Association Between Polymorphisms of VDR, COL1A1, and LCT Genes and Bone Mineral Density in Belarusian Women With Severe Postmenopausal Osteoporosis

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Summary. *Background and Objective. Variation of osteoporosis in the population is the result of an interaction between the genotype and the environment, and the genetic causes of osteoporosis are being widely investigated. The aim of this study was to analyze the association between the polymorphisms of the vitamin D receptor (VDR), type I collagen (COL1A1), and lactase (LCT) genes and severe postmenopausal osteoporosis as well as bone mineral density (BMD).*

Material and Methods. A total of 54 women with severe postmenopausal osteoporosis and 77 controls (mean age, 58.3 years [SD, 6.2] and 56.7 years [SD, 7.42], respectively) were included into the study. The subjects were recruited at the City Center for Osteoporosis Prevention (Minsk, Belarus). Dual-energy x-ray absorptiometry was used to measure bone mineral density at the lumbar spine and the femoral neck. Severe osteoporosis was diagnosed in the women with the clinical diagnosis of postmenopausal osteoporosis and at least 1 fragility fracture. The control group included women without osteoporosis. Polymorphic sites in osteoporosis predisposition genes (ApaI, BsmI, TaqI, and Cdx2 of the VDR gene, G2046T of the COL1A1 gene, and T-13910C of the LCT gene) were determined using the polymerase chain reaction on the deoxyribonucleic acid isolated from dried bloodspots.

Results. The data showed that the ApaI and BsmI polymorphisms of the VDR gene and T-13910C of the LCT gene were associated with severe postmenopausal osteoporosis in the analyzed Belarusian women ($P<0.01$). A statistically significant positive correlation between the VDR risk genotypes ApaI and TaqI and bone mineral density was found ($P<0.05$).

Conclusions. The findings of this study suggest that at least the ApaI and BsmI polymorphisms of the VDR gene and T-13910C of the LCT gene are associated with the risk of postmenopausal osteoporosis in our sample of the Belarusian women.

Introduction

Osteoporosis is a common disease characterized by compromised bone strength predisposing a person to an increased risk of the osteoporotic fracture and leading to significant morbidity, mortality, and high social and economic burden (1). Age, sex, racial, and geographic disparities in the incidence and progression of osteoporosis also testify the multifactorial nature of this disease (2–4). The inheritance of bone mineral density (BMD) varies depending on the characteristics of analyzed population groups and reaches up to 80% (5, 6). Variations of osteoporosis in the population are associated with an interaction between the genotype and the environment (7). Homeostasis of the bone tissue during lifetime is mainly maintained by the balanced processes of bone resorption and formation, resulting from the combined action of multiple genes and environmental factors (8).

Currently, a huge amount of information about the genetic determination of bone mineral density has been accumulated. More than 100 candidate genes associated with BMD and the risk of osteoporosis have already been identified (9–13).

Vitamin D as a part of the endocrine system has a pleiotropic effect on immune modulation, regulation of skeletal metabolism, and cellular proliferation and differentiation (14). The vitamin D receptor (VDR) gene is the most widely studied among other candidate genes determining the development of osteoporosis (15–21). The sequence of the VDR gene was found to be polymorphic in different individuals (18). *Bsm*I, *Apa*I, and *Taq*I polymorphisms are located in the 3'-regulatory region of the VDR gene, so they are often marked as 1 haplotype; these polymorphisms increase the risk of osteoporosis (15–19). *Cdx*2 is a protective polymorphism that reduces the risk of osteoporosis (20, 21).

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The gene encoding type I collagen protein (COL1A1) is considered as a candidate gene in the pathogenesis of osteoporosis. The *G>T* polymorphism of the COL1A1 gene is associated with a decrease in bone mineral density and an increased incidence of the fragility fracture (22, 23).

The lactase (LCT) gene encodes the amino acid sequence of the enzyme lactase. The *T-13910C* polymorphism in this gene leading to the intolerance of lactose may have an indirect impact on calcium supply, bone density, and osteoporotic fractures in the elderly (24, 25).

The aim of this study was to analyze the association of polymorphisms of the VDR, COL1A1, and LCT genes with severe postmenopausal osteoporosis and BMD.

Material and Methods

Study Population

A case-control study including Caucasian postmenopausal women who consecutively visited the City Center for Osteoporosis Prevention in Minsk, Belarus, was carried out.

