ONLINE FIRST

This is a provisional PDF only. Copyedited and fully formatted version will be made available soon.

POLISH GYNECOLOGY
GINEKOLOGIA POLSKA
ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGICZNEGO
THE OFFICIAL JOURNAL OF THE POLISH GYNECOLOGICAL SOCIETY

ISSN: 0017-0011

e-ISSN: 2543-6767

Assessment of the impact of VDR polymorphisms on selected hormonal, metabolic and mineral balance markers in young women with hyperandrogenism

Authors: Izabela Uzar, Anna Bogacz, Elzbieta Sowinska-Przepiera, Anhelli Syrenicz, Adam Kaminski, Marlena Wolek, Bogusław Czerny

DOI: 10.5603/gpl.96519

Article type: Research paper

Submitted: 2023-07-14

Accepted: 2024-07-02

Published online: 2024-12-18

Articles in "Ginekologia Polska" are listed in PubMed.

[ORIGINAL PAPER / GYNECOLOGY]

Assessment of the impact of *VDR* polymorphisms on selected hormonal, metabolic and mineral balance markers in young women with hyperandrogenism

Izabela Uzar[1], Anna Bogacz[2], Elzbieta Sowinska-Przepiera[3], Anhelli Syrenicz[3], Adam Kaminski[4], Marlena Wolek[2], Bogusław Czerny[1, 2]

[1]*Department of Pharmacology and Pharmacoeconomics, Pomeranian Medical University in Szczecin, Poland*

[2]*Department of Stem Cells and Regenerative Medicine, Institute of Natural Fibers and Medicinal Plants, Plewiska, Poland*

[3]*Department of Endocrinology, Metabolic Diseases, and Internal Diseases, Pomeranian Medical University in Szczecin, Poland*

[4]*Department of Orthopaedics and Traumatology, Independent Public Clinical Hospital No. 1, Pomeranian Medical University in Szczecin, Poland*

:Corresponding author

Izabela Uzar

Department of Pharmacology and Pharmacoeconomics, Pomeranian Medical University in Szczecin, 48 Żołnierska St., 71–230 Szczecin, Poland

e-mail: uzari@wp.pl

ABSTRACT

Objectives: Hyperandrogenism is a frequently recognized endocrine imbalance in which there is excessive production of androgens. The purpose of the study was to investigate the impact of vitamin D receptor (VDR) gene polymorphisms on chosen bone metabolism and .biochemical parameters in women with hyperandrogenism

Material and methods: Eighty young females with hyperandrogenism were enrolled in the study, in whom selected parameters of bone turnover, endocrine and metabolic parameters were determined. Two polymorphisms of the *VDR* gene were analyzed: rs731236 (*TaqI*) and rs1544410 (*BsmI*), using real-time polymerase chain reaction (PCR). Statistical tests were .performed in this research with the program SPSS Statistics 17.0 for Windows

Results: The rs731236 and rs1544410 polymorphisms of the *VDR* gene turned out to be statistically significantly related to the concentration of insulin determined in the 60 'glucose tolerance test. There was no relationship between the studied polymorphisms of the *VDR* gene .and the determined parameters of bone metabolism and other biochemical parameters

Conclusions: The research presented that *VDR* gene variants may influence disturbances in .carbohydrate metabolism in young women with hyperandrogenism

Keywords: hyperandrogenism; *VDR* polymorphisms; bone metabolism; biomarkers, hormonal parameters

INTRODUCTION

Hyperandrogenism is characterized by excessive production of androgenic hormones in women and is their most common endocrine disorder, affects up to 10% of females of reproductive age [1]. In about 80% of cases, hyperandrogenism accompanies polycystic ovary syndrome (PCOS). Other causes of excessive androgenization include non-classical congenital adrenal hyperplasia (NCAH), ovarian tumors, and menopause. Idiopathic hyperandrogenemia or idiopathic hirsutism can affect 3.9–15.8% of women [1–3]. In healthy women, androgens are produced in almost equal amounts by the ovaries and adrenal glands. Determining the origin of androgens in hyperandrogenism may be useful in understanding the type and specificity of disorders accompanying hyperandrogenization. The ovaries are the

main source of androgens in idiopathic hyperandrogenism and PCOS, and the adrenal glands in NCAH [4]. Hyperandrogenism in women is characterized by progressive defeminization and the appearance of androgenization symptoms such as seborrhea, acne, hirsutism, androgenic alopecia and metabolic changes [1, 5]. Hyperandrogenism in young females is associated with a wide variation in the degree of expression of individual symptoms, and the degree of their intensity and type do not indicate a specific cause. The symptoms of hyperandrogenization may also appear when the serum concentration of androgens is normal [6, 7].

The active form of vitamin D_3 with hormonal properties is an important factor regulating the calcium-phosphate balance in the human body. However, the role of vitamin D_3 is not limited to its influence on bone metabolism; it is also an important parameter influencing the hormonal balance, and its deficiency plays an important role in the pathogenesis of many endocrine and autoimmune diseases and cancers [8, 9]. It is also known that vitamin D_3 is important for fertility in both women and men by influencing the expression of VDR receptors for vitamin D_3 in such organs as ovaries, uterus, placenta, testes, pituitary and hypothalamus [9, 10]. It has been shown that low vitamin 25 (OH) D levels in women aged 35–51 may be associated with high levels of follicle stimulating hormone (FSH), which leads to lower estrogen levels [10]. The presence of VDR receptor polymorphisms is also associated with metabolic and endocrine disorders in PCOS and hyperandrogenism [11]. Vitamin D_3 plays a role in the synthesis of sex hormones by affecting the expression and activity of enzymes involved in steroidogenesis, and its deficiency may contribute to the occurrence of endometriosis due to its immunomodulatory and anti-inflammatory properties. It has been shown that the active form of vitamin D_3 — calcitriol — increases the production of progesterone by 13%, estradiol by 9%, and estrone by 21% [12]. Calcitriol may also affect the activity of aromatase catalyzing the conversion of androgens to estrogens [9].

The effect of vitamin D on various tissues is modulated by its receptor — VDR. By combining with the VDR receptor, vitamin D activates the transcription of genes dependent on it [11]. The vitamin D receptor gene is highly polymorphic. The *BsmI* polymorphism (rs1544410) seems to be related to metabolic and hormonal disorders in PCOS, but not all authors agree on this. However, it is believed that the presence of this polymorphism increases the risk of PCOS as well as influences the level of vitamin D [11, 13, 14].

In the case of *TaqI* polymorphism of the VDR receptor (rs731236), most authors show its relationship with an increased risk of PCOS and significantly affect the levels of hormonal

and metabolic parameters, as well as an increase in the prevalence of hirsutism and its severity in PCOS [13]. In numerous publications to date, the relationships between *VDR* polymorphisms and metabolic and hormonal parameters in PCOS have been analyzed [13]. There is no similar research on disorders in young women with idiopathic hyperandrogenism.

