Polymorphism of vitamin D3 receptor and its relation to mineral bone density in perimenopausal women

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Abstract
Summary Postmenopausal osteoporosis is the most common metabolic bone disease with important genetic factors. We evaluated the frequency of polymorphism 283G/A of the vitamin D3 \( VDR \) gene receptor. The study included 800 women at the postmenopausal (505) and reproductive (295) age. Statistically significant changes, depending on the genotype, were shown.

Introduction Postmenopausal osteoporosis is the most common metabolic bone disease of strong genetic origin with population variability determined by the interaction of genetic and environmental factors. Recognition of different genetic variants underlying development of osteoporosis would make it possible to administer individual symptomatic treatment as well as early prophylactics of osteoporosis.

Methods The aim of the study was to evaluate the frequency of polymorphism 283G/A of the vitamin D3 \( VDR \) gene receptor and assessment of its relations with the clinical parameters of osseous turnover and degree of postmenopausal osteoporosis.

The study included 800 women at the postmenopausal (505) and reproductive (295) age throughout the Wielkopolska region in Poland. The postmenopausal group included women with osteoporosis and osteopenia and the healthy ones. Women at the reproductive age were healthy. Frequency of the tested gene polymorphism was evaluated in the group where bone mineral density (BMD) was marked and in the control group.

Results The obtained test results pointed to correlation of polymorphism \( VDR \) 283G/A with the BMD scores for the lumbar vertebrae in women with osteopenia and osteoporosis, therefore the ones at risk of fractures. Vitamin D receptor (VDR) polymorphism correlated with reduced BMD values.

Conclusions Polymorphism 283G/A of the vitamin D3 receptor gene has been proved to be the genetic factor of postmenopausal osteoporosis. The polymorphism mentioned above has been proved to be a factor of mineral bone density changes of women.

Keywords Age · Females · Osteoporosis · Polymorphism · \( VDR \) gene

Introduction

Vitamin D is a fat soluble steroid compound performing a variety of functions in the human body. The active form of vitamin D, produced in the kidneys, has a systemic effect. The vitamin produced by other cells performs within the cells or locally. Such effects are the so-called nonclassic functions of vitamin D, such as effect upon proliferation, differentiation, or
apoptosis [1, 2]. Both, the classical functions, i.e., effect upon calcium-phosphate management and the nonclassical ones are imposed by the nuclear receptor (VDR), regulating directly the gene expression [3].

Vitamin D receptor (VDR) belongs to the nuclear receptors, activated by a ligand and performing as transcription factors [4, 5].

Reports of the recent years pointed to the occurrence of vitamin D receptor polymorphism [6]. Initially, VDR polymorphism was analysed in disease associated with bone metabolism, yet the studies were gradually extended to comprise the role of vitamin D3 receptor in the pathogenesis of other disorders, including the neoplastic diseases [6, 7].

The VDR gene is localized in chromosome 12q12-14. It contains polymorphisms with a functional role. Mutations of the VDR gene may be important for the effect of vitamin D upon the cells. The available literature on the effect of concentration and taking vitamin D upon activity, progression, and prognosis in some diseases is scarce, comprises few variables, and often brings contradictory data. The existence of several polymorphisms in the VDR gene has been described using different restriction enzymes. Examples of these include the Tru9I, TaqI, BsmI, EcoRV, and Apal. All these polymorphisms are located between the exons 8 and 9. A different case of RFLP is the so-called FokI. This polymorphism was described in the exon 2.

Polymorphisms of four loci within the VDR gene are associated with biological changes in bone mass and density. Those are polymorphisms BsmI, TaqI, Apal, and FokI.

A dependency was proved to exist between the mineral density of bones and the variants of the VDR gene, coding the receptor protein for vitamin D. The physiological mechanism of the effect of vitamin D receptor genotype polymorphism upon the mineral density of bones has not been fully recognized yet.

Very early effect of vitamin D upon osteogenesis was proved upon discovery of a receptor of this vitamin on the mesenchymal cell. They had been revealed before differentiation to the osseous tissue was started. 1,25-dihydroxycholecalciferol stimulates differentiation of osteoblasts, enhances expression of VDR receptors on a growing bone, and stimulates synthesis of type I collagen. It has also been proved that renal metabolite has an intermediate role in stimulation of osteolysis through processes associated with the RANKL pathway and their effect upon both osteoclastogenesis and bone resorption [8, 9]. It is known that the effect of polymorphism on bone mineral density is not explicit and may interfere with other factors of both genetic and environmental nature. This turned the scientists’ efforts towards effects brought by polymorphisms within the VDR gene, commencing a new era in studies aimed at identification of genes involved in pathogenesis of osteoporosis.

