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Vitamin D receptor gene polymorphisms modulate the skeletal response to vitamin D supplementation in healthy girls

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ABSTRACT

Objectives: Vitamin D receptor (VDR) gene plays an important role in bone mass regulation. We have previously shown a beneficial effect of vitamin D supplementation on bone mass in girls. This study investigated whether the musculo-skeletal response to Vitamin D was modulated by polymorphisms in VDR gene.
Design: Randomized placebo-controlled trial.
Methods: 179 girls (10–17 years), were randomly assigned to placebo or Vitamin D3 for one year. VDR genotypes were determined in 167 girls using BsmI, TaqI and ApaI restriction enzymes. Bone mass at the spine, hip, forearm and total body, and lean mass were measured by DXA at baseline and at one year.
Results: After one year, VDR gene polymorphisms using Bsm1 and TaqI restriction enzymes were associated with percent changes in bone area, BMC and BMD at multiple skeletal sites in the Vitamin D3 group but not in the placebo group. The least increments were observed in the BB and tt genotypes. No similar effect was observed with ApaI enzyme. This relationship between VDR genotypes and changes in BMD and BMC remained significant after adjustment for puberty, changes in lean mass, height and bone area.
Conclusion: VDR gene polymorphisms influence the skeletal response to vitamin D supplementation in healthy adolescent girls.

Introduction

Osteoporosis, a major public health problem worldwide, is a multifactorial disease. Studies showed an association between serum 25 (OH) vitamin D [25-OHD] status and bone mineral density (BMD) and osteoporotic fractures [1–3]. Insufficient levels of 25-OHD are associated with increased risk of osteoporosis and osteoporotic fractures [4–7] and vitamin D supplementation decreases the incidence of osteoporotic fractures, mainly hip fractures [8–10]. Adolescence is a critical time for the acquisition of bone mass, with around 40% of skeletal mass being acquired during pubertal maturation [11]. Vitamin D plays an important role in bone gain during adolescence. Pubertal girls with hypovitaminosis D may be at risk of failure to achieve maximum peak bone mass [12] and our group showed a beneficial effect of vitamin D supplementation on bone mass in girls [13].

Genetic influences account for 50–80% of the inter-individual variability in BMD [14,15]. Candidate genes tested for association with bone mass and/or fractures include sequence variants in the collagen type I alpha 1 gene, estrogen receptor alpha gene, LDL receptor related 5 (LRP5) gene, calcitonin receptor gene, calcium sensing receptor gene, and the nuclear vitamin D receptor (VDR) gene [16,17]. Although VDR gene seems to play an important role in the genetic regulation of bone mass and the pathogenesis of fracture; the findings were somewhat variable depending on age, ethnic group, and restriction enzyme used. Some of the variations between studies may also be explained by gene–gene interactions or gene–environment interactions. Indeed, the beneficial effect of calcium intake on BMD was in part mediated by VDR genotype in a randomized calcium trial conducted in children [18]. We demonstrated a beneficial effect of vitamin D supplementation on bone mass in girls, an effect that was mediated by changes in height, bone area and lean mass [13]. Whether this response to vitamin D in adolescents is modulated by polymorphisms in VDR gene has not been previously assessed.

The objective of this study was to investigate the impact of VDR gene polymorphisms on the musculo-skeletal response to Vitamin D replacement in a randomized double-blind placebo-controlled trial in adolescent girls.

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Methods

Subjects

One hundred and seventy nine girls aged 10–17 years recruited four schools located in the greater Beirut area participated in the study [13]. Exclusion criteria included diseases and use of medications known to affect bone metabolism [13]. The study was approved by the institutional review board, and informed consent was obtained from all study subjects and their parents.

Treatment

The study participants were randomly assigned in a double-blind manner to receive weekly placebo oil or a vitamin D3 preparation (Vigantol oil, Merck KGaA, Darmstadt, Germany). Vitamin D3 preparation were given as 1400 IU weekly, i.e. the equivalent of 200 IU/d (low dose group), or 14,000 IU weekly, i.e. the equivalent of 2000 IU/day (high dose group) for one year [13]. The vitamin D concentration in the three solutions was within 10% of that anticipated based on the label on the bottles and the dilution protocol [13].

Assessments

At study entry, girls underwent careful physical examination including height, weight and pubertal assessment. Calcium intake (mg per day), sun exposure (minutes per week) and exercise frequency (determined as the number of hours spent on sports per week) were assessed with questionnaires [13].

Bone mineral content (BMC, grams) and bone mineral density (BMD, g/cm^2) at the anteroposterior lumbar spine (L1–L4), the left femur (total hip and femoral neck), the forearm (33% radius) and the whole body, as well as lean mass (grams), were measured at baseline and at one year by a dual-energy X-ray absorptiometry (DXA) using a Hologic 4500A device (Hologic, Bedford, MA, USA), in fast array scanning mode. At our center, the mean (SD) CV% of 280 duplicate BMC measurements during the study period were 1.2 ± 1.1 for the spine, 1.5 ± 1.8 for the hip 1.5 ± 0.01 for the femoral neck, 1.1 ± 1.1 for the forearm, 0.89 ± 0.9 for the total body BMC and 0.6 ± 0.7 for lean mass.

At baseline and at one year, blood was drawn for serum calcium and phosphorus which were measured by standard calorimetric methods, using the Hitachi 912 analyzer (Mannheim, Germany), serum 25-OHD and 1,25 $(OH)_2$ D levels were measured by RIA using the IDS (Immunodiagnostic System Limited, UK), and serum PTH measured by ELISA-PTH immunoradiometric assay (CisBio International, Gif-Sur-Yvette, Cedex, France). The intra-assay and inter-assay variability for 25-OHD, 1,25 $(OH)_2$ D and PTH are below 10%.

