Apolipoprotein E gene E2/E2 genotype is a genetic risk factor for vertebral fractures in humans: a large-scale study

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Abstract

Purpose Although many studies have been performed to evaluate whether or not apolipoprotein E gene (*APOE*) polymorphisms are differentially associated with bone mineral density (BMD) and fractures, the results have been conflicting. This large-scale study was performed to investigate whether a relationship exists between *APOE* polymorphisms and risk of fracture.

Methods A hospital-based case–control study was conducted in 3,000 patients with fractures and 3,000 age- and gender-matched healthy controls. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the *APOE* gene polymorphisms.

Results Patients with fractures had a significantly higher frequency of *APOE* E2/E2 genotype [odds ratio (OR)=2.02, 95 % confidence interval (CI)=1.30, 3.14; $P=0.002$] than healthy controls. When stratifying by fracture type, it was found that patients with vertebral fractures had a significantly higher frequency of *APOE* E2/E2 genotype (OR=2.86, 95 % CI=1.73, 4.73; $P<0.001$). No significant differences were found in nonvertebral (hip or wrist or other) fractures.

Conclusions Our study suggests that *APOE* E2/E2 genotype is a potential genetic risk factor for vertebral fractures in humans.

Keywords Apolipoprotein E · Fracture · Gene polymorphism · Case–control study

Introduction

Osteoporosis is a common skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone

tissue with increased susceptibility to fracture [1, 2]. It is a major public health issue. The World Health Organization estimates that 200 million women and men suffer from osteoporosis worldwide [3]. Osteoporosis has a complex etiology and is considered a multifactorial polygenic disease. Genetic factors may play a role in the development of osteoporosis [4–6]. Different polymorphisms have been described in several genes. Although a large number of association studies have been performed, the individual contribution of these polymorphisms to the pathogenesis of osteoporosis remains to be universally confirmed [7–9].

Apolipoprotein E (apoE), a 299-amino acid, arginine-rich glycoprotein, is an integral surface component of chylomicrons, very-low-density lipoproteins, and some subclasses of high-density lipoproteins [10, 11]. The *APOE* gene is polymorphic, consisting of three common alleles (E2, E3, and E4) and six different genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4 and E4/E4) [11]. A recent study strongly underlined the general importance of apoE as a regulator of bone metabolism and identified the *APOE* epsilon2 (E2) allele as a potential genetic risk factor for low trabecular bone mass and vertebral fractures in humans [12–14].

Although many studies have been performed to evaluate whether or not *APOE* polymorphisms are differentially associated with bone mineral density (BMD) and fractures, the results have been conflicting [15–19]. This large-scale study was performed to investigate whether a relationship exists between *APOE* polymorphisms and risk of fracture.

Materials and methods

Study population

A hospital-based case–control study was conducted in 3,000 patients with fractures and 3,000 age- and gender-matched

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healthy controls during the years 2005 to 2013 from the Changhai Hospital Affiliated to the Second Military Medical University, China. All fractures were confirmed by two physicians who reviewed the hospital records and validated each case. For analyses on osteoporotic fractures, we included all fractures, except fractures of the hand, foot, skull, and face. We also excluded pathological and postprocedural fractures. If any of the three vertebral heights (anterior, central, or posterior) showed a minimum decrease of at least 4.6 mm and 15 % in absolute height on the later film, it was considered an incident fracture. The healthy control subjects were matched with the patients for age and sex. Healthy control subjects were recruited from the Changhai Hospital Affiliated to the Second Military Medical University where they were attending a clinic for routine examination. The Ethical Committee of the Changhai Hospital Affiliated to the Second Military Medical University approved the study protocols, and all participants gave written informed consent according to the Declaration of Helsinki.

DNA extraction and genotyping

The commercially available Qiagen kit (QIAGEN Inc., Valencia, CA, USA) was used to extract DNA from peripheral blood leukocytes. Genomic DNA was isolated from 20 g/L ethylenediaminetetraacetic acid (EDTA) or sodium citrate anticoagulated 3–5 ml venous blood and stored at 4 °C. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the *APOE* gene polymorphisms. Based on the GenBank reference sequence, the PCR primers were as follows: sense, ACAGAATTGCGCCCGGCCTG GTACAC; antisense, TAAGCTTGGCACGGCTGTCC AAGGA. The amplified DNA was digested with *Hha* I at 37 °C for two hours. The digested PCR products were analysed by 6 % polyacrylamide gel electrophoresis (PAGE) and stained with ethidium bromide for visualization under UV light. For quality control, all genotyping personnel were blinded to clinical and neuroimaging data.

