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## **Detection the genetic variation of vitamin D binding protein (VDBP) gene in women with osteoporosis in Mosul City**

**Samaa M. AL. Sael**

Mosul university \ Science College \ Biology Department

Email: [samalsial96@gmail.com](mailto:samalsial96@gmail.com)

**Owayes M. Hamed**

Mosul university \ Science College \ Biology Department

Corresponding author email: [owsbio31@uomosul.edu.iq](mailto:owsbio31@uomosul.edu.iq)

**Abstract**--The VDBP amino acid sequence is divided into three domains and has 458 amino acids. Exon 11 in domain III has two SNPs, rs7041 and rs4588, each corresponding to one of the major VDBP kinds (VDBP1S, VDBP1F, and VDBP2). Transport of vitamin D3 to the liver and 25(O.H.)D to the kidney, 1,25(O.H.)2D transport to target organs is mediated by vitamin D-binding protein, which interacts with a particular vitamin D receptor (VDR) to explain biological activities. This study aims to determine whether the VDBP gene has three polymorphisms (rs17467825, rs7041, and rs4588) associated with women with osteoporosis in Mosul city. This study included (96) women, the age range between (35-55) years. The samples were divided into two groups, the first group included (74) women with osteoporosis, and the second group was considered a control group. In this study, DNA was extracted from the blood of all the samples included in the study (95) samples, using the method modified by the researchers. And detection of three different SNP of VDBP, (rs17467825) Polymorphism by ARMS-PCR, and (rs7041T) Polymorphism with (rs4588C) Polymorphism by RFLP-PCR. This study showed a connection between deficiency of vitamin D in women and mutation of the VDBP gene in location rs17467825. Also, the result showed three different genotypes of the VDBP gene. Still, in the different ratios, A.A. 16%, AG 56%, and G.G. 28%, The result of the study also established a link between vitamin D deficiency in women and mutation of the VDBP gene in location rs7041T. Also, the results show three different genotypes of the VDBP gene. Still, in different ratios, TT28%, T.G. 36%, and GG36%, The study's findings revealed a link between vitamin D deficiency and mutation of the

VDBP gene in location rs4588C. Also, the result shows three different genotypes of the VDBP gene but in different ration CC40 %, CA 47%, and AA13%. Conclusion, according to this study, vitamin D binding protein genes have the SNPs rs70141657 A/G, rs7041T/G, and rs4588C/A were associated with a greater incidence of vitamin D insufficiency in otherwise healthy Mosul women.

**Keywords**--VDBP gene, Allele-specific PCR, polymorphism, osteoporosis.

## Introduction

Vitamin D is necessary for calcium homeostasis in the body, which is regulated by the endocrine system (Fleet et al., 2017).and bone mineralization in conjunction with parathyroid hormone (Holick et al., 2006 ). Vitamin D is a fat-soluble secosteroid that has a role in bone metabolism and other biological processes (Almesri et al., 2013). Vitamin D contains biological functions such as calcium homeostasis and various non-classical acts, which are divided into three categories: hormone secretion regulation, cellular regulation, and hormone secretion regulation. Immune function proliferation, differentiation, and regulation (Bikle et al., 2009). Vitamin D is involved in innate and adaptive immunity and bile acid detoxification (Schmidt et al., 2010). Variable kinds of immune cells, such as macrophages, monocytes, lymphocytes, and dendritic cells, have different degrees of expression of the vitamin D receptor (VDR), enzymes, and metabolites (Adams et al., 2014 and Battault et al., 2013). Vitamin D is a steroid hormone that controls the expression of several genes involved in cell activation, differentiation, and proliferation. The active form of VDBP may interfere with differentiation and maintenance of balance of regulatory cells by suppressing of regulatory cells by inhibiting interferon and interleukin 2 (Aktürk et al., 2019). Humans can make VD by consuming it through their diets and supplements and exposure to UVB radiation (Pludowski et al., 2018). D2 (ergocalciferol) is a steroid hormone present in plants but mostly in yeasts and fungus, and D3 (cholecalciferol), which is found in animals, are the two principal forms of the vitamin (Al Mheid et al., 2017 and Jäpelt et al., 2013). Supplementation with vitamin D can be a good source of vitamin D in people. The skin is exposed to UVB; vitamin D3 is generated and manufactured endogenously (Kennel et al., 2010). Transform the 7-DHC to pre-vitamin D3 when exposed to solar UVB, in the cell membrane, which is subsequently thermally isomerized to cholecalciferol (Pludowski et al., 2018). The vitamin D binding protein (VDBP) transports cholecalciferol to the liver, where it is to hydroxylated to in active [25(O.H.)D3] 25-hydroxyVitamin D (O.H.) D3 is the most common vitamin D circulating metabolite, which is hydroxylated by 1-alpha-hydroxylase in the kidney to form D2 (calcitriol)1,25-dihydroxy vitamin D [1,25(O.H.)2D3] the active form which is hydroxylated by 1-alpha-hydroxylase (Bikle et al., 2014). Vitamin D deficiency has been related to cancer, improper bone mineralization, multiple sclerosis diabetes, tuberculosis, rheumatoid arthritis, and cardiovascular disease (Al Mheid et al., 2017). In T.B. patients, low levels of 25-hydroxyvitamin D3 (25(O.H.)D3) have been regularly reported when compared to controls (Garland et

