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# Role of APOE Gene in Bone Mineral Density and Incidence of Bone Fractures in Brazilian Postmenopausal Women

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## Abstract

Osteoporosis is one of the major diseases that affects mostly postmenopausal women. Despite being a multifactorial disease, some genes have been shown to play an important role in osteoporosis. Bone mineral density (BMD) is still largely used to diagnose it, although many other biomarkers are used to better follow the disease onset. It has been shown that the apolipoprotein E (APOE) gene could be a biomarker for risk of fractures as well as to predict lower BMD in patients with osteoporosis. The human APOE gene encodes 3 protein isoforms called ApoE2, ApoE3, and ApoE4, resulting in 4 possible genotypes, because they are a product of a single nucleotide polymorphism found in this gene. So far, the APOE4 allele has been associated with low BMD in postmenopausal women and to incidence of bone breaking in older women. This study aimed to investigate the role of ApoE isoforms in a cohort of 413 postmenopausal Brazilian women. These patients were randomly recruited, clinically examined, and subjected to dual-energy X-ray absorptiometry to measure their BMD. Patients were further grouped as normal BMD (T-score < 0.5) or low BMD (T-score > 1.0, osteopenic or osteoporotic). Patients with osteopenia or osteoporosis were further genotyped for APOE alleles as well as tested for many serum bone turnover biomarkers. Our data showed that presence of the APOE3 allele was associated with both higher BMDs and higher serum concentrations of osteocalcin and alkaline phosphatase, biomarkers for bone formation. On the other hand, the APOE2 and APOE4 alleles were associated with lower BMD as well as higher levels of serum C-terminus collagen peptide and urinary deoxipyridinolines, biomarkers for bone resorption. However, these effects on lower BMD and bone resorption biomarkers observed in either APOE2 or APOE4 alleles were eliminated when patients' genotype carried the APOE3 allele. Codominance of the APOE3 allele was also associated with lesser cases of bone fractures

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in these patients within a 5-year follow-up. In conclusion, our data show that APOE4 may be associated with lower bone formation as well as increased risk of osteoporosis and bone fractures, whereas APOE3 seems to decrease lowering BMD in postmenopausal women, and its presence seemed to lower the incidence of bone breaking in patients with osteoporosis.

**Key Words:** APOE; bone mineral density; isoforms; menopause; osteoporosis.

## Introduction

Aging is an important public health concern nowadays. It is also known that there are a number of chronic diseases closely associated with aging, also known as age-related diseases. One of today's major age-related diseases is osteoporosis *(1)*. Osteoporosis has been proven to be a highly morbid disease in older women because it is closely associated with bone fractures *(2–4)*. It has been shown that 50% of women who experienced bone breakings in their femur or pelvis become unable to walk again, whereas 20% of these women evolve to death in less than 2 years *(4)*. Hence, the improvement of detection of reliable risk factors is fundamental for early diagnosis and therapeutic success in osteoporosis (Table 1).

Among women, this bone loss dramatically increases after menopause, a period marked by progressive ovarian failure, resulting in decrease in plasma concentration of female sex hormones, like estrogen *(3,5)*. Estrogen has a fundamental role in women's physiology. Despite its fundamental role in reproduction, there are many reports pointing out this hormone's action in lipoprotein metabolism and bone formation *(6,7)*. Since 2001, the major type of osteoporosis treatment—hormone replacement therapy—has been identified as a risk factor for cancers of the breast, uterus, and, possibly, ovaries, and therefore was dismissed in clinical practice in many countries *(8)*. Thus, new therapeutic strategies have been pursued to alleviate the signs, symptoms, and aggravation of osteoporosis.

Over the past decade, genetic research has pointed to several other molecules as potential prognostic predictors and possible new therapeutic targets in osteoporosis and tissue healing *(9)*. Mutations or polymorphisms in genes that encode vitamin D receptor, estrogen receptors α and β, interleukin 6, and insulin-like growth factor type 1, show a high correlation with low bone mineral density (BMD) during senescence *(10–12)*. However, the genetic influence on osteoporosis—mainly low-penetrance polymorphic elements—is still controversial. Like any multifactorial disease, osteoporosis is a disease also associated with differences in lifestyle, dietary habits, or even genetic codependency *(13,14)*, making the determination of the sole factor responsible for the disease a hard task.

Although some papers have shown a correlation between low BMD with the apolipoprotein E (ApoE—used when protein is refered) genotype in osteoporotic patients, this is still subject to great controversy *(15–17)*. The human APOE gene is located in chromosome 19, where it presents 3 different single nucleotide polymorphisms (SNPs) that lead to 3 distinct isoforms, namely, ApoE2, ApoE3, and ApoE4. Therefore, combinations of ApoE2-2, 2-3, 2-4, 3-3, 3-4, and 4-4 are found with different distributions in human populations *(18)*. The ApoE4 isoform has been shown to be associated with atherosclerosis, Alzheimer's disease, and osteoporosis *(17–20)*. The hypothesis is that genetic variation at the ApoE locus, known as E2, E3, and E4, can modulate the BMD. So far, the E4 allele has been mostly associated with lower BMD and bone breaking, although recent data have shown that APOE2 may decrease BMD in some populations as well *(13,14,21)*. Although the molecular mechanisms which ApoE apolipoprotein influences the BMD in humans is still poorly understood hypothesis involving transport and/or delivery of vitamins K and D to the bone cells have been discussed *(22,23)*.

Because of this remaining lack of understanding in the role of the APOE gene in osteoporosis onset, this study

**Table 1**
Distribution of APOE Genotypes in Population with Low BMD (Osteopenic or Osteoporotic) According to Their Anthropometric and Body Composition Measurements Obtained by DXA (Average ± Standard Deviation)

| Genotype | E2E2 | E2E3 | E2E4 | E3E3 | E3E4 | E4E4 |
|---|---|---|---|---|---|---|
| Number of patients (% frequency) | 17 (7.9) | 54 (25.2) | 13 (6.0) | 68 (31.8) | 58 (27.1) | 4 (0.2) |
| Height (cm) | 146.4 ± 3.6 | 155.0 ± 5.4 | 153.6 ± 5.9 | 153.8 ± 12.6 | 152.3 ± 5.5 | 151 ± 5.4 |
| Body weight (kg) | 59.7 ± 9.4 | 65.4 ± 16.3 | 66.8 ± 10.1 | 66.7 ± 13.1 | 62.3 ± 9.2 | 58.5 ± 5.4 |
| Age (y) | 70.5 ± 5.7 | 66.8 ± 9.7 | 61.3 ± 4.8 | 63.6 ± 8.4 | 63.7 ± 8.1 | 64.0 ± 3.5 |
| Body mass index (kg/cm$^2$) | 25.2 ± 5.0 | 27.4 ± 6.0 | 29.4 ± 4.0 | 20.0 ± 1.0* | 28.9 ± 7.0 | 25.7 ± 5.1 |
| Total fat mass (%) | 23 | 28 | 31 | 18 | 29 | 23 |
| Body lean mass (%) | 12 | 13 | 8 | 19 | 13 | 11 |

*Abbr:* APOE, apolipoprotein E; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry.
*Stands for statistical significance ($p \leq 0.01$).