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ORIGINAL ARTICLE

Bone mineral density, body height, and vitamin D receptor gene polymorphism in middle-aged men

TERHI REMES[1,2], SARI B. VÄISÄNEN[3,4], ANITTA MAHONEN[1], JOUNI HUUSKONEN[5], HEIKKI KRÖGER[2,6], JUKKA S. JURVELIN[7] & RAINER RAURAMAA[3,7,8]

[1]*Department of Medical Biochemistry, University of Kuopio, Kuopio, Finland,* [2]*Bone and Cartilage Research Unit (BCRU), Mediteknia, University of Kuopio, Kuopio, Finland,* [3]*Kuopio Research Institute of Exercise Medicine, Foundation for Research in Health Exercise and Nutrition, Kuopio, Finland,* [4]*Department of Clinical Chemistry, Kuopio University Hospital, Kuopio, Finland,* [5]*Department of Surgery, North Carelia Central Hospital, Joensuu, Finland,* [6]*Department of Surgery, Kuopio University Hospital, Kuopio, Finland,* [7]*Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland,* [8]*Department of Physiology, University of Kuopio, Kuopio, Finland*

Abstract

BACKGROUND. Polymorphisms of the vitamin D receptor (VDR) gene have been suggested to account for some of the genetic variation in bone mass. However, the relationship has been controversial. It has been suggested that environmental factors such as physical activity may be one of the many reasons for this controversy.

AIM. We investigated the possible interactions of VDR gene polymorphisms and low to moderate intensity exercise on bone mineral density (BMD) in a four-year controlled, randomized intervention trial in 140 middle-aged Finnish men.

METHOD. The TaqI, FokI, and ApaI restriction fragment length polymorphism (RFLP)-markers of the VDR gene were evaluated. BMDs of the lumbar spine (L2–L4), femoral neck, and total proximal femur were measured with dual-energy X-ray absorptiometry (DXA). In addition, the relations of the VDR gene polymorphism with bone turnover markers (serum tartrate-resistant acid phosphatase (TRAP) 5b activity and serum osteocalcin concentration) were evaluated.

RESULTS. At the randomization, the subjects with the VDR TaqI Tt or tt genotype had a greater body height than the subjects with TT genotype (P=0.001). In addition, the association of VDR TaqI polymorphism with femoral BMD was found. The Tt or tt genotype associated with higher femoral neck values than the TT genotype (P=0.003) at randomization. After adjusting the femoral neck for body height, the association remained (P=0.021). We did not find any association between VDR gene polymorphism and bone turnover markers or any interactions of VDR gene polymorphisms and exercise on BMD.

CONCLUSIONS. The TaqI polymorphism may be associated with body height and femoral neck BMD values. The present findings also suggest that the VDR polymorphisms do not modify the effect of regular aerobic exercise on BMD. However, more randomized controlled exercise trials are needed to investigate the role of exercise intensity on VDR gene polymorphisms, and the role of VDR gene polymorphisms on BMD.

Key words: *Bone mineral density, bone turnover, height, male, vitamin D receptor*

Introduction

Osteoporosis is a multifactorial disease with a genetic background in which bone mineral density (BMD) and quality of bone are reduced, leading to weakness of the skeleton and increased risk of fracture. Although most studies concerning gene polymorphisms and BMD have been conducted on women, there is evidence to suggest that genetic factors are also important determinants of osteoporosis in men (1,2).

Vitamin D is one among other important factors required for the bone development and maintenance of bone mass as well as the principal factor required for control of normal calcium and

Correspondence: Terhi Remes, Department of Medical Biochemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. Fax: +358-17-2811510. E-mail: tremes@hytti.uku.fi

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phosphate homeostasis. Nutritional vitamin D status has been linked to BMD in both retrospective (3) and prospective interventional (4) studies. Several studies in women (5–11) and a few studies in men (12–19) have investigated the genetic variants of the gene locus encoding the receptor for the hormonally active form of vitamin D_3 (VDR) and BMD. The human VDR gene consists of 6 non-coding 5′-untranslated exons, and 8 coding exons (exons 2–9). Furthermore, the VDR has also a large 3′-untranslated site (20,21). Several restriction sites have been used to characterize polymorphisms in the VDR gene. The polymorphic sites, BsmI and ApaI at intron 8, and the TaqI at exon 9, are linked to each other (5,21). A FokI site at the translation start site has also been reported as polymorphic and examined in relation to BMD in women (9–11) and in men (12–15). Although VDR gene polymorphisms and BMD have been investigated in several populations, the association of these polymorphisms with BMD is still controversial (5–19). In addition, VDR gene polymorphism has been reported to influence the effect of calcium intake on bone mineral density (22,23). Recent data also indicate that the VDR gene polymorphisms may be related to growth and parameters of body composition (24,25). Vitamin D has direct effects on the skeleton. The active metabolites of vitamin D regulate differentiation, proliferation, and migration of osteoblasts and chondrocytes of the epiphyseal growth plate (26), and deficiency of vitamin D causes growth failure.

