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ORIGINAL RESEARCH

Association between vitamin D receptor polymorphisms and osteoporosis in patients with COPD

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Background: Patients with COPD are at an increased risk of osteoporosis. Although many studies have addressed the relationship between the vitamin D receptor (*VDR*) polymorphisms and bone health, this relationship has not been fully investigated in patients with COPD. In this study, we investigated the association of *VDR* polymorphisms with bone mineral density (BMD) and other clinical parameters in patients with COPD.

Patients and methods: In total, 200 patients with COPD were included in this study. The *VDR* polymorphisms rs1544410 (A/G-*Bsm*I), rs7975232 (A/C-*Apa*I), rs731236 (C/T-*Taq*I), and rs10735810 (C/T-*Fok*I) were determined by Sanger sequencing using blood DNA samples. BMD of the lumbar vertebra and the femoral neck was measured by dual-energy X-ray absorptiometry. Other clinical parameters were also evaluated. Haplotype and multivariate analyses were also performed.

Results: Sex, body mass index, steroid use, percentage of forced expiratory volume in 1 second (FEV_1), alkaline phosphatase, and 25-hydroxyvitamin D significantly influenced the risk of osteoporosis. Patients with osteoporosis were more likely to carry the rs7975232 C allele compared to normal patients with BMD. Haplotypes GCT and GAT were related to osteoporosis. Patients without the haplotype GAT allele showed a significantly lower T-score at the femoral neck and an increased risk of osteoporosis (odds ratio [OR]= 2.78, 95% confidence interval [CI]= 1.20–6.48, P=0.018) compared with carriers in the dominant model.

Conclusion: Genetic variations in *VDR* are significantly associated with osteoporosis among patients with COPD. Further studies are required to confirm the role of the *VDR* polymorphisms in osteoporosis among patients with COPD.

Keywords: chronic obstructive pulmonary disease, osteoporosis, vitamin D receptor gene, polymorphism, haplotype

Introduction

COPD is a major cause of chronic morbidity and mortality worldwide.[1] Predictions from the Global initiative for chronic Obstructive Lung Disease (GOLD) indicate that COPD will rise from the sixth to the third highest mortality rate by 2020 worldwide.[2] Additionally, with aging populations, the burden of COPD has been consistently increasing.[3] Although COPD is primarily a pulmonary disease, it has been linked to many extra-pulmonary comorbidities, leading to a significant burden of disease.[4,5]

Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of the bone, leading to increased fragility and a high risk of fracture.[6] COPD and osteoporosis are strongly related because of common risk factors, such as age, smoking, inactivity, vitamin D deficiency, and the use of systemic corticosteroids.[7–10] In patients with COPD, the prevalence of

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osteoporosis is approximately two to five times higher than that in the same age group without airflow limitation.[8] However, our ability to predict the occurrence of osteoporosis in patients with COPD is still limited.

In general population studies, genetic factors are often suggested as potential risk factors for osteoporosis.[11] Among the multiple candidate genes investigated in osteoporosis, the vitamin D receptor (*VDR*) was the first,[12] and it has been actively discussed in other studies.[11,13–15] The *VDR* gene has four polymorphisms (rs1544410, rs7975232, rs731236, and rs10735810) that are most frequently studied in considering the association of BMD with osteoporosis.[6,16–19] Although many studies have investigated the association between *VDR* polymorphisms and bone health, the results were conflicting. This may be due to heterogeneous study populations, small sample size, different ethnicities, and environmental factors. To find the association of *VDR* polymorphisms with BMD and other clinical parameters in patients with COPD, we narrowed the study population to stable Korean patients with COPD, and the factors that could affect bone metabolism were investigated and controlled. This is the first study of *VDR* gene polymorphism and osteoporosis in patients with COPD.

