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## Molecular and Biochemical Study for (VDBP) gene in women Suffered with osteoporosis in Mosul City


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**Abstract:**
An The GC gene encodes the protein VDBP, which plays an important role in the transport of vitamin D. This protein belongs to the albumin family. The GC gene is located on the long arm of chromosome 4 (4q12-q13), which consists of 1690 nucleotides, and the GC gene has many genetic variations that are related to variation Single nucleotide SNPs and the proteins resulting from these variations differ in their ability to bind with VD and its different forms. due to the presence of two genetic variations, rs7041 and rs4588 for VDBP, three phenotypes were identified which are Gc1s, Gc1f and Gc2, which differ in their ability to bind to the inactive form of vitamin D 25(OH)D3. It has been proven that the variants in this gene change the concentrations of OH-D325 in the plasma, so the defect in these variants of the VDBP gene is closely related to many diseases, including VD deficiency and cancer. The current study included (96) women with ages ranging between (45-35) years who were referred to the private pathological analysis laboratories in the city of Mosul in a period of time that ranged from September to November of 2021. The samples were divided into two groups, the first included 25 women who returned as a control group. The second included 71 women with VD deficiency. The concentration of vitamin D (OH) D325 in the serum is widely used to determine the deficiency in the level of VDBP.



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### Introduction

Vitamin D is a type of steroid hormone with important regulatory functions. It plays an important role in which numerous genes involved in cell differentiation, activation, and proliferation are regulated [1]. Also, VD receptors in the nucleus and cytoplasm are essential for VD to function in cells [2]. VD is found in two main forms: Ergocalciferol (C28H44O) (VD2) which is synthesized in fungi, yeast and plants, and Cholecalciferol (C27H44O) (VD3) which is synthesized in humans in the skin after exposure to sunlight and UVB rays [3]. The metabolic pathway of VD is affected by the genetic variation of a number of genes and thus affects the concentration and function of VD [4]. Studies have indicated that there are genetic variations associated with the concentration of VD and calcium [5]. Genes responsible for encoding proteins that make, transport and activate VD are also associated with changes in the level of VD, including the gene specific to the protein GC. In 1959 Hirschfield named Vitamin D binding protein (VDBP) as a "group-specific component" (GC) after it was isolated. From the

α2-globulin fraction of plasma [6]. It is named VDBP because it is responsible for binding and transporting VD, The GC gene consists of 1690 nucleotides present on chromosome 4 (4q11-q13) and is responsible for encoding the protein VDBP. The most common SNP variations for this gene are rs7041 (c.1296T>G encoding D432E) and rs4588 (c.1307C>A encoding T436K) [7]. The genetic variations of rs7041 and rs4588 are the most common variants of the VD-associated protein gene that affect protein stability. These multiple genetic variances differ in their significance with the active form of vitamin D, 1,25 (OH) 2 D3 [8]. VDBP belongs to a family of proteins (albumin) that are activated in the liver [9]. This VD-binding protein has the ability to bind to the different VD forms, which are VD2 and VD3, where it is transferred to the target tissue [10]. The concentration of vitamin D (OH) D325 in the serum is widely used to determine the deficiency in the level of VDBP [11]. VDBP is essential for the transport of VD throughout the body. In addition to its role in other functions, including transporting fatty acids and stimulating immunity [12]. Studies have indicated that defects in the VDBP gene are linked to a number of diseases, including chronic diseases, and that genetic variations in the forms of the VDBP gene are considered to be one of the causes of these diseases in addition to cancer [13]. The rs17467825 genotype is present in the 3′-UTR region of the VDBP gene. Studies have indicated the negative impact of this genotype on mRNA stability. The mutant allele rs17467825 was found to be associated with an increased risk [14]. Due to the presence of two genetic variations, rs7041 and rs4588 for VDBP, three phenotypes were identified which are Gc1s, Gc1f and Gc2, which differ in their ability to bind to the inactive form of vitamin D 25(OH)D3 [15]. Studies have indicated that the three genetic variations are associated with calcium deficiency and VD concentration in blood serum [16]. Only one gene is responsible for encoding VDR, the VDR gene is located on chromosome 12 (12q13.11) and it has been reported that there are more than 900 variants in the VDR locus [17]. Genetic and environmental factors are responsible for determining the regulation of VDR [18]. Environmental factors include exposure to sunlight, diet, infection, and pollution. Studies have indicated that the response to VD supplementation varies between individuals [19]. Studies have shown that the level of VD-binding protein increases significantly during pregnancy [20].

## Material and methods

### Case Study:

This study included (96) women of an age group ranging from (45-35) years of reviews to the private pathological analysis laboratories in mosul, Iraq for a period ranging from period ranging from August to October of 2021, and it was relied on these clinical cases of the disease to choose Samples. The samples were separated into two groups, based on the biochemical results: The first group consisted of 25 women who did not have any health problems and served as a control group. The second group: This group included 71 women from among the women with VD deficiency, based on biochemical results. (5.0)ml of venous blood was drawn from these women and divided into two parts, the first part was placed in tubes containing EDTA anticoagulant, and the second part was placed in tubes free of any anticoagulant.

### Blood Sample Collection:

(0.5) ml of vein blood was obtained from each of these women and divided into two groups; the first group was placed in tubes containing EDTA anticoagulant to DNA extract, while the second group was put in tubes without any anticoagulant. The tubes were left for one hour until the blood clotted, after which a centrifugation was carried out for a period of (10) ten minutes at a speed of (3000) cycles/minute to obtain the blood serum on which the biochemical tests were conducted.

### Collection of Blood sample:

DNA was extracted from the blood of (96) women who were subjected to this study, using the modified method presented by [21].

