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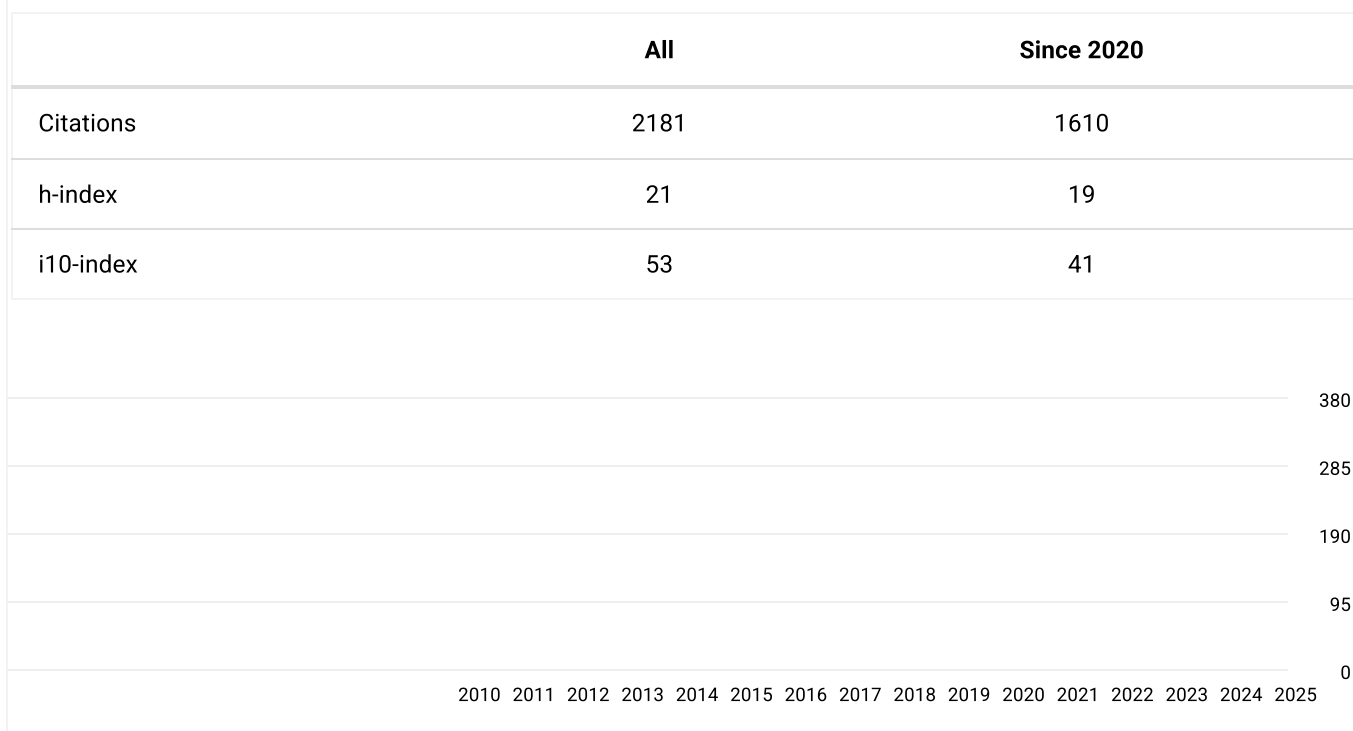
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
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
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
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
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## Section Original Article




# Vitamin D receptor polymorphism associated with obesity in productive age population: A cross-sectional study

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## Abstract

**Background:** Obesity is influenced by genetic factors, lifestyle, and environmental factors. This increase in obesity gives rise to various chronic disease problems.

**Objectives:** This study aimed to identify genetic variations of Vitamin D receptors and determine the relationship to obesity in terms of body mass index and body fat percentage in the working group.

**Methods:** This study is an analytical observational study with a cross-sectional research design approach in the Jembatan Dua area, Angke sub-district, West Jakarta. The research instrument used was a questionnaire to obtain characteristic data, as well as an examination of VDR gene variations rs1544410, rs2228570, rs7975232, and PCR examination to obtain data on gene variation.

**Results:** The results obtained are as follows: the average age of subjects was 42.24, with 58.1% females. A total of 39 (69.1%) are Javanese. Thirty-one (50%) subjects were included in the category of obesity (BMI> 25), and 41 (66.1%) subjects were obese category based on the body fat percentage. The results of bivariate analysis obtained SNP VDR Foxl gene obtained a significant relationship with body mass index with a p-value of 0.047, while the VDR Bsml gene obtained a statistically meaningful relationship with body fat percentage with a p-value of 0.043.

**Conclusion:** Foxl and Bsml VDR polymorphisms are associated with body mass index and body fat percentage.

## Keywords

polymorphism; Vitamin D; obese; gene

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## Vitamin D receptor polymorphism associated with obesity in productive age population: A cross-sectional study

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Original Article

## ABSTRACT

**Background:** Obesity is influenced by genetic factors, lifestyle, and environmental factors. This increase in obesity gives rise to various chronic disease problems.

**Objectives:** This study aimed to identify genetic variations of Vitamin D receptors and determine the relationship to obesity in terms of body mass index and body fat percentage in the working group.

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**Results:** The results obtained are as follows: the average age of subjects was 42.24, with 58.1% females. A total of 39 (69.1%) are Javanese. Thirty-one (50%) subjects were included in the category of obesity (BMI > 25), and 41 (66.1%) subjects were obese category based on the body fat percentage. The results of bivariate analysis obtained SNP VDR FoxI gene obtained a significant relationship with body mass index with a p-value of 0.047, while the VDR BsmI gene obtained a statistically meaningful relationship with body fat percentage with a p-value of 0.043.

**Conclusion:** FoxI and BsmI VDR polymorphisms are associated with body mass index and body fat percentage.

## INTRODUCTION

Obesity is one of the health problems in the world that can occur from children to the elderly. Based on basic health research data held by Ministry of Health Indonesia in 2018, the prevalence of adult obesity increased in a decade from 10.5% to 21.8% using the body mass index (BMI) category  $\geq 27 \text{ kg/m}^2$ . The results of this research show that women suffer more obesity than men and the incidence of obesity in various regions in Indonesia varies depending on economic, demographic, and educational factors.<sup>1</sup> Mortality and morbidity rates will increase with obesity. Chronic degenerative diseases will also increase in number such as diabetes mellitus, cardiovascular disease, osteoarthritis, cancer, and stroke. Obesity is characterized by an increase in the amount of energy stored in the body compared to the amount used. The energy stored in the body can be seen from anthropometric indicators such as body weight, height, abdominal circumference, pelvic circumference, and skinfold thickness.<sup>2</sup> A commonly used indicator to categorize obesity is BMI. Based on the Asia Pacific IMT category, IMT can be classified into obese  $\geq 25 \text{ kg/m}^2$ , overweight 23-24.9  $\text{kg/m}^2$ , normal 18.5-22.9  $\text{kg/m}^2$ , and underweight  $\leq 18 \text{ kg/m}^2$ .



