RESEARCH ARTICLE

Relationship between A163G osteoprotegerin gene polymorphism and other osteoporosis parameters in Roma and non-Roma postmenopausal women in eastern Slovakia

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Background: The study was focused on evaluating the possible correlation between biochemical, anthropometric, and genetic indicators of osteoporosis in postmenopausal women. The frequency of genotypes and differences in measured parameters were evaluated within two ethnically different groups of women in Slovakia.

Methods: The study included 310 postmenopausal women divided into non-Roma and Roma groups. Based on results of densitometry, they were divided into control groups and women with osteoporosis and osteopenia. In all women, a genetic analysis of polymorphism of osteoprotegerin gene promotor region (A163G) was provided along with measurement of indicators of bone tissue metabolism.

Results: There is a particularly low incidence of osteoporosis in Roma women. We found a correlation between bone mineral density (BMD), body mass index, and waist and hip circumference in women with osteoporosis and in Roma women with osteopenia. The frequency of the AG genotype was higher in non-Roma women with osteoporosis, but reached only 10.7% in Roma women with osteopenia. While the presence of the G allele in the non-Roma population was accompanied by higher BMD and markers of osteoformation, it was accompanied by significantly higher concentrations of parathyroid hormone in the Roma population.

Conclusion: The presence of the AG genotype has a different effect on bone metabolism in two ethnically diverse populations of women in Slovakia. In the general population, the presence of the G allele exhibited protective effects consistent with other studies, but in Roma population this appears to be the allele A. However, this requires a further study for confirmation and more detailed characterization of the differences between populations that have this work indicated.

KEYWORDS

biochemical parameters, osteoporosis, osteoprotegerin polymorphism, postmenopausal women, Roma (Gypsy) women

1 | INTRODUCTION

Osteoporosis is a common and complex health problem that affects postmenopausal females, especially the elderly.¹ It is a progressive skeletal disease manifested by decreased bone mineral density (BMD) and collateral damage to the bone microarchitecture, which can subsequently lead to impaired skeletal strength and elevated susceptibility to fractures.² Multiple and varied factors affect bone mineral density (BMD). These have been summarized by Wang et al.³ as nutritional intake factors (eg, calcium and vitamin D, caffeine, alcohol), body weight, physical activity, and exercise. A previous study has shown that among these factors, body weight and body mass index (BMI) exert the greatest impact on the BMD of postmenopausal females.⁴

The bone microenvironment consists of different cells such as stromal (osteoblasts, adipocytes, fibroblasts, and endothelial cells) and nonstromal cells. Osteoclasts and osteoblasts have a vital role in bone remodeling and niche structure.⁵ A disrupted balance between osteoblasts and osteoclasts can lead to decreased bone density and osteoporosis.⁶ Osteoblasts derived from mesenchymal stem cells are responsible for the expression and secretion of molecules and factors that regulate the maturation and proliferation of other cells located in bone marrow. The cytokines formed affect alkaline phosphatase (ALP), osteopontin, osteocalcin, collagen, and fibronectin release, thereby directly affecting bone formation.⁷ Osteoclasts are multinucleate cells that originate from hematopoietic stem cells. Osteoblasts regulate osteoclast maturation and proliferation by several factors such as RANKL and osteoprotegerin (OPG).⁸ RANKL represents the essential osteoblast derived factor required for osteoclast formation and activation and induces bone loss, whereas OPG blocks these effects and prevents bone resorption.⁹ The RANK/RANKL cytokine system, parathyroid hormone (PTH), sex hormones (such as estrogen and testosterone), inflammatory cytokines, GH/IGF-1, BMP2 protein, as well as the wingless related protein/ β -catenin (Wnt/ β -catenin) signaling pathway and iron deposition in the bone marrow are the most common factors that affect the balance between these two cell types.¹⁰ Some studies have indicated that the expressions of OPG and RANKL are decreased and increased, respectively, in MSCs with aging.¹¹ It has also been reported that chronic diseases, disorders of iron deposition in body organs, endocrine disorders, malignant bone tumors, rheumatoid arthritis, and chronic obstructive pulmonary disease disrupt the OPG/RANKL balance resulting in the inhibition of bone reabsorption.^{12–14}

Genetic factors play an important role in the reduction of bone density and development of osteoporosis. One factor is a polymorphism in the SP1 region of the collagen type I alpha 1 gene (*COL1A1*), which has an incidence of 90% in thalassemic patients.^{10,15} Polymorphisms in the coding region of vitamin D receptor such as BSM1 (in intron 8) and FOKL (in exon 2) are also associated with reduced bone density in patients with thalassemia and sickle cell anemia.^{16,17}

The OPG promotor region represents a target sequence for various calciotropic factors regulating OPG gene expression. Polymorphisms in this and nearby regions may contribute to the genetic regulation

of bone mass.¹⁸ The association of the A163G (rs3102735) polymorphism with bone metabolism is very contradictory, as specified in Boroňová et al.¹⁹ One possible explanation for these differences is ethnic background. The purpose of this study was therefore to analyze the anthropometric, biochemical markers of bone metabolism, allelic frequencies, and genotype distribution of A163G polymorphism of the OPG gene (rs 3102735) in the Slovak female population and Roma women from eastern Slovakia. We also monitored the correlation between genotypes and phenotypes in selected groups of postmenopausal women.