The association between the VDR gene polymorphisms and bone turnover has not been widely studied in men (15,27). A novel immunoassay, which is specific for the serum tartrate-resistant acid phosphatase (TRAP) isoform 5b, has recently been developed (28). The activity of this isoform has been shown to be a specific and sensitive marker for monitoring antiresorptive treatment such as hormone replacement therapy in postmenopausal women (28), and thereby may be a promising bone resorption marker. Osteocalcin is a specific product of the osteoblasts during the matrix mineralization phase of their development. It is one of the most abundant non-collagenous proteins of bone, and its synthesis is induced by vitamin D (29).

Not only genetic factors (30,31), but also environmental factors such as physical activity or calcium intake (22,23) may influence BMD (32,33). Physical activity is markedly protective for hip fractures in men (34), but the influence of lifetime physical activity on BMD is controversial (35,36). The present trial (DNASCO) is the first study to investigate the long-term effects of regular low-to-moderate intensity exercise on BMD in a population-based group of middle-aged men. However, no exercise-induced effects on BMD were found during four years of intervention (37). Our aim was to further investigate gene-exercise interactions on BMD or its change by studying the association of the different VDR polymorphisms (TaqI, FokI, and ApaI) and their interactions. In addition, the relations of the VDR gene polymorphism with bone turnover markers and their changes (serum TRAP 5b activity and osteocalcin concentration) were evaluated.

Key messages

- The vitamin D receptor (VDR) TaqI polymorphism may be associated with body height and femoral neck bone mineral density (BMD) values.
- The present findings suggest that the VDR polymorphisms do not modify the effect of regular aerobic exercise on BMD in men.

Materials and methods

Study design

The present data are based on the DNASCO study, which consisted of a 2.5-year lead-in period followed by a randomized controlled clinical trial with regular physical exercise training of low to moderate intensity for six years (38). Here we present results during the first 4 years of exercise intervention, where BMD measures are available. The study cohort was a random sample of Eastern Finnish men aged 50 to 60 years. The subjects were randomized into a reference (n=70) or an intervention (n=70) group after the lead-in period. The men in the intervention group were prescribed aerobic exercise (e.g. walking, jogging, cross-country skiing, swimming, cycling) from 45 to 60 min per session five times a week. BMD, bone mineral content (BMC), the area of proximal femur and lumbar spine, body weight and height, and cardiorespiratory fitness were measured at randomization. BMD, BMC, and area measurements were repeated after 2 years and 3.5 to 4 years from the randomization. Changes in cardiorespiratory fitness (ventilatory aerobic threshold) both in the exercise and reference groups were monitored by annual exercise stress tests (38). An exercise physiologist defined ventilatory aerobic threshold visually as the first non-linear increase of ventilation during breath-by-breath respiratory gas analyses in bicycle ergometer test.

The Ethics Committee of the University of Kuopio approved the study protocol and the participants signed an informed consent. In accordance with the regulations of the Ethics Committee, the reference subjects were advised to make their personal choices whether or not to engage in physical exercise. 136 (97.1%) men came to the second and 132 (94.3%) to the third BMD measurement: four men died and four men dropped out for personal reasons. The study design and methods have been described elsewhere in detail (38,39).

Genotyping

Genomic DNA was extracted and purified from ethylenediaminetetraacetic acid (EDTA) blood samples using QIAmp Blood Kit (Qiagen GmbH, Hilden, Germany). Genomic DNA was amplified in 20 μl of a buffer solution: 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.08% Nonidet P-40, 200 μM each of the four deoxyribonucleotides, 1.5 U of Taq polymerase (Fermentas, Vilnius, Lithuania), and 1.0 μM of each oligonucleotide primer (TaqI/ApaI:5′-CAGAGCATGGACAGGGAGCAAG-3′ and 5′-GCAACTCCTCATGGGCTG-AGGTCTCA-3′; FokI: 5′-ATGGAAACACCTTGCTTCTTC-TCCCTC-3′ and 5′-GATGCC-AGCTGGCCCTGGCAG-TG-3′). Polymerase chain reaction (PCR) was performed with the following steps: 94°C for 5 min and then 94°C for 1 min, 62°C for 1 min, 72°C for 1 min, for 30 cycles, and 72°C for 7 min (UNO-Thermoblock, Biometra, Göttingen, Germany). After amplification, the PCR products were digested with TaqI, ApaI (Fermentas, Vilnius, Lithuania) and FokI (New England BioLabs, USA) restriction endonucleases and electrophorezed in a 2.0% agarose gel. Capital (T, A, F) and small (t, a, f) letters refer to the absence and presence of the restriction site, respectively.