Therefore, the objective of this study was to define the role of relations of the evaluated gene with the process of postmenopausal osteoporosis, bone density as well as other individual and clinical parameters. The aim was to evaluate frequency of gene VDR polymorphism, coding vitamin D3 receptor in the female postmenopausal group. The analysis comprised relation of the evaluated genetic variant to the degree of the osseous changes and the osseous turnover, as well as assessment of the role of the evaluated genetic polymorphisms in the etiopathogenesis of osteoporosis. We hypothesized that the polymorphism 283G/A of the gene receptor of vitamin D3 is closely related to changes in bone density.

Materials and methods

Test group

The study comprised a group of unrelated Caucasian women inhabiting the region of Wielkopolska. Investigations included 800 women at the postmenopausal age (505) and the reproductive one (295). The postmenopausal group included 314 women with osteoporosis, 110 with osteopenia, and 81 healthy individuals. Densometric measurements were performed to define the bone mineral density (BMD) as well as T-score, Z-score, mean bone mineral density index as compared to the mean value for young adult women (YA-young adults) and mean bone mineral density as compared to the mean value for a given age (AM-age matched). Additionally, body weight and height were measured to calculate the body mass index.

Detailed history of each patient was taken to gain information on the diseases developed, medication prescribed, age of first and last menstruation, number of deliveries, birth weight as well as smoking habits.

Qualified for genetic tests were those women in whom menopause occurred at least a year ago, who did not receive therapies possibly influencing the bone mass, including selective estrogen receptor modulators—SERM, calcitonin, bisphosphonates, heparin, steroids, thyroid hormones, antiepileptic drugs, GnRH analogs, tibolon, and underwent no hormonal replacement therapy (HRT). Excluded from the study were patients following bilateral ovariectomy as well as those suffering from endocrine and metabolic disorders, hematological disease, neoplastic conditions, renal disorders, autoimmune diseases, or connective tissue diseases, as the above could possibly influence the bony mass. Additionally, a group of Caucasian women at the reproductive age was examined (mean age 27.5±4.7). Consent to perform the study was obtained from the Bioethical Committee.
Bone mineral density (BMD) was measured at the lumbar vertebrae, from vertebra L2 through L4, employing dual energy X-ray absorptiometry (DXA). Densometric tests were performed with the use of LUNAR DPX 100 unit (by Lunar Corporation, Madison, USA). BMD scores were expressed as grams per square centimeter and presented as T-score and Z-score indices, referring to mean BMD values for a given age group. BMD scoring between one standard deviation from the mean age referring to the peak bone mass, measured by the DEXA method, was considered normal (T-score from +1 to −1).

Tests comprised the influence of the evaluated polymorphism upon T-score, Z-score, L2−L4 AM, L2−L4 YA, L2−L4 BMD, BMI as well as other clinical parameters. Results were presented as arithmetic mean with standard deviation. For this purpose, ANOVA analysis of variance was employed. Values adopted as statistically significant were those higher than p<0.05.

Analysis of gene VDR polymorphism by real-time PCR

Analysis of polymorphism of 283G/A of gene VDR was performed by real-time PCR with the use of LightCycler® 480 designed to ensure quick and accurate polymerase chain reaction. For such procedures, HybProbe hybridization probes were used along with LightCycler® 480 Basic to evaluate the results. Genotyping, i.e., detection of single nucleotide polymorphism of the evaluated genes employed fluorescence measurements taken upon analysis of the melting curve following PCR. LightSNiP set with phials containing proper concentration of starters and probes specific for the amplified fragment was used to test polymorphism of gene VDR. Preparation of LightSNiP used for real-time PCR followed the manufacturers’ instructions.

The reactions were performed, respectively, throughout 45 cycles for the gene evaluated, covering particular stages of amplification, which was followed by melting of the reaction’s products once the temperature reached 95 °C. The genotype was analysed on the basis of the melting curve. The genotype frequency observed upon the recent study was compared to the values expected for genotype distribution at Hardy-Weinberg’s equilibrium, using chi-square—$\chi^2$ test. The formula applied was $p^2+2pq+q^2=1$. Results were presented with 95 % confidence interval (95 % CI).

