Relationship between VKORC1 single nucleotide polymorphism 1173C>T, bone mineral density & carotid intima-media thickness

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Background & objectives: The effects of vitamin K-dependent proteins in bone mineralization and vascular calcification and the implication of vitamin K epoxide reductase gene (VKORC1) 1173C>T polymorphism in warfarin sensitivity are well known. The main objective of the study was to investigate the relationship between *VKORC1* 1173C>T polymorphism, bone mineral density (BMD), and atherosclerosis (evaluated by intima-media thickness of the carotid artery and the presence of calcified plaques) in patients suspected to have osteoporosis or osteopenia and referred for BMD determination.

Methods: *VKORC1* 1173C>T polymorphism was evaluated in 239 consecutive patients referred by their physicians for BMD measurement (dual energy X-ray absorptiometry at L2-L4 lumbar spine, femoral neck and total hip). Ultrasonography of the carotid arteries was performed, intima-media thickness (IMT) was measured and the presence of atherosclerotic calcified plaques was recorded.

Results: In the patients with osteoporosis and osteopenia there was a higher frequency of TT genotype of *VKORC1* 1173C>T (P=0.04). The TT genotype was significantly more frequent in the osteoporotic group compared to the osteopenic group (P=0.01). The mean age and body mass index were lower in the patients with normal BMD and TT genotype (P=0.02, P=0.03). There was no correlation between the IMT and *VKORC1* 1173C>T genotype but the TT genotype had a significant association with the presence of calcified atherosclerotic plaques (P=0.05). This finding was not correlated with normal or pathologic BMD.

Interpretation & conclusions: *VKORC1* 1173C>T polymorphism (TT genotype) was associated with osteoporosis and calcified plaques in the carotid artery in patients referred for BMD measurement. Different mechanisms are probably involved in these associations. TT genotype may serve as a potential genetic marker for the risk of OP and ATS.

Key words Atherosclerotic plaque - bone mineral density - carotid intima-media thickness - gene - osteoporosis - vitamin K epoxide reductase - VKORC1

Osteoporosis (OP) and atherosclerosis (ATS) have a higher prevalence in the ageing population causing serious morbidity and mortality. The common mechanisms in the pathogenesis of OP and vascular calcification suggested are age-related, chronic inflammation, cigarette smoking, diabetes mellitus, estrogen deficiency, hypovitaminosis C, D, and K, oxidized lipids and free radical, or renal failure[1-4]. Many studies have demonstrated the association between OP and ATS: bone mineral density (BMD) and the number of carotid and femoral atherosclerotic plaques[5], BMD and the risk for subclinical coronary atherosclerosis[6], BMD and carotid intima-media thickness (IMT) in postmenopausal women[7], BMD and coronary calcification[8], or calcification of the atherosclerotic plaques and low BMD[9,10].

The role of vitamin K in bone metabolism and vascular health has been demonstrated. The characterization of vitamin K-dependent proteins has demonstrated the implication of vitamin K not only in the blood coagulation but also in the bone metabolism, atherosclerosis and in inflammation, oxidative stress, nerve signaling and even kidney stones[11]. Vitamin K acts as a cofactor for gamma-glutamyl carboxylase, being an electron donor for the conversion of some post-translational glutamyl residues, thus producing the gamma-carboxyglutamate (Gla). The main function of these Gla residues is to actively bind calcium, which is essential for the biological activity of the Gla containing proteins (named Gla-proteins)[12]. The mediator in the recycle process of vitamin K 2,3 epoxide to the vitamin K hydroquinone is vitamin K epoxide reductase (VKOR). The vitamin K epoxide reductase gene (*VKORC1*) is situated on human chromosome 16p11.2 and encodes a protein of 163 amino acids with a mass of 18.2 kD[13]. Numerous single nucleotide polymorphisms (SNPs) were identified, the *VKORC1* gene being highly polymorphic. One of these SNPs, rs 993448 or VKORC1 1173C>T was studied especially in relation to coumarin sensitivity. D'Andrea *et al*[14] found that patients with TT genotype of SNP rs 9934438 (SNP 1173) require a lower dose of warfarin for a safe anticoagulation. This is indicative for a lower activity of the coagulation system partly due to a lower activity of *VKORC1* gene which leads to a less conversion of vitamin K epoxide to its reduced form and so to less carboxylation of the vitamin K-dependent proteins[14]. On the same haplotype with SNP 1173C>T is situated another SNP +2255C>T (rs 2359612) and for this SNP the presence of C allele is associated with almost double the risk for vascular diseases and lower levels of undercarboxylated osteocalcin[15].

