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Preliminary results of ethnic divergence of G1181C (rs2073618) and C290T (rs9525641) OPG gene polymorphisms in groups of postmenopausal Slovak women

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Abstract. – OBJECTIVE: In this study, we focused on observation of the genetic polymorphisms of the OPG genes G1181C (rs2073618) and C290T (rs9525641), their interactions with biochemical markers and anthropometric parameters in groups of postmenopausal Slovak women (Roma and non-Roma, n = 311).

PATIENTS AND METHODS: Genomic DNA was extracted and purified from peripheral blood leukocytes by the kit Ultraclean® Blood non-spin® (Carlsbad, CA, USA) using a standard protocol. Genotyping was performed by the TaqMan SNP genotyping assay. Biochemical markers were measured by the Cobas e411 (Roche Diagnostic, Tokyo, Japan) and Cobas Integra400 plus (Roche Diagnostic, Rotkreuz, Switzerland) analysers.

RESULTS: We recorded a higher frequency of the T allele in the C290T polymorphism of the non-Roma control group (53.846%), in Roma groups: control (T - 56.618%) osteoporotic (T - 51.471%). In the G1181C polymorphism, the CC genotype occurred more in the osteoporotic group (34.286%) compared to the control group (27.885%). In the group of postmenopausal Roma women, a statistically significant difference ($p<0.05$) was found between osteoporotic and control in the biochemical parameters' osteocalcin, C-terminal telopeptide I, and age. Statistically significant differences ($p<0.0001$) were also found in bone mineral density and T-score. The high odds ratio suggests the association of G1181C with osteoporosis. A close relationship was found between haplotypes, BMD, T-score, and IL-6 in control; and BMI, WHR, T-score, and osteocalcin in osteoporotic groups of Roma and non-Roma women.

CONCLUSIONS: The results point to differences in the occurrence of genotypes and associations of haplotypes with the manifestation of osteoporosis in Roma and non-Roma women. However, a larger number of samples is needed to determine whether or not there are differences between the Roma and non-Roma populations.

Key Words:
Biochemical markers, Bone mineral density, Genetic aspects, Menopause, Osteoporosis.

Introduction

Osteoporosis is a multifactorial skeletal disease characterized by the deterioration of bone microarchitecture, resulting in an increased risk of fractures and decreased bone mineral density (BMD)[1-7].

Bone turnover control is provided by the RANK/RANKL/ osteoprotegerin (OPG) system. This physiological mechanism plays an important role in bone metabolism as well as in the immune and cardiovascular systems. Imbalance in this

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system leads to inflammatory, bone and genetic diseases. RANK (receptor activator of nuclear factor-kappa B) is a receptor expressed on preosteoclasts. RANKL (receptor activator of nuclear factor-kappa B ligand), which is a ligand for RANK and OPG, is synthesized in osteoblasts. Interaction between RANKL and RANK stimulates the differentiation and maturation of osteoclasts. The effect of RANKL is blocked by OPG, which acts as a soluble receptor for RANKL, thereby preventing its binding to RANK, leading to a reduction in osteoclast maturation and consequently to a reduction in bone resorption. The RANKL/OPG ratio determines the rate of bone remodeling. Many scientific studies have suggested that the expression of OPG and RANKL is controlled by various cytokines and growth factors, such as transforming growth factor (TGF), insulin-like growth factor (IGF), BMI, and estrogen. Any genes involved in the expression of any component of the RANK / RANKL / OPG pathway are therefore candidate genes for osteoporosis[6,7]. Polymorphisms in the gene for OPG are associated with various phenotypes such as low BMD[8], low T-score in the femoral and lumbar spine, increased risk of fracture[9,10], and influence on biochemical markers of bone turnover[11,12].

In our study, we focused on monitoring the genetic polymorphisms of OPG genes G1181C (rs2073618) and C290T (rs9525641) in groups of postmenopausal Slovak women. This study is specific because of the ethnic divergence of gene polymorphisms and allelic variants and interactions between the gene, biochemical markers and anthropometric parameters. The goal of this study was to determine whether the genotypes of selected polymorphisms are associated with low BMD, anthropometric parameters, or inflammatory markers when comparing the majority population with the Roma population.