### Genotyping:

### Tetra–ARMS-PCR Reactions:

The concentration of DNA in all research samples is adjusted after being measured by biodrop by diluting them with TE buffer solution to get the concentration necessary for PCR reactions and was (25) ng/microliter for every sample

Adding Four primers for every primer reaction (F-outer and R-outer) for the whole gene, forward outer-reverse inner for the normal allele, forward outer-reverse inner) for the mutant allele.

The PCR reaction mixture is prepared by mixing the nucleic acid of each sample and the primer designated for the mutations under study Using the master-mix ingredients in a 0.2-ml tube for PCR produced by the English by Biolaps Company. Mix in the Microfuge for 5-3 seconds to ensure that all of the reaction components are combined. The PCR tubes were then placed in the thermocycler. within the special program for each mutation, then the reaction product is injected at 2% concentration into the pits of the prepared agarose gel, with the inclusion of Biolaps Company's Ladder DNA in one of the initial pits, following which the samples are migrated Running the electrophoresis equipment for 45 minutes, after which the bands are imaged using a gel-documentation device.

**Determination of the genetic variation of the VDBP gene at the locus (rs17467825) using Tetra–ARMS-PCR technology**

The presence of the A G mutation was detected at the site (rs17467825) by adding 4 µl (100 nanogram) from the DNA template and 1 µl (10 picompl) of every mutation specific primer (rs17467825), which was designed by the researcher using Pimer 3 software and used for the first time on this gene, It was prepared by a Korean macrogen firm and added to the master mix's ingredients. The final reaction volume was 20 µl [22].

**Table 1:** demonstrates the primers used to determine genetic variation at the locus (rs17467825) using PCR technology.

| Primer | Sequence | Band size | Annealing |
|---|---|---|---|
| F-outer | TGAGATCAGCTATGGTTGACAGTAAATT | 460 bp | 59 |
| R-outer | CTGGTCCATTTTGGTAAAGTATTTTCAT | | |
| F-inner | TCTGTCAGCGATTCTTAATATAAGAAACAG | 376 bp | |
| R-inner | CAGCACACTCTAAACACATTTCACAAT | 248 bp | |

The reaction tubes were then placed in the thermocycler to perform the multiplication reaction using the reaction's unique program as shown in Table 2:

**Table 2:** Shows the program adopted in the ARMS-PCR technique to identify the mutation (rs17467825)**.**

| No. | Stage | Temperature | Time | Cycle number |
|---|---|---|---|---|
| 1 | Initial denaturation | 95.0. | 5.0. min | 1 |
| 2 | Denaturation | 95.0. | 45.0. sec | 35 |
| 3 | Annealing | 59.0. | 1.0. min | |
| 4 | Extension | 72.0. | 1.0. min | |
| 5 | Final extension | 72.0. | 7.0. min | 1 |
| 6 | Stop reaction | 4.0. | 5.0. min | 1 |

The optimal temperature for the primer bonding in this reaction was determined using the Gradient program on the thermocycler device; the gradient was (5) and the mean temperature was (59) C. The temperature of 59°C was used since it gave the best results. Then the PCR reaction was separated by 2% agarose gel**.**

**PCR-RFLP Reactions:**

After being measured by biodrop, the concentration of DNA in all research samples is adjusted by diluting them using the TE buffer solution to acquire the needed concentration for performing PCR reactions, which was (25) ng/microliter for every sample.

In a 0.2-ml tube of PCR developed by the English Biolaps Company, the DNA of each sample and the primer chosen for the mutations under research are mixed with the components of the master-mix. Mix in the Microfuge for 5–3 seconds to ensure that the reaction components are combined. The PCR tubes were then placed in the thermocycler. within the special program for each mutation After that, the PCR reaction product is incubated with the enzyme trimer for each mutation for 3 hours, and then the sample is placed in pits of a pre-prepared agarose gel at a concentration of 2% with Ladder DNA supplied by Biolaps Company, the samples are then moved using an electrophoresis apparatus. Duration (45) minutes, and finally photographing the gel using the Gel documentation.

**Detection of the genetic variation (rs7041T) of the VDBP gene:**

The presence of the T G mutation was detected at the site (rs7041T) by adding 4 µl (100 Nanogram) from the DNA template and 1 µl (10 picompl) of every mutation specific primer (rs7041T), It was prepared by a Korean macrogen firm and added to the master mix's ingredients [23].

**Table 3:** Shows the primers used to determine genetic variation at the locus (rs7041T) using RFLP-PCR.

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

The PCR tubes are then placed in a thermocycler to perform the polymerase chain reaction, depending on the special program for the reaction, as shown

One cycle of (5) minutes at a temperature of (95) °C for primer denaturation of the DNA strand Each doubling cycle includes (33) cycles, as shown:

(1) minute at (55) °C for initiator binding to template DNA, (45) seconds at (95) °C for double strand denaturation, and (1) minute at (72) °C for primer elongation.

Following the conclusion of the reaction, the tubes were removed from the thermopolymer device with a PCR yield of 286 bp, and (5) microliters were withdrawn and incubated with the trimer enzyme (Hae III) at a concentration of 5 units supplied by Biolaps Company for (3) hours at (37) C.

**Detection of the genetic variation (rs4588C) of the VDBP gene:**

The presence of the C A mutation was detected at the site (rs4588C) by adding 4 µl (100 nanogram) from the DNA template and 1 µl (10 picompl) of every mutation specific primer (rs4588C), It was prepared by a Korean macrogen firm and added to the master mix's ingredients [24].

**Table 4:** Shows the primers used to determine genetic variation at the locus (rs4588C) using RFLP-PCR.

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

The PCR tubes are then placed in a thermocycler to perform the polymerase chain reaction, depending on the special program for the reaction, as shown

One cycle of (5) minutes at a temperature of (95) °C for primer denaturation of the DNA strand Each doubling cycle includes (33) cycles, as shown:

(1) minute at (55) °C for initiator binding to template DNA (45) seconds at (95) °C for double strand denaturation, and (1) minute at (72) °C for primer elongation.

