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Vitamin D receptor (*VDR*) TaqI polymorphism, vitamin D and bone mineral density in patients with inflammatory bowel diseases

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Abstract

Background. A common feature in the etiology of inflammatory bowel disease (IBD) and osteoporosis is a complex genetic background. Moreover, it has been shown that some of the susceptibility loci overlap for both diseases. One of the genes that may be involved in the pathogenesis of IBD as well as decreased bone mass is the vitamin D receptor (*VDR*) gene.

Objectives. The aim of this study was to investigate the association of the TaqI polymorphism (rs731236, c.1056T >C) in the *VDR* gene with serum vitamin D concentration and bone mineral density (BMD) in patients with IBD.

Material and methods. A total of 172 IBD patients (85 with Crohn's disease (CD) and 87 with ulcerative colitis (UC)) and 39 healthy controls were enrolled in the study. Polymorphism was determined with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Bone mineral density was measured at the lumbar spine (L2–L4) and the femoral neck (FN) using dual-energy x-ray absorptiometry (DEXA). Serum concentrations of 25-hydroxyvitamin D were determined using electrochemiluminescence binding assay (ECLIA).

Results. Our studies revealed that serum vitamin D concentration in IBD patients was not lowered in comparison with healthy controls. Patients with CD presented more advanced osteopenia and osteoporosis. Individuals with UC carrying the TaqI tt genotype of *VDR* gene showed significantly higher FN BMD than carriers of TT and Tt genotypes ($p = 0.02$). Moreover, tt genotype was present with higher frequency in UC patients than in controls and CD patients (23% vs 7.7% and 16.5%, respectively).

Conclusions. The tt genotype may have a protective effect on BMD in UC patients.

Key words: vitamin D, bone mineral density, inflammatory bowel diseases, vitamin D receptor gene polymorphism

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Introduction

Inflammatory bowel disease (IBD) is an idiopathic disease caused by uncontrolled mucosal immunity of the gastrointestinal tract, but its pathogenesis is not fully understood.[1] It has been suggested that it originates from a combination of environmental (intestinal microbiota), immunological (impaired regulation of T-helper lymphocytes, Th) and complex genetic factors.[2,3] Inflammatory bowel disease does not concern only the gastrointestinal system and should be treated as a systemic disease with a number of manifestations outside the digestive tract, among which osteopenia and osteoporosis are the most common.[4] Even 22–77% of patients with IBD suffer from osteopenia and 17–41% of suffer from osteoporosis.[5–7] Lean body structure, age over 65, low-protein and low-calcium diet, cigarette smoking, alcohol drinking, vitamin D deficiency, the use of some pharmaceutical drugs, and compound genetic factors are osteoporosis risk factors. Osteoporosis susceptibility genes encode proteins that control bone formation and the maintenance of the normal bone tissue structure. They belong to different families of biological factors such as cytokines, growth factors, matrix components, and calciotropic hormone receptors.[8,9] To date, almost 200 susceptibility loci have been identified for IBD. Some of them are characteristic for Crohn's disease (CD) or ulcerative colitis (UC) and some of them overlap. Moreover, susceptibility loci may be also common for IBD and osteoporosis.[10,11] One of such genes is the vitamin D receptor (*VDR*) gene, encoding the vitamin D ($1,25(OH)_2D_3$) receptor belonging to a family of nuclear receptors and acting as a ligand-activated transcriptional factor. Activation of the *VDR* through direct interaction with $1,25(OH)_2D_3$ prompts the rapid binding of the receptor to regulatory regions of target genes, where it causes directed changes in transcription. This receptor plays a central role in the biological actions of vitamin D; moreover, *VDR* regulates the expression of numerous genes involved in calcium/phosphate homeostasis, cellular proliferation and differentiation, and immune response.[12] Vitamin D is a prohormone that is metabolized to its active form, 1,25-dihydroxycalciferol (calcitriol), having both calcemic and non-calcemic (pleiotropic) effects. The most important calcemic effects include inducing calcium and phosphate absorption in the digestive system, increasing renal calcium reabsorption and inducing bone turnover.[9]

