ROLE OF VITAMIN D RECEPTOR (VDR) GENE POLYMORPHISM

Balwant Chauhan1* and Prashant Sakharkar2

1Department of Biopharmaceutical Sciences, Roosevelt University College of Pharmacy, Schaumburg, IL 60173 USA.
2Department of Clinical and Administrative Sciences, Roosevelt University College of Pharmacy, Schaumburg, IL 60173 USA.

ABSTRACT

Vitamin D deficiency leads to poor bone development and general health. The main source of vitamin D is dermal and amount synthesized depends upon exposure to sunlight. Additional amounts can also be obtained from food or through dietary supplementation. The inactive form of vitamin D is converted to its active form called calcitriol in liver and kidney, which is further utilized by variety of tissues, and its action is mediated via the vitamin D receptor (VDR). Newly converted calcitriol binds to the VDR protein, as encoded for by the VDR gene. VDR is expressed in most tissues of the body and there are several forms of VDR genes depicting polymorphism. There are four most common polymorphic forms found within the VDR gene and these are referred as rs2228570, rs1544410, rs7975232 and rs731236. These are also known traditionally as FokI, BsmI, ApaI and TaqI, respectively. The role of polymorphic forms has been explored in recent years with genes linked to cardiovascular, autoimmune, humoral, pulmonary and neurological diseases. Inadequate levels of vitamin D in the body were found to be associated with various disorders such as Alzheimer, diabetes, heart disease, cancers etc. VDR gene polymorphism also found to influence the allograft outcomes in recipients of renal transplants. The goal of this review is to highlight the role of VDR gene polymorphism in especially in altering Bone Mass Density (BMD), degenerative disc disease, osteoporosis, rickets and other conditions such as breast cancer, allograft survival in renal transplant recipients, new onset diabetes at transplant, hepatitis B infection and chronic periodontitis. Results of the various studies discussed here will broaden our understanding of variability in the Vitamin D and might help us in assessing...
risk of the disease as a predictive marker and in predicting the treatment response.

**KEYWORDS:** Vitamin D; vitamin D receptor; VDR gene; polymorphism; VDR gene polymorphism; allograft outcomes.

**INTRODUCTION**

Vitamin D refers to a group of fat-soluble secosteroids and is important for intestinal absorption of calcium, phosphate, magnesium and zinc. It is well known that the vitamin D deficiency leads to poor bone development and health. Vitamin D maintains healthy calcium and phosphate levels by aiding the absorption of calcium from the intestine in the body as well as by influencing kidney function.[1] Calcium homeostasis maintained by vitamin D improves the strength of human bones by increasing bone density and thereby preventing bone disease such as osteoporosis and rickets.

Skin is the main source of vitamin D. Amount of Vitamin D synthesized depends upon skin’s exposure to sunlight. Most important vitamin D forms are: vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol). Both forms are ingested from the diet and supplements. These forms are biologically inactive and require enzymatic conversion in the liver and kidney. Vitamin D is converted in the liver to calcifediol (=prohormone) and ergocalciferol (vitamin-D$_2$). Vitamin D$_2$ is converted to 25-hydroxyergocalciferol [25-hydroxyvitamin D$_2$-abbreviated as 25(OH)$_2$D$_2$]. Some of the calcifediol which has entered in kidney is converted to calcitriol (1, 25-dihydroxycholecalciferol, abbreviated as 1, 25 (OH)$_2$ D$_3$ (=hormone), is biologically activated form.[2] Calcitriol plays major role in calcium and phosphate homeostasis and also affects immune and neuromuscular functions. Calcitriol is released into the blood circulation. It binds to vitamin D-binding protein (VDBP), which is a career protein in plasma and is transported to various tissues/organs. In addition to skin, liver and kidney, calcitriol is also synthesized by immune system cells like monocyte /macrophages. Calcitriol is a potent ligand of vitamin D receptor (VDR). Hormone binds to VDR in the nucleus. How signal transduction progresses in nucleus and how cellular functions are altered is depicted in Figure 1. VDR belongs to the nuclear receptor superfamily of steroid/thyroid hormone receptors. VDRs are expressed in most of the organs including skin, breast, brain, gonads, heart, parathyroid glands and prostate. Figure 2 summarizes the role possibly played by hormone-VDR complex. Mutations in VDR can affect the regulation of expression of various genes; therefore, VDR gene polymorphism is very important to understand the role played by vitamin-D$_3$ and VDR complex. There are four most common polymorphic forms found...
within the VDR gene. These are referred as rs2228570, rs1544410, rs7975232 and rs731236. These are also traditionally known as FokI, BsmI, ApaI and TaqI, respectively. The BsmI, ApaI and TaqI polymorphisms are strongly associated with one another and the presence of one polymorphism can predict the presence of the others, as they almost always occur together. In this regard they are haplotypes and are tagged. This is a good example of what is known as linkage disequilibrium.

