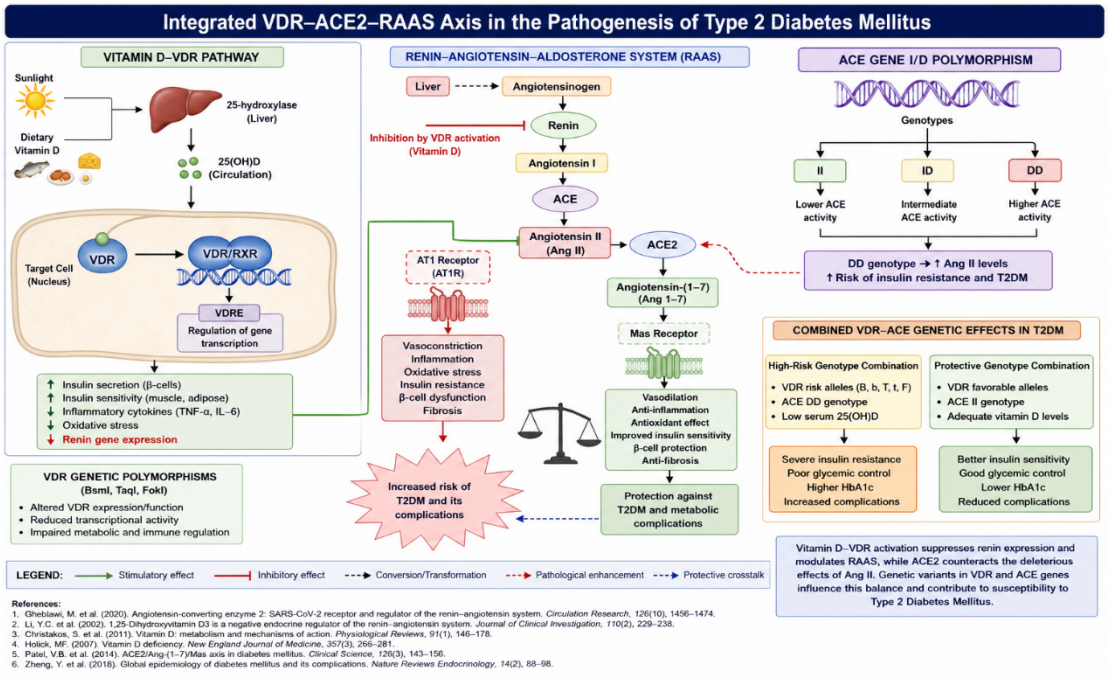


**Figure 1: Combined Axis of VDR–ACE2–RAAS in the Pathophysiology of Type 2 Diabetes Mellitus. The schematic diagram shows the connection between VDR pathways and RAAS. Vitamin D is metabolized in the liver to 25-hydroxyvitamin D (25[OH]D), and this compound acts on VDR and participates in the regulation of gene transcription responsible for insulin production, insulin sensitivity, and inflammation. Activated VDR inhibits renin gene expression, which helps control the activity of RAAS. Angiotensin II, produced by angiotensin I converting enzyme (ACE), increases the risk of insulin resistance, oxidative stress, and inflammation through AT1 receptors. On the other hand, angiotensin II, converted by ACE2 to angiotensin-(1–7), exhibits beneficial effects on insulin sensitivity and inflammation through Mas receptors. Polymorphisms in the genes that encode VDR (BsmI, TaqI, FokI) and ACE (I/D variants) affect this process in such a way that high-risk genotypes lead to increased RAAS activation and, thus, increase the risk of T2DM.**

