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ORIGINAL ARTICLE

Spine bone mineral density and VDR polymorphism in subjects with ulcerative colitis

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Abstract This study established bone mineral density in subjects with ulcerative colitis with respect to disease dissemination and severity and the association between skeletal status and vitamin D receptor (VDR) polymorphism. Forty-seven patients aged 47.6 ± 14.8 years and 47 age- and sex-matched control subjects were evaluated. Disease duration was 8.6 ± 7.2 years. Twenty-four subjects demonstrated mild, 17 moderate, and 5 severe forms of ulcerative colitis; local (proctitis and proctosigmoiditis) changes were present in 26 and disseminated changes in 21. Bone mineral density (BMD, g/cm^2) was assessed at the spine, and distribution of VDR polymorphism was established. In six patients (12.8%) and in two controls (4.25%), *T*-score for BMD was below −2.5, but mean values of BMD did not differ between all patients and controls. Patients with moderate and severe form of disease had lower BMD measurements than patients with a mild form of colitis ulcerosa ($P < 0.05$), and subjects with disseminated intestinal changes had lower BMD measurements than subjects with local changes ($P < 0.001$). Distribution of VDR polymorphism did not differ between patients and controls. Spine *Z*-score was dependent on VDR polymorphism ($P < 0.05$) in male and female patients but not in controls. We concluded that, in patients with ulcerative colitis (UC), spine bone mineral density decreases with progression and dissemination of the disease, and that VDR polymorphism is associated with spine bone mineral density. VDR genotype bb is significantly less likely to cause low BMD in male UC patients, and VDR genotype tt is more likely to cause low BMD in female patients.

Keywords Densitometry · Ulcerative colitis · VDR polymorphism

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Introduction

Skeletal status in subjects with ulcerative colitis (UC) has been a matter of interest of several studies [1–8]. Patients presented *T*-score values in the range for osteoporosis, according to World Health Organization diagnostic criteria (*T*-score lower than –2.5), in a prevalence ranging from 4% [1] to 42% [2], and other authors noted intermediate values from 7 to 22% [3–8]. The pathogenesis of osteoporosis associated with UC is not fully understood, but it is likely to be multifactorial, and possible contributing factors include the influence of intestinal inflammatory process, malnutrition and malabsorption leading to secondary hyperparathyroidism, hypogonadism, corticosteroid treatment, decreased physical activity, and diminished sun exposure. Some studies have also shown an increased risk for fracture in patients with UC [9, 10]. Bone metabolism is controlled by several mechanisms, and among them vitamin D plays an important role. The active form of vitamin D (calcitriol) influences processes within bone tissue, and receptors (vitamin D receptor, VDR) for calcitriol are present in cells of several organs including kidney, parathyroid gland, and muscles. VDRs are also present in the

intestinal tract, and recently a crucial role for the VDR in experimental bowel disease was proven in mice [11]. Relationships between VDR polymorphism and values of bone mineral density (BMD) were studied by several authors, and their findings were contradictory. Some of them observed that genotype BB is associated with lower BMD than genotypes bB and bb [12–16], but others did not confirm these results [12–18].

To our knowledge, VDR polymorphism has not been evaluated in subjects with UC in any study published to date. The aim of this study was to assess whether VDR polymorphism is related to skeletal status in patients with UC and to compare this analysis with data obtained in age- and sex-matched control subjects.

Materials

In the case–control study, 47 Caucasian patients (20 men and 27 women) and 47 control subjects (20 men and 27 women) matched for age and gender were evaluated. Patients were recruited from the Outpatient Gastrology Clinic, and the control group was composed of volunteers from medical staff working in the hospital of the second author of this current study.

To obtain age-matched controls, in the first step patients were evaluated and subsequently controls were recruited (pair-matched). A proposal to participate as a control included every member of the hospital staff who met a preliminary age-matched criterion.

The principal inclusion criterion for patients was diagnosis of UC (details given later). Another inclusion criterion for patients for patients and controls was written informed consent. Exclusion criteria for patients and controls included the presence of any diseases known to affect bone metabolism (such as chronic diseases of endocrine system, stomach surgery, surgical menopause, kidney disease), the use of therapy interfering with bone metabolism [corticosteroids (other than use for UC)], thyroid hormones, anticonvulsants, anticoagulants), and current or past use of antiosteoporotic drugs such as bisphosphonates, hormone replacement therapy, calcitonin, raloxifen, teriparatide, and strontium ranelate.

