A Review on the Role of SNP at rs1544410 of VDR Gene in Different Diseases

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "A Review on the Role of SNP at rs1544410 of VDR Gene in Different Diseases" submitted by Shaidur Rahman (19146092), of Summer, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study did not involve any human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues and field research.

Abstract

Single nucleotide polymorphism (SNP) can take place in the vitamin D receptor (VDR) gene which can cause alteration in normal procedure of the VDR. Thus, ultimately restricting the work of vitamin D which can be a precursor of various diseases. Four major single nucleotide polymorphisms (SNP) of the VDR gene that are mostly worked with are *FokI*, *TaqI, BsmI* and *ApaI*. This study was done by focusing on the SNP of *BsmI* (rs1544410) across different populations by taking information from the studies that performed case-control model studies. It was observed after review of different studies that most of the diseases like rheumatoid arthritis, osteoporosis, rickets, autoimmune diseases and other diseases indicated a relation with *BsmI* polymorphism of VDR gene in different populations with some contradictory reports. But the majority of them got a significant result. Future studies with more detailed work are encouraged considering different ethnic groups.

Keywords: VDR gene; Rheumatoid Arthritis; Polymorphism; Vitamin D; Single Nucleotide Polymorphism, *BsmI* (rs1544410).

Dedication

I want to dedicate my work to my parents who have been relentlessly supporting me throughout entire course of my life. They have been very supportive of me and given me proper guidance to become who I am today. I am very thankful to them and with that Almighty will keep them away from illness and bring happiness in their life.

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List of Acronyms

RA	Rheumatoid Arthritis
VDR	Vitamin D Receptor
SNP	Single Nucleotide Polymorphism
RF	Rheumatic Factors
TNF-α	Tumor Necrosis Factor α
sHPT	Secondary Hyperparathyroidism
PTH	Parathyroid hormone
CRF	Chronic Renal Failure
BMD	Bone Mineral Density
PBC	Primary Biliary Cirrhosis
AIH	Autoimmune Hepatitis
AITD	Autoimmune Thyroid Disease
HVDRR	Hypo-Calcemic Vitamin D-Resistant Rickets

Chapter 1

Introduction

1.1 Background

Vitamin D receptor (VDR) polymorphism has recently been linked with different types of diseases and considered to somewhat responsible for their onset. This has grabbed the interest of the researcher's and plenty of work has been done to find out the relation of polymorphism with many different diseases and the process is still undergoing. Some relevant diseases are; Rheumatoid Arthritis, Osteoporosis, multiple sclerosis, autoimmune diseases etc. It was Hippocrates who first identified rheumatic diseases during the fourth century B.C indicating it as a disease causing partial joint pain. Later, in 1949 Joseph L. Hollander first came with the term 'rheumatology' in his textbook. Rheumatic diseases have been classified in different types but rheumatoid arthritis (RA) is more prevalent than others (Sangha, 2000a). RA is a fatal and widespread autoimmune condition that affects around (0.3% - 1%) of the entire population, and are more common in women compared to males with a ratio of (3:1) (Bagheri-Hosseinabadi et al., 2020). Again, another study on the ancient skeletons have given the scientists an idea that, RA perhaps existed in the North America for about 3000 years ('S, 1990). According to the survey done by the US National Health Examination (1960-2), RA is prevalent in only 0.3% adults whose age is under 35 years, whereas, more than 10% people above 65 years are prevalent to RA. So, it can be said that with the increase of age, the chances of RA in a person also increases ('L, 2005). Multiple pieces of data indicate that genes other than gender may have a role in RA development. Research on families has revealed a strong genetic component to RA. About 40% of first-degree relatives of people with this disease is linked to rheumatoid factor have severe RA, and about 10% of RA patients have a first-

degree family with this disease (Sangha, 2000b). Patients with RA have a higher death rate, as shown by mounting evidence ever since 1950s. Dutch researchers found that the life expectancy of the individuals with RA was decreased by 7 years and 3 years in man and women respectively compared to the general population (Vandenbroucke et al., 1984). Again, osteoporosis is also a similar kind of disease related to the loss of Bone Mineral Density (BMD). In osteoporosis, the osteoclasts become active in a much higher rate, causing a loss in bone density (Yadav et al., 2020). Now, it was observed that the genetical factor plays a great role in case of osteoporosis. For instance, loss of bone density was prevalent on the female child bone from the mother who was suffering from osteoporosis. The cause of having osteoporosis is considered to be because of the loss of BMD. And many studies suggest that, VDR polymorphism is one of reasons for having low BMD (van Leeuwen et al., 1996a).

On personal terms, one of my aunts is prevalent to rheumatoid arthritis and I have grown with seeing her in pain and discomfort all the time. Recently, I was shocked when I got the news that, my cousin, elder son of my aunt is also suffering from predisposition of RA. Being a student of pharmacy and knowing all the terms, it made me curious about the issue. I want to know if there is any genetic association in case of rheumatoid arthritis. Likewise, I also want to check that, if there is also any genetic association with other diseases. This is one of the main reasons for this study to accommodate my curious mind.