BMD measurements

BMD of the lumbar spine (L2–L4) and proximal femur (femoral neck, total proximal femur) was measured using a fan beam dual X-ray absorptiometry (DXA) (EXPERT, Lunar Corp., Madison, WI, USA). The precision (root-mean-square (RMS) average of coefficients of variation (CV)%) (40) of the scanner was determined from lumbar spine and proximal femur DXA scans of ten subjects, and measured by trained laboratory technicians. From each subject, three scans were taken and the subjects were repositioned after each measurement. The CVs were 0.9% for the total proximal femur, 1.8% for the femoral neck, 2.1% for the greater trochanter, 2.6% for the Ward's triangle, and 1.9% for the lumbar spine (L2–L4). From the hip and spine measurements, three (2.1%) and two (1.4%), respectively, were excluded because of severe arthrotic findings in the DXA scans. In addition, eleven (7.9%) of the spine measurements were excluded due to uncertain edge detection, as assessed by an experienced orthopaedic surgeon. A total of 137 hip and 127 spine measurements were included in the analyses.

Due to plane image of DXA, BMD values are expressed as areal BMD (g/cm^2), not true volumetric BMD (g/cm^3). Areal BMD is influenced by the size of bone. We tried to eliminate the effect of bone size by also using the apparent volumetric bone mineral density (BMD_{vol}). In this model, lumbar spine (L2–L4) and femoral neck were assumed to be cylindrically shaped with a circular cross-section. The BMD_{vol} of the spine and the femoral neck were calculated as follows: L2–L4 BMD_{vol}=BMD * (4/(π * mean width of measured vertebral column area L2–L4)), and femoral neck BMD_{vol}=BMD * (4/π) *(height of measured projectional area in the femoral neck/measured projectional area of femoral neck) (41). The variables needed in these calculations were obtained directly from the printouts of the scan analyses. This could only be done for the lumbar vertebrae and femoral neck sites, since the calculation model assumes that the measured site is cylindrical (39).

Determination of bone turnover markers

Serum tartrate-resistant acid phosphatase form (TRAP) 5b activity was measured with a solid-phase immunofixed enzyme activity assay (Bone TRAP® Assay, SBA, Oulu, Finland) as described (28) using TRAP 5b from human osteoclasts as a calibrator (42). The intra-assay variation of the assay is $<6\%$, and inter-assay variation $<8\%$. Serum intact osteocalcin was measured with two-site immunoradiometric assay (IRMA) (Nichols Institute Diagnostics, California, USA). The intra-assay coefficients of variation (CV) were 3.2% and 5.2% at 18.0 μg/L and 84.3 μg/L, and the inter-assay CVs were 5.1% and 7.1% at 15.6 μg/L and 90.0 μg/L, respectively. Serum bone turnover markers were analyzed in 135 subjects.

Anthropometry

Subjects were weighed using a digital scale with light clothing but without shoes (accuracy 0.1 kg). Height was measured in an upright position without shoes

by a stadiometer (accuracy 0.1 cm). Body mass index (BMI, kg/m^2) was calculated by dividing body weight (kg) by the height squared (m^2).

Statistical analyses

Conformance of the different VDR allele frequencies to Hardy-Weinberg equilibrium proportions (43), and differences in genotypes between the study groups were tested by using a chi-square test. Independent samples *t* test, one-way analysis of variance (ANOVA), and one-way analysis of covariance (ANCOVA) were used to test the differences in continuous scale variables with respect to the VDR genotype groups at the randomization and after two and four years. Parameters, not normally distributed, were tested using non-parametric Mann-Whitney test and Kruskal-Wallis test. BMD changes between the VDR genotype groups were tested using ANOVA of repeated measures. The interactions of different VDR genotype groups in BMD and BMD changes were analyzed by ANOVA and ANOVA of repeated measures, respectively. A *P*-value of less than 0.05 was considered statistically significant. Statistical analyses were carried out using the SPSS for Windows versions 9.0 and 10.0 (SPSS Inc. Headquarters, 233 S. Wacker Drive, Chicago, Illinois 60606, USA).

The power calculations were carried out using the nQuery Advisor 5.0. In power calculations, we used FokI genotype as an example. We calculated how large the difference in 4-year BMD change between two extreme genotypes (FF and ff) should be in order to show the statistical difference with the 0.05 significance level and 80% power. The observed differences in 4-year BMD changes (FF *versus* ff) were 1.46% (expected difference 10.50%) in lumbar spine, 1.31% (expected difference 3.90%) in femoral neck, and 0.88% (expected difference 3.10%) in total proximal femur in the exercise group, and 3.77% (expected difference 7.50%) in lumbar spine, 1.32% (expected difference 4.90%) in femoral neck, and 0.09% (expected difference 3.80%) in total proximal femur in the reference group.

