Polymorphism of the vitamin D3 receptor gene and bone mineral density in girls with functional hypothalamic amenorrhea subjected to oestroprogestagen treatment

Zmienność genu receptora witaminy D3 a gęstość mineralna kości u dziewcząt z wtórnym brakiem miesiączki poddanych terapii estroprogestagenowej

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Abstract

Background: We investigated whether the vitamin D3 receptor gene (VDR) polymorphism can modulate therapeutic response of functional hypothalamic amenorrhea (FHA) patients to the oestroprogestagen (EP) treatment.

Material and methods: The study included 84 FHA girls and 50 controls. FHA patients underwent a four-year sequential EP therapy with 17-β oestradiol (2 mg from the 2nd to 25th day of the menstrual cycle) and didrogesterone (10 mg from the 16th to the 25th day). Their hormonal parameters were monitored along with bone turnover marker levels and bone mineral density (BMD). Additionally, the VDR gene BsmI polymorphism was determined.

Results: Hormonal therapy was reflected by a substantial improvement of BMD. However, the values of BMD observed after four years of treatment in FHA patients were still significantly lower than baseline bone mineral density determined in the control group (1.007 ± 0.100 vs. 1.141 ± 0.093 g/cm², respectively; p < 0.001). No significant effects of the VDR genotype were observed on the dynamics of BMD during consecutive years of hormonal treatment and mean bone mineral density determined after completing the therapy (1.006 ± 0.101 vs. 1.013 ± 0.114 vs. 1.006 ± 0.094 g/cm² for BB, bb and Bb genotypes, respectively; p = 0.973).

Conclusions: This study did not confirm that VDR polymorphism can modulate therapeutic outcome of FHA girls subjected to the hormonal treatment. Nonetheless, this study confirmed the effectiveness of EP therapy in the simultaneous treatment of menstrual disorders and the normalisation of bone mineral density in FHA patients. (Pol J Endocrinol 2011; 62 (6): 492–498)

Key words: bone mineral density, vitamin D3 receptor, functional hypothalamic amenorrhea, hormone replacement therapy, osteoporosis

Introduction

Irregular menses are one of the possible consequences of an impairment of the somatosexual development in adolescent girls, which is described by psychologists as “juvenile crisis” [1]. Long-term exposure to stress can lead to functional hypothalamic amenorrhea (FHA), the prevalence of which is estimated at 2.6–8.5% [2], but this rises to 100% in cases of chronic exposure to stress, e.g. in females participating in certain sport disciplines.
[3]. Stress is reflected by an enhanced release of the catecholamines norepinephrine and adrenaline, which via neuronal synapses stimulate a hypothalamic secretion of corticotropin-releasing hormone (CRH). CRH inhibits the pulsatile release of gonadotropin-releasing hormone (GnRH) and activates pro-opiomelanocortin (POMC) and its derivatives, \( \beta \)-endorphin and adrenocorticotropic hormone (ACTH). The activation of the hypothalamic-pituitary-adrenal axis (HPA) is reflected by an enhanced secretion of glucocorticoids, which in turn inhibits the release of GnRH, the pituitary gonadotropins FSH and LH, and oestradiol. High levels of \( \beta \)-endorphin, in turn, stimulate the secretion of prolactin, which further inhibits the pulsatile release of GnRH and increases ACTH and glucocorticoid concentrations [4]. The activation of all the aforementioned mechanisms leads to an impaired proliferation of the ovarian granulosal cells, a decreased synthesis of oestradiol, a disturbed maturation of the ovarian follicles, as well as disturbed ovulation cycles. These disruptions cause menstrual disorders, which are the principal manifestation of FHA [1, 5].

Apart from the direct impairment of sexual maturation, FHA can also affect skeletal development in growing girls. Due to the antiresorptive and anabolic effects of oestrogens, a sufficient serum concentration of the latter is required for reaching peak bone mass [6, 7]. Therefore, menstruation disorders that are related to a decreased oestradiol concentration can be reflected by an inferior peak bone mass and a delayed growth, as well as an increased risk of postmenopausal osteoporosis [7].

