








Genetic association of the BsmI variant of vitamin D receptor gene with risk of morbid obesity

Seyma Ozsoy^{1*} , Serbulent Yigit² , Ayse Feyda Nursal³ , Zeki Ozsoy⁴ , Mehmet Fatih Dasiran⁵ , Emin Daldal⁶ , Akin Tekcan⁷ 

SUMMARY

OBJECTIVE: The aim of this study was to evaluate the vitamin D receptor (VDR) BsmI variant in morbidly obese patients compared with healthy normal controls.

METHODS: The study included 103 patients with morbid obesity and 120 healthy individuals serving as normal controls. The DNA samples obtained from blood were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The gender, age, smoking status, triglycerides, total cholesterol, insulin, mean body mass index, and frequency of allele and genotype of the BsmI variant in the VDR gene in morbidly obese patients were evaluated.

RESULTS: The body mass index of the patients was 47.14 ± 7.19 . The VDR B/B, B/b, and b/b genotype frequencies were 27.2% versus 28.3%; 54.4% versus 50%; and 18.4% versus 21.7% in the morbidly obese patients and the control group, respectively. There was no statistically significant difference between patients and control subjects in the genotype and allele distribution of the VDR BsmI variant ($p > 0.05$). Both patients and control genotype frequencies are consistent with Hardy-Weinberg equilibrium.

CONCLUSION: The BsmI variant in the VDR gene may not seem to predispose to morbid obesity in our study population. Further studies with a larger number of subjects are needed to make a more precise evaluation of this relationship.

KEYWORDS: Morbid obesity. Vitamin D receptor. BsmI. Variant.

INTRODUCTION

The escalating prevalence of obesity worldwide is a matter of significant concern¹. The body mass index (BMI) is used to assess the severity of obesity. BMI is determined by taking a person's body weight in kilograms and dividing it by the square of their height in meters. A BMI ≥ 30 is defined as obesity, and a BMI value exceeding 40 is defined as morbid obesity (obesity class III)². Numerous chronic diseases, including diabetes, gallstones, hypertension, nonalcoholic fatty liver disease, metabolic syndrome, and cardiovascular disorders, have been linked to obesity, according to reports². The etiology of obesity is multifactorial, exhibiting a complex interplay between genes and the environment.

Vitamin D (VitD) is necessary for the homeostasis of bone tissue as well as the minerals such as calcium and phosphorus.

Serum 25-hydroxyvitamin D (25(OH)D) is often used as an index of VitD nutritional status. Circulating 25(OH)D concentrations of obese individuals were found to be lower than their race, socioeconomic status, and age-matched peers³. Once synthesized, VitD exerts its biological effects primarily through the VitD receptor (VDR, NR1H1). VDR belongs to the nuclear hormone receptor superfamily and forms a heterodimer with the retinoid X receptor. Together, they regulate the activity of target genes containing VitD response elements⁴. VDR expression is present in all essential VitD target tissues, including the intestine, kidney, bone, and parathyroid gland. In these tissues, VDR plays a vital role in maintaining calcium and phosphorus homeostasis, ensuring proper mineral balance within the body. VDR knockout mice have been shown to have reduced body weight and are resistant to high-fat diet-induced obesity⁵.

¹Gaziosmanpasa University, Faculty of Medicine, Department of Physiology – Tokat, Turkey.

²Ondokuz Mayıs University, Faculty of Veterinary, Department of Genetics – Samsun, Turkey.

³Hitit University, Faculty of Medicine, Department of Medical Genetics – Çorum, Turkey.

⁴Medical Park Hospital, Department of General Surgery – Tokat, Turkey.

⁵Gaziosmanpasa University, Faculty of Medicine, Department of General Surgery – Tokat, Turkey.

⁶Samsun University, Faculty of Medicine, Department of General Surgery – Samsun, Turkey.

⁷Amasya University, Faculty of Medicine, Department of Medical Biology – Amasya, Turkey.

*Corresponding author: seyma.ozsoy@hotmail.com, seyma.ozsoy@gop.edu.tr

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.

Received on January 29, 2024. Accepted on March 17, 2024.

The *VDR* gene is located on chromosome 12q13 and spans 75 kb of genomic DNA, consisting of 14 exons. Within the *VDR* gene, specific single-nucleotide polymorphisms (SNPs) have been identified, one of which is the rs1544410 SNP located in the intronic region (intron 8 near the 3' end). This SNP, also known as the BsmI variant, represents a restriction fragment length polymorphism (RFLP) of the restriction endonuclease BsmI⁶. Apart from its association with VitD levels, the BsmI polymorphism has been linked to obesity, insulin resistance, and type 2 diabetes in some study cohorts. This suggests that genetic variations in the *VDR* gene may play a role in these conditions, possibly influencing VitD metabolism and related physiological pathways^{7,8}.