Ambulatory women who were at least 2 years postmenopausal and who agreed to participate were included into in the study. Women with the conditions known to affect bone metabolism, i.e., diseases such as Paget's disease, osteogenesis imperfecta, rheumatoid arthritis, etc., or those using medications (glucocorticosteroids) were excluded from the study.

The women with the clinical diagnosis of postmenopausal osteoporosis (the BMD T-score of –2.5 or lower at the femoral neck or the lumbar spine) who sustained at least 1 fragility fracture confirmed by an x-ray examination were defined as the patients with severe osteoporosis. The control group comprised postmenopausal women with the BMD T-score of >–2.5 and without previous fragility fractures. The data of the medical history and the fracture history were obtained by a physician.

The local Research Ethics Committee at the Belarusian Medical Academy of Postgraduate Education approved the study protocol. Written informed consent was obtained from all the participants.

Measurement of Bone Mineral Density

BMD was measured at the lumbar spine and both hips using dual-energy x-ray absorptiometry (Prodigy, GE Lunar, Madisson, WI, USA). The results of the lumbar spine L_1-L_4 BMD and the right and left femoral neck BMD were analyzed.

Genotyping

For genetic analyses, venous blood samples were taken from the cubital vein using the Vacutainer system (Beckton-Dickinson, Franklin Lakes, NJ, USA). DNA was isolated from bloodspots dried on special NucleoSafe cards (Macherey-Nagel, Germany) using the standard proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation (26). The DNA solution was extracted with a phenol-chloroform-isoamyl alcohol mixture to remove protein contaminants and then was precipitated with 100% ethanol. The DNA was pelleted after the precipitation step, washed with 70% ethanol to remove salts and small organic molecules, and resuspended in a buffer at a concentration suitable for further investigation.

Polymorphic sites in osteoporosis predisposition genes (the *Apa*I, *Bsm*I, *Taq*I, and *Cdx*2 polymorphisms of the VDR gene, the *G2046T* polymorphism of the COL1A1 gene, and the *T-13910C* polymorphism of the LCT gene) were determined using the polymerase chain reaction (PCR) analysis with specially designed primers (Table 1).

VDR ApaI, BsmI, TaqI. Polymorphic sites in VDR (*Bsm*I B/b, rs1544410, *Apa*I A/a rs7975232, *Taq*I T/t, and rs731236) were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR amplifications of the related regions were carried out in 20-μL volumes of reaction mixtures containing 2×PCR-buffer (with 3-mM $MgCl_2$), 200 μM of each dNTP, 0.5 μM of each set of specific primers, 1 unit of Tornado-*Taq* DNA polymerase (Primetech, Belarus), and 20–50 ng of the DNA sample. All the PCR reactions were run at 95°C for 15 minutes followed by 30 cycles at 99°C for 1 second, at 62°C for 10 seconds, and extension at 72°C for 20 seconds. The final extension was carried out at 72°C for 2 minutes in an automated thermal cycler (Applied Biosystems 2720, USA). The amplified products were analyzed by electrophoresis on the 8% polyacrylamide gel. The *Bsm*I, *Apa*I, and *Taq*I genotypes were analyzed by *Bsm*I (37°C), *Apa*I (37°C), and *Taq*I (65°C) restriction enzyme digestion (Thermo Scientific, Lithuania), respectively. The *B* allele (191 bp) and the *A* and *T* alleles remained as a single 490-bp band; the *b* allele (115 and 76 bp) and the *a* (280 and 210 bp) and *t* (290 and 200 bp) alleles were observed as 2 bands. All of the digestion products were analyzed by electrophoresis on the 8% polyacrylamide gel.

VDR Cdx2. Two sets of primers were designed for the tetraprimer amplification refractory mutation system PCR (T-ARMS-PCR) (Table 1). G-Rev and A-For are allele-specific primers. These 4 primers generate 3 PCR fragments: the primer set G-For and G-Rev specifically amplifies the *G* allele with a size of 110 bp, A-For and A-Rev specifically amplify the *A* allele with a size of 235 bp, and the out-primer pair (G-For and A-Rev) amplifies the internal control PCR fragment with a size of 297 bp. The PCR amplification was carried out in an automated

Table 1. Polymerase Chain Reaction Primers of VDR, COL1A1, and LCT Gene Polymorphisms Used for the Screening of Genetic Predisposition to Osteoporosis