Following the conclusion of the reaction, The tubes were taken out from the thermopolymer device with a PCR yield of 286 bp, (5) microliters were withdrawn and incubated in the interrupting enzyme (StyI) at a concentration of 5 units provided by Biolaps Company for (3) hours at (37)C.

**Using DNA sequencing technology, determine the nucleotide sequence of amplified pieces:**

The nitrogenous bases' sequence of the gene was determined for the VDBP samples that were included in the study for the purpose of verifying the validity of the designed primer that was used in the ARMS-PCR technique and for the purpose of detecting the presence of any additional discrepancies in the gene. These genes were extracted using a 3130 Genetic Analyzer from the Japanese company Hitachi. These gene sequences were compared to those in the National Center for Biotechnology Information NCBI, and the results were processed using the BLAST tool.

**Table 5:** shows the primers belonging to the VDBP gene and on which the DNA sequencing test was conducted.

| | Primer | Sequence |
|---|---|---|
| VDBP | Forward | 5′ TGAGATCAGCTATGGTTGACAGTAAATT 3′ |
| | Reverse | 5′ CTGGTCCATTTTGGTAAAGTATTTTCAT 3′ |

**Results and discussion**

**Determination of the genetic variation of the VDBP gene at the locus (rs17467825) using Tetra–ARMS-PCR technique**

The results showed, as in Figure (1), the existence of a relationship between women suffering from VD deficiency and the genetic variance of the VDBP gene at the site (rs17467825). As shown in Table

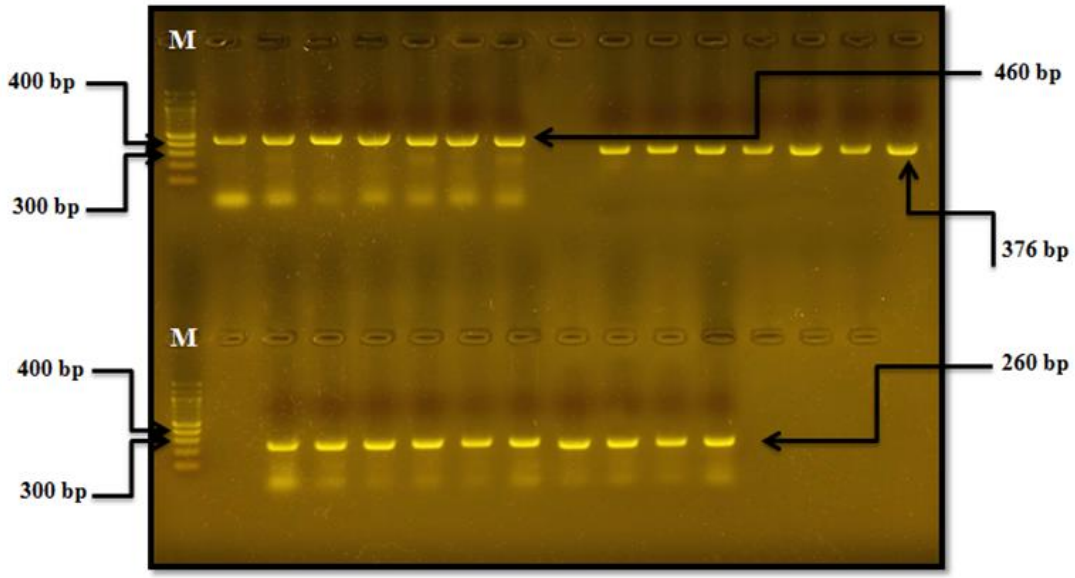



**Figure 1** the existence of a relationship between women suffering from VD deficiency and the genetic variance of the VDBP gene at the site (rs17467825).

**Table (6):** Distribution of the percentage of allelic observations and genotypes of the VDBP gene at the locus (rs17467825) in a group of women that don't have health problems and women with VD deficiency, knowing that the normal allele is the A allele and the mutant allele is the G allele:

| Genotypes | Patients | | Control | | P Value | OR | (95%Cl) |
|---|---|---|---|---|---|---|---|
| | NO. | % | NO. | % | | | |
| AA | 12 | 16 | 18 | 85 | P = 0.0018 | 30.000 | 3.539 to 254.247 |
| AG | 42 | 56 | 2 | 10 | | | |
| GG | 20 | 28 | 1 | 5 | | | |
| Alleles | NO | % | NO | % | P Value | OR | (95%Cl) |
| A | 66 | 44 | 38 | 90 | P < 0.0001 | 11.803 | 4.0080 to 34.7585 |
| G | 82 | 56 | 4 | 4 | | | |

