Association between the vitamin D receptor gene polymorphism and osteoporosis

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Abstract. The influence of the vitamin D receptor (*VDR*) gene for the risk of osteoporosis remains to be elucidated. The aim of the present study was to understand the distribution of various single-nucleotide polymorphisms (SNPs) within the *VDR* gene and its association with the risk of osteoporosis. In total, 378 subjects without a genetic relationship were recruited to the study between January 2013 and July 2015. The subjects were divided into three groups, which were the normal (n=234), osteoporosis (n=65) and osteoporosis with osteoporotic fracture (n=79) groups. Three pertinent SNPs of the *VDR* gene rs17879735 (*Apa*I, Allele A/a, SNP C>A) were examined with polymerase chain reaction-restriction fragment length polymorphism. The bone mineral density (BMD) of the lumbar spine (L2–L4), femoral neck, Ward's and Tro was measured using dual-energy X-ray absorptiometry. The distributions of genotype frequencies aa, AA and Aa were 48.68, 42.86 and 8.46%, separately. Following analysis of each site, BMD, body mass index (BMI) and age, BMD for each site was negatively correlated with age (P<0.01) and positively correlated with BMI (P<0.01). Correction analysis revealed that there were significant differences in the Ward's triangle BMD among each genotype (P<0.05), in which the aa genotype exhibited the lower BMD (P<0.05). No significant difference was identified among the different genotypes in the occurrence of osteoporosis with osteoporotic fracture (P>0.05). In conclusion, these indicated that the *VDR* gene *Apa*I polymorphisms had an important role in the osteoporosis risk.

Introduction

Osteoporosis is a multifactorial skeletal disease characterized by a reduced bone mineral density (BMD) and increased fracture risk, which is an increasing health problem (1). In certain studies, environmental, twin and family studies have shown that BMD and bone turnover are under strong genetic control (2,3). Genetic revelations have started elucidating the complex associations of vitamin D signaling and bone health (4-6). The vitamin D endocrine system has a role in the intestinal absorption of calcium and phosphate. This action of vitamin D is mediated through vitamin D receptor (*VDR*) that specifically binds to 1,25-dihydroxyvitamin D3 for the regulation of skeletal development, maintenance of skeletal architecture, hormone secretion and immune function (7). The gene encoding for *VDR* is considered as a candidate for genetic regulation of bone strength and metabolism. It is localized on chromosome 12 cen-q12, has 11 exons and spans ~75 kb of genomic DNA (8,9).

Morrison *et al* (10) reported a strong association between a *VDR* gene *Bsm*I polymorphism and lumbar and femoral neck (FN) BMDs. Several studies have examined genetic polymorphisms within the *VDR* gene, such as *Bsm*I [allele B/b, single-nucleotide polymorphism (SNP) G>A, rs1544410], *Apa*I (allele A/a, SNP C>A, rs17879735) and *Taq*I (allele T/t, SNP T>C, rs731236), for their influence on BMD and fracture risk (10-13). Current meta-analyses appear to be suggestive rather than conclusive, as certain studies have shown a significant association of the *VDR* gene polymorphism with BMD (14-17), whereas others report contrarily (18-21). These inconsistencies are addressing that the genetic risk involved in osteoporosis may be attributable to genetic heterogeneity, population admixture, gene-environments and gene-gene interactions.

Associations between the *VDR* gene polymorphism and osteoporosis and BMD are affected by ethnicities of the subjects, live environments and the standard and size of the samples. China has a large area and population, as well as various life styles. Therefore, the present study was carried out to examine the influence of rs17879735 within the *VDR* gene on BMD and their coordinated effects as the genetic mediators of osteoporosis in the Chinese population.

Materials and methods

Subjects. The case controls were recruited from the Department of Endocrinology and Metabolism, and the Department of Osteoporosis in Zhongshan Hospital Affiliated of Dalian University (Dalian, Liaoning, China). The local ethic committee

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Table I. Genotype and allele frequencies of vitamin D receptor.

	Genotype, n			Allele, n	
Gender (n)	aa	AA	aa	a	A
Male (101)	47	44	10	138	64
Female (277)	137	118	22	392	162
Total (378)	184	162	32	530	226

Table II. Correlation analysis of bone mineral density among patients with different BMIs, ages and body sections.

	Age		BMI	
Section	r	P-value	r	P-value
Lumbar vertebrae	-0.316	<0.001	0.272	0.000
Femoral neck	-0.349	<0.001	0.174	0.004
Ward's triangle	-0.165	0.003	0.183	0.002
Great trochanter	-0.231	<0.001	0.191	0.001

BMI, body mass index.