Patients and methods

Study participants

In total, 200 patients at St Paul's Hospital who were diagnosed with COPD using the post-bronchodilator pulmonary function test were included in this prospective study. COPD was diagnosed according to the guidelines of the American Thoracic Society/European Respiratory Society[20] and categorized in accordance with the Global initiative for chronic Obstructive Lung Disease guidelines.[21] The inclusion criteria were 1) men over 50 years old and postmenopausal women and 2) patients who had a pulmonary function test and dual energy X-ray absorptiometry (DEXA) 3 months before or after registration. The exclusion criteria were subjects who had 1) an acute exacerbation within 6 weeks of registration; 2) other active pulmonary or infectious diseases; 3) any other diseases that could affect bone metabolism, such as hyperthyroidism, parathyroid disease, diabetes mellitus, malignancy, chronic kidney disease, connective tissue disease, pituitary disease, and adrenal disease; 4) history of using vitamin D, calcium, or bisphosphonate; 5) steroid use for diseases other than COPD and; 6) walking difficulty more than 1 month before DEXA. Patients were enrolled from January 2012 to January 2014. This study was approved by the institutional review board of St Paul's Hospital. All patients provided written informed consent before study registration.

Clinical measurements

Demographic information, sex, age, body mass index (BMI), and smoking history were collected. The enrolled patients' cumulative alcohol and steroid dose were taken from medical records or interviews. Cumulative steroid dose was calculated from COPD diagnosis to study registration. Oral and intravenous steroid dose were combined to give a prednisolone equivalent dosage, and the inhaled steroid dose was assessed to give a budesonide equivalent dosage. Pulmonary function tests were performed using a SensorMedics Vmax 229 (VIASYS Healthcare, Yorba Linda, CA, USA). Biochemical parameters related to bone metabolism, such as calcium, phosphorus, alkaline phosphatase (ALP), 25-hydroxyvitamin D (25(OH)D), and parathyroid hormone were measured.

BMD measurement

BMD (amount of mineral matter per square centimeter of bones) is used as an indirect indicator of osteoporosis and fracture risk. BMD was measured by DEXA using a DPX Bravo (GE Healthcare, Milwaukee, WI, USA) at the lumbar spine and the femoral neck. For the lumbar BMD evaluation, we used the average value for lumbar vertebrae 1–4 (L1–L4), excluding any vertebral fractures, degenerative changes, and calcifications. We classified the subjects into three groups based on the World Health Organization criteria: normal, osteopenia, and osteoporosis. Osteoporosis was defined as a T-score of −2.5 or less, osteopenia was defined as a T-score between −2.5 and −1.0, and normal was defined as a T-score of −1.0 or higher.[22]

Genotyping

Genomic DNA was extracted from peripheral blood using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). Molecular genetic studies were performed by BIOFACT© (Daejeon, South Korea). PCR/DNA sequencing was used to determine the genotype of four single-nucleotide polymorphisms (SNPs) in the *VDR* gene. Primer information for each SNP is given in Table S1. Briefly, the region of the *VDR* gene that encompasses each SNP was amplified by PCR using purified genomic DNA. The DNA amplicons were sequenced using the same PCR primers with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). The products were cleaned with the Big Dye XTerminator Purification Kit (Thermo Fisher Scientific) and resolved with the ABI 3730xl Genetic Analyzer (Thermo Fisher Scientific). The data were analyzed using the ABI Data Collection software v3.0 (Thermo Fisher Scientific), Sequencing Analysis software v5.4 (Thermo Fisher Scientific), and SeqScape software v2.6 (Thermo Fisher Scientific).

Statistical analysis

Means and standard deviation were computed for normally distributed continuous variables, whereas medians and interquartile ranges (25th–75th) were used for non-normally distributed continuous data. Categorical data were described as numbers and percentages. For the genotype distribution, the Hardy–Weinberg equilibrium was tested for each SNP by the standard χ^2 test. For comparison of the continuous variables between subgroups, Student's *t*-test was performed for normally distributed data and Mann–Whitney *U* test was used for non-normally distributed data. Univariate comparison between categorical variables was made using the χ^2 and Fisher's exact tests when appropriate. The χ^2 test or the Fisher's exact test was also used to analyze the frequency of the genotype, the allele, and the haplotype. An independent *t*-test or analysis of variance (ANOVA) test was used to find the relationship between the genotype and the T-score. Logistic regression analysis was performed to evaluate the effects of genetic variations of *VDR* on osteoporosis. Statistical analyses were performed using the SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and R Statistical Programming Language version 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria). A *P*-value <0.05 was considered significant.