Statistical analysis of the results employed the software SPSS 17.0 PL for Windows. The database was supplied with clinical parameters of the patients, the genotype frequency, and the evaluated polymorphism allele. The study was planned according to statement of Human and Animal Rights. It has been approved by the local Bioethical Committee in Poznan (no. 1415/03 (158/06)).

**Results**

Polymorphism VDR 283G/A

Analysis of frequency of homozygous AA and GG genotypes and the heterozygous AG in polymorphism of 283G/A (G283A) of gene VDR showed comparable results in the group with osteoporosis and in healthy postmenopausal females. No statistically significant differences were noted between the groups. More frequent in women with osteoporosis was the heterozygous AG genotype while in healthy women dominating was genotype AA (Tables 1 and 2).

Frequency of AA and GG genotypes and the heterozygous AG genotype in polymorphism 283G/A (G283A) of gene VDR was comparable in the reproductive age group of women with osteoporosis and in the healthy group. No statistically significant differences were noted between the groups. More frequent in women with osteoporosis was the heterozygous AG genotype, while in young, healthy women higher frequency was shown for the AA genotype (Tables 1 and 3).

Frequency of the homozygous GG genotype in polymorphism 283G/A (G283A) of gene VDR differed significantly in women with osteopenia and in the healthy female in postmenopausal age. Higher values were observed in the healthy, postmenopausal group (Table 2). Frequency of AG genotype was slightly higher in osteopenic women. Frequency of AA genotype was comparable in both evaluated groups.

Analysis of frequency of homozygous AA and GG genotypes and the heterozygous AG in polymorphism of 283G/A (G283A) of gene VDR showed the results for genotype AA and AG comparable in women with osteoporosis and in the osteopenic group (Tables 1 and 2). Genotype GG was more frequent in women with osteoporosis. Most frequent in both female groups was genotype AG.

Analysis of frequency of homozygous AA and GG genotypes and the heterozygous AG in polymorphism of 283G/A (G283A) of gene VDR showed the frequency of GG genotype comparable in women with osteopenia and osteoporosis and in the healthy, postmenopausal group (Tables 2 and 3). The homozygous AA genotype was more frequent in healthy women while AG genotype prevailed in ill females. Most frequent in both female groups was the heterozygous AG genotype in ill females and AA in healthy women.

Analysis of frequency of homozygous AA and GG genotypes and the heterozygous AG in polymorphism of 283G/A (G283A) of gene VDR showed comparable results in women with osteopenia and concurrent osteoporosis and in the healthy females at reproductive age (Table 3). No statistically significant differences were noted.

Frequency of homozygous AA and GG genotypes and the heterozygous AG in polymorphism of 283G/A (G283A) of gene VDR was comparable in healthy, postmenopausal women, and in the reproductive age group
In both female groups, genotype AA was more frequent.

Influence of VDR 283G/A polymorphism on parameters of the evaluated women

Results presented in Tables 4 and 5 show the value of BMD L2–L4 YA (%) in the evaluated female osteopenic group was significantly higher in women with genotype AA of polymorphism G283A of gene VDR, as compared to the same parameter in females with genotype AG and GG. The same table shows that the value of BMD L2–L4 AM (%) in patients with genotype AA of the evaluated gene is lower than with genotype GG, i.e., reversely to BMD L2–L4 YA (%).

Results presented in Table 6 point to the values of BMD L2–L4 AM (%) in the evaluated female population with osteoporosis, significantly lower in women with genotype AA of polymorphism G283A of gene VDR, as compared to the same parameter in women with genotype GG.

Discussion

It is estimated that one billion people worldwide, in particular the elderly inhabitants of US and European cities, shows vitamin D deficiency [10–12]. The level of calcidiol is too low even in 30–50 % of inhabitants of such highly sun-exposed regions like the United Arab Emirates, Turkey, or India [13]. People of those regions are used to covering their bodies, restricting therefore synthesis of vitamin D. Biologically active, double hydroxylated form of vitamin D—calcitriol—as well as its analogs effect the target cells through VDR.

