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Comparing the VDR Gene *BsmI* and *CDX2* Polymorphisms in Healthy Turks and Healthy Somalians Living in Turkiye

Ender Coskunpinar[1], Betul Nilgun Engin[2]

[1]Department of Medical Biology, Faculty of Medicine, University of Health Sciences, Istanbul, Turkiye
[2]School of Medicine, University of Health Sciences, Istanbul, Turkiye

ORCID ID: E.C: 0000-0002-1003-5544; B.N.E. 0000-0002-9242-9909

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ABSTRACT

Objectives: This study aimed to evaluate genotypic and allelic differences by comparing the Vitamin D (Vit-D) receptor (VDR) gene *BsmI* (rs1544410) and *CDX2* (rs11568820) polymorphisms in healthy Turks and healthy Somalians living in Turkiye.

Materials and Methods: The study involved 100 healthy Turkish individuals and 60 healthy Somali individuals residing in Turkiye for at least 5 years. The genotyping study was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and allele-specific PCR methods. Statistical analysis was performed to identify the possible differences between groups using the Chi-square and Student's t tests for pair-wise comparisons.

Results: According to the data obtained in the study with regard to the *BsmI* (rs1544410 A/G) and *CDX2* (rs11568820 A/G) genotypes, no statistically significant difference was determined to be present regarding the frequency of carrying the mutant GG genotype in the two groups ($p = 0.95$ and $p = 0.221$, respectively).

Conclusion: The study has found no significant genotypic or allelic difference to be present in terms of the *BsmI* (rs1544410) and *CDX2* (rs11568820) gene variants between healthy Turkish individuals and Somali individuals who have spent most of their lives exposed to less sunlight while living in Turkiye for the past five years.

Keywords: VDR, *BsmI* (rs1544410), *CDX2* (11568820), polymorphism

INTRODUCTION

Vitamin D (Vit-D) is a steroid hormone that is synthesized from cholesterol and binds to its intracellular polypeptide Vit-D receptor (VDR) to start its active mechanisms. The VDR regulates the expression of genes in Vit-D sensitive tissues and initiates the pathways necessary for the formation of biological effects. The VDR gene has eight introns and nine exons and is located in chromosome region 12q13.1. The most well-known function of Vit-D is to regulate bone metabolism through a calcium-phosphorus balance; as such, its deficiency is significant in the development of cardiovascular diseases and hypertension. Vit-D also has immune-regulatory functions, regulating both innate and adaptive immunity (1). Vit-D is also known as an important regulator of innate immune responses to microbial challenges (2) and was accepted at the beginning of the 20th century as having a critical role in preventing rickets (3). Most of the Vit-D the human body consumes is produced by the skin as a result of exposure to sunlight. Vit-D is then processed by the liver and kidney to make it beneficial for bones (4). Sunlight is a very important factor for Vit-D production (5), and Vit-D has two forms: Vitamin D_2 (Ergocalciferol) and vitamin D_3 (Cholecalciferol) (6). When human skin is exposed to solar UVB radiation (wavelengths: 290-315 nm), 7-dehydrocholesterol is converted in the skin first to preVit-D_3, then from preVit-D_3 to Vit-D_3. Meanwhile, Vit-D_2 has yeast and fungal origins and can be converted

Corresponding Author: Ender Coskunpinar **E-mail:** ecoskunpinar@gmail.com
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into Vit-D_3 when consumed with food. During the formation of Vit-D, the 25-hydroxyVit-D (25(OH)D) form is synthesized in the liver, and then the 1α, 25-dihydroxy Vit-D form is synthesized in the kidney (7). The amount of Vit-D production in the skin can vary depending on skin color, latitude, season and, surprisingly according to the time of day (8, 9). Supplementation of 400-1,000 IU per day for infants in the first year of life, 600-1,000 IU per day for children and adolescents between 1-18 years, and 1,500-2,000 IU per day for adults over 18 years is considered appropriate for preventing Vit-D deficiency (10).

Vit-D plays a central role in bone- and calcium-related processes, regulating bone metabolism by stimulating calcium and phosphate absorption in the intestine. Studies have shown a relationship to exist between many acute and chronic diseases such as cardiovascular diseases, liver and kidney diseases, preeclampsia, periodontitis, some autoimmune disorders, cancer, diabetes, and neurological disorders due to a Vit-D deficiency (4, 11). Vit-D also has several effects on bone metabolism, regulating arterial blood pressure, preventing cardiovascular complications, modulating immunological responses, regulating insulin production and preventing diabetes, and protecting against certain cancers (12).