Results

Characteristics of the study population

The present analyses were done in the entire group at randomization, and in the exercise and reference groups separately during the intervention. The characteristics of the study subjects at randomization are shown in Table I. During the 4-year exercise intervention, cardiorespiratory fitness (ventilatory aerobic threshold) increased by 13% in the exercise group and decreased by 2% in the reference group ($P=0.001$) as reported previously (37).

The distribution and allele frequencies of the VDR TaqI, FokI, and ApaI genotypes are shown in Table II. The genotype distributions were in Hardy-Weinberg equilibrium. The division to the VDR genotype groups was equal in the exercise and reference groups in all analyzed genotypes. For the TaqI genotype, the Tt and tt genotype groups were combined since the number of subjects with tt genotype was small (Table II). The TaqI and ApaI polymorphisms were in linkage disequilibrium as previously reported (27,29): higher than expected number (100%) of the subjects with TaqI tt genotype had ApaI AA genotype, and higher than

Table I. Characteristics of the study subjects (mean ± SD) at the randomization in the exercise and reference groups.

Characteristics	Exercise group	Reference group	*P*-value[a]
Number of subjects	70	70	
Age (years)	57.6 ± 2.9	57.8 ± 2.8	0.681
Weight (kg)	80.8 ± 10.5	82.1 ± 12.4	0.529
Height (cm)	172.7 ± 6.0	173.7 ± 5.7	0.292
Ventilatory Aerobic threshold (ml/min)	1150 ± 286	1154 ± 295	0.936
Calcium intake (mg/d)	1084 ± 420	1053 ± 394	0.659
Vitamin D intake (μg/d)	5.82 ± 4.11	5.49 ± 4.06	0.523[b]
Serum TRAP 5b (U/l)	4.62 ± 1.39	4.81 ± 1.67	0.447[b]
Serum osteocalcin (μg/l)	26.4 ± 10.6	30.2 ± 22.2	0.653[b]
L2-L4 BMD (g/cm^2)	1.233 ± 0.178	1.192 ± 0.185	0.190
L2-L4 BMD (g/cm^3)$_{vol}$	0.315 ± 0.051	0.308 ± 0.046	0.386
Femoral neck BMD (g/cm^2)	1.009 ± 0.138	1.024 ± 0.137	0.539
Femoral neck BMD (g/cm^3)$_{vol}$	0.344 ± 0.065	0.346 ± 0.058	0.801
Total proximal femur BMD(g/cm^2)	1.091 ± 0.132	1.103 ± 0.145	0.595

[a]Independent *t*-test; [b]Mann-Whitney test.

Table II. The distribution and allele frequencies of VDR genotypes in the exercise, reference, and entire groups.

VDR genotypes/alleles	Exercise group	Reference group	Entire group
TaqI			
TT	32 (50.0%)	27 (42.9%)	59 (46.5%)
Tt	30 (46.9%)	28 (44.4%)	58 (45.7%)
tt	2 (3.1%)	8 (12.7%)	10 (7.9%)
T	0.73	0.65	0.69
t	0.27	0.35	0.31
c^2	2.60	0.03	0.68
FokI			
FF	34 (53.1%)	24 (38.1%)	58 (45.7%)
Ff	23 (35.9%)	28 (44.4%)	51 (40.2%)
ff	7 (10.9%)	11 (17.5%)	18 (14.2%)
F	0.71	0.60	0.66
f	0.29	0.40	0.34
c^2	1.01	0.32	1.49
ApaI			
AA	19 (29.7%)	21 (33.3%)	40 (31.5%)
Aa	34 (53.1%)	32 (50.8%)	66 (52.0%)
aa	11 (17.2%)	10 (15.9%)	21 (16.5%)
A	0.56	0.59	0.58
a	0.44	0.41	0.43
c^2	0.40	0.14	0.51

expected number (100%) of the subjects with ApaI aa genotype had TaqI TT genotype. We constructed haplotypes for further analyses according to the carrier-status of a At haplotype: 0 (n=59), 1 (n=58), or 2 (n=10) copies of this haplotype.

At randomization, there was a significant association between VDR TaqI polymorphism and body height. The carriers of the t allele had a greater body height than the TT subjects in entire group (P=0.001; Table III) and in reference group (P=0.001; Table IV). However, body height was not associated with FokI or ApaI genotypes. When association between haplotypes and body height was analyzed, the subjects with two At haplotypes were the tallest (176.2 ± 6.6 cm), and the subjects without the At haplotype were the shortest (171.1 ± 6.4 cm), the subjects with one At haplotype being in middle (174.4 ± 4.8 cm; P=0.002). There were no significant differences in age, weight, calcium intake or vitamin D intake between the subjects with the analyzed VDR genotypes or haplotypes (data not shown).