Table III. Correlation between the vitamin D receptor genotype and bone mineral density of different body sections subsequent to correcting the age and body mass index.

	*Apa*I genotype			
Site	AA	Aa	aa	P-value
L2-4	1.082[a]	0.995[a]	0.869	0.032
FN	0.893	0.867	0.762	0.138
Wards	0.697[a]	0.754	0.524	0.047
Tro	0.741	0.711	0.687	0.221

[a]P<0.05, compared with *Apa*I aa genotype.

approved the study, and participants signed informed consent prior to giving their blood sample. Patients were excluded if they had diseases capable of influencing calcium and phosphorus metabolism, such as hyperparathyroidism, renal failure, liver diseases, hyperthyroidism, hypocortisolism, diabetes and other chronic illnesses. A total of 378 subjects (normal BMD, 234 cases; and decreasing BMD, 65 cases) and 79 patients with osteoporosis with osteoporotic fracture were recruited and they were divided into three groups, which were the normal, osteoporosis and osteoporosis with osteoporotic fracture groups. The subjects were aged between 49-84 years old, which included 101 males and 277 females. BMD of the lumbar spine (L2–L4), FN, Ward's and Tro was measured using dual-energy X-ray absorptiometry (Norland Medical Systems, White Plains, NY, USA). Osteoporosis was defined according to the 1994 classification of the WHO (22).

Genotyping. Blood samples were obtained from all the subjects in the morning, after an overnight fast. Venous blood (5 ml) was withdrawn from the vein into Vacutainer tubes containing EDTA as the anticoagulant. Blood samples were centrifuged at 3,000 rpm for 10 min. The buffy coat and red blood cell pellet were used for DNA extraction using the total blood DNA extraction kit (Tiagen Biotechnology Co., Ltd., Beijing, China). Genomic DNA was amplified by polymerase chain reaction (PCR) using *VDR* (rs17879735) specific primers (forward, 5'-CAGAGCATGGACAGGGAGCAA-3' and reverse, 5'-GCAACTCCTCATGGCTGAGGTCTC-3'). The program for the *VDR* PCR assay was as follows: Initial denaturation at 94˚C for 3 min, cycle denaturation at 94˚C for 1 min, cycle annealing at 66˚C for 1 min, cycle extension at 72˚C for 1 min, and final extension at 72˚C for 7 min. There were 40 cycles, and the assay was maintained at 8˚C until the PCR product was removed. Subsequent to PCR, the products were digested with the restriction enzyme *Apa*I to ascertain the *VDR* gene polymorphism. The resulting fragments were subjected to electrophoresis, analyzed on 1.5% agarose gels and visualized with ethidium bromide.

Bone measurements. The measurements in grams per square centimeter were made of the BMDs of the lumbar spine (L2-4), FN, Ward's and Tro with Hologic densitometers (Hologic Inc., Waltham, MA, USA). Bone density scans used in the current analysis were those obtained at cohort baseline, when all women were designated as premenopausal or in early perimenopause on the basis of self-reported menstrual bleeding pattern variation. The cohort baseline BMD values were considered to approximate peak bone mass.

Statistical analysis. All SNP data were evaluated for Hardy-Weinberg equilibrium. Data were analyzed using the χ^2 test and Fisher's exact test where appropriate. Analysis of covariance analysis was used to quantify the associations between lumbar spine-BMD and each of the *VDR* genotypes. All the significant tests were two-sided. Statistical analysis was performed using SPSS version 20.0 software (IBM, Corp., Armonk, NY, USA). For results where P<0.05, exact P-values are given. P<0.05 was considered to indicate a statistically significant difference.

Results

Genotype distributions of VDR. The frequencies distribution of genotype and allele are shown in Table I. By analysis of the 378 subjects, the genotype frequencies were as follows: 48.68% aa, 42.86% AA and 8.46% Aa.

Association between the VDR genotype and BMD of different sections of the body. Following analysis of each site, BMD, body mass index (BMI) and age, the BMD for each site was negatively correlated with age (P<0.01) and positively correlated with BMI (P<0.01), which suggested that age and BMI influenced the different site BMD (Table II). Correction analysis revealed that there were significant differences in the Ward's triangle BMD among each genotype (P<0.05), in which the aa genotype had the lowest BMD (P<0.05) (Table III).

Table IV. Genotype frequencies in the osteoporotic fracture and normal groups.

