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Original Article

APOE polymorphisms in connection with the susceptibility to osteoporotic fracture

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Abstract: Objective: This study was designed to discuss the correlation of apolipoprotein E (*APOE*) polymorphisms with the susceptibility to osteoporotic fracture. Methods: The bone mineral density (BMD) of 50 osteoporotic fracture patients and 51 healthy people were detected. The *APOE* genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Results: The BMD values of different body parts of the patients were apparently lower than those of the healthy people ($P<0.05$). The E4 allele of the *APOE* gene was more frequent in cases than in controls (OR=3.357, 95% CI=1.25-8.99). E4 allele carriers had significantly lower BMDs at the Ward's triangle and lumbar L2-L4 segment ($P<0.05$). Conclusions: *APOE* may be correlated with osteoporotic fracture. The E4 allele of the *APOE* gene may cause a higher likelihood of fracture at Ward's triangle and lumbar L2-L4 segment, and may be a significant marker for osteoporotic fracture.

Keywords: Apolipoprotein E, polymorphism, polymerase chain reaction, osteoporotic fracture

Introduction

The pathogenesis of osteoporotic fracture is described as follows: after the occurrence of osteoporosis, the bone mineral density (BMD) and bone quality can be decreased, which will lead to the reduction of bone strength; and as a result, fracture can be caused by small violence and even by daily activities [1]. As a kind of pathologic fracture, osteoporotic fracture (fragility fracture) is the most serious outcome of osteoporosis; and it appears more frequently among women and the elderly [2]. Being a kind of epidemic disease which develops obscurely, osteoporosis can cause the bone mass of patients gradually reduced without their knowing it and without causing any discomfort or other apparent symptoms; and fracture or other organic lesions in skeleton finally induced by it can greatly affect the life quality of patients [3]. There is still no safe and effective medical method to affect a radical cure of the disease yet. In recent years, with the development of studies on molecular biology technology, we find that genetic factors have important influences on bone mass and osteoporotic fracture [4-7]. A variety of genes, such as vitamin D receptor, estrogen receptor and type I collagen genes, have been observed to be associated with the pathogenesis of osteoporotic fracture [8-10]. Apolipoprotein E (*APOE*) gene is an important gene related to many diseases, and early research has shown that the gene is a risk factor that has the most influence on the disease currently known as the sporadic and tardive Alzheimer's disease [11]. Recent research shows that the APOE4 can affect the bone metabolism by changing the metabolic rate of vitamin K, so carriers of APOE4 have much loss of bone mass, which indicates that APOE4 allele may have associations with osteoporotic fracture [12, 13]. However, some other studies fail to observe the existence of correlations between *APOE* gene and bone mass loss [14, 15]. Therefore, this research designed a case-control study including 50 cases and 51 controls to compare the relationship between their BMD and *APOE* genotypes and further to investigate the correlation between the *APOE* gene and osteoporotic fracture.

Materials and methods

Research subjects

We randomly selected 50 Chinese Han patients newly diagnosed with osteoporotic fracture by X-ray examination from Private hospitals in Shandong Province. The cases (15 males and 35 females) were aged from 54 to 79 years. Meanwhile, 51 healthy old people (15 males and 26 females) with an age range of 45-72 years were voluntarily chosen as the controls, and they had never taken vitamin D and calcium supplements before entering the study. People with diseases that can affect the bone metabolism like diabetes, thyroid disease and kidney disease as well as severe hyperlipidemia were excluded. The cases and controls were not connected by blood and their age and sex status were in equilibrium and comparable. The BMD of all the subjects were detected with NORLAND-XR36DEXA bone sonometer. The diagnostic criterion of osteoporosis was a BMD value lower than the 2.5 standard deviations of the peak bone mass of people with the same sex.

BMD detection

We detected the BMD of five parts of lumbar L2-L4 segment (L2-L4), femoral neck (Neck), greater trochanter (Troch), Ward's triangle (Ward's) and distal radius (Radius) with the dual energy X-ray absorptiometry (DEXA) of American LUNAR company, and the results were presented in g/cm^2. The DEXA was rectified with phantoms after booting each day, and the coefficient of variation (CV) thereof was 1.7%.