Several studies in Asia suggest that being overweight can be a risk factor for diabetes mellitus, hypertension, and stroke.<sup>3</sup>

Increases and decreases in Vitamin D levels in the body are influenced by several factors, namely external factors and internal factors. External factors such as sun exposure, and food intake. At the same time, internal factors are genetic relationships and skin color. In addition, nutritional status is also related to Vitamin D levels in the blood, both levels and metabolism.<sup>4</sup> One study shows obesity is influenced by vitamin D deficiency. Low levels of vitamin D are associated with metabolic syndrome, autoimmune, cancer, and psychiatric disorders. This study is a continuation of the previous one. A preliminary study in the West Jakarta area aimed to see the picture of risk factors for causing vitamin D deficiency in young adults, it was found that 60.6% of 25(OH)D3 levels of subjects were classified as deficiency, and 33.3% were classified as insufficiency. As much as 93.9% of subjects in this mapping study's 25(OH)D3 levels experienced insufficiency to deficiency.<sup>5</sup> Another study conducted in Angke, West Jakarta found a prevalence of 54% of participants experiencing overweight).<sup>6</sup> Vitamin D is a lipid-soluble hormone so it can be used as a sign of metabolic disorders related to adipose tissue of the body.<sup>7</sup> According to sequestration theory, Vitamin D is a fat-soluble vitamin, so when there is an increase in fat in obese patients, there is an increase in the accumulation of Vitamin D in fat resulting in decreased serum vitamin D levels.<sup>8,9</sup>

Various VD genes can undergo polymorphisms in a singular nucleotide gene level, as found in previously stated cases. In these cases, it can lead to a deficiency of vitamin D, which is caused by dysfunctional VDR. A Singapore study showing a link between BMI and the levels of Vitamin D within the subject underlined the low proportion of Vitamin D adequacy in respondents with an increase in BMI of 35.8%.<sup>10</sup> Meanwhile, a study conducted in Taiwan on workers aged 26-65 years stated different results that there was no relationship between BMI and vitamin D levels, but age and season were important factors for hypovitaminosis D.<sup>11</sup>

Another finding was that variations in the *FokI*, *Apal*, and *TaqI* genes were secondary risk factors for dyslipidemia, hyperglycemia, and hyperparathyroidism, but did not correlate with obesity.<sup>12</sup> There are still differences in research results regarding the relationship between genetic variations in Vitamin D receptors with predisposing factors to metabolic and cardiovascular diseases, especially obesity conditions. The prevalence rate of overweight and hypovitaminosis of vitamin D in the West Jakarta area is quite high, so the purpose of this study is to identify genetic variations of Vitamin D receptors and determine the relationship to obesity in terms of body mass index and body fat percentage in the group of workers in the West Jakarta.

## METHODS

This study is an analytical observational study with a cross-sectional. This study involved a group of Dhamamsavana Foundation workers in the Angke Village area, West Jakarta. The included classes for this study were people aged between 20 to 64 years, workers indoors working 8 hours. These subjects had already consented to the study, and are willing to participate in the study. The people excluded from this study are those with a history of malignancy, kidney failure, autoimmune diseases, and limited mobility. This study used consecutive nonrandom sampling techniques.

The sample size used for this study used the infinite population formula:  $n = Z\alpha \cdot 2pxq / d^2$ , with  $Z\alpha = 1.96$   $p = 0.54$   $q = 1 - 0.54 = 0.46$   $d = 0.05$  followed by the finite population formula  $N = n_0 / (1 + n_0 / N)$ , so a minimum of 60 samples are needed. Blood samples are taken to check fasting blood glucose levels, vitamin D, HDL, and triglycerides. Physical examination is also carried out in the form of height, weight, BMI, body fat percentage, and blood pressure checks of systole, diastole, and temperature.

Measurement procedure and how it works: The body height of the subjects is measured using a Seca height gauge with an accuracy of up to 0.1 cm. The subjects are measured in an upright position. BMI is calculated as the quotient of body mass. It is calculated as the body mass in kilograms divided by the height of the subjects as square meters ( $\text{kg}/\text{m}^2$ ). Systolic (SBP) and diastolic (DBP) blood pressure is measured with a digital sphygmomanometer and calculated as

the average of two consecutive readings. For blood chemistry tests, fasting blood glucose will be sent to the clinical laboratory.

### Genetic Analysis

Blood samples were gathered from veins using tubes containing EDTA. Genomic DNA was extracted from 200 µL of each sample using a DNA isolation kit (Zymo Research, Irvine, CA, USA). The extracted DNA samples were then stored in a freezer at -20°C for future analysis. The VDR polymorphism, including ApaI, BsmI, and FokI, was analyzed using RFLP (Restriction Fragment Length Polymorphism) methods by amplifying targeted DNA regions using PCR within a 50 µL reaction volume containing 25 µL of MyTaq™ HS Red Mix (2X) (Meridian Bioscience, Memphis, Tennessee, USA) and 20 µM of each primer.<sup>13</sup> The polymerase chain reaction involves an initial temperature of 95°C for 3 minutes, then, afterward, it is followed by 30 cycles of denaturation at the temperature of 95°C for 30 seconds, After the previously stated process, it is annealed for 30 seconds with a temperature 95°C, before being extended for 1 minute at 72°C, before finally undergoing final extension step for 5 minutes at 72°C.

For ApaI polymorphism (g.59979G > T; rs7975232), a 745-bp PCR product was generated and digested with 1 U of FastDigest ApaI (Thermo Scientific, Hudson, NH, USA) at 37°C for 5 minutes. No cleavage site for the restriction enzyme was present for the A-allele, resulting in a 745 bp product. Conversely, individuals harboring the 'a-allele' exhibited cleavage products measuring 531- and 214-bp. In the case of BsmI polymorphism (A > G; rs1544410), an 870-bp fragment was amplified and treated with BsmI restriction enzyme (New England BioLabs), leading to variable fragment lengths indicative of different alleles. The allele 'B' was identified when an 870 bp product was present, indicating the absence of a cleavage site. Conversely, allele 'b' was identified using the fragments measured as 460, 234, and 176 bp, respectively. FokI polymorphism (C > T; rs10735810) involved amplification of a 273-bp product that was subjected to enzymatic cleavage using FastDigest FokI (Thermo Scientific, Hudson, NH, USA) at 37°C, producing characteristic fragment patterns. The assignment of alleles 'F' or 'f' was predicated upon the presence of a 273 bp fragment (F-allele), or the identified fragments of 198 and 65 bp fragments (f-allele), respectively. Heterozygous individuals (Ff) displayed products measuring 273, 198, and 65 bp concurrently. DNA fragments were cleaved and separated with 2% agarose gel electrophoresis to then be visualized using Blue light illumination following staining with Floro+green (1st Base, Singapore, Singapore). Enzyme and primer sequences were provided in Table 1.<sup>14</sup>

Table.1 Enzyme restriction sequences

Enzyme	Primers sequences	Genotype
ApaI	Forward: 5'- AGA GCA TGG ACA GGG AGCAAG-3' Reverse: 5'-AGAGCATGGACAGGGAGCAAG-3	AA, Aa, aa
BsmI	Forward: 5'- AACCAGCGGGAAGAGGTCAAGGG-3' Reverse: 5'- CAACCAAGACTACAAGTACCGCGTCAGTGA-3'	BB, Bb,bb
FokI	Forward: 5'- GATGCCAGCTGGCCCTGGCACTG-3' Reverse: 5'- ATGGAAACACCTTGCTTCTTCTCCCTC-3	FF,Ff,ff

### Statistical methods

The data (age, blood pressure, fasting glucose, HDL, triglyceride, and level of vitamin D) will be tested for normality, normal data will be presented as mean +/- SD, abnormal data will be presented as median (min, max), and categorical data (gender, ethnic, body mass index, percentage body fat, genotype VDR) will be presented as n (%). The bivariate test analyses the main hypothesis (VDR polymorphism vs BMI and fat percentage) using the Chi-square test if it meets the requirements or the Fisher test if it does not meet the requirements. Statistical analysis using software SPSS 25.0

## Ethical statement

This research has passed the ethical review Number 002/KER/FK/2024 from the Research Ethics Commission of the Faculty of Medicine, Universitas Trisakti.

