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CER 1 gene polymorphism in postmenopausal Roma and non-Roma Slovak women in connection with osteoporosis

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Abstract. – OBJECTIVE: The aim of our study was to investigate the relationship between the *rs74434454* polymorphism of the *CER1* gene and selected biochemical, densitometric and anthropometric markers in Slovak postmenopausal women of two ethnic groups: Roma and non-Roma.

SUBJECTS AND METHODS: The scientific study included 303 postmenopausal women of the non-Roma and Roma populations who were divided into two groups based on densitometric measurements: control group (CG) and osteoporotic group (OG). Genomic DNA was isolated from peripheral blood using a commercial NucleoSpin® Blood kit following a standard protocol. The TaqMan Real-Time PCR method was used for genotyping. Biochemical markers were measured with Cobas e411 and Cobas Integra400 plus analysers.

RESULTS: In the control group of postmenopausal Roma women, the occurrence of the risk genotype GG was not observed. In the group of Roma women with osteopenia and osteoporosis, the GG genotype occurred at a frequency of 3.03%. In the group of non-Roma women (between CG and OG) statistically significant differences were found in all monitored biochemical markers except CTx-I (p<0.66). In contrast, in the group of Roma women, statistical significance was only found in the osteoresorption marker CTx-I (p<0.007). In the population of Roma women, we did not find a statistically significant difference between the AA, AG and GG genotypes in any of the monitored markers.

CONCLUSIONS: The results provide the first and unique insight on the distribution of genotypes and alleles of the *rs74434454 CER1* gene polymorphism and its relationship to markers of bone metabolism in two ethnically distinct groups.

Key Words:
Association, Biomarkers, *CER1* gene, Postmenopausal osteoporosis, Roma.

Introduction

Postmenopausal osteoporosis is a metabolic bone disorder involving both modifiable and uncontrollable risk factors[1]. The main etiological factor in postmenopausal osteoporosis is oestrogen deficiency, which leads to high osteoclast activity and increased osteoresorption[2]. Lack of oestrogens causes an increase in the production of cytokines TNF-α by T-cells, increases the level of interleukins (IL-1, IL-6), and decreases the level of parathormone (PTH) and biosynthesis of vitamin D. Interleukin 7 (IL-7) and TNF-α increase osteoclast activity and are involved in osteoclastogenesis[3].

In these cases, bone resorption predominates over bone formation. The result of this imbalance is a loss of bone mass (BMD-bone mass density), which manifests as a decrease in bone strength,

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disruption of bone microarchitecture, perforation of bone beams from thinning and the formation of fractures[4].

From a molecular-genetic point of view, osteoporosis is a pathological process with a multifactorial background and a significant etiological share of hereditary information[5-7]. Genetic predisposition accounts for approximately 70% of cases of a confirmed diagnosis of osteoporosis. The remaining 30% of cases result from environmental influences[8].

Genetic determination of osteoporosis is challenging due to the involvement of several loci of candidate genes in bone mass regulation and pathogenesis of osteoporotic fractures[9]. Although significant progress has been made in recent decades in identifying candidate genes involved in BMD, fractures, and other related phenotypes, most genetic variants still need to be identified or confirmed in different ethnic groups. However, the results of scientific studies are often controversial, and may be influenced by sample size, ethnicity, gender, age, lack of standardized genotyping methods, gene-gene interactions, binding imbalances with other polymorphisms, epigenetic and/or post-transcriptional gene regulation, or interactions between genes and the environment[10]. Due to the polygenic nature of osteoporosis, the aim of the study is to investigate minor genes which, through interaction, have a continuous effect on bone metabolism and bone homeostasis[11]. We also include the *CER1* gene among these.

The *CER1* gene is one of the cytokines associated with cerberus. Cerberus-related cytokines together with DAN and DRM/Gremlin belong to the group of bone morphogenic protein (BMP) antagonists, they can bind directly to BMP and inhibit its activity[12]. BMP signalling is very important for proper bone development through its involvement in inducing differentiation of mesenchymal cells into osteoblasts. Recombinant human BMP-7 is used to induce bone growth in large bone defects and in the implantation of bone grafts. Changes in BMP antagonists can affect skeletogenesis and changes in human BMD[13].

In our study, we focused on monitoring the *rs74434454* polymorphism of the *CER1* gene in groups of postmenopausal Slovak women and its association with BMD, anthropometric, and biochemical markers. The study uniquely and specifically compares 2 ethnically different groups of postmenopausal women living in the Slovak Republic: Roma and non-Roma.

Subjects and Methods

Subjects

The study included 303 postmenopausal women of the non-Roma (majority, n = 202) and Roma (Gypsy, minority, n = 101) populations of eastern Slovakia. All subjects underwent a clinical examination in Osteocentre, s.r.o. in Prešov (Slovakia) and Hospital AGEL Košice-Šaca (Slovakia) with an initial anamnestic questionnaire. The following criteria were chosen for inclusion of probands in the study: women, postmenopause for $\geq$ 12 months, lived in the territory of the Slovak Republic. Probands visited the osteological ambulance for the first time. They were not previously given any medication that could affect bone metabolism. The criteria for exclusion of probands in the study: diagnosis of rheumatoid arthritis, type I diabetes mellitus, osteogenesis imperfecta, hypogonadism, premature menopause (<45 years), chronic malnutrition, malabsorption, chronic hepatopathy, treatment with corticoid Prednisone 5 mg and above, the body mass index (BMI) <19 $kg.m^{-2}$, anorexia nervosa, primary hyperparathyroidism, diffuse connective tissue disease, chronic inflammatory bowel disease, post-translational syndrome, chronic renal insufficiency, hyperthyroidism, prolonged immobilization, Cushing's syndrome, myeloproliferative diseases, genetic or other metabolic bone diseases. Densitometric measurement was performed using a whole-body densitometer (DXA Hologic Discovery, Hologic Inc., Waltham, Massachusetts, USA) in the lumbar spine region L1-L4.

