Association between bone mineral density and polymorphisms of the VDR, ERα, COL1A1 and CTR genes in Spanish postmenopausal women

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ABSTRACT. Bone mineral density (BMD), the major determinant of osteoporotic fracture risk, has a strong genetic component, and several candidate gene polymorphisms have been implicated in the regulation of this process. In view of the reported associations between the BMD and polymorphisms in the collagen type I α 1 gene (COL1A1), vitamin D receptor (VDR), estrogen receptor (ER)α and calcitonin receptor (CTR) genes, an association study was performed between VDR, COL1A1, CTR and ER genotypes and lumbar spine, femoral neck and Ward’s triangle BMD in postmenopausal Spanish women. We statistically controlled for many confounding factors, such as height, weight, age, years since menopause, use of hormone replacement therapy (HRT), tobacco consumption, use of oral contraceptives, calcium dietary intake or exercise practice. No association between COL1A1 or ER genotypes and BMD was detected. However, we described a statistically significant association between a personal history of fractures and COL1A1 genotype. The ss genotype was found to be over-represented between those women who had a personal history of fractures. The analyses of the VDR polymorphisms showed that FF subjects reached the highest values of BMD at the three measured sites, whereas Ff individuals had an intermediate BMD and ff women had the lowest values. However, the VDR-BsmI gene polymorphism was not found to be associated with adjusted BMD. For the CTR polymorphisms, our study showed that women with the aa genotype had a lower adjusted BMD at the femoral neck. In conclusion, in our postmenopausal Spanish women cohort we found a statistically significant association between the VDR and CTR gene polymorphisms and the BMD. However, we did not find any association between the ER and COL1A1 gene and the BMD. The COL1A1 gene was found to be associated with the prevalence of osteoporotic fractures. Of all the studied gene polymorphisms, the FokI VDR gene polymorphism seems to be the strongest BMD genetic determinant of postmenopausal Spanish women. (J. Endocrinol. Invest. 28: 312-321, 2005) ©2005, Editrice Kurtis

INTRODUCTION
Bone mineral density (BMD) is the best risk predictor of osteoporotic fractures. Twin and family studies have suggested that BMD and age-related bone loss have strong genetic components (1-3). Thus, the identification of genes related to the regulation of bone metabolism and bone mass opens new perspectives in the study of the pathogenesis and treatment of this metabolic bone disorder. The first reported association between BMD and genetic determinants was described in 1994 by Eisman’s group (4). They described an association between the vitamin D receptor (VDR) genotypes and BMD. However, serious controversy exists regarding the association between VDR polymorphisms and osteoporosis (5-7). Moreover, bone density and many other bone strength components are complex phenotypes whose heritability is most likely to be polygenic. Therefore, it is necessary to examine the potential involvement of other candidate genes in

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the pathogenesis of osteoporosis and to study their possible interaction with the VDR polymorphisms. In this regard, other candidate genes associated with osteoporosis have been demonstrated. Type I collagen is the major constituent protein of bone. A guanine-thymidine substitution at the binding site for the Sp1 transcription factor in the first intron of collagen type I α 1 gene (COL1A1) has been associated with a decreased BMD and increased risk of osteoporotic fractures (8, 9), although some other studies did not observe similar associations (10). The estrogen receptor (ER)α is another candidate gene for studying the genetic component of osteoporosis. Several studies reported a significant association of the ER polymorphisms and BMD (11, 12), although this has not been found in others (13). An interactive effect between ER and VDR polymorphisms has also been reported in relation to BMD measurement (14). Another candidate gene for the pathogenesis of osteoporosis is the calcitonin receptor gene. The calcitonin receptor (CTR) is a member of the transmembrane receptor family, and a point mutation polymorphism (C→T) has been identified in the 3′-region of the calcitonin receptor gene which induced a Pro→Leu shift in the third intracellular domain of the protein. This polymorphism is recognized by the Alu I restriction enzyme and it has been reported to be associated with lumbar BMD (15). Although it is generally agreed that several genes contribute to the BMD and to the other fracture risk determinants, each of them has a relatively small effect (16). The estimated heritability of the osteoporosis leaves a considerable influence to the environmental factors, which modify the genetic predisposition. Gene-environment interactions include: tobacco consumption, exercise practice, hormone replacement therapy (HRT), calcium intake, and others. Therefore, environmental and hormonal confounder factors must be taken into account in these association studies. The aim of this project was to study the VDR, ER, COL1A1 and CTR polymorphisms simultaneously in a group of postmenopausal Spanish women, in order to determine their potential association with the lumbar spine, femoral neck and Ward’s triangle BMD. In addition, to determinate the role of these genetic factors and their actual influence on bone mass loss, a statistical control for potential confounding factors, such as hormonal status, age, years since menopause, height and weight was applied.