1.2 Vitamin D

Inadequate level of vitamin D is assumed to be the cause of various diseases. While it is possible to get vitamin D from food sources, it is mostly received through sun exposure, where it is produced in the skin from 7-dehydrocholesterol (Puncevičienė et al., 2021a). Cholecalciferol and vitamin D3 are the two primary types of vitamin D. The liver converts vitamin D3 (cholecalciferol) into (25(OH) D), which is then converted into (1, 25(OH) 2D) in

the kidney. This is the substance that actively increases the gut's absorption of calcium. This increases the calcium transport from the bone, gut, and kidney into the blood. These are the usual target organs. Parathyroid hormone (PTH) encourages 1, 25(OH) 2D synthesis. 1, 25(OH) 2D along with calcium have negative feedback effects on PTH, with the former causing a reduction in PTH levels and the latter having an immediate effect (Lips, 2006). Even though vitamin D is typically thought of as a compound which can only metabolize skeleton and minerals, an immune-regulatory aspect of vitamin D was hypothesized in recent years based on the fact that human lymphocyte activation results in interaction of VDR (Garcia-Lozano et al., 2001). 1.25-dihydroxyvitamin D is the active form of vitamin D, and it helps regulate and differentiate immune cells, which may have anti-inflammatory, immune-modulatory, anti-proliferative, and even gene expression-modulating effects. As an immune system regulator, vitamin D controls apoptosis (cell death) and the activity of suppressor T lymphocytes (T cells that suppress other cells' activities) (Puncevičienė et al., 2021a). Moreover, vitamin D also greatly contribute in other metabolic pathways (Fig. 1) (Valdivielso & Fernandez, 2006). To exert its immune-regulatory effects, vitamin D activates lymphocytes that possess its steroid receptor, VDR and causes them to suppress antibody formation and IL-2 secretion. Finally, these outcomes of vitamin D can directly impact RA (Mukhtar et al., 2019). Premature and dimorphic birth, dark skin, limited exposure to sun, obesity, malabsorption, and old age are all considered as risk factors for vitamin D insufficiency. Additionally, older people's skin generates far less vitamin D than younger people's skin does (Lips, 2006). Hip fracture patients, those living in nursing homes, and the elderly in general have a significantly higher vitamin D deficiency rate than the general population. Europe has a far greater frequency of vitamin D deficiencies than Asia, Australia, or the United States. Contrary to expectations, latitude is positively associated to serum 25(OH) D concentrations throughout Europe (Lips, 2001a). Rickets and osteomalacia are also caused due to vitamin D deficit that is too great to be

corrected with diet alone. Osteomalacia is a condition when concentrated osteoid seams are seen on the trabecular and cortical bone surfaces. When compared to osteoporosis, in which only trace levels of osteoid may be seen, this condition is quite different. Due to decreased level of vitamin D in blood and decreased serum calcium level, vitamin D deficiency induces increased release of PTH, which in turn promotes accelerated osteogenesis and increased bone desorption. The result is a loss of bone, mostly of cortical bone, that can be influential in the development of osteoporosis (Lips, 2006).