2 | MATERIALS AND METHODS

The study protocol was approved by the ethics committee of the University of Prešov. The study was conducted after obtaining written consent from all individuals and in accordance with the ethical principles based on the Helsinki Declaration. Sampling was conducted for 2 years, from June 2010 to June 2012. The study included 310 postmenopausal women based on clinical screening (they had a postmenopausal status, previously with regular menstruation). The basic criteria for enrollment in the study were healthy status, both physically and mentally, and no intake of medications affecting the metabolism of hard tissue (eg, glucocorticoids, heparin, warfarin, cyclosporine, cancer drugs, thyroid hormone), which have resulted in the exclusion of medical conditions as described in Wu and Du.²⁰ Women completed a questionnaire concerning physical activities, intake of milk and dairy products, smoking, and alcohol intake. Women were divided into non-Roma and Roma groups including 209 and 101 individuals. Densitometry measurements (DXA Hologic Discovery; Hologic Inc., Waltham, MA, USA) were performed at the lumbar vertebra (L1–L4) followed by distribution of women according to bone mineral density (BMD) *T*-scores into a control group (≥ 1.0 to -1 , indicating normal status), osteopenia group (< -1.0 to -2.5), and osteoporosis group (≤ -2.5). *T*-score represents comparison of individual BMD values to the scale derived from population of healthy young adults of the same population at peak bone mass allowing objectively division of women into groups. BMD values showed comparison of values measured at the same sites in women. The average age structure and the onset of menopause and frequency distribution of individuals according to BMD *T*-score are shown in Table 1.

Body weight (kg) was measured on a digital personal scale DM-117 Dimarson with accuracy of 100 g, barefoot in light clothing, and body height (cm) was measured by digital height (DIN 862 with a precision of 0.01 mm), both presented as an average of two consecutive measurements. Subsequently, body mass index (BMI, kg m^{-2}) was calculated. Periumbilical waist circumference (in the area above the umbilicus between the lowest rib and the iliac crest) and pertrochanteric hip circumference were measured. From the measured data, waist to hip ratio (WHR) was calculated.

Blood samples for biochemical analysis were collected from the antecubital vein into test tubes without anticoagulant. Blood was

TABLE 1 Mean values of anthropometric and biochemical parameters observed in groups of non-Roma and Roma postmenopausal women

Non-Roma postmenopausal women				Roma postmenopausal women			
Characteristics	Control group	Osteoporotic group	P	Control group	Osteopenic group	Osteoporotic group	P (control groups)
N	104	105	—	68	28	5	—
Age (y)	54.37±7.45	63.02±9.53	.0001***	57.53±9.15	56.04±10.34	58.00±5.79	.014*
Age of onset of menopause (y)	47.91±4.78	47.17±4.86	.267	47.71±6.50	46.00±5.23	45.40±6.31	.810
Body height (cm)	163.19±5.85	160.99±5.97	.008**	153.90±5.95	154.80±5.58	152.90±3.36	.0001***
Body weight (kg)	74.71±12.44	69.02±11.05	.001**	85.10±18.76	85.21±19.08	75.00±15.89	.0001***
BMI (kg m ⁻²)	28.11±4.76	26.65±4.13	.019**	35.90±7.51	35.43±6.92	32.22±7.78	.0001***
Waist circumference (cm)	94.53±12.77	91.78±11.39	.102	111.79±13.95	111.39±15.94	102.40±22.07	.0001...
Hip circumference (cm)	108.26±8.63	104.52±8.87	.002**	116.81±13.05	115.50±13.20	107.00±9.46	.0001***
WHR	0.87±0.08	0.88±0.06	.613	0.96±0.06	0.96±0.07	0.95±0.12	.0001***
BMD (g cm ⁻²)	0.59±0.47	0.56±0.08	.0001***	0.63±0.15	0.62±0.34	0.61±0.30	.498
T-score	-0.25±0.65	-2.9±0.40	—	1.41±2.78	-1.67±0.40	-3.56±0.69	.0001***
ALP (μkat L ⁻¹)	1.121±0.36	1.14±0.71	.872	1.15±0.38	1.26±0.33	1.33±0.47	.692
OC (μg L ⁻¹)	15.93±6.26	13.65±8.12	.024*	17.78±8.40	16.33±7.16	24.25±13.47	.101
PINP (μg L ⁻¹)	34.51±14.62	27.79±21.69	.009**	43.52±19.66	44.07±20.50	60.35±40.66	.001***
CTx-I (ng L ⁻¹)	0.19±0.10	0.20±0.16	.859	0.23±0.13	0.21±0.15	0.50±0.36	.026*
VITD-T (μg L ⁻¹)	16.49±8.63	17.86±10.37	.299	12.29±6.24	12.59±5.40	6.71±2.89	.001***
Ca (mmol L ⁻¹)	2.52±0.14	2.48±0.39	.233	2.41±0.36	2.51±0.15	2.43±0.30	.003**
P (mmol L ⁻¹)	1.32±0.22	1.55±0.94	.016*	1.28±0.22	1.40±0.25	1.36±0.22	.196
Mg (mmol L ⁻¹)	0.88±0.08	0.87±0.12	.518	0.82±0.11	0.84±0.11	0.83±0.11	.000***
PTH (pg mL ⁻¹)	28.73±7.96	40.48±22.20	.0001***	47.82±21.88	42.90±16.87	43.02±2.54	.000***