Hyperandrogenism is a complex disorder with a genetic basis involving natural and epigenetic variables. The explanation of the hereditary background aims to improve diagnosis and treatment options. The aim of this project was to analyze the relationship between the occurrence of rs731236 (*TaqI*) and rs1544410 (*BsmI*) polymorphisms and selected biochemical, hormonal and bone metabolism parameters in young females with hyperandrogenism.

MATERIAL AND METHODS

Patients

The research included 80 young females aged 18 to 35 years with hyperandrogenism examined in 2013 and 2015 in the Department of Endocrinology, Metabolic Diseases and .Internal Medicine at the Pomeranian Medical University in Szczecin, Poland

The following were the inclusion criteria for the study: Caucasian race, absence of menstruation for at least six months followed by at least three months of oligomenorrhea, clinical symptoms of masculinization: acne, seborrhea, hirsutism, without long-term medications, and without significant abnormalities on physical examination. The exclusion criteria were: PCOS, congenital adrenal hyperplasia or premature ovarian failure, low birth weight, prematurity, nutritional disorders, abnormal nutrition during childhood or adolescence, growth and weight gain diseases, intensive sport, metabolic diseases, chronic use of stimulants or drugs that affect bone metabolism**,** and bone disease in the family. All patients also had a concentration of androstenedione (a precursor of androgens), leptin and body mass index (BMI) above reference values. Patients with idiopathic hyperandrogenism (the presence of clinical and biochemical hyperandrogenism in the absence of PCOS features), were included in the study [15, 16].

The severity of hirsutism was determined according to the Ferriman–Gallwey scale (≥ 8 points). We selected two single nucleotide polymorphisms (SNPs): rs731236, rs1544410 on the basis of the following criteria: minor allele frequency > 0.2, functional relevance and

importance, SNPs significantly associated with bone mineral density (BMD) in previous studies.

The research was approved by the Bioethics Committee of the Pomeranian Medical University, number KB-0012/115/15 of 16 November 2015. The study was performed in accord with the Helsinki Declaration (1975, corrected 2000).

Analysis of serum concentrations for selected factors

Each patient had fasting blood collection at 8 am and centrifuged. Immunoenzymatic tests (ELISA — DRG International, Inc.) were used to determine sRANKL (free and bound RANKL), osteoprotegerin (OPG) and 25-OH vitamin D total concentrations. Serum parathormone and calcitonin concentrations were measured with a chemiluminescent assay (Immulite 1000, Siemens). Electrochemiluminescent tests (Cobas, Roche Diagnostic) were used to determine luteinizing hormone (LH), folliculotropic hormone (FSH), 17-hydroxyprogesterone, estradiol, prolactin (PRL), testosterone (T), androstenedione, dehydroepiandrosterone sulfate (DHEA-SO4), sex hormone binding globulin (SHBG), glucose and insulin. Free testosterone concentration was calculated from the free androgen index ((FAI = TT/SHBG × 100%). The analytical sensitivities of the assays were: calcitonin — 2.0 pg/mL, parathormone — 3.1 pg/mL, 25-OH vitamin D total — 5.6 nmol/L, sRANKL — 0.5 pmol/L, OPG — 0.14 pmol/L, leptin — 2.0 ng/mL. Tests were conducted according to the manufacturer's instructions and subjected to quality control using the manufacturer's two-level control set. Samples were performed in duplicate. The microplate reader and microplate washer used for ELISA assays and the precision of the assays were checked using Pathozyme ELISA Sure kit (Omega Diagnostics, UK).

Bone mineral density (BMD) analysis

Bone mineral density (BMD) analysis was performed at the Department of Endocrinology, Metabolic Diseases and Internal Medicine at the Pomeranian Medical University in Szczecin, Poland. BMD was analyzed in the lumbar spine from L2 to L4 vertebrae using Dual Energy X-ray Absorptiometry (DEXA) method with the LUNAR DPX 100 (Lunar Corp., Madison, USA). **The quantitative body mass composition (*i.e.* total body fat — BF), android, gynoid fat, visceral adipose tissue (VAT) and lean body mass were measured in all participants by DEXA using an automatic whole body scanning method. The original manufacturer's software (Body Composition) was used to determine the individual areas of measurements (female, male and visceral region).** All

patients were imaged using the same DEXA Lunar device to minimize inter-device variability. Quality assurance on this device was performed as recommended by the International Society .[for Clinical Densitometry (ISCD) [17

Analysis of the rs731236 and the rs1544410 polymorphisms of the *VDR* gene

Analysis of *VDR* gene polymorphisms was conducted in the Clinical Laboratory at the Department of Endocrinology, Metabolic Diseases, and Internal Medicine at Pomeranian Medical University. Genomic DNA was isolated from peripheral blood using a QIAamp Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's procedure. *VDR* genetic variants: rs731236 (*TaqI*), rs1544410 (*BsmI*) were selected for the study according to the NCBI SNP database http://www.ncbi.nlm.nih.gov/SNP. Genotyping was conducted RT-PCR with TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, USA) on a LightCycler 480 according manufacturer's procedure: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, 40°C for 30 s (20 μL reaction mixture).

Statistical analysis

The results of the study were analyzed statistically using SPSS Statistics 17.0 for Windows. Hardy–Weinberg equilibrium of the polymorphisms was analyzed by the chi-square test. We analyzed the frequency of the studied SNPs and the association with selected biochemical and clinical factors using one-way analysis of variance (ANOVA). Values with normal distribution were presented as means ± SEM (standard error of the mean).

RESULTS

We compared the distribution of genotypes frequencies for the rs731236 and rs1544410 polymorphisms of the *VDR* gene in women with hyperandrogenism. For the rs731236 polymorphism, the *AG* was more common (53.8%) compared to the *AA* (32.5%) and *GG* (13.8%) genotypes was observed. In patients with the *VDR* rs1544410 polymorphism, a higher frequency of the *CT* genotype (52.5%) compared to *CC* (32.5%) and *TT* (15.0%) was observed (Tab. 1).

Analysis of the *VDR* polymorphism rs731236 showed a higher concentration of insulin 60' and insulin 120' in women with the *GG* genotype (insulin 60': *GG* — 172.10 ± 36.29 mg/dL vs *AA* — 156.59 ± 16.48 mg/dL, *AG* — 102.74 ± 9.44 mg/dL, p = 0.008; insulin

120’: *GG* — 119.65 ± 42.56 mg/dL vs *AA* — 100.79 ± 13.78 mg/dL, *AG* — 67.76 ± 7.94 mg/dL, p = 0.063) (Tab. 4). Vitamin D and vitamin 25 (OH) D measurements showed a higher concentration in women with the *AA* genotype (vitamin D: *AA* — 19.32 ± 3.58 ng/mL vs *AG* — 14.98 ± 1.68 ng/mL, *GG* — 8.06 ± 0.68 ng/mL, p = 0.081; vitamin 25 (OH) D: *AA* — 23.82 ± 1.73 ng/mL vs *AG* — 21.56 ± 0.97 ng/mL, *GG* — 17.38 ± 2.39 ng/mL, p = 0.067) (Tab. 5).