The role of vitamin D has been explored in recent years with functions affecting 229 human genes linked to cardiovascular, autoimmune, humoral, pulmonary and neurological diseases. Inadequate levels of vitamin D in the body were found associated with various disorders such as Alzheimer, diabetes, heart disease, cancers etc. VDR gene polymorphism also influences the allograft outcomes in recipients of renal transplants.

Tumor growth of the epithelial cells of the skin, breast, prostate and colon as well as inflammation are regulated by vitamin D through number of signaling pathways. Some of the outcomes (high plasma levels of vitamin D resulting in hypercalcemia) are attributed to the cytokine interferon gamma (IFN-γ), and through macrophage immune function activation, which has been associated with the negative impact on graft outcomes in renal transplant patients. IFN-γ polymorphisms on the other hand have been associated with BK virus nephropathy and cytomegalovirus infection. The goal of this review is to highlight the role of VDR gene polymorphism especially in BMD, degenerative disc disease, osteoporosis, rickets and other conditions such as breast cancer, allograft survival in renal transplant recipients, new onset diabetes at transplant, hepatitis B infection and chronic periodontitis.

**Role and Impact of VDR Gene Polymorphism**

Potential association of VDR gene polymorphisms ApaI, BsmI, FokI and TaqI with bone mineral density (BMD) was examined by Mitra and colleagues in 246 postmenopausal Indian women in one study. Osteoporosis is very common in postmenopausal women. BMD, which is a major determinant of osteoporotic fracture risk, has a particular genetic background. This study revealed that VDR gene polymorphisms are associated with BMD in Indian women. The average BMD at spine and hip of women with genotypes aa, bb (presence of restriction sites for ApaI and BsmI), FF and TT (absence of restriction sites for FokI and TaqI) was found more than 10% higher than those with genotypes AA, BB, ff and tt, respectively. Also, the combinations between BsmI, ApaI and TaqI genotypes showed significant effect on BsmI-ApaI genotypes combination on BMD.
To study the association of VDR gene polymorphisms with bone tissue mineral density and biochemical marker of 25-PO vitamin D in adolescents of both ethnic groups (Aktobe-Kazakh group and Slavonic-Russian group), living in Western Kazakhstan Region, 110 relatively healthy children aged 13-18 years of Aktobe, the representatives of Kazakh ethnic group, 66 (Kazakh children) and Slavonic e 44 (Russian children) were included in this study.\[14\]

Genotype SS was found to be a negative marker in Kazakh adolescents of Western Kazakhstan Region for bone tissue mineral density (BTMD) and 25-PO vitamin D; whereas, in children of Kazakh nationality with osteopathy sign, genotype SS occurred at almost twice the rate in comparison with Slavonic ethnic group and respectively by a factor of 2 less 25-PO vitamin D content, suggesting that disorders of bone mineralization and metabolism depend on ethnic affiliation and presence of defined polymorphic genotypes of VDR gene molecular markers.\[14\]

