CHAPTER- 2

REVIEW OF LITERATURE

2.1 Cancer incidence of the breast

Breast cancer ranks as the foremost prevalent cancer worldwide, and a rising burden has been associated with it for the past couple of years. Breast cancers have been diagnosed in over 2.3 million people and have been fatal for more than 685,000 people by 2020[1]. As per the National Cancer Registry Program, there were 1,62,468 recent reports of breast cancer in India in 2018 and 87,090 breast cancer-related deaths over the same period[36,37]. There is a high degree of geographic variation between countries and regions around the world. The various incidence rates occurring at rates ranging less than forty per 100,000 women in a number of Asian and African regions measured against eighty per 100,000 women in Australia and New Zealand, North America, and some regions of Europe. It is predicted that by 2040, there are likely to be new cases exceeding three million of breast cancer, and one million people die almost every year from these causes of the growing population and aging[38].

2.2 Globocan's Indian Scenario

In India, every year, approximately there are 100,000 new breast cancer diagnoses. Indian population-based registries further estimate that breast cancer onset is one decade earlier in Indian women (aged 50 to 53 years) compared to white/US corelatives (aged 61 years)[39,40]. India is currently seeing an increase in breast cancer cases, which previously outnumbered cervical cancer cases, even though the trend has been increasing for the last two decades. There has been a 15.4% upsurge in mortality and a 25% rise in cancer incidence among women in India (Globocan 2020; http://globocan.iarc.fr/).

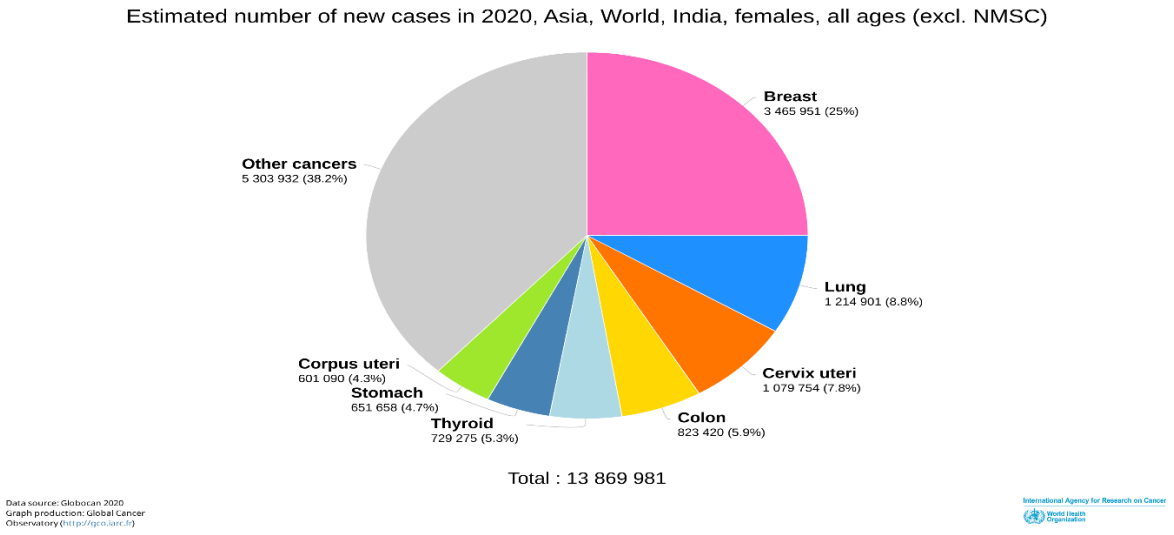

Figure1: Globocon 2020 estimates the cases newly reported in 2020 in Indian females excluding non-melanoma skin cancer(NMSC)

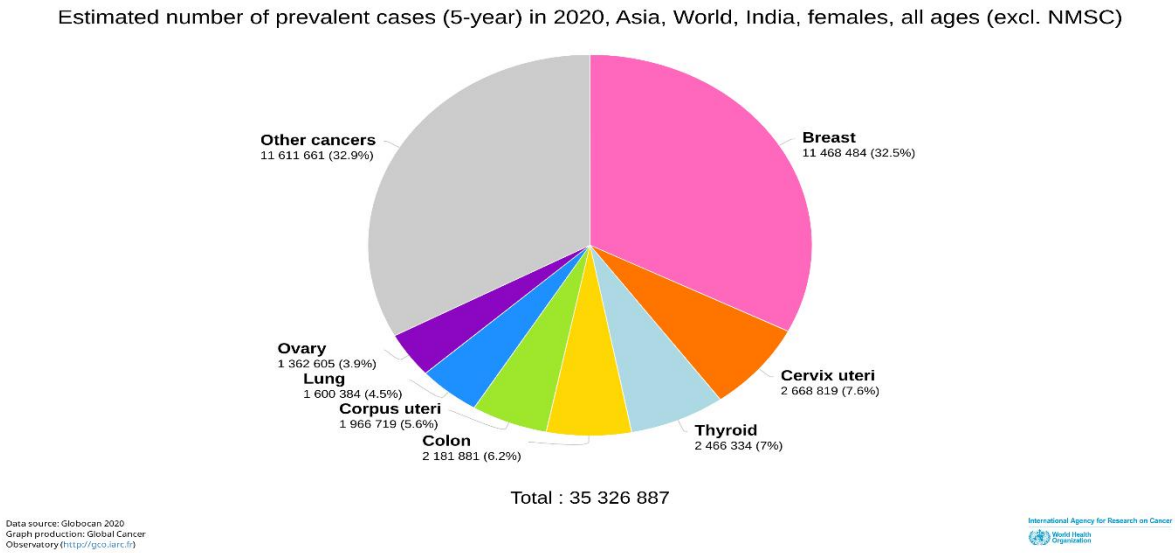

Figure 2: Globocon 2020 estimates the number of Prevalent cases in 2020 in Indian females excluding non-melanoma skin cancer(NMSC)

2.3 Breast Cancer Types

Breast cancer generally referred to as a group of diseases, since it has various biological subtypes that have distinct molecular profiles and clinicopathological features[41–43]. (Figure 3) Invasive and non-invasive types about breast cancer are classified according to their site of origin. The non-invasive form of breast cancer includes an in situ carcinoma of the ducts. “In situ” refers to “in place,” this carcinoma occurs when atypical cancer cells develop within milk ducts without extending to nearby tissues[44].

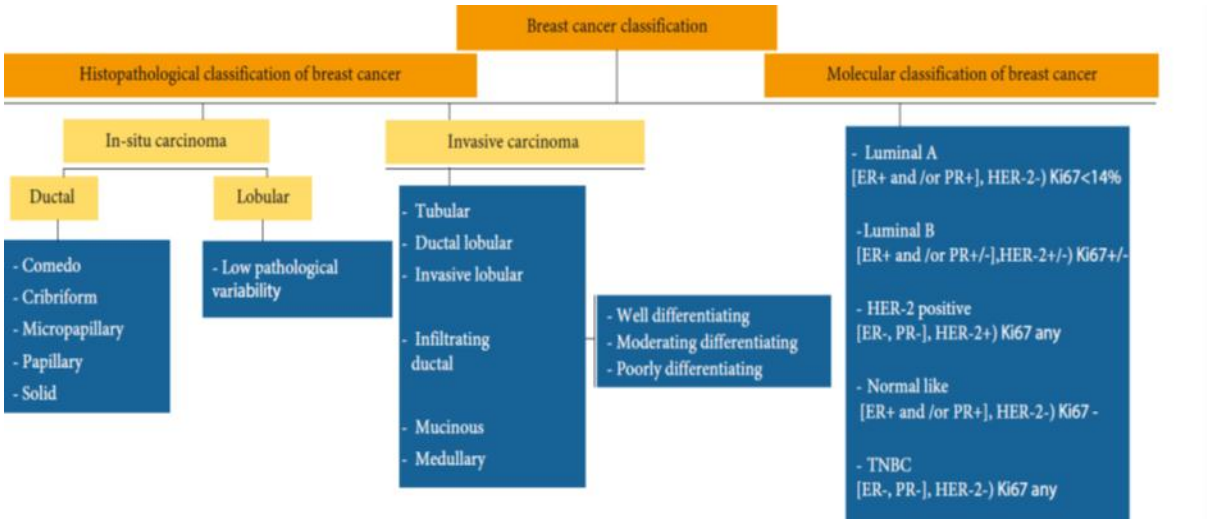

Figure 3: A detailed diagram of histopathological classification and molecular classification of the biological basis regarding breast cancer.

Lobular carcinoma in situ (LCIS) were described by developing breast lobules not extending to outer breast tissue[45]. From the perspective of invasive cancer of the breast, by way of the immune system or systemic circulation, cancer cells can reach various parts or physiological organs. They can also be acclaimed as metastatic breast cancer[46,47]. This type includes infiltrating or invasive lobular carcinoma that often

affects the body's other parts aside from the milk glands of the breasts (lobules)[48]. The infiltrating or invasive ductal carcinoma extends into the breast milk duct wall spreading to breast fatty tissues and probably elsewhere[49]. In medullary carcinoma, normal and medullary tissue were discretely separated[50]. Mutinous or mucinous carcinomas are uncommon breast cancers caused by mucus-forming cells, and these patients possess better prognoses than alternative types[51]. Tubular carcinomas of breast cancer involve a tubular component, which usually has a better outcome than other invasive types[52].

Besides histological subtypes, profiles expression of genes in cancer of the breast have been categorized into diverse molecular subtypes such as positively receptor-responsive (Luminal A, and B types, Normal-like, and Her-2 (Human epidermal growth factor receptor-2) positive, and Negatively responsive receptors as Triple-negative breast cancer (TNBC) or Basal-like[42]. The histopathological and clinical properties of these breast cancer subtypes differ by age and ethnicity, like subtypes of TNBC and HER-2 positive tumors, particularly those seen in younger and premenopausal women, as well as African-American and Asian women, with a higher metastatic potential and recurrence rate[53,54].