Lack of direct association between low serum concentration of vitamin D and aggravation of osteopenia and osteoporosis led to the research into other factors which could account for the mechanism of development of hepatic osteodystrophy. Recently, attention has been paid to polymorphism of the receptor gene of vitamin D as a dependency has been shown between bone mineral density and the VDR gene variant coding the receptor protein for vitamin D. So far, the physiological mechanism of the effect of polymorphism of receptor genotype for vitamin D upon bone mineral density has not been recognized. It is likely to be associated with disorders of intestinal calcium absorption [14]. It is claimed that in some diseases, a large group of patients shows excessive or lower presence of particular variants of the VDR gene. This was proved by Springer et al. [15], who observed relations between vitamin D receptor genotype and development of hepatic dystrophy. In patients with allele T of the VDR gene, 2–3-fold higher risk of spinal fracture was noted.

Recently, some more reports have confirmed the genetic background of bone mineral density. Even if information on the effect of a given gene or genes are contradictory [16], the scientists do agree that bone mineral density depends in 75–80 % on the genetic factors [16, 17]. So far, no single gene was found to prevail over others in the determination of bone mineral density. It is most probably a multi-gene relation [16–18]. Among the number of genes, tested most frequently

Table 1 The frequency of occurrence of polymorphism of the VDR 283G/A genotypes in women with osteoporosis and without osteoporosis (with osteopenia and healthy ones)

| Genotype VDR | Women with osteoporosis | Women without osteoporosis |
|--------------|-------------------------|---------------------------|
|              | Observed value n (%)    | Expected value (%)         | 95 % CI      |
| GG           | 56 (20.1)               | 12.2                      | 15.5–23.4    |
| AG           | 121 (43.5)              | 45.4                      | 37.2–52.0    |
| AA           | 101 (36.3)              | 42.4                      | 29.3–44.7    |
| Total        | 278 (100)               | 100                       |              |

| Observed value n (%) | Expected value (%) | 95 % CI     |
|----------------------|--------------------|-------------|
| GG                   | 23 (14.2)          | 12.7        |
| AG                   | 70 (42.9)          | 45.8        |
| AA                   | 70 (42.9)          | 41.5        |
| Total                | 163 (100)          | 100         |

Table 2 The frequency of occurrence of polymorphism of the VDR 283G/A genotypes in women with osteopenia and healthy in postmenopausal age

| Genotype VDR | Women with osteopenia | Healthy women |
|--------------|-----------------------|---------------|
|              | Observed value n (%)  | Expected value (%) | 95 % CI |
| GG           | 12 (11.4)             | 11.8          | 8.8–16.2 |
| AG           | 48 (45.7)             | 45.0          | 35.6–55.8 |
| AA           | 45 (42.9)             | 43.2          | 34.8–51.3 |
| Total        | 105 (100)             | 100           |          |

| Observed value n (%) | Expected value (%) | 95 % CI     |
|----------------------|--------------------|-------------|
| GG                   | 11 (19.0)          | 12.7        |
| AG                   | 22 (37.9)          | 45.8        |
| AA                   | 25 (43.1)          | 41.5        |
| Total                | 58 (100)           | 100         |
was the vitamin D receptor gene [19, 20] or genes coding collagen [21]. For such reasons, this study chose the gene coding the receptor for active vitamin D metabolites recognized as one with polymorphism likely to affect the osseous tissue density in the patients.

Our studies have shown that females with osteoporosis and osteopenia, with genotype AA, manifest lower values of BMD L2–L4 AM, as compared to other genotypes. This proves the relation of vitamin D receptor polymorphism to lower bone density.

Results of the recent study are comparable to those described in literature. Most of the contributions showed no influence of the VDR genotype upon the mineral density of the lumbar vertebrae [22, 23]. Only Sainz et al. [24], who studied premature Mexican girls, pointed to the effect of the VDR gene on BMD throughout the L2–L4 section.

Frequency of BsmI polymorphism of the VDR gene in groups evaluated during this study was consistent with Hardy-Weinberg equilibrium. It was observed that distribution of frequency of particular BsmI polymorphism genotype was the same as one observed many years ago by Hustmyer et al. [25] who evaluated the Caucasian population. Frequency of heterozygotes observed in the recent study was also close to values obtained in the quoted research. The same team observed that the black and yellow race populations showed significant differences in frequency of both alleles (21 and 6 %, respectively for allele B).

In the present study, the presence of GG genotype of the VDR gene was statistically lower as compared to other genotypes. It is possible that the result is due to elevated supply of vitamin D3, as suggested by Japanese scientists, who noted some relation between vitamin and calcium consumption and the phenotype [26].