Gene	Polymorphism	Primer Sequence
VDR	*Apa*I	VDR_ATF: 5'-CAGAGCATGGACAGGGAGCAA-3' VDR_ATR: 5'-CACTTCGAGCACAAGGGGCGTTAGC-3'
	*Bsm*I	hVDR-3: 5'-AGTGTGCAGGCGATTCGTAG-3' hVDR-4: 5'-ATAGGCAGAACCATCTCTCAG-3'
	*Taq*I	the same as for *Apa*I
	Cdx2	G-For: 5'-AGGATAGAGAAAATAATAGAAAACATT-3' G-Rev: 5'-AACCCATAATAAGAAATAAGTTTTTAC- 3' A-For: 5'-TCCTGAGTAAACTAGGTCACAA-3' A-Rev: 5'-ACGTTAAGTTCAGAAAGATTAATTC-3'
COL1A1	*G2046T*	F: 5'-AATCAGCCGCTCCCATTCTCCTA-3' R: 5'-GGAGGGCGAGGGAGGAGAGAA-3' G2046 probe (FAM): 5'-TCATCCCGCCCCCATTCCCTG-3' 2046T probe (HEX): 5'-TCATCCCGCCCACATTCCCTGG-3'
LCT	*T-13910C*	LCT_FO: 5'-CAGGAAAAATGTACTTAGACCCTACAA-3' LCT_RO: 5'-CGTACTACTCCCCTTTTACCTCGTT-3' LCT_IC: 5'-TGGCAATACAGATAAGATAATGTCGC-3' LCT_IT: 5'-AGAGTTCCTTTGAGGCCAGTGA-3'

VDR, vitamin D receptor gene; COL1A1, type I collagen gene; LCT, lactase gene.

thermal cycler (Applied Biosystems 2720, USA). Ten microliters of the PCR reaction system consisted of 1.0 μL of 10× PCR buffer (1×buffer=10 mM Tris-Cl, pH 8.3; 50 mM KCl; 1.25 mM $MgCl_2$), 1.0 μL of 10×dNTPs (0.2 mM), 0.4 pmol of G-For, 0.6 pmol of G-Rev, 0.6 pmol of A-For, 0.4 pmol of A-Rev, 0.5 U of Super *Taq* (Thermo Scientific, Lithuania), and 10 ng of genomic DNA. The PCR was performed with an initial denaturation at 96°C for 5 minutes, followed by 28 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 45 seconds. The final extension was carried out at 72°C for 5 minutes. The PCR products were size-separated on the 8% polyacrylamide gel at 140 V for 45 minutes. The 100-bp DNA ladder (Thermo Scientific, Lithuania) was used to determine the size of the fragments.

COL1A1 G2046T. The G2046T polymorphism of the COL1A1 gene was detected by using the qPCR method. The amplification reactions were run in the CFX96 (BIO-RAD, USA) Real-Time PCR Detection System. The final reaction volume (20 μL) included 10 μL of 2×qPCR Master Mix (Thermo Scientific, Lithuania), 10 pmol of forward and reverse primers, 5 pmol of G2046 and 2046T probes, and 1 μL of the DNA sample (except the negative control ones).

LCT T-13910C. The position of the *T-13910C* polymorphism was based on the sequence analysis of the LCT gene. Two sets of primers were designed for the ASM-PCR test (Table 1).

The PCR amplification was carried out in an automated thermal cycler (Applied Biosystems 2720, USA). The PCR reaction system consisted of 10-μL 10×PCR buffer (1×buffer=10 mM Tris-Cl, pH 8.3; 50 mM KCl; 1.25 mM $MgCl_2$), 1.0 μL of 10×dNTPs (0.2 mM), 1.0 μL of each primer, 0.5 μL of polymerase, 3.5 μL of mQ water, and 10 ng of genomic DNA. The PCR was performed with an initial denaturation at 95°C for 15 minutes, followed by 28 cycles of denaturation at 99°C for 1 second, annealing at 60°C for 10 seconds, and extension at 72°C for 10 seconds. The final extension was performed at 72°C for 1 minute. The PCR products were size-separated on the 10% polyacrylamide gel at 125 V for 1 hour. The 100-bp DNA ladder (Thermo Scientific, Lithuania) was used to determine the size of the fragments. The amplified products were analyzed by electrophoresis on the 10% polyacrylamide gel. The *C allele* was observed as a 157-bp band; the *T* allele, as a 95-bp band; and the common fragment, as a 205 bp.