2.4 Breast Cancer Risk Factors

Different factors have been implicated in breast cancer incidence along with progression through epidemiological studies. (Table 1)

Table 1: The table summarizes the various factors and their outcomes contributing to breast development risk.

Risk Factors	Outcomes
Early or Delayed puberty	Puberty results in an increase in undifferentiated, proliferative breast cells, as well as increased susceptibility to mutagens, caused by hormonal changes[55].
Early or Delayed menarche	Menarche increases breast cancer risk by increasing breast cell growth and division[56].
Delayed marriage age	Estrogen Hormone exposure for a prolonged period of time[57].
Delayed childbirth age	An absence of breast tissue differentiation and prolonged exposure to estrogen Hormone[58].
Failure of lactation	The absence of differentiation in breast tissue can increase the likelihood of exposure to nonestrogenic mutagens and estrogens[59].
Delayed menopause age	Breast involution occurs late, and estrogen and progesterone exposure is prolonged[60].
Physical inactivity	Due to the higher number of anovulatory cycles, less exposure to sex hormones occurs[61].
Fat-rich diets	Activation of estrogen signaling and cell proliferation are facilitated by cholesterol[62].
Obesity	Enhanced inflammatory cytokines and chemokines production[63].
Alcohol consumption	Enhance estrogen production[64].
Smoking	A DNA adduct and mutations in the p53 gene can be produced[65].
Hormone replacement therapy (HRT)	Long-term exposure to estrogen hormone[66].
Contraceptive Use	The hormones progesterone and estrogen found in contraceptives increase the likelihood[67].
Family history	Mutations in the BRCA1/2 gene[68].
Environmental toxins	The signaling system in the endocrine system can be disrupted by pollutants[69].

2.5 Different Available Approaches for Breast Cancer

Breast cancer screening has been met with much debate due to the uncertainty around its potential advantages and drawbacks. A high percentage of women who test positive turn out not to have the illness, therefore, it is uncertain whether mammographic screening causes more damage than good, according to a 2013 Cochrane analysis. Evidence of benefit was discovered in a 2009 assessment conducted by the Task Force for Preventive Services in the U.S. with people aged 40–70, and the Task Force advises screening every two years for women aged 50–74. Drugs like tamoxifen and raloxifene are given to females facing a higher danger of acquiring cancer of the breast to lower those odds. Some high-risk women choose to have both breasts surgically removed before being diagnosed[70]. A surgical procedure or radiation treatment, targeted therapies, hormone therapy, and chemotherapy are only some options for people with cancer. The two most drastic surgical treatments for breast cancer surgery are breast conservation and mastectomy. People can have breast reconstruction in the event of their mastectomy or eventually later period. Treatments for those whose cancer has spread to other regions of the body focus primarily on making the patient more comfortable and increasing their quality of life in the remaining time they have[71].

2.6 The Synthesis and Signaling of Vitamin D

The lipophilic secosteroid vitamin D3 can be synthesized through the body starting with cholesterol through a procedure beneath the skin that involves multiple steps or potentially ingested by means of diet. Through a photolytic reaction, ultraviolet B (UVB) light converts 7-dehydrocholesterol into vitamin D3 (cholecalciferol) via the intermediate-generation of pre-vitamin D3[72]. Furthermore, an important hydroxylase enzyme associated with CYP450 as CYP-27A1 and CYP-

27B1 facilitates the synthesis of bioactive compound 1,25-dihydroxy vitamin D3 (calcitriol)[73,74]. CYP27A1 assists in the first hydroxylation step in the liver in turn, this leads to intermediate metabolites 25-HydroxyVitamin D (25-OHD3)[75]. In a kidney, CYP27B1 catalyzes the reaction at the second hydroxylation step in which propagating 25(OH)D3 is converted into a highly effective hormone from the steroid family as 1,25(OH)2D3[76]. The interaction of calcitriol which binds to vitamin D receptors (VDR), which have a high affinity for it, is fundamental for carrying out the biochemical functioning of vitamin D.

Studies have shown the vitamin D receptor protein corresponds as part of the nuclear receptor family, which consists of a steroid, thyroid, and vitamin A (retinoid) receptors, and is expressed in multiple tissues of the body[77]. VDR is activated when activated by its ligand, and it acts as factors involved in transcription that frames hetero-dimers along with other proteins, including a receptor for retinoid X (RXR). The functional complex composed of 1,25-OH2D3, VDR, and RXR recognizes vitamin D3 Response Elements (VDREs) with more than 60 target genes in the nucleus and modulates their transcription. (Figure 4) The VDR includes five functional domains: A and B domains containing 20 amino acids and are the shortest. Two Zn fingers motifs reside in the Domain of C (Binding domain for DNA or DBD). Zinc fingers are composed of alpha helices (helixes A and B) representing sites that recognize DNA and bind phosphate. A domain within C and E, the flexible hinge D domain changes structural conformation upon binding of VDR ligands. Vitamin D3 is attached to the domain E (ligand binding domain or LBD) which is composed of twelve alpha helices and three beta strands with short lengths, and there is an activation function (called AF-2) during translation for both N-ter and C-ter[78].

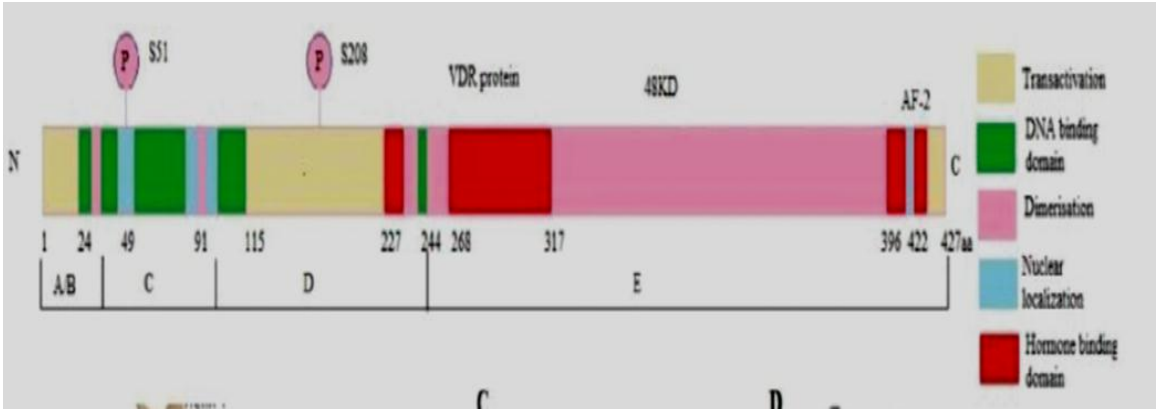

Figure 4: The human VDR molecule is schematically represented, including the receptor's ligand binding domain, nuclear localization domain, heterodimerization domain, transactivation domain, and two major phosphorylation sites. AF-2 is an activation function.

The vitamin-D target genes belong to diverse regulatory processes such as bone metabolism, calcium, and phosphorous homeostasis, immune responses, cell cycle, and differentiation[79]. Cryo-electron microscopic studies have revealed the structure of the 100 kDa-sized ligand-activated VDR-RXR heterodimers bound to the consensus response elements[80]. It provides mechanistic insights into the cooperation and co-regulation between VDR and RXR that facilitate binding the target genes inside the nucleus. The wealth of structural information gathered can be used to understand the effects of mutations in the different parts of VDR protein on the ligand binding, heterodimerization with RXR, and subsequent recognition of the VDREs present on genes with promoter regions are targeted. Figure 5 represents the vitamin D synthesis and downstream signaling pathway diagrammatically[81]. Deficiency and deregulation of the endocrine system of vitamin D are associated with a broad range of disorders, including rickets, osteoporosis, diabetes, cancers (renal, prostate, and breast), auto-immune diseases, hypertension, multiple sclerosis, and cardiovascular diseases[82].

25-OHD3 is 25-hydroxyvitamin D3 and 1,25-OH2D3 is 1, 25-dihydroxy vitamin D3

2.7 Antiproliferative and Anticarcinogenic Effects of Vitamin D and its Associated VDR-Mediated Signaling

Multiple preclinical studies show that vitamin D operates key pathways of cells preventing cancer, namely, apoptosis, differentiation, cell proliferation, and promotes the suppression of vasculogenesis, metastatic development, incursion, and an inflammatory response [83–85]. (Figure 6) Vitamin D and its receptor complex activate CDK inhibitors such as p21 and p27 are transcribed, while cell cycle-promoting agents like cyclins D1, A1, and E1 are suppressed. This alteration in transcription causes the cell cycle to stop at G1, promotes the exit from the cell cycle, and subsequently prevents the cell cycle from expanding further[86]. The signaling from vitamin D decreases the expression of the oncogenes c-myc and c-fos and enhances the manifestation of the tumor suppressor BRCA1, together they govern cell proliferation[87,88]. The knockdown of VDR increased cell proliferation in mammospheres enriched for cancer stem cells, as demonstrated in a preclinical study[89].