Recently a significant association between AA genotype of 9041G>A SNP and a higher mean BMD was found[16] and in VKORC1-deficient mice a reduced length of bone calcification relative to wild-type control mice was demonstrated[17]. From six VKORC1 SNPs genotyped and compared with BMD, rs7292 was found to be associated with OP in non-hispanic white males[18].

It remains undecided whether there is a common VKORC1 SNPs that can be considered a risk factor for both OP and ATS, and if there is a common pathway for the intervention in this polymorphism. Thus, the objective of the study was to evaluate the relationship between BMD, carotid IMT and calcific atherosclerotic plaques (as the risk factor for cardiovascular events due to atherosclerosis)[19], and the *VKORC1* SNPs rs 993448 (*VKORC1* 1173C>T) in patients referred for BMD determination.

Material & Methods

Between September 2009 and September 2010, 239 patients were consecutively enrolled from patients referred by their physicians for BMD measurement in the Rheumatology Clinic of the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. Patients with hormonal replacement therapy or medication affecting the bone metabolism (oral anticoagulants, corticosteroids, or anticonvulsants) were excluded. Subjects with any condition that might interfere with the bone metabolism, such as thyroid disorders, malabsortion, chronic renal and liver diseases or alcoholism were also excluded. Women were eligible if menopause had occurred at least two years prior to their visit. None of the subjects had diseases (fractures, *etc*.) that interfered with the activities of normal daily life. In this group 151 patients had hypertension and 60 patients had non-insulin-dependent diabetes (with 46 having both diseases).

Written, informed consent for participation was obtained from each subject prior to enrolment. The Ethics Committee of University approved the study protocol (ANCS 42107/2008 PNII Grant). All the measurements were performed in the same day.

For each patient demographic and clinical variable were recorded: age, weight, height, body mass index (BMI = weight/height^2, kg/m^2), time since menopause had installed, smoking status. The patients' medication

history and associated diseases including hypertension and diabetes were also recorded. Their blood pressure was measured after a 10 min rest in the supine position using a sphygmomanometer on the right arm of the subject.

Blood samples were drawn from the antecubital vein in the morning after the subject had fasted for 12 h. One sample (7 ml) was used for serum concentration of total cholesterol, triglycerides, and glucose determination by standard laboratory techniques (Cobas Mira Plus analyzer) on the same day. The second sample (5 ml) was used for DNA extraction. The samples obtained were kept frozen at -80°C until use.

BMD measurement: BMD was measured at the lumbar spine (L2-L4) and at the femoral neck by dual energy X-ray absorptiometry (DXA), using a Lunar Prodigy Advance (GE Healthcare, USA) for all BMD measurements. Results were expressed as a T-score (standard deviation from peak adult BMD). Patients were classified as having normal BMD, osteopenia or low bone mass (a value for BMD that was 1-2.5 SD below the mean value for young adults), and osteoporosis (more than 2.5 SD below the mean value for young adults according to the World Health Organization's criteria for diagnosing osteoporosis)[20]. Three sites for assessment of the BMD were considered: L2-L4 at the lumbar spine, femoral neck and total hip and the lower value of these three were used in the statistical analysis[21]. All the measurements were performed by two operators. The inter- and intra-operator coefficient of variation was less than 1.3 per cent.

Measurement of carotid IMT: The wall thickness of the carotid artery was measured bilaterally with Aloka Prosound alpha 10, Tokyo, Japan machine, using 7.5 to 13 MHz linear probe by the same examiner with the patient in supine position. The common carotid arteries were scanned longitudinally. The image was focused on the posterior (far) wall. The perpendicularity between the ultrasound beam and the far wall assured good visualization of the IMT as two parallel echoic lines (the lumen-intima interface and media-adventitia interface). In all cases, three measurements of the common carotid artery far wall were taken 10 mm proximal to the bifurcation. For each subject the mean value of the three measurements was considered to be the current wall thickness of the distal carotid common artery. The mean value taken into account for the study was between the right and left carotid artery IMT. The variability of ultrasonographic measurement was

assessed by performing two measurements in a 15 volunteers group over a one week period. The coefficient of variation between the two IMT measurements was of 10 per cent.

The calcified atherosclerotic plaque was defined as a high echogenic focal widening of the carotid wall with the protrusion into the lumen, associated with posterior shadow. The plaques were recorded as present or absent on the common, internal and external carotid arteries walls.