As indicated in the figure, the production of vitamin D through cutaneous synthesis or dietary sources leads to its hydroxylation by the liver to produce 25-hydroxyvitamin D [25(OH)D] to activate the VDR. The binding of VDR to the vitamin D response element (VDRE) following its formation of a heterodimer with retinoid X receptors (RXR) facilitates the transcription of genes associated with insulin secretion, glucose metabolism, and anti-inflammatory mechanisms (Christakos et al., 2011). The activation of VDR results in improved functioning of pancreatic beta cells and increased insulin sensitivity in peripheral organs, as well as inhibition of pro-inflammatory cytokines like TNF-α and IL-6. One of the important pathways indicated in the figure through which VDR exerts a protective effect against T2DM is the suppression of renin gene expression by VDR-mediated signaling. Under physiological circumstances, renin causes the conversion of angiotensinogen to angiotensin I, and subsequently angiotensin I is converted to Ang II through the action of angiotensin-converting enzyme (ACE). Ang II leads to vasoconstriction, oxidative stress, inflammation, and insulin resistance that adversely affect pancreatic beta cell function in T2DM (Gheblawi et al., 2020). Importantly, ACE2 acts as a counter-regulatory enzyme within RAAS by converting Ang II into angiotensin-(1–7) [Ang-(1–7)], which exerts protective effects via the Mas receptor. This axis promotes vasodilation, anti-inflammatory signaling, antioxidative activity, and

improved insulin sensitivity, thereby mitigating metabolic and vascular complications associated with diabetes (Patel et al., 2014). The balance between the deleterious ACE/Ang II axis and the protective ACE2/Ang-(1–7) axis is therefore crucial in determining metabolic outcomes. Genetic polymorphisms may also affect the function of these pathways. Genetic variations in the VDR gene (BsmI, TaqI, and FokI) can modify the expression and activity of the VDR receptor, resulting in defective insulin signaling and increased T2DM susceptibility (Uitterlinden et al., 2004). The ACE I/D polymorphism can also influence the concentration of ACE in the bloodstream; the DD genotype is linked to high levels of Ang II, insulin resistance, and increased T2DM risk (Zheng et al., 2018). As shown in the figure, there is a complementary interaction between the VDR and ACE pathways, where vitamin D insufficiency and negative genetic polymorphisms may contribute to RAAS stimulation and metabolic disturbances. Low activity of the VDR causes unrestrained renin expression, whereas ACE gene polymorphisms increase the impact of Ang II. In contrast, sufficient vitamin D concentrations and positive genetic factors facilitate ACE2 activity, promoting insulin sensitization and minimizing the risk of T2DM. Therefore, the interconnected VDR–ACE2–RAAS pathway provides a biologically feasible model of T2DM pathogenesis, accounting for genetic susceptibility, endocrine control, and metabolic dysfunction. This pathway can be used to detect new biological markers and design efficient treatment strategies targeting receptor-related pathways in diabetes.

The proposed research aims to explore the interaction between vitamin D receptor (VDR) gene polymorphisms (BsmI, TaqI, and FokI) and angiotensin-converting enzyme (ACE/ACE2) gene polymorphisms for the development of type 2 diabetes mellitus (T2DM). In particular, the study will examine the correlation between the mentioned polymorphisms and the following parameters: glycemic profile, plasma concentrations of 25-OH vitamin D, and disease vulnerability among the subjects. Moreover, this research will seek to assess the synergic influence of VDR and ACE gene polymorphisms on the regulation of receptor-dependent processes, especially VDR-RAAS signaling pathway, which is essential in terms of insulin resistance and metabolic dysfunction.

## Materials and Methods

**Study Population:** This current research was performed post receipt of approval by the Institutional Ethics Committee (Reference Number: GBCM/IEC/2023/11) and in strict adherence to the principles outlined in the Declaration of Helsinki (World Medical Association, 2013). Blood samples were obtained from voluntary subjects visiting Dr. K.K.B.M. Subharti Hospital, Jhajra, Dehradun and informed consent was taken before conducting any procedure. Altogether, 365 subjects were involved in this study, consisting of both males and females. Venous blood samples (about 5 mL) were collected in a sterile manner using venipuncture. A tourniquet was tied at 7-10 centimeters above the puncture point, and the puncture point was cleansed with 70% ethanol. The collected blood samples were separated into three vacutainer tubes: 3 mL was filled in a clot activator tube, 1 mL in EDTA-containing tube, and 1 mL in sodium fluoride tube.