BMI, body mass index; BMD, bone mineral density; WHR, waist to hip ratio; ALP, alkaline phosphatase; OC, osteocalcin; PINP, N-terminal peptide of type I procollagen; CTx-I, C-terminal telopeptide of type I collagen (β cross laps); PTH, parathyroid hormone; VITD-T, vitamin D total.

Statistical significance at * $P < .05$; ** $P < .01$, *** $P < .0001$.

collected at approximately the same time in the morning after fasting. Blood serum for measurements of total vitamin D, procollagen type I amino terminal propetide (PINP), serum cross-linked C-telopeptide of type I collagen (CTx-I), osteocalcin (OC), and parathyroid hormone (PTH) were separated by centrifugation (Selecta R, Spain) at 377 g for 15 minutes and analyzed on the principle of electrochemiluminescence using a fully automated immunochemical analyzer Cobas E411 (Roche Diagnostic, Hitachi HTC, Tokyo, Japan). Other biochemical markers, such as alkaline phosphatase (ALP), and mineral elements, such as Ca (calcium), Mg (magnesium), and P (phosphorus), were measured by a Cobas Integra400 plus biochemical analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland).

Genomic DNA was isolated from peripheral blood collected into tubes containing anticoagulant (K₃EDTA) according to the manufacturer of the commercially manufactured kit Ultraclean® Blood non-spin® DNA Isolation Kit (Carlsbad, CA, USA). A163G polymorphism (rs 3102735) was selected based on scientific studies associated with different phenotypic manifestations. Genotyping was performed by the TaqMan SNP Assay (C__1971047_1_; Applied Biosystems, Foster City, CA, USA) based on a standard protocol. Florescence was detected by

real-time PCR using the StepOut™ Real-Time PCR System. Mutant genotype was confirmed by sequencing and then used as a positive control for each experiment.

The anthropometrical and biochemical parameters were evaluated using the statistical characteristics such as average, median, minimum, maximum, and standard deviation and variation range. To detect the significant differences between groups in each parameter the parametric Student's *t* test was used. For testing the differences between two sets of medians, the nonparametric Mann-Whitney *U* test was used. A nonparametric one-way analysis of variance, Kruskal-Wallis test, was used to test the differences of medium values between multiple sets.

Frequencies of alleles from examined genotype frequencies were calculated using Genotyping software (SNPs; <http://ihg2.helmholtz-muenchen.de/>) for statistical evaluation of the results of individual polymorphisms. Statistical evaluation by chi-squared test to compare allelic frequencies between the two groups of women was used considering a *P*-value of $< .05$ as significant. To determine a statistically significant difference between two parameters, Spearman's correlation coefficient was used.

3 | RESULTS

The total observed group consisted of 209 patients – postmenopausal women from both the urban and rural population of eastern Slovakia. Patients recruited from the general population ($n=209$) were divided into two groups – control and osteoporosis – based on densitometric measurements. Women with osteopenia were not found. These results show that, when comparing body weight, body height, and hip circumference between the control group and the osteoporotic group, no statistically significant differences were found ($P<.01$). The average value of BMI for the whole group was $27.37\pm 4.5\text{ kg m}^{-2}$. Higher values of BMI were observed in the control group and lower values were calculated in the group of osteoporotic patients. Regarding BMI, statistically significant differences were confirmed between the control group and the osteoporotic group ($P<.05$;