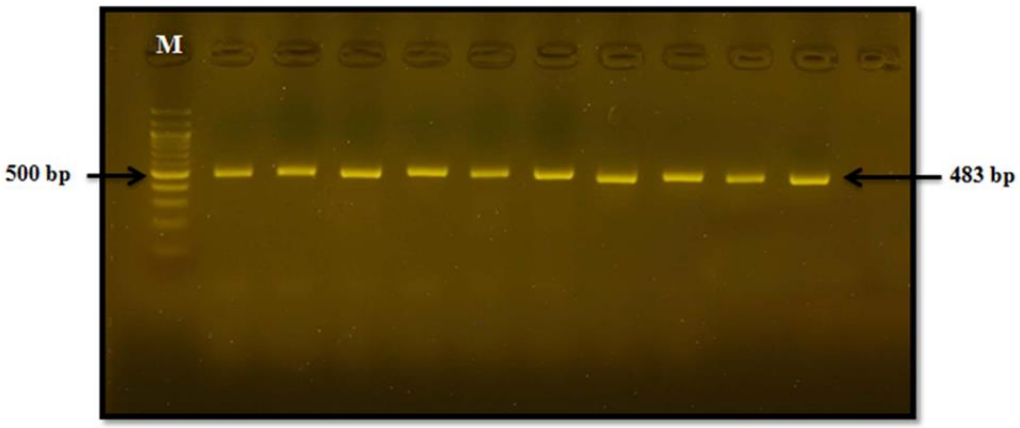
The frequency of different genotypes of the VDBP gene at the locus and the proportion of allelic sightings (rs17467825). The findings for women with VD insufficiency revealed that the value of repeating the abnormal genotype (mutant) GG in the group of women with VD deficiency with a percentage of 28% is the highest In comparison with the control group in the genotype. By 5%, whereas the value of the healthy genotype AA was the lowest between the women with VD insufficiency by 16% In comparison with the healthy (normal) genotype was higher than 85%, while for the heterogeneous genotype AG in the group of women The injured 56% had a higher viewing rate in comparison with the 10% control group In terms of allelic recurrence, the data showed that 56% of patients had the mutant G allele, compared to 4% in the control group, and 44% of patients had the normal allele, compared to 90% in the control group.

Since VD plays a critical role in calcium balance in the whole body along with the thyroid gland in bone mineralization, therefore, mutations in the VDBP gene lead to the loss of these functions. As it is known, VD plays an important role in the human metabolism process, and in controlling the balance of calcium and phosphorous. VDBP binds to and shapes VD and transports it to target tissues. The VDBP gene's genetic variant leads to the synthesis of a protein that is unable to transfer VD to the body tissues and to precipitate the occurrence of physiological disorders, including VD deficiency [25].

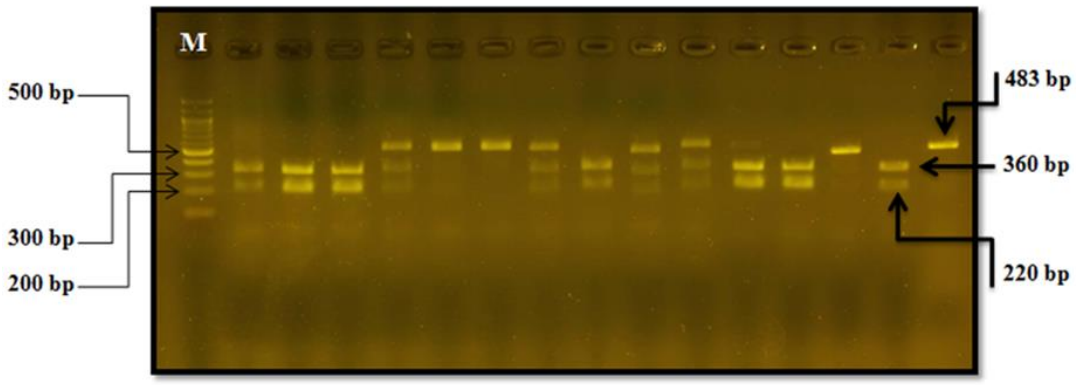
The study's findings also revealed that the OR value of the mutant genotype was very high O.R = 30.0 at level of probability P = 0.0018 and the OR value of the mutant allele was O.R = 11.8 at level of probability P = 0.0001, This is considered a risk factor because it is greater than (1), and hence the amount of V.D decreases.

**Detection of the rs7041T mutation of the VDBP gene:**

The results showed, and as shown in Figures (2) and (3), there is a relationship between women who suffer from VD deficiency and the genetic mutation of the VDBP gene (Asp416Glu) at the site (rs7041). The result of the PCR reaction is in three packages: 483 and 360 for the healthy TT genotype, 309 and 249 for the mutant GG genotype, and 309, 249 and 158 of the heterogeneous TG genotype, and this indicates the appearance of genetic variation for the VDBP gene and in all genotypes and in different percentages




**Figures 2** The PCR product for the VDBP gene variance (rs7041) shows the 483 bp, M reaction product is 100 BP volume guide prepared from Biolabs and separated by 2% agarose gel




**Figures 3** The result of the RFLP-PCR reaction for the rs7041 genetic variant (VDBP) shows that the reaction product contains the first 3 bundles of 483 bp in size for the major gene, the second with a size of 360 bp for the normal allele, and the third bundles with a size of 220 bp for the mutant allele, M is the volume guide size 100 BP prepared from Biolabs Company and separated by 2% agarose gel.

**Table (7):** It shows the percentage of allelic observations as well as the many genotypes of the gene of VDBP at the location (rs7041).

| Genotypes | Patients | | Control | | P Value | OR | (95%Cl) |
|---|---|---|---|---|---|---|---|
| | 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%Cl)** |
| T | 67 | 45 | 23 | 55 | P = 0.2783 | 1.4635 | 0.7352 to 2.9133 |
| G | 81 | 55 | 19 | 45 | | | |

The results of the study for a group of patients revealed that the rate of viewing for the mutated GG genotype was 36%, and the rate of viewing for the normal TT genotype was 28%. In terms of the heterogeneous TG genotype, the rate of viewing for it was 36% compared with the control group, as the rate of observation for the lowest genotype GG was 10%. The rate of observation for the normal genotype TT was 19%, while for the heterogeneous genotype TG, the rate of viewing was the highest, 71%, and the allelic viewing rate was 55% for the mutant G allele and 45% for the normal allele T for women with VD insufficiency compared to the healthy group. The mutant 45% compared to the normal allele 55%.