The VDR is expressed in various parts of the brain throughout embryonic development, and the active form of Vit-D enables the activation of various target genes through the VDR (13). Therefore, some neuropsychiatric diseases have been associated with polymorphisms in the VDR (14). The stability of Vit-D-mediated signaling pathways is important for brain development (15), with functional polymorphisms affecting gene expression having been reported in this gene (16). Mutations in the *BsmI* rs1544410 variant have also been shown to be associated with neurodegenerative diseases (14, 16). As a result of studies, the association of polymorphisms with various diseases draws attention to the effectiveness of these genomic changes; however, the data are still quite limited (17-19). The *CDX2* (rs11568820) variant is associated with transcriptional activity, which can affect calcium absorption (20).

Considering that only 20% of all Vit-D needed by the body is obtained from food, geographical differences are thought to be important in terms of VDR gene polymorphisms. Evaluating the differences in skin color in this context may additionally be appropriate. The number of studies conducted on healthy Somalians in the literature is extremely limited. Moreover, no study is found to have investigated the VDR gene *BsmI* and *CDX2* polymorphisms among Turkish and Somali individuals.

This study aimed to evaluate genotypic and allelic differences by comparing the VDR gene *BsmI* (rs1544410) and *CDX2* (rs11568820) polymorphisms between healthy Turks and healthy Somalians living in Turkiye. This study is the first study to examine these two gene regions (*BsmI* and *CDX2*) together.

MATERIALS AND METHODS

Study Design

The study involved 160 volunteers (90 female and 70 male) between the ages of 18-35 (Table 1). Ethics committee approval was obtained on December 1, 2021 with Decision no. 2015-KAEK-56-21-03 from the Biruni University Clinical Research Ethics Committee. After obtaining informed consent from the volunteers to participate in the study, 2 mL peripheral blood samples were taken. Next, the study conducted genotyping using DNA isolation, polymerase chain reaction (PCR), and then following restriction length polymorphism (RFLP) methods. The gene regions amplified by the PCR and restriction cut results have been visualized using agarose gel electrophoresis.

Volunteer Selection and Sample Obtainment

One hundred healthy Turks and 60 healthy Somalians living in Turkiye for at least 5 years between the ages of 18-35 have been included in the study, with 2 mL samples of peripheral blood being taken from the volunteers who signed the informed consent form. The samples were kept in a refrigerator at +4 ºC until further processing.

DNA Isolation and PCR

DNA samples were extracted according to the kit protocol (High Pure PCR Template Preparation Kit /Product No: 11796828001) and stored in a -20ºC refrigerator for use in subsequent processes. The DNA concentrations obtained as a result of the isolation method were measured using a Denovix DS-11 FX spectrophotometer, with a specific primer design being made for the *BsmI* (rs1544410) and *CDX2* (rs11568820) gene regions (Table 2).

Table 1. Comparison of age and gender in Turkish and Somali volunteers.

	Turkish group					Somali group					
Gender	**(N=100)**			**%**		**(N=60)**			**%**		**p value**
Female	66			66		24			40		***0.001**
Male	34			34		36			60		
Age	**N**	**Mean**	**Min.**	**Max.**	**SD**	**N**	**Mean**	**Min.**	**Max.**	**SD**	**p value**
	100	26	18	35	4.008	60	23	19	26	1.610	0.241

N=Total number, SD=Standard deviation, Min=Minimum, Max=Maximum

Table 2. Primers designed for the VDR Gene *BsmI* (rs1544410) and *CDX2* rs11568820 regions.

Gene	Forward	Reverse
CDX2 (rs11568820)	G-(5′ AGGATAGAGAAAATAATAGAAAACATT 3′) A-(5′ TCCTGAGTAAACTAGGTCACAA 3′)	5′ AACCCATAATAAGAAATAAGTTTTTAC 3′ 5′ ACGTTAAGTTCAGAAAGATTAATTC 3′
BsmI (rs1544410)	5′ CAACCAAGACTACAAGTACCGCGTCAGTGA 3′	5′ AACCAGCGGGAAGAGGTCAAGGG 3′

The PCR mix content for the *BsmI* (rs1544410) gene region was prepared as follows: 18.2 µL dH_2O; 2.5 µL buffer; 2 µL dNTP (2.5 mm); 0.5 µL forward primer; 0.5 µL reverse primer; 0,3 µL Taq polymerase. The PCR mix content was prepared for the *CDX2* (rs11568820) gene region as follows: 15.7 µL dH_2O; 2.5 µL buffer; 1.5 µL $MgCl_2$; 2 µL dNTP (2.5 mm); 0.5 µl forward primer1; 0.5 µL forward primer2; 0.5 µL reverse primer1; 0.5 µLreverse primer2; 0.3 µL Taq polymerase.