Table 1). We found a higher BMD in the control group than in the osteoporotic group (Table 1). Using correlation analysis, no statistically significant effect of BMD on weight, height, BMI, waist circumference, hip circumference, or WHR index was found in the control group. In the osteoporotic group, a substantial dependence was seen ($P<.0001$) between BMD and body weight, BMD and BMI, BMD and waist circumference, BMD and hip circumference, and BMD and age (Table 2). Average values of biochemical parameters determined in Slovak non-Roma population are shown in Table 1. Concentrations of OC and PINP were significantly lower in women with osteoporosis ($P<.05$ and $P<.01$) and P and PTH levels were significantly higher in comparison to the control group ($P<.05$ and $P<.001$). Using correlation analysis, we found statistical significance ($P<.05$) in the control group when comparing ALP and body weight, ALP and BMI, ALP and waist circumference, ALP and hip circumference (Table 2), and ALP

TABLE 2 Collection of statistically significant results from correlation analysis between parameters in groups

Group	Parameter (mean $\pm$ SD)	Parameter (mean $\pm$ SD)	P
Non-Roma women with osteoporosis	Body weight (kg) (69.02 $\pm$ 11.05)	BMD (g cm $^{-2}$) (0.56 $\pm$ 0.08)	.0001
	Body weight (kg) (69.02 $\pm$ 11.05)	Ca (mmol L $^{-1}$) (2.48 $\pm$ 0.39)	.021
	BMI (kg m $^{-2}$)	BMD (g cm $^{-2}$) (0.56 $\pm$ 0.08)	.0001
	Age (y) (63.02 $\pm$ 9.53)	BMD (g cm $^{-2}$) (0.56 $\pm$ 0.08)	.0001
	Age (y) (63.02 $\pm$ 9.53)	PTH (pg mL $^{-1}$) (40.48 $\pm$ 22.20)	.05
	WHR (0.88 $\pm$ 0.06)	OC (μ g L $^{-1}$) (13.65 $\pm$ 8.12)	.033
	Waist circumference (cm) (91.78 $\pm$ 11.39)	BMD (g cm $^{-2}$) (0.56 $\pm$ 0.08)	.0001
	Waist circumference (cm) (91.78 $\pm$ 11.39)	PTH (pg mL $^{-1}$) (40.48 $\pm$ 22.20)	.014
	Hip circumference (cm) (104.52 $\pm$ 8.87)	BMD (g cm $^{-2}$) (0.56 $\pm$ 0.08)	.0001
Roma women with osteopenia	Hip circumference (cm) (104.52 $\pm$ 8.87)	PTH (pg mL $^{-1}$) (40.48 $\pm$ 22.20)	.001
	BMI (kg m $^{-2}$) (35.43 $\pm$ 6.92)	BMD (g cm $^{-2}$) (0.62 $\pm$ 0.34)	.001
	Waist circumference (cm) (111.39 $\pm$ 15.94)	BMD (g cm $^{-2}$) (0.62 $\pm$ 0.34)	.006
Control non-Roma	Hip circumference (cm) (115.50 $\pm$ 13.20)	BMD (g cm $^{-2}$) (0.62 $\pm$ 0.34)	.001
	Body weight (kg) (74.71 $\pm$ 12.44)	ALP (μ kat L $^{-1}$) (1.12 $\pm$ 0.36)	.023
	Age of onset of menopause (y)	ALP (μ kat L $^{-1}$) (1.12 $\pm$ 0.36)	.001
	Age of onset of menopause (y)	PINP (μ g L $^{-1}$) (34.51 $\pm$ 14.62)	.001
	BMI (kg m $^{-2}$) (28.11 $\pm$ 4.76)	ALP (μ kat L $^{-1}$) (1.12 $\pm$ 0.36)	.009
	BMI (kg m $^{-2}$) (28.11 $\pm$ 4.76)	OC (μ g L $^{-1}$) (15.93 $\pm$ 6.26)	.001
	BMI (kg m $^{-2}$) (28.11 $\pm$ 4.76)	Mg (mmol L $^{-1}$) (0.88 $\pm$ 0.08)	.044
	Waist circumference (cm) (94.53 $\pm$ 12.77)	ALP (μ kat L $^{-1}$) (1.12 $\pm$ 0.36)	.009
	Waist circumference (cm) (94.53 $\pm$ 12.77)	VITD-T (μ g L $^{-1}$) (16.49 $\pm$ 8.63)	.005
Control Roma	Hip circumference (cm) (94.53 $\pm$ 12.77)	ALP (μ kat L $^{-1}$) (1.12 $\pm$ 0.36)	.005
	WHR (cm) (0.87 $\pm$ 0.08)	VITD-T (μ g L $^{-1}$) (16.49 $\pm$ 8.63)	.003
	Body weight (kg) (85.10 $\pm$ 18.76)	BMD (g cm $^{-2}$) (0.63 $\pm$ 0.15)	.0001
	Body weight (kg) (85.10 $\pm$ 18.76)	CTx-I (ng L $^{-1}$) (0.23 $\pm$ 0.13)	.011
	Body weight (kg) (85.10 $\pm$ 18.76)	OC (μ g L $^{-1}$) (17.78 $\pm$ 8.40)	.001
	Body weight (kg) (85.10 $\pm$ 18.76)	PINP (μ g L $^{-1}$) (43.52 $\pm$ 19.66)	.024
	BMI (kg m $^{-2}$) (35.90 $\pm$ 7.51)	CTx-I (ng L $^{-1}$) (43.52 $\pm$ 19.66)	.012
	BMI (kg m $^{-2}$) (35.90 $\pm$ 7.51)	PINP (μ g L $^{-1}$) (43.52 $\pm$ 19.66)	.016
	Waist circumference (cm) (111.79 $\pm$ 13.95)	OC (μ g L $^{-1}$) (17.78 $\pm$ 8.40)	.015
	Hip circumference (cm) (111.79 $\pm$ 13.95)	OC (μ g L $^{-1}$) (17.78 $\pm$ 8.40)	.042

