Relation of *BsmI* Vitamin D Receptor Gene Polymorphism to Bone Mineral Density and Occurrence of Osteoporosis in Postmenopausal Chinese Women in Taiwan

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Abstract. Osteoporosis is a common disorder with a strong genetic component. Our aim was to evaluate the correlation of the vitamin D receptor gene intron 8 *BsmI* polymorphism with bone mineral density (BMD) and their relationship to osteoporosis. We determined the vitamin D receptor gene intron 8 *BsmI* polymorphism using polymerase chain reaction-based restriction analysis in 171 postmenopausal Chinese women in Taiwan. The polymorphism was detected using the restriction enzyme *BsmI*, where the B allele indicated absence of the cuttable site and the b allele its presence. BMD of the lumbar spine and proximal femur were measured using dual-energy X-ray absorptiometry. The allelic frequencies for postmenopausal Chinese women in Taiwan were 12.3% for B and 87.7% for b in *BsmI* restriction fragment length polymorphisms. The prevalence of each genotype in the study population was: 6.4% BB, 11.7% Bb and 81.9% bb. The three genotypic groups differed significantly in BMD at the lumbar spine and the femoral neck. These differences corresponded to significant gene-dose effects at the lumbar spine and the femoral neck (*p*<0.001 for both sites). The relative risk for the development of osteoporosis was about 2–3 times as great as that predicted by the differences between genotypes in BMD, and remained significant even after adjustment for age, height and weight. The vitamin D receptor gene intron 8 *BsmI* polymorphism is associated with reduced BMD and predisposes women to osteoporosis.

Keywords: Bone mineral density; Osteoporosis; Polymerase chain reaction-based restriction analysis; Vitamin D receptor gene intron 8 *BsmI* polymorphism

Introduction

Osteoporosis is a common and disabling age-related disease that is characterized by reduced bone mineral density (BMD), disorganization of skeletal integrity and microarchitecture, and increased risk of fragility fracture [1]. BMD is strongly related to genetic control, and as much as 60% of the variance in BMD has been attributed to genetic factors [2]. The results of at least one twin study have further suggested that there are genetic influences on age-related bone loss [3].

Some of the genetic influences on BMD may be mediated by DNA polymorphisms in the vitamin D receptor (VDR) gene, an important regulator of intestinal calcium absorption and bone mineralization [4]. Single nucleotide polymorphisms (SNPs) were used as a tool for mapping the disease gene [5] and in a study of twins in Australia, Morrison et al. found a marked association between the VDR gene intron 8 *BsmI* polymorphism and BMD [6], thereby making it possible to search the candidate genes for the genetic determinants of BMD. However, the relationship between the VDR polymorphism and BMD is controversial [7,8] and,
at best, only partly accounts for the genetic effect on bone mass [9].

Estrogen deficiency in postmenopausal women has been associated with an increase in bone turnover and acceleration of bone loss, leading to an increased susceptibility to bone fractures [10]. To our knowledge, no other study specifically testing the skeletal effects of VDR gene polymorphisms in postmenopausal Chinese women in Taiwan has been conducted. An exploration of the relationship between VDR genotypes and the occurrence of osteoporosis in postmenopausal women was therefore thought to be of interest. In this respect, we studied the correlation between the VDR gene intron 8 BsmI polymorphism and BMD and their relationship to the development of osteoporosis in postmenopausal Chinese women in Taiwan.

Subjects and Methods

Subjects

One hundred and seventy-one postmenopausal women aged 45–74 years (54.32 ± 5.89 years) were randomly enrolled among patients who underwent risk evaluation for osteoporosis at the Department of Obstetrics and Gynecology. Women who met any one of the following criteria were excluded: (1) those who had undergone bilateral ovariectomy or had a natural menopause occurring before the age of 40 years; (2) those who took drugs or had a history of taking drugs which affected bone metabolism, such as glucocorticoids, thyroxine, antiepileptics, bisphosphonates, calcitonin, or hormone replacement therapy for more than 4 months; (3) those who weighed more than 100 kg; (4) those who had disease such as primary hyperparathyroidism, hyperthyroidism, diabetes, cirrhosis and kidney failure. For each woman, a detailed medical history was obtained and her dietary calcium intake was assessed using a sequential questionnaire that included foods accounting for the majority of dietary calcium [11]. This study was approved by the medical ethics committee of China Medical College Hospital, and written informed consent was obtained from all the women who participated in the study.

Polymerase Chain Reaction (PCR)-Based Restriction Analysis

PCRs were carried out in a total volume of 50 μl, containing: genomic DNA, 2–6 pmol of each primer, 1 × Taq polymerase buffer (1.5 mM MgCl2) and 0.25 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). The genomic DNA was prepared from peripheral blood using a DNA Extractor WB kit (Wako, Japan). The VDR gene was amplified using PCR. Similar to previous reports [6,12], detection of the BsmI site was achieved by amplifying a region spanning the site, with the forward primer annealing to starting at the 5’-end of exon 8 (primer 1: 5’-CCGGACACAGCCTGGAGCTG-3’) and the reverse primer annealing to intron 8 (primer 2: 5’-CAGCGG-GAAAGGTCAAGGGG-3’), producing a 570–580 base pair (bp) fragment (Fig. 1). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA). The cycling conditions for intron 8 of the VDR gene were set as follows: one cycle at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 20 s, and one final cycle of extension at 72 °C for 7 min. The 570–580 bp PCR products were mixed with 2 units BsmI (New England BioLabs, Beverly, MA) and reaction buffer according to the manufacturer’s instruction. The reaction was incubated for 3 h at 37 °C. Then, 10 μl of the products was loaded onto 3% agarose gel containing ethidium bromide for electrophoresis. The genotype of the VDR gene intron 8 BsmI polymorphism was determined by observing the number of bands

Fig. 1. A Schematic diagram of the 3’-terminal region of the VDR gene demonstrating the restriction site for BsmI. B The genotype of the VDR gene intron 8 BsmI polymorphism was determined by observing the number of digested bands.