Appropriate binding conditions for the primers designed for the *BsmI* (rs1544410) region are as follows: pre-denaturation at 94ºC for 1 minute, denaturation for 30 seconds at 94ºC, primer attachment for 45 seconds at 58.9ºC, chain elongation for 30 seconds at 72ºC, and elongation at 72ºC for 5 minutes. Appropriate binding conditions for primers designed for the *CDX2* (rs11568820) region are as follows: pre-denaturation at 94ºC for 5 minutes, denaturation at 94ºC for 30 seconds, primer binding at 54.8ºC for 45 seconds, chain elongation at 72ºC for 30 seconds, and elongation at 72ºC for 5 minutes. The template was amplified using the Thermal Cycler (Blue-Ray/ tcst-9620) device over 35 cycles for *BsmI* (rs1544410) and 43 cycles for *CDX2* (rs11568820). Genotyping was done using the PCR and PCR-RFLP methods for the rs1544410 region. PCR and enzyme digestion products were amplified by the PCR method and carried out through restriction enzyme digestion for the *BsmI* (rs1544410) region followed by the agarose gel electrophoresis. These products were visualized in a transilluminator using a CCD camera under UV light. To evaluate the *CDX2* (rs11568820) region of the VDR gene, the study used the allele-specific PCR. The amplified rs11568820 region of the PCR products were separated by size in a 3% agarose gel electrophoresis. Gel staining was performed with ethidium bromide and also visualized in a transilluminator using a CCD camera under ultraviolet light.

Genotyping

Site-specific restriction enzymes were determined in order to detect variant changes in specific regions of the genes from different DNA regions that had been amplified using PCR. The reaction was prepared with the components whose specific amounts were adjusted for each enzyme for a total volume of 15 µL. Specific restriction enzymes were used for restriction cutting. The obtained product was run through the agarose gel electrophoresis to determine whether or not it has a mutation. The cut made by the restriction enzyme in certain regions was visualized in a transilluminator using a CCD camera under ultraviolet light following the agarose gel electrophoresis.

Analyzing the *BsmI* (rs1544410) Variant

The rs1544410 variant in the *BsmI* gene consists of 813 base pairs (bp). Using primers specifically designed for this region, the 813 bp regions were amplified using PCR and visualized over a 2% agarose gel. The amplified PCR products were then digested with the Mva1269I restriction enzyme. The mutant GG allele was displayed as a single 813-bp fragment, with the wild-type AA allele being displayed as two fragments of 478 bp and 335 bp, and the heterozygous AG allele being displayed as 3 fragments of 813 bp, 478 bp, and 335 bp (Figure 1).

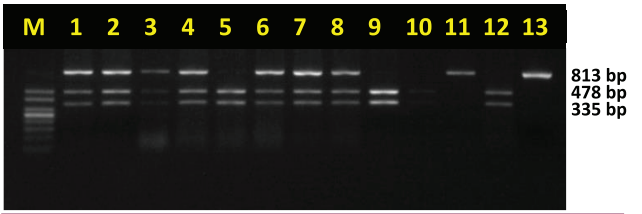

Figure 1. VDR gene *BsmI* digestion polymorphism products: Lane 1,2,3,4,6,7,8: AG heterozygous products, lane 5,9,12: AA wild type products, lane 11: GG homozygous product, lane 13 uncut control PCR product. M: Marker

Analyzing the *CDX2* (rs11568820) Variant

To analyze the *CDX2* (rs11568820) region, the study used the allele-specific PCR, which is a popular method for the DNA typing of SNPs. The single-tube PCR genotyping does not require enzyme cutting or manipulation of the PCR products (Figure 2).

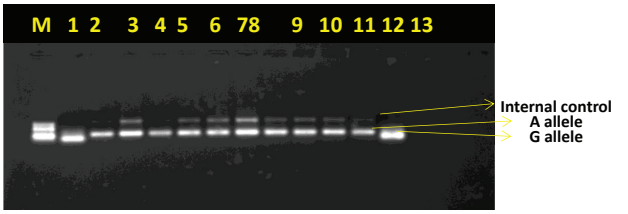

Figure 2. Allele specific PCR analysis of the VDR gene *CDX2* polymorphism. Lane 12: positive control, lane 13: negative control, lane 1,2,4: GG homozygous products, lane 3,5,6,7,8,9,10,11: AG heterozygous products. M: 100 bp ladder.

Statistical Analyses

The program SPSS (ver. 25.0) was used to analyze the data, and the data were evaluated using the chi-square and Student's t tests, with the statistical significance being accepted at $p < 0.05$.

RESULTS

The genotype and allele distributions for both groups are shown in Table 3. According to the data obtained in the study regarding the *BsmI* (rs1544410 A/G) and *CDX2* (11568820 A/G) genotypes, no statistically significant difference was determined in terms of the frequency individuals carrying the mutant GG genotype ($p = 0.95$ and $p = 0.221$, respectively), or alleles ($p = 0.97$ and $p = 0.18$, respectively) in the Somali group compared to the Turkish group.