According to the latest data, patients with IBD suffer from vitamin D deficiency.[13] Decreased exposition to sunlight[14], inappropriate diet, inflammatory changes in the bowel mucosa, and consequences of the digestive tract resection are considered the main reason for this problem.[15–17] This leads to osteomalacia (defective bone mineralization with the maintenance of the normal bone mass) and osteoporosis (a decrease of the properly mineralized bone mass). Moreover, vitamin D may have an important effect on the course of the disease through modulation of the inflammation mechanisms.

It has been shown that polymorphisms in the *VDR* gene are related with changes in bone mineral density (BMD). Furthermore, earlier reports showed that the *VDR* alterations might increase the risk of IBD in Caucasians and Asians.[18,19] The mechanism of this phenomenon is difficult to explain. The 3' region of the *VDR* gene is involved in the regulation of gene expression, which affects the stability of mRNA. In turn, it may lead to a decreased concentration of mRNA resulting in decreased vitamin D levels and a reduction in its inhibitory effects on interleukin 12 (IL-12). Changes in the immune system result in increased Th1-dependent reaction, which may explain the susceptibility to CD. What is also significant is the linkage disequilibrium (LD); the *VDR* gene polymorphisms may be in strong linkage with another, unknown sequence variant, which has a causative effect.[20] Considering the above premises, we decided to deal with this issue. The aim of this study was to analyze the association between the TaqI polymorphism in the *VDR* gene and serum vitamin D concentration as well as BMD in patients with inflammatory bowel disease.

Materials and methods

Patients and clinical data

The study group included 172 IBD patients from the Clinic of Gastroenterology, Human Nutrition and Internal Diseases of the Poznan University of Medical Sciences (Poland), comprising of 85 CD and 87 UC patients. The control group consisted of 39 healthy volunteers without IBD or any bone disorders. Peripheral blood and clinical data were collected from all study subjects. The clinical examination included measurements of BMD of the lumbar spine (L2–L4 levels) and of the femoral neck (FN). The data on weight, height and age at densitometry was also collected. Patients included into the study were not taking any vitamin D supplements. Densitometry measurements were conducted with dual energy X-ray absorptiometry (DEXA) using the DPX-Plus device (Lunar, GE Healthcare, Chicago, USA). Serum concentrations of 25-hydroxyvitamin D (25(OH)D), as markers of vitamin D sufficiency in the organism, were determined using an electro-chemiluminescence binding assay and a Cobas e601 analyzer (Roche, Basel, Switzerland). The functional sensitivity of these assays is 4.01 ng/mL (coefficient of variation: 18.5%). All patients gave their written consent to genetic testing scheduled within this study. The study was approved by the local Ethical Committee of the Poznan University of Medical Sciences (approval No. 92/09).

DNA extraction and *VDR* polymorphism analysis

DNA was isolated from peripheral blood leukocytes using guanidine isothiocyanate and phenol-chloroform

as described elsewhere.[21] DNA fragments including the polymorphic sites of the *VDR* gene were amplified using polymerase chain reaction (PCR). Each amplification reaction contained 100 ng of genomic DNA, 0.25 mM dNTP, 7.5 pmol of each primer, and 0.5 unit of the Taq polymerase (Sigma Aldrich, Saint Louis, USA) in 20 µL total volume. The reaction was conducted in 35 cycles of the following steps: initial denaturation at 94°C for 4 min; denaturation at 94°C for 40 s; primer annealing at 64°C for 40 s; elongation at 72°C for 100 s; and final incubation at 72°C for 180 s. PCR primers: forward CAG AGC ATG GAC AGG GAG CAA and reverse GCA ACT CCT CAT GGC TGA GGT CTC were used.[22] The amplification products were subsequently hydrolyzed using TaqI restriction enzyme (New England Biolabs, Ipswich, USA) at 65°C for 2 h. Digestion products were separated in an agarose gel (1.5%) with ethidium bromide. Alleles of TaqI polymorphism (allele T(c.1056C): 494+251 bp and allele t (c.1056T): 293+251+201 bp) were identified in comparison to the control samples determined with Sanger sequencing.