Based on this information, we aimed to examine whether there is a relationship between the *VDR* BsmI variant and morbid obesity in this study.

METHODS

Study population

A total of 103 morbidly obese patients (30 males and 73 females; mean age: 39.47 ± 12.60 standard deviation [SD] years) who underwent bariatric surgery in the Department of General Surgery, Gaziosmanpaşa University Research Hospital, Tokat, Turkey, were included in this study. An accurately measured BMI is obtained at the first visit to the clinic. All participants were unrelated, with no consanguinity at all, and no spouses were included. BMI was higher than 40 in all patients. Gender and age compatibility of 120 healthy volunteers (55 males and 65 females; mean age: 41.51 ± 12.11 SD years) with a normal BMI were recruited as the control group. Prior to the study, informed consent was obtained from each participant to ensure their understanding and voluntary participation. The study's protocol was reviewed and confirmed by the institutional ethics committee at Ahi Evran University (approval number: 2017-05/36). The research adhered to the principles and guidelines set forth in the Helsinki Declaration to ensure the ethical treatment of the participants and protect their rights and welfare throughout the study.

Genotyping analysis

In this study, approximately 5 mL of peripheral blood was collected from each participant through venipuncture using Vacutainer tubes containing EDTA as an anticoagulant. DNA was removed from leukocytes using a DNA extraction kit (Sigma-Aldrich, Germany) following the manufacturer's instructions. The extracted DNA samples were stored at -20°C until

analysis. To genotype the *VDR* BsmI variant, the polymerase chain reaction-RFLP (PCR-RFLP) method was employed, as previously described⁹. After PCR amplification, the products were subjected to digestion overnight at 37°C using the BsmI restriction enzyme. After the digestion process, the resulting products were separated on a 2% agarose gel, which was stained with ethidium bromide. The visualization of the separated fragments was achieved using ultraviolet transillumination. The BsmI restriction enzyme digestion yields three genotypes: B/B (825 bp), B/b (825, 650, 175 bp), and b/b (650, 175 bp).

Statistical analysis

The data obtained from the study were analyzed using the SPSS version 22.0 software by Windows (SPSS Inc., Chicago, IL, USA). The number of sample groups in the research was determined using the G power Package program. According to the results of the analysis, the total sample size was determined to be 214 with a 95% confidence interval (CI), an alpha error rate of 0.05, and an effect size of 0.5.

To assess the statistical significance of differences between the patient and control groups, a logistic regression analysis was conducted. Odds ratios (OR) and their corresponding 95%CI were computed to quantify the associations. To compare the genotype and allele differences in the *VDR* BsmI between the patient and control groups, the chi-square test was used. In cases where sample sizes were small, Fisher's exact test was applied. The chi-square test was used to assess Hardy-Weinberg equilibrium (HWE). In all statistical analyses, two-tailed tests were used, and differences were considered statistically significant when the p-value was below 0.05 ($p < 0.05$).

RESULTS

In the present study, a total of 223 participants were included, comprising 103 individuals diagnosed with morbid obesity and 120 healthy adult controls. The genotyping for the *VDR* BsmI variant was performed on all subjects. Table 1 presents the characteristics of the participants in both the patient and control groups. Notably, in both study groups, there were more female participants than male participants. While there were 73 (70.9%) female and 30 (29.1%) male patients, the healthy control group consisted of 65 (54.2%) women and 55 (45.8%) men. The mean ages of morbidly obese patients and healthy individuals were 39.47 ± 12.60 and 41.51 ± 12.11 , respectively. The mean BMI of patients was 47.14 ± 7.19 .

The study results, as shown in Table 2, indicate the genotype distributions and allele frequencies of the *VDR* gene BsmI variant in all participants. However, upon analysis, no significant

Table 1. Baseline clinical and demographic features of the patient and control groups.

Characteristics	Control group (n = 120) (%)	Patient group (n = 103) (%)
Gender, females/males, n (%)	65/55 (54.2/45.8)	73/30 (70.9/29.1)
Age, mean \pm SD, years	41.51 \pm 12.11	39.47 \pm 12.60
Smoking status, no/yes, n (%)		74/25 (74.7/25.3)
BMI, mean \pm SD	-	47.14 \pm 7.19
Triglycerides, mean \pm SD, years	-	110.66 \pm 107.98
Total cholesterol, mean \pm SD years	-	130.33 \pm 88.94
Insulin, mean \pm SD years	-	26.80 \pm 52.37

SD: Standard deviation; BMI: body mass index; n: number of samples.