Diagnosis of UC was based on clinical features of the disease and intestinal biopsy performed during colonoscopy followed by histological evaluation. During the study, all patients were in the remission stage of UC. Mean age did not differ between patients and controls. Some differences were noted for body weight and body mass index (Table 1); all patients had lower weight and body mass index (BMI), and females had lower weight and males lower BMI than controls. Among the female patients, 11 were postmenopausal women (14.4 ± 12.7 years since menopause), and among the controls, 12 were postmenopausal (mean, 9.9 ± 4.9 years since menopause). Postmenopausal duration did not differ between female patients and controls. The mean duration of UC was 8.6 ± 7.2 years. During the study all patients were in the remission stage of UC, but during the course of the disease 24 subjects demonstrated mild, 17 moderate, and 6 severe forms of UC. Division was done according to the proposition of Truelove and Witt [19]. The mild form of UC was designated when there were one to four relapses daily without blood or with only a small amount of blood, and heart rate and body temperature were normal; the moderate form was were four to six relapses daily with a significant amount of blood, heart rate of 80–90/min, and body temperature less than 38°C; and the severe form of UC comprised more than six relapses daily, heart rate greater than 90/min, and body temperature more than 38°C. As was shown by colonoscopy, local changes (proctitis or proctosigmoiditis) were present in 26 patients and disseminated changes in intestinal tract (changes observed also in other parts of intestinal tract) in 21 patients. Short-term corticosteroid treatment was used in 9 patients (4 men and 5 women). Mean daily dose equivalent to prednison was 12 ± 6.6 mg, and mean duration of this therapy was 8.0 ± 0.4 months. Surgery was not performed in any

Table 1 Clinical characteristics (mean and standard deviation)

Parameter	Ulcerative colitis subjects			Controls		
	All ($n = 47$)	Males ($n = 20$)	Females ($n = 27$)	All ($n = 47$)	Males (n =20)	Females (n =27)
Age (years)	47.64 ± 14.83	48.80 ± 15.41	46.78 ± 14.63	47.21 ± 10.66	48.55 ± 8.78	46.22 ± 11.93
Weight (kg)	70.81 ± 11.26*	75.10 ± 10.64	67.63 ± 10.81*	78.64 ± 13.89	80.95 ± 13.82	76.93 ± 13.95
Height (cm)	167.15 ± 8.25	173.70 ± 6.61	162.30 ± 5.56	167.81 ± 7.52	171.15 ± 8.58	165.33 ± 5.60
Body mass index (BMI) (kg/m^2)	25.43 ± 4.36**	24.91 ± 3.36***	25.83 ± 5.01	27.84 ± 4.33	27.44 ± 3.33	28.13 ± 4.98

* Value statistically lower than in controls, $P < 0.01$

** Value statistically lower than in controls, $P < 0.001$

*** Value statistically lower than in controls, $P < 0.05$

patient studied. Six female patients had six fractures resulting from minimal trauma (three forearm, two ankle, and one rib); no fractures occurred in male patients and controls. The local ethics committee gave permission for the study, and informed written consent was obtained from all subjects.

Methods

Bone densitometry

Skeletal status was assessed by dual-energy X-ray absorptiometry (DXA). DXA measurements of BMD (g/cm^2) of lumbar spine were performed with Lunar DPX-L (Madison, WI, USA). The coefficient of variation (CV% = SD/mean × 100%) for BMD measurements based on 100 measurements in 20 patients was 1.08%.

DXA variables were expressed also by *T*-score [the number of standard deviations (SD) from peak value for gender)] and *Z*-score (the number of SD from the mean value in age-matched gender group).

All BMD measurements were done by one technician.

VDR genotype analysis

Genomic DNA was extracted from whole blood using the MasterPureGenomic DNA Purification Kit (Epicentre Technologies, Madison, WI, USA). All subjects were genotyped by polymerase chain reaction of the regions that contained the analyzed polymorphisms and digestion with the specific restriction enzymes as previously described [20]. The WDA, WDB, and WDT polymorphisms were genotyped using the *Apa*I, *Bsm*I, and *Taq*I restriction enzymes, respectively. Restriction enzyme digests were analyzed on 1.5% agarose gel.

Statistical analysis

Statistical analysis was performed using Statistica for Windows. The normality of data distribution was established using the Shapiro–Wilk test. Comparisons between variables were performed by analysis of variance (ANOVA) or Mann–Whitney test in a case of nonnormal data distribution. Comparisons were performed between a group of all patients and all controls and gender subgroups. To establish the role of the form of UC or disease dissemination, adequate comparisons were done between subgroups and between controls and subgroups. Relationships between variables studied were performed using Pearson coefficients of correlation. The Chi-square test was used to analyze whether distribution of VDR polymorphism differed in patients and controls. Significance was achieved with $P < 0.05$.