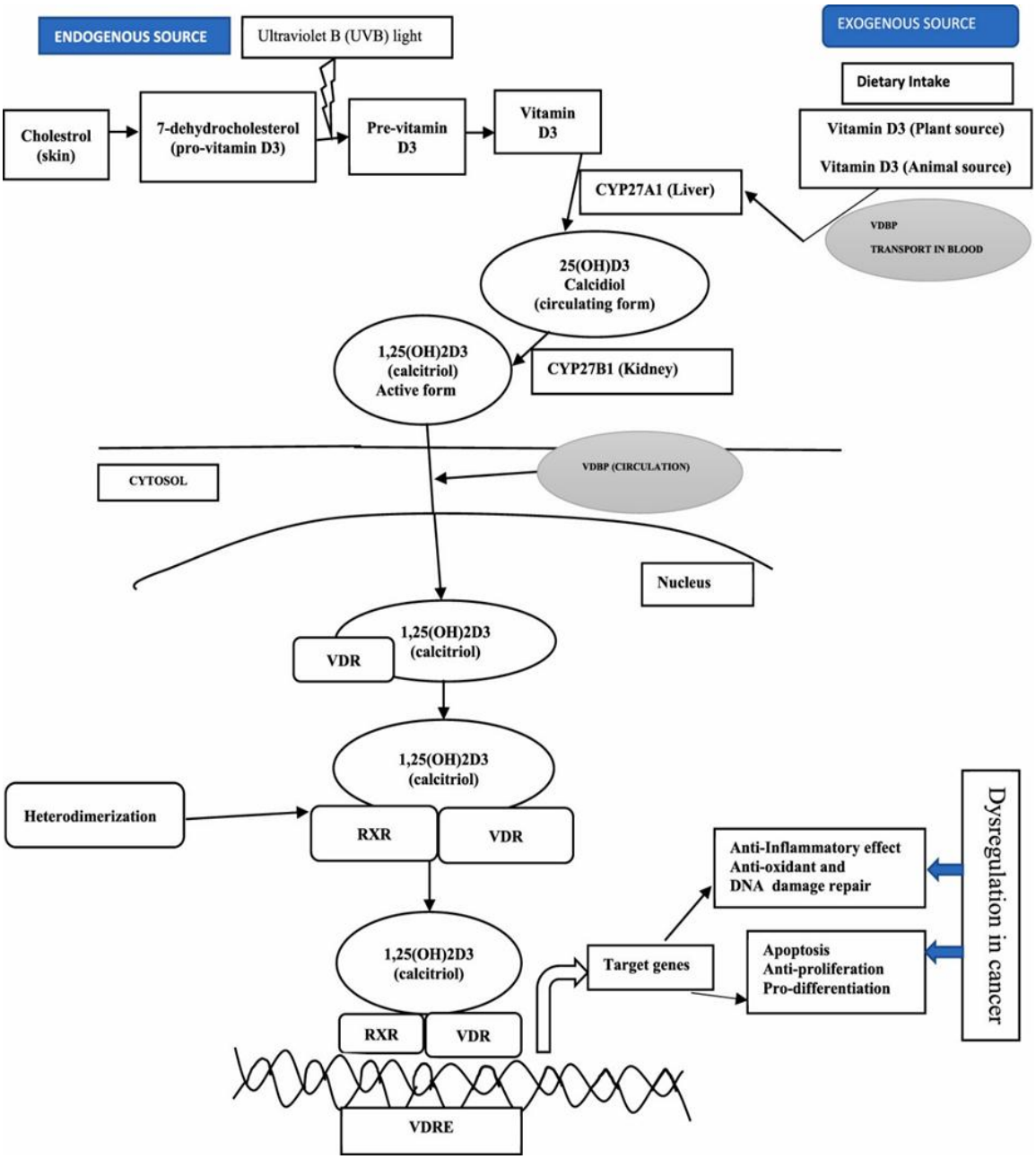

Figure 5: An illustration showing the endogenic synthesis of vitamin D and subsequential signaling pathways. In response to ultraviolet-B (UVB) light, active form (1,25(OH)2D3) being generated from predecessor cholesterol. A ligand for vitamin D receptors called 1,25(OH)2D3 activates their function, which is first involved in heterodimer formation accompanied by retinoid X receptors (RXRs). An identifier of vitamin D response elements (VDRE) is a complex of 100 KDa in size within its nucleus target genes. Transcriptional regulation of genes involved in cellular processes is mediated by vitamin D

signalings, for instance, the proliferation and apoptosis of cells, angiogenesis, and differentiation. There is a crucial role for these pathways in cancer development and progression which is dysregulated in breast cancer. This section describes several vital precursor metabolites and the enzymes involved in the pathway.

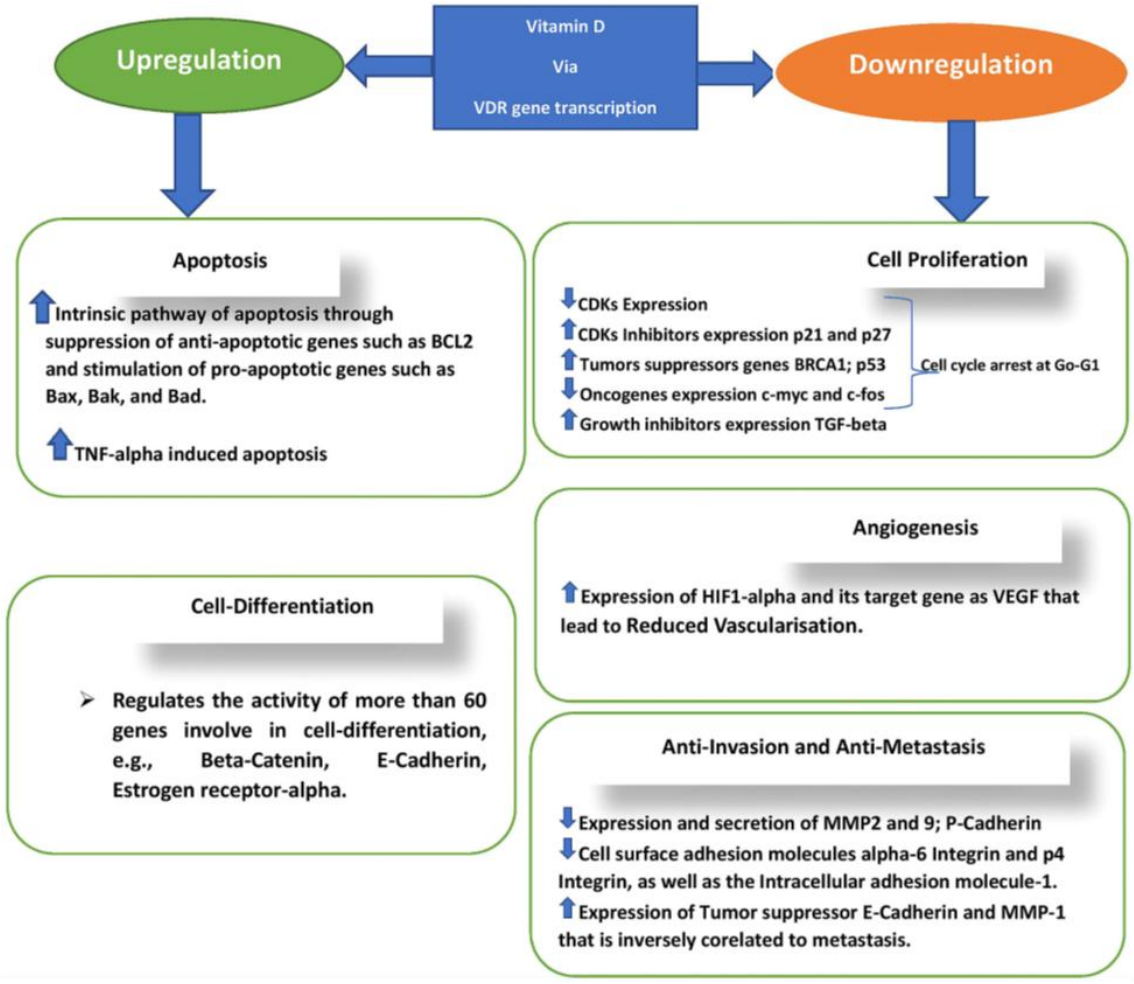

Figure 6: Diagram illustrating Vitamin D's effects on breast cancer[81]. (Upward arrows represent activation, and downward arrows depict suppression in these boxes).

Additionally, 1,25(OH)2D3 stimulates proapoptotic factors like BAX, BAK, BAD and reduces antiapoptotic factors such as BCL-2, BCL-XL to control the apoptosis

process. Besides modulating cellular proliferation, 1,25(OH)2D3 restricts angiogenesis by repressing the pathway of hypoxia-inducible factor (HIF)-1. By suppressing signals triggered by vitamin D, the HIF-1, and its pro-survival targets are suppressed, like the glucose transporter (Glut-I) gene, endothelin-I (ET-I), and vascular endothelial growth factors (VEGF)[90]. Among the anti-invasions or anti-metastasis effects of vitamin D are its suppression of (MMP) matrix metalloproteinases, urokinase-type plasminogen activators (uPA), and P-cadherin expression, that promote epithelial-mesenchymal transitions (EMT) and the occurrence of metastasis is caused by increased expression of molecular components of cell adhesion like E-cadherin and MMP inhibitors[91].

Prostaglandins are pro-inflammation mediators that contribute to cell proliferation and tumor progression via autocrine/paracrine stimulation, and vitamin D reduces cyclooxygenase 2 (COX2) expression and enhances 15-hydroxyprostaglandin dehydrogenase expression; enzymes involved in prostaglandin synthesis and degradation, respectively[92,93]. This finding underscores the significance of defining the anti-inflammatory function of vitamin D by limiting uncontrolled cell growth, specifically in breast malignancies. Studies using VDR null mice validated the direct function of VDR in facilitating growth-arrest and anti-cancer progression. Administration of calcitriol did not induce any effects in cells obtained from mice with knockouts for the VDR, and these addition of VDR agonists failed to inhibit the growth of xenografts derived from VDR null mice[94]. The data suggests that vitamin D is indispensable and enough for the anti-carcinogenic functionality provided by VDR.