DNA isolation and genotyping: DNA was obtained by extraction and purification from 300 μl of peripheral blood, using a comercial extraction kit (Wizard Genomic DNA Purification Kit, Promega, USA).

VKORC1 1173C>T polymorphism genotyping protocol used PCR-RFLP (Restriction fragment lenght polymorphism, based PCR), using a modified protocol described by Kimmel *et al*[22]. Briefly, a 195 bp fragment from the gene that encodes VKORC1, was amplified by PCR, following the amplification programme: initial denaturation of 7 min at 95°C, followed by 45 cycles, each of 40 sec denaturation at 95°C, annealing 50 sec at 59°C and elongation 40 sec at 72°C, with a final elongation of 7 min at 72°C. DNA primer sequences were: Fw_5'- AAGATGAAAAGCAGGGCCTAC- 3' and Rev_5'- CCGAGAAAGGTGATTTCCAA- 3'. DNA samples were amplified by PCR in a final volume of 25 μl, with: $MgCl_2$, 1.5 mM, dNTPmix 0.2 mM each, BSA (Bovine serum Albumine[R], Fermentas MBI, Lituania) 2 mg/ml, 1.25 U of TrueStart DNA polymerase (Fermentas MBI, Vilnius, Lituania), 10 pmoles of each primers (forward and reverse), approximately 150 ng of genomic DNA and nuclease free water up to the final volume of 25 μl. From the obtained amplicon 12 μl was digested overnight, at 37°C with 5U of Eco130I (StyI) (Fermentas MBI, Vilnius, Lituania) restriction enzyme. Digested fragments were migrated in electrophoresis gel, 3 per cent using high resolution MetaPhor agarose (Lonza, Belgium), stained with ethidium bromide. In the presence of T allele, the amplicon is digested by the enzyme in two fragments of 125 and 70 bp. The homozygous genotypes for C allele, present a single fragment of 195 bp, resistant to digestion. The homozygous genotypes for T allele, present 2 fragments, one of 125 bp and the other of 70 bp.

Statistical analysis: For testing normal distribution in the case of a continuous variable Kolmogorov-Smirnov test or Shapiro-Wilk test (for small samples)

was applied. If the variables were normally distributed, the means were compared by t test (in case of two samples) or ANOVA test followed by post-hoc Scheffe analysis (in the case of three samples). If the variables were not normal distributed, the means were compared using Mann-Whitney test (in case of two samples) or Kruskal-Wallis test (in case of three samples) followed by Mann-Whitney test for multiple comparisons with Bonferroni manual adjustment. For testing proportions Chi-square test was used. For multiple comparisons Bonferroni manual adjustment of type 1 error were made.

In the absence of a cut-off value for the mean IMT, the association between mean IMT, BMD and 1173C>T polymorphism were analysed using the ANOVA two-way analysis. The study was made using genotype model (TT, CT, and CC), recessive model (TT versus CT plus CC), and dominant model (TT plus CT versus CC). For the association between a dependent dichotomous variable and multiple nominal or continuous independent variables a multivariate logistic regression was used. Multiple logistic regression analysis was performed with BMD (normal vs. osteopenia or osteoporosis) as a dependent variable and age, BMI, hypertension, diabetes mellitus, the *VKORC1* genotype, and mean IMT as independent variables. Statistical calculations were performed with SPSS 13.0 (Chicago, IL, USA).

Results

The study group consisted of 204 women and 35 men. The characteristics of the study group are summarized in Table I.