Statistical Analysis

The statistical analysis was performed using STATISTICA 10 for Windows (Statsoft Inc., USA). Based on the determined frequencies of genotypes and by using the χ^2 test, the Hardy-Weinberg equilibrium was tested. The association of the genotype alleles between the patients with postmenopausal osteoporosis and the control group was determined by the analysis of Pearson chi-square (χ^2). Crude odds ratios (ORs) were reported with 95% confidence intervals (CI). The differences between the groups were considered statistically significant at $P<0.05$. For BMD, the data are presented as mean (SD) (in g/cm^2).

Results

A total of 54 women with severe postmenopausal osteoporosis were included in this study. The control group consisted of 77 Caucasian women without osteoporosis. The clinical characteristics of the study population are summarized in Table 2. Both groups were matched for age, duration of postmenopausal period, and body mass index, except for height and

weight. The patients with severe osteoporosis had significantly lower BMD than the controls, in both absolute and relative values.

The distribution of the analyzed gene polymorphisms in the controls and in the patients with osteoporosis were in correspondence with the one expected from the Hardy-Weinberg equilibrium ($P>0.05$ in all cases), showing that the analyzed groups were selected correctly. The genotype and allele frequencies of the analyzed polymorphisms are presented in Table 3.

There was a statistically significant difference in the genotype and allele frequencies of the *Apa*I polymorphism between the patients and the controls ($\chi^2=13.74$ and $\chi^2=15.31$, respectively, $P<0.01$). Furthermore, the patients with severe postmenopausal osteoporosis were 3.34 (95% CI, 1.51–7.37) and 2.73 (95% CI, 1.64–4.55) times more likely to carry the *AA* genotype and the *A* allele of the *Apa*I polymorphism, respectively, when compared with the controls.

There also was a statistically significant difference in the genotype and allele frequencies of the

Table 2. Clinical Characteristics of Women Investigated

Characteristic	Patients With Severe Osteoporosis (n=77)	Controls (n=54)	*P* value
Age, years	58.3 (6.2)	56.7 (7.4)	0.13
Height, cm	156.4 (5.2)	162.1 (8.3)	0.0003
Weight, kg	67.4 (12.0)	75.3 (13.7)	0.009
Body mass index, kg/m^2	27.7 (4.7)	28.8 (5.3)	0.36
Years after menopause	10.4 (4.2)	6.1 (2.6)	0.06
Number of fractures	1.3 (0.6)	–	–
Spine BMD, g/cm^2	0.941 (0.134)	1.192 (0.127)	0.0000001
Femoral neck BMD, g/cm^2	0.791 (0.131)	0.947 (0.1)	0.000001
T-score, femoral neck	–2.1 (0.5)	–0.5 (1.2)	0.001
T-score, lumbar spine	–3.3 (0.8)	–0.8 (0.8)	0.001

Table 3. Genotype and Allele Frequencies of Analyzed Osteoporosis Predisposition Gene Polymorphisms in Patients and Controls