The study protocol was approved by the Ethics Committee of the University of Prešov (No. 2/2013) and the Ethics Committee of the Košice-Šaca a.s Hospital (No. 2/2019). The study was conducted after collecting the written informed consent of all individuals and was conducted in accordance with the ethical principles of the Declaration of Helsinki. Sampling was performed over 4 years between 2015-2019.

Biochemical Analyses

Peripheral blood for biochemical analysis was collected in tubes without anticoagulant (5.5 ml). Blood serum was separated by centrifugation at 377 g/15 min (Selecta R, Barcelona, Spain). The following markers were determined in blood serum – ALP (alkaline phosphatase), OC (osteocalcin), CTX (C-terminal telopeptide of procollagen type I), minerals Ca, P and Mg. Analysers were used for biochemical analysis of these markers:

Cobas Integra 400 plus (Roche Diagnostics Ltd., Rotkreutz, Switzerland) and Cobas e411 (Roche Diagnostic, Hitachi HTC Immunochemistry Analyzer, Tokyo, Japan).

Anthropometric Measurements

Selected anthropometric parameters were measured, namely: body weight (kg), body height (cm), waist circumference (cm) and hip circumference (cm). Body weight was measured on an EMOS digital personal scale – PT-718 with an accuracy of 100 g, body height was measured with a digital Soehnle altimeter (accurate to 0.01 mm) as the average of two consecutive measurements. Subsequently, we calculated BMI ($kg.m^{-2}$) from the measured values of body weight and body height. The circumference of the waist was measured periumbilical, and the circumference of the hips was pertrochanteric, using a textile band measure. From the measured data – waist circumference and hip circumference – we calculated the index WHR (Waist Hip Ratio; waist circumference – cm/hip circumference – cm).

Densitometric Measurements

Densitometric measurement of postmenopausal women was performed using a DXA Hologic whole body densitometer (DXA Hologic Discovery, Hologic Inc., Waltham, MA, USA). Body mass density (BMD) measurement was performed in the lumbar spine (L1-L4). Based on the result of densitometric measurement (T-score), postmenopausal women were categorized into the following groups according to WHO guidelines: control group-standard (T-score≥ 1.0 to -1), osteopenic group (<-1.0 to -2.5) and the osteoporotic group (≤-2.5).

Molecular-Genetic Analysis

Genomic DNA was isolated from peripheral blood using a commercial NucleoSpin® Blood kit (MACHERY-NAGEL GmbH & Co. KG, Düren, Germany) according to predetermined procedures. The concentration and purity of the DNA samples were determined using a Nanodrop 2000c instrument (Thermo Fisher, Wilmington, DE, USA). Genotyping analysis of the *rs74434454 CER1* gene polymorphism was performed by Real-Time PCR using a StepOne® Real-Time PCR System (Applied Biosystems®, Foster City, CA, USA). The TaqMan SNP Assay (C__25473795_10) (Applied Biosystems®, Foster City, CA, USA) was selected for genotyping using a standard protocol. The risk genotype was verified by sequencing and subsequently used as a positive control in the following analyses.

Statistical Analysis

The obtained results were statistically processed using Excel 2010 and Statistica ver. 10. We evaluated individual parameters using statistical characteristics: position (average) and variability (standard deviation). To determine the significance of differences in individual parameters between two sets of individuals, we used a parametric Student's *t*-test while a non-parametric Kruskal-Wallis test for analysis of variance was used to determine the differences of the mean values between several sets. The Spearman correlation coefficient was used to determine the statistically significant dependence (relationship) between the two parameters.

Genotyping software (SNPs) (http://ihg2.helmholtz-muenchen.de/) was used to statistically evaluate the results of molecular genetic analysis – allele and genotype frequencies.

Differences in the distribution of genotypes and alleles between the observed groups of individuals were determined based on Pearson's chi-square test (χ^2). A value of $p<0.05$ was chosen as the indicator of statistical significance.

We subsequently analysed the statistical significance of differences in the number of individual alleles and genotypes of the observed polymorphism between sets of patients and controls using the statistical program GraphPad (La Jolla, CA, USA).

In determining the possible association of the allele with osteoporosis, the statistical software MEDCALC® ver. 16.2.1 was used with the OR (odds ratio) value set at 95% CI. Significance levels of $p<0.05$; $p<0.01$; $p<0.001$ were considered to be statistically significant differences.

Results

Table I shows statistically processed values of measured anthropometric and densitometric parameters and biochemical markers in groups of Roma and non-Roma women.

Postmenopausal women in the research group of the majority (non-Roma) population were divided on the basis of densitometric measurement into two groups: control (Control=101) and osteoporotic group (Ost=101). Postmenopausal women of the minority (Roma) population were divided

Table I. Average values of anthropometric and biochemical parameters in the monitored groups of postmenopausal non-Roma and Roma women.