Additionally, to restraining cell proliferation, vitamin D boosts cell differentiation to facilitate normal growth. In order to accomplish this, EGFR and

insulin-like growth factors I (IG-I) are inhibited, which suppresses underlying mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinases (ERK) pathways important for cellular proliferation. Apart from the actions at the genomic level, VDR and 1,25-OH2D3 also carry out protective functions against cancer through non-genomic pathways via interaction with the endoplasmic reticulum stress protein 57 (ERP57)[95,96].

2.8 Vitamin D serum concentration and breast cancer risks

The level of 25-hydroxyvitamin D in the blood has been considered as an accurate measurement concerning vitamin D status[97]. According to the Institute of Medicine, based on 2011 guidelines, a serum concentration of 25-OHD lesser than 20 ng/ml was considered insufficient, and the recommended dietary allowance (RDA) of 600 to 800 international units/day[98]. According to clinical experts, levels within 30 to 50 ng/ml for 25-OHD should be maintained to ensure optimal healthcare (Table 2)[99].

Around the 1980s, Garland and colleagues first described the possibility of sun exposure and breast cancer have been linked[100]. This association has been further analyzed by numerous epidemiology researchers where threshold 25(OH)D levels have been defined before starting respective treatment[101]. A breast cancer diagnosis is often accompanied by vitamin D deficiency[102–104] and are linked to the breast cancerous subtype, thus, patients with high-grade tumors, non-luminal and having estrogen receptors breast cancer exhibit low serum 25-OHD concentrations comparatively to counterparts[102,103].

Table 2: The Vitamin D levels in the blood

Categories	Research Institute for National Health (NIH) ng/ml	Society for Endocrine Research ng/ml
Deficient	Less than 12	Less than 20
Insufficient	Between 12 to <20	Between 21 to 29
Sufficient	≥20	≥30
Excessive	Greater than 50	Greater than 100*

*A serum 25-OHD levels exceeding 150 ng/ml can cause vitamin D intoxication.

During the past few years, concern has grown about vitamin D contribution to breast cancer prevention and treatment[105]. Several epidemiological studies of breast cancer incidence indicate that vitamin D might offer protection contrary to the disease in some groups while not in others, especially in the cases of postmenopausal women. Alternatively, vitamin D hypovitaminosis, common in American populations, increases breast cancer risk for some cohorts[106,107]. Over a five-year follow-up, a research cohort study of US residents including nearly half a million women age-old 35 to 74 revealed a link between a lesser serum 25-OHD level and having a greater chance of post-menopausal cancer of breast[108]. In contrast, an analysis of data from the registry of Danish cancer patients revealed that a relationship was not established between vitamin D levels and cancer of the breast and another type of cancer[109]. In another study of cohorts, 25-OHD levels were strongly regarded for tumor severity in premenopausal women[110]. The results of a pooled analysis of 22 studies were recently

reviewed in a systematic reviews and meta-analysis indicated that 25-OHD deficiency has a net straight association with cancer of the breast, with RRpooled = 1.91, 95% CI: 1.51-24, p-value <0.001[111]. The 25-OHD levels have significantly decreased concentrations in the plasma was noted in premenopausal females evaluated for extremely severe triple-negative breast cancer[112]. An abundant level as for Vitamin D is much more beneficial in preventing breast cancer with triple-negative status, with a 36% reduction the risk increases by 10 nmol/l in levels of Vitamin D[113]. Multivariate analysis also found that levels did not influence survival outcomes for breast cancer survivors[114]. Despite observational studies and meta-analyses suggesting a connection, the apparent causal interrelationship between serum 25(OH)D and cancer risks associated with breasts, and vitamin D deficiency remains a challenge.

A Mendelian randomization study (MR) is another method involving polymorphisms of single nucleotides (SNPs) for evaluating vitamin D stature and risks of breast cancer. There are four gene pathways involved in vitamin D signalling have been connected to plasma vitamin D concentrations in the past: the binding protein for vitamin D (GC), 7-dehydrocholesterol reductase (DHCR7), CYP2R1, and CYP24A1[115]. A genome-wide association study (GWAS) was conducted recently on 79,366 subjects in which a further two sites (SEC23A and AMDHDI) involved in serum 25-OHvitD levels were identified[116]. Further evaluation of these six loci revealed a lack of associations between the variants and breast cancerous risk in 122,977 cases[117]. Researchers from similar groups studied 137 SNPs in accordance with vitamin D in 69 loci, and a causal relationship between 25(OH)D and risk for breast cancer was not found[118]. Even though, the recent improvements in study power, the causal relationship has not yet been established.

Trials conducted by randomization (RCTs) of enough power are required to establish that vitamin D is causally risk-related to breast cancer, responds to treatment, and survival rate. Among the biggest randomization control trials conducted to date, VITAL which is a combination of Vitamin D, and fats rich in omega-3 content were recently completed, involving 25,871 breast cancer patients. Vitamin D3 as a supplement (2000 IU) independently versus alongside marine omega-3 (1 g) was tested in a clinical trial that was unbiased, double-blinded, and compared with placebo. As a result of the vitamin D3 treatment, mean 25-OHvitD levels in the vitamin D3 recipients rose by 30 to 41.8 ng/ml after one year, while placebo levels did not change. According to the VITAL study's initial findings, a comparison was made between vitamin D3 supplemented patients and placebo-controlled participants in which neither group showed a difference in breast cancer incidence[119].

Likewise, an RCT was conducted to investigate the effects of supplementing calcium (1500 mg/day) with vitamin D3 (2000 IU/day) in place of supplements containing fatty acids. Over four years, more than two thousand postmenopausal women underwent study, however, supplementing vitamin D3 with calcium yielded no significant reductions in all-type cancer risk[120]. A further RCT investigated the effects of large dosage vitamin D3 administration (40,000 IU daily) before breast cancer surgery to assess its effects on the proliferation and apoptosis of cells. Although higher amounts of 25(OH)D after two to six weeks of daily supplementation, breast cancer cell proliferation or apoptosis was unaffected[121]. An evaluation of the effectiveness of neoadjuvant vitamin D3 treatment is presently underway on localized breast cancer survivorship for five years (NCT01608451;

ClinicalTrials.gov). In another study, 50,000 IU of vitamin D3 per week is being added to neoadjuvant therapy to assess the effect on pathological response (NCT03986268; ClinicalTrials.gov).

According to studies conducted in vitro and on animals, 1,25-OH2D3 is possibly an anticancer in breast cancer, merely the effect is rarely found in human studies (observational and RCTs)[122–124]. Despite this, vitamin D3 RCTs are likely to have a lot of potential confounders since it is a nutrient. A possible confounding factor is the continuation of self-supplementation and diet during the trial[125]. Research studies in vivo and in vitro have presented favorable outcomes when using 1,25-OH2D3 to reduce breast cancerous progression and growth; however, further clinical trials are warranted.

2.9 The structure and polymorphisms of the Vitamin D Receptor (VDR) gene

2.9.1 VDR gene Structure

There is a nuclear receptor referred to as the vitamin D receptor (VDR) that is responsible for mediating vitamin D's functions, and almost each cellular type in our body is equipped with Vitamin D receptors. Normal mammary glands express VDR, which is crucial to their development and function[126].

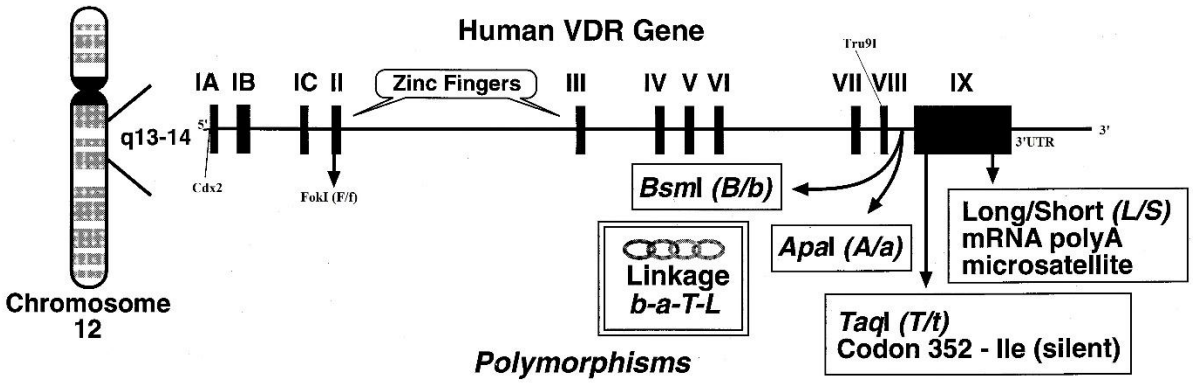

Figure 7: Schematic representation of hVDR chromosomal gene containing 11 exons (black vertical bars) and 3 of which are involved in the transcription of the VDR transcript's 5' UTR.

Human hVDR, a nuclear receptor gene found on chromosome 12 (12q13–14) (Figure 6), resembles to other nuclear receptors genes thereby it encodes a pair of zinc fingers separately through a second exon (II) and a third exon (III), and at the end of 5' has a number of alternate splices and/or translation sites, which adds some complexity[127–129]. An exon (7, 8, and 9) has a site for binding ligands (vitamin D). Three exons (IA-IC) encoding untranslated 5'positional regions are alternately spliced results in three mRNA variants in hVDR, whereas polymorphic sequences in exon II determine whether an alternative translation start site is present or absent[130]. Also, in the 3' UTR, poly(A) repeats are found, which are associated with mRNA stability. An additional exon (V) is a distinctive characteristic of the gene hVDR, hence, absent from other nuclear receptor genes. This region in the hVDR, residues 155–194 are encoded and located near the middle of the gene. VDR has an expansive region compared to other nuclear receptor proteins, suggesting that it may have acquired a novel exon after diverging from other genes related to nuclear receptors[131].