Results

Baseline characteristics of the study group

The basic characteristics of the patients are shown in Table 1. Osteoporosis was present in 27.5%, osteopenia in 49.0%, and normal BMD in 23.5% of the total patients with COPD. The osteoporosis group had significantly more women ($P=0.031$ and $P=0.010$) and a lower average BMI ($P<0.001$, both) compared with the nonosteoporosis

Table 1 Basic characteristics of the study group (n=200)

	Normal BMD (n=47)	Osteopenia (n=98)	Osteoporosis (n=55)	*P*-value	
				Osteoporosis vs nonosteoporosis[a]	Osteoporosis vs normal BMD
Sex					
Male	46 (97.9%)	89 (90.8%)	45 (81.8%)	**0.031**	**0.010**
Female	1 (2.1%)	9 (9.2%)	10 (18.2%)		
Age (years)	73 (70–75)	72.0 (68.0–77.0)	74.0 (69.0–78.0)	0.228	0.429
BMI (kg/m^2)	24.4 (21.9–26.9)	23.0 (20.7–25.5)	20.6 (19.0–23.2)	**<0.001**	**<0.001**
Smoking					
Current	14 (29.8%)	24 (24.5%)	14 (25.5%)	0.321	0.293
Ex	30 (63.8%)	64 (65.3%)	32 (58.2%)		
Never	3 (6.4%)	10 (10.2%)	9 (16.4%)		
Pack-years	37.0 (20.0–52.0)	26.5 (12.8–48.0)	36.0 (15.0–54.0)	0.601	0.732
Alcohol					
Yes	37 (78.7%)	68 (69.4%)	25 (45.5%)	**<0.001**	**0.001**
No	10 (21.3%)	30 (30.6%)	30 (54.5%)		
Cumulative alcohol (kg)[b]	165.2 (23.7–428.0)	86.6 (0–319.7)	0.0 (0–115.1)	**<0.001**	**<0.001**
Steroid use					
Yes	25 (53.2%)	64 (65.3%)	41 (74.5%)	0.097	**0.037**
No	22 (46.8%)	34 (34.7%)	14 (25.5%)		
Cumulative oral or IV (mg)[c]	0.0 (0–40.0)	0.0 (0–200.4)	0.0 (0–560.0)	0.052	**0.028**
Cumulative inhaled (mg)[d]	0.0 (0–671.6)	74.7 (0–850.8)	150.0 (0–1,490.4)	**0.040**	**0.031**
Pulmonary function test					
FEV_1 (% predicted)	83.2±21.1	77.1±20.8	68.0±21.7	**0.001**	**0.001**
FVC (% predicted)	97.3±20.1	95.7±17.1	93.7±17.6	0.371	0.340
FEV_1/FVC (%)	57.2±7.9	53.8±9.9	48.8±13.1	**<0.001**	**<0.001**
Bone metabolic markers					
Calcium (mg/dL)	9.1±0.6	9.2±0.6	9.0±0.6	0.069	0.302
Phosphorus (mg/dL)	3.5±0.7	3.2±0.7	3.3±0.5	0.851	0.144
ALP (U/L)	204.0 (161.0–254.0)	232.5 (190.8–289.5)	272.0 (204.5–311.3)	**0.003**	**<0.001**
25(OH)D (nmol/L)	14.2 (9.7–19.2)	11.7 (8.5–17.3)	9.6 (7.1–15.0)	**0.013**	**0.003**
PTH (pmol/L)	25.2 (19.6–31.0)	25.6 (18.6–35.1)	29.8 (22.4–42.5)	0.061	0.081

Notes: Data represent the mean ± SD, median (IQR) or n (%). *P*-values shown in bold are significant at the 0.05 level. [a]Nonosteoporosis group = normal BMD + osteopenia group. [b]Cumulative alcohol dose was calculated with alcohol degree converter. [c]Cumulative oral or intravenous steroid dose were combined with prednisolone equivalent dosage. [d]Cumulative inhaled steroid dose was calculated with budesonide equivalent dosage.