Patients and Methods

Subjects

The study included 311 postmenopausal women – 209 women of the majority population (non-Roma) and 102 women of the minority population (Roma) (Table I). Both groups of women were divided on the basis of clinical screening (postmenopausal status, regular menstruation, good physical and mental status). Other inclusion criteria for enrollment, according to Mydlárová Blaščáková et al[11], were no medicinal products intake that would affect hard tissue metabolism (such as heparin, warfarin, cyclosporin, glucocorticoids, or thyroid hormones), and densitometric measurement in the lumbar spine region L1 – L4 (DXA HologicDiscovery, HologicInc., Waltham, MA, USA) followed by distribution of women according to bone mineral density (BMD) T-scores into a control group (≥1.0 – -1, normal status), osteopenia (<-1.0 – -2.5) and osteoporosis (≤-2.5). T-score represents a comparison of individual BMD values to the scale derived from the population of healthy young adults of the same population at peak bone mass, allowing objectively division of women into groups. BMD values showed the comparison of values measured at the same sites in women. The study was approved by the Ethics Committee of the University of Prešov no. 2/2013. The study was conducted after obtaining of written informed consent from all individuals and carried out in accordance with the Ethical principles under the Helsinki Declaration. Sampling was carried out over 3 years between 2014–2017.

Anthropological measurements

In the observed groups of women, we measured body weight (kg), body height (cm), waist circumference (cm), and hip circumference (cm) (Table I). Bodyweight was measured on a digital personal scale DM–117 Dimarson (Dimarson Elektroniks, Kyiv, Ukraine), with an accuracy of 100 g, body height was measured barefoot in light clothing by digital height Kinex, 200/0,02 mm, DIN 862 with a precision of 0.02 mm (KINEX Measuring s.r.o., Prague, Czech Republic) as an average of two consecutive measurements. Subsequently, data were taken from weight and height to calculate body mass index – BMI (kg/m^2). Waist circumference was measured periumbilical and hip circumference pertrochanteric using a textile belt. The measured data were used to calculate the waist-hip ratio (WHR) index (waist circumference – cm/hip circumference – cm).

Densitometric measurements

Densitometric measurement of postmenopausal women was conducted with a body densitometer DXA Hologic (DXA HologicDiscovery, Hologic Inc., Waltham, MA, USA). Bone mineral density (BMD) measurement was performed in the lumbar spine (L1 – L4). The device compared the measured values of bone mineral density (g/cm^2) with the average value of the healthy population and provided a T-score. Based on the

densitometric (T-score) results, according to the WHO guidelines, subjects were categorized into the control group - normal (T-score ≥1.0 to -1), osteopenic group (<-1.0 to -2.5) and osteoporotic group (≤-2.5).

Genotyping of polymorphisms

Genomic DNA was isolated from peripheral blood into tubes containing the anticoagulant (K_3EDTA), following the manufacturer's instructions for the Ultraclean® Blood non-spin® DNA Isolation Kit (MO BIO, Laboratories, Inc., Carlsbad, CA, USA). Polymorphisms G1181C (rs2073618) and C290T (rs9525641) were selected based on scientific studies [8-10,12] which are associated with different phenotypic manifestations.

Genotyping was performed by the TaqMan SNP genotyping assay (C___1971047_1_; C__30171941_10) (Thermo Fisher Scientific, Waltham, MA, USA) based on a standard protocol. Fluorescence was detected by the method of Real-Time PCR using the StepOut™ Real-Time PCR System. Mutant genotype was confirmed by sequencing and was then used as a positive control for each experiment. Genotyping was performed by the TaqMan SNP genotyping assay (Thermo Fisher Scientific, Waltham, MA, USA).

Biochemical measurements

Venous blood samples were collected from all individuals from the *vena mediana cubital* into tubes without anticoagulant. From the blood samples, blood serum was separated by 377 g centrifugation over 15 minutes (Selecta R, Spain), where selected biochemical markers (osteocalcin (OC), C-terminal telopeptide I (CTx-I), and IL-6) were then measured by the fully automated Cobas e411 (Roche Diagnostic, Hitachi HTC Immunochemistry Analyzer, Tokyo, Japan). Mineral elements such as Calcium (Ca), Magnesium (Mg) and Phosphorus (P) were measured by the Cobas Integra400 plus biochemical analyser (Roche Diagnostic Ltd., Rotkreuz, Switzerland).