MATERIALS AND METHODS

Subjects

Our study included a total of 177 Caucasian Spanish women, aged from 38 to 74 yr. All of them were postmenopausal, which is defined as an absence of menstruation of at least a year. They were recruited in a routine gynecologic check-up in our center from September 2002 to December 2003. A detailed gynecologic and medical history including medication use and each subject’s health was recorded for all women. All the information was obtained by a personal interview using standardised questionnaires designed at our center, and always carried out by the same doctor. In this interview, participants completed a questionnaire that described their medical history, reproductive history, family and personal history of osteoporotic fractures, medication use including HRT or OCs, smoking and alcohol use, and usual calcium dietary intake. Total calcium intake was calculated using an extensive and detailed account of the intake of calcium containing foods. Physical activity levels were estimated with a recall of hours of physical activity per week. All the patients with chronic diseases or any other conditions which may potentially affect bone mass were excluded from the study. These disorders included chronic diseases involving the vital organs (heart, lung, liver, kidney and brain), serious metabolic diseases (diabetes, hypo- and hyperparathyroidism, etc), other skeletal disease (osteogenesis imperfecta and rheumatoid arthritis, etc) or any chronic use of drugs known to affect bone metabolism (such as corticoid therapy and anti-convulsion drugs). All the patients with associated conditions known to affect bone metabolism were excluded from the study. Women were requested to give written informed consent for genetic analysis. The study was performed in accordance with the Declaration of Helsinki and the European Standards for Good Clinical Practice. The study was approved by the Institutional Ethical Committee.

Anthropometry and BMD measurement

The anthropometric measurements were the height and body weight. The height was measured by a digital Harpenden-type stadiometer (Holttain, UK) and the weight was assessed by professional scales (Seca, Spain). The body mass index (BMI) was calculated as the body weight divided by the height squared (kg/m²). In our study, BMD was determined by dual energy x-ray absorptiometry (DEXA) which is periodically calibrated and has an in vivo precision with a coefficient of variation <1% in all the studied skeletal sites (Hologic QDR 4500; Hologic Inc; Waltham, MA, U.S.A 02154). For all women, anteroposterior lumbar spine (L2-L4), right femoral neck and Ward’s triangle BMD were measured.

DNA analyses

DNA was extracted from a sample of peripheral blood leucocytes using MagnaPure LC system (Roche Molecular Biochemical), and subsequently used in polymerase chain reactions (PCR) to amplify fragments of the VDR, ER, CTR and COL1A1 genes. All reactions were performed in a DNA Thermocycler 9600 (Applied-Biosystems, Norwalk, CT) in a total volume of 50 μl, containing 1x GeneAmp buffer (Applied Biosystems), 1.6 mM MgCl₂, 800 μM deoxynucleotide triphosphates, 2 μM of each primer (see Table 1), 1 U of AmpliTag Gold DNA polymerase (Applied Biosystems) and 100 ng of genomic DNA. Cycling conditions were one cycle of 95°C for 12 min, 40 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 45 sec, and a final extension at 72°C for 7 min. Evaluation of the correct size of the amplified products was assessed through electrophoresis of 10 μl PCR products on 1% agarose gels. Subsequently, 20 μl of the amplified products were digested for 4 h with a restriction enzyme that specifically recognizes the polymorphic site in the amplified DNA fragment (see below), according to the recommendations