In another study, the VDR gene polymorphism in a healthy adult Brazilian population was determined in a group of patients with insulin dependent diabetes mellitus (IDDM) and correlated with the findings with densitometric values in both groups. The VDR genotype was assessed by polymerase chain reaction amplification followed by BsmI digestion on DNA isolated from peripheral blood leukocytes. The IDDM group had a lower BMD compared with the control group. The VDR genotype distribution did not differ from that observed in the IDDM group. In the IDDM group, patients with the Bb genotype had a higher body weight when compared with the BB genotype. However, when age, sex and body mass index was controlled in diabetic patients on regression analysis, BB genotype was associated with a lower mean BMD at lumbar spine and femoral neck than in Bb and bb patients. BB patients had a shorter duration of IDDM than bb and Bb patients. These findings suggested a small influence of VDR gene polymorphism on BMD of a racially heterogeneous population with IDDM.\[15\]

To evaluate the contribution of VDR gene polymorphism in the ethnic groups for bone mass in mother and children of different ethnic origins, the VDR genotypes and bone mass in 43 African-American and white women and their children were studied. All children had a whole body bone mass measurement at age 9. Thirty nine children had follow up measurements at age 11, while all the mothers had a single measurement. Significant ethnic difference in the VDR genotype frequencies among the adults and the children were
observed. No African-American subjects had the genotype “BB”, whereas, there was a 25% frequency of the “BB” genotype in the white adults and 24% in the white children. After pooling the ethnic groups, the mean bone mass in the “bb” genotype was found significantly higher than in the “BB” genotype among the mothers, but this was not found in the children at baseline. However, by age 11, those with the “Bb” or “bb” genotypes had a larger gain in bone mass than those with “BB”. These findings supported the suggestion that the ethnic difference in VDR genotype frequencies may help to explain the well known ethnic differences in bone mass and genotypes. Further, these observations suggested that VDR polymorphism may have an effect on bone mass during puberty as peak bone mass is accumulated during this phase of life.\[16\]

Allelic frequencies of the BsmI, Apal, and TaqI were measured using restriction fragment length polymorphisms (RFLPs) in 144 normal healthy southern Chinese premenopausal women aged between 30 and 40 years and correlation to their peak bone mass with the VDR genotypes was studied in one study. The B allele of the BsmI restriction-site was found only in 5% of the Chinese population compared to western populations. The BBAAtt genotype was found virtually nonexistent in Chinese people. Analysis of the VDR genotype revealed that subjects with BbAaTt and BbAATt haplotypes had the lowest peak bone mass. Although VDR polymorphism is believed to affect calcium absorption, this study failed to confirm a strong relationship between the VDR genotype and peak bone mass in Southern Chinese population with low dietary calcium intake.\[17\]

The effect of the TaqI alleles in vitamin D receptor was assessed on the risk of developing degenerative disc disease in a Southern Chinese population. Lumbar degenerative disc disease was defined by magnetic resonance imaging (MRI) in 804 Southern Chinese volunteers between 18 and 55 years of age. The t allele of TaqI in VDR gene was significantly associated with degenerative disc disease. Further subgroup analysis showed that in individuals younger than 40 years, the likelihood of degenerative disc disease was five times higher. Similarly, disc bulge was significantly associated with t allele in individuals younger than 40 years. This was the largest scale genetics study to date using MRI to define precisely degenerative disc disease in the Southern Chinese population, which has showed that the t allele of vitamin D receptor TaqI is associated with a high risk of degenerative disc disease and disc bulge developing, especially in individuals younger than 40 years.\[18\]
Restriction pattern of the polymerase chain reaction product of the VDR gene with the Bsm1 enzyme and serum osteocalcin in patients with osteoporosis was examined to evaluate if common allelic variants in the gene encoding the VDR are useful in predicting differences in bone mineral density (BMD) and bone turnover rate in Koreans. The prevalence of the BB genotype in the controls was extremely low when compared with that in other reports. Only 2.8% of those patients with osteoporosis had the BB genotype. In contrast, 12.5% had the Bb genotype, and 84.7% had the bb genotype. The prevalence of the BB genotype in patients with severe osteoporosis was found to be extremely low: the BB, Bb, and bb genotypes accounted for 0%, 12.4%, and 87.6%, respectively. Compared with the mean serum osteocalcin level of the pre and post menopausal controls, the level in patients with severe osteoporosis was significantly higher. These results suggested that restriction fragment length polymorphism analysis of the VDR gene with a Bsm1 restriction enzyme in Koreans is not helpful for early detection of patients at risk of developing osteoporosis. 

