

TaqI Polymorphism of the Vitamin-D Receptor Gene and Quality of Life in Postmenopausal Turkish Women

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Aim: We aimed to assess vitamin-D receptor (VDR) gene polymorphism in postmenopausal osteoporotic Turkish women as well as the impact of this on diagnosis, treatment and quality of life.

Materials and Methods: Seventy postmenopausal osteoporotic women and 71 postmenopausal healthy women constituted the disease and control groups, respectively. Genomic DNA was extracted from blood of all participants and analyzed for the *TaqI* gene polymorphism of the VDR gene. The osteoporotic group was taking calcitonin, alendronate, calcitriol and elementary calcium, while the control group was taking only vitamin D and calcium. Quality of life of patients was measured by Nottingham Health Profile (NHP). Bone mineral density (BMD) was measured using DEXA method.

Results: Genotype distribution of disease and control subjects, respectively, were as follows: TT (28.6%; 42.3%), Tt (62.8%; 40.8%), and tt (8.6%; 16.9%). Tt genotype was significantly higher in the study group ($P < 0.005$). There were no significant differences between the distribution of TT and tt genotypes between groups. However, BMD scores were significantly higher in the subjects with active T allele. There were also significant differences in before and after treatment NHP scores ($p:0.001$).

Conclusions: Frequency of Tt variant was found higher in the disease group. The NHP score was significantly lower after treatment. Presence of T allele leads to higher BMD values than observed with t allele. The genetic variations with respect to polymorphism of the VDR gene may be important in determining the diagnosis, treatment outcome and quality of life in osteoporosis.

Key Words: Osteoporosis, quality of life, *TaqI* polymorphism, vitamin D receptor gene

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Postmenapozal Türk Kadınlardaki Vit D Reseptör Genindeki Taq I Polimorfizmi ve Yaşam Kalitesi

Amaç: Postmenapozal osteoporozu olan kadınlardaki olası VDR gen polimorfizminin tanı, tedavi ve yaşam kalitesi üzerindeki etkilerini araştırmaktır.

Yöntem ve Gereç: Yetmiş postmenapozal kadın hasta grubunu ve 71 postmenapozal sağlıklı kadın kontrol grubunu oluşturdu. Çalışmaya katılan tüm bireylerin kan örneklerinden genomik DNA izole edilerek VDR geninin *Taq I* polimorfizmi analiz edildi. Hasta grubuna salmon kalsitonin 200 IU (2 ay) ve alendronat 70 mg haftada, calcitriol 1 g/gün ve elementar calcium 1000 mg/gün verildi. Kontrol grubuna ise calcium 1000 mg ve 880 mg D vitamini verildi. Yaşam Kalitesi Nottingham Sağlık profili (NHP) ile değerlendirildi. Tedavi öncesi ve sonrası vertebra ve femur kemik mineral yoğunluğu DEXA yöntemi ile ölçüldü.

Bulgular: Hasta ve kontrol grubunu genotip dağılımı sırası ile TT (% 28,6; % 42,3), Tt (% 62,8; % 40,8), tt (% 8,6; % 16,9) olarak saptandı. Çalışma grubunda Tt genotipleri kontrol grubuna oranla anlamlı olarak yüksekti. ($P < 0.005$). "TT ve Tt" genotiplerin dağılımına ilişkin gruplar arasında anlamlı bir fark saptanmadı. Ancak T allelini etkin olarak taşıyan olgularda, kemik BMD ve DEXA değerlerinin anlamlı olarak daha iyi olduğu gözlemlendi. Olguların tedavi öncesi ve sonrası yaşam kalitesi skorları arasında anlamlı fark olduğu gözlemlendi ($p:0.001$).