Abbreviations: BMD, bone mineral density; BMI, body mass index; IQR, interquartile range; IV, intravenous; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ALP, alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

(normal BMD + osteopenia group) and the normal BMD group. The osteoporosis group had significantly less alcohol history ($P<0.001$ and $P=0.001$), cumulative alcohol dose ($P<0.001$, both), and forced expiratory volume in 1 second (FEV_1 %, $P=0.001$, both) compared with the nonosteoporosis or the normal BMD group. History of steroid use ($P=0.037$), cumulative dose of oral or intravenous steroid ($P=0.028$), and cumulative dose of inhaled steroid ($P=0.031$) were significantly higher in the osteoporosis group compared with the normal BMD group. With regard to the bone metabolic markers, the osteoporosis group showed a significantly higher ALP ($P=0.003$ and $P<0.001$) and significantly lower 25(OH)D ($P=0.013$ and $P=0.003$) compared with the nonosteoporosis and the normal BMD group.

Association of *VDR* allele frequencies and genotypes with osteoporosis in COPD patients

The minor allele frequency (MAF) of the rs1544410, rs7975232, rs731236, and rs10735810 polymorphisms were 0.04, 0.22, 0.05, and 0.41, respectively, in total patients with COPD. The MAF and genotype frequency of the four SNPs of the *VDR* gene in the subgroups are shown in Table 2. No differences were identified between the osteoporosis group and the nonosteoporosis or normal BMD groups in the MAF of rs1544410, rs731236, and rs10735810. However, the MAF of rs7975232 was significantly different between the osteoporosis group and the nonosteoporosis or normal BMD group ($P=0.041$ and $P=0.016$). All of the genotype distributions were followed using the Hardy–Weinberg equilibrium ($P>0.05$). Genotype distribution of the *VDR* rs7975232 was significantly different in the osteoporosis group compared with the normal BMD group in all models ($P=0.026$, $P=0.042$, and $P=0.044$ for the additive, dominant, and recessive models, respectively). No significant association between the average BMD values (T-score of femoral neck and L-spine) and SNPs in the *VDR* gene was found in any of the models (Table S2). There was no significant relationship between the *VDR* polymorphisms and the level of 25(OH)D in our study (data not shown).

Association of haplotype frequencies and genotypes with osteoporosis in COPD patients

The pairwise linkage disequilibrium (LD) among all four polymorphisms of the *VDR* genes was estimated using the maximum likelihood of D (D′) and a measure of LD association (r^2).[23] Strong LD was observed between two SNPs in the 3′ region, rs1544410 and rs731236. The correlation coefficient r^2 of rs10735810 with the other three SNPs were very low ($r^2=0.002–0.072$); however, the r^2 for rs1544410–rs731236 was 0.836, for rs1544410–rs7975232 was 0.152, and for rs7975232–rs731236 was 0.126. Therefore, we excluded rs10735810 in the haplotype analysis. Several studies have shown that the rs10735810 polymorphism has no linkage to any of the other *VDR* polymorphisms.[24] Haplotypes, other than GCT, GAT, and AAC were excluded, as their frequency was less than 1%. Carrying the GCT

Table 2 Association of *VDR* allele frequencies and genotypes with osteoporosis in patients with COPD

Polymorphism	Genotype			MAF	Subgroup comparison	*P*-value			
						Additive	Dominant	Recessive	MAF
rs1544410	AA	AG	GG		Osteoporosis vs nonosteoporosis*	0.772	NA	0.772	0.776
Normal BMD	0 (0.0%)	5 (10.6%)	42 (89.4%)	0.053					
Osteopenia	0 (0.0%)	6 (6.1%)	92 (93.9%)	0.031	Osteoporosis vs normal BMD	1.000	NA	1.000	1.000
Osteoporosis	0 (0.0%)	5 (9.1%)	50 (90.9%)	0.045					
rs7975232	AA	AC	CC		Osteoporosis vs nonosteoporosis*	0.081	0.193	0.075	**0.041**
Normal BMD	4 (8.5%)	19 (40.4%)	24 (51.1%)	0.287					
Osteopenia	3 (3.1%)	37 (37.8%)	58 (59.2%)	0.219	Osteoporosis vs normal BMD	**0.026**	**0.042**	**0.044**	**0.016**
Osteoporosis	0 (0/0%)	16 (29.1%)	39 (70.9%)	0.145					
rs731236	TT	TC	CC		Osteoporosis vs nonosteoporosis*	0.814	1.000	1.000	1.000
Normal BMD	42 (89.4%)	5 (10.6%)	0 (0.0%)	0.053					
Osteopenia	90 (91.8%)	7 (7.1%)	1 (1.0%)	0.046	Osteoporosis vs normal BMD	1.000	NA	1.000	1.000
Osteoporosis	50 (90.95%)	5 (9.1%)	0 (0.0%)	0.045					
rs10735810	CC	CT	TT		Osteoporosis vs nonosteoporosis*	0.949	0.823	1.000	0.821
Normal BMD	14 (29.8%)	25 (53.2%)	8 (17.0%)	0.436					
Osteopenia	32 (32.7%)	53 (54.1%)	13 (13.3%)	0.403	Osteoporosis vs normal BMD	0.820	0.585	0.832	0.670
Osteoporosis	18 (32.7%)	30 (54.5%)	7 (12.7%)	0.400					