In another study conducted in Nigeria, VDR polymorphisms and susceptibility of some children to develop rickets in the setting of low calcium intake were compared. VDR genotypes were determined by the presence or absence of Bsm I, Apa I, Taq I, and Fok I restriction enzyme cleavage sites. This study involved 105 children with active nutritional rickets and 94 subjects’ representative of the community from which the children with Rickets came from. The ff genotype was less common in the rickets group compared to the community group. Findings of this study raised the possibility that VDR alleles might be important in determining an individual's susceptibility to developing rickets when faced with dietary calcium deficiency.

A study was performed to determine the influence of VDR gene polymorphism on breast cancer risk in Taiwan, which has a low incidence of breast cancer. Polymorphisms in the VDR gene were genotyped for 34 Taiwanese women with sporadic breast cancer, 46 with benign breast tumors and 169 cancer-free female (cohort controls). The ApaI, TaqI, and BsmI polymorphisms in the 3’ end of the VDR gene were associated with breast cancer risk, with a trend for increasing risk with increased numbers of BsmI B> B alleles and ApaI >AA genotypes. These findings indicated that the AA genotype may be associated with an increased risk of breast cancer, while the Aa genotype tends to be associated with decreased risk. These results suggest that polymorphic variation in or near the 3’ end of the VDR gene may influence breast cancer risk in Taiwanese women and justifies further investigation of
the role of VDR polymorphism for sporadic breast cancer in low-incidence areas. These findings may help when designing the targeted therapy in future.

In a study by Vu and colleagues, polymorphisms of VDR genes and Vitamin D binding protein (VDBP) were studied for their association with allograft survival or acute rejection in renal transplant recipients of Hispanic ethnicity. A total of 502 Hispanic renal allograft recipients were genotyped for four different single nucleotide polymorphisms (SNPs) of VDR. Findings of this study indicated that VDBP (rs4588) and VDR gene polymorphisms (rs1544410) are associated with allograft survival or rejection. These findings provided an important insight about Vitamin D polymorphism that affects allograft survival. Identifying this gene polymorphism in patients may prove useful in clinical practice as a predictive marker for triage patients who may have greater success with their allograft survival.

The VDR gene polymorphisms in 379 renal transplant recipients were genotyped for VDR (FokI & ApaI) and the association of each genotype with renal allograft survival and acute rejection were determined in a study by Lavin and colleagues. Significant improved allograft survival was observed for patients who were homozygous or heterozygous for the VDR FokI T allele further suggesting that the chronic allograft rejection can be prevented with the use of right Vitamin D receptor agonists.