Table I. Demographic characteristics of the study group			
	Normal BMD (n=66)	Osteopenia (n=105)	Osteoporosis (n=68)
Age (yr)	61.08 ± 7.62	$62.23 \pm 9.74^+$	$68.01 \pm 9.71^*$
Female (n=204)	61.39 ± 7.34	$64.20 \pm 9.36^+$	$68.22 \pm 9.46^*$
Male (n=35)	59.85 ± 8.86	58.61 ± 10.46	64.75 ± 14.48
Hypertension (%)	66.7	64.8	57.4
Diabetes mellitus (%)	27.3	28.6	17.6
BMI (kg/m^2)	30.71 ± 4.78	$28.56 \pm 5.23^{*+}$	$26.40 \pm 5.58^*$
Mean IMT (mm) (left IMT+ right IMT)/2	0.11 ± 0.07	0.10 ± 0.06	0.11 ± 0.08
Total cholesterol (mg %)	218.40 ± 57.57	215.60 ± 52.76	218.32 ± 47.41
Triglycerides (mg %)	158.13 ± 86.87	145.20 ± 94.61	126.66 ± 74.48
Glucose (mg %)	116.34 ± 33.48	111.38 ± 35.33	103.12 ± 20.87
Lumbar spine BMD (g/cm^2)	1.23 ± 0.16	$1.06 \pm 0.14^{*+}$	$0.83 \pm 0.15^*$
Lumbar spine T score	0.32 ± 1.20	$-1.16 \pm 1.00^{*+}$	$-3.07 \pm 1.21^*$
Lumbar spine Z score	1.37 ± 1.25	$-0.02 \pm 1.22^{*+}$	$-1.55 \pm 1.39^*$
Femoral neck BMD (g/cm^2)	1.00 ± 0.11	$0.84 \pm 0.09^{*+}$	$0.72 \pm 0.10^*$
Femoral neck T score	-0.15 ± 0.95	$-1.36 \pm 0.78^{*+}$	$-2.27 \pm 0.74^*$
Femoral neck Z score	0.94 ± 0.77	$-0.23 \pm 0.69^{*+}$	$-0.70 \pm 0.69^*$
Total hip BMD (g/cm^2)	1.09 ± 0.13	$0.91 \pm 0.11^{*+}$	$0.76 \pm 0.11^*$
Total hip T score	0.50 ± 0.94	$-0.89 \pm 0.71^{*+}$	$-2.24 \pm 2.26^*$
Total hip Z score	1.40 ± 1.00	$0.14 \pm 0.86^{*+}$	$-0.87 \pm 1.83^*$
VKORC1 CC n=43 (17.9%)	13 (30.23%)	20 (46.51%)	10 (23.26%)
CT n=108 (45.1%)	29 (26.85%)	54 (50%)	25 (23.15%)
TT n=88 (36.8%)	24 (27.27%)	31 (35.23)	33 (37.5%)

Values are expressed as mean ± standard deviation
IMT, intima media thickness; BMI, body mass index; CCA, common carotid artery; n, number of patients
$^*P<0.001$ compared to patients with normal BMD; $^+P<0.001$ compared to patients with osteoporosis

Analyzing the relation between BMD and 1173C>T polymorphism, no significant differences were obtained between normal, osteopenic, and ostoeporotic BMD and TT, CT, or CC genotypes (Table II). No significant statistical differences were found when the comparison was made between TT+CT and CC genotypes (TT+CT: 80.30% normal BMD, 80.95% osteopenic patients, 74.36% osteoporotic patients, P=0.70) but when the comparison was made between CC+CT and TT genotypes, a significance was obtained (CC+CT: 63.64% normal BMD, 70.48% osteopenic patients, 53.03% osteoporotic patients, P=0.04). This came from the significant differences obtained by comparing osteopenic and osteoporotic groups and CC+CT and TT genotypes (CC+CT: 70.48% osteopenic patients, 53.03% osteoporotic patients, P=0.03). The association between osteopenic and osteoporotic groups and

CC+CT and TT genotypes was more powerful for the T-score of lumbar spine (P=0.002) than for the T-score of femoral neck (P=0.01). The mean age and mean BMI were lower in the group of patients with TT genotype and normal BMD compared to CC+CT genotype and normal BMD group (P=0.02 and P=0.03, t test) (Tables III and IV).

The relationship between IMT and *VKORC1* 1173C>T genotype did not show any significance when the analysis was made by genotype (Table V, Kruskal-Wallis test) or associations (TT+CT P=0.15, Mann-Whitney test, and CC+CT P=0.22, Mann-Whitney test). As a matter of concern regarding the presence of calcified atherosclerotic plaques, a statistical significance was obtained only to the CC+CT association compared to the TT genotype (P=0.05).