al., 2006). The role of vitamin D in cell growth regulation has prompted much research into the connection between vitamin D and cancers, particularly prostate and colorectal cancers (Aktürk et al., 2019). Because it binds vitamin D and is a serum 2-globulin with a molecular weight of 52–59 kDa, it was named a "group-specific component (Battault et al., 2013). The albumin-binding protein VDBP belongs to the albumin-binding protein family. (Albumin, alpha-albumin/afamin, alfa-fetoprotein). They manifest themselves in the liver (Aktürk et al., 2019). The VDBP amino acid sequence is divided into three domains and has 458 amino acids (Bikle et al., 2009). rs7041 and rs4588 are SNPs in domain III's exon 11 that relate to the three principal VDBP kinds (VDBP1F, VDBP1S, and VDBP2) (Almesri et al., 2013). Vitamin D3 transport to the liver, 25(O.H.)D transfer to the kidneys, and 1,25(O.H.)<sub>2</sub>D transport to the target organs is mediated by VDBP, which interacts with a particular vitamin D receptor (VDR) to explain biological activities (Jäpelt et al., 2013). In humans, VDBP is known to mediate various biological processes, including chemotaxis, osteoclast activation, and fatty acid transport (Adams et al., 2014). It works by interacting with surface immunoglobulins and is found in the cerebrospinal fluid, plasma, and on the membranes of B lymphocytes. Vitamin D binding protein has several activities, including neutrophilic responses, macrophage activation (macrophage activating factor-MAF), and binding to and transporting metabolites. VDBP affects the density of inflammatory reactions in a significant way (Gorham et al., 2005). VDBP has a different range of biological processes, including all vitamin D metabolites that are bound and transported, binding of fatty acids and binding of actin monomers (Holick et al., 2005), leucocyte proteoglycans, complement C5 system activation, binding to membranes as well as Depolymerization of extracellular actin filaments is a significant function of this protein (Al Mheid et al., 2017). The VDBP gene is found on chromosome 4's long arm (4q12-q13) (35 kilobytes of DNA with 13 exons and 12 introns). A mixture of two SNPs, rs7041 and rs4588, results in three different phenotypic alleles (Gc1f, Gc1s, and Gc20). Six distinct phenotypes result from combining these three genes (Holick 2007). The three distinct phenotypic alleles (Gc1f, Gc1s, and Gc20) are caused by a combination of two SNPs in the VDBP gene (rs7041 and rs4588) (Lappe et al., 2009). The role of VDBP gene polymorphisms in determining plasma 25(O.H.)D levels have been studied (Liu et al., 2006). The VDBP gene has a lot of polymorphism and encodes VDBP. The affinity of polymorphic VDBP proteins for the 1,25(O.H.)<sub>2</sub>D metabolite varies. rs4588 (Thr420Lys) and rs7041 (Asp416Glu) are two coding single-nucleotide polymorphisms are on the way. that are linked to circulating vitamin D levels. Furthermore, the two SNPs, rs4588 and rs7041, make three different combinations (Gc1f, Gc1s, and Gc2). Vitamin D metabolites have different binding affinities are provided by these variations. The greatest affinity was discovered in Gc1f, followed by Gc1s and Gc2 (Manousaki and Richards 2017). VDBP SNPs that impact vitamin D levels in the blood plasma may cause changes in blood plasma vitamin D levels, according to a previous research (Garland et a., 2006). Several studies have looked at the possible link between these two SNPs and autoimmune disorders such as osteoporosis, Grave's disease, and diabetes type 2. However, there are scant data on M.S. Also, VDBP polymorphisms in both SNPs rs7041 and rs4588 are strongly linked to 25(O.H.)D status, especially when a large amount of vitamin D must be transported (Dimitrakopoulou et al., 2017). A relationship between SNP rs7041 and breast cancer was discovered in another investigation (Skaaby et al., 2017). Because the