**Estimation of Vitamin D Levels:** The concentrations of 25-hydroxyvitamin D [25(OH)D] in serum samples were measured by means of a commercially available sandwich ELISA kit, according to the manufacturer's instructions. In brief, 10 µL of serum sample and 200 µL of sample diluent were added into wells coated with monoclonal anti-25(OH)D and incubated at room temperature for 20 minutes. Following washings, 100 µL of enzyme conjugate was added and incubated for 10 minutes. Another round of washings was conducted, and 100 µL of chromogenic substrate solution was added to each well before incubating in the dark for 10 minutes. Stop solution (100 µL) was added to terminate the reaction, and the absorbance of the wells was measured spectrophotometrically at 450 nm within 15 minutes. This test is based on the Beer-Lambert principle, whereby the intensity of the colored complex generated is directly proportional to the amount of vitamin D in serum samples (Holick, 2007; Christakos et al., 2011).

**Nucleic Acid Extraction (DNA):** DNA was isolated from peripheral whole blood using the Blood DNA Isolation Kit, which is based on a silica column purification procedure (Norgen Biotek Corp., Lot No. 592769). Specifically, 200 µL of whole blood was lysed in lysis buffer in the presence of Proteinase K and incubated at 55 °C for 10 min to promote cell lysis. The addition of ethanol allowed for DNA isolation using the spin column. Subsequently, the DNA was washed using a series of wash buffers. Finally, DNA was eluted from the column with 200 µL of elution buffer and stored at -20 °C. The quality and purity of the DNA sample was verified using gel electrophoresis and UV spectrometry (Sambrook and Russell, 2001).

**Detection of ACE 2 Gene Polymorphisms (PCR Amplification):** The PCR amplification reactions for the ACE 2 I/D polymorphism were done in a total reaction volume of 25 µL consisting of PCR master mix (10 mM Tris-HCl, 50 mM KCl), dNTPs (200 µM), $MgCl_2$ (1.5 mM), forward and reverse primers (10 pmol each), Taq DNA polymerase (0.5 U), and genomic DNA template (approximately 100 ng). Primer sequences for ACE I/D

polymorphism were 5′-CTGGAGACCACTCCCATCCTTTCT-3′ (forward) and 5′-GATGTGGCCATCACATTCGTCAGAT-3′ (reverse). Thermal cycle parameters for PCR amplification consisted of an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of 94 °C (denaturation step) for 1 minute, 58 °C (annealing step) for 1 minute, 72 °C (extension step) for 1 minute, and finally a 72 °C (extension step) for 10 minutes. After that, the PCR products were analyzed on a 2% agarose gel stained with ethidium bromide and viewed under UV light. Appearance of the 490 bp band implied the presence of I allele, while the appearance of the 190 bp band implied the D allele.

## Results

The present study of 365, including 185 T2DM cases and 180 non-diabetic control individuals. The distribution pattern of VDR gene polymorphisms (BsmI, FokI, and TaqI) as well as ACE2 gene polymorphisms (II, DD, and I/D) has been studied to determine their correlation with T2DM risk.

**Distribution of VDR Gene Polymorphisms:** The analysis of the polymorphisms in VDR genes showed significantly increased polymorphism frequencies in diabetic patients as compared to those in the control group. Polymorphism BsmI was found in 77 diabetic patients and 17 control subjects; FokI was found in 60 diabetic patients and not at all in control subjects; and TaqI was found in 51 diabetic patients and only one in control subjects. All these results show that there is a significantly increased occurrence of polymorphisms of the VDR gene in T2DM patients, which suggests the possible involvement of abnormal VDR gene transcription in the development of the disease as shown in Figure 2.