Bone mineral density and bone turnover

There was a significant association of the TaqI polymorphism with femoral neck BMD values. The carriers of the t allele had higher femoral neck BMD and BMD_{vol} values than the subjects with TT genotype at randomization in the entire group (P=0.003 and P=0.013, respectively; Table III). When height was used as a covariate, the significance of the association decreased in BMD values (ANCOVA; P=0.021) but increased in BMD_{vol} values (ANCOVA; P=0.010). When exercise and reference groups were analyzed separately, the same significant association was seen in the reference group but not in the exercise group in each measurement point (Table IV). No associations were found between the TaqI genotypes and lumbar BMD values (Table IV). No clinically significant BMD changes were found (Table IV).

The lumbar and femoral BMD values and their changes were similar in different ApaI genotype groups (Table V). When association between the haplotypes and BMD values was analyzed, the subjects with one At haplotype had the highest femoral neck BMD values (1.053 ± 0.146 g/cm^2),

Table III. Characteristics (mean ± SD) of the study subjects at randomization among the VDR TaqI genotypes.

Characteristics	TT	Tt and tt	*P*-value[a]
Number of subjects	59	68	
Age (year)	57.9 ± 2.9	57.6 ± 2.9	0.629[b]
Weight (kg)	79.7 ± 13.1	82.7 ± 10.2	0.152
Height (cm)	171.1 ± 6.4	174.7 ± 5.1	0.001
Ca intake (mg/d)	1091 ± 406	1101 ± 404	0.896
Vitamin D intake (μg/d)	5.81 ± 4.05	5.49 ± 3.95	0.745[b]
Serum TRAP 5b (U/l)	4.81 ± 1.47	4.65 ± 1.44	0.258[b]
Serum osteocalcin (μg/l)	27.4 ± 12.7	28.1 ± 12.6	0.817[b]
L2-L4 BMD (g/cm^2)	1.218 ± 0.205	1.205 ± 0.170	0.685
L2-L4 BMD (g/cm^3)$_{vol}$	0.314 ± 0.052	0.308 ± 0.046	0.499
Femoral neck BMD (g/cm^2)	0.978 ± 0.122	1.052 ± 0.142	0.003
Femoral neck BMD (g/cm^3)$_{vol}$	0.333 ± 0.048	0.360 ± 0.068	0.013
Total proximal femur BMD (g/cm^2)	1.076 ± 0.128	1.124 ± 0.144	0.054

[a]Independent samples *t*-test; [b]Mann-Whitney test.

Table IV. VDR TaqI genotypes, characteristics at randomization, and BMD values (mean ± SD) of lumbar spine and proximal femur in the exercise and reference groups.

	Exercise group			Reference group		
Characteristics	TT	Tt and tt	*P*-value[a]	TT	Tt and tt	*P*-value[a]
Number of subjects	32	32		27	36	
Age (year)	58.2 ± 3.0	56.9 ± 2.8	0.085	57.4 ± 2.8	58.2 ± 3.0	0.318
Weight (kg)	79.1 ± 13.1	81.9 ± 6.9	0.293	80.5 ± 13.3	83.5 ± 12.5	0.349
Height (cm)	171.4 ± 7.0	173.7 ± 4.9	0.122	170.8 ± 5.7	175.5 ± 5.2	0.001
Ca intake (mg/d)	1108 ± 460	1126 ± 385	0.860	1073 ± 341	1077 ± 426	0.967
Vitamin D intake (μg/d)	5.65 ± 3.54	5.85 ± 4.23	0.923[b]	6.00 ± 4.63	5.15 ± 3.71	0.733[b]
Serum TRAP 5b (U/l)	4.76 ± 1.45	4.40 ± 1.27	0.182[b]	4.87 ± 1.51	4.86 ± 1.57	0.755[b]
Serum osteocalcin (μg/l)	25.2 ± 10.4	27.4 ± 10.8	0.519[b]	29.9 ± 14.7	28.6 ± 14.1	0.632[b]
BMD values (g/cm^2)						
Lumbar spine (L2–L4)						
Randomization	1.260 ± 0.181	1.211 ± 0.186	0.305	1.173 ± 0.223	1.198 ± 0.156	0.606
4-year	1.291 ± 0.174	1.261 ± 0.201	0.528	1.186 ± 0.206	1.219 ± 0.161	0.496
4-year change (%)	2.93 ± 7.34	4.18 ± 6.69	0.534[c]	1.52 ± 6.75	2.63 ± 4.84	0.348[c]
Femoral neck						
Randomization	0.977 ± 0.124	1.040 ± 0.148	0.069	0.979 ± 0.123	1.062 ± 0.139	0.018
Adjusted			0.156[d]			0.078[d]
BMD_{vol} (g/cm^3)	0.333 ± 0.047	0.358 ± 0.076	0.115	0.333 ± 0.051	0.362 ± 0.062	0.056
4-year	0.966 ± 0.144	1.032 ± 0.158	0.090	0.966 ± 0.110	1.042 ± 0.144	0.028
Adjusted			0.156[d]			0.077[d]
BMD_{vol} (g/cm^3)	0.324 ± 0.054	0.353 ± 0.069	0.069	0.328 ± 0.044	0.353 ± 0.061	0.087
4-year change (%)	−1.19 ± 5.96	−0.91 ± 2.82	0.800[c]	−1.14 ± 4.67	−1.73 ± 4.00	0.237[c]
Total proximal femur						
Randomization	1.071 ± 0.132	1.115 ± 0.132	0.188	1.082 ± 0.126	1.131 ± 0.155	0.186
4-year	1.063 ± 0.143	1.099 ± 0.133	0.308	1.068 ± 0.120	1.123 ± 0.155	0.144
4-year change (%)	−0.78 ± 3.25	−1.44 ± 2.76	0.447[c]	−1.12 ± 3.52	−0.66 ± 3.08	0.431[c]