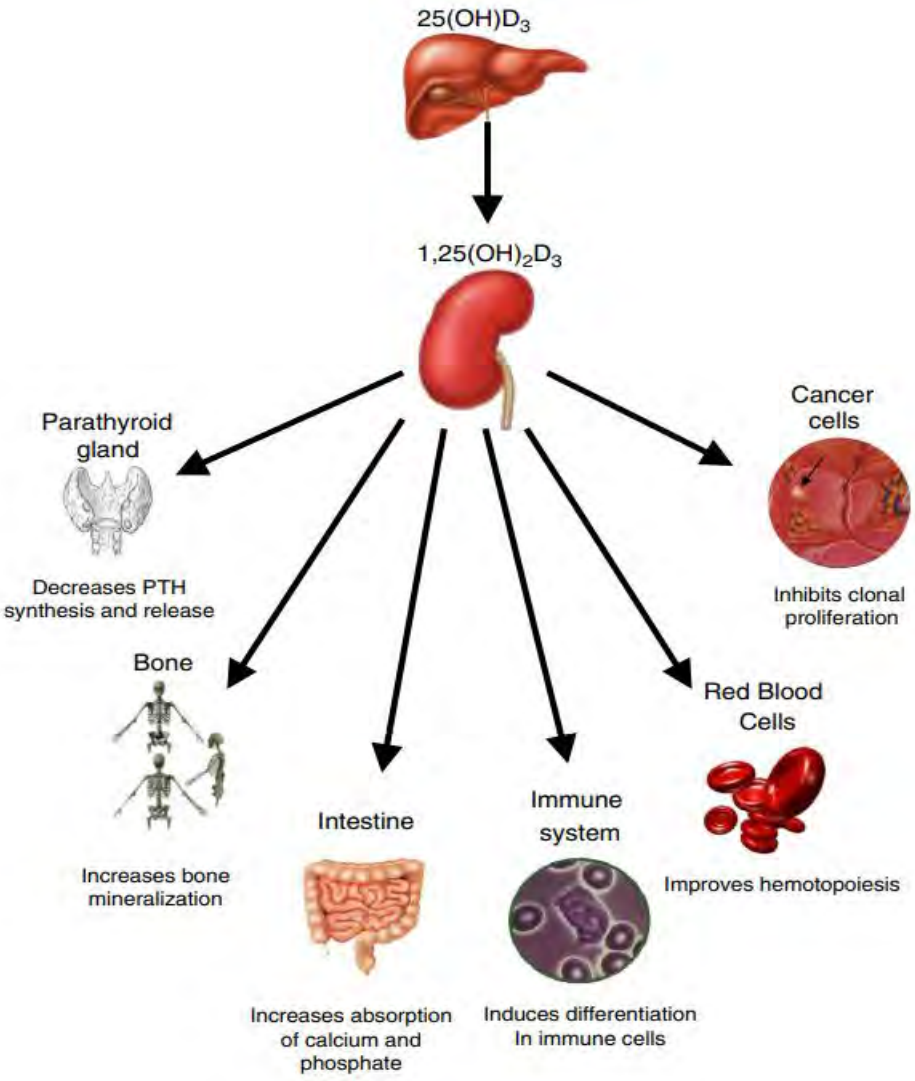

Fig. 1. Effects of Vitamin D in different cells (Valdivielso & Fernandez, 2006).

1.3 Polymorphism

Genetic Polymorphism in genomics refers to variations in DNA sequence between members of the same species. It is commonly acknowledged that genetic polymorphism occurs when a characteristic is inherited from a single genetic locus involving two alleles, and the frequency of the rarer variant is at least 1%. Polymorphism in genetics refers to variations in DNA sequence between members of the same population. Single-nucleotide polymorphisms (SNPs), sequencing repeats, insertions, reductions, and recombination are only some of the sources of genetic variation. Polymorphisms develop as a result of mutations. Instances of insertion, deletion, or rearrangement of nucleotides, as well as changes in nucleotide type, are all potential causes of mutation (Ismail & Essawi, 2012). Alterations like this may happen in the introns of a gene, which are not translated into proteins. The degree to which the gene is expressed would therefore depend on changes in the regulatory genes, which would impact in the amounts of the protein available (Valdivielso & Fernandez, 2006). Once a polymorphism has created, it may be traced from parent to offspring similar to any other DNA sequence. Nearly 50% of SNPs in coding regions lead to in-frame deletions or insertions, known as non-synonymous codon alterations. This alteration of the non-synonymous codon causes the encoded amino acid to change. There are a few different kinds of DNA polymorphisms, but the most common ones include single nucleotide polymorphism (SNPs), copy-number variants, and tandem repeat polymorphisms. At a certain nucleotide site, there is an individual single nucleotide polymorphism. Variation in the composition of the nucleotide pair occupying a given place among the population's DNA molecules is common. This variation is referred as single nucleoside polymorphism. There are two alleles defined by the SNP, and hence three possible genotypes in the population. For example, in a complete set of homozygous chromosomes or a set of heterozygous chromosomes in which one chromosome has the T-A genotype and the other has the C-G genotype (Ismail & Essawi, 2012).

1.4 Polymorphism on Vitamin D Receptor (VDR) Gene

Vitamin D completes its action by attaching with vitamin D receptor that is a nuclear hormone receptor superfamily member encoded by the VDR gene. One of the most researched gene variations in the background of different diseases is the single nucleotide polymorphism (SNP) of the VDR gene. There are 11 exons in this gene, 3 of which comprise a non-coding 50 area, and the remaining 8 are associated with the VDR product. The gene is found in chromosome 12 (locus geni 12q13.11). The VDR genes contains a substantial promoter region that can produce several transcripts specific to various tissues. Over 470 SNPs have been found in the VDR gene so far, although *TaqI, FokI, BsmI*, and *ApaI* have been examined the most, *TaqI*, *BsmI and ApaI* and are located in intron 8 and exon 9, respectively and have been shown to be in significant linkage disequilibrium. In the meanwhile, the start codon polymorphism *FokI* may indirectly affect 25(OH) D levels (Puncevičienė et al., 2021a). Mutations in silent codons caused by these polymorphisms are linked to increased amount of VDR mRNA stability (Bagheri-Hosseinabadi et al., 2020).

Data demonstrates that VDR gene variations affect VDR expression and activity in a variety of complicated ways. The VDR is also a nuclear ligand-dependent transcription mediator that, in conjunction with active vitamin D, controls the expression of several inflammatory and immunological modulatory genes (Tizaoui & Hamzaoui, 2015). Additionally, more than 11,000 probable VDR target genes participate in a variety of physiological processes, including cell proliferation, differentiation, metabolic activity, and others, which increases their involvement to various types of disorders and motivates to perform research into the etiopathogenesis of different diseases. Geographical latitude and ethnic groupings influence RA susceptibility to VDR gene variants differently, showing that *BsmI* (rs1544410) is associated with RA-related bone loss. It is thought that LD and a number of functioning

polymorphisms elsewhere within the VDR gene account for relationships among VDR gene polymorphisms with autoimmune disorders, even though the functional cause of these four VDR SNPs is yet unclear. In some instances, certain VDR polymorphisms have already been linked to RA (Song et al., 2016). The role of VDR signaling in T-cell differentiation and function has been the subject of several research. Recent research has shown that T lymphocytes have a role in etiopathogenesis of RA. Multiple auto-inflammatory diseases, including RA, osteoporosis may be initiated and maintained by polymorphism in the VDR gene and abnormal level of vitamin D (Munger et al., n.d.; Shoenfeld et al., 2009). Yet, some researches are very unclear about these association. The reason might be, low statistical power, clinical heterogeneity, or small sample sizes.