**Distribution of ACE2 Gene Polymorphisms:** The frequency distribution of the genotypes of the ACE2 gene showed marked differences between the diabetic and nondiabetic individuals. The genotype DD was highly common among individuals with T2DM, and its prevalence was found to be 133 cases, whereas the control group did not have a single instance of the genotype. On the other hand, the genotype II was highly common among the control group, whose prevalence was noted to be 169 cases, whereas only 49 diabetic subjects had this genotype. The heterozygous genotype I/D was rare in both groups, with only 3 cases in the diabetic population and 9 cases among the controls as shown in Figure 2.

**Association of VDR and ACE2 Polymorphisms:** VDR and ACE2 gene polymorphism analysis indicated the presence of unique genetic factors that predisposed T2DM individuals. The diabetic population had an increased frequency of polymorphisms in both the VDR genes (BsmI, FokI, and TaqI), together with the dominance of ACE2 DD genotype. However, the healthy control group presented a lack of genetic polymorphisms for VDR along with increased presence of the ACE2 II genotype. These findings suggest the possible interaction of genetic polymorphisms for VDR and ACE2. Therefore, the VDR-RAAS genetic axis is likely to be regulated by such changes through increased expression of angiotensin II activity, inflammation, and insulin resistance, resulting in T2DM progression. The present study shows an evident prevalence of gene polymorphism of the VDR gene, especially the BsmI and FokI polymorphisms, among diabetics. In addition, the genotype ACE2 DD was found to be highly associated with T2DM while the genotype ACE2 II showed protective effects. Followed strong association of VDR polymorphisms and the genotype ACE2 DD that there could have been a genetic susceptibility leading todiabetes.