VDBP gene is expressed poorly or not in lung cancer tissue, there is a link between SNP rs7041 in the VDBP gene and a lower risk of Non-Small Cell Lung Cancer (NSCLC) . According to a study, decreased VDBP levels in the blood may predict lung cancer mortality. Yan et al. discovered a link between the rs2282679 SNP and Rheumatoid Arthritis (Kheiri et al., 2018) in a research, the primary genetic components; Variations in 25(O.H.)D status and VDBP levels are linked to the genes rs4588 and rs7041, and The genetic influence might possibly be linked to a specific pathogenic pathway, including vitamin D (Wenli et al., 2016).

The study aims to:

- 1- This study aimed to see if there was a link between VDBP gene polymorphisms in three SNPs (rs17467825, rs7041, and rs4588) with osteoporosis in Mosul women.

## Material and Method

### Case study

The current study included (95) samples collected from women with a deficiency in vitamin D or osteoporosis, and we divided the sample into (74) samples from women that have a problem with Vitamin D and (21) present as a control group .in the same age category.

### Collection of Blood sample

(5.0) ml from the venous blood from the woman under study and divided into to tow parts, the first part was placed in tubes containing anticoagulant substance EDTA, which was used to extract genomic DNA. and the second part was placed in gel tubes was centrifuged for (10) ten minutes at speed (3000) rpm. For purpose of obtaining blood plasma on which biochemical tests performed

### DNA Extraction

DNA was extracted from the blood of all the sample include in the study, which are (95) samples, using the method modified by the researchers (Iranpur and Esmailzadeh 2010).

### Genotyping

#### Detection of (rs17467825) Polymorphism by ARMS-PCR

to amplify 100 ng of template using the ARMS-PCR system The following primer sequences were used (Wenli et al., 2016):

primer	Sequence	Band size	Annealing
F-outer	TGAGATCAGCTATGGTTGACAGTAAATT	460 bp	59
R-outer	CTGGTCCATTTTGGTAAAGTATTTTCAT		
F-inner	TCTGT CAGCGATTCTTAATATAAGAAACAG	376 bp	
R-inner	CAGCACACTCTAAACACATTTCAAT	248 bp	

### Detection of (rs7041T) Polymorphism by RFLP-PCR

to amplify 100 ng of template using the RFLP-PCR system The following primer sequences were used (Zainab et al., 2015):

Primer	Sequence	Band size	Annealing
Forward	AAATAATGAGCAAATGAAAGAAGA	483 bp	55
Reverse	CAATAACAGCAAAGAAATGAGTAGA		

Then the PCR product digested with *HaeIII* restriction enzyme into two fragment, 360 bp and 220 bp.

### Detection of (rs4588C) Polymorphism by RFLP-PCR

to amplify 100 ng of template using the RFLP-PCR system The following primer sequences were used (Zainab et al., 2015):

Primer	Sequence	Band size	Annealing
Forward	AAATAATGAGCAAATGAAAGAAGA	483 bp	55
Reverse	CAATAACAGCAAAGAAATGAGTAGA		

Then the PCR product digested with *StyI* restriction enzyme into two fragment, 305 bp and 178 bp.