1.5 VDR Gene Polymorphism Related Diseases

Throughout generations many diseases have been widely associated with VDR gene polymorphism. Some of the noteworthy one's are discussed below:

1.5.1 Rheumatoid Arthritis

In the past few decades Rheumatoid Arthritis (RA) has been in the lime light as one of the autoimmune diseases concerning vitamin D. RA defines as an autoimmune inflammatory condition that primarily targets the synovial tissue and severe cases can certainly increase the likelihood of death and disability. A mix of genetic and environmental differences are thought to be possible risk factors for the disease that lead to alterations in the immune system and the development of RA, even though the etiology of the condition has not yet been fully determined (Puncevičienė et al., 2021). Due to a mix of environmental and genetic variables, RA is considered to a multifactorial disease (Mosaad et al., 2014).

Rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA), synovial inflammation and hyperplasia or swelling, cartilage and bone deformity, and systemic cardiovascular, pulmonary, psychological, and skeletal disorders are all hallmark features of RA. The likelihood of developing rheumatoid arthritis in females is more than that in the males has been known for quite some time. Moreover, lifestyle have been also linked to the development of rheumatoid arthritis. Laziness, lack of exercise, excessive smoking all of them can trigger RA. Furthermore, synovitis is also a precursor of RA. Synovitis is swollen condition of the synovial tissue. Leukocytes invade the synovial compartment, causing synovitis. Synovial inflammatory tissue development in rheumatoid arthritis is enabled by various micro-environmental alterations, substantial synovial architectural remodeling, and local fibroblast activation (Mcinnes & Schett, n.d.).

Type-17 helper T cells (Th17), a subtype that generates tumor necrosis factor α (TNF-α), have received a lot of interest recently, despite the fact that rheumatoid arthritis is often thought to be mediated by type-1 helper T cells. Myocardial ischemia, cerebrovascular disease, and heart failure are all more common in those with rheumatoid arthritis, leading to an elevated standardized mortality rate of around 1.5%. Again, inflammation in the joints caused by RA can also hamper the brain (Mcinnes & Schett, n.d.).

1.5.2 Chronic Renal Failure (CRF)

Many research has been performed in the recent decade to observe the effect of VDR polymorphism in the CRF patients. Secondary hyperparathyroidism (sHPT) is considered to be the main cause of building CRF. The calcitriol-VDR complex controls the production of parathyroid hormone (PTH) and the proliferation of the parathyroid cells. Thus, PTH production and parathyroid gland cell growth are both inhibited by the binding of calcitriol through its receptor. Researchers conducted in this field determined the presence of VDR in

the parathyroid cells, which further opened the door for more research (Valdivielso & Fernandez, 2006). Meanwhile, an association between *BsmI* polymorphism with the primary hyperthyroidism was reported in research done in 1995 (’Carling, 1995). Further studies recommended that the patients with minimal PTH level had a presence of B allele in a great number. That is, in each stage of CRF, PTH levels were lower in patients mostly with BB genotype. Additionally, this was also associated with elevated calcitriol levels (Marco et al., 1999). Even after adjusting for calcium and phosphorus levels, we discovered that patients with genotype BB had a greater decrease in PTH concentration than individuals with the genotype bb. It was also proved that patients with the genotype BB might have severe sHPT using the same line of reasoning. Determining the increased onset of developing sHPT linked to a particular *BsmI* genotype is particularly challenging. However, practically all of the published findings suggest that the *BsmI* genotype has some bearing on the development of the sHPT (Valdivielso & Fernandez, 2006).

1.5.3 Osteoporosis

Osteoporosis is referred to a disease that is defined by the loss of the bone mineral density (BMD). Different studies conducted in this sector has shown an association of VDR polymorphism and osteoporosis. Both environmental and genetic factors combine together to generate the disease, osteoporosis. Numerous studies indicate that genetic and environmental influences account for around 70% and 30% of an individual's traits, respectively. Environmental variables have the ability to influence gene expression and, in turn, the mechanism of the disease (’Eagle, 1962). According to research, female children of osteoporotic mothers had lower bone densities than children of mothers with normal bone densities. Similar to this, in comparison to males with average bone density readings, the children of men with idiopathic osteoporosis had greatly reduced BMD. It was found that, the

person with BB genotype had four times more risk of getting a fracture than a person with bb genotype (van Leeuwen et al., 1996b). There are two ways that genes might impact the skeletal system. First, regulate body inputs and outputs to adjust BMD, like the urinary calcium excretion. Secondly, impaired metabolism brought on by genetic mutations. In a study performed it was observed that, *FokI* and *BsmI* polymorphism of the VDR gene was largely linked with the onset of having osteoporosis. In conclusion, it can be said that, along with vitamin D deficiency, genetic variation plays a significant role in changing bone activity, which has a significant impact on osteoporosis incidence (Mohammadi et al., 2014).