Statistical analysis

The measured data were processed with Excel 2010 and Statistica ver. 10 (StataCorp. 2007. Stata Statistical Software: Release 10. College Station, TX, USA). The different parameters were evaluated using the statistical characteristics of the position (average) and variability (standard deviation). To determine the significance of differences between groups in each parameter, a parametric Student's *t*-test was. A Kruskal-Wallis test (nonparametric analysis of variance) was used to detect differences in the median values between multiple sets. To determine the statistically significant dependence between the two parameters, Spearman's correlation coefficient was used.

For statistical evaluation of the results of individual polymorphisms, we calculated the frequencies of alleles from the examined genotype frequencies using the software Genotyping (SNPs) (http://ihg2.helmholtz-muenchen.de/). For statistical evaluation, we used the Chi-squared test to compare allelic frequencies between the two groups of women and determine the difference in the number of alleles between the two groups. A p-value <0.05 was considered statistically significant.

The frequencies of the observed haplotypes were calculated from the number of detected genotypes using an EM algorithm (expectation-maximization algorithm), which is part of the Arlequin 3.1 software package. The statistical significance of differences in the number of individual alleles, genotypes, and haplotypes of polymorphisms between patient and control populations was subsequently analyzed by Fisher's exact test. Using the MEDCALC® statistical software (https://www.medcalc.org/calc/comparison_of_means.php), mean values were compared with the standard deviations of the monitored parameters in individual haplotypes in control and osteoporotic groups among postmenopausal women of the Roma and non-Roma populations.

Results

This study consisted of 311 postmenopausal women of the majority (non-Roma) and minority (Roma) population of eastern Slovakia. The patients of the majority (non-Roma) population were divided into groups: control (Control = 104) and osteoporotic (OG = 105) based on densitometric measurements in the lumbar spine L1 – L4. An osteopenic group was not found. Postmenopausal women of the minority population (Roma) were divided into three groups: control (Control = 68), osteopenic (OP = 29), and osteoporotic (OG = 5). Given that only 5 postmenopausal women were included in the osteoporotic group in the minority population, we consider this group statistically very small (inhomogeneous). On the basis of this and other facts (low BMD, higher mean age, higher risk of fracture, higher genetic predisposition to disease development) and the scientific study

Table I. General characteristics of the subjects.

	Postmenopausal non-Roma women			Postmenopausal Roma women		
	OG n=105	Control n=104	p	OP+OG n=34	Control n=68	p
Age (years)	66.44 ± 9.30	65.99 ± 9.48	0.740	60.18 ± 8.37	55.68 ± 9.40	0.020*
Age of onset of menopause (years)	47.17 ± 4.86	47.91 ± 4.78	0.267	48.26 ± 6.62	46.67 ± 5.95	0.223
BMI ($kg.m^{-2}$)	26.02 ± 4.16	29.17 ± 3.94	0.001**	33.96 ± 6.35	36.30 ± 7.69	0.129
WHR (cm)	0.91 ± 0.08	0.92 ± 0.08	0.270	0.97 ± 0.07	0.95 ± 0.06	0.1377
BMD (g/cm^2)	0.56 ± 0.08	0.72 ± 0.09	0.0001**	0.36 ± 0.08	0.74 ± 0.31	0.0001**
T-score	- 2.92 ± 0.43	- 0.27 ± 0.67	0.0001**	- 1.95 ± 0.80	1.41 ± 2.78	0.0001**
OC ($\mu g.l^{-1}$)	13.65 ± 8.12	15.93 ± 6.26	0.024*	20.50 ± 8.76	16.50 ± 8.10	0.024*
CTx-I ($ng.l^{-1}$)	0.19 ± 0.10	0.20 ± 0.16	0.859	0.29 ± 0.19	0.21 ± 0.14	0.018*
Ca ($mmol.l^{-1}$)	2.48 ± 0.39	2.52 ± 0.14	0.233	2.43 ± 0.17	2.44 ± 0.36	0.878
P ($mmol.l^{-1}$)	1.55 ± 0.94	1.32 ± 0.22	0.016*	1.31 ± 0.23	1.32 ± 0.24	0.841
Mg ($mmol.l^{-1}$)	0.87 ± 0.12	0.88 ± 0.08	0.518	0.82 ± 0.08	0.82 ± 0.12	1.000
IL-6 (pg/ml)	3.82 ± 9.03	2.55 ± 4.06	0.192	3.68 ± 2.90	4.26 ± 4.86	0.524

