ORIGINAL ARTICLE

Correlation of vitamin D receptor gene (*VDR*) polymorphism with osteoporotic changes in Polish postmenopausal women

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Abstract

OBJECTIVE: Recently the significance of genetic traits, influencing hormonal and environmental factors, in susceptibility to osteopenia and osteoporosis development has been indicated. Much attention to the polymorphic variants of vitamin D receptor (*VDR*) gene was paid. The restriction polymorphisms in *VDR* gene could be involved in the modulation of vitamin D action and modulate the level of bone mineral density (BMD) and the risk to develop osteopenia and osteoporosis.
MATERIALS AND METHODS: Total 321 postmenopausal women (mean age 63.26 ± 8.90 years), including women with osteoporosis (163 patients) and osteopenia (95) have been compared to 63 women with normal *t*-score value. For detection of VDR polymorphisms PCR/RFLP (polymerase chain reaction/restriction fragment length polymorphism) assay have been used.
RESULTS: The frequency of *Bsm*I, *Apa*I, and *Taq*I polymorphic variants of *VDR* gene detected in investigated groups was not statistically different. The slight, not significant tendency to prevalence of *a* allele (*Apa*I polymorphism) in the controls comparing to women with osteoporosis and osteopenia have been noted. Higher prevalence of homozygous *TT* genotype (*Taq*I polymorphism) the in the both groups with lower BMD value (47.9 : 49.5 *vs.* 34.9% in the controls) and higher prevalence of *T* allele in these both groups (65.9 : 68.4 *vs.* 57.9) was been also observed.
CONCLUSIONS: The presence of *T* allele of *Taq*I polymorphism could predict the higher risk to develop osteoporosis in postmenopausal woman; consequently

t allele could have protective effect. The presence of *A* allele (*Apa*I polymprphism) seems to be weakly connected with osteoporosis susceptibility.

1. INTRODUCTION

Osteoporosis, a common multifactorial skeletal disease with a consequent increase in bone fragility and susceptibility to fractures, is one of the most essential reasons of health care problems in elderly age, particularly in women post menopause. It was indicated that osteoporosis development is influenced through multiple hormonal, environmental and genetic factors (Shen *et al.* 2003, Carbonell Sala and Brandi, 2007). Many etiological observations are related to the hormonal regulation of the bone mineralisation mediated through estrogens, parathormon, calcitonin, and vitamin D (Sutto and MacDonald, 2003, Ralston, 1997). Numerous observations underline importance of genetic polymorphism influencing hormonal response and bone metabolism (Ferrari *et al.* 1995, Howard *et al.* 1995, Gennari *et al.* 2002).

The most important hormonal regulator of the bone mineralisation and mineral homeostasis is vitamin D endocrine system in which vitamin D, its metabolites, and vitamin D receptor (VDR) plays a major role (Christakos *et al.* 1997, Christakos *et al.* 2007). Moreover, this system is also integrated in immune response, cell proliferation, cell differentiation, and cancer development (Newcomb *et al.* 2002, Blazer *et al.* 2000, Cui and Rohan, 2006).

VDR is nuclear hormone receptor (NHR) belonging to the superfamily of ligand-dependent transcription factors binding to the specific DNA sequences (response elements) located in the promoter regions of target genes and able to regulate their function by activation or repression. Most important biological action of VDR is mediation of the complex biological actions of 1,25-dihydroxyvitamin D3 important for the regulation of calcium and phosphate homeostasis, skeletal metabolism and bone remodeling (Christiakos *et al.* 2007). The main steps connected with the control of gene transcription by *VDR* include: ligand binding, heterodimerization with retinoid receptor (RXR), binding of the heterodimer to vitamin D response elements (VDREs) and recruitment of other coactivators into the transcription preinitiation complex (Sutton and MacDonald, 2003).