Results

Table 2 gives results of densitometric measurements in patients and controls. All values were lower in patients, but the only significant difference was established between Z-score in female patients and controls. The World Health Organization criteria for diagnosis of osteoporosis, a value of *T*-score for hip or spine ≤–2.5, was observed in six patients (12.8%) and two controls (4.25%). Duration of disease and corticosteroid therapy did not affect BMD values (data not shown). Tables 3 and 4 present results of spine densitometric measurements in patients divided into subgroups according to the form and dissemination of the disease, respectively. Subgroups did not differ in respect to mean age, body weight and height, postmenopause duration, duration of the disease, and duration of steroid administration. Densitometric variables differed significantly between patients with mild and moderate forms of UC and between patients with mild and severe form of UC ($P < 0.01$). Densitometric values in patients with moderate and severe form of UC did not differ. Comparisons between values obtained in patients with local and disseminated changes in intestinal tract showed that all values were significantly lower in subjects with disseminated changes ($P < 0.001$).

Comparisons between all controls and patients with different stages of the disease showed that skeletal variables in patients with the mild form of UC did not differ and were significantly lower in patients with moderate and severe form of UC than in controls. Measured bone variables were significantly lower in patients with disseminated

Table 2 Results of densitometric measurements in patients and controls (mean and standard deviation)

Parameter	All patients ($n = 47$)	Male patients ($n = 20$)	Female patients ($n = 27$)	All controls ($n = 47$)	Male controls ($n = 20$)	Female controls ($n = 27$)
Bone mineral density (BMD) (g/cm^2)	1.11 ± 0.18	1.12 ± 0.19	1.11 ± 0.18	1.18 ± 0.17	1.15 ± 0.18	1.20 ± 0.17
T-score	–0.88 ± 1.49	–1.00 ± 1.58	–0.78 ± 1.44	–0.32 ± 1.47	–0.73 ± 1.48	–0.01 ± 1.41
Z-score	–0.60 ± 1.27	–0.69 ± 1.51	–0.53 ± 1.07*	–0.20 ± 1.41	–0.67 ± 1.46	0.15 ± 1.29

* Value statistically lower than in controls, $P < 0.05$

Table 3 Results of densitometric measurements in patients according to form of ulcerative colitis (mean and standard deviation)

Parameter	Mild form ($n = 24$)	Moderate form ($n = 17$)	Severe form ($n = 6$)	All controls ($n = 47$)
BMD (g/cm^2)	1.19 ± 0.16*	1.05 ± 0.17$^{\#}$	0.96 ± 0.16***	1.18 ± 0.17$^{\#\#}$
T-score	–0.22 ± 1.25**	–1.43 ± 1.38$^{\#}$	–2.23 ± 1.44***	–0.32 ± 1.47$^{\#\#}$
Z-score	–0.10 ± 1.08*	–0.91 ± 1.09$^{\#}$	–2.01 ± 1.44***	–0.20 ± 1.41$^{\#\#}$

Values between patients with moderate and severe form of ulcerative colitis (UC) did not differ

*Value statistically higher than in patients with moderate form of UC, $P < 0.05$

**Value statistically higher than in patients with moderate form of UC, $P < 0.01$

***Value statistically lower than in patients with mild form of UC, $P < 0.01$

$^{\#}$ Value statistically lower than in controls, $P < 0.05$

$^{\#\#}$ Value statistically higher than in patients with severe form of UC, $P < 0.01$

Table 4 Results of densitometric measurements in patients according to dissemination of ulcerative colitis (mean and standard deviation)

Parameter	Local changes ($n = 26$)	Disseminated changes ($n = 21$)	All controls ($n = 47$)
BMD (g/cm^2)	1.20 ± 0.15*	1.01 ± 0.16$^{\#\#}$	1.18 ± 0.17
T-score	0.03 ± 1.40*	−1.77 ± 1.29$^{\#}$	−0.32 ± 1.47
Z-score	−0.04 ± 0.10*	−1.32 ± 0.12$^{\#}$	−0.20 ± 1.41

Values between patients with local changes of ulcerative colitis (UC) and controls did not differ

*Value statistically higher than in patients with disseminated changes, $P < 0.001$