1.5.4 Rickets

Rickets is a disorder associated with the developing child that is mostly prevalent in the last levels of vitamin D deficit and is brought on by a malfunction in the mineralization of bone matrix-1 (Mao & Huang, 2014a). Children's vitamin D deficiency is mostly caused by inadequate vitamin D consumption and inadequate sun exposure, especially in dark-skinned populations (Lips, 2001b). Previous different studies have proved some relationship with the VDR polymorphism and onset of rickets. To properly mineralize bone, vitamin D makes sure that calcium and phosphate are delivered in sufficient amounts to the locations where it forms. Consequently, the VDR gene polymorphisms may have an impact on how calcium and phosphate are delivered. Genetic vitamin D-resistant rickets, is condition of severe rickets that first appears shortly after birth, is caused by mutations in the gene carrying VDR by preventing it from performing its usual role (Malloy et al., 1999). Studies indicates that, the polymorphism at B allele/BB genotype for *BsmI*, F allele/FF genotype for *FokI* and subsequently AA genotype for *ApaI* was linked to the risk of rickets (Mao & Huang, 2014a).

1.5.5 Nephrolithiasis

Nephrolithiasis is a condition where stones are basically formed in the kidney due to excessive concentration of the calcium and oxalate level in the urine. Nephrolithiasis is a complex disease that develops due to the consequences of the interaction of hormonal and genetic variations along with environmental factors (Valdivielso & Fernandez, 2006). Thousands of chemicals are normally excreted by the kidneys through urine, which also contains different ions. These urine solutes can precipitate as microcrystals inside the renal pelvis, grow in size, and eventually crystallize into clinically important stones when they crystallize. Different studies suggest that, the variations in the allele of different VDR genes are associated with nephrolithiasis. To be precise, VDR polymorphism of *FokI, TaqI* and *BsmI* are considered to be responsible for the growth of nephrolithiasis (González-Castro et al., 2019). It was seen that, a person with *BsmI* polymorphism of phenotype bb had a much-increased rate of calcium excretion through the urine. Thus, increasing the risk of forming stones (Ruggiero et al., n.d.).

1.5.6 VDR Polymorphism and Cancer

Although, the relation of VDR polymorphism and cancer do not have any concrete evidence. But different studies have found some association with cancers like: colon cancer, breast cancer and prostate cancer (Valdivielso & Fernandez, 2006). One of the earliest studies linking prostate cancer within the US population with polyA variant of the VDR gene (UTR polymorphisms) was reported in 1997. Another report published a year ago demonstrated the association among *TaqI* polymorphism with an elevated risk of prostate cancer (Ingles et al., 1997). Again, some studies showed a relationship among *ApaI* polymorphism and breast cancer, whereas; some showed a relationship among colon carcinoma and *BsmI* polymorphism (Valdivielso & Fernandez, 2006).

1.5.7 Autoimmune Diseases of Liver

Both primary biliary cirrhosis (PBC) and Autoimmune hepatitis (AIH) are considered to be the major type of autoimmune disease of the liver. They are both considered to be chronic liver inflammation which are caused by the immune system. The etiology of both of this autoimmune disease is still unidentified and has been linked with the genetic polymorphism (Vogel et al., 2002). AIH is different from other chronic hepatitis and can be found all over the world regardless of the race or ethnicity. AIH is seen in both children and adult but it is significantly prevalent among the women. The body of the person suffering from AIH starts to see the antigen of their own liver as foreign particles (Mieli-Vergani et al., 2018). Whereas, PBC is assumed to be a degenerative liver disease that destroys the tiny intrahepatic bile ducts, causing periportal inflammation, fibrosis, and eventually liver failure. It is also more prevalent in women compared to men. Environmental and genetic factors are supposed to the reason of having PBC (Carey et al., 2015). Moreover, in a study it was shown that, first degree relatives of the patient having PBC are susceptible to this disease. This study also reported that, about 2% of the offspring of the PBC patients were having the same disease (Gershwin et al., 2005). So, this explains a lot about the possible link of genetic predisposition.

1.5.8 Other Diseases

Recently VDR gene polymorphism is the most worked topic and has been linked with different types of diseases. For instance, one study suggests that, a person with b allele will have a much-increased level of blood pressure than a normal person. Moreover, research on autoimmune diseases like primary biliary cirrhosis, autoimmune hepatitis, Graves' disease, and multiple sclerosis have been performed to find out association with VDR polymorphism (Valdivielso & Fernandez, 2006). Additionally, it has been claimed that polymorphism in *TaqI* and *FokI* have

an impact on the likelihood of developing multiple sclerosis. Furthermore, FokI polymorphism was more prevalent in diseased patients in the Japanese population (Smolders et al., 2009).

1.6 Purpose/ Objective of the Study

The purpose/ objective of this study is to completely analyze and determine if there is any relationship between VDR polymorphism in the *BsmI* (rs1544410) with different diseases throughout different populations, does the relationship varies due to different race and ethnicity and to understand its effect and its intensity.