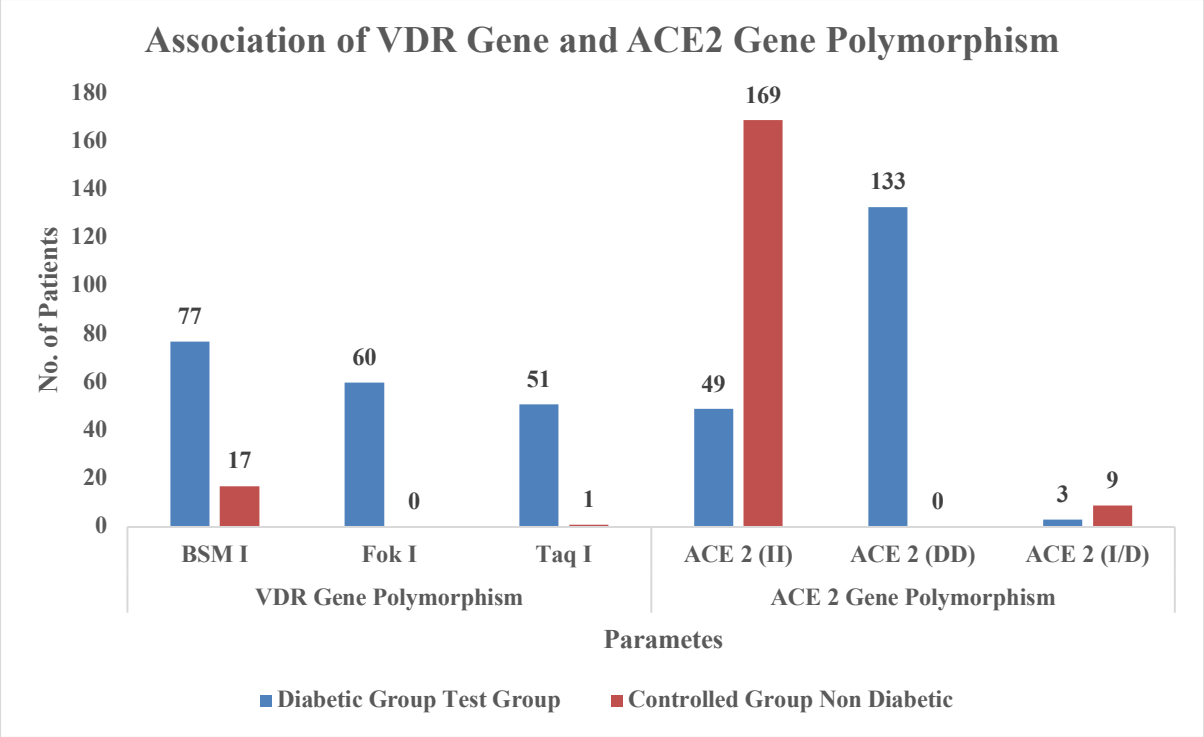


**Figure 2: Association of VDR Gene and ACE 2 Gene Polymorphism**

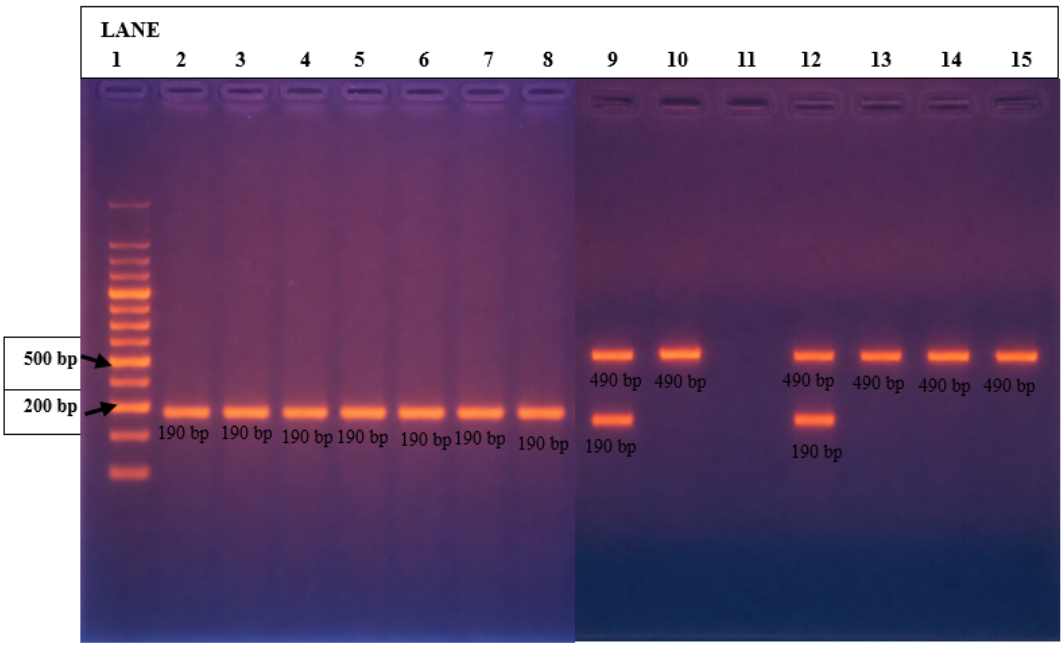


**Figure 3: Agarose gel electrophoresis result of the PCR amplification of the ACE2 gene polymorphism.**

Agarose gel electrophoresis was conducted to visualize the amplification of the ACE2 gene polymorphism. Lane 1 corresponds to the DNA molecular weight marker (100 bp ladder) showing reference bands of ~200 bp and ~500 bp for determining the sizes of fragments. Expected bands for the PCR product that corresponds to the ACE2 gene polymorphism are around ~490 bp (insertion allele, I) and 190 bp (deletion allele, D). Lanes 2-8mostly show one predominant band of ~190 bp denoting samples homozygous for deletion alleles or DD genotypes. On the other hand, lanes 9-15 show varying results where lanes having only one band of ~490 bp indicate homozygous samples or II genotypes, while lanes that have two bands are denoted as ID genotypes.