## RESULTS

An overview of the characteristics of the subject of study can be seen in Table 2. Based on Table 2, the average age of subjects was 42.24, female as much as 58.1%. A total of 39 (69.1%) are Javanese. Blood pressure obtained average values of 121.82  $\pm$  17.51 mmHg for systolic and 78.24  $\pm$  11.01 for diastolic. Based on body mass index of 31 (50%) included in the category of obesity (BMI > 25) and 41 (66.1%) based on body fat percentage included in the category of obesity. The SNP of the VDR gene of most subjects had an Ff genotype of 34 (54.8%) for FoxI, 42 (67.7%) of the Bb genotype for BsmI, 34 (54.8%) of the Aa genotype for ApaI and 60 (96.8%) of the TT genotype for TaqI.

Table 2. Subject Characteristics (n=62)

Variable	n(%)	mean $\pm$ SD
<b>Gender</b>		
Man	26 (41.9)	
Woman	36 (58.1)	
<b>Ethnic group</b>		
Javanese	39 (62.90)	
Sundanese	13 (20.97)	
Betawi	2 (3.22)	
Batak	3 (4.84)	
Minang	5 (8.07)	
<b>Age</b>		42.24 $\pm$ 8.82
<b>Blood pressure</b>		
Systolic		121.82 $\pm$ 17.51
Diastole		78.24 $\pm$ 11.01
<b>Fasting glucose</b>		89.33 $\pm$ 13.39
<b>HDL (high-density lipoprotein)</b>		53.42 $\pm$ 13.36
<b>Triglyceride</b>		120.76 $\pm$ 78.46
<b>Body mass index</b>		
Obese	31 (50)	
Not obese	31 (50)	
<b>% Body Fat</b>		
Obese	41 (66.1)	
Not obese	21 (33.9)	
<b>Level Vitamin D</b>		16.50 $\pm$ 6.54
<b>GDR</b>		
<b>FoxI genotype</b>		
FF (homozygote )	12 (19.4)	
Ff (heterozygote)	34 (54.8)	
ff	16 (25.8)	
<b>BsmI genotype</b>		
BB (homozygote)	10 (16.1)	
Bb (heterozygote)	42 (67.7)	
bb	19 (16.1)	

Table 2. Subject Characteristics (n=62) (continued)

Variable	n(%)	mean± SD
<b>Apal (rs7975232) genotype</b>		
AA (homozygote polymorphic)	12 (19.4)	
Aa (heterozygote polymorphic)	34 (54.8)	
aa	16 (25.8)	
<b>TaqI genotype</b>		
TT (homozygote polymorphic)	60 (96.8)	
Tt (heterozygote polymorphic)	0 (0)	
tt	2 (3.2)	

Table 3. Bivariate Analysis of Association of VDR Gene SNPs with Body Mass Index (n=62)

SNP VDR	Body mass index			OR	95% CI	p
	Not obese n (%)	Obese N (%)	Total n (%)			
<b>FoxI</b>						
FF (Reference)	6 (19.4)	6 (19.4)	12 (19.4)			0.047*
Ff	15 (48.4)	19 (61.3)	34 (54.8)	5.06	0.91-28.15	
ff	10 (32.3)	6 (19.4)	16 (25.8)	0.67	0.07-6.11	
<b>BsmI</b>						
BB (Reference)	4 (12.9)	6 (19.4)	10 (16.1)			0.781
Bb	22 (71.0)	20 (64.5)	42 (67.7)	0.67	0.16-2.71	
bb	5 (16.1)	5 (16.1)	10 (16.1)	0.67	0.11-3.92	
<b>Apal</b>						
AA (Reference)	6 (19.4)	6 (19.4)	12 (19.4)			0.832
Aa	16 (51.6)	18 (58.1)	34 (54.8)	0.71	0.19-2.76	
aa	9 (29.0)	7 (22.6)	16 (25.8)	0.17	0.17-3.02	
<b>TaqI</b>						
TT	29 (93.5)	31 (100)	60 (96.8)			0.151**
Tt	0	0	0			
tt	2 (6.5)	0	2 (3.2)	0.88	0.10-15.33	

CI: confidence interval; OR: odds ratio; \*p<0.05; \*\* fisher

The results of bivariate analysis showed that SNP of the FoxI VDR gene obtained a significant relationship with body mass index, with a p-value of 0.047 (Table 3), while the BsmI VDR gene obtained a statistically meaningful relationship with body fat percentage, with a p-value of 0.043 (Table 4). Figure 1 presents representative gel electrophoresis results of PCR products digested with *FokI*, *BsmI*, and *Apal* enzymes, clearly showing the banding patterns corresponding to different genotypes.

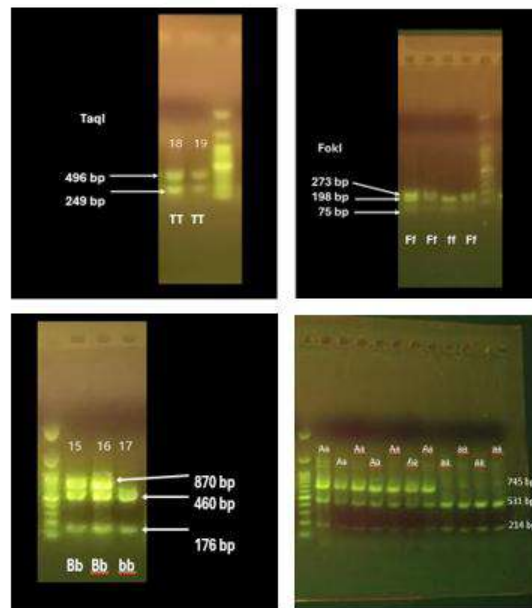


Figure 1. Restriction Fragment Length Polymorphism VDR

Table 4. Bivariate Analysis of SNP Relationship of VDR Gene with Body Fat Percentage (n=62)

SNP gen VDR	Body Fat Percentage			OR	95% CI	p
	Not Obese n (%)	Obese n (%)	Total n (%)			
<b>FoxI</b>						
FF (reference)	4 (19)	8 (19.5)	12 (19.4)			0.937
Ff	11 (52.4)	23 (56.1)	34 (54.8)	3.78	0.78-18.13	
ff	6 (28.6)	10 (24.4)	16 (25.8)	1.2	0.19-7.44	
<b>BsmI</b>						
BB (reference)	1 (4.8)	9 (22)	10 (16.1)			0.043*
Bb	18 (85.7)	24 (58.5)	42 (67.7)	1.37	0.30-6.32	
bb	2 (9.5)	8 (19.5)	10 (16.1)	0.43	0.07-2.68	
<b>Apal</b>						
AA (reference)	4 (19)	8 (19.5)	12 (19.4)			0.96
Aa	12 (57.1)	22 (53.7)	34 (54.8)	0.3	0.56-1.62	
aa	5 (23.8)	11 (26.8)	16 (25.8)	0.8	0.12-5.20	
<b>TaqI</b>						
TT	20 (95.2)	40 (97.6)	60 (96.8)			0.624
Tt	0 (0)	0 (0)	0 (0)			
tt	1 (4.8)	1 (2.4)	2 (3.2)	0.5	0.03-8.42	