The gene encoding for the *VDR* was one of the first examined in connection with osteoporosis since relation of its genetic effect to bone mass in a twin and general population studies (Ralston, 1997, Ferrari *et al.* 1999, Spector *et al.* 1995). In humans, VDR is a product of a single gene located on the long arm of the chromosome 12 (12q12-q14) which cDNA was cloned in 1988 (Baker *et al.* 1988). The VDR gene consists of 11 exons that together with introns span approximately 7 kB of genomic DNA and code for VDR protein made up of 427 aminoacides. It must be emphasized that *VDR* is the candidate polymorphic gene that actually undergoes numerous investigations dedicated to the molecular genetics of osteoporosis. In addition to the rare deleterious mutations, which cause the 1,25-dihydroxyvitamin D-resistant rickets, *VDR* contains over 100 single nucleotide polymorphisms (SNP). Currently several marker loci within the *VDR* have been identified as related to biological variations in bone mass (Liu *et al.* 2003). Among them most frequently studied are polymorphisms localized at the 3'-end of the gene – *Bsm*I, *ApaI* (intron 8), and *Taq*I (exon 9; silent codon change *ATT* for *ATC* which both code for isoleucine) (Hustmyer *et al.* 1993, Haussler *et al.* 1998). Since different studies assessing *VDR* polymorphisms provide conflicting results (Zintzaras *et al.* 2006, Uitterlinden *et al.* 2006, Fang *et al.* 2006) larger studies in different populations as well as the use of other genetic approaches like linkage studies in extended pedigree are still needed.

The aim of present study was to determine the frequency of *Apa*I, *Bsm*I and *Taq*I polymorphisms in the gene coding for vitamin D receptor and its influence to bone mineral density in the group of Polish postmenopausal women with established bone mineral density.

2. MATERIALS AND METHODS

2.1. Patients: We have analysed 321 postmenopausal women (average age in investigated group was 63.26 ± 8.90 years, average age of last menses 48.85 ± 4.24 and average period since menopause (YSM) 14.37 ± 9.44 years). From each woman the clinical data about age, age of last menses appearance, body weight, height, and years since menopause (YSM) have been collected. The body mass index (BMI) according to WHO criteria (normal BMI 18.9–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obesity – more than 30.0 kg/m^2) have been analysed. All subjects passed densitometry examination at lumbar spine (L1–L4) by dual energy X-ray absorptiometry (DXP 100). Bone mineral density (BMD), *t*-score, the percent of young adults (YA) index, and aged matched (AM) index measured in L1–L4 were given as result of densitometric evaluation.

We have excluded women with endocrine disorders (diabetes mellitus, thyroid diseases), autoimmunologic diseases, cancer disease (current or in anamnesis), and also women taking medicaments inducing osteoporosis. All women were Caucasians of Polish origin. All of them were informed about the goal of study and given their written consent. Approval of Ethical Committee of Medical University in Poznan was given.

2.2. Genotyping Methods: From each patient 6–7 ml of venous blood was collected. The samples were stored in the temperature of minus 20°C. Genomic DNA was extracted from peripheral blood leukocytes (QIAamp DNA mini Kit; Qiagen, Hilden, Germany) using recommended procedures. For determination of

*Apa*I, *Bsm*I and *Taq*I restriction sites of the gene coding for VDR the PCR/RFLP (polymerase chain reaction/ restriction fragment length polymorphism) assay has been performed.

For the detection of *ApaI* (intron 8) and *TaqI* (exon 9) polymorphic sites following specific primers were used: F 5`-CAg AgC ATg gAC Agg gAg CAA-3`, and R 5`-gCA ACT CCT CAT ggC TgA ggT CTC-3` as described previously by Riggs et al (1995). The *BsmI* polymorphic site (intron 8) was amplified using primers F 5`-ggC AAC CTg AAg ggA gAC gTA-3`; R 5`CTC TTT ggA CCT CAT CAC CgA C-3` as described by Ye et. al (2000). Reaction was carried out in a volume of 25 µl, and containing 25 ng of genomic DNA, 0,45 µM of each primer (TibMolbiol, Poland), 2,5 µl of the 10x reaction buffer (750 mM Tris-HCl pH 8,8; 200 mM $(NH_4)_2SO_4$, 0,1% Tween 20), 1,5 mM $MgCl_2$, 0,25mM dNTP, and 1U Taq polymerase (Fermentas, Lithuania). Amplification was started with an initial denaturation at 95°C for 5 min. followed by 35 cycles of 60 s at 95°C, 60 s at 65°C (*Apa*I and *Taq*I), or 57°C for *Bsm*I, extension 90 s at 72°C, and after them one final elongation cycle at 72°C for 10 minutes Thermal cycling was performed by using the PTC 200 Programmable Thermal Controller, (MJ Research Inc., USA). The expected length of the PCR products were 745 base pairs for (*Apa*I, *Taq*I) and 461 bp for *Bsm*I. Mutations in amplified fragments were recognized by restriction enzymes produced by Fermentas, Lithuania (*Mva*1269I for *Bsm*I and *Taq*I) and EURx, Poland for *Apa*I polymorphism using standard methods. VDR genotypes were named by capital letters in the absence of the restriction site and small letters when the restriction site was present. After digestion depending on genotype, we have gotten following fragments: for *Apa*I (AA 745 bp, Aa 745, 528, 217 bp, aa 528, 217 bp), *Taq*I (TT 245, 495 bp, Tt 495, 290, 245, 205 bp, tt 290, 245, 205), and for *Bsm*I (BB 461 bp, Bb 461, 203, 258 bp, bb 203, 258 bp). Digested products were visualised by electrophoresis method on 2% agarose gel with ethidium bromide and visualized on an ultraviolet transilluminator and documented by UVI-KS4000i/Image PC system.