Abbreviations: BMI – body mass index; WHR – waist to hip ratio; BMD – bone mineral density; OC – osteocalcin; CTx-I – C- terminal telopeptide of type I collagen (β cross laps); Ca – calcium; P – phosphorus, Mg – magnesium; IL-6 – interleukin 6. Statistical significance at *p<0.05; **p<0.01.

by Mamolini et al[13], the decision was made to combine the osteopenic and osteoporotic groups (OP+OG). Table I shows the statistical values of selected anthropometric, densitometric and biochemical markers.

These results indicate that significantly higher mean values in all anthropometric, biochemical and densitometric parameters (except for age) were found in the control group of postmenopausal non-Roma women. By means of Student's *T*-test, a statistically significant difference was found between control and OG of postmenopausal women in parameters of BMI, BMD, T-score and statistically significant differences were found in the biochemical parameters OC and phosphorus. A highly statistically significant correlation (p<0.01) was declared after correlation analysis between BMD and BMI. Kruskal-Wallis nonparametric analysis of variance determined statistically significant dependence (p<0.05) between the age and BMD in OG postmenopausal women. The value of BMD gradually declined with age.

All calculated mean values of biochemical parameters were within the range of reference values in all groups of postmenopausal Roma and non-Roma women. In postmenopausal Roma women, a statistically significant difference was found between OP+OG and control (p<0.05) in the biochemical parameters OC and CTx-I and in age. Statistically significant differences (p<0.0001) were also found in the parameters BMD and T-score.

In our research, we also dealt with the representation of individual genotypes and the G1181C (rs2073618) and C290T (rs9525641) alleles of the OPG gene in the monitored groups of women (postmenopausal non-Roma women *vs.* postmenopausal Roma women). Table II provides a statistical analysis of the results.

Regarding the C290T (rs2073618) OPG gene polymorphism, the heterozygous CT genotype was represented at the highest frequency in the two monitored groups (postmenopausal non-Roma and Roma women). Statistical significance in the representation of genotypes between control and osteoporotic groups was not found in either group. The distribution of genotypes and alleles in the osteoporotic group was found to be consistent with the Hardy-Weinberg equilibrium. The frequency of the alleles in both monitored groups of postmenopausal non-Roma women was balanced. A higher incidence of the T allele was noted in the control group (53.846%), while the frequency of the C allele was higher in the osteoporotic group (46.190%) compared to the control group. In the group of postmenopausal Roma women, a higher incidence of the T allele was found in control (C – 43.382%, T – 56.618%), and also in OP+OG (C – 48.529%; T – 51.471%).

Table II. Genotype frequencies of the SNP polymorphisms C290T (rs9525641) and G1181C (rs2073618) in the control/osteoporotic non-Roma groups and control/osteopenic and osteoporotic groups of Roma women.

			Genotype										
					CC		CT		TT				
Polymorphism	**Population**	**Group**	**N**	**N**	**Frequency (%)**	**N**	**Frequency (%)**	**N**	**Frequency (%)**	**HWE (p)**	**X^2**	***p***	**OR**
C290T rs9525641	Postmenopausal non-Roma women	Control	104	16	15.385	64	61.538	24	23.077	0.075			
		OG	105	26	24.762	45	42.857	34	32.381	0.158	0.00	0.994	0.999
	Postmenopausal Roma women	Control	68	13	19.117	33	48.530	22	32.353	0.920			
		OP+OG	34	7	20.588	19	55.882	8	23.530	0.489	0.49	0.486	0.813
			Genotype										
					GG		**GC**		**CC**				
Polymorphism	**Population**	**Group**	**N**	**N**	**Frequency (%)**	**N**	**Frequency (%)**	**N**	**Frequency (%)**	**HWE (p)**	**X^2**	***p***	**OR**
G1181C rs2073618	Postmenopausal non-Roma women	Control	104	24	23.077	51	49.038	29	27.885	0.862			
		OG	105	22	20.952	47	44.762	36	34.286	0.364	0.77	0.381	1.188
	Postmenopausal Roma women	Control	68	10	14.706	36	52.941	22	32.353	0.443			
		OP+OG	34	5	14.706	17	50	12	35.294	0.796	0.04	0.840	1.063