The genetic variation rs7041 is one of the most common kinds of the VDBP gene that has a close association with the level of25 (OH)D3 in plasma, as it was previously reported that the rs7041 Polymorphism is linked to an increase in risk factors in chronic diseases and various cancers. Also, previous studies showed that the genetic variation rs7041 for VDBP interferes with the natural activity of VD, which contributes to an increased risk of chronic diseases, including osteoporosis and breast cancer in women [26]. Other studies have not demonstrated an association of rs7041 VDBP profiles with rectal cancer in the US population. The uncoordinated results are as a result of physiological and pathological effects of the rs7041 VDBP isoform, which contributed to an increased risk of HCC on different races and tissues [27].

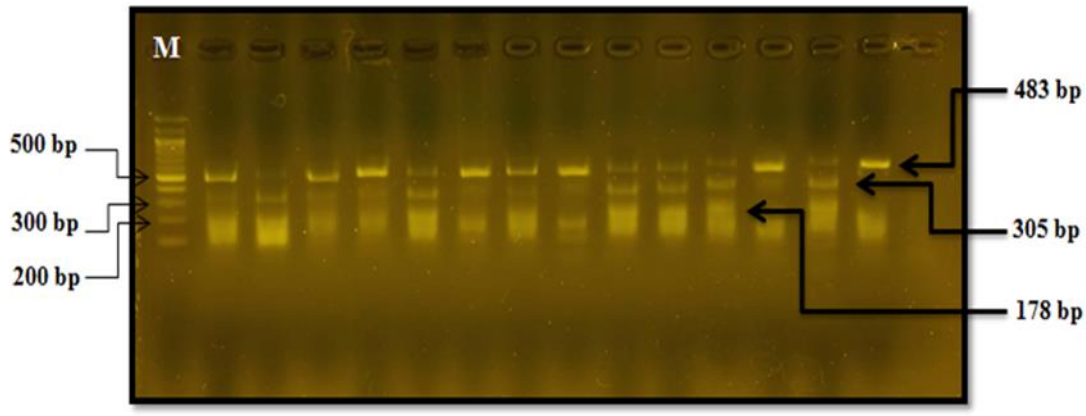
The study's findings also revealed that the OR value of the mutant genotype was very high O.R = 2.7000 at the level of probability P = 0.2776 and the OR value of the mutant allele was O.R = 1.4635 at the level of probability

P = 0.2783, which and this is a risk factor and one of the causes for the lack of VD deficiency About the change in the action of the protein VDBP.

**Detection of the (rs4588C) mutation of the VDBP gene:**

The results showed, and as shown in Figure (4), there is a relationship between women suffering from VD insufficiency and a VDBP gene mutation (Thr420Lys) at the site rs4588C. 309 and 158 for normal CC genotype, 309 and 249 for mutant AA genotype, and 309, 249 and 158 for AC heterogeneous genotype, and these indicate the appearance of genotype variation for the VDBP gene with all genotypes and with different percentages

**Figures (4):** shows the result of the PCR reaction for the genetic variance (rs4588C) of the VDBP gene and the three-band reaction product is 483bp 178, bp 305, bp, M is the volume guide of 100 BP and was prepared by Biolabs and separated by 2% agarose gel:




**Figures 4** The result of the RFLP-PCR reaction for the genetic variation (rs4588C) shows the VDBP gene. The reaction product contains 3 packets, the first with a size of 483 bp for the major gene, the second with a size of 305 bp for the normal allele, and the third bundle with a size of 178 bp for the mutant allele, M is the volume guide of 100 BP was prepared from Biolabs Company and separated by 2% agarose gel.

**Table (8):** shows the percentage of allelic observations and the several VDBP gene genotypes at the (rs4588C) locus:

| **Genotypes** | **Patients** | | **Control** | | **P Value** | **OR** | **(95%Cl)** |
|---|---|---|---|---|---|---|---|
| | **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%Cl)** |
| **C** | 95 | 65 | 20 | 47 | P = 0.0547 | 0.5072 | 0.2537 to 1.0137 |
| **A** | 53 | 35 | 22 | 53 | | | |

The results of the study for a The percentage of patients in the group revealed that observation for the AA mutated the genotype had the lowest (13%) percentage and the viewing rate for the typical CC genotype was 40%, while for the heterogeneous genotype CA, the rate of viewing for it was the highest 47% compared with the control group, as the rate of the observed genotype was AA mutation is 20% and the rate for the normal CC genotype

observation was the lowest 14%, while for the heterogeneous genotype CA, the rate of sighting is the highest 66%. The rate of observation for the normal allele was 35% for the mutant allele A and 65% for the normal allele C in women with VD deficiency in comparison with The healthy group, as the rate of viewing of the abnormal or mutant allele was 53%, In comparison with 47% of the normal allele.

Furthermore, low calcium and phosphorus concentrations will lead to impaired bone mineralization and rickets [28]. VD is also a regulator of the immune system, and some studies have suggested that VDBP is associated with bone health. The C rs4588 alleles are associated with higher concentrations of VDBP and BMD. Lower levels of VDBP with lower bone mineral density in the rs4588 “A” allele were associated with lower levels of the inactive form of vitamin D 25(OH)D3 and an increased risk of bone fracture. In the obese, the rs4588 “C” allele was associated with lower levels of the active form of vitamin D 25(OH)D3 and Females have greater BMIs. The Rs4588 "T" and rs7041 "G" alleles increased the risk of PCOS among VD-deficient women [29].

The study's findings also revealed that the OR value of the mutant genotype was very high O.R = 0.2250 at the level of probability P=0.0804 and the value of the mutant allele was O.R = 0.5072 at the level of probability P =0.0547, which and this is considered a risk factor and one of the causes of VD deficiency About the change in the action of the protein VDBP.