OR: odds ratio; CI: confidence interval; \*p&lt;0.05

## DISCUSSION

The levels within the subjects of this study have an average of 16.50 ng/ml, and can be categorized as vitamin D deficient, this may be because the average employee works indoors so that less outdoor activities and inadequate exposure to natural sunlight. Low exposure to sunlight is the main cause of reduced vitamin D intake, which leads to deficiency of vitamin D. Vitamin D has various physiological roles in the body such as hemostatic calcium and phosphate, regulating the process of proliferation and differentiation of the human cells, immune system and also affecting the effectivity of the cardiovascular system. Vitamin D deficiency conditions are found

to affect a variety of acute and chronic diseases. Vitamin D deficiency is associated with various metabolic disorders. These include cases of type 2 diabetes, common obesity, and other kinds of metabolic syndromes as well.<sup>15</sup>

The findings of this study suggest a potential association between VDR gene polymorphisms, particularly FokI VDR gene SNPs, and BMI. This suggests that the presence of VDR genetic variation may potentially hold influence over the regulation of body fat distribution, and the body weight. It seems reasonable to suggest that vitamin D plays an important role in calcium and phosphate metabolism, as well as in several other biological functions, including the modulation of immune and inflammatory responses. These functions may affect body weight regulation.<sup>16,17</sup> Furthermore, vitamin D plays an important role in body weight regulation and energy metabolism, as it is understood that VDR is expressed in various tissues, including the liver, muscle, and adipose tissue, all of which are involved in energy metabolism. This study offers further insight into the potential influence of VDR gene polymorphisms on the body's response to vitamin D, which may in turn affect BMI. The observed association between VDR FokI SNPs and BMI suggests that these genetic variations could be considered potential genetic markers for predicting the risk of obesity or weight loss.<sup>16,18,19</sup>

This study lends further support to the findings of previous studies, which have indicated that VDR may play a role in regulating body weight and body composition. It seems that individuals with VDR gene polymorphisms may tend either a lower or a higher BMI, depending on the specific gene variation in question. It seems that the interaction between genes and environmental factors, such as exposure, may depend on the type of variation involved. Additionally, some studies have suggested that the interaction between the VDR gene and environmental factors, such as dietary vitamin D intake and sun exposure, may potentially influence BMI.<sup>19,20</sup>

One study in China suggests that there may be a potential association between FokI polymorphism and increased susceptibility to metabolic syndrome in the pediatric age group.<sup>21</sup> There is some evidence to suggest that the VDR polymorphism may be associated with increased BMI. This could be due to the location of genes that are more related to vitamin D receptor function, which may affect gene transcription activity, mRNA stability, or interactions with co-factors that are important in metabolic processes. Furthermore, it is possible that changes in receptor structure due to these polymorphisms could alter the affinity or binding effectiveness of vitamin D, which could then affect various metabolic pathways, including those involved in body weight regulation and body fat distribution.<sup>22,23</sup> It seems likely that the interaction between VDR polymorphisms and several environmental factors such as vitamin D intake, sun exposure, and lifestyle may contribute to BMI variability and be associated with the onset of several diseases.<sup>24,25</sup>

The Chinese study found that VDR SNP variations did not correlate with BMI and WC, but these VDR variations were related to triceps skin fold thickness and body fat percentage. In the GT Apal genotype is associated with higher cholesterol and LDL levels. The VDR variant has an important influence on adipose and adipose network activity in Han China.<sup>2</sup> VDR is expressed in the adipose tissues, examples include 3-TC-LI adipocyte, the SAT and VAT presence in humans, the human preadipocytes, and the other differentiated adipocytes as well. This adipose tissue has various functions for lipid synthesis, fatty acid transport, and adipokine secretion<sup>21</sup>. The study by Fang found that there is no relationship between VDR SNP and BMI or WC, the difference can be influenced by different ethnic backgrounds. The increase in body fat percentage is linked with the presence of the T allele of FokI and the T allele of Apal, meanwhile, the cases of triceps skin fold thickness are linked with the G allele of rs2239179 and the T allele of Apal.<sup>2</sup>

It seems that there might be a link between the BsmI SNP polymorphism in the VDR gene and body fat percentage. This could be due to some factors, including the location of the BsmI SNP in an area that affects the vitamin D receptor in functionality and expressionality. It is thought that the VDR gene intronic region is where the BsmI SNP is located, which could potentially affect the mRNA splicing process and VDR protein expression. It is thought that variations in the splicing process may result in the production of different vitamin D receptor isoforms, which could potentially lead to variations in biological activity. It is thought that variations in BsmI SNPs may

affect the binding affinity of vitamin D to the receptor, which could potentially influence the transcriptional activity of genes regulated by VDR. This could have implications for the regulation of genes involved in body fat distribution and fat metabolism. It is also possible that BsmI polymorphism may affect the stability of mRNA, which could in turn affect the amount of VDR protein present in adipose cells.<sup>26,27</sup>

Polymorphism ApaI and BsmI genotypes showed significant differences in WC, WHR, body fat percentage, TG, and HDL-C levels, implying that these specific VDR genotypes are linked particularly to the lipid profile and distribution. Dyslipidemia, encompassing hypertriglyceridemia and low HDL-C, constitutes a facet of metabolic syndrome. Vitamin D's potential impact on lipid metabolism has been a subject of interest. Numerous epidemiological investigations have explored the nexus between vitamin D levels and dyslipidemia. Vitamin D status has a positive correlation between vitamin D with serum HDL-C levels, along with a negative correlation with TG within the context of lipid regulation, adipose tissue takes on a pivotal role. It is noteworthy that this tissue expresses the VDR along with enzymes involved in vitamin D metabolism and associated signaling pathways, thus emerging as the principal reservoir for vitamin D storage.<sup>28-30</sup>

It seems that genetic variation in the form of BsmI polymorphism may influence leptin levels in the body. The adipocyte cells produce the Leptin hormone, which affects energy metabolism and is thought to regulate appetite. It seems possible that the VDR gene polymorphism may affect how vitamin D interacts with adipose tissue, affecting leptin production. It is reasonable to suggest that leptin plays an important role in energy regulation and body weight management. It is also reasonable to suggest leptin levels affect body mass index (BMI) and body fat percentage. It is thought the hypothalamus in the brain affects leptin's function to inhibit appetite and increase energy expenditure. In individuals with a normal body weight, there is a fluctuation in leptin levels by the body's fat reserves. However, in individuals who are obese, leptin levels are typically elevated, yet they may experience leptin resistance, which is when the body does not respond effectively to leptin signals. This can result in disturbances in the regulation of appetite and energy expenditure. Furthermore, leptin resistance in obese individuals can contribute to the difficulty in losing weight despite high leptin levels.<sup>31-34</sup>

Vitamin D deficiency can cause an increased risk of diabetes mellitus.<sup>35</sup> VDR gene variations that have been known to have a relationship with obesity are *FokI* (rs 2228570), *BsmI* (rs1544410), *ApaI* (rs7975232), and *TaqI* (rs 731236). Expression of mRNA and proteins from VDRs such as BsmI is associated with an increased incidence of obesity.<sup>26</sup> Several studies provide pro and con results on the relationship of VDR gene polymorphism with obesity. The results of the study of Tobias et al. showed that vitamin D 25 OH levels were lower in subjects with more weight and obesity compared to subjects with normal and less weight.<sup>36</sup> This is associated with a very slow vitamin D release rate, caused by body fat, which will lead to low serum 25(OH)D levels.