DNA was extracted from whole blood and polymorphisms in the VDR gene were assessed by agarose gel electrophoresis following digestion with three restriction enzymes Bsm I, Taq I and Apa I after polymerase chain reaction amplification of specific region of the VDR gene [16]. VDR genotyping using the FokI restriction enzyme because that technique was not available at our institution when the study was conducted. Alleles were designated according to the presence or absence of the specific restriction enzyme cleavage site, with capital letters denoting the absence and small letters denoting the presence of the restriction site. DNA was available for VDR genotypes in 167 girls only, the subset on whom the analyses were conducted.

Statistical analyses

We first assessed the relationship between musculo-skeletal parameters (i.e BMC, BMD, bone area) and VDR genotype at study entry before and after adjustment for determinants of bone mass in children, i.e age, height, pubertal status, lean mass, exercise, calcium intake, 25-OHD and PTH. We then calculated the percent change in these musculo-skeletal parameters after one year of treatment and we assessed the relationship between these changes and VDR genotype, before and after adjustment for mediators of the musculo-skeletal response to vitamin D. These include age, menarcheal status, changes in lean mass, height or bone area (as surrogate of changes in bone size, 13).

In the bivariate analyses, the relationships between VDR genotypes and continuous variables were assessed using one-way analysis of variances (ANOVA).

In the overall group, the association between VDR genotype and BMC or BMD was adjusted for vitamin D level at baseline and for treatment group (placebo versus low dose versus high dose vitamin D) using multivariate linear regression model.

Because there was no significant difference in the changes in the musculo-skeletal parameters between the low and high dose vitamin D, and there was no interaction between treatment and genotypes at any skeletal site, data from the two groups were then combined in the analyses [13]. In the vitamin D treated group, adjusted analyses through linear regression with two models with BMC and BMD of a specific skeletal site were performed. In the first model, the outcome was the percent change in BMD or BMC at one year and the predictors were VDR genotype (using one restriction enzyme at a time), age, menarcheal status, percent changes in lean mass and percent changes in height. In the second model, percent changes in height was replaced with percent changes in bone area of the specific bone site. Dummy variables were created for VDR genotypes and for treatment groups before entering them in both models.

Analyses were done using STATA version 10. P-values <0.05 were considered significant.

Results

Clinical and musculo-skeletal characteristics

In the overall group, the mean age was 13.0 ± 2.1 years, BMI 20.6 ± 3.9, the calcium intake was 670.6 ± 359 mg/day. The mean 25-OHD level was 14.1 ± 8.1 ng/ml and the mean 1,25 $(OH)_2$ D was 81.3 ± 29.1 pg/ml. The mean serum calcium and PTH levels were within the normal ranges. There was no difference in any of the above parameters between the treatment groups nor in any of the musculo-skeletal baseline characteristics, i.e BMC, BMD, bone area and lean mass (data not shown) [13]. There was no significant difference in pubertal stage distribution between genotypes (Table 1) or between treatment groups (data not shown). The frequency distributions of VDR polymorphisms in the study group were as follows: For BsmI: 29% bb, 53% Bb and 18% BB; for ApaI: 13% aa, 49% Aa and 38% AA; for TaqI: 15% tt, 53% Tt and 32% TT. The alleles were in the Hardy–Weinberg equilibrium.

Relationship between VDR genotypes and anthropometrical and musculo-skeletal parameters at study entry

There was no difference in mean age, height, weight, sun exposure, exercise, calcium intake between the three VDR genotype groups. There was also no difference in mean serum calcium, phosphorus 25-OHD levels or 1,25 $(OH)_2$ D levels between genotypes. This was consistent when using BsmI, ApaI or TaqI (Table 1).

There was also no difference between VDR genotypes in the mean lean mass, BMC or BMD of any skeletal site. This finding was consistent whether BsmI, ApaI and TaqI restriction enzymes were used. The lack of relationship between VDR genotype and bone mass persisted after adjusting for classical determinants of bone mass in children, i.e age, height, pubertal status, lean mass, 25-OHD and PTH [data not shown].

Table 1
Baseline characteristics of the study group according to VDR genotype.