Gene, Polymorphism	Genotype, Allele	Frequency of Genotypes and Alleles, %		χ^2	*P* value	OR (95% CI)
		Patients With Osteoporosis N=54	Controls n=77			
VDR, *Apa*I	*aa*	13	37.7	13.74	0.001	0.25 (0.10–0.62)
	Aa	44.4	44.2			1.01 (0.50–2.04)
	AA	42.6	18.1			3.34 (1.51–7.37)
	a	35.2	59.7	15.31	0.00009	0.37 (0.22–0.61)
	A	64.8	40.3			2.73 (1.64–4.55)
VDR, *Bsm*I	*bb*	20.4	51.9	13.35	0.001	0.24 (0.11–0.53)
	Bb	57.4	33.8			2.64 (1.29–5.42)
	BB	22.2	14.3			1.71 (0.69–4.24)
	b	49.1	68.8	10.39	0.001	0.44 (0.26–0.73)
	B	50.9	31.2			2.29 (1.38–3.81)
VDR, *Taq*I	*TT*	31.5	50.6	5.21	0.07	0.45 (0.22–0.93)
	Tt	48.1	31.2			2.05 (1.00–4.21)
	tt	20.4	18.2			1.15 (0.48–2.77)
	T	55.6	66.2	3.07	0.08	0.64 (0.38–1.06)
	t	44.4	33.8			1.57 (0.95–2.6)
VDR, *Cdx*2	*GG*	75.9	68.8	0.79	0.67	1.43 (0.65–3.14)
	GA	24.1	31.2			0.70 (0.32–1.54)
	AA	0	0			NA
	G	88	84.4	0.66	0.42	1.35 (0.65–2.79)
	A	12.0	15.6			0.74 (0.36–1.53)
COL1A1, *G2046T*	*GG*	75.9	72.7	3.51	0.17	1.18 (0.53–2.63)
	GT	20.4	27.3			0.68 (0.30–1.57)
	TT	3.7	0			NA
	G	86.1	86.4	0.0	0.95	0.98 (0.48–2.00)
	T	13.9	13.6			1.02 (0.50–2.09)
LCT, *T-13910C*	*TT*	13.0	29.9	10.04	0.007	0.35 (0.14–1.89)
	TC	46.3	51.9			0.80 (0.40–1.60)
	CC	40.7	18.2			3.09 (1.4–6.84)
	T	36.1	55.8	9.91	0.002	0.45 (0.27–0.74)
	C	63.9	44.2			2.24 (1.35–3.71)

VDR, vitamin D receptor gene; COL1A1, type I collagen gene; LCT, lactase gene, NA, not applicable.

*Bsm*I polymorphism between the patients and the controls; however, for this polymorphism, significant associations were observed for the heterozygous genotype *Bb*. The patients with severe postmenopausal osteoporosis were 2.64 (95% CI, 1.29–5.42) and 2.29 (95% CI, 1.38–3.81) times more likely to carry the *Bb* genotype and the B allele of the *Bsm*I polymorphism, respectively, when compared with the controls.

When the genotype frequency of other polymorphisms of the VDR gene – *Taq*I and *Cdx*2 – was compared between the patients and the controls, no statistically significant differences in the frequency of genotypes and alleles were found. The same picture was observed in the analysis of the *G2046T* polymorphism of the COL1A1 gene (Table 3).

The analysis of the data also revealed a statistically significant difference in the genotype and allele frequencies of the *T-13910C* polymorphism in the LCT gene. The patients with severe postmenopausal osteoporosis were 3.09 (95% CI, 1.40–6.84) and 2.24 (95% CI, 1.35–3.71) times more likely to carry the *CC* genotype and the *C* allele of the *T-13910C* polymorphism, respectively, when compared with the controls.

The relationship between the genotypes and BMD is shown in Table 4. The data showed that for all the gene polymorphisms, with the exception of the LCT gene polymorphism, the BMD was lower in the individuals carrying the osteoporosis predisposition genotypes as compared with the individuals with favorable genotypes. The statistically significant association was shown in the VDR *Apa*I polymorphism: the BMD of the left femoral neck (LF) in the carriers of the *Aa* genotype and the BMD of the right femoral neck (RF) in the carriers of the *aa* genotype were greater when compared with the carriers of the *AA* genotype. For VDR *Taq*I, a statistically significant difference in the BMD of the RF was observed between the bearers of the *TT* and *tt* genotypes.

Discussion

In the patients with severe postmenopausal osteoporosis, all the polymorphism genotype frequencies did not differ from the expected Hardy-Weinberg equilibrium. This allows us to use the multiplicative model of inheritance in the statistical analysis, performing the analysis of not only genotypes, but also alleles.

In the present study, a statistically significant association between postmenopausal osteoporosis and at least 3 analyzed gene polymorphisms was demonstrated. Interestingly, the statistically significant association was observed for 2 most studied polymorphisms of the VDR gene, i.e., *Apa*I and *Bsm*I. Currently, the VDR gene is the best studied one in the set of the candidate genes that determine the

Table 4. Association Between Genotype and Bone Mineral Density at the Lumbar Spine, Left Femoral Neck, and Right Femoral Neck