$^{\#\#}$ Value statistically lower than in controls, $P < 0.001$

$^{\#}$ Value statistically lower than in controls, $P < 0.01$

Table 5 Distribution of vitamin D receptor (VDR) polymorphism (number and percentage)

Polymorphism	Genotype	Ulcerative colitis subjects			Controls		
		All ($n = 47$)	Males ($n = 20$)	Females ($n = 27$)	All ($n = 47$)	Males ($n = 20$)	Females ($n = 27$)
WDA	AA	12 (25.5%)	6 (22.2%)	6 (30%)	9 (19.1%)	6 (22.2%)	3 (15.0%)
	Aa	26 (55.4%)	14 (51.9%)	12 (60%)	29 (61.8%)	16 (59.3%)	13 (65.0%)
	aa	9 (19.1%)	7 (25.9%)	2 (10%)	9 (19.1%)	5 (18.5%)	4 (20.0%)
WDB	BB	8 (17.0%)	4 (14.8%)	4 (20%)	8 (17.0%)	5 (18.5%)	3 (15.0%)
	Bb	22 (46.8%)	14 (51.9%)	8 (40%)	23 (49.0%)	14 (51.9%)	9 (45.0%)
	bb	17 (36.2%)	9 (33.3%)	8 (40%)	16 (34.0%)	8 (29.6%)	8 (40.0%)
WDT	TT	17 (36.2%)	12 (44.45%)	5 (25%)	20 (42.6%)	11 (40.7%)	9 (45.0%)
	Tt	23 (48.9%)	12 (44.5%)	11 (55%)	22 (46.8%)	13 (48.2%)	9 (45.0%)
	tt	7 (14.9%)	3 (11.0%)	4 (20%)	5 (10.6%)	3 (11.1%)	2 (10.0%)

intestinal changes compared with adequate results in controls, and in patients with local changes, skeletal status was not affected.

Table 5 presents the distribution of VDR polymorphism in patients and controls, respectively. As was shown by chi-square test, distribution of VDR polymorphism did not differ between patients and controls. To verify whether skeletal status differs in relationship to VDR polymorphism in subgroups for receptor WDA, WDB and WDT values of spine BMD *Z*-score were compared (to avoid differences of mean age in subgroups). Analyses have shown differences in spine BMD *Z*-score for patient gender subgroups for WDT in females (Fig. 1) and for WDB in males (Fig. 2) and the lack of such observations in controls (Figs. 3, 4). 13% of all subjects studied and 22% of female patients studied had a prior fracture.

Discussion

The only study available at the moment on the potential role of VDR in ulcerative colitis was the experiment performed in mice [11]. To our knowledge, the current study is the first showing associations between VDR polymorphism with BMD measurements in subjects with UC. VDR genotype bb is significantly less likely to cause low BMD

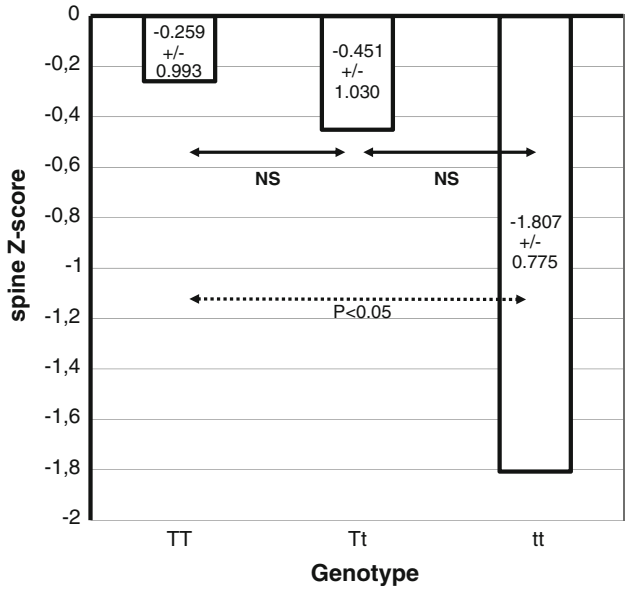

Fig. 1 Mean value of spine *Z*-score in relationship to genotype WDT in female patients