Characteristics	Postmenopausal non-Roma women			Postmenopausal Roma women		
	Control	Ost	*p*	R-Control	R-Osteop+R-Ost	*p*
N	101	101	–	68	33	–
Age (years)	65.99 ± 9.47	66.43 ± 9.29	0.736	55.67 ± 9.40	60.15 ± 8.49	0.022*
Age at onset of menopause (years)	47.91 ± 4.78	47.9 3± 4.90	0.977	47.19 ± 4.96	48.36 ± 5.82	0.296
Body height (cm)	161.90 ± 5.91	160.99 ± 6.16	0.285	154.72 ± 5.51	152.83 ± 6.03	0.121
Body weight (kg)	76.52 ± 11.49	67.31 ± 10.68	0.001***	86.96 ± 19.34	79.85 ± 16.51	0.073
BMI (kg.m^{-2})	29.17 ± 3.94	26.02 ± 4.16	0.001***	36.29 ± 7.69	34.07 ± 6.42	0.155
Waist circumference (cm)	97.16 ± 10.49	92.57 ± 11.76	0.004**	111.32 ± 15.50	111.00 ± 13.88	0.919
Hip circumference (cm)	105.30 ± 9.36	101.01 ± 9.15	0.001***	116.98 ± 13.80	113.85 ± 11.10	0.258
WHR	0.92 ± 0.07	0.91 ± 0.09	0.588	0.95 ± 0.06	0.97 ± 0.07	0.117
BMD L1-L4 (g cm^{-2})	1.00 ± 0.22	0.79 ± 0.09	0.001***	0.74 ± 0.31	0.36 ± 0.09	0.001***
T-score L1-L4	-0.05 ± 1.24	-2.28 ± 0.90	0.001***	1.41 ± 2.77	-1.96 ± 0.81	0.001***
Z-score L1-L4	1.59 ± 1.29	-0.53 ± 1.19	0.001***	1.65 ± 1.30	-0.51 ± 1.13	0.001***
ALP (µkat.l^{-1})	1.12 ± 0.37	0.81 ± 0.25	0.001***	1.15 ± 0.34	1.28 ± 0.35	0.077
OC (µg.l^{-1})	17.75 ± 7.77	14.47 ± 7.87	0.003**	16.65 ± 8.42	20.15 ± 8.67	0.055
CTx-I (ng.l^{-1})	0.34 ± 0.20	0.32 ± 0.18	0.661	0.22 ± 0.14	0.30 ± 0.18	0.007**
Ca (mmol.l^{-1})	2.38 ± 0.20	2.23 ± 0.20	0.001***	2.47 ± 0.20	2.43 ± 0.17	0.274
P (mmol.l^{-1})	1.18 ± 0.24	1.08 ± 0.17	0.001***	1.32 ± 0.24	1.30 ± 0.22	0.717
Mg (mmol.l^{-1})	0.85 ± 0.09	0.91 ± 0.11	0.001***	0.82 ± 0.12	0.83 ± 0.08	0.819

BMI, body mass index; WHR, waist to hip ratio; BMD, bone mineral density; ALP, alkaline phosphatase; OC, osteocalcin; CTx-I, C-terminal telopeptide of type I collagen (β-CrossLaps); Ca, calcium; P, phosphorus; Mg, magnesium; Ost, osteoporotic group of non-Roma women; R-Control, control group of Roma women; R-Osteop+R-Ost, osteopenic and osteoporotic group of Roma women; statistical significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

into three groups: control (R-Control=68), osteopenic (R-Osteop=28) and osteoporotic (R-Ost=5) groups. Given the inclusion of only 5 postmenopausal women in the osteoporotic group of the minority population based on densitometric measurement, this group is considered very small and inhomogeneous. For this reason, with reference to the scientific study of Mamolini et al[14], the decision was made to combine the osteopenic and osteoporotic groups.

Based on the BMI classification (according to WHO), it was found that the group of non-Roma women with diagnosed osteoporosis (BMI – 26.02 ± 4.16 kg.m^{-2}) and the control group of non-Roma women (BMI – 29.17 ± 3.94 kg.m^{-2}) were classified as overweight (BMI=25.00-29.9 kg.m^{-2}). The control group of Roma women was included in the category of obesity I (BMI – 36.29 ± 7.69 kg.m^{-2}) and the R-Osteop+R-Ost group in the category of obesity II. Based on the WHR index (fat distribution), the control group and the R-Osteop+R-Ost group and the osteoporotic group of non-Roma women can be classified as high risk (for women >0.85).

A higher mean BMD value was found in control group of non-Roma women (1.00 ± 0.22 g.cm^{-2}) compared to R-control women (0.74 ± 0.31 g.cm^{-2}). Furthermore, in the group of non-Roma women diagnosed with osteoporosis, a higher average value of BMD (0.79 ± 0.09 g.cm^{-2}) was calculated compared to the R-Osteop+R-Ost group of the Roma population (0.36 ± 0.09 g.cm^{-2}). A statistically significant difference (p<0.001) between the control group of Roma and non-Roma women was found in densitometric parameters of total BMD and total T-score. We found a statistically significant difference in the densitometric parameter of BMD between the groups of women diagnosed with osteoporosis of the non-Roma population and the R-Osteop+R-Ost group of women from the Roma population (p<0.001).

In the study, we also focused on the analysis of selected mineral elements, osteoformation and osteoresorption markers associated with the disease osteoporosis. The results of the biochemical markers are shown in Table I. Statistically significant differences were found in the group of non-Roma women (between Control and Ost; p<0.001; p<0.01) in all monitored biochemical markers except CTx-I (p<0.661). In contrast, in the group of Roma women between R-Control

and R-Osteop+R-Ost, statistical significance was only found in the osteoresorption marker CTx-I (p<0.007) (Table I). The calculated average values of biochemical markers in the group of Roma and non-Roma postmenopausal women were in accordance with the reference values for the Slovak population. When comparing the observed biochemical markers between the control groups (Control versus R-Control) and the groups of women diagnosed with osteoporosis (Ost versus R-Osteop+R-Ost), statistically significant differences were found, shown in Table II.

