Apolipoprotein E Polymorphism and the Characteristics of Diseased Vessels in Male Chinese Patients With Angiographic Coronary Artery Disease: A Case-Case Study

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ABSTRACT

Background: Variations in the apolipoprotein E (apo E) gene may predict the incidence of coronary artery disease (CAD). However, the correlation between apo E polymorphism and the severity of CAD is still unclear.
Hypothesis: Apolipoprotein E polymorphism can predict CAD.
Methods: Used a case-case study of 213 Chinese angiographically-defined CAD patients who were screened for apo E genotypes. The characteristics of their diseased vessels were recorded.
Results: Apolipoprotein E4 carriers had >75% stenosis, more wide-ranging and longer vessel disease, a greater number of diseased vessels, and a higher Gensini score than apo E2 carriers or individuals with the apo E3/3 genotype. Apolipoprotein E2 carriers had ⩽75% stenosis and a shorter length of vessel disease than individuals with the apo E3/3 genotype or apo E4 carriers. The severity of stenosis, length of vessel disease, and number of diseased vessels were affected by the interaction between genotype and body mass index, family history of CAD, total plasma cholesterol level, smoking history, and hypertension history.
Conclusion: The apo E4 allele may serve as an independent genetic marker predicting severity of CAD. Other CAD risk factors may accelerate the process of pathogenesis. The apo E2 allele may play a protective role.

Introduction

Apolipoprotein E (apo E) polymorphism leads to disorders in lipidemia metabolism and is related to atherosclerosis. Individuals with different apo E genotypes have different susceptibilities to coronary artery disease (CAD). Studies have shown that the apo E4 allele is a genetic marker for CAD and an independent risk factor that predicts the incidence of cardiovascular disease[1]; apo E genotypes are also significantly associated with the severity of angiographically-defined CAD in women.[2] However, research on the correlation of apo E polymorphism with CAD severity has found large variations among different ethnic populations, between the sexes, and among groups with different exposures to environmental factors.[2,3] In this research, we designed a case-case study to analyze the relationship between apo E alleles and the characteristics of diseased vessels in 213 male patients diagnosed by selective coronary angiography (CAG) in China.

Materials and Methods

Study Population

A total of 213 male CAG-defined CAD patients between the ages of 34 and 83 were enrolled in this study. Genetic samples were obtained with informed consent and the study was approved by the institutional review board of the Fourth Military Medical University. Detailed medical data were collected, including the course and duration (in yrs) of chest pain, smoking behavior (yes/no), hypertension (yes/no), diabetes mellitus (yes/no), family history of CAD (yes/no), and body mass index (BMI; kg/m^2). Fasting total plasma cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined as described by Ilveskoski et al.[4] The concentration of low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald formula.[5]

Samples were divided into 3 groups according to genotype: E2 (including apo E2/2 and apo E2/3), E3 (apo E3/3), and E4 (including apo E3/4 and apo E4/4). Since the apo E2 and apo E4 alleles play opposite roles in lipid metabolism and CAD incidence, individuals with the apo E2/4 genotype (a total of 11 cases) were excluded.

Coronary artery disease was diagnosed on the basis of CAG (⩾50% stenosis in any major pericardial coronary artery). Major exclusion criteria included cardiomyopathy, New York Heart Association (NYHA) class IV congestive heart failure, myocardial infarction, and significant valvular or congestive heart disease. We recorded the characteristics of diseased vessels, including the severity of the most serious stenosis (⩽75% or >75%), the number of diseased vessels (1, 2, or 3 vessels), the longest length of vessel

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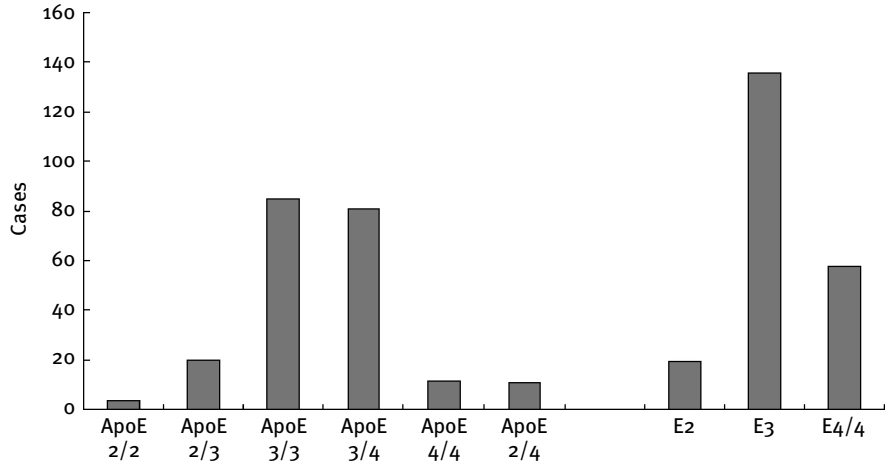