**Haplotype analysis:**

The analysis of haplotype results plays an important role in identifying new genetic associations between the alleles of different genetic variations for a particular gene. Through this study, six possible genetic patterns resulting from the allelic associations of genetic mutations were identified (rs4588C, rs7041, rs17467825) and as shown in the following table (9):

**Table (9):** shows the genotypes and their frequencies resulting from the Haplotype test

| Genotype | Frequency (patient) | Frequency (control) | Odd. Ratio | CI (95%) |
|---|---|---|---|---|
| AA | 23.00(0.155) | 68.99(0.548) | 0.153 | (0.087~0.270) |
| AC | 42.02(0.284) | 51.01(0.405) | 0.588 | (0.355~0.975) |
| GA | 31.00(0.209) | 0.01(0.000) | 2284.256 | (143.770~36292.906) |
| GC | 50.98(0.344) | 5.99(0.048) | 10.645 | 4.378~25.879)( |
| A\CC | 0.98(0.007) | 0.00(0.000) | - | - |
| G\CC | 0.02(0.000) | 0.00(0.000) | - | - |

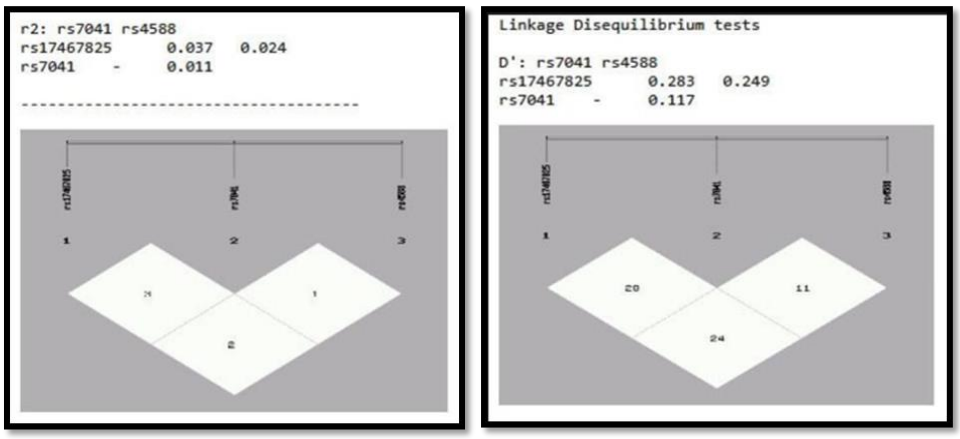
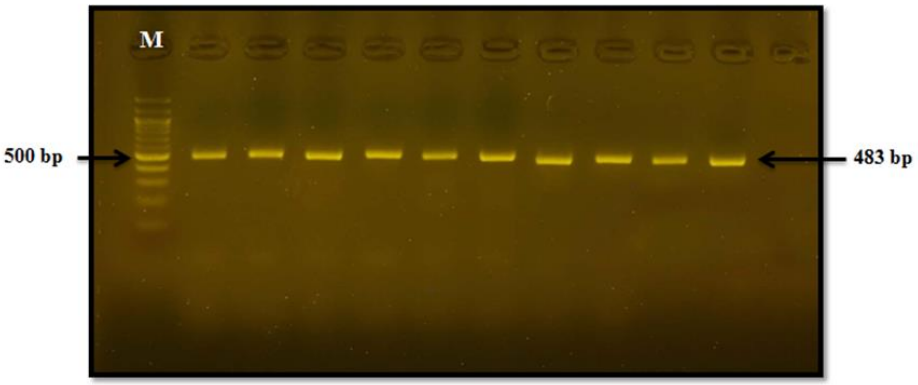



**Figure 5** It shows the overlap the VDBP gene by haplotype analysis

It is clear from the above table that the GC genotype had greater importance than the rest of the phenotypes, as its frequency in patients was (50.98 (0.344)) compared to its frequency in the control group 5.99 (0.048)) and when conducting the Hardy-Weinberg equilibrium test for genetic variance At the rs17467825 and rs7041 loci, the p value = 0.201 and p = 0.023 respectively is less than 0.5, and this indicates that this group is unstable and not subject to Hardy equilibrium because of these mutations that affected the gene VDBP, while when this test was performed on the mutation rs4588C the value of P=0.84, which is higher than 0.5, and this indicates that the group is balanced according to Hardy's equilibrium.

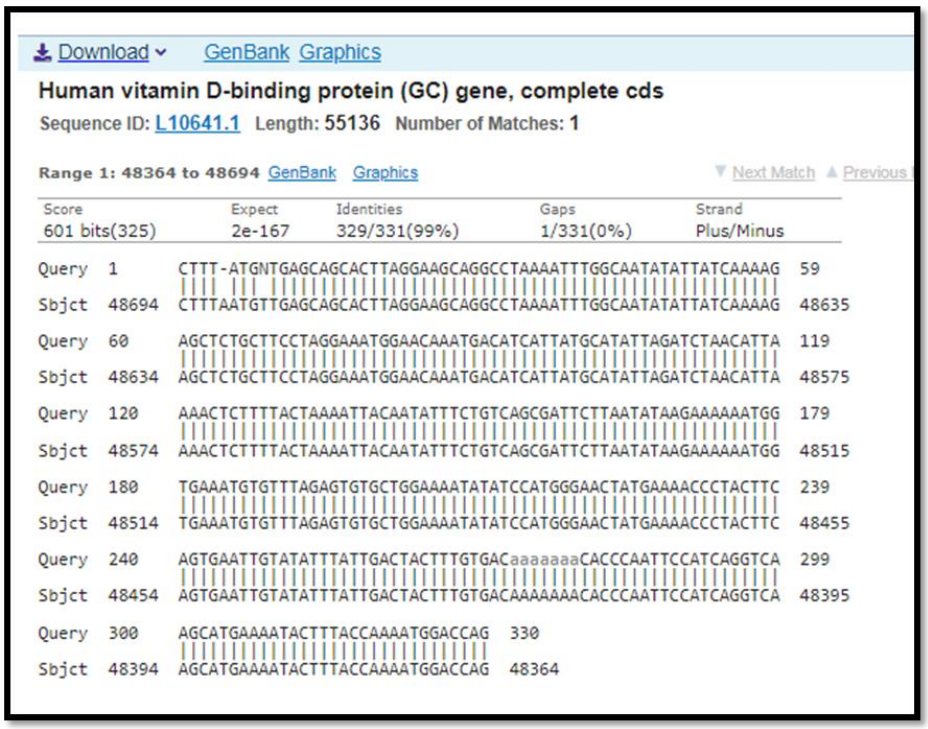
**Determination of nucleotide sequencing of amplified fragments utilizing DNA sequencing technology:**