Notes: *Nonosteoporosis group = normal BMD + osteopenia group. *P*-values shown in bold are significant at the 0.05 level.
Abbreviations: *VDR*, vitamin D receptor; BMD, bone mineral density; MAF, minor allele frequency.

haplotype was associated with osteoporosis (Table 3), as was not carrying the GAT haplotype. The BMD values (T-score of the femoral neck and the L-spine) according to haplotype genotypes are shown in Table S3. The T-score of the femoral neck was significantly lower in the subgroup that was not carrying the GAT haplotype in the dominant model (P=0.040).

Multivariate analysis of *VDR* genotypes and haplotypes in patients with COPD

Multivariate logistic regression analysis for osteoporosis in patients with COPD was performed. BMI, alcohol use, steroid use, FEV_1 (%), ALP, and 25(OH)D were all significantly different in the osteoporosis group compared with the normal group and were therefore included as covariates. After a stepwise selection, BMI, cumulative alcohol dose, and FEV_1 (%) were entered as variables into the model. In the GAT haplotype dominant model, the group without the GAT haplotype included significantly more patients with osteoporosis (odds ratio [OR] =2.783, P=0.018) compared with the group containing the GAT haplotype (Table 4). The rs7975232 recessive model (OR =2.093, P=0.057) and GCT haplotype recessive model (OR =2.117, P=0.059) showed statistically borderline results.

Discussion

Studies investigating twins and families have shown that osteoporosis has a strong genetic component, and up to 50% of the variance in BMD can be attributed to genetic factors.[25,26] The *VDR* gene is composed of a 5′ promotor (from exon 1a to f), coding exons (exon 2–9), and a 3′ untranslated region in chromosome 12q13.1.[27] Morrison et al first showed that the polymorphisms located at the 3′ end of the *VDR* gene, as determined by the restriction enzymes *Bsm*I, *Apa*I, and *Taq*I, were related to BMD.[12] As polymorphisms at the *VDR* gene locus have been suggested to be associated with bone mass, the *VDR* gene polymorphisms were considered important for osteoporosis.[24,28]

In this study, we investigated the role and influence of four *VDR* polymorphisms in patients with COPD: rs1544410 (A/G-*Bsm*I), rs7975232 (A/C-*Apa*I), rs731236 (C/T-*Taq*I), and rs10735810 (C/T-*Fok*I). The rs7975232 (A/C-*Apa*I), haplotype GCT, and GAT polymorphisms were related to osteoporosis in our study. Patients not carrying the GAT haplotype showed a significantly lower T-score at the femoral neck in the dominant model. Furthermore, not carrying the GAT haplotype was associated with osteoporosis in the dominant model after adjustment of confounders.

Low body weight, low FEV_1, smoking, older age, female sex, inactivity, systemic inflammation, systemic corticosteroids, and vitamin D deficiency are known COPD-related risk factors for osteoporosis.[8,29,30] In our study, sex, BMI, steroid use, FEV_1, and vitamin D deficiency were confirmed as risk factors of osteoporosis in patients with COPD. Meanwhile, smoking was unrelated, and alcohol consumption was significantly lower in COPD patients with osteoporosis. This was mainly due to the high proportion of females in the osteoporosis group. Korean society traditionally places a taboo on women who smoke or drink. Among females in our study, there was one current, four ex-, and 15 never-smokers, compared with 51 current, 122 ex-, and seven never-smokers among the males. Additionally, all females in our study had no history of alcohol consumption, whereas 72.2% of men had a history of alcohol. Serum ALP is the most commonly