Statistical analysis

We conducted an analysis of the distribution of genotype concordance with the Hardy–Weinberg equilibrium. First, the normality of the distribution and the homogeneity of variable variances were conducted in the studied groups using the Shapiro–Wilk t test and Levene's test, respectively. In the event of non-concordance with 2 or at least 1 condition, the non-parametric Kruskal–Wallis H test was used to compare the groups. In the event of a statistically significant heterogeneity between the groups, multiple comparisons were conducted using the Dunn's test in order to evaluate an association between qualitative variables (the 3 study groups vs groups carrying different *VDR* genotypes). All analyses were conducted using STATISTICA v. 10.0 software (StatSoft, Tulsa, USA) and the calculator on the http://ihg.gsf.de/cgi-bin/hw/hwa1.pl website. P-values below 0.05 were considered as indicative of a statistical significance.

Results

The basic characteristics of the study subjects have been summarized in Table 1. The comparison of investigated groups has shown differences in BMI, which occurred significantly reduced in IBD patients than in controls. Moreover, CD patients presented lower BMI than UC patients. We observed decreased BMD of lumbar spine and FN in patients with CD and UC. Individuals with CD presented more advanced osteopenia and osteoporosis than patients with UC. However, we did not find statistically significant differences in vitamin D concentration between studied groups (Table 1). The genotypes distribution in examined groups was concordant with the Hardy–Weinberg equilibrium.

Table 1. Basic characteristics and clinical parameters of the study subjects.

Parameter mean	CD patients n = 85	UC patients n = 87	Controls n = 39	p-value
Age [years]	35.24 (SD 12.12)	39.40 (SD 14.43)	30.74 (SD 8.6)	$^{c}p = 0.040$
Weight [kg]	63.20 (SD 13.86)	68.63 (SD 14.92)	73.62 (SD 13.65)	$^{b}p < 0.001$
Height [cm]	171.39 (SD 10.30)	170.80 (SD 9.74)	172.69 (SD 9.35)	$p = 0.70$
BMI [kg/m^2]	21.36 (SD 3.62)	23.43 (SD 4.32)	24.57 (SD 3.45)	$^{a}p < 0.001$ $^{b}p < 0.001$
L2–L4 BMD [g/cm^2]	1.12 (SD 0.18)	1.16 (SD 0.14)	1.22 (SD 0.08)	$^{b}p < 0.001$ $^{c}p = 0.01$
L2–L4 T-score	–0.82 (SD 1.45)	–0.46 (SD 1.15)	0.09 (SD 0.70)	$^{b}p < 0.001$ $^{c}p = 0.007$
L2–L4 Z-score	–0.41 (SD 1.32)	–0.18 (SD 1.19)	0.09 (SD 0.66)	$^{b}p = 0.03$
FN BMD [g/cm^2]	0.95 (SD 0.18)	0.98 (SD 0.15)	1.07 (SD 0.16)	$^{b}p < 0.001$ $^{c}p = 0.003$
FN T-score	–0.65 (SD 1.28)	–0.31 (SD 1.14)	0.41 (SD 1.03)	$^{b}p < 0.001$ $^{c}p = 0.001$
FN Z-score	–0.27 (SD 1.08)	0.06 (SD 1.04)	0.38 (SD 0.99)	$^{b}p = 0.002$
25-OHD [ng/mL]	21.43 (SD 12.32)	22.06 (SD 9.27)	21.56 (SD 9.11)	$p = 0.77$

BMD – bone mineral density; CD – Crohn's disease; FN – femoral neck; ns – non-significant; SD – standard deviation; UC – ulcerative colitis. All results are presented as means with standard deviations (SD); ^{a}CD vs UC; ^{b}CD vs controls; ^{c}UC vs controls.