Concentrations of the other parameters analyzed: estradiol, FEI (Free Estradiol Index), prolactin, 17-OH-progesterone, LH, FSH, SHGB, testosterone, BAT, FAI, androstendione, DHEA-SO4, glucose 0’, glucose 60’, glucose 120’, insulin 0’, calcitonin, parathormone, OPG, sRANKL, BMD L1-L4, T-score, Z-score, BMD total, BMI, BMC, AG, VF and TBS in context of the of the rs731236 showed no significant associations.

Analysis of the second *VDR* polymorphism rs1544410 showed higher concentrations of insulin 60’ and insulin 120’ in women with the *TT* genotype (insulin 60’: *TT* — 172.10 ± 36.29 mg/dL vs *CC* — 156.59 ± 16.48 mg/dL, *CT* — 102.74 ± 9.44 mg/dL, p = 0.008; insulin 120’: *TT* — 119.65 ± 42.56 mg/dL, CC — 100.79 ± 13.78 mg/dL, *CT* — 67.76 ± 7.94 mg/dL, p = 0.063) (Tab. 4). The concentration of vitamin D was higher in women with the *CC* genotype (*CC* — 19.32 ± 3.58 ng/mL vs *CT* — 14.98 ± 1.68 ng/mL, *TT* — 8.06 ± 0.68 ng/mL, p = 0.081) (Tab. 5).

Measurements of the other parameters analyzed: estradiol, FEI, prolactin, 17-OH-progesterone LH, FSH, SHGB, testosterone, BAT, FAI, androstendione, DHEA-SO4, glucose 0’, glucose 60’, glucose 120’, insulin 0’, calcitonin, parathyroid hormone, osteoprotegerin, sRANKL, BMD L1-L4, T-score, Z-score, BMD total, BMI, BMC, AG, VF and TBS in women with hyperandrogenism in relation to the *VDR* rs1544410 polymorphism distribution of genotypes did not show any significant differences.

Moreover, the statistical power for the two only statistically significant comparisons was 0.2396 for *AA* vs *GG* and 0.2592 for *CC* vs *TT*, respectively. These are preliminary results to be confirmed in a larger group. Nevertheless, the highly significant p value (p = 0.008) for both comparisons indicates that such differences may exist.

DISCUSSION

The analysis of the *VDR* gene polymorphisms showed a relationship for the *TaqI* rs731236 and BsmI rs1544410 polymorphisms and the insulin concentration determined at 60

minutes (insulin 60') in the glucose tolerance test. In the case of the rs731236 *VDR* polymorphism, *GG* homozygotes showed a significantly higher concentration of 60' insulin compared to *AA* homozygotes and *AG* heterozygotes. For the rs1544410 *VDR* polymorphism, a significantly higher concentration of 60' insulin was observed in *TT* homozygotes compared to *CC* and *CT* genotypes. For both polymorphisms, a significant trend was observed in relation to the insulin concentration determined in the 120' glucose tolerance test (insulin 120'). For the rs731236 *VDR* polymorphism, the 120' insulin concentration was higher for the *GG* genotype compared to the *AA* and *AG* genotypes. In the case of the rs1544410 *VDR* polymorphism, the 120' insulin concentration was higher for the *TT* genotype compared to the *CC* and *CT* genotypes. In addition, the *GG* homozygotes of the rs731236 polymorphism occurred with a similar frequency to the *TT* homozygotes of the rs1544410 polymorphism, which may enhance carbohydrate disturbances in patients. In all the patients with hyperandrogenism, the 60' and 120' insulin concentration in the glucose tolerance test was above the reference range. In the literature, Xavier et al. [18] indicate that the rs731236 and rs1544410 polymorphisms of the *VDR* gene are related to PCOS, in which carbohydrate disorders are frequent. It has been shown that vitamin D deficiency may be related to insulin resistance. Insulin secretion by pancreatic beta cells is regulated by the concentration of calcium. Vitamin D, by regulating calcium concentration and polymorphisms of the VDR gene for vitamin D, may influence insulin secretion by pancreatic beta cells [8]. It has been shown that low vitamin D levels are also associated with type 2 diabetes in PCOS, although the mechanism is not fully known [19]. In a study on the Brazilian population, Rodrigues et al. [20] also observed an association of type 2 diabetes with low 25(OH)D levels. However, they did not observe an association between the frequency of the genotype or alleles of the rs1544410 and rs731236 polymorphisms, and type 2 diabetes. This study included 101 patients of the Brazilian population with type 2 diabetes. However, many publications showed an association between *VDR* polymorphisms and carbohydrate disorders [20]. Mayer et al. [21] indicate that low vitamin D levels are often associated with altered glucose metabolism. They observed a significant relationship between the level of 25(OH)D and fasting blood glucose and insulin sensitivity. They also described the relationship of low vitamin 25(OH)D level and decreased insulin resistance with the rs2228570 polymorphism of the VDR receptor. Shaat et al. [22] demonstrated the relationship between the rs1544410 *VDR* polymorphism and the increase in insulin secretion in women after pregnancy complicated by gestational diabetes mellitus (GDM). In studies on 13-year-olds (72% of girls) living in tropical countries, Rahmadhani et al. [23] noticed a relationship between the rs1544410 VDR