	Genotype			
Site	AA	Aa	aa	Total, n
Osteoporotic, n (%)	43 (54.43)	9 (11.39)	27 (34.18)	79
Normal, osteoporotic, n (%)	105 (44.87)	18 (7.69)	111 (47.44)	234
Total, n	148	27	138	313

χ^2=4.433, P=0.109.

Genotype frequencies in the osteoporotic fracture and normal groups. No significant difference was identified among the different genotypes in the occurrence of osteoporosis with osteoporotic fracture (P>0.05) (Table IV).

Discussion

Osteoporosis sickness (osteoporotic, OP) is suffering from injury to the bone tissue microstructure, the bone ingredient and the bone. The proportion continuously reduces, the bone qualitative change is thin, the bone brittleness increases and easy bone fracture is one type of whole body metabolism barrier disease (23). At present, the incidence of OP is continuously increasing and has the trend of rejuvenation. OP complicated various fractures seriously influenced people life quality. The relative gene polymorphisms were not only the important disease factors of OP (24), but are also beneficial to the early screening and prevention, as well as early diagnosis of molecular genetics (25). Various candidate genes polymorphisms may have an association with BMD, such as vitamin D, the estrogen receptor (*ER*) gene, interleukin-6 and transforming growth factor (26-29).

Presently, the *ER* and *VDR* genes have received the most attention, in which *VDR* may be the main regulation gene (30). The human *VDR* gene is located in chromosome 12, with 44,000 bases and consists of 9 exons. Previous studies have examined genetic polymorphisms within the *VDR* gene, such as *Fok*I (allele F/f, SNP C>T, rs2228570), *Bsm*I (allele B/b, SNP G>A, rs1544410), *Apa*I (allele A/a, SNP C>A, rs17879735), and *Taq*I (allele T/t, SNP T>C, rs731236) for their influence on BMD and fracture risk. A number of domestic studies have reported the association between the *VDR* gene polymorphisms and BMD, and there were significant differences in results among countries, which also had mutual conflicts with each other.

The Australian study by Bell *et al* (30) reported that the *Apa*I genotype exhibited an association with lumbar BMD in two ethnicities, in which male lumbar BMD was lower in males with the aa compared to the AA genotype by 6.7%. Dundar *et al* (31) reported that the lumbar BMD was lower in patients with the aa compared to the AA genotype in 136 cases of menopausal women. Additionally, Korean (32) and Spanish studies also revealed that the *VDR* gene *Apa*I gene polymorphisms had an association with BMD in menopausal women (33). However, no effect was identified for the association of *Apa*I with BMD in the Chilean (34) and Finnish (35) studies. The study by Huang *et al* (36) suggested that the *Apa*I genotype had an association with the BMD of L1-4M, FN and Ward's triangle, as well as the BMC value, while no association with the BMD of Trochanter sites and BMC value was identified, which suggested that *Apa*I polymorphisms may influence the bone loss of cancellous bone and cortical bone in older males. Zhao *et al* (37) suggested that lumbar BMD was significantly lower in Han menopausal women with aa compared to the Aa genotype, while Lau *et al* (38) identified no association between the *Apa*I genotypes and BMD.

The genotype frequencies of the *VDR* gene were 48.68% aa, 42.86% AA and 8.46% Aa, similar to its distribution in the Shanghai province, however, these were significantly different from Italian and Greek populations. The BMDs of the different sites were higher in the AA compared to the Aa and aa genotypes, and these were significantly negatively correlated with the age and significantly positively correlated with BMI. Following correction of age and BMI, significant differences were identified between lumbar and Ward's triangle BMD and different genotypes, which were in agreement with the Korean and Shanghai studies. These indicated that the *VDR* gene *Apa*I polymorphisms had an important role in the osteoporosis risk in the Chinese Han population.

The study of the association between the *VDR* gene polymorphisms and BMD found that patients with certain genotypes had low BMD, and their BMD significantly decreased with the aa genotype, which provided a theoretical basis for gene diagnosis and treatment for the Chinese Han population. In addition, subjects with a high risk of osteoporosis could be treated and prevented earlier.

Certain potential limitations of the present study may have influenced the results, such as the participants in the present study were Chinese Asians; and hence, the inferences may not be generalized to other populations. Furthermore, functional SNPs within the promoter region of *VDR* gene that may influence the transcriptional efficacy are not taken into account, which may either mask or augment the expression of this genotype. Further studies of other SNPs with regard to osteoporosis using additional genetic markers may provide new information on the genetic background underlying osteoporosis in China.

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