We observed that tt genotype was present with higher frequency in UC patients than in controls and CD patients (23% vs 7.7% and 16.5%, respectively). Comparing the whole group of IBD patients with controls we pointed out that tt genotype was almost 3 times more frequent than TT+Tt genotypes (OR = 2.96), but this observation was borderline significant (p = 0.07). The same analysis performed in UC group revealed that in these patients tt homozygotes occurred even more frequently (OR = 3.58, CI = 1.00–12.87, p-value = 0.04) (Table 2).

In the next step, we carried out the analysis of bone mass parameters and serum vitamin D levels in correlation with TaqI genotypes of *VDR* gene. Patients with CD did not significantly differ in lumbar spine (L2–L4) and FN BMD, T-score, Z-score, nor in 25(OH)D serum concentration (p > 0.05 for all comparisons); however, we have noticed slightly higher bone mass parameters values in tt genotype carriers, while in TT homozygotes they were the most decreased. Considering UC patients, lumbar spine values of BMD, T-score and Z-score as well as FN, have emerged higher in tt homozygotes even more clearly; the differences between FN BMD values in different genotypes carriers were statistically significant (overall p = 0.02, post hoc p: [tt] vs [Tt] = 0.02) (Table 3). In controls, we made the same observations, although in post hoc tests they remained borderline significant or insignificant.

Table 2. Alleles and genotypes frequency in *VDR* TaqI (rs731236, c.1056T>C) loci.

Group	Genotype frequencies (%)	Allele frequencies (%)			
	TT	Tt	tt	T	t
IBD (all patients), n = 172	59 (34.3)	79 (45.9)	34 (19.8)	197 (57.3)	147 (42.7)
UC patients, n = 87	31 (35.6)	36 (41.4)	20 (23.0)	98 (56.3)	76 (43.7)
CD patients, n = 85	28 (32.9)	43 (50.6)	14 (16.5)	99 (58.2)	71 (41.8)
Controls, n = 39	13 (33.3)	23 (59.0)	3 (7.7)	49 (62.8)	29 (37.2)
Group	**Comparisons of allelic and genotypic frequencies between groups under study**				
	[tt] vs [TT+Tt]	[TT] vs [Tt+tt]	[tt] vs [TT]	[T] vs [t]	[t] vs [T]
IBD vs controls OR, 95% CI p-value	OR = 2.96 (0.86–10.18) p = 0.07	OR = 1.04 (0.50–2.18) p = 0.91	OR = 2.45 (0.66–9.39) p = 0.17	OR = 0.79 (0.48–1.32) p = 0.37	OR = 1.26 (0.76–2.09)
UC vs controls OR, 95% CI p-value	**OR = 3.58 (1.00–12.87) p = 0.04**	OR = 1.11 (0.50–2.46) p = 0.80	OR = 2.80 (0.71–11.06) p = 0.13	OR = 0.76 (0.44–1.32) p = 0.33	OR = 1.31 (0.76–2.27)
CD vs controls OR, 95% CI p-value	OR = 2.37 (0.64–8.77) p = 0.19	OR = 0.98 (0.44–2.20) p = 0.97	OR = 2.17 (0.53–8.87) p=0.28	OR = 0.83 (0.48–1.43) p = 0.49	OR = 1.21 (0.70–2.10)
CD vs UC OR, 95% CI p-value	OR = 0.66 (0.31–1.41) p = 0.28	OR = 0.89 (0.47–1.67) p = 0.71	OR = 0.78 (0.33–1.82) p = 0.56	OR = 1.08 (0.71–1.66) p = 0.72	OR = 0.93 (0.60–1.42)

In bold were marked statistically significant ($p < 0.05$) and borderline results. CI – confidence intervals; OR – odds ratio.