Sonuç: "Tt" variantının hastalarda anlamlı olarak yüksek olduğu saptandı. Yaşam kalitesinin tedavi öncesi ve sonrası skorlarının da anlamlı olarak düştüğü gözlemlendi. T allelinin varlığının kemik BMD ve DEXA değerlerinde t alleleline göre anlamlı olarak daha iyi olduğu gözlemlendi. Osteoporoz hastalarında VDR gen polimorfizminin saptanması, hastaların tanısında, tedavinin planlanmasında ve yaşam kalitesinin değerlendirilmesinde önemli olduğu kanısına varıldı.

Anahtar Sözcükler: Osteoporoz, Yaşam kalite, *TaqI* polimorfizmi, Vitamin D reseptör geni

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Introduction

Osteoporosis (OP) is a common disease associated with reduced bone mineral density, affecting up to 40% of women at some point during their life span. Although OP is a multifactorial disease, genetic factors play an important role in its pathogenesis (1-3).

Although a strong association between vitamin-D receptor (VDR) genotype and bone mineral density (BMD) was suggested in OP (4,5), there are conflicting findings on the possible association between postmenopausal bone loss and the VDR genotype (6,7). The association of VDR genotype and BMD may be different in various ethnic and geographical groups (8-10). Therefore, we assume that it would be worthwhile to assess the VDR polymorphism among the Turkish population.

The action of vitamin D depends on the functional status of the VDR, which has polymorphic variants. The original claim that the polymorphic variation at the VDR locus may account for up to 75% of the genetic contribution to bone mass has been contested, with subsequent work yielding conflicting data (4,11).

OP interferes with the patient's quality of the life. The Nottingham Health Profile (NHP) is widely accepted as a useful index for the generic health status measures, and possibly to assess quality of life in OP patients (12).

The purpose of this study was to assess the *TaqI* polymorphism on the VDR gene among Turkish osteoporotic women in view of its importance in the diagnosis and treatment of OP as well as on the quality of life of these patients.

Materials and Methods

Seventy women with postmenopausal OP who consecutively admitted to our Physical Medicine and Rehabilitation Clinic were included into this study. Their ages ranged from 46 to 69 years (mean 60.5 ± 5.5). The diagnosis of OP in the study group was made according to BMD values of the patients detected in the lumbar and hip regions.

OP was diagnosed according to the World Health Organization (WHO) criteria (13). The BMD, expressed as density of the area in grams per square centimeter, was measured in the anterior-posterior lumbar spine (L_{2-4}) and femur with dual-energy X-ray absorptiometry (DEXA) using a QDR-1500 Hologic instrument.

Seventy-one healthy postmenopausal women whose ages ranged from 44 to 69 years (mean 60.4 ± 5.2) were also included in the study and comprised the control group. Their BMD values were less than -1 SD and more than -2.5 SD that of healthy young adult women (14).

The routine laboratory investigations (complete blood count, blood chemistry, thyroid function tests) were performed in both groups. None of the subjects had bone fractures related to OP or any other disease that might affect the bone metabolism.

The serum estradiol (E2) levels of the patients and the control subjects were less than 30 pg/ml. Their postmenopausal periods ranged from 3 to 17 years (mean 8.8 ± 2.9) in study group and 2 to 15 years in controls (mean 7.4 ± 3.2).

One of the standard therapy protocols was administered to the patients in the study group and consisted of intranasal salmon calcitonin 200 IU/day (total 2 months), alendronate 70 mg/week, elementary Ca 1000 mg/day and calcitriol 1 μ g/day. The subjects in the control group were given 1000 mg elementary Ca 1000 mg/day and 880 IU vitamin D orally. Both groups were advised to undertake regular exercise daily and to increase their dietary intake of milk or milk products.

The NHP is a questionnaire that helps to assess quality of life. It is a widely accepted outcome measurement, and has also been validated in our country. Briefly, NHP is comprised of 38 statements (answered "yes" or "no") that assess subjective distress in six sections. The scores for each section range from 0 (no problem) to 100 (all problems listed are present) (12). Since it would be difficult to separate emotional reactions and social isolation statements in our population, assessment of the latter was not performed. The NHP questionnaire was applied twice for each patient, with the first application before initiation of the medical therapy and the second at the 8th month of therapy.