	BsmI			TaqI		
	BB $N=48$	Bb $N=88$	BB $N=31$	tt $N=24$	Tt $N=89$	TT $N=54$
Age (years)	12.8 ± 2.1	13.2 ± 2.1	13.8 ± 2.1	13.9 ± 2.2	13.2 ± 2.1	12.9 ± 2.0
Height (cm)	151.8 ± 9.1	152.4 ± 10.5	154.1 ± 9.5	153.9 ± 9.7	152.5 ± 10.5	152.2 ± 9.0
Weight (kg)	46.3 ± 11.2	47.3 ± 11.1	49.8 ± 13.1	47.8 ± 11.2	47.5 ± 11.6	47.3 ± 11.8
Calcium intake (mg/day)	709 ± 338	692 ± 411	602 ± 251	558 ± 255	685 ± 374	722 ± 386
Sun exposure (min/week)	426 ± 335	424 ± 308	522 ± 372	500 ± 383	464 ± 329	382 ± 296
Exercise (hrs/week))	3.4 ± 4.8	3.9 ± 4.8	4.2 ± 5.0	3.8 ± 3.7[a]	4.1 ± 5.2[a]	3.3 ± 4.6[a]
Serum calcium (mg/dl)	9.8 ± 0.3	9.9 ± 0.3	9.9 ± 0.4	9.9 ± 0.3	9.9 ± 0.3	9.8 ± 0.3
Serum 25 (OH) D (ng/ml)	14.3 ± 9.4	14.0 ± 7.3	14.5 ± 8.6	14.0 ± 8.5	15.0 ± 8.6	12.8 ± 6.9
Serum 1,25(OH)2D (pg/ml)	79.4 ± 28.9	83.5 ± 29.5	78.1 ± 30.0	76.2 ± 26.8	84.4 ± 29.4	81.1 ± 32.5
PTH (pg/ml)	23.0 ± 34.6	17.8 ± 22.1	12.1 ± 13.2	12.1 ± 13.2	17.8 ± 21.9	21.8 ± 33.4
LS BMC (grams)	42.1 ± 12.6	37.3 ± 10.4	39.2 ± 12.3	37.5 ± 10.4	42.3 ± 12.7	39.4 ± 12.4
Lumbar Spine BMD (g/cm^2)	0.820 ± 0.17	0.767 ± 0.14	0.789 ± 0.15	0.770 ± 0.14	0.812 ± 0.17	0.793 ± 0.15
Total hip BMC (grams)	23.8 ± 6.0	23.1 ± 5.1	22.9 ± 5.8	23.4 ± 5.2	23.5 ± 5.8	22.9 ± 5.8
Total hip BMD (g/cm^2)	0.790 ± 0.13	0.784 ± 0.11	0.782 ± 0.13	0.790 ± 0.11	0.781 ± 0.13	0.782 ± 0.12
Femoral neck BMC (grams)	3.3 ± 0.8	3.2 ± 0.6	3.3 ± 0.6	3.2 ± 0.6	3.2 ± 0.7	3.3 ± 0.6
Femoral neck BMD (g/cm^2)	0.739 ± 0.12	0.717 ± 0.10	0.718 ± 0.11	0.725 ± 0.10	0.726 ± 0.13	0.719 ± 0.11
Trochanter BMC (grams)	6.1 ± 1.8	5.9 ± 1.3	6.0 ± 1.7	5.9 ± 1.3	5.9 ± 1.7	6.0 ± 1.7
Trochanter BMD (g/cm^2)	0.615 ± 0.10	0.617 ± 0.08	0.613 ± 0.10	0.621 ± 0.09	0.609 ± 0.10	0.612 ± 0.10
Radius BMC (grams)	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	1.3 ± 0.2
Radius BMD (g/cm^2)	0.584 ± 0.06	0.617 ± 0.08	0.574 ± 0.07	0.570 ± 0.06	0.582 ± 0.07	0.575 ± 0.07
Subtotal body BMC (grams)	1199 ± 333	1102 ± 315	1146 ± 333	1117 ± 320	1171 ± 323	1152 ± 336
Tanner I to II	42%	34%	20%	21%	30%	46%
Tanner III to V	58%	66%	80%	79%	70%	53%
Placebo group	31.3%	33.0%	32.3%	20.8%	37.1%	29.6%
Vitamin D treated group	68.8%	67.0%	67.7%	79.2%	62.9%	70.4%

Values are mean ± SD.
[a] Significant difference between genotypes ($p<0.05$).

Relationship between VDR and musculo-skeletal response at one year

In the overall group

There was no significant difference in the changes in vitamin D levels between genotypes (Table 2).

As shown in Table 2, VDR gene polymorphisms using BsmI restriction enzyme were associated with increments in BMC at one year at the lumbar spine ($p=0.04$), the femoral neck ($p=0.007$) and the total body (0.01) and with increments in BMD at the forearm ($p=0.01$) and the total body ($p=0.05$). They were also associated with increments in bone area at the femoral neck ($p=0.03$) and total body ($p=0.01$). The least increments were observed in the BB (−/−) genotype (Table 2).

Similarly, using TaqI restriction enzyme, VDR gene polymorphisms were associated with changes in the lumbar spine BMC ($p=0.06$), the femoral neck BMC ($p=0.01$), the forearm BMD ($p=0.04$) and the femoral neck area ($p=0.02$). The least increments were obtained in the tt (+/+) genotype (Table 2).

No such effect was observed when VDR was determined using ApaI restriction enzyme.

There percent change in BMC was significantly higher in the vitamin D treated group compared to placebo at the total body, total hip and trochanter in Bb genotype ($p<0.05$), with similar trend in bb genotype. No similar effect was observed in BB genotype (Fig. 1). Similar results were obtained at the total hip and the trochanter when VDR genotype was determined using TaqI enzyme (data not shown).

Table 2
Percent changes in musculo-skeletal parameters and 25 (OH) vitamin D in the overall study group (vitamin D and placebo), according to VDR genotype using BsmI or TaqI restriction enzyme.