Table 2. Genotype and allele frequencies of the *VDR BsmI* gene variant in the patient and control groups.

<i>VDR BsmI</i>	Patient group n = 103 (%)	Control group n = 120 (%)	χ^2	p-value	OR (95%CI)
Genotypes					
B/B	28 (27.2)	34 (28.3)	1.62	>0.05	0.855 (0.40–1.78)
B/b	56 (54.4)	60 (50.0)		>0.05	0.864 (0.50–1.47)
b/b	19 (18.4)	26 (21.7)		>0.05	0.854 (0.35–2.04)
B/B+B/b:b/b	84:19	94:26		>0.05	1.222 (0.63–2.39)
B/B:B/b+b/b	28:75	34:86		>0.05	0.944 (0.52–1.70)
HWE	0.33	0.96			
Alleles					
B	112 (19.6)	128 (24)		>0.05	1.042 (0.71–1.51)
b	94 (80.3)	112 (76)			

n: number of samples; p-value: the statistical significance; HWE: Hardy-Weinberg equilibrium.

differences were observed between morbidly obese patients and healthy individuals concerning the *VDR BsmI* genotype and allele frequencies ($p > 0.05$). This implies that the presence of the *VDR BsmI* variant does not appear to be associated with morbid obesity in this study population. Both patients and control genotype frequencies are consistent with HWE ($p > 0.05$).

DISCUSSION

Obesity has emerged as a pressing global health concern, affecting a growing number of countries due to its high prevalence, substantial economic costs, and significant health implications². The excess of macronutrients in fatty tissues stimulates the release of inflammatory mediators such as $\text{TNF}\alpha$ and IL-6 ¹⁰. VitD deficiency is positively associated with serum levels of inflammatory markers such as IL-6 , $\text{TNF}\alpha$, and C-reactive protein in obese individuals¹¹. VitD may have immunomodulatory effects with its anti-adipogenic properties. This may help reduce inflammation in fatty tissues¹². It has been reported that there is a relationship between VitD deficiency during pregnancy and gestational diabetes¹³.

Metabolic regulation of VitD in adipose tissue is dependent on VDR. VDR belongs to the steroid hormone receptor superfamily, and it has been found to play a significant role in the development of obesity. Data show a widespread expression of VitD-related metabolic enzymes and VDR in human adipose tissue¹⁴. Since VDRs are present in various body tissues, their gene polymorphisms may influence the risk of VitD-related metabolic disorders and regulate the receptor's effectiveness based on VitD status¹⁵.

Genetic changes in the *VDR* gene can cause defects in gene activation. But they also support cell proliferation, differentiation, calcium metabolism, immune function, etc¹⁶. Among the various polymorphisms identified in the *VDR* gene to date, *BsmI*, *Apal*, *TaqI*, and *FokI* have been studied the most¹⁷. Various *VDR* polymorphisms have been associated with type 2 diabetes mellitus, insulin release¹⁸, and metabolic parameters related to obesity¹⁹. The *VDR BsmI* variant is believed to affect the gene's mRNA stability and gene transcription, potentially altering the expression of other genes. Studies conducted on different populations have reported varying associations between the *BsmI* variant and obesity. In a study conducted

in France, it was shown that the *VDR* BsmI b/b genotype is predisposed to obesity compared to B/B and B/b genotypes²⁰. Another study reported an association between body weight and BsmI variant in men in age-adjusted analysis²¹. In the study conducted with Saudi men, it was found that the BsmI b/b genotype was higher in the obese group compared to the lean group²². Additionally, those with the b/b genotype had higher BMI and HOMA-IR than those with the B/B and B/b genotypes. A study in obese children in Turkey found that BsmI polymorphism had a positive effect on the formation of obesity, metabolic syndrome, and hepatosteatosis²³. However, some studies have not observed significant relationships between the *VDR* BsmI variant and obesity-related parameters. For example, a Polish cohort of postmenopausal women did not find any association of the *VDR* BsmI variant with BMI, total fat volume, or visceral fat²⁴. Similarly, a study on obese and non-obese women with polycystic ovary syndrome did not show a significant association with the *VDR* BsmI variant²⁵.