Similar results were obtained following evaluation of the female population in the USA, which showed relations between the VDR genotype and the risk of osteoporous bone fractures [27–29]. On the other hand, no relation between bone fractures and the VDR gene polymorphism was found in European populations [30]. Evaluation of Dutch population showed that allele B of BsmI polymorphism of the VDR gene is associated with predispositions to bone fractures [29]. Results confirming correlation of the evaluated alleles with BMD and higher risk of bone fractures were delivered by another Dutch research center [27].

### Table 3

The frequency of occurrence of polymorphism of the VDR 283G/A genotypes in women with osteoporosis and osteopenia and healthy in reproductive age

| Genotype | Women with osteoporosis and osteopenia | Healthy women |
|----------|----------------------------------------|---------------|
|          | Observed value | Expected value | 95 % CI | Observed value | Expected value | 95 % CI |
| GG       | 68 (17.8)      | 15.9          | 16.0–21.9 | 51 (17.4)      | 13.6          | 13.2–22.2 |
| AG       | 169 (44.1)     | 47.9          | 37.3–55.3 | 113 (38.9)     | 46.5          | 32.7–41.6 |
| AA       | 146 (38.1)     | 36.2          | 31.4–47.8 | 128 (43.7)     | 39.9          | 35.9–50.2 |
| Total    | 383 (100)      | 100           |          | 292 (100)      | 100           |          |

### Table 4

Characteristic of the population of healthy women in postmenopausal age taking part in the study of the VDR 283G/A polymorphism

| Genotype | GG        | AG       | AA       |
|----------|-----------|----------|----------|
| T-score  | −0.09±0.60| 0.06±0.68| 0.18±0.59|
| Z-score  | 0.65±0.90 | 0.70±0.53| 0.60±0.77|
| Body mass (kg) | 66.93±8.54 | 70.28±8.37 | 68.08±10.33 |
| Height (cm)  | 163.73±4.58 | 164.04±5.02 | 162.42±4.16 |
| BMI (kg/m²) | 25.01±4.50 | 25.70±3.98 | 25.06±4.13 |
| Age      | 55.70±9.02 | 55.60±8.87 | 52.17±8.00 |
| Birth weight | 3508.71±492.32 | 3408.66±500.32 | 3819.79±473.15 |
| Reproductive years | 37.01±5.01 | 37.56±4.65 | 39.06±5.06 |
| Age of menarche | 13.13±1.55 | 13.02±1.72 | 14.08±1.37 |
| Last menstrual period age | 50.18±4.73 | 50.07±5.06 | 51.37±4.40 |
| Number of pregnancies | 1.91±1.29 | 1.97±1.07 | 1.99±1.33 |
| Years after menopause | 7.01±4.82 | 7.03±5.20 | 8.03±4.92 |
| BMD L2–L4 (g/cm²) | 1.19±0.07 | 1.29±0.08 | 1.10±0.19 |
| BMD L2–L4 YA (%) | 101.02±7.71 | 96.02±6.84 | 106.51±7.34 |
| BMD L2–L4 AM (%) | 100.96±8.73 | 104.63±10.71 | 113.54±9.57 |
For a long time now, the VDR gene has been accepted as one of the major 'candidate genes' involved in development of osteoporosis. Evidence of genetic susceptibility to osteoporosis could appear as one of the methods used to identify individual predispositions to this disease and as an early prophylactic measure. It should be remembered, however, that in many cases it is the environmental factor that determines development of such disorders. Environmental factors may affect the osseous mass in a variety of ways. Therefore, the evaluated populations should be tested to find any links between the genotype and the phenotype. Some contributions discussing such correlations have already been published [31, 32].