	BsmI			TaqI		
	BB ($n=31$)	Bb ($n=88$)	bb ($n=48$)	TT ($n=54$)	Tt ($n=89$)	tt ($n=24$)
Spine BMC[@]	8.7 ± 8.1[a,b]	13.1 ± 10.9[b]	14.4 ± 10.5[a]	14.5 ± 10.3[a]	12.7 ± 11.1	8.4 ± 7.7[a]
Total hip BMC	8.5 ± 10.0	10.9 ± 9.2	11.3 ± 9.3	11.3 ± 8.6	10.5 ± 9.8	9.2 ± 9.8
Femoral neck BMC[@]	0.9 ± 6.6[a,b]	4.5 ± 7.2[b]	6.5 ± 8.1[a]	6.5 ± 7.7[a]	4.1 ± 7.3	1.0 ± 7.2[a]
Forearm BMC[@]	4.7 ± 5.6	6.6 ± 7.6	7.6 ± 5.9	7.5 ± 5.9	6.4 ± 7.5	5.0 ± 5.7
Total body BMC	5.7 ± 7.8[a,b]	8.6 ± 8.2[b]	10.9 ± 8.4[a]	10.3 ± 8.4	8.4 ± 8.4	5.5 ± 7.9
Spine BMD	5.7 ± 5.7	7.9 ± 7.0	9.0 ± 6.7	9.2 ± 7.0	7.5 ± 6.7	5.9 ± 6.2
Total hip BMD	4.6 ± 4.0	6.2 ± 4.9	6.7 ± 4.7	6.7 ± 4.4	5.9 ± 5.1	5.2 ± 3.9
Femoral neck BMD	2.3 ± 3.7	4.0 ± 4.8	4.7 ± 5.2	4.7 ± 4.9	3.7 ± 4.8	2.8 ± 4.1
Forearm BMD	2.6 ± 2.3[a]	4.5 ± 3.2[a]	3.6 ± 3.3	3.7 ± 3.3	4.4 ± 3.1[a]	2.6 ± 2.7[a]
Total body BMD	1.4 ± 3.3[a,b]	2.9 ± 3.4[b]	3.3 ± 3.6[a]	3.3 ± 3.7	2.9 ± 3.4	1.3 ± 3.3
Spine area	2.7 ± 4.1	4.6 ± 5.4	4.8 ± 6.1	4.8 ± 5.8	4.6 ± 5.6	2.3 ± 3.5
Total hip area	3.5 ± 6.4	4.2 ± 4.6	4.1 ± 5.2	4.1 ± 4.8	4.2 ± 5.0	3.5 ± 6.3
Femoral neck area	−1.3 ± 5.7[a]	0.4 ± 4.3	1.7 ± 5.4[a]	1.7 ± 5.2[a]	0.3 ± 4.4	−1.7 ± 6.0[a]
Forearm area	2.1 ± 4.1	2.3 ± 4.3	3.8 ± 4.9	3.6 ± 5.1	2.2 ± 4.2	2.5 ± 4.0
Total body area	4.0 ± 5.3[a]	5.4 ± 5.4[b]	7.5 ± 4.9[a, b]	6.9 ± 5.0	5.3 ± 5.5	4.7 ± 5.4
Height	1.8 ± 2.0	2.3 ± 2.1	3.0 ± 2.4	2.9 ± 2.4	2.3 ± 2.1	2.0 ± 2.1
25 (OH) vitamin D	93.3 ± 155	89.3 ± 150	77.5 ± 107	67.1 ± 90	105 ± 179	123 ± 299

[a,b]Variables with same superscript are significantly different from each other; No difference in percent changes in height, BMD, BMC or bone area at any skeletal site with ApaI enzyme (data not shown).
[@]Implies a significant interaction between treatment and VDR genotype.

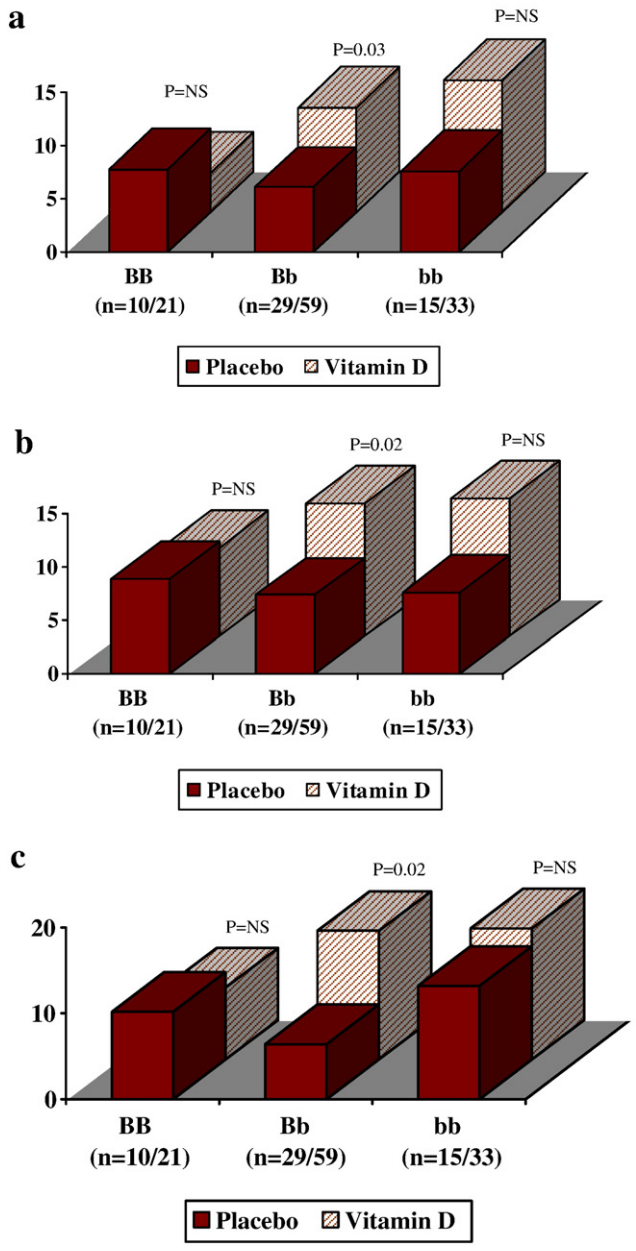

Fig. 1. Percent changes in total body BMC (a), total hip BMC (b) and trochanter BMC (c), according to VDR genotype using BsmI enzyme and treatment group. A significant difference in percent changes in BMC between placebo and vitamin D treated group was observed in Bb ($p<0.05$), with similar trend in bb but not in BB genotype.

Significant interaction between treatment (placebo versus vitamin D treated group) and VDR was obtained at the spine, femoral neck and forearm, ($p<0.05$). Therefore, the analyses were conducted separately in the vitamin D treated group and in the placebo group.