Studies in Iranian populations are known to show a relationship between variations in *ApaI* and obesity, and become one of the risk factors for obesity. However, the *TaqI* gene was found to have no meaningful association with obesity.<sup>25</sup> Rayinda et.al found the *BsmI* gene has a connection to insulin resistance and the lack of vitamin D within the body. However, this had no association with the occurrence of obesity cases in tropical populations. The study also suggested that the *FokI* gene has been associated with an increased incidence of diabetes mellitus in Asian populations.<sup>27</sup> The *FokI* gene was found to be associated with increased blood glucose levels and BMI of more weight and/or obese in Turkey.

The findings that this study had shown have the potential bring contribution toward developing an effective strategy for the control of obesity and body weight. VDR polymorphisms have been shown to influence vitamin D metabolism, highlighting the significance of consuming nutritional and vitamin D supplementation approaches and other environmental factors. However, there are still limitations of this study, including the absence of certain crucial environmental factors, such as sun exposure, dietary habits, and vitamin D supplementation.



## CONCLUSION

Based on this study, the polymorphisms of Vitamin D receptors are associated with body mass index (BMI) and the percentage of body fat. FoxI and BsmI were associated with obesity and body weight. Other strategies are needed to prevent obesity such as physical activity, dietary restriction, and vitamin D supplementation. Further studies are needed to explore other VDR gene polymorphisms as the larger sample.

## CONFLICT OF INTEREST

The authors should make a conflict-of-interest disclosure statement or a declaration that they do not have any conflicts of interest. They should disclose at the time of revision any financial arrangement they may have with a company whose product is pertinent to the submitted manuscript or with a company making a competing product. Such information will be held in confidence - while the paper is under review - and will not influence the editorial process.

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The authors declare that there is no conflict of interest in this study.

## DATA AVAILABILITY

Data sharing does not apply to this article.

## SUPPLEMENTAL DATA

None.

## AUTHOR CONTRIBUTIONS

Conceptualization: Y, M. Data curation: Y, M, MD, EM. Format analysis: M, EM, RZ. Methodology: Y, M, MDH writing of the original draft: Y. Writing of review and editing: M, MD, RZ.

## DECLARATION OF USING AI IN THE WRITING PROCESS

We are using the Grammarly application for English grammar and Mendeley for arranging references.

## LIST OF ABBREVIATIONS

VDR= Vitamin D Receptor; SNP= Single Nucleotide Polymorphism; WC= Waist Circumference; BMI= Body Mass Index

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# Vitamin D receptor polymorphism associated with obesity in productive age population: A cross-sectional study

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## Vitamin D receptor polymorphism associated with obesity in productive age population: A cross-sectional study

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Original Article

## ABSTRACT

**Background:** Obesity is influenced by genetic factors, lifestyle, and environmental factors. This increase in obesity gives rise to various chronic disease problems.

**Objectives:** This study aimed to identify genetic variations of Vitamin D receptors and determine the relationship to obesity in terms of body mass index and body fat percentage in the working group.

**Methods:** This study is an analytical observational study with a cross-sectional research design approach in the Jembatan Dua area, Angke sub-district, West Jakarta. The research instrument used was a questionnaire to obtain characteristic data, as well as an examination of VDR gene variations rs1544410, rs2228570, rs7975232, and PCR examination to obtain data on gene variation.

**Results:** The results obtained are as follows: the average age of subjects was 42.24, with 58.1% females. A total of 39 (69.1%) are Javanese. Thirty-one (50%) subjects were included in the category of obesity (BMI > 25), and 41 (66.1%) subjects were obese category based on the body fat percentage. The results of bivariate analysis obtained SNP VDR FokI gene obtained a significant relationship with body mass index with a p-value of 0.047, while the VDR BsmI gene obtained a statistically meaningful relationship with body fat percentage with a p-value of 0.043.

**Conclusion:** FokI and BsmI VDR polymorphisms are associated with body mass index and body fat percentage.

## INTRODUCTION

Obesity is one of the health problems in the world that can occur from children to the elderly. Based on basic health research data held by Ministry of Health Indonesia in 2018, the prevalence of adult obesity increased in a decade from 10.5% to 21.8% using the body mass index (BMI) category  $\geq 27 \text{ kg/m}^2$ . The results of this research show that women suffer more obesity than men and the incidence of obesity in various regions in Indonesia varies depending on economic, demographic, and educational factors.<sup>1</sup> Mortality and morbidity rates will increase with obesity. Chronic degenerative diseases will also increase in number such as diabetes mellitus, cardiovascular disease, osteoarthritis, cancer, and stroke. Obesity is characterized by an increase in the amount of energy stored in the body compared to the amount used. The energy stored in the body can be seen from anthropometric indicators such as body weight, height, abdominal circumference, pelvic circumference, and skinfold thickness.<sup>2</sup> A commonly used indicator to categorize obesity is BMI. Based on the Asia Pacific IMT category, IMT can be classified into obese  $\geq 25 \text{ kg/m}^2$ , overweight 23-24.9  $\text{kg/m}^2$ , normal 18.5-22.9  $\text{kg/m}^2$ , and underweight  $\leq 18 \text{ kg/m}^2$ .



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Several studies in Asia suggest that being overweight can be a risk factor for diabetes mellitus, hypertension, and stroke.<sup>3</sup>

Increases and decreases in Vitamin D levels in the body are influenced by several factors, namely external factors and internal factors. External factors such as sun exposure, and food intake. At the same time, internal factors are genetic relationships and skin color. In addition, nutritional status is also related to Vitamin D levels in the blood, both levels and metabolism.<sup>4</sup> One study shows obesity is influenced by vitamin D deficiency. Low levels of vitamin D are associated with metabolic syndrome, autoimmune, cancer, and psychiatric disorders. This study is a continuation of the previous one. A preliminary study in the West Jakarta area aimed to see the picture of risk factors for causing vitamin D deficiency in young adults, it was found that 60.6% of 25(OH)D3 levels of subjects were classified as deficiency, and 33.3% were classified as insufficiency. As much as 93.9% of subjects in this mapping study's 25(OH)D3 levels experienced insufficiency to deficiency.<sup>5</sup> Another study conducted in Angke, West Jakarta found a prevalence of 54% of participants experiencing overweight).<sup>6</sup> Vitamin D is a lipid-soluble hormone so it can be used as a sign of metabolic disorders related to adipose tissue of the body.<sup>7</sup> According to sequestration theory, Vitamin D is a fat-soluble vitamin, so when there is an increase in fat in obese patients, there is an increase in the accumulation of Vitamin D in fat resulting in decreased serum vitamin D levels.<sup>8,9</sup>

Various VD genes can undergo polymorphisms in a singular nucleotide gene level, as found in previously stated cases. In these cases, it can lead to a deficiency of vitamin D, which is caused by dysfunctional VDR. A Singapore study showing a link between BMI and the levels of Vitamin D within the subject underlined the low proportion of Vitamin D adequacy in respondents with an increase in BMI of 35.8%.<sup>10</sup> Meanwhile, a study conducted in Taiwan on workers aged 26-65 years stated different results that there was no relationship between BMI and vitamin D levels, but age and season were important factors for hypovitaminosis D.<sup>11</sup>

Another finding was that variations in the *FokI*, *Apal*, and *TaqI* genes were secondary risk factors for dyslipidemia, hyperglycemia, and hyperparathyroidism, but did not correlate with obesity.<sup>12</sup> There are still differences in research results regarding the relationship between genetic variations in Vitamin D receptors with predisposing factors to metabolic and cardiovascular diseases, especially obesity conditions. The prevalence rate of overweight and hypovitaminosis of vitamin D in the West Jakarta area is quite high, so the purpose of this study is to identify genetic variations of Vitamin D receptors and determine the relationship to obesity in terms of body mass index and body fat percentage in the group of workers in the West Jakarta.

