Apa1 VDR polymorphism and osteoporosis risk in postmenopausal Mexican women

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Abstract

Aim: To analyze the association between Apa1 VDR polymorphism and osteoporosis in Mexican mestizo postmenopausal women. Methods: A cross-sectional study was conducted in 534 postmenopausal mestizo women from Mexico City to determine the association of the Apa1 Vitamin D Receptor gene polymorphism (rs7975232) with osteoporosis and osteoporosis plus fracture. Bone mineral density (BMD) was assessed using dual-energy X-ray absorptiometry. Genotyping was performed using real-time PCR with an allelic discrimination assay. Results: The Apa1 allele frequencies were no different between groups. No association was found between Apa1 genotypes and osteoporosis (AA, OR: 1.08; 95% CI: 0.62-1.87; AC, OR: 0.70; 95% CI: 0.45-1.07). Similar results were obtained for osteoporosis plus fracture (AA, OR: 0.93; 95% CI: 0.50-1.71; AC, OR: 0.70; 95% CI 0.45-1.07). After adjusting for age, the result remained. Conclusion: These findings are in agreement with previous studies reporting no association of Apa1 VDR polymorphism with osteoporosis. (Gac Med Mex. 2015;151:443-7)

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Introduction

Osteoporosis is a common metabolic bone disease that affects nearly 200 million people worldwide and it is characterized by low bone mineral density (BMD) and deterioration of the bone tissue microarchitecture, which leads to increased bone fragility and a subsequent increased risk of fracture1,2. The main clinical outcome measure is bone fracture. Evidence originating from studies of twins has demonstrated that genetic factors can account for up to 85% of variation in peak bone mass3-5. In addition, studies have demonstrated an association between a family history of fracture and bone mass reduction6,7. Different candidate genes have been proposed to identify genetic variants that increase the susceptibility to develop osteoporosis8. VDR is one of the most widely studied; this gene codifies for the nuclear hormone receptor for Vitamin D3. The human VDR gene has been mapped in chromosome 12q13.11 and is composed of 11 exons. VDR interacts with its ligand to play an important role in calcium homeostasis by regulating bone growth and cell differentiation, intestinal calcium absorption and parathyroid hormone secretion9. Therefore, VDR is a natural place to start looking for genetic variations that might confer...
susceptibility to osteoporosis. In 1994, Morrison, et al. showed the relationship between the VDR genotype and bone mass\textsuperscript{10}. Ever since then, many studies have been conducted on the association of VDR polymorphisms and BMD, including Gdx2 (rs11568820), Taql (rs731236), BSMI (rs1544410), Fokl (rs10735810) and VDL Apa1 (rs7975232)\textsuperscript{11,12}. However, the results have been inconsistent in all populations, included those of Mexican origin\textsuperscript{13-19}. Most Mexicans are the result of miscegenation between Indigenous Americans, Spaniards and, to a lesser degree, western Africans. This miscegenation may confer variants of risk for diseases that are not observed in other populations. To better understand the impact of VDR polymorphisms, the purpose of this study was to analyze the association of the VDR Apa1 polymorphism with osteoporosis in postmenopausal mestizo Mexican women.

Material and methods

Participants

Three-hundred and eighty-seven postmenopausal, osteoporosis-diagnosed, unrelated mestizo women from Mexico City were included. In addition, 147 postmenopausal mestizo women with normal BMD were asked to participate. The women were recruited in the Instituto Nacional de Rehabilitación de Mexico City. Osteoporosis was diagnosed when BMD was lower than -2.5 standard deviations (SD) by means of dual-energy x-ray absorptiometry (DEXA). All women were interviewed with the help of a structured questionnaire in order to obtain clinical characteristics. Women who met any of the following criteria were excluded: having undergone bilateral oophorectomy or having natural menopause before the age of 40 years; use of medications or history of use of medications that affect bone metabolism, such as corticosteroids, thyroxine, antiepileptic drugs, bisphosphonates, calcitonin or estrogen plus progestogen, for more than 4 months; having diseases such as primary hyperparathyroidism, hyperthyroidism, bone dysplasia and osteogenesis imperfecta. Additionally, patients were asked their history of fractures. All fractures were verified in clinical records of the institution. Only verified fractures were included for analysis. To be considered Mexican mestizo, the participants’ families had to have lived in Mexico for at least 3 generations and speak the Spanish language. The study was approved by the Research Commission and the Ethics and Biosafety Committees of the Hospital Infantil de México Federico Gómez, and was conducted following the ethical principles for medical research in human beings of the Declaration of Helsinki.