Table 3 Association of haplotype frequencies and genotypes with osteoporosis in patients with COPD

Haplotypes	Genotype			Haplotype frequency	Subgroup comparison	*P*-value			
						Additive	Dominant	Recessive	Haplotype frequency
GCT	GCT/GCT	GCT/–	–/–		Osteoporosis vs nonosteoporosis*	0.063	0.110	0.055	**0.030**
Normal BMD	24 (51.1%)	19 (40.4%)	4 (8.5%)	67 (71.3%)					
Osteopenia	57 (58.2%)	37 (37.8%)	4 (4.1%)	151 (77.0%)	Osteoporosis vs normal BMD	**0.027**	**0.042**	**0.044**	**0.016**
Osteoporosis	39 (70.9%)	16 (29.1%)	0 (0.0%)	94 (85.5%)					
GAT	GAT/GAT	GAT/–	–/–		Osteoporosis vs nonosteoporosis*	0.054	**0.028**	0.325	**0.018**
Normal BMD	2 (4.3%)	18 (38.3%)	27 (57.4%)	22 (23.4%)					
Osteopenia	3 (3.1%)	30 (30.6%)	65 (66.3%)	36 (18.4%)	Osteoporosis vs normal BMD	**0.028**	**0.018**	0.210	**0.013**
Osteoporosis	0 (0.0%)	11 (20.0%)	44 (80.0%)	11 (10.0%)					
AAC	AAC/AAC	AAC/–	–/–		Osteoporosis vs nonosteoporosis*	0.772	0.772	NA	0.776
Normal BMD	0 (0.0%)	5 (10.6%)	42 (89.4%)	5 (5.3%)					
Osteopenia	0 (0.0%)	6 (6.1%)	92 (93.9%)	6 (3.1%)	Osteoporosis vs normal BMD	1.000	1.000	NA	1.000
Osteoporosis	0 (0.0%)	5 (9.1%)	50 (90.9%)	5 (4.5%)					

Notes: SNPs in haplotypes are in order of rs1544410, rs7975232, and rs731236. *Nonosteoporosis group = normal BMD + osteopenia group. *P*-values shown in bold are significant at the 0.05 level.

Abbreviations: BMD, bone mineral density; SNPs, single-nucleotide polymorphisms.

Table 4 Multivariate logistic regression analysis for the GAT dominant type in patients with COPD (n=200)

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR	*P*-value
BMI (kg/m^2)	0.748 (0.661–0.845)	**<0.001**	0.739 (0.648–0.842)	**<0.001**
Cumulative alcohol (kg)[a]	0.998 (0.997–1.000)	**0.018**	0.998 (0.997–1.000)	**0.009**
FEV_1 (% predicted)	0.975 (0.960–0.991)	**0.002**	0.974 (0.956–0.993)	**0.006**
Not carrying GAT haplotype[b]	2.304 (1.097–4.840)	**0.027**	2.783 (1.196–6.476)	**0.018**

Notes: [a]Cumulative alcohol dose was calculated with alcohol degree converter. [b]Reference = GAT/GAT or GAT/–. *P*-values shown in bold are significant at the 0.05 level.
Abbreviations: BMI, body mass index; CI, confidence interval; FEV_1, forced expiratory volume in 1 second; OR, odds ratio.

used biomarker of bone formation, showing a higher value in patients with osteoporosis compared with normal subjects.[31] In our study, serum ALP was significantly increased in the osteoporosis group.