## METHODS

This study is an analytical observational study with a cross-sectional. This study involved a group of Dhamamsavana Foundation workers in the Angke Village area, West Jakarta. The included classes for this study were people aged between 20 to 64 years, workers indoors working 8 hours. These subjects had already consented to the study, and are willing to participate in the study. The people excluded from this study are those with a history of malignancy, kidney failure, autoimmune diseases, and limited mobility. This study used consecutive nonrandom sampling techniques.

The sample size used for this study used the infinite population formula:  $n = Z_{\alpha}^2 \cdot p \cdot q / d^2$ , with  $Z_{\alpha} = 1.96$   $p = 0.54$   $q = 1 - 0.54 = 0.46$   $d = 0.05$  followed by the finite population formula  $N = n_0 / (1 + n_0 / N)$ , so a minimum of 60 samples are needed. Blood samples are taken to check fasting blood glucose levels, vitamin D, HDL, and triglycerides. Physical examination is also carried out in the form of height, weight, BMI, body fat percentage, and blood pressure checks of systole, diastole, and temperature.

Measurement procedure and how it works: The body height of the subjects is measured using a Seca height gauge with an accuracy of up to 0.1 cm. The subjects are measured in an upright position. BMI is calculated as the quotient of body mass. It is calculated as the body mass in kilograms divided by the height of the subjects as square meters (kg/m<sup>2</sup>). Systolic (SBP) and diastolic (DBP) blood pressure is measured with a digital sphygmomanometer and calculated as



the average of two consecutive readings. For blood chemistry tests, fasting blood glucose will be sent to the clinical laboratory.

### Genetic Analysis

Blood samples were gathered from veins using tubes containing EDTA. Genomic DNA was extracted from 200 µL of each sample using a DNA isolation kit (Zymo Research, Irvine, CA, USA). The extracted DNA samples were then stored in a freezer at -20°C for future analysis. The VDR polymorphism, including Apal, BsmI, and FokI, was analyzed using RFLP (Restriction Fragment Length Polymorphism) methods by amplifying targeted DNA regions using PCR within a 50 µL reaction volume containing 25 µL of MyTaq™ HS Red Mix (2X) (Meridian Bioscience, Memphis, Tennessee, USA) and 20 µM of each primer.<sup>13</sup> The polymerase chain reaction involves an initial temperature of 95°C for 3 minutes, then, afterward, it is followed by 30 cycles of denaturation at the temperature of 95°C for 30 seconds. After the previously stated process, it is annealed for 30 seconds with a temperature 95°C, before being extended for 1 minute at 72°C, before finally undergoing final extension step for 5 minutes at 72°C.

For Apal polymorphism (g.59979G > T; rs7975232), a 745-bp PCR product was generated and digested with 1 U of FastDigest Apal (Thermo Scientific, Hudson, NH, USA) at 37°C for 5 minutes. No cleavage site for the restriction enzyme was present for the A-allele, resulting in a 745 bp product. Conversely, individuals harboring the 'a-allele' exhibited cleavage products measuring 531- and 214-bp. In the case of BsmI polymorphism (A > G; rs1544410), an 870-bp fragment was amplified and treated with BsmI restriction enzyme (New England BioLabs), leading to variable fragment lengths indicative of different alleles. The allele 'B' was identified when an 870 bp product was present, indicating the absence of a cleavage site. Conversely, allele 'b' was identified using the fragments measured as 460, 234, and 176 bp, respectively. FokI polymorphism (C > T; rs10735810) involved amplification of a 273-bp product that was subjected to enzymatic cleavage using FastDigest FokI (Thermo Scientific, Hudson, NH, USA) at 37°C, producing characteristic fragment patterns. The assignment of alleles 'F' or 'f' was predicated upon the presence of a 273 bp fragment (F-allele), or the identified fragments of 198 and 65 bp fragments (f-allele), respectively. Heterozygous individuals (Ff) displayed products measuring 273, 198, and 65 bp concurrently. DNA fragments were cleaved and separated with 2% agarose gel electrophoresis to then be visualized using Blue light illumination following staining with Floro+green (1st Base, Singapore, Singapore). Enzyme and primer sequences were provided in Table 1.<sup>14</sup>

Table.1 Enzyme restriction sequences

Enzyme	Primers sequences	Genotype
Apal	Forward: 5'- AGA GCA TGG ACA GGG AGCAAG-3'	AA, Aa, aa
	Reverse: 5'-AGAGCATGGACAGGAGCAAG-3	
BsmI	Forward: 5'- AACCAGCGGGAAGAGGTCAAGGG-3'	BB, Bb,bb
	Reverse: 5'- CAACCAAGACTACAAGTACCGGTCAGTGA-3'	
FokI	Forward: 5'- GATGCCAGCTGGCCCTGGCACTG-3'	FF,Ff,ff
	Reverse: 5'- ATGGAAACACCTTGCTTCTTCTCCCTG-3	

### Statistical methods

The data (age, blood pressure, fasting glucose, HDL, triglyceride, and level of vitamin D) will be tested for normality, normal data will be presented as mean +/- SD, abnormal data will be presented as median (min, max), and categorical data (gender, ethnic, body mass index, percentage body fat, genotype VDR) will be presented as n (%). The bivariate test analyses the main hypothesis (VDR polymorphism vs BMI and fat percentage) using the Chi-square test if it meets the requirements or the Fisher test if it does not meet the requirements. Statistical analysis using software SPSS 25.0

## Ethical statement

This research has passed the ethical review Number 002/KER/FK/2024 from the Research Ethics Commission of the Faculty of Medicine, Universitas Trisakti.

## RESULTS

An overview of the characteristics of the subject of study can be seen in Table 2. Based on Table 2, the average age of subjects was 42.24, female as much as 58.1%. A total of 39 (69.1%) are Javanese. Blood pressure obtained average values of 121.82  $\pm$  17.51 mmHg for systolic and 78.24  $\pm$  11.01 for diastolic. Based on body mass index of 31 (50%) included in the category of obesity (BMI > 25) and 41 (66.1%) based on body fat percentage included in the category of obesity. The SNP of the VDR gene of most subjects had an Ff genotype of 34 (54.8%) for FoxI, 42 (67.7%) of the Bb genotype for BsmI, 34 (54.8%) of the Aa genotype for ApaI and 60 (96.8%) of the TT genotype for TaqI.

Table 2. Subject Characteristics (n=62)

Variable	n(%)	mean $\pm$ SD
<b>Gender</b>		
Man	26 (41.9)	
Woman	36 (58.1)	
<b>Ethnic group</b>		
Javanese	39 (62.90)	
Sundanese	13 (20.97)	
Betawi	2 (3.22)	
Batak	3 (4.84)	
Minang	5 (8.07)	
<b>Age</b>		42.24 $\pm$ 8.82
<b>Blood pressure</b>		
Systolic		121.82 $\pm$ 17.51
Diastole		78.24 $\pm$ 11.01
<b>Fasting glucose</b>		89.33 $\pm$ 13.39
<b>HDL (high-density lipoprotein)</b>		53.42 $\pm$ 13.36
<b>Triglyceride</b>		120.76 $\pm$ 78.46
<b>Body mass index</b>		
Obese	31 (50)	
Not obese	31 (50)	
<b>% Body Fat</b>		
Obese	41 (66.1)	
Not obese	21 (33.9)	
<b>Level Vitamin D</b>	16.50 $\pm$ 6.54	
<b>GDR</b>		
<b>FoxI genotype</b>		
FF (homozygote )	12 (19.4)	
Ff (heterozygote)	34 (54.8)	
ff	16 (25.8)	
<b>BsmI genotype</b>		
BB (homozygote)	10 (16.1)	
Bb (heterozygote)	42 (67.7)	
bb	19 (16.1)	