Table 5 Characteristic of the women population with osteopenia taking part in the study of the VDR 283G/A polymorphism

| Genotype | GG | AG | AA |
|----------|----|----|----|
| T-score  | −1.78±0.53 | −1.94±0.32 | −1.60±0.47 |
| Z-score  | −0.90±0.52 | −0.80±0.39 | −0.75±0.46 |
| Body mass (kg) | 64.39±9.51 | 67.01±8.52 | 65.58±9.84 |
| Height (cm) | 161.02±4.76 | 164.13±4.72 | 163.38±4.87 |
| BMI (kg/m²) | 23.02±3.73 | 25.62±4.03 | 24.82±3.92 |
| Age | 53.01±8.15 | 56.13±4.78 | 54.82±3.92 |
| Birth weight | 3298.12±523.48 | 3225.81±491.73 | 3280.15±527.75 |
| Reproductive years | 39.26±4.82 | 37.82±5.12 | 37.11±4.72 |
| Age of menarche | 13.86±1.02 | 12.47±1.78 | 13.01±1.60 |
| Last menstrual period age | 47.77±4.78 | 50.36±5.01 | 52.82±4.49 |
| Number of pregnancies | 1.90±1.41 | 1.95±1.15 | 1.91±1.38 |
| Years after menopause | 7.71±4.92 | 7.20±5.19 | 7.03±4.77 |
| BMD L2–L4 (g/cm²) | 0.99±0.05 | 0.97±0.06 | 0.92±0.04 |
| BMD L2–L4 YA (%)* | 76.01±4.05 | 77.28±4.80 | 90.21±4.35 |
| BMD L2–L4 AM (%)* | 95.62±8.67 | 88.35±8.44 | 82.66±7.45 |

Significance of bold entries was observed by post hoc analysis *p<0.05

Evaluation of Polish population showed prevailing allele T of TaqI polymorphism of the VDR gene in patients with osteoporosis [33]. As claimed by the authors, this suggests relation between VDR polymorphism and the risk of osteoporosis. Data regarding the European and Mexican population bring similar results to confirm such relation [34]. Some reports point, however, to the lack of relation between the VDR gene polymorphism and bone density [35]. Also, evaluation of Chinese population has not proved significant dependencies between the VDR gene and BMD index. Such diversity of results needs to be clarified, including differences between populations or the environmental factors which could alter genetic

Table 6 Characteristic of the women population with osteoporosis taking part in the study of the VDR 283G/A polymorphism

| Genotype | GG | AG | AA |
|----------|----|----|----|
| T-score  | −3.02±0.44 | −3.32±0.92 | −3.10±0.26 |
| Z-score  | −1.60±0.60 | −1.65±0.52 | −1.58±0.40 |
| Body mass (kg) | 60.01±5.47 | 62.02±7.58 | 64.14±6.82 |
| Height (cm) | 160.96±4.73 | 162.29±4.19 | 160.30±5.00 |
| BMI (kg/m²) | 24.04±4.15 | 24.47±3.70 | 23.80±4.01 |
| Age | 56.11±7.52 | 56.03±8.38 | 58.23±7.23 |
| Birth weight | 2930.01±528.73 | 3072.82±466.02 | 3306.28±506.28 |
| Reproductive years | 36.03±4.62 | 36.92±5.13 | 36.00±4.15 |
| Age of menarche | 13.59±1.87 | 13.06±1.29 | 12.52±1.06 |
| Last menstrual period age | 48.98±4.65 | 48.62±4.73 | 51.06±5.03 |
| Number of pregnancies | 2.62±1.29 | 1.89±1.00 | 1.96±1.14 |
| Years after menopause | 10.02±5.14 | 11.36±6.03 | 13.27±5.87 |
| BMD L2–L4 (g/cm²) | 0.83±0.06 | 0.83±0.04 | 0.80±0.02 |
| BMD L2–L4 YA (%) | 68.23±5.01 | 72.53±5.31 | 70.81±4.88 |
| BMD L2–L4 AM (%)* | 81.15±8.33 | 76.28±7.63 | 66.61±6.73 |

Significance of bold entries was observed by post hoc analysis *p<0.05
predispositions, eventually manifested in different phenotypes [36, 37].

Remarkable discrepancies have been noted upon comparison of results gained by various teams, investigating this polymorphism of vitamin D receptor, with regard to bone conditions. The major reason, however, is the number of patients comprising the studies. Let us emphasize that our tests have included 800 patients, which places the study among a few projects covering such a large population. It should also be pointed out that the population evaluated was highly homogenous. Besides, the women included in the test groups have lived in the area comprised by the study, practically from birth.

Gene mutation may result in altered activation of other genes and/or their products having a major role in the same metabolic pathways. Such a nature of effect has been shown in studies where participation of allele B of the VDR gene was associated with the increased risk of osteoporosis. Other studies revealed low BMD scores upon subordination to allele B of the VDR gene [31, 32]. Future studies are needed to confirm the validity of these observations.

Conflicts of interest None.

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