In the current study, we investigated the genetic association between the *VDR* BsmI variant and morbid obesity. The genotype and allele frequencies of this variant showed no statistically significant difference between the morbidly obese patients and the healthy controls (Table 2). These findings were consistent with some previous studies.

This study has some limitations. The first limitation is that it evaluates only one variant of the *VDR* gene. Other variants of this gene may also affect the development of morbid obesity. Additionally, gene-environment and gene-gene interactions

were not evaluated. The final limitation is that the *VDR* blood level was not assessed. However, the strength of our study is that only patients from a single region were included.

CONCLUSION

Identifying the relationship between genetic variants and obesity is crucial to understanding pathogenesis. This study represents a valuable step in investigating the potential link between *VDR* BsmI and obesity susceptibility. Large samples and replication in different ethnic groups are required to evaluate the results. The results may help develop personalized therapeutic approaches.

AUTHORS' CONTRIBUTIONS

SO: Conceptualization, Formal Analysis, Supervision, Project administration, Writing – original draft, Writing – review & editing. **SY:** Conceptualization, Data curation, Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing. **AFN:** Conceptualization, Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing. **ZO:** Conceptualization, Data curation, Supervision, Writing – review & editing. **MFD:** Conceptualization, Supervision, Writing – review & editing. **ED:** Conceptualization, Supervision, Writing – review & editing. **AT:** Conceptualization, Data curation, Supervision, Writing – review & editing.

REFERENCES

1. Khan SM, Hajj Chehadeh S, Abdulrahman M, Osman W, Safar H. Establishing a genetic link between FTO and VDR gene polymorphisms and obesity in the Emirati population. *BMC Med Genet.* 2018;19(1):11. <https://doi.org/10.1186/s12881-018-0522-z>
2. Lin X, Li H. Obesity: epidemiology, pathophysiology, and therapeutics. *Front Endocrinol (Lausanne).* 2021;12:706978. <https://doi.org/10.3389/fendo.2021.706978>
3. Vranić L, Mikolašević I, Milić S. Vitamin D deficiency: consequence or cause of obesity? *Medicina (Kaunas).* 2019;55(9):541. <https://doi.org/10.3390/medicina55090541>
4. Martínez-Sena T, Soluyanov P, Guzmán C, Valdivielso JM, Castell JV, Jover R. The vitamin D receptor regulates glycerolipid and phospholipid metabolism in human hepatocytes. *Biomolecules.* 2020;10(3):493. <https://doi.org/10.3390/biom10030493>
5. Wang PQ, Pan DX, Hu CQ, Zhu YL, Liu XJ. Vitamin D-vitamin D receptor system down-regulates expression of uncoupling proteins in brown adipocyte through interaction with Hairless protein. *Biosci Rep.* 2020;40(6):BSR20194294. <https://doi.org/10.1042/BSR20194294>
6. El-Beshbishy HA, Tawfeek MA, Taha IM, FadelElahi T, Shaheen AY, Bardi FA, et al. Association of vitamin D receptor gene BsmI (A>G) and FokI (C>T) polymorphism in gestational diabetes among Saudi Women. *Pak J Med Sci.* 2015;31(6):1328-33. <https://doi.org/10.12669/pjms.316.7525>
7. Akter R, Afrose A, Sharmin S, Rezwan R, Rahman MR, Neelotpol S. A comprehensive look into the association of vitamin D levels and vitamin D receptor gene polymorphism with obesity in children. *Biomed Pharmacother.* 2022;153:113285. <https://doi.org/10.1016/j.biopha.2022.113285>
8. Nam SW, Choi J, Jeon HJ, Oh TK, Lee DH. The associations between vitamin D receptor BsmI and Apal polymorphisms and obesity in Korean patients with type 2 diabetes mellitus. *Diabetes Metab Syndr Obes.* 2021;14:557-64. <https://doi.org/10.2147/DMSO.S293032>
9. Yildiz S, Tumer MK, Yigit S, Nursal AF, Rustemoglu A, Balel Y. Relation of vitamin D and BsmI variant with temporomandibular diseases in the Turkish population. *Br J Oral Maxillofac Surg.* 2021;59(5):555-60. <https://doi.org/10.1016/j.bjoms.2020.08.101>
10. Ellulu MS, Patimah I, Khaza'i H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci.* 2017;13(4):851-63. <https://doi.org/10.5114/aoms.2016.58928>