Figure 1. Genotypes: apo E2/2, apo E2/3, apo E2/4, apo E3/3, apo E3/4, apo E4/4. Apo E alleles: E2, E3, E4. Distribution of apo E genotypes in a Hardy-Weinberg equilibrium ($\chi^2 = 2.113$, $P = 0.833$).

disease, and characteristics of the stenosis (limited, tubular, or wide-ranging).

We also quantitatively evaluated the diseased vessels using the Gensini scoring system.[6]

Apolipoprotein E Genotype Determination

High-molecular-weight DNA was extracted from whole blood as described elsewhere.[7] Apolipoprotein E genetic polymorphism was investigated according to the described techniques.[8,9] The genetic sequences, including the 2 apo E polymorphic sites (codons 112 and 158), were amplified by a polymerase chain reaction. To maintain subject anonymity, blind genotyping was performed.

Statistical Analysis

Differences in the mean values of age, BMI, course of disease, and lipid levels among the 3 groups were compared using analysis of variance (ANOVA). Differences in smoking, hypertension, diabetes, and family CAD histories were compared using a χ^2 test. Apolipoprotein E allele frequencies were calculated by allele counting. Analysis of variance was used to test for a significant linear trend in the association of apo E polymorphism with the characteristics of diseased vessels. Multiple linear regression was then used to determine the odds ratio (OR) with 95% confidence interval (CI); the analysis was adjusted for significant CAD risk factors. Multiple regression analyses were also used to test for the effects of interactions between apo E genotypes and environmental factors on characteristics of diseased vessels. A 2-tailed test result of $P < 0.05$ was deemed to be significantly different.

Results

Distribution of Apo E Genotypes

The samples were found to have 6 different genotypes (apo E2/2, apo E2/3, apo E3/3, apo E3/4, apo E4/4, and apo E2/4) and 3 different alleles (E2, E3, and E4). The most frequent genotypes were apo E3/3 and apo E3/4. The frequencies of apo E alleles were 9.2%, 63.9%, and 27.3% for E2, E3, and E4, respectively. E3 was the most frequent allele. Figure 1 shows the distribution of apo E genotypes in a Hardy-Weinberg equilibrium.

Characteristics of the Samples

As shown in Table, the apo E2 carriers had a significantly lower BMI than individuals with the apo E3/3 genotype ($P < 0.001$) or the apo E4 carriers ($P = 0.005$). The apo E4 carriers had a higher prevalence of family history of CAD. A total of 11 cases with the apo E2/4 genotype were excluded.

Apoliprotein E Polymorphism and Lipid Levels

Lipid levels among the 3 groups are shown in Table 1. Levels of TC ($F = 9.603$, $P < 0.001$), HDL-C ($F = 3.552$, $P = 0.031$), and LDL-C ($F = 13.980$, $P < 0.001$) differed significantly among the 3 groups. The TC levels ($r = 0.253$, $P < 0.001$), TG levels ($r = -0.146$, $P = 0.038$), and LDL-C levels ($r = 0.250$, $P < 0.001$) were correlated with the apo E groups. The apo E4 carrier had a higher TC level than the apo E2 carrier ($P = 0.018$) or apo E3/3 genotype ($P < 0.001$), and a higher LDL-C level than the apo E3 carrier ($P < 0.001$). Individuals with the apo E3 genotype had a higher TG level than apo E4 carriers ($P = 0.028$). The apo E2 allele had a lower HDL-C than the apo E3/3 genotype ($P = 0.012$) or the apo E4 allele ($P = 0.012$).