Based on a correlation analysis, in the group of postmenopausal non-Roma women diagnosed with osteoporosis, an interdependence was found between OC and WHR (p<0.05). In the control group of non-Roma women, an interdependence we found between BMD and Ca, WHR and Ca, WHR and P, CTx-I and WHR (p<0.05). Through correlation analysis, a statistically significant relationship was identified between BMI and OC, and between BMI and CTx-I in the R-Osteop+R-Ost group of Roma women. A statistically significant dependence (p<0.05) between BMI and OC was also found in the control group of Roma women.

The aim of this scientific study was to monitor the distribution of genotypes and alleles of the *rs74434454 CER1* gene polymorphism in postmenopausal women of the Roma and non-Roma populations of the Slovak Republic. Table III shows the representation of individual genotypes and alleles in all monitored groups of women. The highest frequency of AA genotype was found in all groups of non-Roma and Roma women. The genotypic distribution of the *rs74434454 CER1* gene polymorphism between the study groups (Control versus Ost; R-Control versus R-Osteop+R-Ost) was not statistically significant (p<0.716; p<0.306). When determining the OR (relative risk value), no possible association was found between the risk allele G and the increased risk of osteoporosis in either group of women (Roma/non-Roma). The frequency of occurrence of allele A was 88.61% in the control group of non-Roma women and 87.62% in Ost, the frequency of occurrence of allele G was 11.39% in control and 12.38% in Ost. In the control group of postmenopausal Roma women, we did not observe the occurrence of the GG genotype. In the R-Osteop+R-Ost group, the GG genotype occurred at a frequency of 3.03%. The most fre-

Table II. Average values of anthropometric and biochemical parameters in the monitored groups of postmenopausal non-Roma and Roma women.

Characteristics	Groups					
	Control	R-Control	p	Ost	R-Osteop+R-Ost	p
N	101	101	–	68	33	–
Age (years)	65.99 ± 9.47	55.67 ± 9.40	0.001***	66.43 ± 9.29	60.15 ± 8.49	0.001***
Age at onset of menopause (years)	47.91 ± 4.78	47.19 ± 4.96	0.295	47.93 ± 4.90	48.36 ± 5.82	0.698
Body height (cm)	161.90 ± 5.91	154.72 ± 5.51	0.001***	160.99 ± 6.16	152.83 ± 6.03	0.001***
Body weight (kg)	76.52 ± 11.49	86.96 ± 19.34	0.001***	67.31 ± 10.68	79.85 ± 16.51	0.001***
BMI (kg.m^{-2})	29.17 ± 3.94	36.29 ± 7.69	0.001***	26.02 ± 4.16	34.07 ± 6.42	0.001***
Waist circumference (cm)	97.16 ± 10.49	111.32 ± 15.50	0.001***	92.57 ± 11.76	111.00 ± 13.88	0.001***
Hip circumference (cm)	105.30 ± 9.36	116.98 ± 13.80	0.001***	101.01 ± 9.15	113.85 ± 11.11	0.001***
WHR	0.92 ± 0.07	0.95 ± 0.06	0.014*	0.91 ± 0.09	0.97 ± 0.07	0.001***
BMD L1-L4 (g.cm^{-2})	1.00 ± 0.22	0.74 ± 0.31	0.001***	0.79 ± 0.09	0.36 ± 0.09	0.001***
T-score L1-L4	-0.05 ± 1.24	1.41 ± 2.77	0.001***	-2.28 ± 0.90	-1.96 ± 0.81	0.065
Z-score L1-L4	1.59 ± 1.29	1.65 ± 1.30	0.765	-0.53 ± 4.00	-0.51 ± 1.13	0.928
ALP (µkat.l^{-1})	1.12 ± 0.37	1.15 ± 0.34	0.591	0.81 ± 0.25	1.28 ± 0.35	0.001***
OC (µg.l^{-1})	17.75 ± 7.77	16.65 ± 8.42	0.386	14.47 ± 7.87	20.15 ± 8.67	0.001***
CTx-I (ng.l^{-1})	0.34 ± 0.20	0.22 ± 0.14	0.001***	0.32 ± 0.18	0.30 ± 0.18	0.672
Ca (mmol.l^{-1})	2.38 ± 0.20	2.47 ± 0.20	0.003**	2.23 ± 0.20	2.43 ± 0.17	0.001***
P (mmol.l^{-1})	1.18 ± 0.24	1.32 ± 0.24	0.001***	1.08 ± 0.17	1.30 ± 0.22	0.001***
Mg (mmol.l^{-1})	0.85 ± 0.09	0.82 ± 0.12	0.099	0.91 ± 0.11	0.83 ± 0.08	0.001***

BMI, body mass index; WHR, waist to hip ratio; BMD, bone mineral density; ALP, alkaline phosphatase; OC, osteocal-cin; CTx-I, C-terminal telopeptide of type I collagen (β-CrossLaps); Ca, calcium; P, phosphorus; Mg, magnesium; Ost, osteoporotic group of non-Roma women; R-Control, control group of Roma women; R-Osteop+R-Ost, osteopenic and os-teoporotic group of Roma women; statistical significance *p < 0.05, **p < 0.01, ***p < 0.001.

Table III. Frequencies of genotypes and alleles of the *rs74434454 CER1* gene polymorphism in the observed groups of postmenopausal women.