2.3. Statistical Analysis: The statistical analysis has been performed by SPSS 14.O PL for Windows statistical software. As statistically significant we have assumed two-sided p value lower than 0.05. Frequencies of genotypes were compared by chi-square test; mean values for clinical parameters were compared by one-way ANOVA.

3. RESULTS

3.1. Bone Mineral Density and Demographic and Clinical Parameters.

Investigated group of 321 postmenopausal women was separated, after WHO criteria according to *t*-score value: 163 women with *t*-score value lower than -2.5 (average *t*-score -3.36 and *z*-score -1.65) diagnosed as osteoporosis; 95 with *t*-score between -1.5 and -2.5 (average *t*-score -2.07 and *z*-score -0.72) classified as osteopenia, and 63 women have *t*-score at normal range (more than -1.5; average *t*-score -0.55 and *z*-score 0.39). Detailed assessment of demographic and clinical data of these groups is shown in Table 1. Mean age of investigated groups was not statistically different (64.27 *vs.* 61.65 *vs.* 63.08 years, in the group with osteoporosis, osteopenia and controls, respectively). The same observation was connected with average age of last menses appearance (48.64 *vs.* 48.47 *vs.* 49.97 years, respectively). We have not observed statistically significant differences after comparing these values for both investigated groups together (osteoporosis and osteopenia) against the controls. The mean weight and BMI were higher in the group of healthy women (mean weight: 69.83 *vs.* 60.09 and 63.42 kg in the group with osteoporosis and osteopenia; mean BMI: 25.66 *vs.* 23.95 and 24.81 kg/ m^2 in the group with osteoporosis and osteopenia). The average height was the highest in the group of women with t-score more than –1.5 (162.21 *vs.* 158.42 and 159.45, respectively). In the control group the higher L1 – L4 BMD level (1.11 g/cm^2), higher L1 – L4%YA (94.43%) and L1 – L4%AM index (105.16%) if compared to the group with osteoporosis and osteopenia have been observed (see Table 1).

3.2. Frequency of VDR genotypes and alleles

The presence of all *Bsm*I, *Apa*I and *Taq*I polymorphic variants of *VDR* gene detected in studied groups is summarised in Table 2. Appearance of *Bsm*I polymorphism was similar in all investigated groups. Analysing *Apa*I polymorphism we have noticed the slight tendency to prevalence of *a* allele in the controls than in the women with decreased BMD value (not significant). Interestingly, *AA* genotypes and *A* alleles were overrepresented in the osteopenia group. Investigation of *Taq*I polymorphism showed the high prevalence of homozygous *TT* genotype in the groups with osteoporosis and osteopenia (47.9 : 49.5 *vs.* 34.9% in the controls, p = ns). Consequently, higher prevalence of *T* allele in the both groups with osteoporosis and osteopenia (65.9 : 68.4 *vs.* 57.9 in controls, p = ns) has been also observed (see Table 2).

3.3. Correlation of VDR genotypes with clinical parameters

We have analysed the mean values of demographic and clinical parameters with obtained *VDR* genotypes applying one-way ANOVA Test. Most interesting was observation that in total group menopausal age was significantly higher (50.1 +/- 4.1) for *tt* genotypes of *Taq*I polymorphism (p = 0.024) than for *TT* (48.9 +/- 4.1) or *Tt* (48.2 +/- 4.3). The body weight in whole group was also connected with *Taq*I polymorphism: *TT* – 61.4 +/- 9.0 kg; *Tt* – 64.2 +/- 11.9 kg; *tt* – 64.7 +/- 8.7 kg (p = 0.035). Consequently, similar observation was valid for BMI: *TT* – 24.1 +/- 3.1; *Tt* – 25.2 +/- 4.0; *tt* – 25.3 +/- 3.5 (p = 0.024). For particular genotypes only

Tab. 1. Demographic data of investigated groups. Mean in given with standard deviation (±SD).