Chapter 2

Materials and Methods

To maintain the authenticity and integrity of the work, all the information collected was taken from different verified and well-established journals. The database was collected from well-known sources like: Google Scholar, PubMed, Springer, Nature, Science Direct etc. The author's name, the journal and the date of publication, the country of origin, the subjects' ethnicity, the number of participants in both case and control groups, the genotyping method, and the genotype numbers in both case and control group were all extracted in a standardized process. The articles used were full-text papers and it was kept in mind to not use any half text papers.

All the collected studies were studied thoroughly before using their information in this work. The needed information was pointed out and used in this work. During the work proper citation was maintained (APA-7th). Moreover, proper referencing was also done in this work. This work tried to find out the relation of VDR gene polymorphism in *BsmI* (rs1544410) in case of different diseases.

The key words used to search for the related articles were; (“Rheumatoid Arthritis” OR “RA”), (“Vitamin D”), (“Vitamin D receptor” OR “VDR”), (“Polymorphism”), (“VDR polymorphism”), (“*BsmI*”), (“rs1544410”), (“VDR polymorphism related diseases”), (“Diseases associated with *BsmI* Polymorphism”), (“Osteoporosis”). Furthermore, more effective studies were obtained from the reference list that was provided in the articles of the primary search.

The inclusion of the studies was done on the basis of the provided criteria: (i) articles having association studies between VDR polymorphism and *BsmI* (rs1544410); (ii) all the studies

performed case-control design; (iii) provided information about genotype or phenotype in the cases and controls of different diseases. On contrary to that, half-text papers, information from the abstract, pirated data, studies not containing information of *Bsm*I polymorphism were all excluded respectively.

Chapter 3

Result & Discussion

3.1 Result

The result was developed after all the thorough analysis of the related studies that was performed in different population. A defined model for *BsmI* SNP is: Allelic type (b vs. B), homozygote (bb vs. BB), dominant type (bb + Bb vs. BB), recessive type (bb vs. Bb + BB), & heterozygote (Bb vs. BB) (Bagheri-Hosseinabadi et al., 2020).

In case of rheumatoid arthritis, a study was performed over Pakistani population consisting of about 300 RA case and about 412 controls. In this test, after performing a genetic analysis on *BsmI* (rs1544410) it was found that, the 'AA' & 'AT' genotypes were in a much greater number in the RA cases than in the healthy group. So, a significant relation among *BsmI* (rs1544410) and RA was found in Pakistani population (Mukhtar et al., 2019). Another study that was performed in the Lithuanian population comprising of 206 RA patients and 180 control groups showed a very different result. No genotype or allele of the *BsmI* VDR loci evaluated was significantly linked to the expected chance of RA disease. The arrangement of both genotypes and allele frequencies of *BsmI* did not significantly differ between the control group and the RA patients ($p > 0.05$). Thus, no significant relation between *BsmI* polymorphism (rs1544410) and RA was found in Lithuanian population (Puncevičienė et al., 2021a). A similar study was performed in Spain containing 120 RA cases and 200 control groups. Between patients and control groups, no variations in the frequency of genotypes for the *BsmI* polymorphism (rs1544410) was seen. Although it was stated that, a strong linkage disequilibrium was observed between genotypes of *BsmI* and *TaqI* site. This could have a very minor onset on RA (Garcia-Lozano et al., 2001). However, a study done among 128 patients and 200 control

groups have also identified a different result. The allelic frequency of the *BsmI* was significantly different in cases compared to the control group (P 0.001). The frequency of 'b' allele in *BsmI* was more prevalent in the cases than in the control group (OR = 2.16). The frequency of the genotype's 'bb' of *BsmI* was found to be higher in RA patients and 'BB' of *BsmI* was found to be higher in control group when the genotype distribution of RA patients and control group was analyzed (Pc = 0.05) (Mosaad et al., 2014b). A meta-analysis performed in 2020 that consisted of 17 studies done among 2153 RA cases and 2326 control groups. This study included the European, Asian and African populations. In the comprehensive population study, the results showed no correlation between the *BsmI* SNP and the probability of developing RA. However, analysis of subgroup discovered a significant relationship among SNP of *BsmI* and onset of RA among Africans in the dominant variant (P = 0.01), the recessive variant (P = 0.01), the allelic variant (P = 0.001) (Bagheri-Hosseinabadi et al., 2020). Similar findings were seen in another meta-analysis done on the Europeans. In all study participants, meta-analysis found no correlation among RA and B allele of *BsmI* (OR =1.065, 95% CI =0.911-1.245, p=0.427). The analysis of all subjects' homozygote comparison, recessive and dominant models were inconclusive to find *BsmI* polymorphism's connection with RA (Song et al., 2016b). Another meta-analysis done on the Caucasian, Caucasian European and Asian contradicted this information revealing that in dominant (P = 0.057), recessive (P = 0.071), as well as allele (P = 0.069) models, the relationship between the *BsmI* polymorphism and the risk of RA was slightly significant. There was a very small but significant association among *BsmI* polymorphism and RA in the Caucasian Population (Tizaoui & Hamzaoui, 2015).