## Result and Discussion

### Determination of genetic variance of rs17467825

The findings of the investigation revealed a correlation between vitamin D deficiency and a mutation in the clock gene at rs17467825, as indicated when observing in Fig. 1 and the PCR product has a three bands (460 for gene target, 376 for wild allele and for mutant 248 bp),

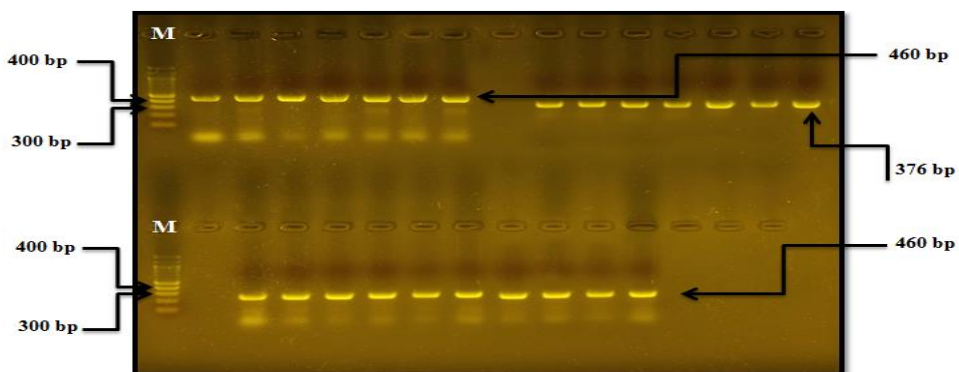


Fig (1): also, the result showed three different genotypes of VDBP gene but in different ration A.A. 16%, AG 56% and G.G. 28%, table 1

Table 1: Distribution of polymorphism for allele and genotype frequency of rs17467825 for VDBP gene

Genotypes	Patients		Control		P Value	OR	(95%CI)
	NO.	%	NO.	%			
AA	12	16	18	85	P = 0.0018	30.000	3.539 to 25 4.247
AG	42	56	2	10			
GG	20	28	1	5			
Alleles	NO.	%	NO.	%	P Value	OR	(95%CI)
A	66	44	38	90	P < 0.0001	11.803	4.0080 to 3 4.7585
G	82	56	4	10			

In addition to this study, also be found the value of odd ration is (30) that is more 1, thus, it is considered a risk factor for this disease in  $P=0.0018$ . On another hand the result showed for observation of allele, the wild allele A existence 90% in healthy women and 44% in patients, and mutant allele G existence 10% in healthy women and 56% in patients, and the odd ration for allele be 11.803 like that considered a risk factor for disease.

#### Determiration of genetic variance of the rs7041T

The findings of the investigation revealed a correlation between vitamin D deficiency and a mutation in the VDBP gene at rs7041T, as indicated when observing in Fig. 2 the PCR product 483 bp and after digestion by HAEIII enzyme has two bands (220,360bp).

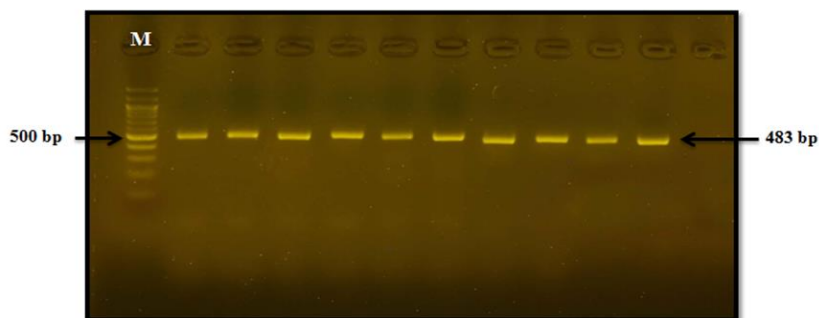


Fig. (2): PCR product for VDBP gene with 483 bp, M ladder wech separated with 2% agarose gel electrophoresis.