polymorphism and the risk of vitamin D deficiency, and that the AA genotype shows a significantly lower level of vitamin 25 (OH) D compared to other genotypes. This was also observed in our study, but it was not statistically significant. The AA genotype was associated with a higher risk of vitamin D deficiency and insulin resistance compared to the GG genotype. In the meta-analysis by Han et al. [24] presented the relationship between the rs1544410 *VDR* polymorphism and the metabolic syndrome (MetS) and the rs731236 *VDR* polymorphism with PCOS. In addition, they observed an association of *BsmI* and *Taq1* polymorphisms with diseases associated with insulin resistance in Caucasians with dark pigmentation. Many studies show that vitamin D deficiencies are higher in dark-pigmented Caucasians and Asians because of the lower ability to produce this vitamin in the skin. On the other hand, Apaydin et al. [25] studied *BsmI* and *TaqI* polymorphisms in pregnant Turkish women and found no relationship between them and gestational diabetes melitus. Thus, the results of studies on the effect of *VDR* polymorphisms on carbohydrate metabolism are not always unambiguous and not always easy to interpret, due to the multitude of factors affecting glycemia and diseases dependent on insulin resistance. However, it is known that vitamin D, via the VDR receptor, plays a key role in the insulin metabolic pathway and insulin secretion both by controlling calcium levels and affecting pancreatic beta cells, and it also inhibits the immune response in type 2 diabetes [20, 25]. Therefore, it seems justified to focus research on metabolic disorders also in young women with hyperandrogenism. Because their early diagnosis would enable the prevention of more serious disorders. As indicated by the previously cited publications, this is also important during pregnancy, which may be complicated by diabetes [22, 25]. The results of our research, although requiring confirmation on a larger group of patients, seem to be consistent with the earlier observations of many authors and indicate a possible relationship between VDR polymorphisms and disturbances in carbohydrate metabolism also in young women with hyperandrogenism. They open up a new aspect of interest in a group of women who have not been studied so often in this area. It is likely that vitamin D supplementation may increase insulin sensitivity in hyperandrogenemia and PCOS [26]. It is known that insulin resistance is common in PCOS, and that hyperandrogenism itself is associated with insulin resistance [27–29]. Insulin also enhances LH-stimulated androgen production. On the other hand, androgens may reduce insulin sensitivity [29]. Hyperinsulinemia and insulin resistance, as suggested by Talaei et al. [30], may be associated with the occurrence of hirsutism in patients with PCOS and idiopathic hirsutism. Vitamin D concentration is also associated with clinical hyperandrogenism in women [18]. A trend was observed for vitamin D concentration for both polymorphisms. For

the rs731236 *VDR* polymorphism, this concentration was higher for the *AA* genotype compared to the *AG* and *GG* genotypes. For the rs1544410 *VDR* polymorphism, the vitamin D concentration was higher in carriers of the *CC* genotype compared to the *CT* and TT genotypes. For the rs731236 *VDR* polymorphism, a trend was also shown for the vitamin 25 (OH) D concentration, which was higher in *AA* homozygotes compared to *AG* heterozygotes and *GG* homozygotes. In all the participants with hyperandrogenism, vitamin D concentration was below the reference value range in deficit (< 20 ng/mL). These results may indicate a relationship of vitamin D concentration to the *TaqI* and *BsmI* polymorphisms of the VDR gene in young women with hyperandrogenism, but this requires further research. It has been proven in numerous studies that the effect of vitamin D is not limited only to the regulation of mineral balance but is also of great importance for hormonal and carbohydrate metabolism. Vitamin D interacts through VDR receptors located in various tissues and organs involved in the regulation of calcium and carbohydrate metabolism and reproductive functions and modulates their functions. *VDR* gene polymorphisms have been identified as affecting androgen secretion disorders in PCOS [31]. Vitamin D is also of key importance in bone metabolism in PCOS patients [32]. However, the results regarding the relationship between VDR gene polymorphisms and the risk of PCOS are not clear [33]. Some authors confirm this relationship [34], while others do not show it [31, 35, 36]. Vitamin D deficiency may also be associated with an increased risk of PCOS, and vitamin D alone may protect PCOS patients against osteoporosis [26]. No statistically significant relationship between the rs731236 and rs1544410 *VDR* gene polymorphisms and bone mineral density and other parameters of bone metabolism was found in the studies carried out in women with hyperandrogenism, similarly to the studies by Bander et al. [37] or Seremak-Mrozikiewicz et al. [38] for the *TaqI* polymorphism. This may be related to the relatively small group of women and their young age. Also, the results of a meta-analysis by Shen et al. [39] did not confirm the relationship between the *VDR* gene polymorphisms *BsmI, TaqI* as well as *ApaI* and FokI with the risk of fractures in postmenopausal women. On the other hand, Stathopolou et al. [40] did not observe an association of the *BsmI* and *TaqI* polymorphisms with BMD, osteoporosis, and the risk of osteoporotic fractures in Greek postmenopausal women. The results of many studies are often ambiguous because osteoporosis is a disorder with a complex etiopathogenesis and influenced by numerous factors, for example, the type of population studied, ethnic and geographical differences, as found in the meta-analysis of Zintzaras et al. [41]. However, many authors confirm the relationship of *VDR* gene polymorphisms with bone mineral density, for example Banjabi et al. [42], who showed a significantly higher risk of developing

osteoporosis for *TaqI* polymorphism carriers. Similarly, Ahmad et al. [43] point to the *TaqI* polymorphism as an important risk factor for the development of osteoporosis and significantly associated with BMD in menopausal women. Douroudis et al. [44] indicate a significant correlation between *VDR* polymorphisms and lower bone mineral density in postmenopausal women.

In this project, no relationship between the rs731236 and rs1544410 polymorphisms of the *VDR* gene was found with other determined biochemical and clinical parameters. On the other hand, Ranjzad et al. [45] demonstrated the association of *VDR* gene polymorphisms with the concentration of LH and SHBG in women with PCOS. Women with the *GG* variant of the *VDR* gene rs1544410 SNP had a lower level of SHBG in relation to the *AA* variant. In contrast, the *GG* genotype turned out to be a likely risk factor for PCOS because it was associated with an increase in bioavailable androgens in women with PCOS. In this study, a correlation between the *CC* genotype of the *VDR* gene rs731236 polymorphism and the LH concentration was also observed [45]. Earlier studies showed an association between insulin resistance and hyperandrogenism, and that PCOS was associated with a significant decrease in insulin sensitivity, independent of obesity. The influence of the active form of vitamin D — calcitriol — on FSH secretion has also been suggested [42]. Jukic et al. [10] observed that a low concentration of vitamin 25 (OH) D leads to an increase in FSH secretion and a decrease in the concentration of estrogen in premenopausal women aged 30–49, and that the concentration of vitamin 25 (OH) D is positively correlated with the concentration of anti-Müllerian hormone (AMH), which is an ovarian reserve marker.

This study was designed to determine the effects of VDR receptor variants on bone metabolism, metabolic and hormonal factors in young females with hyperandrogenizm. Our research also has limitations. The main potential limitation is a relatively small number of participants without a control group. More studies involving more women are needed as idiopathic hyperandrogenization is an increasingly common disorder contributing to metabolic and mineral imbalances and to social problems. There are few reports on this in the literature, as the focus of interest is hyperandrogenization associated with PCOS. This project is part of the search for the relationship between genetic conditions and the type of disorders observed, as well as the possibility of using molecular tests in the diagnosis and therapy of hyperandrogenism and coexisting disorders and their prevention.

CONCLUSIONS

The research presents that *VDR* gene polymorphisms may be related to disturbances in carbohydrate metabolism in young women with hyperandrogenism. The rs731236 and rs1544410 polymorphisms of the *VDR* gene turned out to be statistically significantly related to the concentration of insulin determined in the 60 'glucose tolerance test. This may suggest the possibility of changes in carbohydrate metabolism, but more research is needed on a larger group of patients. The relationship of these polymorphisms with the parameters of bone turnover and other biochemical and clinical parameters has not been demonstrated.