A meta-analysis was performed on osteoporosis consisting of 65 different studies. This study worked with 6880 patients and 8049 control groups. Surprisingly, in allelic model there was no association or relation found between *BsmI* (1544410) polymorphism and osteoporosis. It was also similar in the genetic model (Yadav et al., 2020). A similar result was also seen in

another meta-analysis of 41 studies. The allelic model shows that, patients diagnosed with osteoporosis, the general population had a rate of B allele distribution that averaged out to be 35.74 percent, with the average frequency among the controls coming in at 35.25 percent. There was no difference found in the BB genotype of both the patients and control group. Thus, no association was observed (Qin et al., 2013). This result was contradicting with a study performed in Egypt on postmenopausal women having osteoporosis. In patients, the frequency of the BB genotype was 54%, the frequency of the Bb genotype was 30%, and the frequency of the bb genotype was 16%. While in the controls, the frequency of these three factors was, respectively, 5%, 10%, and 85%. Patients had a much greater frequency of the BB genotype than controls did (P = 0.001), whereas controls had a significantly higher frequency of the bb genotype than patients did. So, the *BsmI* polymorphism was found to be hugely associated with osteoporosis in this study (Mansour et al., 2010).

A meta-analysis performed on rickets was published on 2013 consisting a study of 86 articles. This study for *BsmI* (rs1544410) polymorphism was done on 717 rickets patient and 565 control groups. The study demonstrated; a frequency of the B allele was found to be an average of 16.5% in cases but only 6.45% in the control group. Furthermore, p value of B allelic group was 0.017 and that of BB genotype was found to be 0.044. This confirms the association of BB genotype and B allele of *BsmI* polymorphism with rickets (Mao & Huang, 2014b). Another study performed in Middle East shows that, BB and Bb genotype of the *BsmI* was responsible for lower level of serum 25(OH)D. Moreover, the bb genotype was also linked to be responsible for low uptake of calcium in case of case group. This can show predisposition of *BsmI* polymorphism in case of rickets (Baroncelli et al., 2008). It was also seen in case of hypo-calcemic vitamin D-resistant rickets (HVDRR). Bone abnormalities, hypocalcemia, secondary hyperparathyroidism, and increased levels of blood vitamin D are the prominent features of this extremely uncommon autosomal recessive disorder. The frequency of the *BsmI* genotypes

were different among the case and control group (p = 0.029). Thus, showing an association with the *BsmI* polymorphism (Nicolaidou et al., 2007)

A study on primary biliary cirrhosis (PBC) containing 31 cases and 51 controls was published in 2000. The study shows that the genotype frequency differed in the case and control group (BB= 45.2%/ 16%, Bb= 32.3%/ 48%, bb= 22.5%/ 48%) with a p value of 0.01. The showed that a significant association of the *BsmI* polymorphism along with PBC (Halmos et al., 2000). In another study performed on 101 patients and 160 control groups of Chinese with PBC similar results were found. In case of PBC patients, the Bb allele frequency reduced drastically (P = 0.021, OR = 0.26) and bb alleles were found more frequently (P = 0.01, OR = 4.41). The demonstrated the link between *BsmI* polymorphism and PBC in Chinese population (Lieying et al., 2005). Furthermore, A study conducted in Germany has also shown an association of *BsmI* polymorphism and PBC in their population. The frequency of Bb allele and bb allele differed from the control groups very prominently (p= 0.004, OR = 0.44; p = 0.01, OR = 2.1) (Vogel et al., 2002).

Autoimmune thyroid disease (AITD) like Graves' disease (GD) has also been recently linked with *BsmI* polymorphism of VDR gene. A study performed on 90 patients showed that there was a statistically substantial difference between GD patients and controls when comparing *BsmI* polymorphisms (P = 0.004). As a result, the proportion of patients with the BB genotype was significantly lower than predicted. In addition, individuals with GD had a considerably lower frequency of the "B" allele (33.3%) (el Gawad et al., 2012). Another meta-analysis performed on AISD among 1158 patients and 1049 control groups also showed an association between *BsmI* (rs1544410) polymorphism with GD (Feng et al., 2013).

BsmI polymorphism (rs1544410) of VDR gene has been associated with multiple sclerosis (MS) in quite an interesting way. In a study consisting of 270 MS patients and 303 control

group it was seen that; the BB genotype was much more prevalent on the control group. And recessive model confirmed the idea that BB genotype is significantly decreasing the chance of MS onset (Čierny et al., 2016).

In a study performed in 2012 on breast cancer showed a very significant association with the *BsmI* polymorphism. Upon study it was seen that, the genotypes bb and Bb has increased the risk of having breast cancer (p =0.01). Thus, suggesting that, b allele of *BsmI* is responsible for increased susceptibility of the breast cancer (Shahbazi et al., 2013). A meta-analysis has also shown association of renal cell and ovary cancer with *BsmI* polymorphism (Raimondi et al., 2009).

3.2 Discussion

The study performed a review on the relationship of different diseases in association with BsmI (rs1544410) polymorphism of VDR gene. This study demonstrated various types to relation with different disease.