Although we did not investigate the relationship between *VDR* polymorphism and osteoporosis in healthy population without COPD, several studies have investigated the association of the *VDR* gene with BMD, especially using *Bsm*I, *Apa*I, *Taq*I, and *Fok*I. However, the results have been inconsistent.[6,28,32,33] Morita et al investigating *Apa*I, *Taq*I, and *Fok*I found that the effect of the *VDR* genotype on bone mass was negligible in Japanese women.[32] Several other studies reported that the *VDR* gene has no significant effect in BMD.[11,28,33–35] However, other studies have shown a relationship between the *VDR* genotype and osteoporosis.[13,36–40] A meta-analysis by Zintzaras et al showed that the *Bsm*I polymorphism was more closely related to osteoporosis than were the *Apa*I, *Taq*I, and *Fok*I polymorphisms.[6] In Korea, the *Fok*I polymorphism has been suggested as the factor associated with osteoporosis in postmenopausal women.[41,42] These inconsistent results are mainly due to different study populations, insufficient sample size, different ethnicities, high allelic heterogeneity, variable LD across population groups, and environmental factors that would have confounded the underlying genetic effect.[23] In our study, the patient population was selected from stable Korean patients with COPD, and the factors that could affect bone metabolism were investigated and controlled. Although direct comparisons are difficult, there are a few *VDR* polymorphism genotyping data in Korean healthy subjects regardless of osteoporosis diagnosis.[43–47] Compared with our results, rs1544410, rs731236, and rs10735810 showed the almost similar genotype distribution in normal BMD, osteopenia, and osteoporosis group. However, rs7975232 genotype distribution showed a difference in osteoporosis group.

In this study, we performed haplotype analysis. When there are multiple polymorphisms within the same gene, information contained at each polymorphic site is "linked" to its neighbors. Therefore, inheriting one polymorphism means a high likelihood of inheriting the neighboring polymorphism.[48] Compared with single SNP analysis, a haplotype study gives more information and has an advantage in disease association studies, as it gives the cumulative effect of all SNPs in that gene.[23] Although there was no association between the *Bsm*I–*Apa*I–*Taq*I haplotype and any osteoporotic type in the GENOMOS study,[11,49] Thakkinstian et al reported an association between the BAT haplotype and osteoporosis (OR =4.21 [CI =2.2–8.10], $P<0.001$).[48] Uitterlinden et al showed an association between the bAT haplotype and low BMD and subsequently, an overrepresentation of the baT haplotype among fracture cases.[50] In our study, the GCT (baT) and GAT (bAT) haplotypes were related to osteoporosis. Especially, not carrying the GAT haplotype was a risk factor for osteoporosis. Taken together, the results from the haplotype analysis suggest the strong effect of rs7975232.

We are aware of the several limitations of this study. First, the sample size was not large enough to reach definitive conclusions. However, there has been no prior *VDR* polymorphism research in patients with COPD with osteoporosis. Repetitive, large-scale population studies are warranted in the future. Second, although the prevalence of osteoporosis is high in severe patients with COPD, most patients in this study were considered to be suffering from mild-to-moderate COPD. Many severe patients with COPD were excluded from the study due to recent acute exacerbations, comorbidities, and pulmonary infections. Third, in our study, the population was weighted toward men. Sex differences in COPD have been suggested by other studies;[51,52] therefore, subgroup analysis by sex in a large population is needed. Fourth, no index representing physical activity was included in our study. Physical activity is one of the important factors in both COPD and osteoporosis. A detailed physical activity questionnaire or a 6-minute walk test is warranted in future studies.

Conclusion

Genetic variations in *VDR* are significantly associated with osteoporosis among patients with COPD. In this study, the

rs7975232 C allele and GCT haplotype were risk factors for osteoporosis in patients with COPD. Furthermore, not carrying haplotype GAT was associated with osteoporosis prevalence and low BMD in the dominant model. Further studies are needed to confirm the role of *VDR* polymorphisms in osteoporosis among patients with COPD.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Primers of vitamin D receptor (*VDR*) polymorphisms

Name of gene	Sequence of primer
rs1544410	Forward: TACTTTGCTGGTTTGCAGAGCC
	Reverse: CTTTGGACCTCATCACCGACA
rs7975232	Forward: TCTGGATCATCTTGGCATAGAG
	Reverse: TAGCAGAGCAGAGTTCCAAGC
rs731236	Forward: TCTGGATCCTAAATGCACGGAG
	Reverse: AGTCAGGAGATCTCATTGCCAA
rs10735810	Forward: CATCTGAAACCAGGCAGCTGA
	Reverse: TGTGGCTGTGAGCGCCGCAT

Table S2 Association of *VDR* genotypes and bone mineral density (T-score) in patients with COPD