Consequently, the implementation of an effective therapy of FHA-type menstrual disorders is of vital importance. Administration of oestrogens and progestagens in the form of oestroprogestagen (EP) therapy is the standard management in such cases. The principal objective of this treatment is to restore the proper concentrations of oestradiol and thus stimulate regular menstrual cycles [8]. Our previous studies revealed that if implemented sufficiently early, EP therapy also results in the normalisation of bone mineral density. However, skeletal response to hormonal treatment was heterogeneous in patients who received EP [9–11]. The variability of the outcomes was partly explained by the polymorphisms of the oestrogen receptor gene [10, 11], but undoubtedly this factor is not the sole predictor of therapeutic response.

The vitamin D3 receptor (VDR) gene is another gene which could potentially be responsible for the degree of bone mineral density normalisation in response to the hormonal treatment. Meta-analyses of the VDR gene polymorphism function suggested its possible associations with bone mineral density (BMD) and bone mineral content (BMC), as well as the risk of fractures in postmenopausal women [12, 13]. Vitamin D regulates the calcium and phosphorus metabolism, thus modulating BMD levels [14]. In turn, the VDR gene is responsible for proper interactions between the receptor and its ligand; furthermore, the VDR is the absolute determinant of the biological activity of 1,25(OH)\(_2\)D\(_3\) [15].

The results of previous studies suggest that the VDR gene polymorphism is associated with BMD, due to the fact that this receptor is a crucial mediator of the intestinal absorption of calcium, and thus of bone mineralisation in the lumbar spine in children, adolescent girls, and mature individuals of both genders, and also modulates the risk of fractures in elderly men and women [13, 16–19]. Additionally, some authors have claimed an interaction between oestrogen receptor-\( \alpha \) and VDR genotypes with respect to BMD [20].

In view of this evidence, we assumed that the VDR polymorphism can also modulate therapeutic response of FHA patients to the hormonal treatment implemented to normalise menstrual disorders and prevent skeletal mass loss. The aim of this study was to verify this hypothesis on the basis of a four-year longitudinal study.

Material and methods

Participants

The study included 134 girls aged 16 and 17 years who were divided into two groups: 1) 84 patients with FHA, treated at the Department of Gynaecology, University of Medical Sciences in Poznan, Poland, between 2004 and 2009 (Group A); and 2) 50 girls whose menstrual cycles were normal (controls, Group C).

All procedures were approved by the Local Ethics Committee of the University of Medical Sciences in Poznan. Both the subjects and their parents gave their informed consent before the start of any procedure.

Qualifying criteria for Group A included: 1) at least six months of amenorrhea preceded by at least three years of oligomenorrhea; 2) psychological problems (learning disability and/or family problems) confirmed by a clinical psychologist. Group A exclusion criteria, based on medical history and a standardised questionnaire survey [9], included the following: 1) polycystic ovary syndrome, congenital adrenal hyperplasia or premature ovarian failure; 2) low birth weight or preterm birth; 3) at least one confirmed episode of an eating disorder; 4) poor diet during childhood or puberty; 5) episodes of impaired growth and body mass gain; 6) extensive participation in sports that may have influenced bone mineralisation; 7) metabolic disorders that may be associated with decreased bone mineralisation;
8) prolonged use of stimulants or drugs that may affect bone metabolism; 9) familial history of osteoporosis; and 10) incomplete four-year follow-up (return of regular menstrual cycles, cessation of treatment due to medical indications or other reasons).

The control group comprised healthy, normally menstruating girls, who gave their consent to participate in this study due to prophylactic reasons.

Clinical and laboratory tests

Anthropometric measurements (height, body weight) were taken for all participants and their body mass index (BMI) was calculated. The development of secondary sexual characteristics was assessed using the Tanner scale [21]. Baseline values of hormonal parameters were determined for thyrotropin (TSH), follicle-stimulating hormone (FSH), luteinising hormone (LH), total serum testosterone (T), sex hormone binding globulin (SHBG), oestradiol (E2), and prolactin (PRL). The following bone turnover markers were also measured in both groups: serum concentration of the bone fraction of alkaline phosphatase (BALP) and the urine concentration of cross-linked n-telopeptide of type I collagen (NTx). Bone mineral density (BMD) measurements were also performed. Subjects in Group A were additionally tested for: blood morphology, erythrocyte sedimentation rate, and blood concentrations of calcium, phosphorus, creatinine, total protein, alkaline phosphatase, vitamin D3, and parathyroid hormone.