Genotypic frequencies										
		AA		AG		GG				
Group	N	N	Frequency (%)	N	Frequency (%)	N	Frequency (%)	HWE (*p*)	χ^2	*p*
Control	101	82	81.19	15	14.85	4	3.96	0.01	0.669	0.716
Ost 101	79	78.22	19	18.81	3	2.97	0.18			
R-Control	68	60	88.82	8	11.76	0	0	0.61	2.360	0.306
R-Osteop+R-Ost	33	27	81.82	5	15.15	1	3.03	0.25		

Allele frequencies								
	A		G					
Group	N	Frequency (%)	N	Frequency (%)	χ^2	*p*	OR	95% CI
Control	179	88.61	23	11.39	0.095	0.759	1.099	0.601-2.001
Ost	177	87.62	25	12.38				
R-Control	128	94.12	8	5.88	1.442	0.236	1.898	0.658-5.481
R-Osteop+R-Ost	59	89.40	7	10.60				

N, number of individuals; N, number of genotypes, alleles; HWE (*p*), value of statistical significance - result of comparison of actual genotype frequencies with theoretical frequencies (calculated according to Hardy-Weinberg's law); *p*, statistical significance; Ost, osteoporotic group of non-Roma women; OR, odds ratio (degree of risk); R-Control, control group of Roma women; R-Osteop+R-Ost, osteopenic and osteoporotic group of Roma women; χ^2, chi-square test; CI, interval reliability.

quent genotype was AA (R-Control – 88.82%; R-Osteop+R-Ost – 81.82%) followed by genotype AG (R-Control – 11.76%; R-Osteop+R-Ost – 15.15%). We can state that higher frequencies of the G allele were found in the control and osteoporotic group of non-Roma women compared to women of the Roma population. Using Student's t-test, we found statistically significant differences in non-Roma women in genotypes AA, AG and GG when comparing the results of anthropometric parameters, densitometric parameters and biochemical markers, shown in Table IV. As the GG genotype was not detected in the R-Control of postmenopausal women, we could not compare the observed markers.

Using a Kruskal-Wallis nonparametric analysis of variance, statistically significant differences were found between genotypes AA and GG in densitometric markers BMD (L1-L4), T-score (L1-L4) and biochemical marker ALP ($p<0.02$) in the Ost group of non-Roma women. In the Roma female population, no statistically significant difference was found in R-Control and R-Osteop+R-Ost between genotypes AA, AG and GG in any of the monitored markers.

Discussion

At present, there are few scientific studies dealing with Roma morbidity compared to other populations. Roma forms a genetic isolate; in comparison with the majority (non-Roma) population they differ in anthropometric characteristics, constant population growth, especially in less developed settlement communities, high frequency of consanguinity, and inbreeding, which is 10 to 100 times higher than in the non-Roma population. Estimates from 2014 suggest that approximately four hundred thousand individuals of the Roma ethnic group live in the Slovak Republic, with the highest concentration in the Eastern part of the republic. The health status of the Roma population is unsatisfactory and affected by multiple factors, including: inadequate health care for their own health and for that of their children, non-compliance with hygiene, frequent alcoholism, smoking, and inappropriate housing. Within the Roma ethnic group, there is greater inter-population genetic variability compared to non-Roma populations. Based on these arguments, we decided in this study to compare two ethnically distinct groups of postmenopausal women related to osteoporosis.

Aghaei Meybodi et al[15] pointed out that some anthropometric parameters, such as individual weight should be used to increase the significance of the diagnostic value of BMD in women at risk of osteoporotic fractures.

There are currently conflicting views on the impact of BMI on bone quality. De Laet et al[16] indicated that women with low body mass index are at greater risk of developing osteoporosis and osteoporotic fractures occur in the shoulders, arms and ankles. Changes in body weight also affect the rate of bone loss. Women with a BMI<18.5 $kg.m^{-2}$ lose bone mass faster (0.8% per year) than women with a stable body weight. In women whose body weight has gradually increased, there is no significant loss of bone mass (0.1% per year)[17]. Obesity is considered a protective factor for skeletal development and osteoporosis. Nevertheless, clinical studies suggest that obesity may also have a negative effect on bone quality despite normal BMD values, as measured by densitometry. One reason may be an increase in mechanical pressure on the bone[18]. Ravn et al[19] found that low BMI and low body weight are risk factors that affect low BMD and increased bone loss in postmenopausal women. Yasar et al[20] reported that BMI was positively correlated with BMD and that individuals who had a BMI in the range of 20-25 $kg.m^{-2}$ had a higher rate of bone loss. Yasar et al[20] and Nagi et al[21] reported a positive correlation between BMI and BMD in control and osteoporotic groups of women. In our sample of Roma and non-Roma women, we did not find a statistically significant correlation between BMD and BMI. Statistically significant differences in the monitored densitometric parameters and anthropogenic indices are shown in Table I.

Yoo et al[22] monitored risk factors for osteoporosis (age, body weight, body height, BMI and waist circumference) in a group of Korean women (n=1674). The authors reported the following average values of the monitored parameters in the osteoporotic group: age 52.90 ± 8.20 years, waist circumference 80.80 ± 9.20 cm, BMI 23.30 ± 3.10 $kg.m^{-2}$, body height 150.30 ± 5.7 cm, body weight 52.90 ± 8.20 kg. By comparing these results with the same parameters in our OG group of non-Roma women, we found higher average values in all monitored parameters. Based on the Student's t-test, the authors confirmed a statistically significant difference ($p<0.001$) between the control and osteoporotic groups of Korean women in the parameters of age, BMI, body height and weight. In our study in non-Roma postmenopaus-

Table IV. Comparison of anthropometric, biochemical and densitometric markers in different genotypes of polymorphism *rs74434454 CER1* gene between control and osteoporotic/osteopenic groups of women.