Figures (7) show the 483 bp PCR product of the VDBP gene, on which the nucleotide sequence determination test was performed




**Figures 6** The PCR product of the VDBP gene and reaction product 483 bp, M is the volume guide with a size of 100 bp and was prepared by Biolabs and separated by 2% agarose gel

The results of matching the nucleotide sequences belonging to the VDBP gene for the samples included in the study showed that they are 100% identical with the nucleotide sequences in the NCBI site, and this indicates the accuracy of the designed primer for the first time, which was used in this study, as shown in the following figures:



Download GenBank Graphics

**Human vitamin D-binding protein (GC) gene, complete cds**

Sequence ID: L10641.1 Length: 55136 Number of Matches: 1

**Range 1: 48364 to 48694** GenBank Graphics ▼ Next Match ▲ Previous

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 601 bits(325) | 2e-167 | 329/331(99%) | 1/331(0%) | Plus/Minus |

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Query  60     AGCTCTGCTTCCTAGGAAATGGAACAAATGACATCATTATGCATATTAGATCTAACATTA  119
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Sbjct  48634  AGCTCTGCTTCCTAGGAAATGGAACAAATGACATCATTATGCATATTAGATCTAACATTA  48575

Query  120    AAACTCTTTTACTAAAATTACAATATTTCTGTCAGCGATTCTTAATATAAGAAAAAATGG  179
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Sbjct  48574  AAACTCTTTTACTAAAATTACAATATTTCTGTCAGCGATTCTTAATATAAGAAAAAATGG  48515

Query  180    TGAAATGTGTTTAGAGTGTGCTGGAAAATATATCCATGGGAACTATGAAAACCCTACTTC  239
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Query  300    AGCATGAAAATACTTTACCAAAATGGACCAG  330
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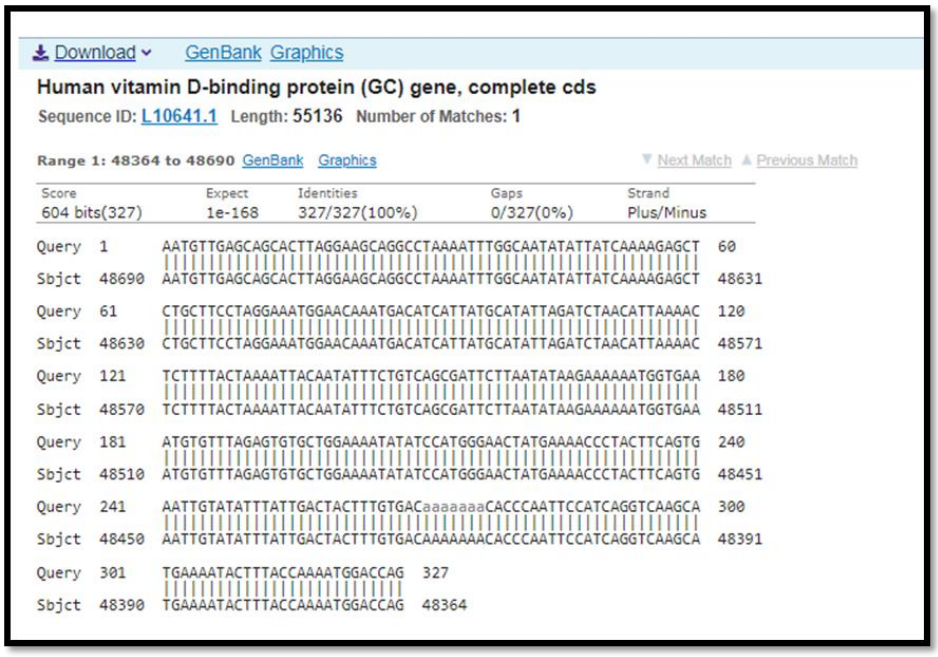

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**Human vitamin D-binding protein (GC) gene, complete cds**

Sequence ID: L10641.1 Length: 55136 Number of Matches: 1

**Range 1: 48364 to 48690** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 604 bits(327) | 1e-168 | 327/327(100%) | 0/327(0%) | Plus/Minus |

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Query  121    TCTTTTACTAAAATTACAATATTTCTGTCAGCGATTCTTAATATAAGAAAAAATGGTGAA  180
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Query  301    TGAAAATACTTTACCAAAATGGACCAG  327
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Sbjct  48390  TGAAAATACTTTACCAAAATGGACCAG  48364
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**Human vitamin D-binding protein (GC) gene, complete cds**

Sequence ID: L10641.1 Length: 55136 Number of Matches: 1

**Range 1: 48364 to 48688** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 601 bits(325) | 2e-167 | 325/325(100%) | 0/325(0%) | Plus/Minus |