DNA extraction

We collected the peripheral venous blood of the subjects, and used 0.2% (w/v) ethylenediamine tetraacetic acid disodium salt (EDTA-2Na) as the anticoagulant. Conventional phenol-chloroform extraction method was adopted to collect genome DNA from leukocytes of the subjects.

PCR-RFLP analysis

The forward and reverse primer sequences were respectively 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'. Each 50 μl PCR (polymerase chain reaction) reaction system contained leukocyte DNA (1 μg), forward and reverse primers (20 pmol each), dimethyl sulfoxide (DMSO) (10% (w/v)), four kinds of dNTPs (200 μmol/L each), $MgCl_2$ (1.5 mmol/L), and Taq DNA polymerase (1.5 u). The PCR reaction conditions were: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 2 min; and at last extension at 72°C for 10 min. 30 μl PCR amplification products were added with 8 u HhaI (Promega company, USA) and the mixture was digested at 37°C for 8 h. Then, we performed 15% (w/v) native polyacrylamide gel electrophoresis at 4 V/cm voltage for 4 h and ethidium bromide staining for 40 min. The results were observed under an ultraviolet lamp and lengths of fragments obtained after digestion with HhaI incision enzyme were determined according to the DNA marker (pUC18/MspI).

Determination of analytic results

After digestion of the 299 bp PCR production fragment of the *APOE* gene by HhaI incision enzyme, there appeared six kinds of combinations of fragments with different lengths, which represented six genotypes respectively. Genotypes E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4 were respectively represented by three fragments of 91 bp, 83 bp and 61 bp, four fragments of 91 bp, 83 bp, 61 bp and 48 bp, five fragments of 91 bp, 83 bp, 72 bp, 61 bp and 48 bp, three fragments of 91 bp, 61 bp and 48 bp, four fragments of 91 bp, 72 bp, 61 bp and 48 bp, and three fragments of 72 bp, 6l bp and 48 bp.

Statistical analysis

The data were processed by SPSS18.0 software and presented in the form of $\bar{x} \pm s$. We utilized χ^2 test to compare distribution frequencies of *APOE* genotypes and alleles of the two groups. Hardy-Weinberg equilibrium (HWE) test was used to evaluate whether the genotype and allele distributions were in equilibrium. Statistical significance existed when $P<0.05$.

Results

Basic information

This study involved 50 cases and 51 controls. The genotype distributions of the control group

Table 1. BMD comparison of different body parts in two groups

Body part	Case	Control	*P*
Ward's triangle	0.612±0.214	0.792±0.102	<0.05
L2-L4	0.778±0.124	1.054±0.019	<0.05
Troch	0.593±0.182	0.701±0.089	<0.05
Neck	0.614±0.018	0.792±0.103	<0.05
Radius	0.614±0.018	0.792±0.103	<0.05
Ca	2.419±0.118	2.582±0.103	>0.05
P	2.130±0.095	2.451±0.115	>0.05
AKP	76.831±10.471	64.791±13.854	>0.05

Table 2. Comparison of *APOE* genotypes and alleles distributions in two groups

Group	*APOE* genotype						*APOE* allele		
	E2/E2	E3/E3	E4/E4	E2/E3	E2/E4	E3/E4	E2	E3	E4
Case n=50	2	9	15	7	10	7	19	30	51
Control n=51	3	10	9	13	10	6	31	41	30
P	1.00	1.00	0.17	0.21	1.00	0.78	0.17	0.20	0.02

Table 3. Correlation of *APOE* alleles with BMD

Body part	E2 n=50	E3 n=71	E4 n=81	*P*
Ward's (BMD)	0.668±0.114	0.654±0.132	0.513±0.213	<0.05
L2-L4 (BMD)	1.103±0.125	0.987±0.315	0.801±0.241	<0.05
Troch (BMD)	0.683±0.214	0.668±0.124	0.603±0.06	>0.05
Neck (BMD)	0.798±0.132	0.745±0.113	0.727±0.151	>0.05
Radius (BMD)	0.358±0.014	0.342±0.120	0.332±0.181	>0.05

were in conformity with the HWE (*P*>0.05) and the two groups were comparable.

BMD and bone metabolism indicators of different body parts in two groups

As described in **Table 1**, the BMD values of different body parts were obviously lower in case group than in control group (*P*<0.05); but differences of the values of blood calcium, blood phosphate and alkaline phosphatase (AKP) in two groups were not statistically significant (*P*>0.05).