Article information and declarations

Ethics statement

The study was approved by the Ethical Committee of the Pomeranian Medical University (no. KB-0012/115/15 of 16 November 2015).

Author contributions

Conceptualization, methodology, preparation of the manuscript — IU; validation and formal analysis — AB; writing: review and editing — ESP; validation and formal analysis — AS; writing: review — AK; analysis of data — MW; supervision, project administration — BC.

Conflict of interest

All authors declare no conflict of interest.

Funding

The study was supported by statutory projects from the Institute of Natural Fibers and Medicinal Plants in Poznań and Pomeranian Medical University in Szczecin, Poland.

References

1. Sowińska-epiera E, Niedzielska M. Hiperandrogenism in women as a clinical diagnosthic and therapeutical problem. Prz Lek. 2018; 75(8): 405–410.
2. Carmina E, Rosato F, Jannì A, et al. Extensive clinical experience: relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism. J Clin Endocrinol Metab. 2006; 91(1): 2–6, doi: 10.1210/jc.2005-1457, indexed in Pubmed: 16263820.

3. Sanchón R, Gambineri A, Alpañés M, et al. Prevalence of functional disorders of androgen excess in unselected premenopausal women: a study in blood donors. Hum Reprod. 2012; 27(4): 1209–1216, doi: 10.1093/humrep/des028, indexed in Pubmed: 22343706.

4. Carmina E. Ovarian and adrenal hyperandrogenism. Ann N Y Acad Sci. 2006; 1092: 130–137, doi: 10.1196/annals.1365.011, indexed in Pubmed: 17308139.

5. Ajmal N, Khan SZ, Shaikh R. Polycystic ovary syndrome (PCOS) and genetic predisposition: a review article. Eur J Obstet Gynecol Reprod Biol X. 2019; 8(3): 100060, doi: 10.1016/j.eurox.2019.100060, indexed in Pubmed: 31403134.

6. Unluhizarci K, Kaltsas G, Kelestimur F. Non polycystic ovary syndrome-related endocrine disorders associated with hirsutism. Eur J Clin Invest. 2012; 42(1): 86–94, doi: 10.1111/j.1365-2362.2011.02550.x, indexed in Pubmed: 21623779.

7. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016; 37(5): 467–520, doi: 10.1210/er.2015-1104, indexed in Pubmed: 27459230.

8. Wehr E, Trummer O, Giuliani A, et al. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. Eur J Endocrinol. 2011; 164(5): 741–749, doi: 10.1530/EJE-11-0134, indexed in Pubmed: 21389086.

9. Muscogiuri G, Altieri B, de Angelis C, et al. Shedding new light on female fertility: the role of vitamin D. Rev Endocr Metab Disord. 2017; 18(3): 273–283, doi: 10.1007/s11154-017-9407-2, indexed in Pubmed: 28102491.

10. Jukic AM, Steiner AZ, Baird DD. Association between serum 25-hydroxyvitamin D and ovarian reserve in premenopausal women. Menopause. 2015; 22(3): 312–316, doi: 10.1097/GME.0000000000000312, indexed in Pubmed: 25093721.

11. Song DoK, Lee H, Hong YS, et al. Vitamin D receptor and binding protein polymorphisms in women with polycystic ovary syndrome: a case control study. BMC Endocr Disord. 2019; 19(1): 145, doi: 10.1186/s12902-019-0477-x, indexed in Pubmed: 31870342.

12. Parikh G, Varadinova M, Suwandhi P, et al. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. Horm Metab Res. 2010; 42(10): 754–757, doi: 10.1055/s-0030-1262837, indexed in Pubmed: 20711952.

13. Vulcan T, Filip GA, Lenghel LM, et al. Polymorphisms of vitamin D receptor and the effect on metabolic and endocrine abnormalities in polycystic ovary syndrome: a review. Horm Metab Res. 2021; 53(10): 645–653, doi: 10.1055/a-1587-9336, indexed in Pubmed: 34544196.

14. Al Thomali A, Daghestani MH, Daghestani MH, et al. Polymorphic variations in VDR gene in saudi women with and without polycystic ovary syndrome (PCOS) and significant influence of seven polymorphic sites on anthropometric and hormonal parameters. J Med Biochem. 2018; 37(4): 415–425, doi: 10.2478/jomb-2018-0007, indexed in Pubmed: 30584400.

15. Ortiz-Flores AE, Martínez-García MÁ, Nattero-Chávez L, et al. Iron overload in functional hyperandrogenism: in a randomized trial, bloodletting does not improve metabolic outcomes. J Clin Endocrinol Metab. 2021; 106(4): e1559–e1573, doi: 10.1210/clinem/dgaa978, indexed in Pubmed: 33462622.

16. Luque-Ramírez M, Ortiz-Flores AE, Martínez-García MÁ, et al. Effect of iron depletion by bloodletting vs. observation on oxidative stress biomarkers of women with functional hyperandrogenism taking a combined oral contraceptive: a randomized clinical trial. J Clin Med. 2022; 11(13): 3864–3880, doi: 10.3390/jcm11133864, indexed in Pubmed: 35807149.

17. Shuhart CR, Yeap SS, Anderson PA, et al. Executive summary of the 2019 ISCD position development conference on monitoring treatment, DXA cross-calibration and least significant change, spinal cord injury, peri-prosthetic and orthopedic bone health, transgender medicine, and pediatrics. J Clin Densitom. 2019; 22(4): 453–471, doi: 10.1016/j.jocd.2019.07.001, indexed in Pubmed: 31400968.

18. Xavier LB, Gontijo NA, Rodrigues KF, et al. Polymorphisms in vitamin D receptor gene, but not vitamin D levels, are associated with polycystic ovary syndrome in Brazilian women.

Gynecol Endocrinol. 2019; 35(2): 146–149, doi: 10.1080/09513590.2018.1512966, indexed in Pubmed: 30182771.

19. Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab. 2007; 92(6): 2017–2029, doi: 10.1210/jc.2007-0298, indexed in Pubmed: 17389701.

20. Rodrigues KF, Pietrani NT, Bosco AA, et al. Lower vitamin D levels, but not VDR polymorphisms, influence type 2 diabetes mellitus in brazilian population independently of obesity. Medicina (Kaunas). 2019; 55(5): 188, doi: 10.3390/medicina55050188, indexed in Pubmed: 31121922.

21. Mayer O, Seidlerová J, Černá V, et al. Serum vitamin D status, vitamin D receptor polymorphism, and glucose homeostasis in healthy subjects. Horm Metab Res. 2018; 50(1): 56–64, doi: 10.1055/s-0043-122144, indexed in Pubmed: 29183090.