Discussion

In this study, we analyzed *VDR* gene TaqI polymorphic variants and their relation to BMD parameters and serum vitamin D levels in a particular group of patients with IBD. The *VDR* gene was one of the first genes studied with regard to its possible role in the development of osteoporosis.[23–25] Vitamin D binds to a specific steroid receptor that has a transcription factor activity. The formation of vitamin D steroid receptor complex results in the activation or silencing of target gene expression. This leads to synthesis regulation of proteins that actively participate in bone metabolism and calcium homeostasis.[26] Alterations in the *VDR* gene may result in alterations of the structure, function and/or activity of the VDR protein, i.e., affect the transcription factor participating in the signal transduction from vitamin D to genes that are under its control. Studies on *VDR* gene polymorphisms showed divergent results in the analyzed groups of patients and populations.[27] A meta-analysis carried out by Xue et al. in 2013 describes 9 studies conducted in the years 1995–2011. What follows is that ff genotype of the FokI polymorphism in *VDR* gene is associated with a significant risk of UC in Asians, while the TaqI polymorphism (tt genotype) was associated with an increased risk of CD in Europeans as well as in Asians.[18] In turn, Wang et al. did not report any correlation between ApaI, BsmI and FokI polymorphisms and IBD. Wang et al. paid particular attention to differences in the genetic profiles of studied patients regarding ethnic differences between them. They have demonstrated no significant differences in the distribution of allele frequencies between the examined groups of IBD and controls, either any relationship between *VDR* polymorphisms or the occurrence of the disease.[20] In turn, other studies carried out in the European populations (UK, Germany) revealed the association of the TaqI polymorphism tt genotype with the occurrence of IBD. The authors found that tt genotype was more frequent in patients with CD with numerous fistulas and stenoses.[28] Interestingly, the latest meta-analysis conducted by Zhang et al. involved 17 studies on patients with postmenopausal osteoporosis and showed no significant relationship between *VDR* TaqI polymorphism and osteoporosis susceptibility in Caucasians and the overall populations as well.[29] An earlier report by Sikorska et al. is in concordance with Zhang's findings, although the Polish scientists pointed out the relation of *VDR* alteration with osteoporotic fractures susceptibility.[24] Another paper concerning Polish patients with UC has shown no differences in the distribution of *VDR* TaqI polymorphism in comparison to healthy control subjects.[30]

In our study, we observed the protective effect of the *VDR* gene TaqI t (c.1057T) allele on BMD in IBD patients and controls. Particularly, UC patients with tt genotypes had a significantly higher FN bone mass. Our observations are contrary to those reported by Morrison et al., who showed that tt homozygotes had a lower bone mass.[25] In the study by Noble et al., no association between TaqI *VDR* polymorphisms and bone mass was shown, and the only independent factor associated with osteoporosis was low BMI.[28] In our study, we did not show significant differences

Table 3. Analysis of the TaqI polymorphism genotypes with respect to BMD, T-score, Z-score and 25(OH)D concentration in the study groups