[a]Independent samples *t*-test; [b]Mann-Whitney test; [c]ANOVA of repeated measures; [d]ANCOVA; Height and weight were used as a covariate.

and the subjects without the At haplotype had the lowest (0.978 ± 0.122 g/cm^2), the subjects with two At haplotypes having the middle values (1.046 ± 0.129 g/cm^2; P=0.011). The same but a weak association was detected in femoral neck BMD_{vol} values (no At: 0.333 ± 0.048 g/cm^3, one At: 0.361 ± 0.070 g/cm^3, two At: 0.352 ± 0.056 g/cm^3; P=0.041).

At randomization, the subjects with the FokI ff genotype had higher lumbar BMD than the subjects with FF genotype in the reference group (Table VI). However, the association was not seen in the exercise (Table VI) or entire groups (data not shown). No difference was found between FokI genotypes and femoral BMD values or BMD changes (Table VI).

The ApaI polymorphism was related to serum osteocalcin concentration in the reference group (P=0.005; Table V). There were no associations between the analyzed TaqI or FokI polymorphisms or At haplotypes and any of the markers of bone turnover (serum TRAP 5b activity, osteocalcin concentration) in the entire or in exercise and reference groups (Tables III–IV and VI).

Discussion

In the present study, the carriers of the TaqI t allele were significantly associated with greater body height and higher femoral neck BMD values than other subjects. With regard to the FokI and ApaI polymorphism, no statistically significant differences were found.

There are few studies investigating the association of VDR gene polymorphisms with BMD in male populations (12–19,27), and only one study has evaluated interaction effects between VDR polymorphism and exercise training on bone metabolism in men (13). A number of previous studies have used the restriction enzyme BsmI. The absence of the BsmI restriction site (B allele) is very closely linked with the presence of the TaqI restriction site (t allele) (44). Therefore, TaqI genotypes analyzed in this study could be reclassified as BsmI genotypes taking the tt and TT genotypes as the BB and bb genotypes, respectively. The frequency of the tt genotype was lower compared to other Caucasian studies, but was similar with the frequency of the tt genotype in postmenopausal women in Finland (6).

Table V. VDR ApaI genotypes, characteristics at randomization, and BMD values (mean ± SD) of lumbar spine and proximal femur in the exercise and reference groups.

	Exercise group				Reference group			
Characteristics	AA	Aa	aa	*P*-value[a]	AA	Aa	aa	*P*-value[a]
Number of subjects	19	34	11		21	32	10	
Serum TRAP 5b (U/l)	4.61 ± 1.45	4.39 ± 1.18	5.13 ± 1.69	0.483[b]	4.56 ± 1.40	5.11 ± 1.77	4.74 ± 0.79	0.365[b]
Serum osteocalcin (μg/l)	30.1 ± 13.1	23.7 ± 7.7	27.9 ± 12.2	0.248[b]	22.4 ± 4.8	33.4 ± 17.8	29.8 ± 9.0	0.005[b]
Ca intake (mg/d)	1119 ± 390	1091 ± 413	1202 ± 525	0.769	1020 ± 334	1063 ± 419	1223 ± 388	0.394
Vitamin D intake (μg/d)	6.41 ± 4.80	5.40 ± 3.47	5.73 ± 3.43	0.825[b]	5.98 ± 3.31	4.82 ± 3.55	6.79 ± 6.65	0.424[b]
BMD values (g/cm²)								
Lumbar spine (L2–L4)[1]								
Randomization	1.258 ± 0.194	1.210 ± 0.174	1.268 ± 0.201	0.541	1.165 ± 0.156	1.205 ± 0.209	1.171 ± 0.184	0.747
4-year	1.296 ± 0.230	1.254 ± 0.158	1.306 ± 0.196	0.632	1.181 ± 0.172	1.217 ± 0.193	1.210 ± 0.182	0.795
4-year change (%)	2.80 ± 7.02	4.07 ± 6.83	3.46 ± 7.89	0.982[c]	1.23 ± 5.09	2.22 ± 6.34	3.52 ± 5.41	0.224[c]
Femoral neck[2]								
Randomization	0.993 ± 0.145	1.022 ± 0.142	0.996 ± 0.128	0.741	1.080 ± 0.165	1.003 ± 0.095	0.991 ± 0.167	0.091
4-year	0.988 ± 0.154	1.012 ± 0.162	0.978 ± 0.137	0.763	1.054 ± 0.163	0.991 ± 0.095	0.971 ± 0.159	0.165
4-year change (%)	−0.60 ± 2.98	−1.04 ± 5.54	−1.80 ± 3.72	0.841[c]	−2.44 ± 3.28	−0.70 ± 4.97	−1.80 ± 3.72	0.392[c]
Total proximal femur[2]								
Randomization	1.067 ± 0.125	1.109 ± 0.139	1.087 ± 0.131	0.545	1.153 ± 0.163	1.084 ± 0.125	1.103 ± 0.153	0.233
4-year	1.050 ± 0.131	1.103 ± 0.144	1.063 ± 0.130	0.386	1.140 ± 0.164	1.074 ± 0.119	1.091 ± 0.157	0.270
4-year change (%)	−1.52 ± 3.10	−0.56 ± 2.91	−2.17 ± 2.99	0.404[c]	−1.18 ± 2.04	−0.54 ± 4.12	−1.14 ± 2.41	0.750[c]