Molecular Analysis

DNA extraction and analysis

After obtaining informed consent, blood samples were drawn from each subject. Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes. DNA was extracted from whole blood by salting out procedure (6).

Genotypic analysis of polymorphisms of the VDR gene

The genotype for four restriction fragment length polymorphisms (RFLP) of the VDR gene was determined by polymerase chain reaction (PCR) amplification and enzymatic digestion of the products with *Apal*, *Bsml*, *TaqI* and *FokI*.

A region of the receptor gene carrying the three reported polymorphic restriction sites *Apal*, *Bsml*, *TaqI* [situated in intron 8 and the 5' end of exon 9; 2000 base pairs (bp)] was amplified by PCR.

The specific primers for the VDR were upstream primer positioned in exon 8 (5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3') and downstream primer positioned in exon 9 (5'-CACTTCGAGCACAAGGGGCGTTAGC-3'). PCR was performed in a 50 µL volume with 20-100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1 mM MgCl₂, 1x PCR buffer with (NH₄)₂SO₄ and 1U *Taq* polymerase (MBI Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Genius, Cambridge, England). PCR conditions were 3 min for initial denaturation at 94.5°C; 35 cycles at 94.5°C for 1 min for denaturation, 1 min at 61°C for annealing and 2 min at 72°C for extension, followed by 7 min at 72°C for final extension. With the enzymes *Apal*, *Bsml* and *TaqI*, respective genotypes were defined as A, B, T (indicating the absence of the restriction site) or a, b, t (indicating the presence of restriction site). The digest products were resolved at 100 V for 20-30 min on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. A 100 bp marker (100 bp DNA Ladder, MBI Fermentas, Vilnius, Lithuania) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, France).

PCR product for *Apal*, *Bsml* and *TaqI* polymorphism was 2000 bp long; the lengths of the fragment and genotyping after digestion for 3 h at 30°C with *Apal* (Roche Molecular Biochemicals, GmbH, Germany), digestion for 3 h at 37°C with *Bsml* isoschizomer *MvaI269I* (MBI Fermentas, Vilnius, Lithuania) and digestion for 3 h at 65°C *TaqI* (MBI Fermentas, Vilnius, Lithuania) were AA 2000 bp; Aa 2000 bp, 1700 bp and 300 bp; aa 1700 bp and 300 bp; BB 2000 bp; Bb 2000 bp, 1350 bp and 650 bp; bb 1350 bp and 650 bp; TT 2000 bp; Tt 2000 bp, 1800 bp and 200 bp; tt 1800 bp and 200 bp.

In addition, another polymorphism (*FokI*) of the VDR gene was analyzed in our study. The primers and PCR conditions for amplifying exon 2 of the VDR gene were described previously [7-9]. The primers 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' and 5'-ATGGAACACCTTGCTTCTTCTCCCTC-3' were used to amplify exon 2. PCR was performed in a 25 µL volume with 20-100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1 mM MgCl₂, 1x PCR buffer with (NH₄)₂SO₄ and 1U *Taq* polymerase. After amplification PCR products were digested by restriction endonuclease *FokI* isoschizomer *BseGI* (MBI Fermentas, Vilnius, Lithuania) for 3 h at 55°C, the genotyping of VDR gene was determined by fragment separation on a 3% agarose gel. The genotypes were classified as: FF, homozygotes, absence of the Fok I site results in one fragment of 265 bp; Ff, heterozygotes exhibiting fragments of 265 bp, 196 bp, and 69 bp; ff, homozygotes, presence of the site results in two fragments of 196 bp and 69 bp.

Statistical Analyses

The data were analyzed using SPSS 13.0 for Windows. The associations of BMD and the other parameters in each VDR genotype were assessed using ANOVA. ANOVA and paired t test were used to compare NHP scores and VDR genotype.