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Table 1. Comparison of Characteristics

	E2 (n = 24)	E3 (n = 85)	E4 (n = 93)	*P* Value
Age (y)	54.96 ± 12.84	58.19 ± 11.16	55.85 ± 9.66	0.242
Course of disease (y)	2.58 ± 2.81	3.40 ± 4.88	3.55 ± 5.56	0.698
BMI (kg/m²)	22.28 ± 1.37	25.8 ± 3.98	24.43 ± 2.99	0.001
Smoking (y/n)	14/10	51/34	49/44	0.605
Hypertension (y/n)	10/14	41/44	55/38	0.183
Diabetes (y/n)	4/20	21/64	35/58	0.056
Family history (y/n)	5/19	43/42	47/46	0.023
TC (mmol/L)	4.05 ± 0.90	3.93 ± 1.24	4.76 ± 1.43	<0.001
TG (mmol/L)	1.75 ± 0.96	1.75 ± 1.49	1.33 ± 1.10	0.066
HDL-C (mmol/L)	0.83 ± 0.33	1.11 ± 0.26	1.11 ± 0.57	0.031
LDL-C (mmol/L)	2.57 ± 1.70	2.04 ± 1.31	3.14 ± 1.50	<0.001

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total plasma cholesterol; TG, triglycerides.

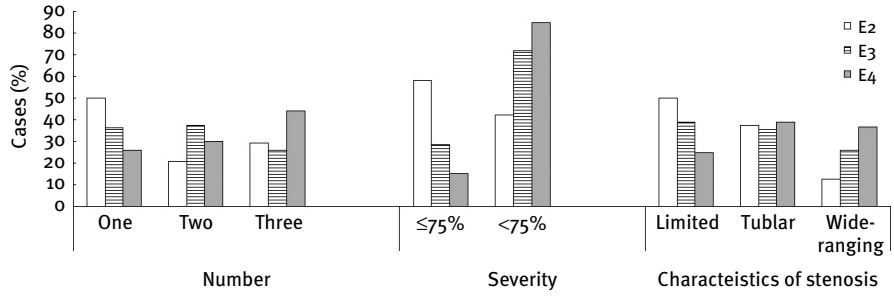

Figure 2. There are significant differences among the 3 groups in the number of stenosis (E3 vs E4, $P = 0.038$; E2 vs E4, $P = 0.043$), severity of stenosis (E2 vs E3, $P = 0.008$; E3 vs E4, $P = 0.043$; E2 vs E4, $P < 0.001$), and characteristics of stenosis (E3 vs E4, $P = 0.043$; E2 vs E4, $P = 0.023$).

Comparison of the Characteristics of Diseased Vessels

Significant differences were found in the number of diseased vessels ($\chi^2 = 10.206, P = 0.037$), severity of stenosis ($\chi^2 = 19.170, P < 0.001$), characteristics of stenosis ($\chi^2 = 10.514, P = 0.033$), length of vessel disease ($F = 8.085, P < 0.001$), and Gensini scores ($F = 13.480, P < 0.001$; Figures 2 and 3). Compared to individuals with the apo E3/3 genotype and apo E2 carriers, apo E4 carriers had significantly higher numbers of diseased vessels, more serious (>75%) stenosis, more wide-ranging vessel disease, longer vessel disease, and higher Gensini scores. However, the apo E2 carriers were more likely to have ⩽75% stenosis and shorter vessel disease compared to individuals with the apo E3/3 genotype. We also found a different disease tendency among the 3 groups. Compared to individuals with the apo E3/3 genotype, apo E4 carriers tended to develop 3 diseased vessels (OR: 2.258, 95% CI: 1.197–4.260, $P = 0.012$), wide-ranging vessel

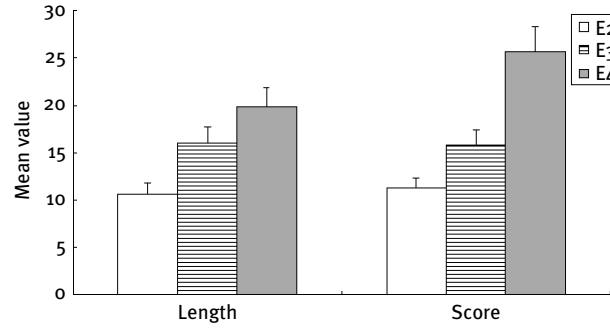

Figure 3. There are significant differences among the 3 groups in the length (mm) of diseased vessels (E2 vs E3, $P = 0.012$; E3 vs E4, $P = 0.038$; E2 vs E4, $P < 0.001$) and Gensini score (E3 vs E4, $P < 0.001$; E2 vs E4, $P < 0.001$).