Oestradiol, TSH, FSH, and LH were measured in the serum using a solid-phase chemiluminescence immunoassay (Immulite; Diagnostic Products Co, Los Angeles, CA, USA). The assay sensitivity was 20 pg/ml for oestradiol, 0.01 mU/ml for TSH, 0.1 mU/ml for FSH, and 0.1 mIU/ml for LH. The intraassay and interassay coefficients of variation (CVs) for oestradiol were 9.3% and 10.5%, respectively, 1.9% and 5.0% for FSH, respectively, and 3.6% and 5.0% for LH, respectively. Total serum testosterone was measured using an RIA (Orion Diagnostica, Espoo, Finland) with a 0.1 nmol/l limit of detection, and both intraassay and interassay CVs were of 6%. Sex hormone binding globulin (SHBG) was measured using a fully automated system (Immulite: DPC, Inc., Los Angeles, CA, USA), which uses a solid-phase two-site chemiluminescence enzyme immunoassay, and has an interassay CV of less than 8%. Prolactin concentrations were determined by a microparticle enzyme immunoassay using the automated Abbott AxSYM system (Abbott Laboratories, Chicago, IL, USA). For prolactin, the within run CV was less than 3%, and the total CV was less than 6%.

Serum BALP was measured using a specific monoclonal antibody (Alkphase-B; Metra Biosystems, Mountain View, CA, USA), with a 0.7 U/l sensitivity, and the intraassay and interassay CVs at 28 U/l amounted to 3.3% and 7.9%, respectively. Ntx was measured in the morning sample of urine and was corrected for urinary creatinine. Measurements were carried out by means of an enzyme-linked immunoassay (VitrosTM NTX reagent pack; Ortho-Clinical Diagnostics, Amersham, UK), with a detection limit of 20 nmol bone collagen equivalent and an interassay CV of 4.1%.

BMD measurements were performed on the basis of the DXA method (GE Lunar Prodigy Advance, Madison, WI, USA; software enCORE version 8.8), using an automatic scan mode. Five measurements of the lumbar spine (L2-L4) BMD were carried out in the course of the treatment: 0 — as a baseline, and then after one, two, three and four years. Results were presented as absolute values (g/cm²) and relative changes (%).

Additionally, the VDR gene BsmI polymorphism was determined in subjects in Group A. In order to extract genomic DNA from peripheral blood mononuclear cells using a non-enzymatic inorganic method [22], 10 ml of venous blood was collected and stored with an EDTA anticoagulant. BsmI polymorphism of the VDR gene was analysed according to Morrison [23]. DNA amplification was performed by means of the PCR method with pairs of the following primers flanking the polymorphic site: 5’-CAACCAAGACATCAAGTACCggCTCAGTGA-3’ as a sense VDR-se primer, and 5’-AACCAGCGGGAAGAGGTCAAGGG-3’ as an antisense VDR-as primer. An 800 bp-long product was obtained, and incubated at 37°C for three hours with a 2.5 U BsmI restriction enzyme (MBI Fermentas). This incubation resulted in three possible products: 1) bb genotype (confirmed presence of BsmI restriction site) — PCR product digested into 150 bp- and 650 bp-long fragments of cDNA; 2) BB genotype (lack of BsmI restriction site) — undigested 800 bp-long PCR product; 3) heterozygous Bb genotype — combination of both aforementioned homozygous types (800 bp-, 650 bp- and 150 bp-long products). DNA samples (40 ng) were amplified in a 20 μl solution containing 4 mM of either primer (VDR-se and -as), 2.0 mM of each deoxyribonucleotide triphosphate (dATP, dTTP, dCTP, dGTP; MBI Fermentas), PCR buffer (final concentration of magnesium: 1.5 mM MgCl₂; MBI Fermentas) and 0.5 units of Taq polymerase (MBI Fermentas). The amplification was carried out under the following conditions: 1) initial denaturation at 94°C for 5 min; 2) 35 cycles of denaturation at 94°C, 25 s each; 3) binding of primers, 50 s at 60°C; and 4) elongation of the chain at 72°C for 50 s. PCR was completed by a 10-minute elongation of the chain at 72°C. After digestion by the BsmI enzyme, PCR products were separated from the VDR primers by electrophoresis on a 3% ethidium bromide stained agarose gel.
**Intervention**