22. Shaat N, Katsarou A, Shahida B, et al. Association between the rs1544410 polymorphism in the vitamin D receptor (VDR) gene and insulin secretion after gestational diabetes mellitus. PLoS One. 2020; 15(5): e0232297, doi: 10.1371/journal.pone.0232297, indexed in Pubmed: 32407388.

23. Rahmadhani R, Zaharan NL, Mohamed Z, et al. The associations between VDR BsmI polymorphisms and risk of vitamin D deficiency, obesity and insulin resistance in adolescents residing in a tropical country. PLoS One. 2017; 12(6): e0178695, doi: 10.1371/journal.pone.0178695, indexed in Pubmed: 28617856.

24. Han FF, Lv YL, Gong LL, et al. VDR Gene variation and insulin resistance related diseases. Lipids Health Dis. 2017; 16(1): 157, doi: 10.1186/s12944-017-0477-7, indexed in Pubmed: 28822353.

25. Apaydın M, Beysel S, Eyerci N, et al. The VDR gene FokI polymorphism is associated with gestational diabetes mellitus in Turkish women. BMC Med Genet. 2019; 20(1): 82, doi: 10.1186/s12881-019-0820-0, indexed in Pubmed: 31096931.

26. Guraya SS, Alhussaini KA, Shaqrun FM, et al. Correlation of clinical, radiological and serum analysis of hypovitaminosis D with polycystic ovary syndrome: A systematic review and meta-analysis. J Taibah Univ Med Sci. 2017; 12(4): 277–283, doi: 10.1016/j.jtumed.2017.02.005, indexed in Pubmed: 31435252.

27. Zeng X, Xie YJ, Liu YT, et al. Polycystic ovarian syndrome: correlation between hyperandrogenism, insulin resistance and obesity. Clin Chim Acta. 2020; 502: 214–221, doi: 10.1016/j.cca.2019.11.003, indexed in Pubmed: 31733195.

28. Otto-Buczkowska E, Grzyb K, Jainta N. Polycystic ovary syndrome (PCOS) and the accompanying disorders of glucose homeostasis among girls at the time of puberty. Pediatr Endocrinol Diabetes Metab. 2018; 24(1): 40–44, doi: 10.18544/PEDM-24.01.0101, indexed in Pubmed: 30083660.

29. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016; 37(5): 467–520, doi: 10.1210/er.2015-1104, indexed in Pubmed: 27459230.

30. Talaei A, Adgi Z, Mohamadi Kelishadi M. Idiopathic hirsutism and insulin resistance. Int J Endocrinol. 2013; 2013: 593197, doi: 10.1155/2013/593197, indexed in Pubmed: 24228029.

31. Santos BR, Lecke SB, Spritzer PM. Apa-I polymorphism in VDR gene is related to metabolic syndrome in polycystic ovary syndrome: a cross-sectional study. Reprod Biol Endocrinol. 2018; 16(1): 38, doi: 10.1186/s12958-018-0355-9, indexed in Pubmed: 29669566.

32. Di Bari F, Catalano A, Bellone F, et al. Vitamin D, bone metabolism, and fracture risk in polycystic ovary syndrome. Metabolites. 2021; 11(2), doi: 10.3390/metabo11020116, indexed in Pubmed: 33670644.

33. Krul-Poel YHM, Snackey C, Louwers Y, et al. The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. Eur J Endocrinol. 2013; 169(6): 853–865, doi: 10.1530/EJE-13-0617, indexed in Pubmed: 24044903.

34. Niu YM, Wang YD, Jiang GB, et al. Association between vitamin D receptor gene polymorphisms and polycystic ovary syndrome risk: a meta-analysis. Front Physiol. 2019; 10(9): 1902, doi: 10.3389/fphys.2018.01902, indexed in Pubmed: 30687119.

35. Jedrzejuk D, Łaczmański Ł, Milewicz A, et al. Classic PCOS phenotype is not associated with deficiency of endogenous vitamin D and VDR gene polymorphisms rs731236 (TaqI), rs7975232 (ApaI), rs1544410 (BsmI), rs10735810 (FokI): a case-control study of lower Silesian women. Gynecol Endocrinol. 2015; 31(12): 976–979, doi: 10.3109/09513590.2015.1062865, indexed in Pubmed: 26422783.

36. Dasgupta S, Dutta J, Annamaneni S, et al. Association of vitamin D receptor gene polymorphisms with polycystic ovary syndrome among Indian women. Indian J Med Res. 2015; 142(3): 276–285, doi: 10.4103/0971-5916.166587, indexed in Pubmed: 26458343.

37. Bander D, Parczewski M, Urbańska A, et al. Osteoporosis associated selected single nucleotide polymorphisms frequency in HIV-infected and non-infected Polish population. Endokrynol Pol. 2017; 68(5): 541–549, doi: 10.5603/EP.a2016.0043.

38. Seremak-Mrozikiewicz A, Drews K, Mrozikiewicz PM, et al. Correlation of vitamin D receptor gene (VDR) polymorphism with osteoporotic changes in Polish postmenopausal women. Neuro Endocrinol Lett. 2009; 30(4): 540–546, indexed in Pubmed: 20010502.

39. Shen H, Xie J, Lu H. Vitamin D receptor gene and risk of fracture in postmenopausal women: a meta-analysis. Climacteric. 2014; 17(4): 319–324, doi: 10.3109/13697137.2013.856401, indexed in Pubmed: 24156276.

40. Stathopoulou MG, Dedoussis GVZ, Trovas G, et al. The role of vitamin D receptor gene polymorphisms in the bone mineral density of Greek postmenopausal women with low calcium intake. J Nutr Biochem. 2011; 22(8): 752–757, doi: 10.1016/j.jnutbio.2010.06.007, indexed in Pubmed: 21115334.

41. Zintzaras E, Rodopoulou P, Koukoulis GN. BsmI, TaqI, ApaI and FokI polymorphisms in the vitamin D receptor (VDR) gene and the risk of osteoporosis: a meta-analysis. Dis Markers. 2006; 22(5–6): 317–326, doi: 10.1155/2006/921694, indexed in Pubmed: 17264402.

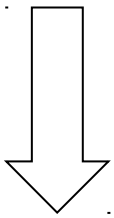
42. Banjabi AA, Al-Ghafari AB, Kumosani TA, et al. Genetic influence of vitamin D receptor gene polymorphisms on osteoporosis risk. Int J Health Sci (Qassim). 2020; 14(4): 22–28, indexed in Pubmed: 32694969.