Results

There were no significant differences in the demographic data between the groups ($P > 0.05$). The demographic data and T-scores are shown in Table 1.

Table 1. Demographic data and DEXA values.

Parameters	Patients	Controls
Age (year)	60.5 ± 5.50	60.4 ± 5.2
BMI (m/kg ²)	31.7 ± 4.50	32.5 ± 4.5
VBMD	0.98 ± 0.20	1.1 ± 0.12
FBMD	0.87 ± 0.20	0.9 ± 0.12
WBMD	0.63 ± 0.16	0.7 ± 0.10
TS	-2.8 ± 1.40	-0.5 ± 1.0
TF	-2.2 ± 1.30	-0.8 ± 0.9
TW	-2.7 ± 1.20	-0.7 ± 0.9

BMI: Body mass index. V_{BMD}: BMD value of spine. F_{BMD}: BMD value of femur. W_{BMD}: BMD value of Wards triangle of femur. Ts: DEXA-T value of spine. T_F: DEXA-T value of femur. T_w: DEXA-T value of Wards triangle of femur.

The VDR genotype distribution in patients and control groups were TT (28.6%; 42.3%), Tt (62.8%; 40.8%), and tt (8.6%; 16.9%), respectively. There was a significant difference in the Tt genotype between patients and controls ($P < 0.05$), with the Tt genotype overrepresented in the study group (Table 2). Genotyping was based upon independent scoring of the outcomes by two reviewers who were unaware of the case/control status (Figure 1).

Table 2. Results of molecular analyses of VDR gene polymorphism.

Genotype	TT	Tt*	tt
Patients (n) (%)	20 (28.6%)	44 (62.8%)	6 (8.6%)
Controls (n) (%)	30 (42.3%)	29 (40.8%)	12 (16.9%)

* The difference was significant, $P < 0.05$.

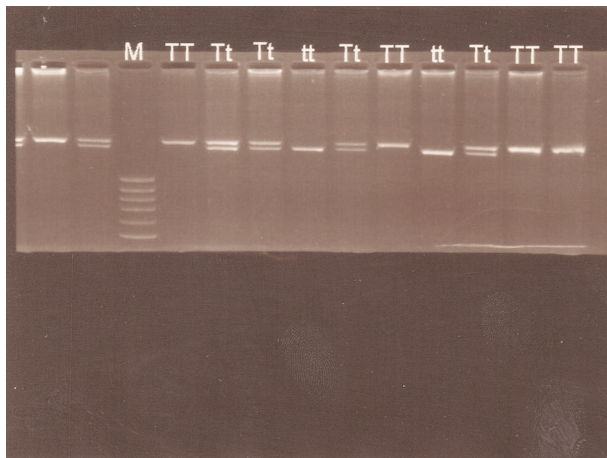


Figure 1. TaqI polymorphism of vitamin-D receptor gene of groups.

The differences between frequencies of TT and tt variants in patients and controls were not significant. To understand which allele (T or t) was of importance with respect to OP and quality of the life, we compared total T and t alleles between the patient and control groups, and the difference again was not significant ($p: 0.246$). However, we believe that presence of T alleles has a positive effect on BMD and DEXA values as well as quality of life. Presence of tt genotype did not have a positive effect on bone or on the quality of life. This may be interpreted as the feature of tt genotype or may have been due to the limited number of the patients or controls who had it (Table 3).

NHP parameters were significantly lower after the therapy when compared to values before the treatment ($P < 0.001$) (Table 4).

Discussion

Osteoporosis, which has become an important issue in this century as a consequence of improved health care, may be considered a complex interaction between subtle genetic polymorphisms and environmental influences (16). The presence of familial concordance of OP appears to be suggestive of a genetic basis (17,18).