Gene, Polymorphism	Region of Measurement	BMD, mean (SD), g/cm^2			*P* value
		Genotype	Genotype	Genotype	
		aa	*Aa*	*AA*	*aa* vs. *AA*
VDR *Apa*I	L_1–L_4	1.036 (0.057)	0.982 (0.032)	0.909 (0.042)	>0.05
	LF	0.858 (0.039)	0.871 (0.023)	0.783 (0.029)	<0.05
	RF	0.867 (0.042)	0.860 (0.028)	0.757 (0.028)	<0.05
		bb	*Bb*	*BB*	*bb* vs. *BB*
VDR *Bsm*I	L_1–L_4	1.019 (0.042)	0.949 (0.033)	0.945 (0.056)	>0.05
	LF	0.843 (0.03)	0.849 (0.025)	0.804 (0.032)	>0.05
	RF	0.848 (0.033)	0.831 (0.028)	0.781 (0.036)	>0.05
		TT	*Tt*	*tt*	*TT* vs. *tt*
VDR *Taq*I	L_1–L_4	1.006 (0.031)	0.952 (0.038)	0.911 (0.065)	>0.05
	LF	0.853 (0.022)	0.845 (0.030)	0.786 (0.031)	>0.05
	RF	0.844 (0.024)	0.835 (0.034)	0.756 (0.035)	<0.05
		GG	*GA*	*AA*	*GG* vs. *GA*
VDR *Cdx*2	L_1–L_4	0.959 (0.028)	0.987 (0.044)	–	>0.05
	LF	0.828 (0.018)	0.873 (0.039)	–	>0.05
	RF	0.814 (0.021)	0.859 (0.043)	–	>0.05
		GG	*GT*	*TT*	*GG* vs. *GT*
COL1A1 *G2046T*	L_1–L_4	0.986 (0.025)	0.911 (0.054)	–	>0.05
	LF	0.852 (0.019)	0.810 (0.037)	–	>0.05
	RF	0.838 (0.021)	0.794 (0.043)	–	>0.05
		TT	*TC*	*CC*	*TT* vs. *CC*
LCT *T-13910C*	L_1–L_4	0.905 (0.067)	0.990 (0.034)	0.958 (0.037)	>0.05
	LF	0.8 (0.035)	0.865 (0.025)	0.825 (0.029)	>0.05
	RF	0.8 (0.045)	0.845 (0.029)	0.814 (0.031)	>0.05

BMD, bone mineral density; L_1–L4, lumbar spine; LF, left femoral neck; RF, right femoral neck; VDR, vitamin D receptor gene; COL1A1, type I collagen gene; LCT, lactase gene.

development of osteoporosis. The 3.3-times higher risk of low BMD in the presence of the VDR *Apa*I *AA* genotype shows the importance of the *A* allele in the development of postmenopausal osteoporosis. Moreover, there is a statistically significant decrease in the osteoporosis risk for the *aa* genotype carriers.

The *Bsm*I polymorphism contains a restriction site marked as the *b* allele. Up to 16% of the Caucasians are homozygotes for a functionally defective allele of this gene (*BB*) and are at risk of osteoporosis and osteoporotic fractures (16). In the present study, the frequency of the *BB* allele in the control group (14.3%) was very similar. The frequency of the *Bb* genotype was higher in the group of the patients with postmenopausal osteoporosis compared with the control subjects. The probability of having this genotype was more than 2.6 times greater for the patients compared with the control population. In total, the risk of osteoporosis for risk (*B*) allele bearers was 2.3 times higher compared with control subjects.

The LCT is the third gene, the polymorphism of which showed a statistically significant association with postmenopausal osteoporosis. The frequencies of both risk allele and homozygous genotypes were shown to be higher in the patients with postmenopausal osteoporosis as compared with the control group.

In the present study, we failed to show any association between *Taq*I and *Cdx*2 of the VDR gene and *G2046T* of the COL1A1 gene, and the risk of the development of postmenopausal osteoporosis. The relationship of these gene polymorphisms and osteoporosis has been well established in the European population (16, 20–23). Moreover, for the COL1A1 gene, the haplotype analysis has demonstrated that the relationship with the risk of osteoporosis is specific for the *G2046T* polymorphism rather than for others (27).

For the VDR *Cdx*2 *A* allele, a statistically significant protective effect was not observed potentially reflecting a lack of statistical power, in particular for a very rare *AA* genotype group, which was absent in the present study. Therefore, further studies with a higher number of individuals have to be performed to determine its protective effect. The same possible explanation could be used for the absence of any association of the COL1A1 gene with postmenopausal studies, as no homozygous risk genotypes in the present study were found.