43. Ahmad I, Jafar T, Mahdi F, et al. Association of vitamin D receptor gene polymorphism (TaqI and Apa1) with bone mineral density in North Indian postmenopausal women. Gene. 2018; 659(4): 123–127, doi: 10.1016/j.gene.2018.03.052, indexed in Pubmed: 29559350.

44. Douroudis K, Tarassi K, Ioannidis G, et al. Association of vitamin D receptor gene polymorphisms with bone mineral density in postmenopausal women of Hellenic origin. Maturitas. 2003; 45(3): 191–197, doi: 10.1016/s0378-5122(03)00148-8, indexed in Pubmed: 12818464.

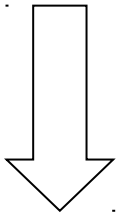
45. Ranjzad F, Mahban A, Shemirani AI, et al. Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. J Assist Reprod Genet. 2011; 28(3): 225–232, doi: 10.1007/s10815-010-9506-4, indexed in Pubmed: 21082232.

minor allele frequency (*VDR*) > 0.2



SNPs significantly associated with BMD

in previous studies.



***TaqI* rs731236, *BsmI* rs1544410**

.Figure 1

Table 1. The frequency of alleles and genotypes of the *VDR* polymorphisms in women with hyperandrogenism

VDR TaqI rs731236			**VDR BsmI rs1544410**		
Genotype	**Observed value n [%]**	**Expected value [%]**	**Genotype**	**Observed value n [%]**	**Expected value [%]**
AA	26 (32.5)	35.3	*CC*	26 (32.5)	34.5
AG	43 (53.8)	48.2	*CT*	42 (52.5)	48.5
GG	11 (13.8)	16.5	*TT*	12 (15.0)	17.0
Total	80 (100)	100	**Total**	80 (100)	100
Allele			**Allele**		
A	95 (59.38)	–	*C*	94 (58.75)	–
G	65 (40.62)	–	*T*	66 (41.25)	–
Total	160 (100)	–	**Total**	160 (100)	–

Table 2. Analysis of hormonal factors vs. *VDR* gene variants in women with hyperandrogenism

Parameter	**VDR Taq I rs731236**	**Mean ± SEM**	**p value**	**VDR Bsm I rs1544410**	**Mean ± SEM**	**p value**
Estradiol [pg/mL]	*AA*	50.11 ± 7.78	0.794	*CC*	50.11 ± 7.78	0.608
	AG	53.43 ± 7.23		*CT*	51.79 ± 7.29	
	GG	59.57 ± 10.40		*TT*	63.02 ± 10.11	
FEI [pmol/nmoL]	*AA*	7.30 ± 1.29	0.330	*CC*	7.30 ± 1.29	0.330
	AG	5.96 ± 1.09		*CT*	5.96 ± 1.09	
	GG	9.06 ± 1.56		*TT*	9.06 ± 1.56	
Prolactin [ng/mL]	*AA*	14.78 ± 1.91	0.290	*CC*	14.78 ± 1.91	0.290
	AG	20.31 ± 3.09		*CT*	20.31 ± 3.09	
	GG	16.78 ± 2.20		*TT*	16.78 ± 2.20	
17-OH-Progesterone [ng/mL]	*AA*	1.57 ± 0.45	0.481	*CC*	1.57 ± 0.45	0.529
	AG	1.19 ± 0.11		*CT*	1.19 ± 0.11	
	GG	1.60 ± 0.32		*TT*	1.54 ± 0.29	
LH [mIU/mL]	*AA*	9.79 ± 1.21	0.133	*CC*	9.79 ± 1.21	0.336
	AG	12.18 ± 1.94		*CT*	11.86 ± 1.10	
	GG	5.97 ± 1.25		*TT*	7.51 ± 1.89	
FSH [mIU/mL]	*AA*	5.60 ± 0.33	0.168	*CC*	5.60 ± 0.33	0.397
	AG	12.02 ± 3.53		*CT*	10.47 ± 3.29	
	GG	5.54 ± 0.61		*TT*	10. 20 ± 4.69	

FEI — Free Estradiol Index; FSH — Follicle Stimulating Hormone; LH — Luteinizing Hormone

Table 3. Analysis of hormonal factors vs *VDR* gene variants in women with hyperandrogenism

Parameter	**VDR Taq I rs731236**	**Mean±SEM**	**p value**	**VDR Bsm I rs1544410**	**Mean±SEM**	**p value**
Testosterone [ng/mL]	*AA*	0.54 ± 0.05	0.379	*CC*	0.54 ± 0.05	0.368
	AG	0.46 ± 0.04		*CT*	0.46 ± 0.04	
	GG	0.45 ± 0.07		*TT*	0.44 ± 0.06	
BAI [%]	*AA*	45.47 ± 2.89	0.182	*CC*	45.47 ± 2.89	0.182
	AG	39.01 ± 2.44		*CT*	39.01 ± 2.44	
	GG	45.56 ± 4.65		*TT*	45.56 ± 4.65	
FAI [%]	*AA*	1.94 ± 0.12	0.183	*CC*	1.94 ± 0.12	0.183
	AG	1.66 ± 0.10		*CT*	1.66 ± 0.10	
	GG	1.94 ± 0.20		*TT*	1.94 ± 0.20	
Androstendione [ng/mL]	*AA*	4.32 ± 0.37	0.574	*CC*	4.32 ± 0.37	0.574
	AG	3.95 ± 0.36		*CT*	3.95 ± 0.36	
	GG	3.68 ± 0.25		*TT*	3.68 ± 0.25	
DHEA-SO4 [µg/dL]	*AA*	271.77 ± 31.34	0.785	*CC*	271.77 ± 31.34	0.959
	AG	252.86 ± 26.67		*CT*	260.63 ± 26.35	
	GG	284.13 ± 29.67		*TT*	261.46 ± 35.32	
SHBG [nmol/L]	*AA*	35.49 ± 4.88	0.442	*CC*	35.49 ± 4.89	0.442
	AG	48.18 ± 6.88		*CT*	48.18 ± 6.88	
	GG	41.99 ± 15.81		*TT*	41.99 ± 15.81	

BAI — Body Adiposity Index; DHEA-SO4 — dehydroepiandrosterone sulfate; FAI — Free Androgen Index; SHGB — sex hormone binding globulin

Table 4. Analysis of clinical factors vs. *VDR* gene variants in women with hyperandrogenism