11. Rodríguez-Rodríguez E, Aparicio A, Andrés P, Ortega RM. Moderate vitamin D deficiency and inflammation related markers in overweight/obese schoolchildren. *Int J Vitam Nutr Res.* 2014;84(1-2):98-107. <https://doi.org/10.1024/0300-9831/a000197>
12. Athanassiou L, Mavragani CP, Koutsilieris M. The immunomodulatory properties of vitamin D. *Mediterr J Rheumatol.* 2022;33(1):7-13. <https://doi.org/10.31138/mjr.33.1.7>
13. Eller ABP, Ejzenberg D, Monteleone PAA, Soares JM, Baracat EC. Vitamin D and in vitro fertilization: a systematic review. *J Assist Reprod Genet.* 2023;40(4):735-43. <https://doi.org/10.1007/s10815-023-02767-2>
14. Lu S, Cao ZB. Interplay between vitamin D and adipose tissue: implications for adipogenesis and adipose tissue function. *Nutrients.* 2023;15(22):4832. <https://doi.org/10.3390/nu15224832>
15. Benito LAO, Kogawa EM, Silva CMS, Melo FF, Sales-Peres SHC, Silva ICRD, et al. Bariatric surgery and vitamin D: trends in older women and association with clinical features and VDR gene polymorphisms. *Nutrients.* 2023;15(4):799. <https://doi.org/10.3390/nu15040799>
16. Akter R, Afrose A, Sharmin S, Rezwan R, Rahman MR, Neelotpol S. A comprehensive look into the association of vitamin D levels and vitamin D receptor gene polymorphism with obesity in children. *Biomed Pharmacother.* 2022;153:113285. <https://doi.org/10.1016/j.biopha.2022.113285>
17. Saad M, Ahmad EA. Vitamin D receptor gene polymorphism among egyptian obese children. *Asian J Clin Nutr.* 2017;9:24-9. <https://doi.org/10.3923/ajcn.2017.24.29>
18. Aravindhan S, Almasoody MFM, Selman NA, Andreevna AN, Ravali S, Mohammadi P, et al. Vitamin D receptor gene polymorphisms and susceptibility to type 2 diabetes: evidence from a meta-regression and meta-analysis based on 47 studies. *J Diabetes Metab Disord.* 2021;20(1):845-67. <https://doi.org/10.1007/s40200-020-00704-z>
19. Karonova T, Grineva E, Belyaeva O, Bystrova A, Jude EB, Andreeva A, et al. Relationship between vitamin D status and vitamin D receptor gene polymorphisms with markers of metabolic syndrome among adults. *Front Endocrinol (Lausanne).* 2018;9:448. <https://doi.org/10.3389/fendo.2018.00448>
20. Ye WZ, Reis AF, Dubois-Laforgue D, Bellanné-Chantelot C, Timsit J, Velho G. Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. *Eur J Endocrinol.* 2001;145(2):181-6. <https://doi.org/10.1530/eje.0.1450181>
21. Correa-Rodríguez M, Carrillo-Ávila JA, Schmidt-RioValle J, González-Jiménez E, Vargas S, Martín J, et al. Genetic association analysis of vitamin D receptor gene polymorphisms and obesity-related phenotypes. *Gene.* 2018;640:51-6. <https://doi.org/10.1016/j.gene.2017.10.029>
22. Al-Hazmi AS, Al-Mehmadi MM, Al-Bogami SM, Shami AA, Al-Askary AA, Alomery AM, et al. Vitamin D receptor gene polymorphisms as a risk factor for obesity in Saudi men. *Electron Physician.* 2017;9(10):5427-33. <https://doi.org/10.19082/5427>
23. Özhan B, Bilgihan E, Çetin O, Ağıladioğlu K. The relation of vitamin D receptor gene polymorphisms with risk of obesity, metabolic syndrome, hepatostetosis in Turkish children. *Pamukkale Med J.* 2023;16(2):274-80. <https://doi.org/10.31362/patd.1249471>
24. Tworowska-Bardzińska U, Lwow F, Kubicka E, Łaczmanski Ł, Jedrzejuk D, Dunajska K, et al. The vitamin D receptor gene BsmI polymorphism is not associated with anthropometric and biochemical parameters describing metabolic syndrome in postmenopausal women. *Gynecol Endocrinol.* 2008;24(9):514-8. <https://doi.org/10.1080/09513590802302985>
25. Kumar AN, Satyanarayana U, Naidu JN, Ramalingam K, Anitha M. Vitamin D receptor gene polymorphism in obese and non obese Indian women with polycystic ovary syndrome. *Int J Curr Res.* 2015;7(8):19686-91.