Table 3. Genotype and allele distributions of *BsmI* (rs1544410) and *CDX2* (11568820) gene variants.

	Somali Group		Turkish Group			
VDR *BsmI*	**N=60**	**%**	**N=100**	**%**	**X^2**	**P value**
Genotyping						
AA	18	30	29	29.37		
AG	31	51.67	54	53.13	0.089	0.95
GG	11	18.33	17	17.5		
Allele						
A	67	55.83	112	56	0.0008	0.97
G	53	44.17	88	44		
VDR *CDX2*						
Genotyping						
AA	22	36.67	50	50		
AG	25	41.67	30	30	3.01	0.221
GG	13	21.66	20	20		
Allele						
A	69	57.5	130	65	1.79	0.18
G	51	42.5	70	35		

DISCUSSION

Vit-D deficiency arises when a person does not get enough Vit-D through the skin (from sun exposure) or food, as well as when their liver and/or kidneys have pathologies processing Vit-D. Each individual has different Vit-D requirements, and Vit-D expression and levels of the VDR gene may vary in different geographies. VDR gene polymorphisms have been investigated all over the world due to their association with susceptibility to various diseases, as Vit-D plays a very important role in preventing multifactorial pathologies including osteoporosis and bone metabolism disorders in particular. The biological action of Vit-D occurs through its receptor encoded by the VDR gene. Therefore, VDR gene polymorphisms can affect bone mineral density, susceptibility to osteoporosis, and response to Vit-D supplementation.

Vit-D deficiency can cause bone problems in both children (e.g., rickets, osteomalacia) and adults (osteoporosis). Many studies have reported links between Vit-D deficiency and various health problems including depression, fractures, diabetes, heart diseases, kidney diseases, cancer, and infection. However, most of these connections do not emit the same results in each society, and the publications in the literature are quite inconsistent with each other (4). In Marozik et al. (21) published an article in 2021 and reported the results that the rs1544410 T/T polymorphism increases the risk of osteoporosis and decreases bone mineral density, while the rs11568820 polymorphism has a significant dose effect with 25(OH)D. González Rojo et al.'s 2022 study (22) on 246 cardiovascular disease patients from Southern Spain and 246 controls of Caucasian origin reported *BsmI* (rs1544410) and *CDX2* (rs11568820) polymorphisms to increase susceptibility to cardiovascular disorders when Vit-D intake varies. Liu et al. published in the article in 2015 (23) reported rs11568820 and rs1544410 polymorphisms in the VDR to be associated with gout in the Chinese Han male population. Nevertheless, despite that study obtaining no genotypic significance, a predisposition was observed in allelic frequencies. In an article by Maciejewski et al. (24) investigated the relationship between thyroid-related orbitopathy, an autoimmune disease that typically occurs in the course of Graves' disease, and VDR gene polymorphisms and, while different publications in the literature have stated these polymorphisms to provide a predisposition, Maciejewski et al. (24) reported the gene polymorphisms of the relevant region to not be associated with the disease, adding a new example to the contradictory results. Tantawy et al. (25) published in 2016 article on Egyptian pediatric acute lymphocytic leukemia (ALL) patients found no association for the rs1544410 and rs11568820 genotypes with ALL, despite the significant genetic heterogeneity present in the VDR gene. Studies conducted in recent years have also shown Vit-D deficiency to be at very low levels in Somali individuals who have migrated to Northern European countries when compared to local individuals (26-28). Nelson et al. (29) studied on African men and reported no association between rs1544410 polymorphism and aggressive prostate cancer, while also reporting 25(OH)D levels and the rs11568820 polymorphism to be associated with the disease. One study conducted with healthy volunteers living in the Emirati population stated the Emirati population genotype and allele distribution of VDR gene polymorphisms to not differ from Caucasians living in the USA and France but to have a significant difference with Asian populations (30). As seen in the examples above, Vit-D receptor gene polymorphisms have shown inconsistent results in both comparisons of healthy volunteers and when comparing individuals with various diseases to

healthy volunteers. The current study found no significant genotypic or allelic difference in terms of the *BsmI* (rs1544410) and *CDX2* (rs11568820) gene variants between healthy Turkish individuals and Somali individuals who have spent most of their lives exposed to less sunlight and been living in Turkiye for the last five years.

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Ethics Committee Approval: Ethical approval was taken from the Biruni University Clinical Research Ethics Committee (Decision Number: 2015-KAEK-56-21-03). All procedures were followed in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Peer-review: Externally peer-reviewed.

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