Table II. Distribution of patients depending on BMD and 1173 C>T genotype

1173 C>T Genotype	Normal BMD (P=0.36)					Osteopenia (P=0.69)					Osteoporosis (P=0.33)				
	n	Mean	SD	95% CI		n	Mean	SD	95% CI		n	Mean	SD	95% CI	
				Lower bound	Upper bound				Lower bound	Upper bound				Lower bound	Upper bound
TT	29	-0.40	0.76	-0.69	-0.11	54	-1.81	0.38	-1.92	-1.71	25	-3.30	0.62	-3.56	-3.04
CT	24	-0.37	0.63	-0.63	-0.10	31	-1.80	0.41	-1.95	-1.65	33	-3.96	2.77	-4.94	-2.98
CC	13	-0.68	0.53	-1.00	-0.37	20	-1.72	0.49	-1.95	-1.49	10	-3.38	0.64	-3.84	-2.92

n, number of patients; SD, standard deviation; CI, confidence interval

Table III. Distribution of age for the patients depending of BMD and 1173C>T genotype

1173 C>T Genotype	Normal BMD (P=0.02)					Osteopenia (P=0.07)					Osteoporosis (P=0.98)				
	N	Mean	SD	95% CI		n	Mean	SD	95% CI		n	Mean	SD	95% CI	
				Lower bound	Upper bound				Lower bound	Upper bound				Lower bound	Upper bound
TT	24	58.17	9.11	54.32	62.01	31	66.06	9.99	62.40	69.73	33	67.73	8.79	64.61	70.84
CT+CC	42	62.69	6.19	60.76	64.62	74	62.23	9.54	60.02	64.44	35	68.29	10.63	64.64	71.94

n, number of patients; SD, standard deviation

Table IV. Distribution of BMI for the patients depending of BMD and 1173C>T genotype

1173 C>T Genotype	Normal BMD (P=0.03)					Osteopenia (P=0.89)					Osteoporosis (P=0.53)				
	n	Mean BMI	SD	95%CI		n	Mean BMI	SD	95%CI		n	Mean BMI	SD	95%CI	
				Lower bound	Upper bound				Lower bound	Upper bound				Lower bound	Upper bound
TT	24	28.92	4.68	26.95	30.90	31	28.41	6.92	25.87	30.95	33	26.85	6.31	24.61	29.09
CT+CC	42	31.61	4.57	30.18	33.03	74	28.57	4.36	27.56	29.58	35	25.98	4.85	24.32	27.65

n- number of patients, SD- standard deviation

Table V. Correlation between mean IMT and *VKORC1* 1173 C>T genotype

1173 C>T Genotype	n (%)	Average for mean IMT P=0.27	SD for mean IMT	95% CI for the average of mean IMT		Calcified plaques P=0.14 (%)
				Lower Bound	Upper Bound	
TT	88 (36.8)	0.104	0.063	0.092	0.116	55 (62.50)
CT	108 (45.1)	0.111	0.077	0.095	0.127	55 (50.93)
CC	43 (17.9)	0.099	0.066	0.078	0.119	20 (46.51)

n- number of patients, SD- standard deviation, CI- confidence interval

Table VI. Multivariate logistic regression model with dependent variable - BMD (osteoporosis or osteopenic patients=1) and independent variables - age, BMI, hypertension, diabetes mellitus, VKORC1 genotype, and mean IMT

	B	SE	P	OR	95.0 % CI for OR	
					Lower bound	Upper bound
Age	0.05	0.02	0.003	1.06	1.02	1.09
BMI	-0.10	0.03	0.001	0.90	0.85	0.96
Mean IMT	-0.46	2.16	0.83	0.63	0.01	43.47
Hypertension	0.18	0.35	0.61	1.20	0.61	2.37
DM	-0.06	0.36	0.87	0.94	0.47	1.89
VKORC1			0.97			
VKORC1(TT)	0.10	0.42	0.82	1.10	0.48	2.52
VKORC1(CC)	0.03	0.44	0.95	1.03	0.43	2.44
Constant	0.55	1.61	0.73	1.73		

CI, confidence interval; SE, standard error; OR, odds ratio; BMI, body mass index; DM, diabetes mellitus; BMD, bone mineral density; IMT, intima-media thickness

When analyzing the influence of BMD and *VKORC1* 1173 C>T genotype on mean IMT (two-way ANOVA, dependent variable means IMT), none of the following factors had a significant influence on IMT: VKORC1 *P*=0.81, BMD *P*=0.32 or the composed factor VKORC1 - BMD *P*=0.42.

The association between BMD and age, BMI, hypertension, diabetes mellitus, the *VKORC1* genotype, mean IMT analyzed with multivariate logistic regression (Enter method) revealed that only age (OR 1.06 95% CI 1.02 to 1.09, *P*=0.003) and BMI (OR 0.90, 95% CI 0.85 to 0.96, *P*=0.001) had significant influence on BMD (Table VI).