APOE genotypes distributions in two groups

The *APOE* genotypes and alleles distribution frequencies of the 50 osteoporotic fracture patients and the 51 healthy people are shown in **Table 2**. As observed in **Table 2**, the distributions of the six genotypes of *APOE*, namely E2/E2, E3/E3, E4/E4, E2/E3, E2/E4 and E3/E4, had no statistically significant differences between the two groups (*P*>0.05). Of the three alleles, the E4 allele has the highest frequency in the case group, and the frequency was much higher compared to that in the controls (*P*<0.05), suggesting that people with E4 allele may have an apparently elevated risk of developing osteoporotic fracture (OR=3.357, 95% CI=1.25-8.99). The distributions of E2 and E3 alleles were not significantly different between the two groups (*P*>0.05). Further exploration on the relationship between the BMD values of different body parts and E2, E3 and E4 alleles suggested that people with E4 allele had lower BMD at Ward's triangle and lumbar L2-L4 segment than carriers of the other two alleles, and statistical significance was found in the differences (*P*<0.05) (**Table 3**).

Discussion

Osteoporosis has a significant influence on the occurrence and healing of fractures, and it can reduce the strength of the fractured bone as well as the quality of bone healing [1]. Because of the concealed onset and long history of osteoporosis as well as the high incidence and great harm of the fracture caused by it, it is rather difficult to achieve early diagnosis and treatment of osteoporotic fracture [16]. Osteoporotic fracture affects the quality and length of life of the patients so seriously that it has become a public health problem that should not be ignored [17, 18]. Smith et al. discovered the importance of genetic factors in the bone mass acquisition process for the first time in 1973; and since then, people gradually pay more and more attention to influences of

genetic factors on the onset of osteoporotic fracture [19].

Recent researches have pointed out that the APOE4 allele is related to the increased risk of many chronic diseases such as cardiovascular disease, Alzheimer's disease, hyperlipidemia and osteoporosis [6, 20-22]. This also reflects that the *APOE* gene may be one of the main genetic factors affecting human aging and health. The associations of allelic variations of the *APOE* gene with BMD, bone loss and osteoporotic fracture have been reported as well. Masataka et al. have proved that APOE4 allele is intimately associated with lower BMD of Japanese postmenopausal women, and the APOE4 allele carriers have relatively lower BMDs [6]. Cauley et al. studied the connection between *APOE* alleles and the bone mass loss rate of osteoporotic fracture patients, and found that APOE4 allele might be an important genetic risk factor of hip fracture [23]. It has also been confirmed by many scientists that APOE4 allele together with APOE4 protein are closely correlated with primary osteoporosis and osteoporotic fracture [24-26]. In addition, some other scholars have demonstrated that APOE4 allele and APOE4 protein are markers for primary osteoporosis and especially for osteoporotic fracture [27, 28].

The present study showed that the BMDs of Ward's triangle, lumbar L2-L4 segment, greater trochanter, femoral neck and distal radius were apparently decreased after the occurrence of osteoporotic fracture, the contents of blood calcium and blood phosphate were also lower in people with lower BMD levels, but the AKP level was higher in individuals having lower BMDs. All these indicated that the occurrence of osteoporotic fracture might be in close connection with the decreasing of BMD, and patients with osteoporosis might have significantly reduced BMDs.

Furthermore, as could be known from our study, the case group had obviously higher APOE4 allele frequency than the control group, and E4 allele significantly reduced the BMD at Ward's triangle and lumbar L2-L4 segment. Because the decreased bone mass is a risk factor for fracture occurrence, APOE4 allele carriers may suffer from higher risk of developing fractures. Therefore, the APOE4 allele may have close correlations with primary osteoporosis and osteoporotic fracture.

Osteoporotic fracture is regulated by multiple genes and polymorphisms as well as race and environmental factors. However, the present study only concluded the possible relationship of the APOE4 allele with osteoporotic fracture. Therefore, the genetic factors influencing the osteoporotic fracture susceptibility are still not fully clear at present. Due to the fact that APOE4 allele may increase the risk of osteoporotic fracture, its carriers should pay attention to the early prevention and treatment of the disease. What's more, future studies about this issue should also take race and environmental factors into consideration.

Disclosure of conflict of interest

None.

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