and age of onset of menopause. The values of ALP increased in the control group with increasing age, later age of onset of menopause, higher body weight, higher BMI, and with larger waist and hip circumference. We found a statistically significant relationship using correlation analysis ($P < .05$) when comparing the concentration of PINP and the age of onset of menopause in the control group. It was also found that the average values of N-terminal peptide of type I procollagen were rising at the later age of onset of menopause in the control group. Correlation analysis of the relationship between OC and WHR index revealed statistical significance in the osteoporotic group ($P < .05$). The values of osteocalcin increased with increasing WHR index in the osteoporotic group. We found statistical significance ($P < .05$) in the osteoporotic group with relation to PTH and age, and PTH and hip circumference at the significance levels of $P < .05$ and $P < .001$ using correlation analysis (Table 2). The average values of mineral elements calcium and phosphate were within the reference range (up to 0.3 mmol L^{-1} for Ca and 1.5 mmol L^{-1} for P).

According to the parameters determined in Roma women, they were divided into three groups – control, osteopenic, and osteoporotic (Table 1). Only five women with osteoporosis were identified in the Roma group, not representing a number suitable for further statistical evaluation. However, statistical differences were not detected in anthropological parameters, either. The statistical evaluation of the Roma women's biochemical parameters (ALP, OC, P1NP) based on Student's *t*-test did not reveal statistically significant differences between the control group and osteopenic group (Table 1). A statistical significance in the Roma control group was found in the relationship between OC and body weight, and CTx and BMI. According to correlation analysis, statistical significances were detected between BMD and BMI, waist circumference and BMD, hip circumference and BMD, and BMD and phosphate levels (Table 2). Furthermore, we found a correlation of at $P < .05$ between the waist circumference and OC, OC and hip circumference, P1NP and body weight, BMI and P1NP, and body weight and CTx (Table 2). Osteocalcin and the N-terminal procollagen peptide type 1 concentrations in the Roma control group decreased with increasing body weight values.

A comparison of non-Roma and Roma control groups showed significant differences in biochemical parameters (Table 1) except in ALP activity and OC concentrations. Differences in any anthropometric parameters between the both control groups of non-Roma and Roma women were statistically significant (Table 1).

In the study group, we observed the representation of individual genotypes and alleles of polymorphism A163G (rs 3102735) in the postmenopausal group (Table 3). Statistical significance was not detected in represented genotypes between control and osteoporotic group. The distribution of genotypes and alleles was within Hardy-Weinberg equilibrium in the control group and opposite to the distribution in the osteoporotic group. Statistically significant difference ($P < .05$) was found among the obtained frequency of genotypes and frequencies calculated theoretically for a given population group (observed vs expected number). Analysis of association of polymorphism A163G (rs3102735) with selected anthropometric and biochemical indicators showed significant differences between BMD and AA and GG genotypes ($P < .05$) in the control group as well as genotypes AA and AG ($P < .01$) in the osteoporotic group (Table 4). Almost all measured biochemical parameters in different genotypes of osteoporotic and control groups were within the reference range and did not differ significantly. Neither concentration of osteoformation marker OC (osteocalcin) was in genotypes AG and GG of control and osteoporotic group. Osteoresorption marker CTx and total vitamin D were below the reference range in all genotypes in both groups ($15\text{--}40 \mu\text{g L}^{-1}$, $0.330\text{--}0.782 \text{ ng L}^{-1}$, and $30\text{--}100 \mu\text{g L}^{-1}$).