Discussion

In this cross-sectional study we used a recessive model (TT versus CT or CC genotype for the *VKORC1* 1173C>T genetic polymorphism) and showed that in patients with osteoporosis and osteopenia there was a more frequent TT genotype of *VKORC1* 1173C>T. Further, the TT genotype was significantly more frequently found in the osteoporotic group compared to the osteopenic group. For the whole group of patients no correlation could be established between the IMT and *VKORC1* 1173C>T genotype but, when we analyzed the presence of calcified atherosclerotic plaques, there was a significant association with the TT genotype (*P*=0.05). This finding was not associated with normal or pathologic BMD.

Teichert *et al*[23] found that the T-allele of the *VKORC1* 1173C>T polymorphism was associated with a small but significantly increased risk of aortic calcification, explaining this situation by the dominant effect of the T-allele. When they analyzed the presence of 4 to 6 calcified plaques in the carotid artery, no association with the T-allele was found. In our study the aortic calcifications had not been recorded and so a correlation with the carotid calcifications has not been searched for. We took in consideration the presence of calcified plaques in the carotid arteries, regardless of their number. The association of calcified plaques to *VKORC1* genotype was established only in the presence of TT genotype suggesting that T allele had a recessive effect.

D' Andrea *et al*[14] stated that the TT genotype was associated with a less carboxylation of vitamin K-dependent proteins. Osteocalcin and matrix Gla protein, two members of Gla-proteins family for which vitamin K acts as a cofactor for gamma-glutamyl carboxylase (involved in the bone turnover and the prevention of arterial calcification)[12], should be, in this condition, at a lower level of carboxylation. The higher prevalence of TT genotype that was found in the osteoporotic group could be explained by this mechanism. Wang *et al*[15] found in subjects with TT genotype a higher level of undercarboxilated osteocalcin but they assumed that the association found between the higher risk of vascular diseases (stroke, coronary heart disease, and aortic dissection) and the C allele of the +2255 locus might not be related to the effect on the gamma-carboxilation of the vitamin K-dependent proteins. Another yet-known function of VKORC1 could be responsible for this association[15]. Due to the lack of association between all the three variables (*VKORC1* genotype, BMD, and IMT/calcified plaques) the hypothesis of different mechanisms of VKORC1 involvement in OP and ATS is suggested by our study: TT genotype influences the BMD via vitamin K-dependent proteins and the presence of calcified plaques via another unknown function. The effects of *VKORC1* genetic polymorphism on hepatic expression in patients receiving warfarin had been studied but little is known about expression of the gene in other tissues[24]. More studies are needed in this direction.

The frequency of *VKORC1* 1137TT genotype was found to be significantly lower in patients with venous thromboembolism than in matched controls[25]. It was suggested that the VKORC1 haplotype group A (including the T allele of the *VKORC1* 1137C>T polymorphism) might be associated with a lesser procoagulant effect[25]. In these settings, haplotype B carriers (including the C allele of the *VKORC1* 1137C>T polymorphism) might have an elevated level of vitamin K-dependent proteins and protein C and protein S plasma levels would also expected to be elevated. This hypothesis was not confirmed by the study of Kimura *et al*[26] raising the possibility of different mechanisms involved in this association.

We found an association between age and BMI, TT genotype, and normal BMD. The patients with TT genotype and normal BMD were younger than the other genotypes, suggesting that their BMD had become abnormal earlier and OP had appeared at a younger age. Also, the lower BMI of TT genotype patients compared to other genotypes should be interpreted as

a risk factor for developing OP. The positive relation between BMI and OP is well known[27,28].

One of the limitations of the study was the absence of the data regarding genotype distribution of *VKORC1* 1173C>T in the Romanian population. The interethnic variability of *VKORC1* genotype was demonstrated. The frequency of 1173TT in Asian patients is higher than in Caucasian and African populations[29,30]. The frequency of TT genotype in the Caucasian population was 17 per cent[31] and 15.1 per cent[24] in the earlier studies compared to 36.8 per cent in our study. In the previously mentioned study, all the subjects were healthy volunteers, unlike the subjects in our study who were consecutively enrolled from patients presenting for BMD measurement. Large populational studies are required to establish the real proportion of *VKORC1* genotype in the Romanian population. Another limitation of the study was the low sample size that might be responsible for the lack of statistical power.

In conclusion, *VKORC1* polymorphism (TT genotype) was associated with OP and the presence of calcified plaques in the carotid artery. Probably different mechanisms are involved in these associations.

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