In the vitamin D3 treated group

As shown in Table 3, VDR gene polymorphisms using BsmI restriction enzyme were associated with changes in height ($p=0.02$) and with changes in BMC at the lumbar spine ($p=0.02$), the femoral neck ($p=0.04$) and the total body ($p=0.001$). They were also associated with changes in BMD at the total body ($p=0.02$) and the forearm ($p=0.009$), and with changes in bone area at the total body ($p=0.01$). The least increments were observed in the BB genotype (Table 3). Similarly, when VDR were determined using TaqI restriction enzyme, there was an effect of VDR on the skeletal response at some skeletal sites, but not effect on lean mass or height was observed. This effect was noted at the lumbar spine BMC ($p=0.02$), the total body BMC ($p=0.02$), the forearm BMD ($p=0.04$) and the total body BMD ($p=0.03$). The least increments were observed with the tt genotype (Table 3).

Similar results were obtained when the analyses were implemented in the low dose and the high dose vitamin D treated groups separately (Table 4).

There was no significant difference in serum 25-OHD, 1,25 $(OH)_2$ D at one year between genotypes when using BsmI, TaqI or ApaI restriction enzyme. There was also no significant difference in the changes in vitamin D levels between genotypes (Table 3). No significant relationship was observed between percent changes in BMC or BMD at any skeletal site and 25 (OH) vitamin D level at one year or with percent changes in 25 (OH) vitamin D level in the overall group or within genotypes. This was consistent when VDR genotype was determined using BsmI and TaqI restriction enzymes. The relationships between VDR genotypes and BMC or BMD persisted after adjustment for serum 25-OHD level at one year and after adjustment for percent changes in 25-OHD.

No such relationships were observed when VDR genotypes were determined using ApaI restriction enzyme [data not shown].

Volumetric bone density was calculated at the spine and at the femoral neck using the following formulas: $BMAD = BMC / A^{3/2}$ and femoral neck $BMAD = BMC / A^2$, where BMC is the bone mineral content and A is the projected area, and the percent changes in volumetric BMAD were calculated. There was no significant difference in the percent changes in volumetric BMAD between genotypes, whether using BsmI, TaqI or ApaI enzyme [data not shown].

No relationship was found between VDR and changes in lean mass (Table 3).

In the placebo group

There was no effect of VDR genotype, whether using BsmI, TaqI, or ApaI restriction enzymes, on the musculo-skeletal response in the placebo group (data not shown).

Multivariate analyses

In the overall group, the association between VDR genotypes and BMC and BMD persisted after adjustment for baseline vitamin D level, and for treatment group (placebo versus low dose versus high dose vitamin D) [data not shown].

In the vitamin D treated group, the association between VDR genotypes and both BMC and BMD persisted also after adjustment for age, menarcheal status, percent changes in lean mass and percent changes in height (Table 5). Subjects with the bb genotype had the largest increments in BMC at the lumbar spine, femoral neck, and the largest increments in forearm and total body BMD. Similarly, subjects with the TT genotype had the largest increments in BMC at the spine and femoral neck, and the as largest increments in total body BMD. Similar results were obtained when changes in height were replaced with changes in bone area in the model (Table 5).

Discussion

VDR genotype affected the skeletal response to vitamin D at one year in healthy adolescent girls with low 25-OHD levels at study entry. This effect persisted after adjustment for age, pubertal status and for changes in lean mass, height and bone area. The lowest response was observed in the BB and tt genotypes (as expected since results are mirror images of each other), but no similar effect was observed when VDR genotype was determined using Apa1 restriction enzyme.

The frequency distribution of the various VDR genotypes in this study group is similar to previously published figures [19–22]. There was no relationship between bone mass and VDR gene polymorphisms at baseline either before or after adjustment for parameters known to

Table 3
Percent changes in musculo-skeletal parameters and 25 (OH) vitamin D levels in the vitamin D treated group according to VDR genotype using BsmI or TaqI restriction enzyme.

	BsmI			TaqI		
	BB ($n=21$)	Bb ($n=59$)	bb ($n=33$)	TT ($n=38$)	Tt ($n=56$)	tt ($n=19$)
Spine BMC	8.0 ± 7.4[b]	14.1 ± 11.9[b]	16.4 ± 10.7	16.5 ± 10.4[b]	13.7 ± 12.2[a]	7.8 ± 6.9[a, b]
Total hip BMC	8.3 ± 8.9	12.6 ± 10.0	13.0 ± 9.9	12.9 ± 8.8	11.9 ± 10.4	9.8 ± 10.3
Femoral neck BMC	1.4 ± 5.7[b]	4.7 ± 7.7	6.9 ± 8.5[b]	6.7 ± 8.1	4.2 ± 7.9	2.4 ± 6.3
Forearm BMC	4.1 ± 5.0	7.1 ± 8.6	8.6 ± 6.1	8.4 ± 6.2	6.8 ± 8.6	4.6 ± 5.4
Total body BMC	3.7 ± 7.1[a,b]	9.8 ± 8.6[b]	12.4 ± 8.7[a]	11.5 ± 8.7[b]	9.6 ± 8.8[a]	4.7 ± 7.4[a, b]
Spine BMD	7.3 ± 5.7	9.0 ± 7.8	9.7 ± 7.0	10.1 ± 7.2	8.5 ± 7.6	5.7 ± 6.0
Total hip BMD	2.1 ± 2.3	4.7 ± 3.4	3.8 ± 3.6	3.9 ± 3.6	4.6 ± 3.3[b]	2.3 ± 2.7[b]
Femoral neck BMD	4.6 ± 4.1	6.8 ± 5.2	7.3 ± 4.9	7.3 ± 4.5	6.4 ± 5.5	5.1 ± 4.1
Forearm BMD	2.5 ± 4.1[a]	4.4 ± 4.9	5.3 ± 5.3[a]	5.2 ± 4.9[a]	4.0 ± 5.2	3.3 ± 4.3[a]
Total body BMD	1.0 ± 3.3[a,b]	3.1 ± 3.5[a]	3.8 ± 3.8[b]	3.8 ± 3.8[b]	3.0 ± 3.6	1.1 ± 3.2[b]
Spine area	2.6 ± 4.2	4.5 ± 5.9	6.0 ± 6.3	5.7 ± 4.9	4.7 ± 6.2	2.0 ± 3.5
Total hip area	3.4 ± 5.6	5.2 ± 5.0	5.0 ± 5.4	5.0 ± 4.9	4.9 ± 5.0	4.1 ± 6.6
Femoral neck area	−1.0 ± 5.0	0.3 ± 4.6	1.4 ± 5.8	1.4 ± 5.5	0.14 ± 4.6	−0.9 ± 5.3
Forearm area	2.0 ± 3.6	2.8 ± 4.7	4.5 ± 5.2	4.3 ± 5.4	2.6 ± 4.4	2.2 ± 3.7
Total body area	3.5 ± 5.3[b]	6.0 ± 5.8	8.0 ± 5.1[b]	7.1 ± 5.3	6.1 ± 5.8	4.2 ± 5.6
Height	1.6 ± 2.0[b]	2.6 ± 2.2	3.4 ± 2.5[b]	3.2 ± 2.5	2.6 ± 2.2	1.9 ± 2.2
Lean mass	7.4 ± 8.2	9.7 ± 8.5	10.5 ± 8.9	10.0 ± 8.5	9.9 ± 8.6	7.4 ± 8.7
25 (OH) vitamin D	143 ± 314	122 ± 172	112 ± 111	150 ± 197	94 ± 101	154 ± 331