Table 2. Subject Characteristics (n=62) (continued)

Variable	n(%)	mean± SD
<b>Apal (rs7975232) genotype</b>		
AA (homozygote polymorphic)	12 (19.4)	
Aa (heterozygote polymorphic)	34 (54.8)	
aa	16 (25.8)	
<b>TaqI genotype</b>		
TT (homozygote polymorphic)	60 (96.8)	
Tt (heterozygote polymorphic)	0 (0)	
tt	2 (3.2)	

Table 3. Bivariate Analysis of Association of VDR Gene SNPs with Body Mass Index (n=62)

SNP VDR	Body mass index			OR	95% CI	p
	Not obese n (%)	Obese N (%)	Total n (%)			
<b>FoxI</b>						
FF (Reference)	6 (19.4)	6 (19.4)	12 (19.4)			0.047*
Ff	15 (48.4)	19 (61.3)	34 (54.8)	5.06	0.91-28.15	
ff	10 (32.3)	6 (19.4)	16 (25.8)	0.67	0.07-6.11	
<b>BsmI</b>						
BB (Reference)	4 (12.9)	6 (19.4)	10 (16.1)			0.781
Bb	22 (71.0)	20 (64.5)	42 (67.7)	0.67	0.16-2.71	
bb	5 (16.1)	5 (16.1)	10 (16.1)	0.67	0.11-3.92	
<b>Apal</b>						
AA (Reference)	6 (19.4)	6 (19.4)	12 (19.4)			0.832
Aa	16 (51.6)	18 (58.1)	34 (54.8)	0.71	0.19-2.76	
aa	9 (29.0)	7 (22.6)	16 (25.8)	0.17	0.17-3.02	
<b>TaqI</b>						
TT	29 (93.5)	31 (100)	60 (96.8)			0.151**
Tt	0	0	0			
tt	2 (6.5)	0	2 (3.2)	0.88	0.10-15.33	

CI: confidence interval; OR: odds ratio; \*p&lt;0.05; \*\* fisher

The results of bivariate analysis showed that SNP of the FoxI VDR gene obtained a significant relationship with body mass index, with a p-value of 0.047 (Table 3), while the BsmI VDR gene obtained a statistically meaningful relationship with body fat percentage, with a p-value of 0.043 (Table 4). Figure 1 presents representative gel electrophoresis results of PCR products digested with *FokI*, *BsmI*, and *Apal* enzymes, clearly showing the banding patterns corresponding to different genotypes.

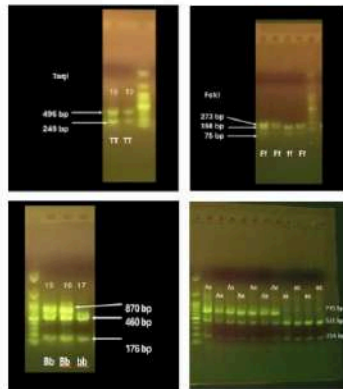


Figure 1. Restriction Fragment Length Polymorphism VDR

Table 4. Bivariate Analysis of SNP Relationship of VDR Gene with Body Fat Percentage (n=62)

SNP gen VDR	Body Fat Percentage			OR	95% CI	p
	Not Obese n (%)	Obese n (%)	Total n (%)			
<b>FoxI</b>						
FF (reference)	4 (19)	8 (19.5)	12 (19.4)			0.937
Ff	11 (52.4)	23 (56.1)	34 (54.8)	3.78	0.78-18.13	
ff	6 (28.6)	10 (24.4)	16 (25.8)	1.2	0.19-7.44	
<b>BsmI</b>						
BB (reference)	1 (4.8)	9 (22)	10 (16.1)			0.043*
Bb	18 (85.7)	24 (58.5)	42 (67.7)	1.37	0.30-6.32	
bb	2 (9.5)	8 (19.5)	10 (16.1)	0.43	0.07-2.68	
<b>ApaI</b>						
AA (reference)	4 (19)	8 (19.5)	12 (19.4)			0.96
Aa	12 (57.1)	22 (53.7)	34 (54.8)	0.3	0.56-1.62	
aa	5 (23.8)	11 (26.8)	16 (25.8)	0.8	0.12-5.20	
<b>TaqI</b>						
TT	20 (95.2)	40 (97.6)	60 (96.8)			0.624
Tt	0 (0)	0 (0)	0 (0)			
tt	1 (4.8)	1 (2.4)	2 (3.2)	0.5	0.03-8.42	

OR: odds ratio; CI: confidence interval; \*p&lt;0.05

## DISCUSSION

The levels within the subjects of this study have an average of 16.50 ng/ml, and can be categorized as vitamin D deficient, this may be because the average employee works indoors so that less outdoor activities and inadequate exposure to natural sunlight. Low exposure to sunlight is the main cause of reduced vitamin D intake, which leads to deficiency of vitamin D. Vitamin D has various physiological roles in the body such as hemostatic calcium and phosphate, regulating the process of proliferation and differentiation of the human cells, immune system and also affecting the effectivity of the cardiovascular system. Vitamin D deficiency conditions are found

to affect a variety of acute and chronic diseases. Vitamin D deficiency is associated with various metabolic disorders. These include cases of type 2 diabetes, common obesity, and other kinds of metabolic syndromes as well.<sup>15</sup>

The findings of this study suggest a potential association between VDR gene polymorphisms, particularly FokI VDR gene SNPs, and BMI. This suggests that the presence of VDR genetic variation may potentially hold influence over the regulation of body fat distribution, and the body weight. It seems reasonable to suggest that vitamin D plays an important role in calcium and phosphate metabolism, as well as in several other biological functions, including the modulation of immune and inflammatory responses. These functions may affect body weight regulation.<sup>16,17</sup> Furthermore, vitamin D plays an important role in body weight regulation and energy metabolism, as it is understood that VDR is expressed in various tissues, including the liver, muscle, and adipose tissue, all of which are involved in energy metabolism. This study offers further insight into the potential influence of VDR gene polymorphisms on the body's response to vitamin D, which may in turn affect BMI. The observed association between VDR FokI SNPs and BMI suggests that these genetic variations could be considered potential genetic markers for predicting the risk of obesity or weight loss.<sup>16,18,19</sup>

This study lends further support to the findings of previous studies, which have indicated that VDR may play a role in regulating body weight and body composition. It seems that individuals with VDR gene polymorphisms may tend either a lower or a higher BMI, depending on the specific gene variation in question. It seems that the interaction between genes and environmental factors, such as exposure, may depend on the type of variation involved. Additionally, some studies have suggested that the interaction between the VDR gene and environmental factors, such as dietary vitamin D intake and sun exposure, may potentially influence BMI.<sup>19,20</sup>

One study in China suggests that there may be a potential association between FokI polymorphism and increased susceptibility to metabolic syndrome in the pediatric age group.<sup>21</sup> There is some evidence to suggest that the VDR polymorphism may be associated with increased BMI. This could be due to the location of genes that are more related to vitamin D receptor function, which may affect gene transcription activity, mRNA stability, or interactions with co-factors that are important in metabolic processes. Furthermore, it is possible that changes in receptor structure due to these polymorphisms could alter the affinity or binding effectiveness of vitamin D, which could then affect various metabolic pathways, including those involved in body weight regulation and body fat distribution.<sup>22,23</sup> It seems likely that the interaction between VDR polymorphisms and several environmental factors such as vitamin D intake, sun exposure, and lifestyle may contribute to BMI variability and be associated with the onset of several diseases.<sup>24,25</sup>