Parameter	**VDR Taq I rs731236**	**Mean ± SEM**	**p value**	**VDR Bsm I rs1544410**	**Mean ± SEM**	**p value**
Glucose 0' [mg/dL]	*AA*	90.64 ± 1.89	0.449	*CC*	90.64 ± 1.89	0.449
	AG	89.88 ± 2.17		*CT*	89.88 ± 2.17	
	GG	96.89 ± 9.53		*TT*	96.89 ± 9.53	
Glucose 60' [mg/dL]	*AA*	130.61 ± 6.44	0.843	*CC*	130.61 ± 6.44	0.843
	AG	135.20 ± 5.41		*CT*	135.20 ± 5.41	
	GG	135.21 ± 9.80		*TT*	135.21 ± 9.80	
Glucose 120' [mg/dL]	*AA*	109.31 ± 5.55	0.949	*CC*	109.31 ± 5.55	0.949
	AG	107.16 ± 3.92		*CT*	107.16 ± 3.92	
	GG	109.13 ± 11.40		*TT*	109.13 ± 11.40	
Insulin 0' [µIU/mL]	*AA*	22.49 ± 2.77	0.722	*CC*	22.49 ± 2.77	0.722
	AG	24.55 ± 6.56		*CT*	24.55 ± 6.56	
	GG	31.48 ± 7.71		*TT*	31.48 ± 7.71	
Insulin 60' [µIU/mL]	*AA*	156.59 ± 16.48	**0.008**	*CC*	156.59 ± 16.48	**0.008**
	AG	102.74 ± 9.44		*CT*	102.74 ± 9.44	
	GG	172.10 ± 36.29		*TT*	172.10 ± 36.29	
Insulin 120' [µIU/mL]	*AA*	100.79 ± 13.78	0.063	*CC*	100.79 ± 13.78	0.063
	AG	67.76 ± 7.94		*CT*	67.76 ± 7.94	
	GG	119.65 ± 42.56		*TT*	119.65 ± 42.56	
AG	*AA*	1.05 ± 0.06	0.855	*CC*	1.05 ± 0.06	0.655
	AG	1.08 ± 0.05		*CT*	1.09 ± 0.05	
	GG	1.03 ± 0.07		*TT*	1.01 ± 0.07	
VF	*AA*	969.80 ± 242.17	0.701	*CC*	969.80 ± 242.17	0.909
	AG	843.70 ± 146.86		*CT*	872.12 ± 149.74	
	GG	1114.71 ± 347.58		*TT*	988.50 ± 326.40	

,*$p < 0.05$ — comparison between genotypes and the parameters analyzed (one-way ANOVA test); values normally distributed are expressed as means ± SEM; AG — distribution of android and gynoid fat; VF — visceral fat indication

Table 5. Analysis of bone metabolism and clinical factors vs. *VDR* gene variants in women with hyperandrogenism

Parameter	**VDR Taq I rs731236**	**Mean±SEM**	**p value**	**VDR Bsm I rs1544410**	**Mean±SEM**	**p value**
Vitamin D [ng/mL]	*AA*	19.32 ± 3.58	0.081	*CC*	19.32 ± 3.58	0.081

	AG	14.98 ± 1.68		*CT*	14.98 ± 1.68	
	GG	8.06 ± 0.68		*TT*	8.06 ± 0.68	
Vitamin 25 [OH] D [ng/mL]	*AA*	23.82 ± 1.73	0.067	*CC*	23.82 ± 1.73	0.153
	AG	21.56 ± 0.97		*CT*	21.28 ± 0.95	
	GG	17.38 ± 2.39		*TT*	18.84 ± 2.61	
Calcitonin [pg/mL]	*AA*	3.30 ± 2.13	0.563	*CC*	3.30 ±2.13	0.565
	AG	1.73 ± 0.21		*CT*	1.64 ± 0.20	
	GG	1.47 ± 0.31		*TT*	1.82 ± 0.45	
PTH [pg/mL]	*AA*	38.60 ± 3.96	0.875	*CC*	38.60 ± 3.96	0.822
	AG	41.46 ± 3.64		*CT*	41.97 ± 3.69	
	GG	41.13 ± 6.62		*TT*	39.36 ± 6.29	
OPG [pmol/L]	*AA*	3.40 ± 0.24	0.127	*CC*	3.4 ± 0.24	0.171
	AG	3.57 ± 0.15		*CT*	3.57 ± 0.16	
	GG	4.37 ± 0.72		*TT*	4.27 ± 0.66	
sRANKL [pmol/L]	*AA*	243.18 ± 48.12	0.603	*CC*	243.18 ± 48.12	0.641
	AG	196.99 ± 23.21		*CT*	199.70 ± 23.64	
	GG	221.65 ± 37.99		*TT*	209.56 ± 36.42	

BMI — body mass index; OPG — osteoprotegerin, PTH — parathyroid hormone

Table 6. Analysis of bone metabolism and clinical factors vs. *VDR* gene variants in women with hyperandrogenism

Parameter	VDR Taq I rs731236	Mean ± SEM	p value	VDR Bsm I rs1544410	Mean ± SEM	p value
BMD total	*AA*	1.18 ± 0.03	0.488	*CC*	1.18 ± 0.03	0.780
	AG	1.19 ± 0.02		*CT*	1.19 ± 0.02	
	GG	1.23 ± 0.04		*TT*	1.21 ± 0.05	
BMD L1-L4	*AA*	1.20 ± 0.03	0.622	*CC*	1.20 ± 0.03	0.465
	AG	1.23 ± 0.02		*CT*	1.24 ± 0.02	
	GG	1.24 ± 0.07		*TT*	1.20 ± 0.07	
T score	*AA*	0.12 ± 0.32	0.506	*CC*	0.12 ± 0.32	0.263
	AG	0.59 ± 0.20		*CT*	0.69 ± 0.17	
	GG	0.53 ± 0.58		*TT*	0.18 ± 0.61	
Z score	*AA*	0.16 ± 0.27	0.652	*CC*	0.16 ± 0.27	0.219
	AG	0.32 ± 0.20		*CT*	0.40 ± 0.19	
	GG	0.11 ± 0.48		*TT*	0.39 ± 0.50	
BMI	*AA*	30.84 ± 2.32	0.312	*CC*	30.84 ± 2.32	0.513
	AG	27.93 ± 1.44		*CT*	28.18 ± 1.46	
	GG	32.54 ± 3.04		*TT*	30.99 ± 3.06	
BMC [g]	*AA*	2338.44 ± 67.51	0.281	*CC*	2338.44 ± 67.51	0.341
	AG	2446.16 ± 36.65		*CT*	2442.55 ± 37.69	
	GG	2361.57 ± 89.25		*TT*	2386.13 ± 81.10	
TBS	*AA*	1.40 ± 0.03	0.327	*CC*	1.40 ± 0.03	0.452
	AG	1.34 ± 0.03		*CT*	1.34 ± 0.03	
	GG	1.40 ± 0.06		*TT*	1.37 ± 0.06	

BMC — bone mineral content; BMD L1-L4 — bone mineral density L1-L4; BMD total — bone mineral density total; TBS — Trabecular Bone Score; T-score — the ratio of the bone mineral density (BMD) of the test person to the average bone density of the young person; Z score — bone mineral density index