[a,b]Variables with same superscript are significantly different from each other; No difference in percent changes in height, BMD, BMC or bone area at any skeletal site with ApaI enzyme (data not shown).

affect bone mass in this age group. Since the first report on the relationship between VDR genotype and BMD in 1994, a large number of studies were conducted. Most of these studies included adults and a few were conducted in children. Results in both age groups have been conflicting. In children, the results differed according to gender, pubertal status and restriction enzyme used [22–27]. Our findings at baseline were similar to those reported by some, but not all studies assessing the relationship in adolescent girls [22–27].

There was also no relationship between VDR gene polymorphisms and lean mass at baseline. The relationship between VDR genotypes and muscle strength and/or lean mass has been studied in adults but, to our knowledge, not in children. The findings differed according to gender [28–30] and to the site of muscle studied [30].

The relationship between serum PTH levels and VDR genotype is equally inconsistent across studies [30,20–22]. A possible resistance to vitamin D action at the parathyroid tissue and/or at the intestines has been suggested with some VDR genotypes [31,32]. In the current study, PTH at baseline showed significant association with VDR gene polymorphisms when genotype was determined using ApaI restriction enzyme only. We do not think that the difference in PTH levels had significant implications, since it was not associated with differences in BMD at any skeletal sites. Whether this difference in PTH levels was due to a difference in calcium absorption and therefore would have a negative effect on bone density later on in life could not be assessed in the current study.

Vitamin D supplementation was associated with significant bone gain compared to placebo. This bone gain was affected by VDR genotype using BsmI and TaqI in the vitamin D treated group only, thus suggesting that polymorphisms in VDR gene do not have an effect on changes in bone mass on their own, but may modulate the skeletal response to vitamin D during childhood and adolescence. This effect was independent of the serum vitamin D level achieved at one year and was independent of the demonstrated mediators of the skeletal response to vitamin D, namely changes in bone size and lean mass [13]. Hunter et al. [33] measured the effect of two-year vitamin D3 supplementation on bone density in twin young postmenopausal women. They showed no clear significant effect of VDR gene polymorphisms on BMD response to treatment but there was a modest trend toward a positive treatment

Table 4
Percent changes in musculo-skeletal parameters and 25 (OH) vitamin D levels in the low dose and high dose vitamin D, according to VDR genotype using BsmI *or* TaqI restriction enzyme.