Patients from Group A underwent a four-year sequential EP therapy with a preparation composed of the natural female sex hormone 17β-oestradiol (2 mg from the 2nd to 25th day of the menstrual cycle) and didrogesterone (10 mg from the 16th to the 25th day of the menstrual cycle). The goal of the treatment was for the patients to resume regular menstrual bleeding. Spontaneous menstruations did not resume in any of the participants and therefore the therapy was continued for four years. In addition to hormonal treatment, patients were encouraged to modify their lifestyles and dietary habits. They were prescribed calcium and vitamin D3 preparations at individual doses adjusted for their dietary content and for the season of the year. Moreover, regular physical activity (15 minutes of recreational gymnastics twice a day) was suggested.

**Follow-up**

Follow-up measurements of TSH, FSH, LH, T, SHBG, E2, and PRL, BALP and Ntx were performed after six months of EP treatment in patients belonging to Group A. Moreover, BMD measurements were carried out each year, starting 12 months after the initiation of EP therapy.

**Statistical analysis**

Continuous variables were presented as arithmetic means and their standard deviations (SD). Their normal distribution was tested using the Kolmogorov-Smirnov test. A logarithmic transformation was used for the Ntx variable. Arithmetic means between Groups A and C and among certain genotypes of the vitamin D3 receptor gene in Group A were compared with ANOVA and the Tukey post-hoc test. Mean values of parameters determined during consecutive treatment phases in Group A were compared with Friedman ANOVA. Calculations were performed using Statistica 9.0PL (StatSoft® Inc. Tulsa, OK, USA) software, and statistical significance was defined as \( p \leq 0.05 \).

**Results**

Prior to initiating the hormonal therapy, Group A patients differed significantly from the control group girls in terms of all analysed parameters other than BALP concentration (Table I). No significant effects of the VDR gene polymorphism were noted on anthropometric characteristics, hormonal profile, the levels of bone turnover markers, or BMD in Group A. In turn, the only significant association between VDR gene polymorphism and analysed parameters in the control group pertained to BMD, whose level was markedly higher in girls with BB genotype compared to other participants (1.246 ± 0.117 vs. 1.110 ± 0.081 vs. 1.145 ± 0.084 g/cm² for BB, bb and Bb genotypes, respectively; \( p = 0.010 \)).

After six months of treatment, a significant improvement of all studied hormonal parameters was observed in Group A; however, these values were still significantly lower when compared to controls. Furthermore, a significant decrease in Ntx along with

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**Table I. Baseline characteristics of patients with functional hypothalamic amenorrhea (FHA) and the controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 84)</th>
<th>Group C (n = 50)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body height [cm]</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>162.27</td>
<td>6.63</td>
<td>166.82</td>
</tr>
<tr>
<td>Body weight [kg]</td>
<td>49.21</td>
<td>6.23</td>
<td>59.70</td>
</tr>
<tr>
<td>Menarcheal age [years]</td>
<td>14.05</td>
<td>1.12</td>
<td>12.46</td>
</tr>
<tr>
<td>Oestradiol [pg/ml]</td>
<td>22.75</td>
<td>8.41</td>
<td>60.06/133.86*</td>
</tr>
<tr>
<td>Testosterone [ng/ml]</td>
<td>0.42</td>
<td>0.14</td>
<td>0.67</td>
</tr>
<tr>
<td>FSH [mIU/ml]</td>
<td>3.52</td>
<td>1.29</td>
<td>6.81</td>
</tr>
<tr>
<td>LH [mIU/ml]</td>
<td>1.63</td>
<td>1.18</td>
<td>9.49</td>
</tr>
<tr>
<td>PRL0 [ng/ml]</td>
<td>8.10</td>
<td>2.84</td>
<td>9.82</td>
</tr>
<tr>
<td>PRL60 [ng/ml]</td>
<td>144.30</td>
<td>49.59</td>
<td>81.99</td>
</tr>
<tr>
<td>BALP [U/ml]</td>
<td>39.44</td>
<td>13.81</td>
<td>40.27</td>
</tr>
<tr>
<td>Ntx [mEBCE/mg/ml CR]</td>
<td>407.59</td>
<td>230.89</td>
<td>49.39</td>
</tr>
<tr>
<td>BMD [g/cm²]</td>
<td>0.822</td>
<td>0.088</td>
<td>1.141</td>
</tr>
</tbody>
</table>