	Total group	Osteoporosis	Osteopenia	Controls (normal t-score value)	p value
n	321	163	95	63	
Average age (years) median range	63.26 ± 8.90 63 45 – 90	64.27 ± 8.72 63 46 – 90	61.65 ± 9.97 60 45 – 82	63.08 ± 7.24 64 49 – 82	ns
Average age of last menses (years) median range	48.85 ± 4.24 50 38 – 60	48.64 ± 4.28 50 38 – 60	48.47 ± 4.18 49 38 – 59	49.97 ± 4.07 50 40 – 60	ns
Average period since menopause (YSM) (years) median range	14.37 ± 9.44 14 1 – 41	15.65 ± 8.85 15 1 – 40	13.36 ± 10.80 11 1 – 41	12.54 ± 8.79 14 1 – 38	ns
Weight (kg) median range	62.99 ± 10.24 62 41 – 114	60.09 ± 8.16 60 41 – 85	63.42 ± 9.40 63 41 – 95	69.83 ± 12.77 68 50 – 114	<0.001
BMI (kg/m^2) median range	24.74 ± 3.63 24.46 16.38 – 43.44	23.95 ± 3.08 23.61 16.38 – 33.30	24.81 ± 3.35 24.54 17.51 – 40.58	25.66 ± 4.09 25.06 19.03 – 35.43	<0.001
Height (cm) median range	159.47 ± 5.62 160 140 – 176	158.42 ± 5.36 158 140 – 172	159.45 ± 5.68 160 144 – 172	162.21 ± 5.33 162 152 – 176	<0.001
L1-L4 BMD (g/cm^2) median range	0.89 ± 0.15 0.88 0.513 – 1.403	0.78 ± 0.08 0.79 0.51 – 0.95	0.93 ± 0.05 0.93 0.76 – 1.08	1.11 ± 0.11 1.08 0.99 – 1.40	<0.001
L1-L4%YA (%) median range	75.43 ± 12.92 74 43 – 119	65.95 ± 7.11 67 43 – 86	79.11 ± 3.43 79 73 – 88	94.43 ± 9.39 91 82 – 119	<0.001
L1-L4%AM (%) median range	88.11 ± 13.84 86 54 – 153	79.57 ± 8.51 79 54 – 100	91.46 ± 8.73 91 65 – 112	105.16 ± 13.38 102 77 – 153	<0.001
t-score median range	-2.43 ± 1.26 -2.52 -5.56 – 1.86	-3.36 ± 0.67 -3.28 -5.56 – (-2.51)	-2.07 ± 0.31 -2.16 -2.50 – (-1.51)	-0.55 ± 0.92 -0.85 -1.49 – 1.86	<0.001
z-score median range	-0.97 ± 1.14 -1.12 -3.86 – 3.99	-1.65 ± 0.72 -1.62 -3.86 – (-0.05)	-0.72 ± 0.75 -0.74 -2.04 – 0.89	0.39 ± 1.13 0.10 -1.85 – 3.99	<0.001

BMI – body mass index, YSM – years since menopause, BMD – bone mineral density, YA – young adults, AM – aged matched

menopausal age remains significantly different for *Taq*I polymorphism in the groups with normal t-score and osteoporosis. For the BMD significant differences were found only for women with normal t-score for *Apa*I polymorphism where values for BMD L1-L4, BMD age matched, and t-score were significantly different (p values 0.046, 0.046, and 0.030, respectively), but these differences were due to higher values noted for heterozygous *Aa* genotypes, whereas homozygotes *AA* and *aa* have similar values.

4. DISCUSSION

Numerous twin and family studies showed that genetic factors play an important role in osteoporosis development. The genetic effects influence to bone mass and structure were the subjects of many investigations (Morrison *et al.* 1994, Kikuchi *et al.* 1999). Currently it is estimated that genetic contribution to the pathogenesis of osteoporosis account from 50% to 80% of individual bone mass (Sutton and MacDonald, 2003). The gene encoding for the VDR receptor was one of the first examined and its role on genetic susceptibility to osteoporosis in twins and in general population was shown (Morrison *et al.* 1994). Recently several marker loci within the *VDR* gene have been identified as related to biological variations in bone mass. Incorrect answer to vitamin D action results osteomalacia defect in bone mineralisation as well as reduction in BMD – the major determinant of bone strength. Morrison *et al.* suggested the strong association between VDR polymorphisms and BMD. This authors shown that the higher BMD was connected with *bb* genotype (Morrison *et al.* 1994). This suggestion was supported by many other reports

Tab. 2. The frequency of genotypes and alleles of *Bsm*I, *Apa*I and *Taq*I polymorphisms in *VDR* gene in investigated groups.