[a]One-way analysis of variance; [b]Kruskal-Wallis test; [c]ANOVA of repeated measures; [1]Exercise group: randomization $n=62$, 4-year $n=62$; Reference group: randomization $n=60$, 4-year $n=59$; [2]Exercise group: randomization $n=63$, 4-year $n=63$; Reference group: randomization $n=62$, 4-year $n=61$.

We found a strong relationship between body height and VDR TaqI polymorphism. In the entire group, the subjects with Tt (Bt) and tt (BB) genotypes were taller than the subjects with TT (bb) genotype. When the At haplotypes were analyzed, the same significant association was

Table VI. VDR FokI genotypes, characteristics at randomization, and BMD values (mean ± SD) of lumbar spine and proximal femur in the exercise and reference groups.

	Exercise group				Reference group			
Characteristics	FF	Ff	ff	P-value[a]	FF	Ff	ff	P-value[a]
Number of subjects	34	23	7		24	28	11	
Serum TRAP 5b (U/l)	4.68 ± 1.23	4.51 ± 1.68	4.32 ± 0.87	0.413[b]	4.97 ± 1.41	4.70 ± 1.03	5.05 ± 2.64	0.330[b]
Serum osteocalcin (μg/l)	26.3 ± 9.4	26.7 ± 13.4	25.0 ± 6.0	0.736[b]	29.9 ± 14.1	27.5 ± 11.8	31.9 ± 20.1	0.921[b]
Ca intake (mg/d)	1097 ± 425	1180 ± 440	988 ± 327	0.567	980 ± 455	1135 ± 340	1129 ± 334	0.331
Vitamin D intake (μg/d)	5.64 ± 3.37	6.43 ± 4.77	3.86 ± 2.17	0.343[b]	5.93 ± 4.27	5.42 ± 4.11	4.92 ± 4.15	0.657[b]
BMD values (g/cm²)								
Lumbar spine (L2–L4)[1]								
Randomization	1.256 ± 0.191	1.202 ± 0.153	1.239 ± 0.244	0.571	1.115 ± 0.133	1.200 ± 0.199	1.307 ± 0.203	0.021
4-year	1.300 ± 0.194	1.246 ± 0.181	1.254 ± 0.188	0.562	1.152 ± 0.155	1.213 ± 0.199	1.294 ± 0.166	0.116
4-year change (%)	3.78 ± 6.64	3.66 ± 6.39	2.32 ± 10.76	0.269[c]	3.29 ± 5.28	2.13 ± 4.91	−0.48 ± 8.29	0.257[c]
Femoral neck[2]								
Randomization	0.994 ± 0.136	1.018 ± 0.145	1.051 ± 0.147	0.584	1.037 ± 0.129	1.022 ± 0.160	1.021 ± 0.093	0.917
4-year	0.981 ± 0.138	1.011 ± 0.181	1.049 ± 0.135	0.522	1.013 ± 0.129	1.005 ± 0.153	1.012 ± 0.106	0.974
4-year change (%)	−1.29 ± 3.61	−1.03 ± 6.17	0.02 ± 2.80	0.928[c]	−2.16 ± 4.45	−1.11 ± 3.96	−0.84 ± 4.86	0.303[c]
Total proximal femur[2]								
Randomization	1.080 ± 0.120	1.103 ± 0.148	1.122 ± 0.149	0.689	1.105 ± 0.146	1.111 ± 0.163	1.122 ± 0.084	0.952
4-year	1.068 ± 0.115	1.087 ± 0.162	1.122 ± 0.163	0.628	1.097 ± 0.131	1.096 ± 0.174	1.114 ± 0.071	0.942
4-year change (%)	−1.04 ± 2.72	−1.51 ± 3.54	−0.16 ± 2.43	0.475[c]	−0.49 ± 3.40	−1.28 ± 3.05	−0.58 ± 3.60	0.847[c]