*depending on cycle phase
A significant increase in BALP level were observed as a result of EP therapy; mean values of both bone turnover markers were significantly higher in Group A when compared to the control group (Table II). No significant effects of the VDR polymorphism were observed in Group A with regards to mean values of the parameters analysed after six months of therapy, nor their percentage change from respective baseline levels.

Hormonal therapy was reflected by a substantial improvement of BMD determined in consecutive years (Table III). However, the values of BMD observed after four years of treatment in Group A were still significantly lower than baseline bone mineral density determined in the control group (1.007 ± 0.100 vs. 1.141 ± 0.093 g/cm², respectively; p < 0.001). No significant effects of the VDR genotype were observed on the dynamics of BMD during consecutive years of hormonal treatment and mean bone mineral density determined after completing the therapy (1.006 ± 0.101 vs. 1.013 ± 0.114 vs. 1.006 ± 0.094 g/cm² for BB, bb and Bb genotypes, respectively; p = 0.973).

### Table II. Hormonal and bone turnover parameters after six months of EP therapy in Group A (n = 84) compared to baseline values in this group and baseline values of the controls (n = 50)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Absolute value</th>
<th>Relative change (%)</th>
<th>p value (baseline)</th>
<th>p value (control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Oestradiol [pg/ml]</td>
<td>68.42</td>
<td>11.72</td>
<td>423.1</td>
<td>376.6</td>
</tr>
<tr>
<td>FSH [mIU/ml]</td>
<td>5.35</td>
<td>1.27</td>
<td>70.4</td>
<td>80.5</td>
</tr>
<tr>
<td>LH [mIU/ml]</td>
<td>6.64</td>
<td>4.57</td>
<td>264.7</td>
<td>550.6</td>
</tr>
<tr>
<td>BALP [U/ml]</td>
<td>61.22</td>
<td>18.93</td>
<td>64.3</td>
<td>51.4</td>
</tr>
<tr>
<td>Ntx [mEBCE/mg/ml CR]</td>
<td>195.73</td>
<td>131.82</td>
<td>-51.4</td>
<td>18.6</td>
</tr>
</tbody>
</table>

*depending on cycle phase

### Table III. Relative changes (%) in bone mineral density (BMD) during consecutive years of EP administered in Group A patients (n = 84)

<table>
<thead>
<tr>
<th>Year</th>
<th>Overall (n = 84)</th>
<th>BB genotype (n = 16)</th>
<th>bb genotype (n = 30)</th>
<th>Bb genotype (n = 38)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.85</td>
<td>7.91</td>
<td>5.47</td>
<td>5.15</td>
<td>7.58</td>
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<tr>
<td>2</td>
<td>7.54</td>
<td>9.73</td>
<td>10.63</td>
<td>9.05</td>
<td>9.45</td>
</tr>
<tr>
<td>3</td>
<td>5.22</td>
<td>6.97</td>
<td>6.46</td>
<td>6.98</td>
<td>6.99</td>
</tr>
<tr>
<td>4</td>
<td>2.39*</td>
<td>3.75</td>
<td>4.18</td>
<td>2.09*</td>
<td>3.14</td>
</tr>
<tr>
<td>p value</td>
<td>0.009</td>
<td>0.193</td>
<td>0.045</td>
<td>0.046</td>
<td>–</td>
</tr>
<tr>
<td>Overall</td>
<td>23.38</td>
<td>13.79</td>
<td>24.78</td>
<td>15.07</td>
<td>23.47</td>
</tr>
</tbody>
</table>

*significantly different compared to previous years; SD — standard deviation

### Discussion

This study confirmed our previous observations, which suggest that the administration of EP therapy can be reflected by improved bone mineral density in FHA girls [9, 11]. As early as six months into the treatment, significant improvement was observed in all hormonal parameters analysed in our group. Normalisation of the hormonal profile was accompanied by significant changes in the levels of bone turnover markers: decrease of Ntx and increase of BALP concentrations. These findings suggest an initiated process of normalisation of bone formation. Changes in the laboratory parameters were reflected by a significant increase of BMD, observed during consecutive years of the treatment. However, similar to our previous studies [9, 11], marked individual variability in the extent of treatment response was observed in our patients.