2.9.2 Gene Polymorphisms

The term polymorphism refers to a phenotypic variant that exists in two or more forms of individual sequences of DNA from the same population of a particular kind. A single nucleotide polymorphism occurs when base pair variations occur at a single site and occurs within about one percent of the population. Introns or non-coding regions of genes can contain these types of changes, which would not affect protein translation, but could influence gene expression and protein levels. Alternatively,

changes in DNA coding regions or exons can cause altered protein sequences. As a result of these changes, restriction sites are created or eliminated where endonucleases result in DNA digestion. This results in different-sized DNA fragments that can be detected by electrophoresis using gels, and known as restriction fragment length polymorphism (RFLP). The fragments generated are undigested, therefore, dominant homozygous type, while the fragments digested by digestion are heterozygous or homozygous recessive type[132,133].

In some cases, polymorphic alleles may be linked with one another and inherited together in a nonrandom proportion within a population, called linkage disequilibrium (LD). Haplotypes are combinations of alleles (blocks) or SNPs that tend to be inherited together on the same chromosome. These blocks vary in size from 10 to 20 kb and are valuable in determining genetic disorders[134,135].

2.9.3 Polymorphisms in the VDR gene

Sequence variations amounting to more than 200 have been documented in the gene of VDR, this is what is known as polymorphism, for instance, restriction fragment length polymorphism (RFLP) and a variable tandem repeat (VNTR)[136]. Several of these allelic variants are linked to various diseases such as prostate cancer, osteoarthritis, diabetes, auto-immune diseases, and renal diseases like nephrolithiasis, highlighting the possible use of VDR polymorphisms as diagnostic biomarkers for these diseases[137–139]. Interestingly, nearly all the VDR polymorphisms are inherent in the regulatory areas, hinting at possible effects on gene expression, mRNA stability, and protein translation rate (Figure 6)[140]. Among the most prevalent polymorphisms located within the coding regions are FokI and TaqI in exons II and IX, exclusively, while a polymorphism in Cdx2 in exon I, BsmI, and ApaI in intron VII, and the

polymorphic (A) repeats near the 3' UTR are reported in regulatory areas. Intron VIII contains Tru91 and exon I contains A-1012G close to the transcription start codon, which are rare polymorphisms. (Table 3)[141–143].

Table 3: List of the common and rare polymorphisms identified in the promoter, exonic, intronic, and 3' UTR regions of the VDR gene and their corresponding genotypes.

	Gene Polymorphism	Gene Location/Base Change	SNP ID	Genotypes
Common Polymorphisms				
1	Fok1	Exon 2 start site/ (C to T)	rs10735810/ rs 2228570	Homozygotes/Heterozygotes (ff,FF/Ff)
2	Apa1	Intron 8/ (G to T)	rs7975232	Homozygotes/Heterozygotes (aa,AA/Aa)
3	Bsm1	Intron 8/ (A to G)	rs1544410	Homozygotes/Heterozygotes (bb,BB/Bb)
4	Taq1	Exon 9/ (T to C)	rs731236	Homozygotes/Heterozygotes (tt,TT/Tt)
5	Cdx2	Exon 1e (promoter region)/ (G to A)	rs11568820	G allele, A allele
6	Poly (A)	3'-UTR region	rs17878969	S allele: <18 As
				L alllele: >18 As
Rare Polymorphisms				
7	A-1012G	Exon 1a (promoter region)/ (A to G)	rs4516035	G allele, A allele
8	Tru9I	Intron 8/ (G to A)	rs757343	U allele, u allele

Polymorphisms in Fok1 are also called start codon polymorphisms (SCPs). In this polymorphism, thymine is changed into cytosine in the DNA binding domain ten bp upstream of exon 2, resulting in a more active transcription factor with a shorter length of three amino acids[144]. Translation commences on the secondary site of the ATG in an individual with an ACG sequence encoding the codon of the start sequence (labeled as F), causing a decrease of 3 amino acids in the NH2 terminus, which contains 424 amino acids. The entire stretch of VDR protein has approximately 427 amino acids if the onset of an ATG sequence initiates the transcription (labelled as f). Also, an allele that has a restriction site is designated as 'f', whereas one without a restriction site assigned the form 'F' (active)[145]. So far, Fok1 is the sole observed polymorphism of the VDR gene that causes an altered protein to be produced. Based on one study, African Americans had an ff genotype frequency of 4%, while Asians and Caucasians had a 13-18%[146].

Bsm1, Apa1, and Taq1 SNPs accounted for the majority of variations in functional sequences detected on the 3' terminus around the gene VDR. There is linkage disequilibrium between these SNPs, which belong to the similar haplotype block, and thus, SNPs of this type may affect mRNA stability[147]. The Bsm1 and Apa1 genes were positioned in intron eight, while the Taq1 gene is in exon nine. In the VDR gene, Apa1 and Bsm1 polymorphisms are silent single nucleotide polymorphisms. In these SNPs, restriction enzyme sites are indicated by lowercase letters, these include b, a, and t, while absences are indicated by uppercase letters[148].

The poly(A) tail is composed of tandem repeats (VNTRs), as well as short tandem repeats (STRs), around 3' end with adenine nucleotides. The polymorphism can be classified as biallelic, as individuals possess either short (S, with fewer than 18

As) or long (L, with more than 18 As) poly (A) stretches. It is considered that the S allele is the most active VDR allele[149]. The polymorphism poly(A) also links to polymorphisms identified in Bsm1, Apa1, and Taq1 and influences VDR mRNA's stability. There is a significant disequilibrium across the linkages among the four genetic variants of the VDR gene, which are localized close to one another. Among the commonly occurring haplotypes is the haplotype for baTL with BsmI and ApaI restricted sites, while TaqI is not present as well as poly(A) repeats of long length, and among the BAtS haplotypes, there are no Bsm1, Apa1 restriction sites, while Taq1 site with short Poly (A) repeats exist[150].

The VDR Cdx2 polymorphism is one nucleotide change, that has been identified through examining sequences of the promoter region. This vitamin D receptor gene contains an SNP in the exon 1e region and is associated with adenine to guanine change (A/G). According to initial reports, it is located in a region of 3731 bp upstream of exon 1a of the VDR promoter regions in Japanese women[151]. Many ethnic populations later found that it was situated at 1739 kb upstream from exon 1e, about two kb apart from exon 1a[152]. Specifically, it contains the attachment site of the protein Cdx2, which is a crucial caudal-related homeodomain protein associated with the intestinal tract, and is responsible for increasing the transcription of the VDR gene. The Cdx2 protein attached increasingly firmly at the time the A allele resides in the Cdx2 promoter than with the presence of the G allele. In contrast, the G allele inhibits transcription initialization, while the A allele stimulates it[153].

Lastly, two other polymorphisms in the VDR are seldom explored, the polymorphism in A-1012-G and the Tru 9I polymorphism. A-1012-G is defined in terms of substituting adenine nucleotide (A) for guanine (G) near the transcription

start site of exon 1a at 1012 bp. An analysis of the conformational state of single-strand polymorphisms on the promoter side of the VDR has identified[154]. VDR polymorphism Tru9I in which the Gaunine (U allele) is converted to the Adenine (u allele) has been reported in intron 8 of the VDR[155].

2.9.4 Breast cancer and VDR gene polymorphism

There are numerous epidemiological studies on different ethnical populations that examine the link between various polymorphisms in the VDR gene and breast cancerous risk, as mentioned under and summarized up to recently in table form (Table 4).

2.9.5 Fok1 gene polymorphism and breast cancer

According to studies about Fok1 polymorphisms and breast cancer, Fok1 is a highly controversial SNP. It was reported that the study in the United Kingdom identified the FF allele of VDR complemented by long-Poly (A) could pose a risk indicator[156], whereas the Nursing's health survey in the US identified VDR ff as a risk factor[157]. A meta-analysis done in 2009 according to Tangs et al. found a firm association relating genetic variant VDR ff and risk of breast cancer in European women[158]. In an analysis of Iranian patients with cancerous breasts, Shahbazi et al. (2013) incabable to establish a statistically significant correlation implicating FokI genotypes and breast cancer[159]. Accordingly, et al., Mishra (2013) found alleles for VDR Fok1 f being related to early breast cancer development, and F alleles possibly involved in tumor progression and patient outcome[160]. According to Nemenqani et al. (2015), it has been shown that the Fok1 polymorphism has an impact on breast cancer's ER and PR status[161]. Amadori et al. (2017) conducted a study of populations

with mixed backgrounds (Africans plus Caucasians), although, this only had an effect found in Africans, and the proportionally small sample size is likely to have affected the findings[162]. Research has mostly been conducted on Afro-Caucasian populations and has yielded non-conclusive results[163–169]. Due to the diversity of Indian subcontinental populations and ethnic groups, little is widely known about variations in the vitamin receptor gene for FokI. Accordingly, among two studies in India, the Fok1 ff genotype[170] and Ff ,FF genotypes[171] were strongly correlated with cancer of the breast. Accordingly, a meta-analysis that included thirty-four studies, covering 26,372 cases and 32,883 controls, Fok1, in combination with Bsm1, Apa1, and poly (A) variants of VDR, contributes towards breast cancer development[172]. Further studies are needed to determine how functional polymorphisms affect breast cancer risk in additional ethnic groups.

2.9.6 Bsm1 gene polymorphism and breast cancer

Various research studies on Bsm1 gene polymorphisms have been conducted, with varying conclusions among ethnic groups and authors. A majority of findings did not get any association concerning Bsm1 and breast cancer[160,163,165,166,173–176]. There have been few studies examining the connection between Bsm1 bb and Bb genotypes[159,164,169,177,178]. In Iranians, Shahbazi reported was statistically significant a more likely that women with the Bsm1 bb genotype in combination with the BB genotype would develop breast cancer (ORs 1.74 and CI 1.06-2.97) when BRCA1 and 2 mutations of non-carrier subgroups were involved[159]. The results of a study of 130 cases and 100 controls in Egypt revealed having the B alleles protects against cancer of the breast, with a significantly reduced risk by forty-four percent and sixty percent, accordingly, when genotypes Bb and BB are comparable to bb genotype [169].