The Chinese study found that VDR SNP variations did not correlate with BMI and WC, but these VDR variations were related to triceps skin fold thickness and body fat percentage. In the GT Apal genotype is associated with higher cholesterol and LDL levels. The VDR variant has an important influence on adipose and adipose network activity in Han China.<sup>2</sup> VDR is expressed in the adipose tissues, examples include 3-TC-LI adipocyte, the SAT and VAT presence in humans, the human preadipocytes, and the other differentiated adipocytes as well. This adipose tissue has various functions for lipid synthesis, fatty acid transport, and adipokine secretion<sup>21</sup>. The study by Fang found that there is no relationship between VDR SNP and BMI or WC, the difference can be influenced by different ethnic backgrounds. The increase in body fat percentage is linked with the presence of the T allele of FokI and the T allele of Apal, meanwhile, the cases of triceps skin fold thickness are linked with the G allele of rs2239179 and the T allele of Apal.<sup>2</sup>

It seems that there might be a link between the BsmI SNP polymorphism in the VDR gene and body fat percentage. This could be due to some factors, including the location of the BsmI SNP in an area that affects the vitamin D receptor in functionality and expressionality. It is thought that the VDR gene intronic region is where the BsmI SNP is located, which could potentially affect the mRNA splicing process and VDR protein expression. It is thought that variations in the splicing process may result in the production of different vitamin D receptor isoforms, which could potentially lead to variations in biological activity. It is thought that variations in BsmI SNPs may

affect the binding affinity of vitamin D to the receptor, which could potentially influence the transcriptional activity of genes regulated by VDR. This could have implications for the regulation of genes involved in body fat distribution and fat metabolism. It is also possible that BsmI polymorphism may affect the stability of mRNA, which could in turn affect the amount of VDR protein present in adipose cells.<sup>26,27</sup>

Polymorphism ApaI and BsmI genotypes showed significant differences in WC, WHR, body fat percentage, TG, and HDL-C levels, implying that these specific VDR genotypes are linked particularly to the lipid profile and distribution. Dyslipidemia, encompassing hypertriglyceridemia and low HDL-C, constitutes a facet of metabolic syndrome. Vitamin D's potential impact on lipid metabolism has been a subject of interest. Numerous epidemiological investigations have explored the nexus between vitamin D levels and dyslipidemia. Vitamin D status has a positive correlation between vitamin D with serum HDL-C levels, along with a negative correlation with TG within the context of lipid regulation, adipose tissue takes on a pivotal role. It is noteworthy that this tissue expresses the VDR along with enzymes involved in vitamin D metabolism and associated signaling pathways, thus emerging as the principal reservoir for vitamin D storage.<sup>28-30</sup>

It seems that genetic variation in the form of BsmI polymorphism may influence leptin levels in the body. The adipocyte cells produce the Leptin hormone, which affects energy metabolism and is thought to regulate appetite. It seems possible that the VDR gene polymorphism may affect how vitamin D interacts with adipose tissue, affecting leptin production. It is reasonable to suggest that leptin plays an important role in energy regulation and body weight management. It is also reasonable to suggest leptin levels affect body mass index (BMI) and body fat percentage. It is thought the hypothalamus in the brain affects leptin's function to inhibit appetite and increase energy expenditure. In individuals with a normal body weight, there is a fluctuation in leptin levels by the body's fat reserves. However, in individuals who are obese, leptin levels are typically elevated, yet they may experience leptin resistance, which is when the body does not respond effectively to leptin signals. This can result in disturbances in the regulation of appetite and energy expenditure. Furthermore, leptin resistance in obese individuals can contribute to the difficulty in losing weight despite high leptin levels.<sup>31-34</sup>

Vitamin D deficiency can cause an increased risk of diabetes mellitus.<sup>35</sup> VDR gene variations that have been known to have a relationship with obesity are *FokI* (rs 2228570), *BsmI* (rs1544410), *ApaI* (rs7975232), and *TaqI* (rs 731236). Expression of mRNA and proteins from VDRs such as BsmI is associated with an increased incidence of obesity.<sup>26</sup> Several studies provide pro and con results on the relationship of VDR gene polymorphism with obesity. The results of the study of Tobias et al. showed that vitamin D 25 OH levels were lower in subjects with more weight and obesity compared to subjects with normal and less weight.<sup>36</sup> This is associated with a very slow vitamin D release rate, caused by body fat, which will lead to low serum 25(OH)D levels.

Studies in Iranian populations are known to show a relationship between variations in *ApaI* and obesity, and become one of the risk factors for obesity. However, the *TaqI* gene was found to have no meaningful association with obesity.<sup>25</sup> Rayinda et.al found the *BsmI* gene has a connection to insulin resistance and the lack of vitamin D within the body. However, this had no association with the occurrence of obesity cases in tropical populations. The study also suggested that the *FokI* gene has been associated with an increased incidence of diabetes mellitus in Asian populations.<sup>27</sup> The *FokI* gene was found to be associated with increased blood glucose levels and BMI of more weight and/or obese in Turkey.

The findings that this study had shown have the potential bring contribution toward developing an effective strategy for the control of obesity and body weight. VDR polymorphisms have been shown to influence vitamin D metabolism, highlighting the significance of consuming nutritional and vitamin D supplementation approaches and other environmental factors. However, there are still limitations of this study, including the absence of certain crucial environmental factors, such as sun exposure, dietary habits, and vitamin D supplementation.



## CONCLUSION

Based on this study, the polymorphisms of Vitamin D receptors are associated with body mass index (BMI) and the percentage of body fat. FoxI and BsmI were associated with obesity and body weight. Other strategies are needed to prevent obesity such as physical activity, dietary restriction, and vitamin D supplementation. Further studies are needed to explore other VDR gene polymorphisms as the larger sample.

## CONFLICT OF INTEREST

The authors should make a conflict-of-interest disclosure statement or a declaration that they do not have any conflicts of interest. They should disclose at the time of revision any financial arrangement they may have with a company whose product is pertinent to the submitted manuscript or with a company making a competing product. Such information will be held in confidence - while the paper is under review - and will not influence the editorial process.

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The authors declare that there is no conflict of interest in this study.

## DATA AVAILABILITY

Data sharing does not apply to this article.

## SUPPLEMENTAL DATA

None.

## AUTHOR CONTRIBUTIONS

Conceptualization: Y, M. Data curation: Y, M, MD, EM. Format analysis: M, EM, RZ. Methodology: Y, M, MDH writing of the original draft: Y. Writing of review and editing: M, MD, RZ.

## DECLARATION OF USING AI IN THE WRITING PROCESS

We are using the Grammarly application for English grammar and Mendeley for arranging references.

## LIST OF ABBREVIATIONS

VDR= Vitamin D Receptor; SNP= Single Nucleotide Polymorphism; WC= Waist Circumference; BMI= Body Mass Index

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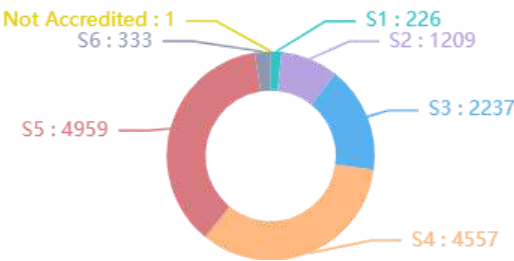
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