The absence of association between the *Taq*I polymorphism of the VDR gene and severe postmenopausal osteoporosis in the Belarusian women was very surprising because this polymorphism is very well studied, and its association with osteoporosis has been shown in many European populations (13, 28), including Russian (29). Its mechanisms mostly involve the hormonal regulation of osteogenesis, inactivating the vitamin D receptor.

Therefore, it is important to mention that the absence of a significant association of VDR *Taq*I and *Cdx*2 and the COL1A1 polymorphism with postmenopausal osteoporosis in the present study may be explained by the number of persons analyzed, which is insufficient for the investigation of rare alleles, and this does not reduce the interest in further studies. Further molecular and genetic analysis of these gene polymorphisms on the Belarusian population may help obtain more information on their association with postmenopausal osteoporosis.

The analysis of the relationship between gene polymorphisms and bone mineral density in both investigated groups revealed a statistically significant association between the femoral neck BMD and the frequency of the VDR *Apa*I and *Taq*I polymorphisms. No statistically significant association was observed between the lumbar spine BMD and the gene polymorphism genotypes; however, for all the genes, except LCT, there was lower BMD in the individuals carrying osteoporosis-predisposing genotypes.

For VDR *Apa*I, we have found a statistically significant association between the femoral neck BMD and the genotypes: in the subjects with the *Aa* genotype, the RF BMD was higher by 14.5%; and in the women with the *aa* genotype, the LF BMD was higher by 11.2% than in those with the *AA* genotype. Thus, taking into consideration a significant association between VDR *Apa*I and severe postmenopausal osteoporosis in this case-control study, a conclusion might be drawn that this polymorphism may be a useful marker for osteoporosis screening at least in Belarusian women.

The findings of this study suggest that the VDR *Apa*I gene polymorphism has a statistically significant association with the BMD level and severe postmenopausal osteoporosis in the analyzed group of the Belarusian women.

Surprisingly, another polymorphism demonstrating a statistically significant association between the bone mass and the genotype was VDR *Taq*I, which did not reveal even a trend for osteoporosis predisposition in a genetic case-control study. In a comparative analysis, the carriers of the *TT* genotype had the RF BMD by 11.6% higher compared with the individuals with the *tt* genotype.

No statistically significant association between bone mineral density and the polymorphisms of the VDR *Bsm*I and *Cdx*2, COL1A1 and LCT genes may be explained by an insufficient number of the subjects investigated. In another study, a relationship between the BMD level and osteoporosis predispo-

sition genotypes is most evident among individuals with a relatively low calcium intake (30). This is especially important for the LCT gene *T-13910C* polymorphism, pathogenesis of which includes a reduced calcium intake as a result of lactose intolerance.

VDR *Cdx*2 was reported to have a significant association between the *A* allele and a higher BMD in postmenopausal women, predicting the association of this allele with a decreased risk of fracture (20, 21). In the present study, we found no statistically significant association between BMD and the VDR *Cdx*2 polymorphism genotypes, possibly as a result of insufficient statistical power.

According to Uitterlinden et al. (17), the difference in BMD between women carrying the *G* or *T* alleles of the COL1A1 *G2046T* polymorphism increases with age. Interestingly, in their study, there was no significant difference in BMD between the *GG* and *TT* genotypes in the subjects aged 55–69 years; a significant difference in BMD was detected only in the subjects aged 70–80 years. Thus, it is possible that our study group was too young and represented a too narrow range of age to show any difference in the BMD level between the COL1A1 genotypes.

The findings of this study suggest that at least VDR *Apa*I and *Bsm*I and LCT *T-13910C* polymorphisms are likely to be associated with the risk of postmenopausal osteoporosis and make the greatest contribution to its development in the Belarusian population. At the same time, the VDR *Cdx*2 and COL1A1 *G2046T* polymorphisms demonstrated a nonsignificant association with osteoporosis, and further molecular and genetic analysis is required.

Screening of these genetic markers may enable an early identification of risk groups to perform preventive measures in a timely manner and also to improve treatment effectiveness, avoid complications, reduce disability and mortality rates in these patients, as well as cut down the treatment costs.

Conclusions

The findings of this study suggest that the *Apa*I polymorphism of the VDR gene has the greatest association with the BMD level and severe postmenopausal osteoporosis in the analyzed group of the Belarusian women. The present study prompts a further large-scale study of postmenopausal osteoporosis predisposition genes with larger sample sizes and in relation to fracture risk and environmental factors.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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