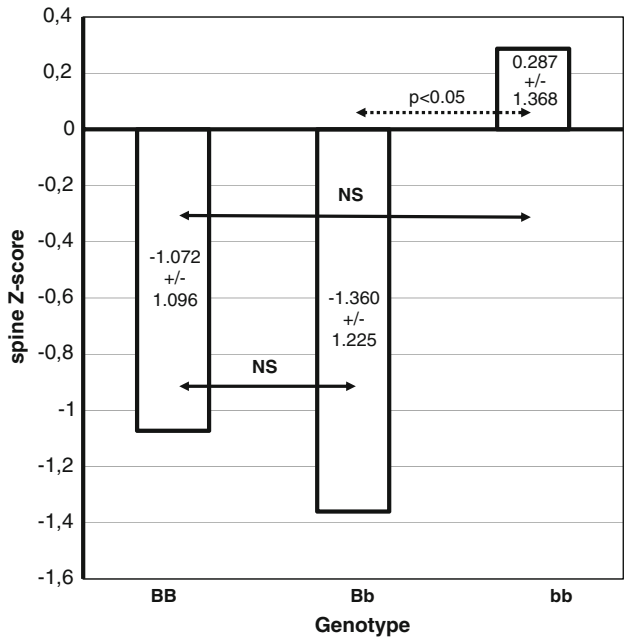

Fig. 2 Mean value of spine *Z*-score in relationship to genotype WDB in male patients

in male UC patients, and in female patients the VDR genotype tt is more likely to cause low BMD.

Distribution of VDR polymorphism did not differ in patients and controls, but an association between BMD and VDR genotypes was observed only in patients. Certainly, the current results should be treated as preliminary, and further studies are warranted. We consider that another significant clinical finding of the current study is the observation of aggravation in skeletal status with disease severity and

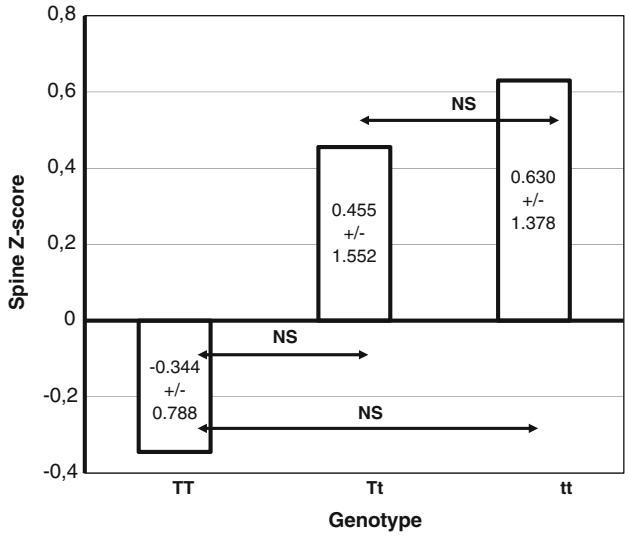

Fig. 3 Mean value of spine *Z*-score in relationship to genotype WDT in female controls

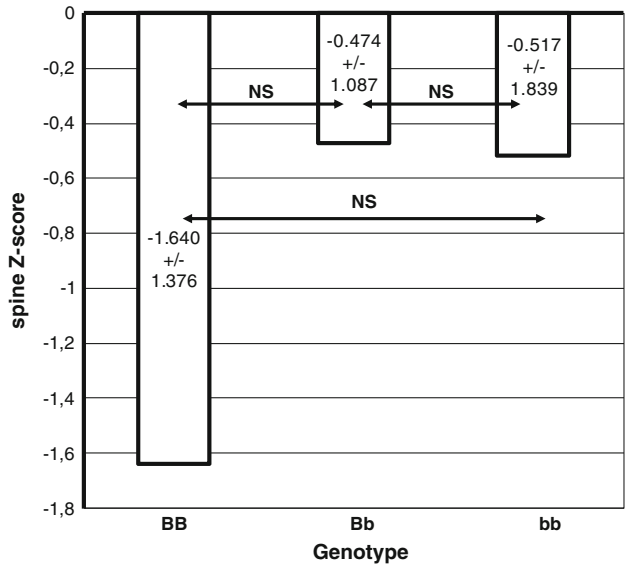

Fig. 4 Mean value of spine *Z*-score in relationship to genotype WDB in male controls

dissemination. Generally, only some authors assessed the skeletal status in patients with UC according to a clinical form of the disease or disease dissemination. Ulivieri et al. [21] have shown, in a group of 43 patients, that the mild form of UC is not a risk for decreased BMD. This observation is comparable with current results. The authors noted no differences at the baseline for spine and whole-body BMD, and also after 6 years BMD did not differ between patients and controls. In their description of materials, the authors [21] described the dissemination of UC, but this factor was not taken into consideration, and further comparison with current data is not possible. In a study by Bjarnason et al. [4],

disease severity and localization of intestinal changes did not affect BMD measurements.