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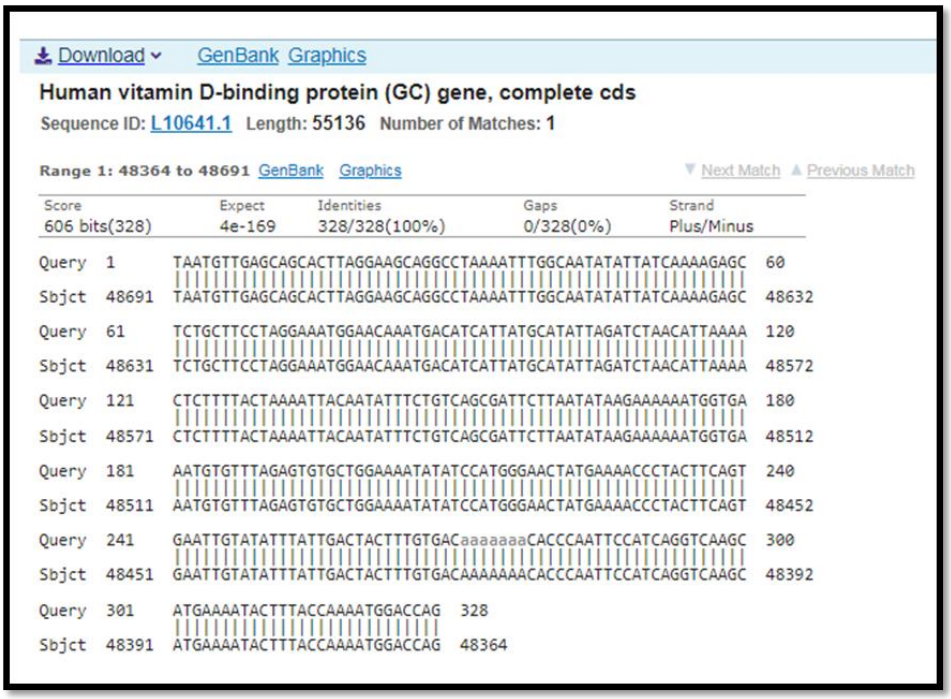

Download GenBank Graphics

**Human vitamin D-binding protein (GC) gene, complete cds**

Sequence ID: L10641.1 Length: 55136 Number of Matches: 1

**Range 1: 48364 to 48691** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Identities | Gaps | Strand |
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| 606 bits(328) | 4e-169 | 328/328(100%) | 0/328(0%) | Plus/Minus |

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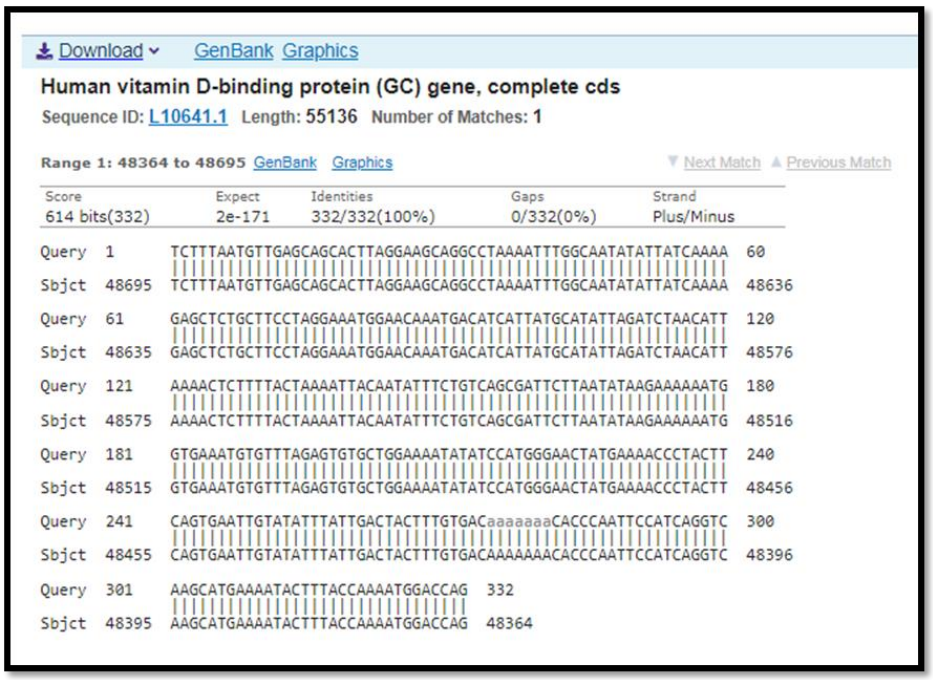


**Figure 7** The product shows the conformation of the nucleotides belonging to the VDBP gene in the study samples with the nucleotide sequence of the main VDBP gene NCBI stands for National Center for Biotechnology Information.

**Table (10):** It depicts the locations and types of VDBP gene mutations observed in women with vitamin D insufficiency

| ID sequence | Nucliotide | Location | Mutation type | Idendity | Gaps |
|---|---|---|---|---|---|
| L10641.1 | A --⟶ | 48690 | Deletion | 99% | 0 |

When the table (10) was observed, it was found that there was a new genetic variation of the type of deletion in the nucleotide sequences of the gene VDBP in the study samples when compared with the sequences of the gene at the NCBI site.

It is a multifunctional protein that plays a role in the transport of the inactive VD form 25(OH) D3 and the active form of vitamin D, 25(OH) D31, found in the rotational apparatus (Speeckaert et al., 2006). Several studies confirmed that VDBP regulates the half-life of active VD (OH) D325 in the circulatory system by stabilizing the hormone and maintaining the level of VD. The presence of these genetic variations in the nucleotide sequences of the VDBP gene leads to the synthesis of a protein that is less efficient in its work to bind with VD and then transfer it to the target tissues and thus increase the health problems resulting from VD deficiency, including osteoporosis [30].

## Conclusion

It was found from our current study that the cases of deficiency in the level of VD did not depend on a specific factor, as it was found that there are several factors, including genetic and environmental factors. We conclude

from this study the existence of a new genotype of VDBP gene due to mutations that have been registered on the NCBI global gene site.

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