Abbreviations: 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 (*$p<0.05$), OR, odds ratio – rate risk.

In the G1181C polymorphism (rs2073618), the heterozygous genotype GC (Control – 49.038%; OG – 44.762%) was represented in the highest frequency group in postmenopausal non-Roma women and the CC genotype was more prevalent in the osteoporotic group (34.286%) than the control group (27.885%). The distribution of genotypes and alleles was consistent with the Hardy-Weinberg equilibrium. No statistical significance of genotypes and alleles was found between control and osteoporotic groups in either population studied. In the populations of postmenopausal non-Roma women, a higher frequency of the G allele was found in the control group (47.596%) than in the osteoporotic group (43.333%). The frequency of the C allele was higher in the osteoporotic group (56.667%) compared to the control group (52.404%). Similarly, it was also found in the populations of postmenopausal Roma women, where we found a higher frequency of occurrence of the C allele in Control (C – 41.176%, G – 58.824%) and in OP+OG (C – 60.294%, T – 39.706%).

By comparing the haplotype frequencies of SNPs C290T and G1181C, no statistical significance was found in the haplotypes of patients diagnosed with osteoporosis compared with control groups (non-Roma population of postmenopausal women – χ^2=5.153, p<0.161; Roma population of postmenopausal women – χ^2=1.203, p<0.752) (Table III).

In our monitored set of postmenopausal Roma and non-Roma women, we found the presence of all 9 genotypes (Table III). Combined genotypes in the two populations of postmenopausal women were not statistically significantly different between patients with osteoporosis and controls (non-Roma population of postmenopausal women - χ^2=11.715, p<0.164; Roma population of postmenopausal women – χ^2=2.821, p<0.945).

Comparison of allele, genotypes and haplotypes frequencies between groups of postmenopausal women with diagnosed osteoporosis and healthy individuals revealed no statistically significant differences, suggesting that these polymorphisms are not associated with postmenopausal osteoporosis in the Slovak population of non-Roma and Roma women.

Based on these results, we decided to compare the mean values and standard deviations of the monitored parameters in individual haplotypes in the control and osteoporotic groups of postmenopausal women of the Roma and non-Roma populations using the MEDCALC® statistical software (Ostend, Belgium). Table IV shows the statistical p-value calculated by the T-test. Statistically, significant differences are marked with the monitored parameters.

Discussion

The Slovak Republic is a multinational state. According to the last census of the Slovak population in 2011, 1.96% of citizens claimed the Roma ethnic group. The health status of the Roma population is unsatisfactory and conditional on multiple factors: inadequate health care for own health as well for the health of children, lack of hygiene, frequent alcoholism and smoking, inappropriate housing, etc.

Within the Roma ethnic group, there is a greater inter-population genetic variability than in non-Roma populations. It is assumed that the present diversity of the Roma ethnic group reflects, at least partially, the situation before leaving their original homeland[14].

At present, there are few scientific studies dealing with Roma morbidity compared to other populations. The main reason for this being that this ethnic group lives in isolation and often changes the location of residence. Roma constitutes a genetic isolate that is internally differentiated into more or less isolated groups, with a small inter-population flow of genetic information and with a significant role of the primary, secondary, and tertiary effects of the founder. For these reasons, we also decided to include postmenopausal Roma women in our research group.