Genotypes of postmenopausal Roma women									
Parameters	AA			AG			GG		
	CG	OG	*p*	CG	OG	*p*	CG	OG	*p*
N	82/101	79/101	–	15/101	19/101	–	4/101	3/101	–
Age (years)	66.17 ± 9.36	66.89 ± 9.28	0.621	62.73 ± 10.22	64.42 ± 8.02	0.593	74.50 ± 5.44	67.00 ± 16.64	0.426
Age at onset of menopause (years)	47.61 ± 4.80	47.72 ± 5.04	0.887	49.00 ± 4.87	48.63 ± 4.21	0.814	47.33 ± 3.51	47.66 ± 6.65	0.935
Body height (cm)	161.72 ± 5.98	160.87 ± 5.68	0.360	162.86 ± 4.58	160.42 ± 7.92	0.266	162.00 ± 9.79	167.67 ± 9.29	0.474
Body weight (kg)	76.21 ± 11.42	67.83 ± 10.29	0.001***	78.33 ± 13.05	64.10 ± 11.69	0.002**	76.00 ± 7.78	73.66 ± 13.05	0.777
BMI ($kg.m^{-2}$)	29.12 ± 3.91	26.24 ± 3.94	0.001***	29.51 ± 4.65	25.07 ± 5.15	0.014*	28.95 ± 1.36	26.06 ± 2.82	0.129
Waist circumference (cm)	97.05 ± 10.85	93.32 ± 11.21	0.034*	98.13 ± 9.06	87.78 ± 11.91	0.010**	95.75 ± 7.13	103.00 ± 18.02	0.487
Hip circumference (cm)	104.75 ± 8.93	102.05 ± 6.91	0.034*	109.13 ± 10.76	95.89 ± 14.36	0.005**	102.25 ± 11.08	106.33 ± 11.59	0.655
WHR	0.92 ± 0.08	0.9 1± 0.08	0.299	0.90 ± 0.05	0.92 ± 0.11	0.454	0.94 ± 0.03	0.96 ± 0.07	0.554
BMD L1-L4 ($g.cm^{-2}$)	0.99 ± 0.23	0.79 ± 0.09	0.001***	1.01 ± 0.15	0.77 ± 0.08	0.001***	1.10 ± 0.21	1.02 ± 0.15	0.580
T-score L1-L4	-0.07 ± 1.17	-2.31 ± 0.82	0.001***	0.03 ± 1.60	-2.48 ± 0.78	0.001***	0.12 ± 1.46	-0.23 ± 1.36	0.755
Z-score L1-L4	1.55 ± 1.26	-0.53 ± 1.12	0.001***	1.61 ± 1.05	-0.88 ± 0.92	0.005**	2.40 ± 2.47	1.60 ± 2.42	0.687
ALP ($\mu kat.l^{-1}$)	1.09 ± 0.38	0.78 ± 0.23	0.001***	1.17 ± 0.28	0.89 ± 0.25	0.005**	1.52 ± 0.28	1.17 ± 0.10	0.107
OC ($\mu g.l^{-1}$)	17.20 ± 7.11	2.20 ± 0.20	0.001***	18.94 ± 8.99	2.31 ± 0.16	0.398	24.42 ± 13.99	25.40 ± 17.85	0.938
CTx-I ($ng.l^{-1}$)	0.33 ± 0.19	0.31 ± 0.18	0.493	0.31 ± 0.23	0.36 ± 0.16	0.516	0.50 ± 0.29	0.43 ± 0.28	0.785
Ca ($mmol.l^{-1}$)	2.39 ± 0.21	2.20 ± 0.21	0.001***	2.38 ± 0.18	2.32 ± 0.17	0.301	2.24 ± 0.20	2.42 ± 0.23	0.308
P ($mmol.l^{-1}$)	1.16 ± 0.24	1.09 ± 0.18	0.018*	1.28 ± 0.27	1.06 ± 0.16	0.005**	1.15 ± 0.15	1.21 ± 0.20	0.666
Mg ($mmol.l^{-1}$)	0.85 ± 0.09	0.91 ± 0.12	0.001***	0.85 ± 0.10	0.92 ± 0.11	0.064	0.79 ± 0.11	0.92 ± 0.16	0.291

Continued

Table IV *(Continued)*. Comparison of anthropometric, biochemical and densitometric markers in different genotypes of polymorphism *rs74434454 CER1* gene between control and osteoporotic/osteopenic groups of women.