Genotyping

Genomic DNA was extracted from peripheral white blood cells (WBC) using a Puregene Gentra blood kit (QIAGEN, Mississauga, Ontario, Canada). The presence of the polymorphism was determined by real-time PCR using the TaqMan allelic discrimination C\textsubscript{28977635} assay for VDR Apa1 (Applied Biosystems, Foster City, CA, USA), following standardized protocols. The plate was turned on at 95 °C for 10 min, at 92 °C for 15 s and then at 60 °C for 1 min for 40 cycles. To verify genotyping quality, 267 samples were duplicated in a blinded fashion; agreement between samples and duplicates was 100%.

Statistical analysis

Sample size was calculated to obtain 80% statistical power using the Quanto 1.2.4 software (University of Southern California, Los Angeles, CA, USA)\textsuperscript{20}. To assess the VDR Apa1 genotypes Hardy-Weinberg equilibrium, the program available at http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl was used\textsuperscript{21}. 95% confidence intervals (CI) for alleles and phenotypes were estimated with GraphPad QuickCalcs (GraphPad Software, Inc., San Diego, CA). Demographic characteristics were expressed as means and standard deviations. Odds ratios (OR) (with 95% CI) for the development of osteoporosis were estimated for subjects with different types of VDR Apa1. In addition, ORs were adjusted for age using a multiple logistic regression. Statistical calculations were carried out with SPSS v.20 (IBM, Chicago, IL).

Results

Demographic characteristics are shown in Table 1. Women with osteoporosis were older than those in

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<th>Table 1. Demographic characteristics</th>
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<td>Normal BMD</td>
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<td>Age (years)</td>
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<td>Weight (kg)</td>
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the control group. Height and weight were greater in the control group. VDR Apa1 genotype and allele frequencies are shown in Table 2. No Hardy-Weiberg equilibrium deviations were observed in the control group. No association was found between VDR genotypes and the risk for osteoporosis (Table 3). Similar results were observed when the association with women with osteoporosis who had sustained a fracture was assessed. To understand if age was a confounder, the OR value was adjusted, and the lack of association of VDR Apa1 genotypes with osteoporosis and osteoporosis with fracture was maintained.

Discussion

Osteoporosis is a multifactorial disease with a strong genetic component. Low BMD has been observed to have a familiar pattern in the Mexican population\textsuperscript{22,23}. This is the first study to explore the association of the VDR Apa1 polymorphism with the risk for osteoporosis in a Mexican mestizo population. The result shows that there is no association between the presence of this variant and the risk for osteoporosis. This result is consistent with previous reports in different populations\textsuperscript{24-31}. Lack of association has also been described in two meta-analyses that have assessed the association of Apa1 and the risk for osteoporosis and fractures\textsuperscript{32,33}. This lack of association between Apa1 and peak BMD has also been observed in Chinese young adults and obese Spaniards older than 55 years\textsuperscript{34}. On the other hand, no BMD increase was observed in young women supplemented with vitaminD3, a benefit observed in women who had the Bsm1 and Taql polymorphisms\textsuperscript{35}. However, there are reports associating the Apa1 variant with increased risk for osteoporosis\textsuperscript{36-39}. Recently, a meta-analysis that has included three studies in Chinese women has shown an association of the Apa1 variant with a decrease in BMD\textsuperscript{40}; therefore, Apa1 association with low BMD and osteoporosis remains controversial. While the relationship between DMO and Apa1 genotypes is not clear, an association between genotypes and the osteocalcin and parathyroid hormones levels has been observed to exist in healthy adolescents\textsuperscript{41}. With regard to the treatment of osteoporosis, Apa1 does not influence on the success of calcitriol therapy for recurrent fractures\textsuperscript{42}. Several studies have demonstrated the association of Apa1 with different genotypes, such as breast, ovarian

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<th>Table 2. VDR Apa1 (rs7975232) allelic and genotypic frequencies</th>
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<td>Genotypes</td>
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<th>Table 3. VDR gene Apa1 polymorphism (rs7975232) association with the risk for osteoporosis and osteoporosis plus fracture</th>
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<td>AA + AC</td>
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*Adjusted by age
or sporadic prostate cancer, type 1 Gaucher disease, systemic lupus erythematosus, Crohn’s disease, coronary artery disease in type 2 diabetes and asthma. The relationship between these diseases and Apa1 has not been studied in the Mexican population.

Apa1 allelic frequencies in the mestizo population of Mexico City are similar to those previously reported for the Californian Mexican population included in the HapMap Project. This fact suggests that Apa1 allelic frequency is constant in Mexican mestizo populations.

In summary, this is the first study to explore the association between the VDR Apa1 polymorphism and the risk for osteoporosis and fractures in Mexican mestizo population. Our results broaden the information on an association of the VDR Apa1 polymorphism with osteoporosis and confirm previous evidence suggesting there is no relationship between the Apa1 variant and the risk for developing osteoporosis.

Acknowledgements

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Conflicts of interest

The study has no conflicts of interest.

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