Only a few scientific studies are devoted to the C290T polymorphism. Mencej et al[15], found that the polymorphisms rs9525641 and rs9533156 are associated with low BMD in the femoral and lumbar spine. Mencej-Bedrač et al[16] found that the C290T polymorphism is associated with low BMD value in osteoporotic postmenopausal women in a population of 641 Slovenian individuals divided into four groups - osteoporotic (239 postmenopausal women), control (228 postmenopausal women), premenopausal women (57 women), and a group of older men (117 individuals), based on statistical analysis. The C290T polymorphism has not shown an effect on BMD in premenopausal women or elderly men. The genotype frequencies in the control group were CC (24.1%), CT (46.5%), TT (29.4%). The genotype frequencies in the postmenopausal osteoporotic group were – CC (20%), CT (49.2%), TT (30.8%). The results of our study, in a group of postmeno-

Table III. Frequencies of haplotypes and combined genotypes of SNP polymorphisms of C290T (rs9525641) and G1181C (rs2073618).

		Non-Roma postmenopausal women					Roma postmenopausal women				
Haplotype group		**OG**		**Control**		**Significance OG vs. Control**	**OP+OG**		**Control**		**Significance OP+OG vs. Control**
C290T	G1181C					χ^2=5.153 p=0.161					χ^2=1.203 p=0.752
C	G	46	43.81%	61	58.65%		17	50.00%	33	48.53%	
T	G	23	21.90%	14	13.46%		5	14.71%	13	19.12%	
C	C	25	23.81%	19	18.27%		9	26.47%	13	19.12%	
T	C	11	10.48%	10	9.62%		3	8.82%	9	13.24%	
C	G or C	71	67.62%	80	76.92%	χ^2=2.256 p=0.133	26	76.47%	46	67.65%	χ^2=0.850 p=0.357
T	G or C	34	32.38%	24	23.08%		8	23.53%	22	32.35%	
C or T	G	69	65.71%	75	72.12%	χ^2=0.999 p=0.318	22	64.71%	46	67.65%	χ^2=0.088 p=0.766
C or T	C	36	34.29%	29	27.88%		12	35.29%	22	32.35%	
Combined genotypes											
C290T	G1181C										
CC	GG	3	2.86%	4	3.85%	χ^2=11.715 p=0.164	0	0.00%	2	2.94%	χ^2=2.821 p=0.945
CT	GC	19	18.10%	33	31.73%		9	26.47%	17	25.00%	
TT	CC	11	10.48%	10	9.62%		3	8.82%	9	13.24%	
CC	GC	12	11.43%	9	8.65%		4	11.76%	7	10.29%	
CC	CC	11	10.48%	3	2.88%		3	8.82%	4	5.88%	
CT	GG	12	11.43%	15	14.42%		4	11.76%	7	10.29%	
CT	CC	14	13.33%	16	15.38%		6	17.65%	9	13.24%	
TT	GG	7	6.67%	5	4.81%		1	2.94%	1	1.47%	
TT	GC	16	15.24%	9	8.65%		4	11.76%	12	17.65%	

Table IV. Comparison of the mean values of monitored parameters between individual haplotypes in control and osteoporotic groups among postmenopausal women of the Roma and non-Roma populations.