[a]One-way analysis of variance; [b]Kruskal-Wallis test; [c]ANOVA of repeated measures; [1]Exercise group: randomization n=62, 4-year n=62; Reference group: randomization n=60, 4-year n=59; [2]Exercise group: randomization n=63, 4-year n=63; Reference group: randomization n=62, 4-year n=61.

detected. However, the opposite association has been found in Caucasian boys at birth, during and after puberty (24). Since we studied middle-aged men, the results are not comparable. Considering the relationship between body size and bone size, as well as the influence of calcium intake on both body height and bone area (45), it is possible that VDR alleles together with dietary calcium have an indirect and complex influence on peak bone mass through the regulation of skeletal growth.

The TaqI t allele associated with high femoral neck BMD at randomization also after adjustment for height. We also eliminated the effect of bone size by using the apparent volumetric bone mineral density (BMD_{vol}) (41), and BMD_{vol} associated with the TaqI t allele similarly as femoral neck BMD. In the reference group, the association of TaqI polymorphism with femoral neck BMD was related to difference in body height since the association decreased after adjusting for height. Our results agree with the Rotterdam study (16) and previous findings in women (7,8) and men (17). The bAT haplotype associated with low femoral BMD in 880 men (mean age 67.5 years) and 902 women, while no differences were seen in mean femoral BMD values among BsmI and TaqI genotypes (16). A trend of an increased femoral neck BMD was reported in the BB men (17). However, most of the studies observed the opposite association between TaqI or BsmI genotypes and BMD in men (14,15,17–19,27) as well as in women (5,6,17). With regard to the FokI and ApaI polymorphisms, controversial results have also been reported (12–14,16,25,27).

There are several possible reasons for the controversy in reported associations of VDR polymorphisms with BMD. There may be allelic heterogeneity at the VDR locus among different populations or linkage disequilibrium with another bone metabolism-related gene. Several environmental factors (racial differences, age, hormonal status, body composition, nutrition, and important lifestyle variables such as smoking, caffeine, alcohol use, and the level of physical activity) may influence. In addition, the BsmI, TaqI, and ApaI polymorphisms do not lead to any differences in coding regions. However, the start codon polymorphism, FokI, which encodes three amino acids shorter VDR protein, has been shown to generate functional differences (46–48). It has also been proposed that transcriptional activity of gene, resulting in varying VDR mRNA levels, is influenced by allelic variations. The BsmI B haplotype leads to 40% greater gene transcription or mRNA stability than the b haplotype (5).

To our knowledge, no previous data about associations between VDR gene polymorphisms and serum TRAP 5b activity, which is a bone resorption marker, have been published. In the reference group, the ApaI polymorphism was related to serum osteocalcin concentration only in the reference group, which suggests a chance finding. Van Pottelbergh and co-workers (27) found that the highest value for osteocalcin was observed with At-At genotype in elderly men, but not in younger men. No difference between FokI genotypes and serum osteocalcin concentrations was detected (27). The functional difference between FokI alleles and osteocalcin was found in young men (13). In agreement with our study, Fassbender and co-workers found no difference in serum osteocalcin levels between TaqI or FokI genotypes in men and women (49).

In the present study, we did not find any strong bone responsiveness to physical activity in different VDR genotype groups. In young men, a functional difference was found between FokI alleles and bone turnover after one month of weight training (13). Two studies in women have investigated the response of the VDR BsmI gene-exercise interaction on BMD with controversial results (50,51). However, the number of subjects was small in both studies.

This intervention study was successful, since the exercise group actually changed their life-style, which was seen as an increase in ventilatory aerobic threshold compared with the reference group. In addition, the drop-out rate was very low (6%) indicating an excellent adherence and compliance (37). However, there were a few limitations of this study. We could not analyze the nutritional vitamin D status (circulating 25(OH)D levels in serum) due to the blood sample deficiency. The statistical power of 4-year BMD change in genotype groups was limited. The training program at low to moderate intensity was planned to be applicable to the general population of middle-aged people. Therefore, we cannot exclude the possible modifying effects of the VDR polymorphisms on exercise response to BMD at higher intensity exercise or in women.

In conclusion, we observed associations between body height, femoral BMD, and VDR gene TaqI genotypes and At haplotypes. We suggest that the TaqI polymorphism may affect body height and femoral neck BMD values. The significance of these findings and their applicability to a larger population needs further studies. The present findings also suggest that the VDR polymorphisms do not modify the effect of regular aerobic exercise on BMD in men.

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