The VDR is involved in the regulation of calcium homeostasis in many tissues (1). It is known that there are three polymorphic sites, *BsmI*, *Apal* and *TaqI*, on the VDR gene (2,19). In a study regarding *TaqI* polymorphism, the frequencies of the different forms of the VDR genotypes were found to be 39.5%, 41%, and 19.5%, for the TT, Tt and tt variants, respectively (20). In our study, however, the frequencies of TT, Tt and tt variants were 28.6%, 62.8% and 8.6% in the study group, and 42.3%, 40.8%, 16.9% in the control group, respectively. The data above indicate that the frequency of each genotype varies between different populations.

Table 3. Results of multiple comparisons between genotype subgroups, BMD and T-scores in the patient group.

Comparison of Genotype	VBMD	FBMD	WBMD	Ts	TF	TW
TT-Tt	P: 0.001	P: 0.001	P: 0.001	P: 0.001	P: 0.001	P: 0.001
TT-tt	P: 0.001	P: 0.004	P: 0.001	P: 0.001	P: 0.006	P: 0.001
Tt-tt	P: 0.419	P: 0.956	P: 0.680	P: 0.364	P: 0.962	P: 0.726

VBMD : BMD value of spine. FBMD : BMD value of femur. WBMD: BMD value of Wards triangle of femur. Ts: T-score of spine. TF : T-score of femur. TW : T value of Wards triangle of femur.

Table 4. Results of before and after therapy NHP scores according to subgroups of genotype.

NHP Parameters	Subgroups of Genotype	Before Therapy NHP Scores	After Therapy NHP Scores
Pain	TT	67.4 ± 6.2	35.9±4.7
	Tt	76.4 ± 5.5	37.8 ± 4.0
	tt	75.0 ±7.8	36.7 ± 2.6
Fatigue	TT	67.4 ±6.2	35.7 ± 4.7
	Tt	76.4 ± 4.8	36.6 ± 4.9
	tt	77.5 ±8.8	37.5 ±4.2
Physical Activities	TT	66.1 ± 5.2	36.3 ± 5.7
	Tt	74.1 ± 5.1	35.6 ± 4.9
	tt	75.8 ± 3.8	36.7 ± 4.1
Sleeping	TT	68.9 ± 5.8	34.4 ± 4.1
	Tt	74.7 ± 4.9	33.1 ± 6.1
	tt	74.2 ± 2.1	36.7 ± 2.6
Emotional Reactions	TT	67.1 ± 5.8	37.8 ± 4.0
	Tt	75.2 ± 5.3	36.6 ± 5.2
	tt	74.8 ± 3.6	36.7 ± 2.8

It was shown that the bone densities did not differ according to *TaqI* genotype. In addition, there was a trend toward higher values for vertebral and femoral bone density in girls with the "TT" genotype and toward lower values in girls with the homozygous "tt" genotype (21). However, we found a significant difference in frequency of "Tt" variants between the patients and controls.

The BMD values were reported to be low in subjects with the tt genotype (15). It was suggested that BMD scores of subjects with TT genotype are better than those with Tt genotype (22). We found that the influence of variants of TT-Tt and TT-tt, which consisted mainly of "T" alleles, was significantly better than that of the variants of Tt-tt, which consisted of mainly "t" alleles, on all BMD and DEXA values (Table 4). This condition may also be interpreted by ethnic diversity.

In our study, all patients who had all genotypes had significant improvement in the quality of life as determined by NHP scores. There was no significant

difference between genotypes of the patients on the quality of life as determined by NHP scores.

Previous studies addressed mainly VDR gene polymorphism and BMD changes. However, to our knowledge, this is the first study in which the quality of life was assessed as a function of VDR gene polymorphism. Although we determined a significant difference in distribution of the Tt genotype between groups, there was no impact of this on evaluation of health quality and treatment. This may be associated with the limited number of cases. However, patients with the "t" allele should be given particular attention with respect to treatment and prophylaxis of OP.

In conclusion, genetic variations may be important in determining the treatment outcome in OP, such as the polymorphism of the VDR gene that codes for calcium-related receptors. The genetic patterns of the patients may be of importance both in treatment planning and patient evaluation in OP.

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