4 | DISCUSSION

It is well documented that bone tissue begins to change functional and mechanistic properties at about age 40 on the basis of a negative balance between two basic processes taking place in bone tissue: osteoformation and bone resorption. It was found that bone mineral density also varies depending on race and ethnicity.²¹ In our study, we observed SNP polymorphism in the promotor region of the osteoprotegerin gene which has already been confirmed in several

TABLE 3 Genotype frequencies of A163G polymorphism in the control/osteoporotic non-Roma groups and control/osteopenic groups of Roma women

		Genotype									
		AA		AG		GG					
Group	N	n	Frequency (%)	n	Frequency (%)	n	Frequency (%)	HWE (P)	χ ²	P	OR
Control	104	77	74.038	24	23.077	3	2.885	.506	.79	.373	1.269
Women with osteoporosis	105	68	64.762	37	35.238	0	0	.028*			
Control-Roma	68	57	83.823	10	14.706	1	1.471	.478	.66	.56	0.585
Roma women with osteopenia	28	25	89.286	3	10.714	0	0	.765			

HWE (P), value of statistical significance – the result of a comparison of the actual frequencies of genotypes with theoretical frequencies (calculated according to the Hardy-Weinberg law); statistical significance (* $P < .05$); OR, odds ratio – rate risk.

TABLE 4 Comparison of anthropometric parameters and BMD in different genotypes A163G (rs3102735) in the control and osteoporotic/osteopenic groups

Group	Genotypes (non-Roma women)					Genotypes (Roma women)				
	Items	AA	AG	GG	P	AA	AG	GG	P	
Control group	N	77/104	24/104	3/104	–	57/101	10/101	1/101	–	
	Age (y)	54.29±7.53	55.33±7.34	49.00±5.57	.385	56.82±8.60	62.30±11.29	50.00±0.00	.241	
	Age of onset of menopause (y)	47.60±4.80	49.00±4.87	47.33±3.51	.464	47.44±6.66	49.20±5.96	48.00±0.00	.633	
	Height (cm)	163.24±6.10	162.63±5.10	166.33±5.86	.584	154.04±5.80	153.75±7.08	148.00±0.00	.423	
	Weight (kg)	74.32±12.83	74.33±11.07	87.67±6.81	.125	86.42±19.32	78.60±15.15	75.00±0.00	.457	
	BMI (kg/m ²)	27.96±4.96	28.12±4.19	31.75±3.20	.312	36.37±7.59	33.40±7.26	34.24±0.00	.451	
	Waist circumference (cm)	93.96±13.30	95.04±11.22	105.00±6.93	.221	113.11±14.23	105.90±10.58	96.00±0.00	.141	
	Hip circumference (cm)	108.00±8.87	108.21±8.14	115.33±3.22	.214	117.39±13.02	113.60±14.17	116.00±0.00	.786	
	WHR	0.87±0.08	0.88±0.08	0.91±0.03	.529	0.96±0.06	0.94±0.09	0.83±0.00	.123	
	BMD (g cm ⁻²)	0.54±0.06	0.58±0.10	0.71±0.17	.042*	0.61±0.30	0.68±0.35	0.36±0.00	.363	
	ALP (μkat L ⁻¹)	1.12±0.31	1.09±0.46	1.57±0.55	.907	1.16±0.39	1.13±0.30	0.75±0.00	.346	
	OC (μg L ⁻¹)	16.65±6.84	14.04±3.32	12.52±5.8	.216	16.83±8.30	23.33±7.49	15.98±0.00	.042*	
	PINP (μg L ⁻¹)	35.78±15.56	30.92±10.26	30.63±18.80	.329	41.80±19.24	54.02±20.68	36.73±0.00	.208	
	CTX-I (ng L ⁻¹)	0.20±0.11	0.19±0.08	0.14±0.15	.494	0.23±0.14	0.28±0.12	0.17±0.00	.261	
	VITD-T (μg L ⁻¹)	16.64±9.12	16.57±6.93	11.86±9.54	.597	11.96±5.53	14.44±0.21	2.23±0.00	.682	
	Ca (mmol L ⁻¹)	2.53±0.14	2.50±0.15	2.53±0.13	.687	2.41±0.38	2.42±0.21	2.23±0.00	.494	
	P (mmol L ⁻¹)	1.34±0.21	1.25±0.24	1.34±1.14	.148	1.29±0.23	1.23±0.20	1.23±0.00	.796	
	Mg (mmol L ⁻¹)	0.87±0.08	0.89±0.06	0.84±0.03	.378	0.82±0.12	0.80±0.10	0.77±0.00	.340	
	PTH (pg mL ⁻¹)	28.73±8.83	28.33±4.70	31.66±5.10	.676	47.48±23.49	50.11±11.15	44.23±0.00	.283	