Control group	**CG**		**TG**		**CC**		**TC**	
	***t*-test**	***p***	***t*-test**	***p***	***t*-test**	***p***	***t*-test**	***p***
Age (years)	1.599	0.117	2.912	0.004	1.008	0.315	1.479	0.141
Age of onset menopause (years)	0.942	0.351	1.338	0.183	0.821	0.413	0.439	0.662
BMI ($kg.m^{-2}$)	0.447	0.657	14.618	0.0001	11.427	0.0001	6.978	0.0001
WHR (cm)	1.649	0.106	5.889	0.0001	6.437	0.0001	2.288	0.023
BMD ($g.cm-^{2}$)	6.154	0.0001	4.052	0.0001	4.809	0.0001	2.482	0.014
T-score	6.202	0.0001	7.586	0.0001	5.164	0.0001	2.347	0.020
OC ($\mu g.l^{-1}$)	1.284	0.206	0.674	0.501	0.207	0.837	1.699	0.091
CTx-I ($ng.l^{-1}$)	1.663	0.103	0.725	0.469	1.874	0.063	3.087	0.002
Ca ($mmol.l^{-1}$)	0.525	0.602	8.415	0.0001	0.720	0.473	3.797	0.0002
P ($mmol.l^{-1}$)	1.706	0.090	4.903	0.0001	0.285	0.776	0.859	0.392
Mg ($mmol.l^{-1}$)	1.786	0.076	8.287	0.0001	5.453	0.0001	3.976	0.0001
IL-6 ($pg.ml^{-1}$)	5.033	0.0001	5.718	0.0001	9.869	0.0001	2.008	0.046
Osteoporosis group	**CG**		**TG**		**CC**		**TC**	
	***t*-test**	***p***	***t*-test**	***p***	***t*-test**	***p***	***t*-test**	***p***
Age (years)	1.755	0.082	5.009	0.0001	0.959	0.339	1.521	0.131
Age of onset menopause (years)	0.341	0.734	0.739	0.461	0.267	0.790	1.714	0.089
BMI ($kg.m^{-2}$)	7.244	0.0001	8.302	0.0001	9.030	0.0001	10.022	0.0001
WHR (cm)	8.986	0.0001	7.291	0.0001	5.068	0.0001	7.114	0.0001
BMD ($g.cm^{-2}$)	2.775	0.006	7.904	0.0001	8.570	0.0001	6.040	0.0001
T-score	10.694	0.0001	20.303	0.0001	6.397	0.0001	10.906	0.0001
OC ($\mu g.l^{-1}$)	3.621	0.0004	5.124	0.0001	5.241	0.0001	8.347	0.0001
CTx-I ($ng.l^{-1}$)	2.644	0.0091	4.548	0.0001	1.445	0.151	4.849	0.0001
Ca ($mmol.l^{-1}$)	1.443	0.151	1.476	0.142	0.317	0.752	1.217	0.226
P ($mmol.l^{-1}$)	1.138	0.257	1.326	0.187	1.783	0.077	6.942	0.0001
Mg ($mmol.l^{-1}$)	0.489	0.625	0.000	1.000	1.815	0.071	0.447	0.656
IL-6 ($pg.ml^{-1}$)	0.405	0.686	0.504	0.615	1.089	0.278	17.067	0.0001

pausal Slovak non-Roma women, do not coincide with the results of Mencej-Bedrač et al[16] regarding genotype frequencies within the C290T polymorphism (rs9525641). Results of genotype frequencies in the control group of postmenopausal Roma women (CC 19.12%, CT 48.53%, TT 32.35%) are similar to those reported by Mamolini et al[13] who conducted research on postmenopausal Italian women (CC 17.95%, CT 48.72%, TT 33.33%). The results of the allele frequencies were the same in both groups of postmenopausal non-Roma women as in the SNP polymorphism database (www.ncbi.nlm.nih.gov). In the OP+OG group of the postmenopausal Roma women, differences were found in the representation of the allele frequencies (C 44.64%, T 55.36%).

Takács et al[17] observed a higher BMD value within the TT genotype in their study of 360 postmenopausal Hungarian women (C290T polymorphism). The authors report that a higher proportion of genotypes containing at least one mutated allele (C) was measured in the osteoporotic group (27%) *vs.* the control group (20.3%). In our study, we also observed higher bone density (non-Roma control: 0.59 ± 0.11 g/cm^2, Roma control: 0.76 ± 0.39 g/cm^2) in the TT genotype in Roma and non-Roma control groups. Based on our findings and scientific studies by Takács et al[17] and Mamolini et al[13] we can state that the TT genotype may represent a protective antagonist against bone resorption.

On the basis of the statistical evaluation of the multiple comparisons of values by Kruskal-Wallis non-parametric analysis of variance, we found a statistically significant difference ($p<0.05$) in BMI parameters between genotypes CC – TT, CT – CC, in the osteoporotic group of postmenopausal non-Roma women. We also found a significant relationship between the C290T polymorphism and BMI in the osteoporotic group of the majority population. For the remaining monitored parameters, no relationship was confirmed with the C290T polymorphism in either group.

While authors of other studies[18-20] have not found any relationship of polymorphism G1181C (rs2073618) with postmenopausal osteoporosis, they point to the discovery of different outcomes within ethnic groups as well as between the areas of the same state within the same ethnic group. The authors' association studies point to the association of G1181C polymorphism with osteoporotic phenotype expression[10,21-24]. In contrast to these studies, other authors revealed a non-significant association of G1181C polymorphism with the expression of this phenotype[9, 25-27].