Parameter mean (SD)	Crohn's disease			
	TT n = 28	Tt n = 43	tt n = 14	p-value
L2–L4 BMD [g/cm^2]	1.11 (0.19)	1.12 (0.18)	1.15 (0.19)	p = 0.98
L2–L4 T-score	−0.87 (1.45)	−0.85 (1.44)	−0.61 (1.61)	p = 0.97
L2–L4 Z-score	−0.44 (1.41)	−0.44 (1.26)	−0.23 (1.40)	p = 0.90
FN BMD [g/cm^2]	0.94 (0.15)	0.95 (0.19)	0.96 (0.19)	p = 0.99
FN T-score	−0.71 (1.00)	−0.66 (1.36)	−0.52 (1.58)	p = 0.98
FN Z-score	−0.30 (0.82)	−0.28 (1.14)	−0.17 (1.41)	p = 0.92
25 OHD [ng/mL]	20.04 (12.44)	21.95 (11.47)	22.62 ±15.07	p = 0.62
Parameter	Ulcerative colitis			
	TT n = 31	Tt n = 36	tt n = 20	p-value
L2–L4 BMD [g/cm^2]	1.15 (0.12)	1.15 (0.16)	1.21 (0.13)	p = 0.14
L2–L4 T-score	−0.55 (1.09)	−0.52 (1.36)	−0.23 (0.78)	p = 0.41
L2–L4 Z-score	−0.21 (1.26)	−0.24 (1.32)	−0.03 (0.78)	p = 0.71
FN BMD [g/cm^2]	0.97 (0.14)	0.95 (0.16)	1.05 (0.12)	p = 0.02 [tt] vs [tt] p = 0.02 [tt] vs [tt] p = 0.08 [tt] vs [tt] p = 1.00
FN T-score	−0.36 (1.02)	−0.49 (1.30)	0.10 (0.94)	p = 0.09
FN Z-score	0.01 (0.99)	−0.06 (1.15)	0.37 (0.86)	p = 0.18
25 OHD [ng/mL]	23.60 (10.48)	20.97 (8.77)	21.63 (8.22)	p = 0.84
Parameter	Controls			
	TT n = 13	Tt n = 23	tt n = 3	p-value
L2–L4 BMD [g/cm^2]	1.25 (0.09)	1.20 (0.07)	1.31 (0.04)	p = 0.03 [tt] vs [tt] p = 0.08 [tt] vs [tt] p = 0.75 [tt] vs [tt] p = 0.21
L2–L4 T score	0.32 (0.66)	−0.13 (0.68)	0.80 (0.30)	p = 0.03 [tt] vs [tt] p = 0.07 [tt] vs [tt] p = 0.81 [tt] vs [tt] p = 0.15
L2–L4 Z score	0.34 (0.51)	−0.11 (0.70)	0.54 (0.52)	p = 0.03 [tt] vs [tt] p = 0.19 [tt] vs [tt] p = 1.00 [tt] vs [tt] p = 0.06
FN BMD [g/cm^2]	1.04 (0.12)	1.10 (0.18)	1.03 (0.14)	p = 0.58
FN T score	0.28 (0.76)	0.53 (1.19)	0.12 (0.81)	p = 0.77
FN Z score	0.31 (0.66)	0.46 (1.18)	0.10 (0.62)	p = 0.93
25 OHD [ng/mL]	21.44 (7.11)	22.04 (10.65)	18.34 (2.86)	p = 0.77

All results are presented as means with standard deviations (SD); BMD – bone mineral density; FN – femoral neck; ns – non-significant; SD – standard deviation.

in serum vitamin D concentrations between individuals carrying different polymorphic variants; moreover, our IBD patients did not presented any differences in serum 25(OH)D in comparison to controls. However, in the study conducted on a group of 308 patients with CD in New Zealand, a country with one of the highest incidence rates for this disease in the world, lower vitamin D concentrations were found in IBD patients compared to healthy individuals. These authors correlated low concentrations of 25(OH)D with age and low exposure to sunlight.[31]

Polymorphism analyzed in our study has been frequently studied with regard to its contribution to the development of osteoporosis in populations all over the world. The results remained equivocal or even contradictory. Molecular diagnostics based on analyzing allelic variants of candidate genes potentially associated with altered bone turnover in IBD patients would allow us to estimate the predisposition to developing osteoporosis long before the first symptoms appear. This would make it possible to apply prophylaxis or therapies, thus delaying osteoporosis development, which would considerably improve the quality of IBD patients' lives and reduce the cost of healthcare.

Conclusions

In conclusion, the *VDR* gene TaqI polymorphism may be related to BMD. We suggest that tt genotype has a protective effect on BMD particularly in UC patients.

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