Environmental and genetic variables can have a significant impact on the development of the rheumatoid arthritis. Multiple populations' association studies involving *BsmI* polymorphisms and risk of RA have produced mixed findings; some have shown a substantial link, while others have not been statistically significant. Low statistical analysis, small sample numbers, and/or clinical heterogeneity may be the root reasons of this disparity (Maalej et al., 2005; Mosaad et al., 2014b). For instance, few studies have shown positive relation between *BsmI* polymorphism and onset of RA but others have found no relations. In some studies, although, no association was found but it was mentioned that the linkage disequilibrium (LD) of *BsmI* with the actual disease-causing genes, may be connected to the etiology of RA (Lemos et al., 2008). The positive relation among *BsmI* polymorphism and susceptibility to RA was reported

by many studies (Mosaad et al., 2014b; Mukhtar et al., 2019). *BsmI* is a functioning site of VDR gene and has shown a potential for greater level of vitamin D in the *BsmI* bb genotype in the RA groups (Punceviciene et al., 2021a). Another study shows, Patients who are homozygous for the *TaqI* site and the *BsmI* site (BB/ tt) exhibit an early onset of rheumatoid arthritis. These genotypes were linked to accelerated bone loss in RA patients who are female (Garcia-Lozano et al., 2001). To be exact, no relation between *BsmI* polymorphism and RA was found in Caucasians, Europeans and Asian population, if found it was very minimal. It is noteworthy to mention that, relation among *BsmI* polymorphism and RA was strongly seen in the African population (Bagheri-Hosseinabadi et al., 2020). The reasons for these contraindications can be many. One of the important reasons might be that the studies of the VDR *BsmI* polymorphisms did not provide statistically significant findings. Secondly, ethnicity-specific study was used to get patient data, therefore the findings are only relevant to that specific ethnic group (Song et al., 2016b).

Similar results were found in case of osteoporosis. Different studies gave different contradicting results. For instance, *BsmI* polymorphism was associated with osteoporosis in some studies (Mansour et al., 2010) and was not associated in many studies (Qin et al., 2013; Uysal et al., n.d.; Yadav et al., 2020). The function and expression of VDR may be significantly influenced by environmental interactions and population ethnicity. It is possible that the study of the link between the VDR *BsmI* gene polymorphism and osteoporosis susceptibility would be impacted by geographical and racial differences (Qin et al., 2013). This can be a huge cause contraindicating result. The studies performed consisted of very small population, this was mentioned by many studies as a reason.

In rickets the association of *BsmI* polymorphism was very clear and robust. The BB genotype of *BsmI* was seen significantly in the patients compared to the control groups (Baroncelli et al.,

2008; Mao & Huang, 2014b). Similar results were seen in PBC patients. A difference in Bb genotype and bb genotype was observed among case and control groups showing the association of *BsmI* polymorphism with PBC (Lieying et al., 2005; Vogel et al., 2002). Again, Autoimmune thyroid disease like Graves' disease has shown that B allele is present in huge frequency in the patient having graves' disease. This change in the gene is quite significant and considered to be responsible for the disease onset (el Gawad et al., 2012).

In case of multiple sclerosis (MS), no significant association was found in many studies (Tizaoui et al., 2015). But one study reported that instead of increasing the risk of MS, *BsmI* polymorphism is decreasing the risk of MS. The BB genotype was more significant in the control groups along with the absence of b allele. This condition can increase the VDR mRNA expression which in turn improves the receptor function of VDR, which somewhat decreased the onset of MS (Čierny et al., 2016).

In our study, breast cancer, renal cell cancer and ovary cancer has been associated with the *BsmI* polymorphism. *BsmI* polymorphism is assumed to increase the onset of these cancers (Raimondi et al., 2009; Shahbazi et al., 2013). But rather an interesting report was observed in studying cancer and *BsmI* polymorphism. Some *BsmI* polymorphism was involved in deduction of the risk of cancer. Such as, in prostate cancer, the person having Bb genotype instead of bb genotype had a 17% less chance of having the cancer. And the margin was of 9% in case of the colorectal cancer. Furthermore, an overall analysis on different cancers showed a 7% reduction in the onset of having cancer (Raimondi et al., 2009).

Chapter 4

Conclusion

To conclude, it is confirmed that VDR polymorphism can affect the level of vitamin D in an individual's body which can then lead to the different related diseases. And, studies have shown that intake of Vitamin D supplementation can decrease the risk of onset of RA. However, by the genetic analysis of the *BsmI* polymorphism, the relationship between VDR polymorphism of *BsmI* (rs1544410) was confirmed in different diseases with some contradictory result was found. The study confirms that there is association of *BsmI* polymorphism with different disease in different populations. Although, the result was found to both positive and negative in some cases. All of this contra indicatory studies shows that the studies performed till date are not conclusive and needs to be worked in a broad margin. Moreover, the ethnicity and race play a significant role on this onset and future works should be done considering this in mind.

Future Aspects of the Study

The study has shown that, there some contradictory results and some are not conclusive. In the future more works should be done in this field. And, during those studies few aspects should be kept in mind. The work should be designed considering the ethnicity and race of the population since the results have varied in different ethnicity. Moreover, the work should be performed in a great number and try to identity the cause of the polymorphisms in them.

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