Statistical analysis

Comparisons between groups were made with chi-square test (nominal data) or Student t-test (interval data). The allele and genotype frequencies of the folate metabolism gene in patients were compared to controls using the chi-square test. The Hardy–Weinberg equilibrium was tested for goodness-of-fit chi-square test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. A *P*-value was considered significant at a level of <0.05. All analyses were performed with Stata software (College Park, TX).

Results

The demographical and clinical features of the participants are shown in Table 1. The mean age was 61.0 (±9.1) years for the fractures cases and 60.8 (±8.9) years for the controls. The 42.4 % participants were male for the fracture cases and 42.3 % for the controls. No significant differences were found between the cases and the controls in gender, age, body mass index (BMI), physical activity scale for the elderly (PASE), total cholesterol, triglycerides and smoking status (Table 1).

Patients with fractures had a significantly higher frequency of *APOE* E2/E2 genotype [odds ratio (OR)=2.02, 95 % confidence interval (CI)=1.30, 3.14; *P*=0.002] than healthy controls (Table 2). When stratifying by fracture type, it was found that patients with vertebral fractures had a significantly higher frequency of *APOE* E2/E2 genotype (OR=2.86, 95 % CI=1.73, 4.73; *P*<0.001) (Table 3). No significant differences were found in nonvertebral (hip or wrist or other) fractures (Table 3).

Discussion

Although many studies have been performed to evaluate whether or not *APOE* polymorphisms are differentially associated with BMD and fractures, the results have been conflicting, possibly because of variations in study design, small sample sizes, and heterogeneous populations, among other issues [19]. Some studies supported that there was an association between *APOE* polymorphisms and fractures. A study in 284 unrelated postmenopausal Japanese found that the *APOE* E4 allele was associated with a low bone mass [20]. A study in 219 hemodialysis patients showed that *APOE* genotype appears to be an important genetic risk factor for bone fracture

Table 1 The demographical and clinical features of the participants

Features	Cases	Controls	<i>P</i>
Total no.	3,000	3,000	
Gender (male/female)	1,272/1,728	1,269/1,731	0.94
Age, years	61.0±9.1	60.8±8.9	0.39
BMI, kg/m ²	26.1±3.1	26.0±3.0	0.20
PASE	144.2±6.1	144.0±6.0	0.20
Total cholesterol, mg/dL	190.0±3.8	189.9±3.7	0.30
Triglycerides, mg/dL	141.0±7.2	140.8±7.1	0.28
Smoking status (ever/never)	650/2,350	648/2,352	0.95
Vertebral fractures	1,140		
Nonvertebral fractures	1,860		
Hip fractures	900		
Wrist fractures	500		
Other fractures	460		

BMI body mass index, *PASE* physical activity scale for the elderly

Table 2 Frequencies of *APOE* gene polymorphisms in fractures cases and controls groups

Genotype	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR (95%CI)	<i>P</i>
E2/E2	60 (2.0)	30 (1.0)	2.02 (1.30,3.14)	0.002
E2/E3	339 (11.3)	360 (12.0)	0.93 (0.80,1.09)	0.40
E2/E4	40 (1.3)	45 (1.5)	0.89 (0.58,1.36)	0.59
E3/E3	1,926 (64.2)	1,932 (64.4)	0.99 (0.89,1.10)	0.87
E3/E4	610 (20.3)	605 (20.2)	1.01 (0.89,1.15)	0.87
E4/E4	25 (0.8)	28 (0.9)	0.89 (0.52,1.53)	0.68
E2 allele frequency	499 (8.3)	465 (7.8)	1.08 (0.95,1.23)	0.25
E3 allele frequency	4,801 (80.0)	4,829 (80.5)	0.97 (0.89,1.06)	0.52
E4 allele frequency	700 (11.7)	706 (11.7)	0.99 (0.89,1.11)	0.87

OR odds ratio, CI confidence interval

[21]. A prospective study in 1,750 women showed that the *APOE* polymorphism may be a candidate gene for hip fractures among community dwelling nondemented women [22]. A community-based study of 899 older adults found that the *APOE* E4 allele appears to be a risk factor for hip fracture [23]. But other studies did not support such an association between *APOE* polymorphisms and fractures. A study of 327 women showed no association between bone mass parameters, hip or lower forearm fracture and *APOE* genotypes in a population of postmenopausal women and age-matched controls [17]. The Rotterdam study did not support the hypothesis that the *APOE* E4 allele risk allele was associated with BMD, increased bone loss, or an increased risk of osteoporotic fractures [18].