	Low dose vitamin D group						High dose vitamin D group					
	BB ($n=12$)	Bb ($n=31$)	bb ($n=15$)	TT ($n=19$)	Tt ($n=28$)	tt ($n=11$)	BB ($n=9$)	Bb ($n=28$)	bb ($n=18$)	TT (n=19)	Tt (n=28)	tt (n=8)
LS BMC	6.9 ± 5.8[a]	16.4 ± 13.0	16.3 ± 11.4	16.7 ± 11.7[a]	15.7 ± 13.4	7.1 ± 6.0	9.5 ± 9.3[a]	11.6 ± 10.3	16.5 ± 10.5	16.2 ± 10.1	11.8 ± 10.8	8.7 ± 8.2
TH BMC	6.4 ± 5.2	13.2 ± 10.3	10.7 ± 8.3	11.9 ± 7.6	12.5 ± 10.9	6.4 ± 5.4	10.8 ± 12.2	12.0 ± 9.7	14.9 ± 11.0	14.0 ± 10.1	11.4 ± 10.0	14.5 ± 13.7
FNBMC	0.4 ± 4.7[a]	5.9 ± 8.8	4.5 ± 6.4	5.2 ± 6.3	5.3 ± 9.1	0.7 ± 4.9	2.9 ± 7.1[a]	3.4 ± 6.3	8.8 ± 9.7	8.3 ± 9.4	3.1 ± 6.4	5.0 ± 7.8
FA BMC	2.5 ± 3.4[a]	8.7 ± 6.5	8.2 ± 6.6	8.5 ± 6.5[a]	8.3 ± 6.6	2.5 ± 3.5	6.3 ± 6.2	5.3 ± 10.3	8.8 ± 5.8	8.3 ± 6.0	5.2 ± 10.2	7.4 ± 6.4
TB BMC	3.5 ± 7.2[a]	10.4 ± 8.8	10.6 ± 8.2	10.2 ± 8.4[a]	10.2 ± 9.0	4.1 ± 7.2	4.0 ± 7.4[a]	9.1 ± 8.4	10.8 ± 8.3	12.9 ± 9.1	9.0 ± 8.7	5.4 ± 8.1
LS BMD	5.3 ± 5.4	10.5 ± 8.5	8.4 ± 5.2	8.9 ± 6.0	10.2 ± 8.5	5.4 ± 5.7	5.2 ± 6.4	7.4 ± 6.6	7.9 ± 5.6	11.2 ± 8.2	6.7 ± 6.4	6.1 ± 6.7
THBMD	5.1 ± 4.1	7.3 ± 5.5	6.5 ± 4.0	7.1 ± 4.0	6.9 ± 5.6	5.2 ± 4.3	3.9 ± 4.2	6.2 ± 4.9	6.3 ± 6.4	7.6 ± 5.0	6.0 ± 5.5	5.0 ± 4.2
FNBMD	2.8 ± 4.9	5.0 ± 5.9	4.2 ± 3.3	4.5 ± 3.3	4.7 ± 6.2	3.2 ± 5.0	2.0 ± 2.9	3.7 ± 3.6	3.7 ± 3.6	5.9 ± 6.1	3.3 ± 3.9	3.6 ± 3.4
FA BMD	1.8 ± 1.9[a]	4.5 ± 2.7	3.9 ± 3.7	4.2 ± 3.3[a]	4.3 ± 2.9	1.8 ± 2.0	2.4 ± 2.8	5.0 ± 4.1	4.5 ± 3.8	3.6 ± 4.0	4.9 ± 3.8	3.0 ± 3.4
TBBMD	1.7 ± 3.0	3.5 ± 3.5	3.0 ± 3.8	3.5 ± 3.8	3.1 ± 3.5	1.9 ± 3.1	0.1 ± 3.6[a]	2.7 ± 3.6	5.1 ± 6.4	4.0 ± 4.0[a]	2.9 ± 3.7	0.1 ± 3.2
LS area	1.5 ± 3.1	5.2 ± 6.8	7.1 ± 6.3	6.9 ± 5.8	4.8 ± 7.1	1.7 ± 3.1	4.0 ± 5.3	3.7 ± 4.7	6.3 ± 5.9	4.5 ± 6.0	4.5 ± 5.3	2.4 ± 4.1
TH area	1.1 ± 2.4[a]	5.3 ± 4.8	3.6 ± 4.5	4.2 ± 3.8[a]	5.0 ± 5.3	1.1 ± 2.6	6.4 ± 7.3	5.1 ± 5.2	2.3 ± 6.1	5.8 ± 5.8	4.9 ± 4.8	8.3 ± 8.3
FN area	−2.2 ± 4.1	0.7 ± 4.6	0.2 ± 5.3	0.7 ± 4.9	0.4 ± 7.1	−2.3 ± 4.3	0.8 ± 5.9	−0.2 ± 4.7	4.9 ± 5.5	2.1 ± 6.1	−0.2 ± 4.6	1.3 ± 6.3
FA area	0.7 ± 2.0	3.6 ± 5.0	4.0 ± 5.0	4.1 ± 5.4	3.3 ± 4.6	0.8 ± 2.1	3.8 ± 4.5	1.9 ± 4.2	8.7 ± 5.6	4.5 ± 5.5	1.9 ± 4.2	4.2 ± 4.5
TB area	3.4 ± 5.5	6.6 ± 6.0	7.2 ± 4.6	6.2 ± 5.3	7.0 ± 5.8	3.7 ± 5.6	3.6 ± 5.5[a]	5.4 ± 5.5	3.7 ± 2.4	8.1 ± 5.3	5.3 ± 5.9	4.8 ± 5.8
Height	1.3 ± 1.6	2.9 ± 2.1	3.1 ± 2.8	3.0 ± 2.5	2.9 ± 2.2	1.3 ± 1.7	2.0 ± 2.4	2.4 ± 2.3	10.5 ± 9.7	3.5 ± 2.5	2.3 ± 2.2	2.7 ± 2.6
Lean mass	5.0 ± 7.0	10.7 ± 8.7	10.5 ± 8.1	9.9 ± 7.3	11.2 ± 9.1	4.1 ± 6.6	10.6 ± 9.0	8.6 ± 8.3	158 ± 106	10.1 ± 9.7	8.5 ± 7.9	11.9 ± 9.7
25-OHD	43.9 ± 59.9	46.2 ± 54.8	57.6 ± 92.6	73 ± 81	35 ± 54	37 ± 58	275 ± 455	208 ± 215	158 ± 106	226 ± 247	152 ± 105	316 ± 474

[a] Implies significant difference between genotypes, using the same restriction enzyme and within the same treatment group; No difference in percent changes in height, BMD, BMC or bone area at any skeletal site with ApaI enzyme (data not shown).

Table 5
Multivariate analyses showing the independent impact of VDR genotype on changes in BMC or BMD in response to vitamin D supplementation adjusted for age, menarcheal status, changes in lean mass and changes in height or in bone area[1].