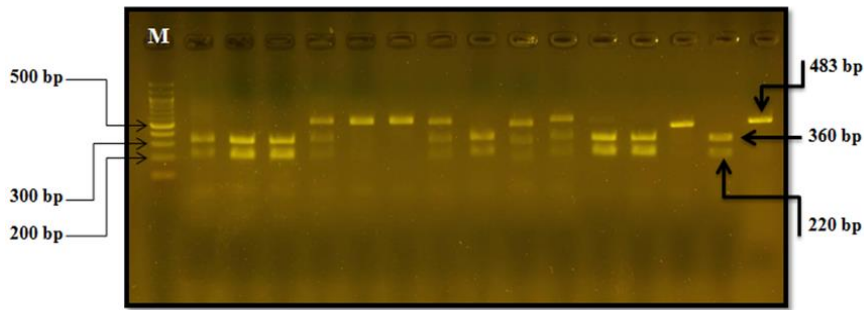


Fig. (3): detection of rs7401 mutation by RFLP-PCR is digested by HaeIII, M ladder, lane (5,6,13,15) have wild genotype T.T. with PCR product 483 bp, (4,7,9,10) have heterozygote T.G. with three bands of PCR product 483, 360, 220, and (1,2,3,8,11,12,14) have mutant genotype G.G. with PCR product have two bands 360 bp and 220 bp, with separated by 2 % agarose gel electrophoresis. Also the result show three different genotype of VDBP gene but in different ration TT28%, T.G. 36%, GG36%, table 2

Table 2: Distribution of polymorphism for allele and genotype frequency of rs7401 for VDBP gene

Genotypes	Patients		Control		P Value	OR	(95%CI)
	NO.	%	NO.	%			
TT	20	28	4	19	P = 0.2776	2.7000	0.4494 to 16.2233
TG	27	36	15	71			
GG	27	36	2	10			
Alleles	NO.	%	NO.	%	P Value	OR	(95%CI)
T	67	45	23	55	P = 0.2783	1.4635	0.7352 to 2.9133
G	81	55	19	45			

In addition to this study, also be found the value of Odd ration be (2.7000) that is more 1, thus, it is considered a risk factor for this disease in P=0.2776. On another hand the result showed for observation of allele, the wild allele T existence 55% in healthy women and 45% in patients, and mutant allele G existence 45 % in healthy women and 55% in patients, and the odd ration for allele be 1,4635 like that considered a risk factor for disease.

#### Determination of genetic variance of the rs4588C

The findings of the investigation revealed a correlation between vitamin D deficiency and a mutation in the VDBP gene at rs4588C, as indicated when observing in Fig. 4 the PCR product has two bands (178, 305 bp).

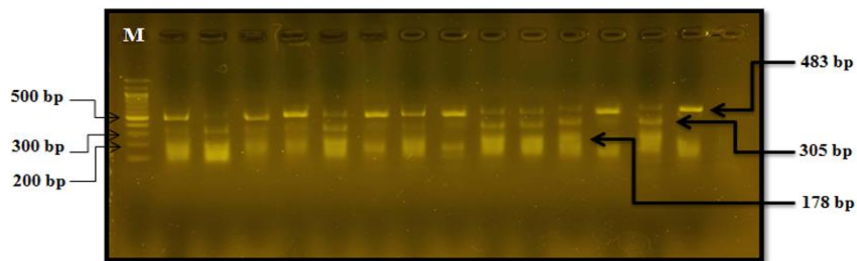


Fig. (4): detection of rs4588C mutation by RFLP-PCR, M ladder, lane (1,4,6,8,12,14) have wild genotype CC with PCR product 483 bp, (5,9,10,11,13) have heterozygous C.A. with three bands of PCR product 483, 305,178 bp, and (2,3,7) have mutant genotype A.A. with PCR product have two bands 305 bp and 178 bp, with separated by 2 % agarose gel electrophoresis.