Group	Genotypes (non-Roma women)					Genotypes (Roma women)				
	Items	AA	AG	GG	P	AA	AG	GG	P	
Osteoporotic group/ osteopenic group	N	68/105	37/105	0/105	—	25/101	3/101	0/101	—	
	Age (y)	63.72±9.85	61.73±8.90	—	.35	55.24±10.34	62.67±9.29	—	.169	
	Age of onset of menopause (y)	47.16±4.55	47.19±5.44	—	.941	46.36±3.89	43.00±13.11	—	.089	
	Height (cm)	160.26±5.84	162.37±6.05	—	.128	154.08±5.17	160.83±6.29	—	.075	
	Weight (kg)	67.71±11.43	71.45±10.01	—	.592	83.04±18.11	103.33±20.60	—	.053	
	BMI (kg m ⁻²)	26.37±4.29	27.15±3.80	—	.306	34.89±6.79	39.93±7.72	—	.265	
	Waist circumference (cm)	90.99±11.87	93.24±10.46	—	.317	109.92±15.37	123.67±18.50	—	.265	
	Hip circumference (cm)	103.60±9.02	106.22±8.44	—	.195	114.04±12.53	127.67±14.84	—	.110	
	WHR	0.88±0.06	0.88±0.06	—	.848	0.96±0.07	0.97±0.04	—	.970	
	BMD (g cm ⁻²)	0.60±0.20	0.70±0.16	—	.007**	0.66±0.33	0.34±0.23	—	.110	
	ALP (μkat L ⁻¹)	1.14±0.81	1.13±0.50	—	.524	1.23±0.33	1.54±0.24	—	.069	
	OC (μg L ⁻¹)	13.03±7.57	14.79±9.05	—	.358	16.15±7.52	17.84±3.19	—	.528	
	PINP (μg L ⁻¹)	26.70±23.18	29.80±18.78	—	.205	43.41±21.35	49.85±12.01	—	.738	
	CTX-I (ng L ⁻¹)	0.19±0.16	0.20±0.16	—	.883	0.20±0.15	0.33±0.16	—	.128	
VITD-T (μg L ⁻¹)	17.96±11.49	17.70±8.08	—	.615	13.20±5.34	7.52±2.89	—	.058		
Ca (mmol L ⁻¹)	2.47±0.32	2.48±0.49	—	.409	2.51±0.15	2.47±0.17	—	.710		
P (mmol L ⁻¹)	1.50±0.87	1.65±0.07	—	.931	1.42±0.25	1.22±0.23	—	.220		
Mg (mmol L ⁻¹)	0.87±0.11	0.87±0.13	—	.667	0.83±0.11	0.86±0.06	—	.823		
PTH (pg mL ⁻¹)	41.40±24.37	38.79±17.73	—	.788	38.96±10.01	75.80±28.64	—	.029*		

Statistical significance at *P<0.05; **P<0.01.

studies as a potential indicator of the connection between impaired bone structure and phenotype as well as related anthropometric and biochemical parameters in both ethnically different populations of women from Slovakia.

Two hundred and nine postmenopausal women were divided into two groups (control and women with osteoporosis) based on clinical screening and densitometry measurements. Statistically significant differences were found in age, body height, body weight, hip circumference, and BMI between groups. The mean age was significantly higher in women with osteoporosis, but the other differing parameters were lower in comparison with control (Table 1). Interestingly, Benčová et al.²² found higher BMI in women Slovak aged 60–69 years compared to women from Italy at the same age. The average BMI in the control group of the majority of the population is partly consistent with the results of Piedra et al.²³ in the Caucasian population. It was interesting that the prevalence of osteoporosis among Roma women was so low (5 out of 101 investigated), making it impossible to statistically compare investigated parameters between populations. However, it should be pointed out that Roma women who could be evaluated in the study (control group and women with osteopenia) are of approximately the same average age even when compared to a control group of non-Roma women. Based on *T*-score, approximately 28% of examined Roma women were discovered to have osteopenia. In comparison between control groups of Roma and non-Roma women, statistically significant differences were determined in anthropometric parameters such as body weight, BMI, and waist and hip circumference (WHR), which were higher on average among Roma women (Table 1). In fact, both groups of non-Roma women were overweight though all groups of Roma women fall into the category of obesity. Finkelstein et al.²⁴ indicated that obesity plays a protective role in bone loss rate in late perimenopausal and postmenopausal women. The possible association between obesity and BMD in women as well as men is the conversion of androgen to estrogen followed by oxidative metabolism pathways of estrogen²⁵ allowing the maintenance of appropriate levels of insulin and other regulating factor as insulin-like growth factor-1, leptin, and adiponectin.²⁶ The absolute BMD values (Table 1) of all Roma women groups were superior to those of non-Roma confirming that the effect of BMI on fracture risk is not linear but rather a U shaped.²⁷ Finkelstein et al.²⁴ and Sowers et al.²⁸ pointed to the finding that body size attenuated differences in the changes in bone remodeling between ethnic groups. Finally, correlation analysis of our results clearly confirms a significant association between BMD and BMI, and waist and hip circumference both in women with osteoporosis and Roma women with osteopenia (Table 2). Even though the eating habits of Roma women were generally related to higher intake of fatty foods, lower proportions of vegetables (except potatoes), and very few dairy products, unlike non-Roma women. In the context of the generally higher average absolute values of BMD in the Roma population as well as lower average values in the control groups may be mentioned that different skeletal sites are under distinct genetic and environmental influences. The genetic variants (in 56 loci) influencing bone acquisition differ from the ones involved in bone maintenance and bone loss across the life course.²⁹ An interesting fact according to