Skeletal site	Models with VDR using BsmI enzyme[a]				Models with VDR using TaqI enzyme[a]			
	Genotype[b]	β (SE)	*P*-value	95% CI	Genotype[c]	β (SE)	*P*-value	95% CI
LSBMC	Bb (+/−)	1.8 (1.5)	0.1	[−0.3; 5.9]	Tt (+/−)	2.2 (1.5)	0.07	[−0.23; 5.8]
	bb (+/+)	2.3 (1.7)	0.01	[1.1; 5.8]	TT (−/−)	2.8 (1.6)	0.01	[2.0; 5.5]
FNBMC	Bb (+/−)	2.9 (1.2)	0.02	[0.4; 5.4]	Tt (+/−)	2.5 (1.3)	0.06	[−0.15; 5.3]
	bb (+/+)	3.0 (1.7)	0.009	[0.9;6.4]	TT (−/−)	3.9 (1.4)	0.009	[0.97; 6.8]
FABMC	Bb (+/−)	1.5 (0.6)	0.01	[0.3; 2.7]	Tt (+/−)	1.5 (0.6)	0.02	[0.16; 2.8]
	bb (+/+)	0.4 (0.7)	0.5	[−0.9; 1.7]	TT (−/−)	0.4 (0.7)	0.5	[−0.97; 1.9]
TBBMC	Bb (+/−)	0.4 (0.5)	0.1	[−0.7; 1.3]	Tt (+/−)	0.5 (0.5)	0.09	[0.18; 1.9]
	bb (+/+)	0.7 (0.6)	0.03	[0.6; 1.5]	TT (−/−)	0.8 (0.5)	0.03	[0.56; 1.6]
	Models with VDR using BsmI enzyme[d]				Models with VDR using TaqI enzyme[d]			
	Genotype[b]	β (SE)	*P*-value	95% CI	Genotype[c]	β (SE)	*P*-value	95% CI
LSBMC	Bb (+/−)	0.9 (1.0)	0.09	[−0.6; 3.4]	Tt (+/−)	0.7 (1.1)	0.04	[0.4; 3.0]
	bb (+/+)	1.5 (1.1)	0.01	[0.7; 3.8]	TT (−/−)	1.4 (1.2)	0.02	[0.9; 3.8]
FNBMC	Bb (+/−)	1.8 (0.9)	0.04	[0.01; 3.7]	Tt (+/−)	0.8 (1.0)	0.04	[0.2; 2.8]
	bb (+/+)	2.1 (1.0)	0.03	[0.11; 4.2]	TT (−/−)	1.4 (1.1)	0.02	[0.7; 3.6]]
FABMC	Bb (+/−)	0.5 (1.0)	0.2	[−0.7; 1.8]	Tt (+/−)	0.4 (1.8)	0.07	[−0.8; 1.8]
	bb (+/+)	1.4 (0.9)	0.01	[0.3; 2.6]	TT (−/−)	1.1 (0.6)	0.04	[0.8; 2.4]
TBBMC	Bb (+/−)	0.6 (0.6)	0.1	[0.6; 1.6]	Tt (+/−)	0.7 (0.5)	0.1	[−0.26; 1.9]
	bb (+/+)	0.8 (0.5)	0.03	[0.2; 1.8]	TT (−/−)	0.8 (0.6)	0.02	[0.50; 1.8]

[1]Only data with significant results were reported.
[a] Adjusted for age, menarcheal status, changes in lean mass and changes in height.
[b] Reference genotype was BB (−/−).
[c] Reference genotype was tt (+/+).
[d] Adjusted for age, menarcheal status, changes in lean mass and changes in bone area.

effect for total hip BMD for the TT genotype [34]. Another study conducted in Japanese women showed that the BMD response to vitamin D analogue at the lumbar spine was VDR genotype dependent, the best response was observed in the bb genotype [35]. Findings from these two studies in adults are in accordance with the current findings in adolescent girls. Conversely, Graafmans et al. [36] demonstrated that the BMD response at the femoral neck was lower in bb genotype compared to Bb and BB genotypes in elderly women. On the other hand, Morrison et al. examined the influence of VDR genotype on vertebral fracture during therapy using either calcium or calcitriol and showed the response to calcitriol therapy to be most pronounced in patients carrying the *TaqI* t allele in combination with the *FokI* f allele [37]. To our knowledge, there are no studies assessing the relationships between VDR gene polymorphisms and the musculo-skeletal response to vitamin D in adolescents. However, Ferrari et al. [18] showed a significant effect of calcium on BMD at various sites in girls with the Bb but not the bb genotype, whereas spontaneous BMD gain was higher in girls on placebo in the bb genotype subgroup. Participants in the current study had low 25-OHD levels and calcium intake at baseline and Dawson-Hughes et al. showed that women with BB allelic variants of the VDR have reduced calcium absorption efficiency on low calcium intake compared to women with the bb variants, suggesting a functional defect in the intestinal VDR (34). Therefore, the poor skeletal response in BB genotype to vitamin D supplementation may in part be mediated through poorer intestinal calcium absorption. The results here-in may not apply to adolescents with optimal calcium and/or serum 25-OHD levels.

Although there were no statistically significant differences in height, weight and pubertal status among genotypes at baseline, girls with BB and tt genotypes tended to be taller and heavier and there was a trend toward a higher proportion of advanced pubertal stage in these girls compared to other genotypes, suggesting that these girls were closer to pubertal maturity, the time when peak BMC velocity declines, which might, at least in part, explain their lower BMC changes over one year. On the other hand, although the effect of VDR on BMC and BMD persisted after adjustment for bone area, there was no significant difference in the percent changes in volumetric BMAD between genotypes, implying that the effect of genotype may be, in part, related to changes in bone size.

There are several limitations to our study. It was not population based. It did not evaluate FokI, the particular polymorphism that has been associated with calcium absorption [18,38], and did not evaluate interaction with other genes such as the estrogen receptor gene or other relevant genes polymorphisms such as calcium sensing receptor gene. Finally, the interpretation of polymorphic variations in the VDR gene is hindered by the fact that most but not all polymorphisms used have unknown functional effects [39,40]. The likely explanation for an observed association of polymorphisms with unknown functional effects with BMD outcomes is assumed to be because of the presence of a truly functional sequence variation elsewhere in the gene which is in linkage with such non-functional genes.

VDR gene polymorphisms influence the skeletal response to vitamin D supplementation in health adolescent girls with low 25-OHD and low calcium intake. Whether this effect is mediated by difference in calcium absorption needs to be investigated. If confirmed, this would justify tailoring calcium and vitamin D supplementation using VDR genotyping in children and adolescents.

Conflict of interest
The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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