Genotypes of postmenopausal Roma women									
Parameters	AA			AG			GG		
	R-Control	R-Osteop+R-Ost	*p*	R-Control	R-Osteop+R-Ost	*p*	R-Control	R-Osteop+R-Ost	*p*
N	60/101	27/101	–	8/101	5/101	–	0/101	1/101	–
Age (years)	55.55 ± 9.35	59.85 ± 7.77	0.039*	56.60 ± 10.35	59.60 ± 12.42	0.649	-	71	–
Age at onset of menopause (years)	47.11 ± 4.95	48.7 4 ± 5.49	0.173	47.75 ± 5.31	46.20 ± 8.25	0.685	–	49	–
Body height (cm)	154.60 ± 5.58	153.70 ± 5.17	0.494	155.62 ± 5.12	148.50 ± 7.03	0.057	–	151	–
Body weight (kg)	86.13 ± 19.98	80.44 ± 17.38	0.205	93.12 ± 12.79	73.80 ± 10.96	0.018*	–	94	–
BMI ($kg.m^{-2}$)	36.00 ± 7.99	34.01 ± 6.87	0.266	38.43 ± 4.64	32.94 ± 2.95	0.039*	–	41.23	–
Waist circumference (cm)	111.25 ± 16.11	111.70 ± 14.99	0.901	111.87 ± 10.49	106.20 ± 6.41	0.304	–	116	–
Hip circumference (cm)	116.38 ± 14.23	113.92 ± 11.85	0.436	121.50 ± 9.48	112.60 ± 8.17	0.112	–	118	–
WHR	0.95 ± 0.06	0.97 ± 0.07	0.132	0.92 ± 0.07	0.94 ± 0.06	0.556	–	0.98	–
BMD L1-L4 ($g.cm^{-2}$)	0.75 ± 0.32	0.36 ± 0.09	0.001***	0.64 ± 0.21	0.39 ± 0.03	0.025*	–	0.17	–
T-score L1-L4	1.51 ± 2.87	-1.96 ± 0.82	0.001***	0.60 ± 1.87	-1.64 ± 0.32	0.025*	–	-3.6	–
Z-score L1-L4	1.69 ± 1.34	-0.42 ± 1.23	0.001***	1.37 ± 0.93	-0.96 ± 0.37	0.001***	–	-0.6	–
ALP (μkat.l-1)	1.16 ± 0.35	1.27 ± 0.35	0.159	1.09 ± 0.32	1.39 ± 0.43	0.177	–	1.01	–
OC ($\mu g.l^{-1}$)	16.90 ± 8.61	19.91 ± 8.37	0.131	14.79 ± 7.08	22.38 ± 11.61	0.167	–	15.52	–
CTx-I (ng.l-1)	0.22 ± 0.15	0.30 ± 0.14	0.017*	0.19 ± 0.09	0.34 ± 0.37	0.300	–	0.28	–
Ca ($mmol.l^{-1}$)	2.50 ± 0.19	2.42 ± 0.17	0.093	2.32 ±0.28	2.45 ± 0.14	0.367	–	2.51	–
P ($mmol.l^{-1}$)	1.33 ± 0.23	1.29 ± 0.22	0.457	1.18 ± 0.27	1.27 ± 0.23	0.571	–	1.56	–
Mg ($mmol.l^{-1}$)	0.83 ± 0.09	0.82 ± 0.08	0.579	0.71 ± 0.24	0.85 ± 0.06	0.211	–	0.72	–

BMI, body mass index; WHR, waist to hip ratio; BMD, bone mineral density; ALP, alkaline phosphatase; OC, osteocalcin; CTx-I, C-terminal telopeptide of type I collagen (β-CrossLaps); Ca, calcium; P, phosphorus; Mg, magnesium; Ost, osteoporotic group of non-Roma women; R-Control, control group of Roma women; R-Osteop+R-Ost, osteopenic and osteoporotic group of Roma women; statistical significance *$p < 0.05$, *$p < 0.01$, ***$p < 0.001$.

al women, we also found a statistically significant difference ($p<0.001$; $p<0.004$) between Control and Ost groups in body weight, BMI and waist circumference.

A statistically significant difference in BMI, age and body weight between the control and osteoporotic groups of Macedonian women was confirmed by Jovčevska et al[23]. Yoo et al[22] have shown that various factors are involved in the development of osteoporosis, such as height, age at onset of menopause, duration of breastfeeding, presence of diseases such as hyperlipidemia, hypertension, osteoarthritis and diabetes mellitus. Tian et al[24] did not confirm a statistical significance between the control and osteoporotic groups in the parameters of age and age at onset of menopause in a sample of postmenopausal Chinese women. This statistical significance was not found in our group of non-Roma women either. In the group of Roma women, we found a statistical significance ($p<0.022$) between R-Control and R-Osteop+R-Ost in the age parameter using Student's t-test (Table I). In Table II we can see that the average age at onset of menopause in women in the R-Osteop+R-Ost group was 0.43 ± 0.92 years later than in the osteoporotic group of non-Roma women.

Analysis of specific biochemical markers provides us with an overview of bone turnover dynamics in many metabolic bone disorders. An imbalance between the process of osteoformation and osteoresorption can be observed in aging and pathological conditions such as osteoporosis[25].

Tian et al[24] found higher mean values of OC, CTx-I, Ca and P in the population of Chinese postmenopausal women in the group of women with osteoporosis and point to a statistical difference ($p<0.001$) in these biochemical markers between Control and Ost groups. The authors did not find a mutual association between BMD (total) and osteocalcin. BMD decreased as the osteocalcin osteformation marker increased. Similar results in the biochemical markers OC and CTx-I are reported by Naeem et al[26], who found statistically significantly higher levels of these markers in the osteoporotic group of postmenopausal Pakistani women (control group – OC 17.82 ± 10.00 ng.l^{-1}; CTx-I 0.20 ± 0.3 ng.ml^{-1}; osteoporotic group – OC 21.40 ± 7.50 ng.l^{-1}; CTx-I 0.25 ± 0.40 ng.l^{-1}) ($p<0.001$). In our research group of Roma women, specifically in the R-Osteop+R-Ost group, a higher average value was found in the biochemical marker OC (20.15 ± 8.67 ng.l^{-1}). The mean CTx-I value was higher in the ROP+ROS group. Jenkins et al[27] stated that CTx-I levels increase with age based on their scientific research (Australian women).

A higher OC value was found by Jovčevska et al[23] in the osteoporotic group of postmenopausal Mecedonian women and by Hamdi[28] in the osteoporotic group of Iraqi women. These mentioned authors point out that the diagnosis of osteoporosis through the measurement of OC is not sufficient, but rather it is necessary to include in the portfolio of biochemical markers such as osteoresorption, osteoformation markers and mineral elements. The Elecsys ß-CrossLaps (CTx-I) serum assay is a potentially useful tool for assessing bone resorption, including its response to oestrogen[29]. In postmenopausal women, osteoporosis occurs relatively frequently because lower estrogen levels reduce the ability to retain calcium (Ca) in the bones[30]. A decrease in bone mineral concentration leads to bone degeneration, which affects fractures[31].