In some studies, differences in skeletal measurements in patients with UC compared with healthy individuals (irrespective of the form and dissemination of the disease) were not shown. In a study by Jahnsen et al. [22], a case–control analysis of a group of 60 patients with UC showed no differences in BMD values in comparison to healthy controls, and a prospective observation of 44 patients over 2 years did not reveal changes in mean lumbar spine BMD [23]. However, significant bone loss was observed in 27% of patients with UC. Comparable results were given by Schoon et al. [24], who observed no differences in total body, spine, and hip BMD in a group of patients with recently diagnosed inflammatory bowel disease (both Crohn disease and UC) versus controls. In multivariate regression analysis, duration of complaints longer than 6 months was associated with low BMD. Also, in this study analysis of disease activity or dissemination was not included. Other authors obtained contradictory results and noted differences in mean BMD values between patients with UC and controls [6].

In the study, we obtained approximately 13% prevalence of osteoporosis at the spine in patients with UC. This value is in the range of data given by other authors, 4–42% [1–8]. Pollak et al. [2] had a fracture prevalence of 5.8%, Ulivieri et al. [21] observed patients without fractures, and some other authors did not give this information [3, 4].

In some studies, fracture risk in subjects with inflammatory bowel disease was assessed [9, 10, 25, 26]. van Staa et al. [10] and Card et al. [26] showed increased fracture risk, whereas Loftus et al. [25] stated that fracture risk was not elevated in comparison to control subjects. Moreover, corticosteroid use was a contributor to fracture risk [10, 26], and other authors [25] achieved contradictory results.

In our study, we did not obtain a relationship between duration of steroid use and BMD results, and BMD values did not differ between corticosteroid users and non-users. It is not easy to comment on this observation, and several issuses should be taken into consideration. First, our patients were relatively young, and the ratio of bone formation and bone resorption in their age group is usually an advantage for bone metabolism; second, the steroid dose and duration of the therapy was rather short; and third, the unrecognized phenomenon indicates individual sensitivity for bone steroid side effects in some patients while others are entirely immune. Finally, steroid therapy affects bone tissue with respect to microarchitecture whereas bone quantity expressed by bone densitometry is rather stable. Steroid use is a widely accepted risk factor for fractures irrespective of its influence on BMD values. These problems are nowadays intensively studied in many research centers, but the conclusion is unknown.

In a study by Ulivieri et al. [21], at both baseline and follow-up, densitometric measurements did not differ between corticosteroid users and non-users. In our retrospective analysis we were not able to establish cumulative lifetime steroid intake, but despite this limitation we also noted the lack of effect of corticosteroid use, expressed as the mean duration of the therapy, on BMD measurements. In a small prospective study by Roux et al. [3] in 21 patients with UC, no correlation between coricosteroid treatment and BMD changes in follow-up was observed. Also in a study by Pollak et al. [2], cumulative dose of corticosteroids did not influence spine and femoral neck BMD. However, this study analyzed a group of patients with UC and patients with Crohn's disease. Also, Bjarnason et al. [4] did not observe an influence of corticosteroid use on densitometric variables. In a recent 2-year prospective study by Jahnsen et al. [23], corticosteroid administration did not affect the densitometric measurements. Other authors observed a negative effect of corticosteroid use on BMD values [6].

The current study has several limitations, including case–control design, the relatively low number of subjects studied, especially in the severe form of the disease, densitometric measurements performed only in one skeletal site, and the lack of laboratory data on bone metabolism. One of the weaknesses of the study was some differences in body size between patients and controls. These differences might have interfered with the results of the study in regard to direct comparisons of BMD values, but these did not influence the relationship of VDR polymorphism and BMD because latter analyses were performed separately in patients and controls.

Current results should be treated as preliminary, and further studies are warranted. Recently, Lee and Tucker [27], in an editorial article published in *Annals of Internal Medicine*, clearly stated that despite hundreds of association studies and retrospective meta-analyses of polymorphism in more than 30 genes that are associated with BMD and fractures, no convincing conclusions have emerged. The VDR polymorphism is no exception, and understanding the pathogenesis of osteoporosis will require revealing several different aspects, also including genetic investigations.

In conclusion, in patients with ulcerative colitis, spine BMD decreases with progression and dissemination of the disease, and VDR polymorphism is associated with spine BMD. VDR genotype bb is significantly less likely to cause low BMD in male UC patients, and in female patients the VDR genotype tt is more likely to cause low BMD.

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