Many previous studies by other authors have documented an association between the polymorphism of the VDR gene and the level of BMD in adolescent...
girls [24–26]; therefore we assumed that the genotypic variability of this receptor can also modulate the level of therapeutic response in our group. However, this hypothesis was not confirmed by the results of our study: patients with various genotypes of VDR did not differ significantly in terms of baseline bone mineral density, or the dynamics of its changes in the course of hormonal treatment.

In our opinion, the nature of the underlying disease, along with the low representation of certain VDR genotypes in our patients, constitute the principal reasons for the outcome of this study. FHA is a hormonal dysfunction, associated with a deficiency of oestrogens that are responsible for osteogenesis, among other functions [6,7]. Consequently, one can assume that the effectiveness of EP therapy is mostly determined by the ability to respond to hormonal substitution. This was confirmed both in our previous studies [9–11] and in experiments by other authors [27–29], all suggesting the involvement of the oestrogen receptor-α gene polymorphisms in determining response to EP therapy. The principal function of VDR is to maintain normal levels of vitamin D3 metabolism and compensate for its potential deficiencies [30]. In turn, all FHA patents participating in this study had normal serum levels of this vitamin, and received mineral and vitamin supplementation throughout the study period, fully covering recommended requirements for vitamin D3 [31].

One should remember that none of the previous studies confirming the role of the VDR gene polymorphism in determining bone mineral density identified the exact mechanisms responsible for this phenomenon [15]. This mostly results from the complexity of the cascade responsible for the maintenance of the peak BMD. The process of bone mass normalisation is determined either by genetic factors or by environmental influences, and the mutual relationships between these two groups of factors [32,33]. Apart from VDR, BMD is determined by other factors, such as the level of endogenous vitamin D (mostly dependent upon environmental influences), dietary supply and bioavailability of calcium, hormones involved in skeletal metabolism (parathyroid hormone, oestrogens and a variety of tissue hormones), as well as many others [34]. Also functional regulation of the vitamin D3 receptor is a complex process — it is determined not only by the VDR genotype, but also by the type of skeletal tissue showing its expression [30].

Barring the complexity of biological processes involved in BMD determination, methodological aspects can constitute another potential reason for discrepancies in the results of the previous research. The variability of the different sites at intron 8/exon 9 of the VDR gene, BsmI, Apal and TaqI, and the FokI sites at exon 2.3, has been analysed during previous experiments, and different restriction enzymes were used. The most relevant data regarding the involvement of the VDR gene in the determination of bone mineral density pertains to the polymorphism of the FokI region. Several authors have observed that an FF genotype is associated with a better absorption of calcium, while an ff genotype frequently co-exists with bone mass deficiencies. However, the existing evidence regarding the role of BsmI polymorphism, analysed in our study, is conflicting. Some authors have observed the highest BMD levels among carriers of homozygous genotypes of this polymorphism, BB or bb, while others have revealed Bb genotype as the predictor of high bone mineral density [24–26,35–38]. Also our study did not elucidate the role of the BsmI polymorphism: we did not observe significant effects of this polymorphism on BMD in FHA patients; however, among patients from the control group, bone mineral density was significantly higher among carriers of BB genotype. While this latter finding is consonant with the observations of several authors [24], it nonetheless contrasts with the results obtained in a variety of other studies revealing this genotype to be predisposed to lower BMD [25,26,35–38].

Conclusions

Given the aforementioned facts, our current knowledge on the role of polymorphism of the VDR receptor gene in adolescent girls is still insufficient to be utilised in clinical practice.

However, this does not diminish the principal outcome of this study, which is to confirm the effectiveness of EP therapy in the simultaneous treatment of menstrual disorders and the normalisation of bone mineral density in FHA girls. Nevertheless, further research is needed in order to find potential predictive factors that could be used for the identification of patients who could benefit most from the simultaneous supplementation of vitamin D and oestrogens.

References