Additionally, BB genotypes remarkably minimize the risk of breast cancer by 32, 33, and 47 percent, especially, according to studies in Kazakhstan, Pakistan, and Iran[174,177,178]. Briefly, it appears that Bsm1 has no association pertaining to breast cancer risk primarily. Therefore, differentials of significance were observed between ethnically similar pools in this concern, and analyses of subgroups would be conducted in order to investigate these further.

2.9.7 Taq1 gene polymorphism and cancer of the breast

The TaqI genetic polymorphism in very few studies is strongly related to breast cancer[179]. The results of case-control study involving a population-based sample, VDR Taq1 Tt is associated with a significant association among premenopausal women (ORs =1.33; CI of 95%: 1.01–1.74) instead it wasn't with vitamin D and calcium intake[163]. Regarding, an analysis of haplotypes in nested case-control studies, Taq1, which is part of block B, contributes to a lowered breast cancerous risk (ORs =0.5)[167]. A study comparing Africans-Americans and Hispanics found that TaqI appears to have a more greater linkage disequilibrium associating Apa1 in Hispanics compared to Africans-Americans[160]. According to the Jordanian inhabitants, Taq1 genotypes Tt, TT, Tt are significantly associated with 25(OH)D levels[180]. Consequently, many studies haven't shown correlation between Taq1 VDR polymorphism and breast cancer exposure. Further, to obtain more accurate results, large sample sizes as well as different environmental and genetic factors must be investigated.

2.9.8 Apa1 gene polymorphism and breast cancer

The VDR Apa1 polymorphism was found to be significantly associated with tumor differentiation. An extensive study of case-control populations indicates an intensified cancer risk of the breast in women comprising the AA genotype[163]. However, nesting case-control studies reveal that linkage disequilibrium exists for ApaI and is linked to a fifty percent lowered risk of breast cancer[167]. In a study involving Caucasian women in Marin country, genotype AA of the ApaI gene frequency was more than twice as high as the general population, which could be referring to a more risk of breast cancer. The genotypes aa and Aa were also present, although at a lower frequency[181]. Conversely, based on the work of Mishra and colleagues demonstrates an absence of association between ApaI and breast cancerous risk, but still a prominent relationship combining the genotype of VDR ApaI aa and tumors with poor differentiation (p-value 0.04). Furthermore, the aa allele rate of occurrence was higher in Hispanics or Latinas than African-Americans among younger people, possibly indicating that VDR-Apa1 is involved in advancing breast cancer in young individuals[160].

Additionally, Ahmed et al., did not disclose a relatedness between the African population[168] and Guo et al., in the Chinese population[175]. As noted by Abd-Elsalam et al., having an ApaI aa genotype increases breast cancer risk significantly (Odd Ratios 2.2)[169]. In an Iranian study, a stratified analysis of Apa1 variations found that the size of tumors and lymph nodes metastasis do not correlate significantly[179]. Consequently, studies had generally found that the Apa1a allele is linked with a greater risk of cancer of the breast, despite the lack of extensive research across races.

2.9.9 cdx2, poly (A), Tru91 gene polymorphisms and breast cancer

The polymorphism in vitamin D receptor poly (A) was linked as a contributor to developing cancer of the breast. It was reported in 2000 by Ingles et al. that Latina females from different cultures with the SS genotype possess an elevated susceptibility to breast cancer versus the LL polyA genotype[182]. Correspondingly, studying case-control populations, shows that the polymorphic (A) genotype is not likely to increase the risk of developing cancer of the breast, nonetheless rather through increased vitamin D intake. Females bearing poly(A) genotypes LS and SS had the highest risk for cancer of the breast having more vitamin D consumption (Odds Ratios 1.41)[173]. Conversely, in Iran's female population, there is a higher risk of cancer of the breast associated with those carriers of poly(A) alleles LL or LS (Odds Ratios 1.8), and 25(OH)D-deficient women and L carriers are more likely to develop breast cancer[183]. However, an analysis by Jinjiang and colleagues (2014) meta-analyses manifested an absence of a kinship between polymorphism of the VDR polymorphic (A) and breast cancer risk[184]. An observational study based on case-control samples in a population disclosed the Cdx2 AG genotype involved a lower risk for cancer of the breast (ORs =0.83), whereas the Cdx2 AA genotype was associated with a higher risk of cancer (OR =1.36)[163] In another investigation of Yao et al. (2012), the Cdx2 G allele found a marginal association toward breast cancerous risk[185] Similarly, comparative analysis of women in southern Pakistan with polymorphism of cdx2, a relationship was established between the GG genotype and breast cancer susceptibility (Odd Ratios 1.8)[186], and in another study, the Cdx2 AA genotype was only associated with breast cancer susceptibility in Africans[162]. According to the study about VDR Tru9I polymorphism, premenopause females with

breast cancer are not significantly associated with it, and compared with other genotypes, Tru9I 'uu' showed a prominent cancer risk in breasts (Odd Ratios 1.1)[187].

Therefore, it can be concluded, that polymorphic (A) appears debatable, although breast cancer risks are increased by an allele variant called L. Still, furthermore, a study beyond the shadow of a doubt is needed to confirm this. Moreover, continuing research is needed in the case of polymorphisms for cdx2 and Tru9I due to the limited database.

Table 4: Research studies in different VDR gene polymorphisms and Breast cancer[188].

Research studies of the author (Year)	Populations being studied	Research Type	Analyzed polymorphisms	Counts for Cases/ Controls	Research findings
(2011) Anderson *et al.*	Caucasians	Population based-case control Study(PCCS)	Cdx2, Fok1 , Taq1 Bsm1, and Apa1	1,777/1,839	Risks of Breast cancer inversely correlates to the Fok1 ff genotype. Bsm1 and Taq1 show a lack of association. Breast cancer risk is raised by Cdx2 AA genotype.
(2012) Engel *et al.*	Caucasian	Nested-case control study(NCCS)	27 SNPs, which include Fok1	270/554	Fok1, Taq1, and Apa1 did not show any association.
(2012) Rollinson *et al.*	Hispanics and Non-Hispanics	PCCS	Poly(A), Bsm1, and Fok1	Hispanics-1,527/791 Non-Hispanics-1,599/922	Fok1, Bsm1, Poly(A) shown no relationship.
(2012) Dalessandri *et al.*	Caucasians	CCS-Pilot	ApaI	164/174	There is a significant rise in the risk of breast cancer with the AA genotype.
(2012) Yao *et al.*	Africans-Americans and Caucasians	(PCCS)	Cdx 2	928/843	Among African Americans, Cdx2 G allele reduces breast cancer risk.
(2013) Fuhrman *et al.*	Caucasians	(PCCS)	Six SNPs in CYP24A1 which includes Bsm1 and Fok1	484/845	Neither Fok1 nor Bsm1 show any association.
(2013) Mishra *et al.*	Africans-Americans and Hispanics	(CCS)	Apa1, Fok1, Bsm1, and Taq1	232/349	Risks of breast cancer is higher with the Fok1 f allele. Bsm1, Taq1, and Apa1 show no association.
(2013) Shahbazi *et al.*	Iranis	(CCS)	Bsm1, and Fok1	140/156	Fok1 shows no association. Breast cancer is significantly associated with BsmI bb and Bb genotypes.

(2014) **Akilzhanova *et al.***	**Others**	**(CCS)**	**BsmI, and Fok1**	**315/604**	**Elevated breast cancer risk was correlated with the Fok1 ff genotype. Bsm1 shows a lack of association.**
(2015) **Abd-Elsalam *et al.***	**Africans**	**(CCS)**	**Bsm1, Fok1, Taq1, and Apa1**	**130/100**	**Fok1 and Taq1 show no association. BsmI bb genotype and Apa1 aa genotype are significantly associated to a higher breast cancerous risk.**
(2015) **Nemenqani *et al.***	**Asians**	**(CCS)**	**Taq1, and Fok1**	**95/100**	**Unlikely, Taq1 is unrelated to breast cancer risk, but Fok1 ff is.**
(2015) **Rashid *et al.***	**Others**	**(CCS)**	**BsmI, and Fok1**	**463/1,012**	**Fok1 shows no association. The BsmI bb genotype increases breast cancer risk.**
(2015) **Guo *et al.***	**Chinese**	**(CCS)**	**Apa1,Taq1, and Bsm1**	**219/391**	**Bsm1, Taq1, and Apa1 show no association**
(2015) **Iqbal *et al.***	**Others**	**(CCS)**	**Cdx2**	**103/161**	**Breast cancer susceptibility, particularly in the Cdx2 GG genotype.**
(2015) **Colagar *et al.***	**Others**	**(CCS)**	**Polymorphic (A)**	**134/127**	**Long poly (A) L alleles are significantly a risk factor for breast cancer.**
(2016) **Deshasaux *et al***	**Caucasians**	**(NCCS)**	**Bsm1, and Fok1**	**233/466**	**Fok1 and Bsm1 show no association**
(2017) **Amadori *et al.***	**Africans and Caucasians**	**(PCCS)**	**Cdx2, Fok1, and A1012G**	**53/50**	**The Fok1 ff genotypes leads to increased breast cancerous risk. (Africans). Cdx2 AA genotypes are linked to breast cancer vulnerability.**
(2017) **El-Shorbagy *et al.***	**Africans**	**(CCS)**	**Taq1, Bsm1, and Apa1**	**100/50**	**Bsm1, Apa1, and Taq1 show no association**
(2017) **Atoum *et al.***	**Others**	**(CCS)**	**TaqI**	**122/100**	**The association does not exist.**
(2018) **Shahabi *et al.***	**Iranian**	**(CCS)**	**Bsm1, and Fok1**	**203/214**	**Fok1 shows no association. Breast cancer risk is significantly increased among BsmI bb and Bb genotypes.**
(2018) **Iqbal *et al.***	**Others**	**(CCS)**	**Tru9I**	**228/503**	**A high risk of breast cancer is connected to the Tru91 uu genotype.**
(2019) **Shaker *et al.***	**Africans**	**(CCS)**	**Bsm1, and Fok1**	**115/120**	**Fok1 and Bsm1 shows non-association.**
(2019)	**Indians**	**(CCS)**	**FokI**	**125/125**	**The Fok1 ff variant is related to increased breast cancerous risk.**