In our group of postmenopausal Roma and non-Roma women, we also focused on the analysis of the mineral elements Ca, P and Mg. Higher levels of magnesium were recorded in Ost (0.91 ± 0.11 mmol.l^{-1}) compared to Control (0.85 ± 0.09 mmol.l^{-1}) of non-Roma postmenopausal women. Table I shows statistically significant differences between Control and Ost, R-Control and R-Osteop+R-Ost of postmenopausal non-Roma and Roma women. In the control group of postmenopausal Roma women, we found higher average values in the parameter phosphorus and calcium. However, no statistical significance was found in these parameters between R-Control and R-Osteop+R-Ost. Higher phosphorus levels may be due to impaired blood Ca:P ratio or latent disease in some women in the study group. Results may vary due to nutrition, ethnicity, genetic predisposition, environmental factors, or other reasons.

The *121T>C (*rs74434454*) *CER1* gene polymorphism was addressed by Koromila et al[32,33], who studied postmenopausal Greek women. The sample group of Koromila et al[32] consisted of three hundred postmenopausal women (Control – 100 women; Ost – 200 women). In their study, the authors focused on the analysis of factors – age, BMI, duration of menopause, smoking, calcium intake, T-score, vertebral fractures, hip joint, etc. between the control and osteoporotic groups. The subject of their study was genotypic analysis of five polymorphisms of the *CER1* gene (*rs1494360*, *rs17289263*, *rs7022304*, *rs3747532*, *rs74434454*).

The results of the analysis of the *rs74434454* polymorphism of the *CER1* gene showed the presence of only two genotypes both in the control group (TT 90.10%, TC 9.90%) and in the group of women with osteoporosis (TT 65.60%, TC 34.40%). In our group of non-Roma postmenopausal women, the GG genotype was also represented (3.96% in control and 2.97 in osteoporotic groups). The GG genotype did not occur in the control group of postmenopausal Roma women. In the R-Osteop+R-Ost group, it was at a frequency of 3.03%. The distribution of genotypes and alleles of the *rs74434454 CER1* gene polymorphism in the examined groups of our research group is shown in Table III. In determining the OR (relative risk value) we found no possible association between the G allele and increasing risk of osteoporosis in either group of women (Roma and non-Roma).

Koromila et al[32] reported the frequency of the minor allele C 0.14% and the SNP polymorphism database (NCBI) lists the frequency of allele G as 0.12%. In all groups of our study, the frequency of the G allele was lower than 0.12% and 0.14%; only in Ost group was the frequency of the G allele 0.124%. The authors also report a statistically significant difference in the T-score parameter between the control and osteoporotic groups in all monitored polymorphisms. Table IV shows statistically significant differences in anthropometric parameters, densitometric parameters and biochemical markers in individual genotypes of the *rs74434454 CER1* gene polymorphism in the Roma and non-Roma groups of postmenopausal women.

Koromila et al[33] discussed the relationship of the *CER1* gene polymorphisms (*rs7022304*, *rs17289263*, *rs3747532*, *rs1494360*, *rs74434454*) and the *DDK1* gene with biochemical markers and bone mineral density in postmenopausal Greek women. The authors found that, in the group of women diagnosed with osteoporosis, the onset of menopause was 2.5 years earlier than in the control group of women. In the Ost group of the non-Roma population of our research group, no earlier onset of menopause was found, but interestingly a later onset of menopause was indicated in the R-Osteop+R-Ost group of postmenopausal Roma women, although this was not statistically significant. In our group of postmenopausal non-Roma women, we found statistically significant differences between control and Ost groups in the biochemical markers ALP, OC, Ca, P, and Mg in genotype AA. Statistically significant differences in biochemical markers ALP and P were found in genotype AG (Table IV). In the Roma group of postmenopausal women, a statistically significant difference ($p<0.01$) was found only in the osteoresorption marker CTx-I in genotype AA.

Conclusions

Our results provide the first and unique information on the distribution of genotypes and alleles of the *rs74434454 CER1* gene polymorphism in two ethnically different groups of the Slovak population – Roma and non-Roma. At present, there are few studies dealing with Roma morbidity compared to other populations. Roma constitutes a genetic isolate that is internally differentiated into more or less isolated groups, with a small interpopulation flow of genetic information and with a significant role of the primary, secondary, and tertiary founder effects. Within the Roma population we found only 5 postmenopausal women with osteoporosis (a small inhomogeneous group), leading to join the osteopenic and osteoporotic groups into one (R-Osteop + R-Ost).

Statistically significant differences were found between the Roma and non-Roma women in BMD. In the group of Roma women between R-Control and R-Osteop+R-Ost, statistical significance was only found in the CTx-I. An interdependence between OC and WHR in the group of postmenopausal non-Roma women diagnosed with osteoporosis, and between BMD and Ca, WHR and Ca, P, CTx-I were found. A statistically significant correlation was identified between BMI and OC, CTx-I in the R-Osteop+R-Ost group of Roma women.

No association between the risk allele G and the increased risk of osteoporosis in any group of women was found. The higher frequencies of the G allele in the control and osteoporotic group of non-Roma compared to Roma women were found. Statistically significant differences were found between genotypes AA and GG in densitometric markers T-score (L1-L4), BMD (L1-L4) and ALP in the non-Roma women with osteporosis. In the Roma female population, no statistically significant differences were found either in control or osteoporotic/osteopenic groups between genotypes AA, AG and GG in any of the monitored markers.

Conflict of Interest
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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