VDR polymorphism			Osteoporosis *(n = 163)*	Osteopenia *(n = 95)*	Osteoporosis and osteopenia *(n = 258)*	Normal t-score (controls) *(n = 63)*
			n (%)	**n** (%)	**n** (%)	**n** (%)
***Bsm*I**	***Genotypes***	***BB***	**27** (16.6)	**17** (17.9)	**44** (17.1)	**10** (15.9)
		Bb	**66** (40.5)	**39** (41.1)	**105** (40.7)	**27** (42.9)
		bb	**70** (42.9)	**39** (41.1)	**109** (42.2)	**26** (41.3)
	Alleles	***B***	**120** (36.8)	**73** (38,4)	**193** (37.4)	**47** (37.3)
		b	**206** (63.2)	**117** (61.6)	**323** (62.6)	**79** (62.7)
***Apa*I**	***Genotypes***	***AA***	**35** (21.5)	**27** (28.4)	**62** (24.0)	**12** (19.1)
		Aa	**82** (50.3)	**42** (44.2)	**124** (48.1)	**32** (50.8)
		aa	**46** (28.2)	**26** (27.4)	**72** (27.9)	**19** (30.2)
	Alleles	***A***	**152** (46.6)	**96** (50.5)	**248** (48.1)	**56** (44.4)
		a	**174** (53.4)	**94** (49.5)	**268** (51.9)	**70** (55.6)
***Taq*I**	***Genotypes***	***TT***	**78** (47.9)	**47** (49.5)	**125** (48.4)	**22** (34.9)
		Tt	**59** (36.2)	**36** (37.9)	**95** (36.8)	**29** (46.0)
		tt	**26** (16.0)	**12** (12.6)	**38** (14.7)	**12** (19.1)
	Alleles	T	**215** (66.0)	**130** (68.4)	**345** (66.9)	**73** (57.9)
		t	**111** (34.0)	**60** (31.6)	**171** (33.1)	**53** (42.1)

(Fleet *et al.* 1995). In the contrast to them some published findings has not found any association between osteoporosis, osteopenia and genetic factors (Spotila *et al.* 1996, Alahari *et al.* 1997, Tsai *et al.* 1996, Garneo *et al.* 1995).

It is known that *Taq*I polymorphism does not show any clinical function, but many authors suggest that the VDR restriction polymorphisms are in linkage disequilibrium with another functional polymorphism that is involved in bone turnover and bone tissue metabolism. It was also clearly shown that the frequency of *Bsm*I restriction polymorphism of VDR gene should be always connected with racial differences. In Caucasian the most frequent (40–50%) was the *Bb* genotype (study from the populations in USA, Australia, and West Europe) (Morrison *et al.* 1994, Salamone *et al.* 1996). In the contrast in Asian population *bb* genotype is common (95% *bb* and 1% *BB* genotypes) (Tsai *et al.* 1996). It is known that *Bsm*I polymorphism probably does not appear any clinical function, but could be in linkage disequilibrium with functional polymorphism.

Gong et al. (Gong *et al.* 1999) analysed 95 clinical studies investigated the correlation between *Bsm*I, *Apa*I, *Taq*I and *Fok*I polymorphisms of VDR gen and BMD. The authors indicated that 34,3% (23 publications) of clinical studies suggested positive correlation between one of the allele (*b, a, T* and *F* for *Bsm*I, *Apa*I, *Taq*I and *Fok*I polymorphisms, relatively) and higher BMD, BMC, higher calcium absorption, such as lower bone loss, and lower risk of bone fractures when the BMD was investigated in the lumbar L2 – L4. Meta-analysis made by Thakkinstan et al. (Thakkinstan *et al.* 2004) (39 clinical studies, pre- and post menopausal women) only *Bsm*I genetic polymorphism was investigated in correlation with BMD. The presence of bb and Bb genotypes in postmenopausal women were correlated with BMD higher value in lumbar L2 – L4. Interestingly, this observation was not confirmed in premenopausal women. Nevertheless, in the prospective multicenter large-scale association study performed by the The Genetic Markers for Osteoporosis (GENOMOS) consortium involving 9 European research teams (26242 participants; 7837 men and 18405 women) different polymorphisms were genotyped (Cdx2-promoter, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I) and statistical analyses revealed that these polymorphisms were not associated with lumbar spine or femoral neck BMD. Only Cdx2 A-allele was associated with 9 % reduced risk for vertebral fracture in this study (Uitterlinden *et al.* 2006).