Raza *et al.*					
(2019) **Ahmed *et al.***	**Africans**	**(CCS)**	**Apa1, Taq1, and Fok1**	**392/193**	**Apa1 and Taq1 show no association. Relationship exists between the Fok1 ff genotype and breast cancer risk.**
(2020) **Matini *et al.***	**Others**	**(CCS)**	**Apa1, and Taq1**	**150/150**	**The TaqI tt genotype and Apa1 aa genotype show a significant increase in breast cancerous risk.**
(2023) **Chakraborty M *et al.***	**Indians**	**(CCS)**	**FokI**	**130/130**	**FF and Ff genotypes of Fok1 are associated with breast cancerous risk.**

NCCS is a nested case-control study, PCCS is a population-based case-control study, and CCS is a case-control study.

2.10 Steroid Receptors State in Cancer of the Breast

The steroid receptors (SRs) comprise polypeptides belonging to the nuclear receptor superfamily, which are cytoplasmatic proteins that can invade the nucleus, and gene expression is modulated by transcription factors (TFs). They are evolutionarily and structurally related cytoplasmatic proteins[189]. These receptors include Estrogen, progesterone, androgen, Human epidermal growth factor 2 (Her2), glucocorticoids, and mineralocorticoids receptors, entire of which are presented in the mammary glands of humans also within different kinds of breast cancer[190]. The binding of the various ligands involved like androgen, estrogen, Her2, and progesterone assists receptor dimer formation and nuclear transposition. As a result of identifying the promoter regions of hormone-responsive genes (HREs), the transcriptional modulation by the hormone-receptor complex regulates downstream target gene expression [191].

2.10.1 Estrogen Receptor

Several studies have shown that nuclear hormonal receptors recognizing estrogen are implicated in breast cancer as a robust prognostic cancer biomarker since the 1980s[192]. Breast cancer cells are stimulated and enriched by female estrogen, estradiol (E2). However, the presentation of estrogen receptors in human breast cancerous tumor growth persists a mystery despite this well-established prognostic value[193]. It has been reported that estrogen metabolism contributes to carcinogenesis by damaging DNA within mammalian cells[194]. Overall, breast cancer patients who express estrogen receptors have a better disease-free and overall survival[195]. Breast cancers that have estrogen receptors in their primary tumor are also more likely to develop estrogen receptors in contralateral tumors[196]. An extensive incidence involving hormone-positive receptor cancer of the breast has been shown in relation to nulliparity, early menarche, and breastfeeding absence[197].

Receptors for estrogen are classified into two types that have been determined – ER α and ER β. ERα deals with an essential contribution to breast cancer progression, while ER β is shown to be associated with an aggressive form of the disease[198]. There are five ER β variants (ER β1–ER β5) and five ER α isoforms (45, 62, 46, 53, and 36 Kdaltons) identified in breast cancer[199]. A balance between the ER α and ER β activity in an organ stimulates or inhibits estrogen signaling. ER α function has now been well characterized, making it widely used for imaging and verifying medication approaches[200]. In addition to the uterus and pituitary gland, ER α is expressed extensively within the liver, bones, mammary glands, testis, kidney, heart, cervix, skeletal muscles, hypothalamus, and vaginal lining. Approximately 30% of healthy breast glandular epithelium cells express ER α[201]. It has been shown that

ER α activation promotes tumorigenesis in different categories of cancer, inclusive of breast cancer[202]. Additionally, ER β is unique in its functions and has the potential to become a novel target for pharmacological interventions[203]. In the normal breast, ER β is found in luminal and myoepithelial cells, as well as in the subcutaneous adipose and testicular tissues, as well as the prostate, the ovary, the uterus, and the brain[204]. In ER β breast cancer, PI3K/Akt pathway (Phosphatidylinositol 3-kinase) is activated, leading to 3,4,5 phosphatidylinositol triphosphate synthesis resulting in leading to Akt serine-threonine kinase activation that involved in physiological and pathological processes[205]. Accordingly, estrogen receptor β may be useful as a predictor both positively and negatively[206]. Despite its importance, ER β further provides false positives results for estrogen receptor α, and its significance still needs to be investigated.

2.10.2 Progesterone Receptor

A receptor for progesterone (PR), that is coded around the gene PGR residing on the 11q22.1 loci, is another essential receptor in normal and cancerous mammary glands. The PR A and PR B isoforms that are known to exist. Both are derived from the PGR gene, but PRA lacks 164 amino acids at the N-terminus because it is transcribed using alternative promoters[207]. Normally, the PRA and PRB isoforms coexist almost equally, but breast cancer cells usually have a distorted PRA: PRB ratio[208]. In a healthy mammary gland, progesterone stimulates the proliferations and differentiation of cellular processes of cause breast cancer growth and development. This mechanism usually occurs during puberty and lactation[209]. In rodent studies, only twenty to forty percent of cells in the luminal epithelium expressed progesterone receptor, indicating that most cells are not directly affected by progesterone. Thus,

progesterone induces proliferative activity in two stages. A progesterone-responsive cell proliferates and synthesizes paracrine mitogenic factors within the first 24 hours after exposure to progesterone, promoting the remainder of the cells to multiply[210]. Breast carcinogenesis is well known to be driven by progestin induction of PR activity. In many epidemiological studies, utilization of progestins in birth control pills and hormonal replacement therapy significantly boosts the risk of breast cancer[211–213]. In contrast, as the tumor develops, a lack of PR expression leads to less differentiated and more aggressive phenotypes, resulting in a worse prognosis[214]. Further, in mouse model studies, an estimate of the relative number of cells that express PR has increased amounting to 20-40 percent inside healthy breast tissue to relatively 50 percent in invasive cancer, suggesting changing the signaling system of paracrine into autocrine as a primary factor of tumors progression[210].

2.10.3 Estrogen/Progesterone Receptor across Breast Cancer

In breast cancer, for many years, there has been debate over whether combining the presence of PR with ER is a requirement for the definition of hormone receptor positivity. For instance, a study recommended that PR testing fails to provide helpful information for clinical decision-making because most ER-positive tumors exhibit PR positivity[215]. Other publications responded by emphasizing the importance of PR detection[216,217]. A key argument focuses on cases with PR positives and ER negatives. Further, among ER-positive tumors, the PR-positive ones respond more sensitively based on hormone therapy, the subgroups thus defined are clinically plausible. Therefore, PR remains an essential biomarker along with ER[218].

There have been several ways of testing the estrogen and progesterone receptors; currently, IHC (immunohistochemistry) is the most recent standard of practice[219]. The Clinical Oncology Society of America (ASCO), and American College of Pathologists (ACP) congregated a panel of international experts that set a threshold of 1% nuclei expression or higher for positive results when normative epithelium and exterior factors were present[220]. A significant biomarkers role for a breast cancer's ER and PR receptors lies in its ability to predict hormonal therapy/endocrinal therapy blockade. Initially, hormonal treatment for cancer of the breast was used to lessen the threat of precancerous lesions called in situ carcinoma of the ducts (DCIS). The application of selectable modulators of estrogen receptors (SERMs), namely tamoxifen, raloxifene, and toremifene, has significantly reduced the risk of development of anyone ipsilaterally or contralaterally tumorous cancer of the breast among women before and after menopausal condition[221–223]. Also, SERMs were used to reduce the recurrence rate of patients with prior cancer of breast who were positive for hormone receptors. In general, SERMs and aromatase inhibitors (AIs) reduce breast cancer recurrence rates significantly when used over a five-year period, having a reduced relative risk of 50 to 66 percent, preventing secondary cancers rates unlike anything an active agent available in oncology today[224,225].

Furthermore, ER or PR status, in conjunction based on genetic evaluation was able to help in selecting adjuvant therapy decisions in node-negative cancer of the breast detected early. Patients with low OncotypeDX ® recurrence scores, for example, may require only endocrine therapy, don't respond biologically towards drug treatment, as a result, experience toxic effects otherwise benefiting clinically[226,227]. A hormone receptor blocking agent may be used as neoadjuvant therapy for patients

with higher-level cancer of the breast with hormone receptors that are large in size, lobular histology, or involves lymph nodes. Further evaluation ensures the determination of results in the clinical practice of this therapy[228,229]. In addition to chemotherapy, these biomarkers can also be used to determine whether endocrine therapy will be effective in the first-line, second-line, or third-line of treatment by patients with recurring or metastatic cancer of the breast with ER or PR positivity. However, therapeutic endocrine intervention did not show inadequacy over chemotherapeutic medications in patients with advanced ER/PR-positive breast cancer. More specifically, endocrine therapeutic agents are effective especially in patients with recurrent cancer of the breast despite adjuvant endocrine therapy[230,231]. Therefore, those who have breast cancer advanced or metastases disease are treated through various agents that target the hormone receptor alone or in combination with other treatments, and several research milestones have established their credibility.