A lot of studies have been conducted to examine the association of genetic polymorphism and osteoporotic fracture. A meta-analysis of 5,834 women from 30 study groups suggested that XX homozygotes of the estrogen receptor may have higher BMD and also a decreased risk of fractures when compared with carriers of the x allele, whereas the PvuII polymorphism was not associated with either BMD or fracture risk [24]. A meta-analysis of 26 published studies including 7,849 participants suggested that the *COL1A1* Sp1 alleles were associated with a modest reduction in BMD and a significant increase in risk of osteoporotic fracture, particularly vertebral fracture [25]. A meta-analysis of 13 studies including 1,279 cases and 6,069 controls conducted by Lei et al. suggested a modest but statistically significant association

between the *ESR1* PvuII pp genotype and vertebral fracture [26]. The meta-analysis of 17 studies including 2,112 fracture cases and 4,521 controls by Ji et al. suggested that there was a modest but statistically significant association between the BsmI bb genotypes of the vitamin D receptor gene and fracture [27].

The *APOE* gene polymorphisms were also associated with many other diseases. A meta-analysis of 28 case-control studies provided evidence for an association between the *APOE* E4 allele and frontotemporal lobar degeneration [28]. A meta-analysis of experimental and human studies suggested an association between *APOE* gene expression and its gene polymorphism with nephrotic syndrome susceptibility [29]. A meta-analysis of 29 studies suggested that *APOE* gene polymorphisms was associated with the risk of vascular dementia [30]. A case-control study showed that the *APOE* epsilon polymorphism has the expected impact on the plasma lipid profile, and the rs4420638 G allele may counterbalance the deleterious effect of the E4 allele on low-density lipoproteins-cholesterol levels in an Algerian population [11]. A case-control study found that *APOE* gene polymorphism was associated with maximal oxygen uptake levels after exercise training in Chinese young adults [31]. A hospital-based case-control study suggested that *APOE* E3/E4 genotype was associated with a higher lower-extremity deep venous thrombosis risk [32]. A meta-analysis of 45 studies found that *APOE* gene polymorphisms were associated with essential hypertension [33]. A meta-analysis of seven studies suggested

Table 3 Stratification analysis of *APOE* genotype frequency in fractures cases

Fracture type	Cases	E2/E2		E2/E3		E2/E4		E3/E3		E3/E4		E4/E4	
		<i>n</i> (%)	<i>P</i>	<i>n</i> (%)	<i>P</i>	<i>n</i> (%)	<i>P</i>	<i>n</i> (%)	<i>P</i>	<i>n</i> (%)	<i>P</i>	<i>n</i> (%)	<i>P</i>
Vertebral fractures	1,140	32 (2.8)	<0.001	126 (11.1)	0.40	16 (1.4)	0.82	729 (64.0)	0.79	228 (20.0)	0.91	9 (0.7)	0.66
Nonvertebral fractures	1,860	28 (1.5)	0.12	213 (11.4)	0.57	24 (1.3)	0.55	1,197 (64.4)	0.98	382 (20.5)	0.76	16 (0.9)	0.79
Hip fractures	900	15 (1.7)	0.10	108 (12.0)	1.00	12 (1.3)	0.72	568 (63.1)	0.48	189 (21.0)	0.59	8 (0.9)	0.90
Wrist fractures	500	7 (1.4)	0.42	55 (11.0)	0.52	6 (1.2)	0.61	327 (65.4)	0.67	101 (20.2)	0.99	4 (0.8)	0.77
Other fractures	460	6 (1.3)	0.55	50 (10.9)	0.49	6 (1.3)	0.75	302 (65.7)	0.60	92 (20.0)	0.93	4 (0.9)	0.89

an association between *APOE* E4 mutation and increased risk of recurrent pregnancy loss [34]. A meta-analysis of seven studies suggested that the *APOE* polymorphisms were associated with the risk of psoriasis, especially E2 and E3 alleles [35]. A meta-analysis included 29 studies which suggested that the *APOE* E4 allele was associated with an increased risk of developing cerebral infarction in a Chinese population [36]. A meta-analysis of 6,977 subjects provides evidence that *APOE* E2 mutation is associated with multiple sclerosis risk [37].

A strength of this study was the relatively large sample size. There are some limitations to the present study that should be mentioned. First of all, although this study suggested that *APOE* E2/E2 genotype was a potential genetic risk factor for vertebral fractures in humans, more biological background data are needed to explain our results. Secondly, this study only considers a Chinese population that may limit the application of these findings to other ethnic populations. Thirdly, the current finding might involve gene-to-environment interactions, which were not explored in the present study. Finally, this is a hospital-based case–control study, so the selection bias cannot be avoided and the subjects may not be representative of the general population.

In conclusion, this study suggests that *APOE* E2/E2 genotype is a potential genetic risk factor for vertebral fractures in humans. However it is highly desirable that our findings are validated through replication in other case–control series.

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