	Additive			*P*-value	Dominant		*P*-value	Recessive		*P*-value
rs1544410	AA (n=0)	AG (n=16)	GG (n=184)							
Femoral neck	NR	−1.4±1.3	−1.3±1.1	0.655	NA	NA	NA	NA	NA	NA
L-spine	NR	−1.5±1.4	−1.3±1.7	0.571	NA	NA	NA	NA	NA	NA
rs7975232	AA (n=7)	AC (n=72)	CC (n=121)		AC + CC (n=193)	AA (n=7)		CC (n=121)	AA + AC (n=79)	
Femoral neck	−0.7±1.2	−1.2±1	−1.4±1.2	0.184	−1.3±1.1	−0.7±1.2	0.119	−1.4±1.2	−1.2±1.0	0.189
L-spine	−0.4±0.8	−1.2±1.9	−1.4±1.5	0.220	−1.4±1.7	−0.4±0.8	0.399	−1.4±1.5	−1.1±1.8	0.215
rs731236	TT (n=182)	TC (n=17)	CC (n=1)		TT + TC (n=199)	CC (n=1)		TT (n=182)	TC + CC (n=18)	
Femoral neck	−1.3±1.1	−1.4±1.3	−0.7	0.801	−1.3±1.1	−0.7	0.580	−1.3±1.1	−1.3±1.3	0.812
L-spine	−1.3±1.7	−1.5±1.4	−1.8	0.879	−1.3±1.7	−1.8	0.771	−1.3±1.7	−1.5±1.4	0.638
rs10735810	CC (n=64)	CT (n=108)	TT (n=28)		CC + CT (n=172)	TT (n=28)		CC (n=64)	CT + TT (n=136)	
Femoral neck	−1.2±1.2	−1.4±1.1	−1.3±1.1	0.637	−1.3±1.1	−1.3±1.1	0.739	−1.2±1.2	−1.4±1.1	0.446
L-spine	−1.4±1.6	−1.4±1.7	−1.0±1.6	0.541	−1.4±1.7	−1.0±1.6	0.275	−1.4±1.6	−1.3±1.7	0.624

Note: Data represent the mean ± SD.
Abbreviations: L-spine, lumbar spine 1–4; NA, not applicable; NR, no result; *VDR*, vitamin D receptor.

Table S3 Association of haplotype genotypes and BMD (T-score) in patients with COPD

	Additive			*P*-value	Dominant		*P*-value	Recessive		*P*-value
GCT	GCT/GCT (n=120)	GCT/– (n=72)	–/– (n=8)		GCT/GCT + GCT/– (n=192)	–/– (n=8)		GCT/GCT (n=120)	GCT/– + –/– (n=80)	
Femoral neck	−1.4±1.2	−1.2±1.0	−0.7±1.1	0.151	−1.4±1.1	−0.7±1.1	*0.096*	−1.4±1.2	−1.2±1.0	0.165
L-spine	−1.4±1.5	−1.2±1.9	−0.6±0.9	0.293	−1.4±1.7	−0.6±0.9	*0.200*	−1.4±1.5	−1.1±1.8	0.232
GAT	GAT/GAT (n=5)	GAT/– (n=59)	–/– (n=136)		GAT/GAT + GAT/– (n=64)	–/– (n=136)		GAT/GAT (n=5)	GAT/– + –/– (n=195)	
Femoral neck	−1.1±1.1	−1.1±0.9	−1.4±1.2	0.123	−1.1±0.9	−1.4±1.2	**0.040**	−1.1±1.1	−1.3±1.1	0.597
L-spine	−0.4±0.8	−1.1±2.0	−1.5±1.5	0.161	−1.0±1.9	−1.5±1.5	*0.085*	−0.4±0.8	−1.3±1.7	0.228
AAC	AAC/AAC (n=0)	AAC/– (n=16)	–/– (n=184)							
Femoral neck	NR	−1.4±1.3	−1.3±1.1	0.655	NA	NA	NA	NA	NA	NA
L-spine	NR	−1.5±1.4	−1.3±1.7	0.572	NA	NA	NA	NA	NA	NA

Notes: SNPs in haplotypes are in order of rs1544410, rs7975232 and rs731236. Data represent the mean ± SD. *P*-values shown in bold are significant at the 0.05 level.
Abbreviations: BMD, bone mineral density; L-spine, lumbar spine 1–4; NA, not applicable; NR, no result; SNPs, single-nucleotide polymorphisms.