VDR gene polymorphism, Taq1 A/G located in exon 9 and its association with the development of new onset diabetes at transplant (NODAT) in Hispanic renal transplant recipients (RTRs) was examined. NODAT is an important metabolic complication that increases risk of cardiovascular disease and is associated with lower allograft and patient survival in RTRs. A total of 129 RTRs with no evidence of pre-existing diabetes who developed NODAT and 186 controls with no history of diabetes were included in this study. The Taq1 A/G (rs731236) polymorphism was genotyped using polymerase chain reaction-restriction fragment length polymorphism analysis. The genotype frequency of the Taq1 A/G polymorphism differed significantly between NODAT patients and controls. Kaplan-Meier survival analysis also suggested more than 1.9 fold increased risk of allograft failure in NODAT patients. After 3 years, graft survival began to decrease in the NODAT group compared with control group. Findings of this study indicated that the Taq1 polymorphism of VDR is significantly associated with NODAT.
A possible association between the VDR, SNPs, and hepatocellular carcinoma (HCC) in patients with chronic hepatitis B virus (HBV) infection was explored in this study. A total of 968 chronic HBV infection patients were enrolled, of which 436 patients were diagnosed HCC patients, and 532 were non-HCC patients. The clinical and pathological characteristics of HCC and the genotypes of VDR gene at FokI, BsmI, ApaI, and TaqI were determined. The genotype frequencies of VDR FokI C>T polymorphism differed significantly between HCC and non-HCC groups. HCC patients had a higher prevalence of FokI TT genotype than non-HCC subjects. With FokI CC as reference, the TT carriage had a significantly higher risk for development of HCC after adjustments with age, sex, HBV infection time, a-fetoprotein, smoking status, and alcohol intake. In addition, the TT genotype carriage of FokI polymorphisms were associated with advanced tumor stage, presence of cirrhosis, and lymph node metastasis. The SNP at BsmI, ApaI, and TaqI did not show any positive association with the risk and clinical and pathological features of HCC. Findings of this study suggested that the FokI C>T polymorphisms may be used as a molecular marker to predict the risk and to evaluate the disease severity of HCC in those infected with HBV. [24]

In another study, association of polymorphisms in VDR gene exons with the incidence of Chronic Periodontitis (CP) was examined. CP is caused by enhanced resorption of the alveolar bone supporting the teeth and is associated with intraoral inflammation after infection with certain bacteria. The VDR gene polymorphism was reported recently to be significantly related to the occurrence of tuberculosis and infection of chronic hepatitis B virus. This may be interpreted to indicate a close relationship between VDR gene polymorphism and the immunological action, because vitamin D activates monocytes, stimulates cell-mediated immunity, and suppresses lymphocyte proliferation. [25]

This was a case-controlled study with a group of 168 unrelated Japanese subjects whose ages ranged from 35 to 65 years. The TaqI I polymorphism in the VDR gene was found to be associated significantly with CP. The TT genotype was found to be associated with CP, and with well-recognized risk factors, smoking and diabetes on multiple logistic regression analyses. This indicated that the VDR gene polymorphism (TT genotype) is a risk factor for CP, independent of smoking and diabetes. [25]
Table 1: VDR gene polymorphism and its impact on disease conditions.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>SNP</th>
<th>Disease Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>FokI</td>
<td>rs2228570</td>
<td>Breast cancer, allograft survival in renal transplant, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Apa I</td>
<td>rs 7975232</td>
<td>BMD, breast cancer</td>
</tr>
<tr>
<td>Taq I</td>
<td>rs 731236</td>
<td>BMD, degenerative disc diseases, breast cancer, new onset diabetes at transplant, chronic periodontitis</td>
</tr>
<tr>
<td>Bsm I</td>
<td>rs 1544410</td>
<td>BMD, breast cancer, allograft survival in renal transplant</td>
</tr>
</tbody>
</table>

SNP- Single nucleotide protein; BMD- Bone mineral density.

Figure 1: Vitamin D Synthesis, Activation and Cellular Response.

CONCLUSIONS

Polymorphisms in the VDR gene have been linked to several diseases (Table 1). Recent studies have indicated that many polymorphisms exist in the VDR gene, however their influence on VDR protein function are largely unidentified. Research is therefore focused on documenting additional polymorphisms across the VDR gene and trying to understand the functional consequences of such variations. Eventually, results of these research studies will broaden our understanding of variability in the Vitamin D receptor (VDR) gene and might help us in assessing risk for the disease as a predictive marker and in predicting the treatment response.

REFERENCES