Also the result show three different genotype of clock gene but in different ration CC40 %, CA 47%, AA13%, table 3

Table 3: Distribution of polymorphism for allele and genotype frequency of rs4588C for VDBP gene

Genotypes	Patients		Control		P Value	OR	(95%CI)
	NO.	%	NO.	%			
CC	30	40	3	14	P = 0.0804	0.2250	0.0423 to 1.1977
CA	35	47	14	66			
AA	9	13	4	20			
Alleles	NO.	%	NO.	%	P Value	OR	(95%CI)
C	95	65	20	47	P = 0.0547	0.5072	0.2537 to 1.0137
A	53	35	22	53			

Addition to this study, also be found the value of Odd ration be (0,2250) that is less than 1. Thus, it is didn't considered a risk factor for this disease in P=0,0804. On the other hand, the result showed for observation of alleles, the wild allele C existence 47% in healthy women and 65% in patients, and mutant allele A existence 53 % in healthy women and 35% in patients, and the odds ratio for allele be 0,5072 it is didn't considered a risk factor for this disease.

## Discussion

In Iraq, polymorphisms had never been investigated before. This study aimed to examine the relationship between vitamin D status and various genetic polymorphisms such as rs17467825, rs7041, and rs4588 on the VDBP gene, which codes for VDBP and vitamin D levels in osteoporotic women in Mosul. VDBP polymorphism has been linked to various diseases, including diabetes, obesity, osteoporosis, cardiovascular disease, respiratory diseases, and pregnancy complications. according to recent research, Compared to controls, women with osteoporosis had decreased total serum 25(O.H.)D concentrations, according to our findings (Lucato et al., 2017). Another research found no significant differences between patients with osteoporosis and healthy controls in serum bioavailable or free 25(O.H.)D levels. vitamin D and VDBP have a significant influence on human health (Manousaki and Richards 2017). Polymorphisms in

the VDBP gene have been linked to a variety of illnesses, including osteoporosis and cancer (Holick et al., 2005). To the best of this study's knowledge, only two SNPs have been investigated in relation to the VDBP gene and osteoporosis in women (Kheiri et al., 2018). Because severe periodontitis has high family aggregation, recent disease investigations have focused on single nucleotide polymorphisms (SNPs), suggesting that genetic predisposition may be an essential etiological element (Pludowski et al., 2018). Most SNP genotyping techniques rely almost entirely on PCR amplification of the target DNA sequence, but they differ in how they distinguish between alleles. SNPs are found throughout the genome, and their high levels of variation make them important genetic markers for disease vulnerability (Al Mheid et al., 2017). The VDBP gene's rs17467825 is found in the 3-UTR region. These SNPs with functional differences may be in significant linkage disequilibrium (L.D.) that impacts DBP mRNA stability. These three locations have been linked to bone mineral density, obesity, and 25-hydroxyvitamin D levels, all of which are critical contributors to periodontitis development. Xiong et al. genotyped 1873 participants from 405 white nuclear families across 20 genes and observed a connection between VDBP rs17467825 and spine bone mineral density (Manousaki and Richards 2017). In Arab Asians, individuals with rs17467825 G.G. have the lowest 25-hydroxyvitamin D concentration (Bikle et al., 2009). Suaini et al. discovered found the minor allele rs17467825 was connected to a greater risk of vitamin D deficiency (Dimitrakopoulou et al., 2017). According to the same study, this site showed the strongest link to fat mass percentage. This study has investigated the importance of genetic polymorphisms of VDBP (rs17467825, rs7041, rs4588C) in women with osteoporosis (Wenli et al., 2016). In this study, only two SNPs have investigated the association between VDBP gene and women with osteoporosis. the most comprehensive understanding of in this work, we discovered that rs4588C is statistically linked to a decreased risk of osteoporosis in general in the recessive model, although the genotype distributions of rs17467825 and rs7041 are not verified to be associated with generalized osteoporosis. The nonsynonymous SNPs rs7041 and rs4588 were shown to have a high correlation with the vitamin D status of the healthy participants in the research population. Serum concentrations of 25-(O.H.) V.D. are low were linked to the T allele of rs7041 and the A allele of rs4588 (p 0.05).

### **Conclusion**

According to this study, vitamin D binding protein gene have the SNPs rs70141657 A/G, rs7041T/G, and rs4588C/A were associated to a greater incidence of vitamin D insufficiency in otherwise healthy Mosul women.

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