Many interesting studies concerning the association between *VDR* polymorphisms and bone mineral density have been performed in Polish population. Horst-Sikorska et al. (Horst-Sikorska *et al.* 2005) investigated 187 patients with osteoporosis (161 women, 26 men) and 19 healthy subjects from Poland. The statistically significant relationship between BMD value and *T* allele of *Taq* I VDR gene was found. Genotypes: *aa*, *bb*, *TT* of VDR gene occur more frequently in polish osteoporotic population in Wielkopolska region within patients with higher risk of bone fractures. In further researches Horst-Sikorska et al. (Horst-Sikorska *et al.* 2007) evaluated the associations of the *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms *VDR* gene with the bone mineral density (BMD) of the lumbar part of the spinal column (BMD LS) and the neck of the femur (BMD FN), and with the occurrence of fractures from 239 women and

40 men from the region of western Poland. The authors observed that three polymorphisms of the *VDR* gene (*Bsm*I, *Apa*I, *Taq*I) indicated strong linkage disequilibrium. Association analysis of the *VDR* gene *Fok*I polymorphism with BMD LS showed a dose effect of allele *f*. The association of the *Apa*I polymorphism with the occurrence of fractures was observed. Associations were also observed between the occurrence of fractures and the *baT* haplotypes of the *VDR* gene. Our group (Seremak-Mrozikiewicz *et al.* 2007) observed statistically higher frequency of *B* allele, the lower frequency of *b* allele and *bb* genotype in the investigated group of 34 postmenopausal women with low BMD and concluded that theses observations could suggest the important role of *B* allele of the *VDR* gene in the pathogenesis of osteoporosis in the group of women with low mineral density and possible protective role of b allele in this disease.

In our study the most interesting results were connected with *Taq*I polymorphism: observed genotypes distribution in the group with *t*-score lower than –2.5 (*TT : Tt : tt* = 47.85 : 36.20 : 15.95%) and with *t*-score range between –2.5 and –1.5 (*TT : Tt : tt* = 49.47 : 37.90 : 12.63%) were similar to the results received in the some other studies in Caucasians (Spector, 18). We have detected the higher frequency of *TT* genotypes in the group of women with osteoporosis (47.85%) and osteopenia (49.47%). These women had also lower mean weight (60.09 and 63.42 kg, respectively) and BMI (23.95 and 24.81 kg/cm^2, respectively).

Lower BMD level and *t*-score value (also YA and AM index) have indicated the *TT* genotype as a risk factor of decreased BMD value. In the study performed by Spector and co-workers (Spector *et al.* 1995), the *Taq*I polymorphism was also correlated with osteoporosis appearance in postmenopausal women, and additionally the authors noted the strong linkage disequilibrium between the *Taq*I and *Bsm*I polymorphisms. On the other hand controversial to our results it was determined that the postmenopausal women with *TT* genotype have higher BMD at the lumbar spine and at the proximal femur if comparing to *tt* genotype (Peacock *et al.* 1995). Fang et al. evaluated the association between the genetic profile – *Bsm*I and *Taq*I polymorphisms and the risk of vertebral fracture. The thirteen studies with a total of 20 eligible comparisons (1632 fracture cases and 5203 controls) were included in this meta-analysis. There was significant between-study heterogeneity and no evidence of relationship between the VDR *Bsm*I or *Taq*I polymorphism and fracture risk was observed with any genetic model (Fang *et al.* 2006).

In our study we have not confirmed the significant role of *Bsm*I and *Apa*I polymorphisms in osteoporosis development. The presence of *T* allele of VDR polymorphism could predict the lower bone mineral density and *t*-score in the group of postmenopausal women and suggested the protective role of *tt* genotype and *t* allele. The over representation of *T* allele in women with lower BMD value should be check to risk of bone fracture. For founding a clearly correlation between *Taq*I polymorphism and osteoporotic bone fracture further investigations are still required.

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