Seremak-Mrozikiewicz et al[28] did not detect significant differences in genotype frequencies of G1181C (rs2073618) polymorphism in their study of 310 postmenopausal women divided into three groups (osteoporotic, osteopenic, control). The frequency of genotypes in the osteoporotic group was as follows: GG (19.40%), GC (52.50%), CC (28.10%), frequency of the G allele was 45.7%, and the C allele 54.3%. The effect of this polymorphism on bone density, according to the authors, remains unclear. They assume that the third amino acid, lysine, is exchanged for asparagine. This may cause changes in kinetics and the secretion of this protein. In our study, in the osteoporotic group of non-Roma women, different genotype rates were found (GG 20.95%, GC 44.76%, CC 34.29%) and allele rates (G 43.33% and C 56.57%) as reported by Seremak-Mrozikiewicz et al[28]. When comparing the frequency representation of the G1181C polymorphism allele (rs2073618) according to the SNP polymorphism database (www.ncbi.nlm.nih.gov), we found a higher incidence of the C allele in the osteoporotic group of postmenopausal non-Roma women and in both monitored Roma groups (non-Roma control: 56.67%, Roma control: 61.03%, Roma OP+OG: 57.14%).

Differences in allelic frequencies were reported by Moffett et al[29], Richards et al[30], Styrkarsdottir et al[31], Styrkarsdottir et al[32], Paternoster et al[33], Kim et al[23] and Piedra et al[34] pointing to a higher BMD value in the lumbar spine region of women with the CC genotype than women with the GC or GG genotypes. Also, Choi et al[22] found that women with the CC genotype had a 7% higher BMD value in the distal radius and a 10% higher bone area in the hepatic area (*os calacaneus*) compared to a group of women with GG genotype in postmenopausal Korean women. In our study, there was no confirmed association of CC genotype with higher BMD in any group.

We found a statistically significant association ($p<0.01$) between BMD and BMI, and between age and BMD ($p<0.05$) in OG of postmenopausal non-Roma women. The results of our study show that differences in genotype and allele frequency between populations (Roma *vs.* non-Roma) and groups (control group *vs.* osteoporotic group) are not significant in the investigated polymorphisms C290T (rs9525641) and G1181C (rs2073618). However, we decided to perform a haplotype analysis. The T-test results calculated by software MEDCALC® (https://www.medcalc.org/calc/comparison_of_means.php) showed statistically significant difference

(p<0.0001) in the parameters BMD, T-score, and IL-6 analysed in all haplotypes (CG, TG, CC, TC) comparing the control group of postmenopausal Roma and non-Roma women. When comparing the osteoporotic group of the non-Roma population with the OP+OG of Roma population, we found a statistically significant difference (p<0.0001) in parameters BMI, WHR, BMD, T-score, and OC in all observed haplotypes (Table IV). The results of our study are very difficult to compare with the results of other scientific studies dealing with the same issue as the authors investigating other haplotypes did not address the issue of the Roma population in Slovakia. However, they point to differences between Roma and non-Roma populations and the association G1181C polymorphism with osteoporosis manifestation.

Conclusions

The results of our study provide preliminary information and point to significant differences in the representation of genotypes and alleles of the polymorphisms C290T (rs9525641) and G1181C (rs2073618) among the populations (Roma *vs.* non-Roma) and the monitored groups (control group *vs.* osteoporotic group) of postmenopausal Slovak women. There were significant differences between some anthropometric parameters in Roma and non-Roma control and osteoporotic groups in BMD, T-score and OC. Within Roma groups, CTx-I and P levels also differed. According to the odds ratio, there is an association between G1181C polymorphism and osteoporosis. We found a closer relationship between BMD, T-score and IL-6 levels in control groups and BMI, WHR, T-score, OC, and haplotypes in osteoporotic groups of Roma and non-Roma women. As allele frequencies showed no significant difference in either Roma or non-Roma control groups, it will be more appropriate to extend the study by a significantly larger number of analysed samples to confirm if there are population differences.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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