Trémollières et al.³⁰ and regarding questionnaire data could be parity of Roma women having four kids or more. Other comparisons of BMD values with regard to reference values used for women in Europe we consider yet to be less appropriate due to the relatively low number of women in groups and high standard deviations. Under the other options, the values (BMD and *T*-score) could be affected by positioning anatomic variations within the measurements, the calculation method, and most likely and indeed interesting by the values of the regional reference population of young people used.

Between commonly used markers of bone formation, serum alkaline phosphatase was shown to be in negative correlation with serum calcium levels in postmenopausal women.³¹ Blood calcium level is, in turn, related to the PTH-mediated calcium release from bones and vitamin D-activated intestinal resorption. Levels of magnesium affect the secretion of PTH in the same way as calcium, but blood phosphorus levels are regulated reciprocally to that of calcium. A statistical test comparison revealed differences between non-Roma and Roma control groups, although measured values were within reference range. Ca and Mg levels appeared to be lower and apparently PTH level were significantly higher in Roma women in control group in comparison to non-Roma control group (Table 1). ALP and vitamin D levels showed positive correlation in the non-Roma control group (Table 2). In contrast, serum osteocalcin in non-Roma women, PINP, and especially C-terminal telopeptide of type I collagen levels (in both groups) were lower than reference range. Mencej-Bedrac et al.³² did not find any effect of the same biochemical parameters when comparing bone mass in Slovene women. As can be noted from our study, when comparing the values of both women with osteoporosis, osteopenia, and controls, there were statistically significant differences (Table 1), and serum osteocalcin and PINP levels showed positive correlation with body weight and waist and hip circumference in Roma women (Table 2).

Earlier, Landahl et al.³³ described 12 polymorphisms in the osteoprotegerin gene. At least three of them (A163G, T245G, C1181G) are shown linked with risks of fractures. However, the OPG gene contains several binding sites that could mediate stimulation of OPG gene expression by various calciotropic factors.³⁴ Studies on promoter OPG polymorphism A163G were put into the association with increased risk of osteoporosis. The allele A was associated with osteoporosis in the Chinese population,³⁵ although an obvious connection with the G allele was introduced by meta-analysis of Luo et al.³⁶ and Guo et al.³⁷ In the Caucasian population of the Czech Republic, actually very close to Slovakia, Zajíčková et al.¹⁸ demonstrated such an association. However, in Slovakia, Krajčovicová et al.³⁸ reaffirmed that the G allele was not associated with an increased incidence in bone fractures. We found the most significant representation of genotypes AA in all examined subgroups, 74% in non-Roma, 83.8% in Roma control groups, 64.8% in women with osteoporosis, and 89.3% in women with osteopenia. A higher frequency of G allele (17.6%) was found in non-Roma women with osteoporosis when compared to the control group (14.4%). However, in Roma women with osteopenia, the frequency of G allele reached only 2.9% (Table 3).

Some differences between non-Roma and Roma control groups could be noticed in measured determinants in association with

genotypes. First, the highest mean values of height, vitamin D, Ca, P, Mg were found in genotype AA in non-Roma group. Conversely, in Roma groups the highest mean values of age, weight, BMI, waist and hip circumference, WHR, BMD, ALP, OC, PINP, and CTx-I associated with genotype AA were found (Table 4). Despite relatively high values of BMD in Roma control groups, the highest values of all were associated with genotype GG in non-Roma women and again in connection with genotype AG the highest values of OC, PINP, and parathyroid hormone in Roma women were measured. A similar trend in the accumulation of higher levels of mentioned parameters in genotype AA were observed in the groups of women with osteoporosis and osteopenia. The highest mean values of BMD in osteoporotic non-Roma women and PTH levels in osteopenic Roma women associated with genotype AG represented another noticeable difference between Roma and non-Roma groups.

A comparison of genotype distribution and the values of measured anthropometric and biochemical parameters focused on genotype GG in the non-Roma control group. Genotype AG in non-Roma women with osteoporosis was associated with highest values of BMD among measured ($P < .05$ and $P < .001$, respectively), while GG genotype was not detected. In the examined Roma women, there is no indication of the protective effects of G allele. Its presence is associated with low BMD and significantly elevated levels of parathyroid hormone. Within the studied polymorphisms of OPG, we can summarize that the presence of the G allele has a different effect on metabolism and structure and consequently susceptibility to bone damage in the two ethnically diverse populations of women in Slovakia.

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