## Discussion

This study highlights a strong correlation between polymorphisms within the genes for Vitamin D Receptor (VDR) (BsmI, FokI, and TaqI) and angiotensin-converting enzyme 2 (ACE2) and their relationship with the onset of type 2 diabetes mellitus. It was noted that there is a significantly high prevalence of VDR polymorphisms among diabetic patients than non-diabetic controls, especially concerning BsmI (77% vs. 17%), FokI (60% vs. 0%), and TaqI (51% vs. 1%). It clearly implies that mutations within the VDR gene play a pivotal role in causing metabolic derangements and insulin resistance. This finding supports evidence from past research suggesting that VDR polymorphisms alter transcription factor activity and signaling pathways, thereby impacting insulin secretion and action (Uitterlinden et al., 2004; Mitri and Pittas, 2011). Moreover, low vitamin D levels and defective VDR signaling are also associated with excessive cytokine generation and poor pancreatic β-cells function, which are key factors in the development of T2DM (Christakos et al., 2011; Holick, 2007).

The prevalence of ACE2 gene polymorphism showed a significant predominance of the DD genotype in diabetic patients (133 patients), whereas the II genotype was highly prevalent in the control population (169 cases). The lack of the DD genotype in the control group and its high frequency in diabetics suggests a strong genetic predisposition related to RAAS regulation imbalance. In particular, the DD genotype of the ACE gene is well known to be positively correlated with the increase of angiotensin II concentration, which triggers oxidative stress, endothelial dysfunction, and insulin resistance (Zheng et al., 2018). On the other hand, the II genotype of the ACE2 gene can play a protective function because it intensifies the activity of the ACE2/Ang-(1–7)/Mas receptor pathway, responsible for vasodilation, anti-inflammatory action, and insulin sensitivity (Patel et al., 2014; Gheblawi et al., 2020).

Indeed, the joint evaluation of VDR and ACE2 genotypes in this study implies possible cooperation between these two genetic mechanisms. The concomitant occurrence of VDR genotypes and ACE2 DD allele in patients with diabetes mellitus can explain the theory of impaired VDR-RAAS interaction, which causes progression of T2DM. The function of VDR is well described by the fact that it is involved in the inhibition of renin gene expression (Li et al., 2002). Genetic mutations associated with decreased activity of VDR will contribute to the development of excessive renin synthesis, increased activity of angiotensin II and thus metabolic disorders. In combination with ACE2 DD genotype, this effect may increase, leading to enhanced inflammation, insulin resistance, and malfunction of pancreatic cells.

Additionally, the findings of this study are backed up by epidemiologic and molecular studies, which have highlighted the importance of genetic susceptibility in the development of T2DM. The lack of any significant differences in terms of the distribution of the VDR polymorphism alleles and prevalence of the ACE2 II genotype in the control group adds weight to their role in protecting the body against diseases by ensuring metabolic homeostasis. Thus, it can be said that these findings offer convincing evidence regarding the involvement of molecular pathways involving receptors in the pathology of diabetes.

## Conclusion

This study shows conclusive evidence linking VDR gene polymorphisms (BsmI, FokI, and TaqI) and ACE2 gene polymorphisms to Type 2 Diabetes Mellitus. The higher frequency of VDR gene polymorphisms and ACE2 DD genotype in diabetic subjects indicates the presence of a powerful genetic disposition through the dysfunction of VDR pathway activation and RAAS system imbalance. The beneficial effect of the ACE2 II genotype and lack of VDR gene polymorphism in control subjects underscores their importance in glucose metabolism regulation. This study emphasizes the significance of the VDR-ACE2-RAAS pathway in T2DM development, indicating a novel target for early detection in high-risk patients. This study adds to existing literature by providing an additional genetic angle that can be considered a promising biomarker in diabetes mellitus.

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