2.10.4 Androgen Receptors across Breast Cancer

In humans, the androgen receptor (AR) is a 110 kDalton polypeptide whose gene is found in chromosome Xq11-Xq12. The ligands of this receptor include low-potency testosterone, dihydrotestosterone (DHT), as well as other metabolites of the pathway of the androgen present in minimal amounts in the blood[232]. Despite expressing primarily in organs of masculine genitalia, it appears in varying levels of the cervix, vaginal lining, breast acini, and ducts. The healthy breast epithelium expresses AR in approximately 20% of the cells and 60 to 80% of breast cancers[233]. Breast cancers with estrogen receptor positives (ER+) are more likely to co-express AR (70–90%), although it is also expressed in ER-negative cancers (40%)[234,235]. The study of in vitro models suggests that AR signaling may be influenced by tumor ER

expression in breast cancers. As a result, AR signaling drives tumor growth and inhibits apoptosis in ER-negative breast cancers, whereas it antagonizes ER signaling pathways in ER+ breast cancers[236,237]. Several epidemiological studies have consistently shown there is an association between AR expression and an improved prognosis for ER-positive breast cancerous patients, which is in agreement with in vitro studies. Conversely, epidemiologic studies conducted on women showing estrogen receptor-negative cancer of the breast vary considerably[238]. As a result of these findings, AR is likely to function as a tumor suppressant in estrogen-positive cancer of the breast.

Breast tumors with HER2 + ve/ER-ve were found to be AR-positive in approximately 70% of cases[239]. In comparison, researchers found that Patients who have androgen receptor+ with HER2+/ER+ features had clinically poorer outcomes than those with ER+ve subtypes. According to previous research, AR was associated with inadequate disease-free survival (DFS) and overall survival (OS) in patients expressing HER2-positive or ER-negative breast cancer[240]. The DFS and OS of patients with HER2+ve/ER-ve were associated with higher mRNA levels of AR in another study[241]. According to these studies, androgen receptors serve a role in oncogenesis for HER2-positive cancers of the breast.

There is a subgroup of cancer of the breast called TNBC that contributes to 10 percent of breast cancer, and is a very threatening and recurrence-prone subtype of breast cancer. TNBC expressing AR was found in 10-50% of cases[242]. Clinical studies have shown that those with TNBC who are AR-positive had a reduced rate of survivorship than AR-ve triple-negative breast cancerous patients[243]. The results analyses of 559 cancer patients with TNBC showed that androgen receptors

expression was associated with adverse outcomes regarding overall survival; AR+ve patients with no nodes of lymph metastases had a worse OS and DFS, the highest mortality rate, and recurrence rate, compared to AR-negative patients, with three times higher mortality and recurrence risks than AR-ve patients[244]. In clinical research, AR has been linked to a poor response to neoadjuvant chemotherapy, suggesting that it contributes to drug resistance[245]. Based on a statistical analysis of more than two thousand cancer patients suffering from TNBC, androgen receptor representation was associated with an improved DFS and low grade of tumors, yet not with an increased likelihood of lymph node metastasis or overall survival[246]. Despite this, another study conducted analyses of more than four thousand TNBC patients accessed by twenty-seven studies found that AR did not correlate with OS, DFS, or disease relapse-free survival[247]. These contradictory results are still being investigated, and considering these factors together, a better directive approach is preferable to determine activity related to AR in TNBC patients helping assess its relevance clinically.

2.10.5 Her2/neu Receptor in Breast Cancer

The Her2/neu (ErbB2) transmembrane glycoprotein receptor is part of the EGFR receptor family, which also includes HER1, HER2, HER3, and HER4. All members of the family have similar kinase tyrosine (TK) intracellular locations, but they each have separate extracellular domains of binding. HER 2 receptors lack the domains that bind to extracellular ligands; thus they must homo or heterodimerize with HER 3 and HER 1 to be activated[248,249]. Many cancers exhibit HER2/neu overexpression, involving breasts, lungs, heads and necks, and gastrointestinal tracts, but breast cancer is predominantly often affected[250]. Approximately 20-25% of breast

cancers overexpress HER2/neu, and as a result, this type of overexpression has been associated to unfavorable prognosis, irrespective of coexpression of PR and ER[251,252]. It was estimated that patients in this group exhibited three-fold increased death risks and distant metastases, and well-known chemotherapies do not yield better outcomes in comparison with patients without HER2 overexpression[253,254].

HER2 receptor overexpression assays include IHC and FISH, which are the most widely accepted. A discordance rate of up to 20% exists between IHC and FISH results, and performing FISH tests is regarded as the best method of determining genotype.[255,256]. Since trastuzumab was discovered, an increase in HER2/neu expression was observed in breast cancer no longer an indicator still, a predictive one[257]. The first phase III trial of trastuzumab was conducted three years after its development in the case of individuals exhibiting metastasized Her 2 receptor overexpression cancerous breast. In trials, when combined with chemotherapy or alone, trastuzumab can be an effective treatment that significantly improved survivorship rates for patients with cancer of the breast who have metastatic HER2 overexpression, and FDA approval for trastuzumab was given in 1998[258,259]. However, this trastuzumab therapy is not effective in all HER2-overexpressed breast cancers due to the development of resistance to both Anti-HER2 and endocrine therapies[258,260]. A significant and inverse relationship has also been observed between ERBB2 gene copy number and ER/PR protein expression levels for cancer of the breast, potentially also contributing to the relative opposition of HR+/HER2+ tumors to endocrine therapies[261]. However, steroid hormone receptors are not inactive in HER2+ tumors even when their expression is quantitatively lower. In response to the overexpression of HER2, downstream signaling cascades are activated, resulting in

post-translational modification (phosphorylation) of the ER, rendering it constitutively active, even in the absence of a ligand[262]. Despite substantial interplay involving HER2, ER, and PR signaling routes, HR expressions remain a predictive biomarker for endocrine therapy response when HER2+ patients are involved in early-stage cancer of the breast, and both estrogen receptors and HER2 drive tumor growth[263].

Preclinical and clinical research indicates that dual-receptor targeting attributed to hormonal receptor+/HER2+ cancerous breast may have a more significant benefit than single-receptor targeting since crosstalk between ER and HER2 contributes to resistance advancement designed for both endocrine and Her2-directed agents[264]. These combinations may also be effective in treatment regimens without chemotherapy, which may reduce treatment burden and adverse events while improving patient quality of life[265].

2.11 Breast Cancer VDR Polymorphisms and hormone receptor status

Breast cancerous diagnosis, treatment options, and prognosis depend significantly accordingly to the degree of differentiation and type of histopathology. It is the receptor for vitamin D that has been found in correlation to breast cancer prognosis[266]. As VDR has anticancer properties, a polymorphism or Single nucleotide change might reduce the protective effect. However, this possibly paves the way for a relationship involving gene polymorphisms in VDR and clinical or histo-pathological characteristics[267].

A UK Caucasian population investigated the Fok I, Poly A, and Bsm I polymorphisms that were associated with specific clinical/pathological characteristics

and found there were no VDR genotypes associated with lymph node involvement or ER expression in the tumor. However, in tumors of grades II and III, The genotypes of Bsm1 were significantly affiliated with tumor grade, with an excess of genotype bb, and also, the genotype poly A displayed similar results. As a result, it appears that BsmI/poly (A) is involved in the progression of tumors and disease risk[268]. In a Study on the UK Caucasian population in invasive patients of breast cancer, grading of the tumor, lymph nodes infiltration, and expression of estrogen receptors were unrelated to BsmI, FokI, or poly(A) genotypes, separately or in combination. This result remains unchanged when age, HRT use, and menopause status are adjusted[269]. Another study of a similar population of postmenopausal women analyzed the polymorphisms of Cdx2, Taq1, Fok1, and VDR-5132. There was, however, an effect modification related to ER status of the tumor, such that carrying the allele t of Taq I polymorphic variation resulted in a substantially elevated cancer risk in breasts when contrary to non-carriage only for positively affected by ER tumors[270]. A case-control study demonstrated that alleles possessing the 'f' allele on Fok1 polymorphisms affect estrogen receptor+ve growth of cancer among Saudis female patients[161].

A some other retrospective, cohort case-control study conducted on premenopausal Pakistani females investigated the Cdx2 VDR polymorphism and ruled out that additional factors that affect the development of cancerous breasts, for example, tumor grading, ER or PR stature, and HER2/ Neu stature, have been determined and there are differences among cases. The Cdx2 GG genotype was observed slightly commonly seen in low-grade and ER+ve patients, but the findings were not statistically significant[186]. A study done on African- Egyptian females with breast cancer confers that neither ER nor PR expression in the tumor, stage of the

tumor, or invasiveness of lymph nodes were associated with any VDR genotype (BsmI, ApaI, and TaqI)[176]. The study included a case-control design as well was conducted on Iranian females with germline mutations of BRCA1/2 and found no association between VDR FokI and ApaI SNPs and histopathological characteristics of breast tumors[178].

However, a study was conducted on Pakistani breast cancerous patients to investigate vitamin D concentration and the genotypic frequency of vitamin D receptors single nucleotide polymorphisms (SNPs) FokI and TaqI alongside different clinicopathological features. The results indicate a substantial connection for the FokI SNP and tumors type (p-value 0.012) but not with TaqI SNP (p-value 0.188). ER or PR status and tumor grade were both significantly associated with FokI and TaqI SNPs (p-value 0.001), and the FokI genotype a significant relationship existed for Her2 status (p-value 0.001), whereas the TaqI SNP showed no statistical significance was found (p-value 0.221)[271]. Similarly, another recent study done on the same population reveals that a statistically relevant association was not indicated between VDR TaqI, BsmI genotypes, and expressions of ER, PR, and Her2/Neu and clinicopathological following factors, notably the size of the tumors, types, grades, lymph nodes, and distal sites metastases[272].

Hence, because of a lack of power and a smaller sample size, fewer studies examined the hormonal receptor's stature in assessing the potential associations between polymorphisms in VDR and cancer of the breast. Further research in larger Asian populations are warranted to support these findings.