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disease (OR: 1.873, 95% CI: 0.973–3.606, $P = 0.042$), and >75% stenosis (OR: 2.220, 95% CI: 1.060–4.648, $P = 0.043$). However, the apo E2 carriers tended to have ⩽75% stenosis (OR: 3.558, 95% CI: 1.391–9.099, $P = 0.008$).

Analysis of the Correlation Between Genotype and the Characteristics of Diseased Vessels

The apo E groups were significantly associated with the length of vessel disease ($F = 12.55$, $P < 0.001$), characteristic of stenosis ($F = 8.520$, $P = 0.004$), number of diseased vessels ($F = 5.47$, $P = 0.020$), severity of stenosis ($F = 15.79$, $P < 0.001$), and Gensini scores ($F = 22.28$, $P < 0.001$). The severity of stenosis was significantly associated with the apo E genotype ($F = 39.99$, $P = 0.001$) even after other factors were accounted for. But no relationship was found between lipid levels and characteristic of diseased vessels. Additionally, we found that BMI was associated with length of vessel disease ($F = 4.10$, $P = 0.044$), number of diseased vessels ($F = 10.47$, $P = 0.001$), characteristics of diseased vessels ($F = 4.20$, $P = 0.042$), quantitative scores ($F = 5.70$, $P = 0.018$), and TG levels ($F = 9.02$, $P = 0.003$).

Significant interaction effects were observed between genotype and BMI ($F = 5.215$, $P = 0.023$), CAD family history ($F = 19.856$, $P < 0.001$), and TC levels ($F = 4.475$, $P = 0.035$); between TC levels and BMI ($F = 5.959$, $P = 0.015$), smoking ($F = 4.728$, $P = 0.030$); between LDL-C levels and BMI ($F = 17.165$, $P < 0.001$), smoking ($F = 6.514$, $P = 0.011$), family history ($F = 5.037$, $P = 0.025$), on the severity of stenosis; between genotype and BMI ($F = 4.710$, $P = 0.031$), smoking history ($F = 4.350$, $P = 0.038$), and TC levels ($F = 3.746$, $P = 0.045$); between TC levels and smoking history ($F = 4.788$, $P = 0.029$), family history ($F = 5.199$, $P = 0.023$), on the length of vessel disease; between genotype and BMI ($F = 3.852$, $P = 0.050$), hypertension history ($F = 5.702$, $P = 0.017$), and TC levels ($F = 6.187$, $P = 0.013$); between TC, LDL-C levels and BMI ($F = 12.231$, 6.394; $P < 0.001$, 0.012) on the number of diseased vessels. In addition, the characteristics of stenosis was affected by a significant interaction between genotype and hypertension history ($F = 7.023$, $P = 0.008$).

Discussion

Apolipoprotein E is a constituent of lipoprotein. It plays an important role in transportation and metabolism of lipid. Studies have shown that apo E alleles have a greater influence on lipid metabolism than any other alleles.[10] Apolipoprotein E polymorphism can explain as much as 11% of the variation in lipid parameters.[11] Increased plasma TC levels play an important role in the occurrence of CAD, and apo E alleles are a marker for susceptibility to increased lipid levels and atherosclerotic disease.[12] But some studies have shown that there is no correlation between lipid levels and CAD severity and that apo E polymorphism was still a CAD risk factor even after lipid factors were accounted for.[1,13] And the E4 allele is also associated with a higher restenosis rate after percutaneous transluminal coronary angioplasty.[14] Though the association of apo E polymorphism with the severity of CAD was recently discovered, study results are still controversial.[1,2,13] So we designed a case-case study to research the relationship between apo E polymorphism and characteristics of diseased vessels in male CAD patients defined by CAG in China.

We found that apo E3/3 and apo E3/4 were the most frequent genotypes (39.91% and 38.03%). The least frequent genotype was apo E2/2. The frequency of the apo E4 allele was 27.3%. This distribution was not the same as the distribution in healthy people or in patients with CAD that was not defined by CAG.[3,15]

Our study showed that apo E4 carriers had more serious stenosis, longer vessel disease, a greater number of diseased vessels, more wide-ranging vessel disease, and higher Gensini scores than apo E2 carriers or individuals with the apo E3/3 genotype. The risk of having a >75% stenosis, 3 diseased vessels, and a wide-ranging vessel disease were 2.22-fold, 2.258-fold, and 1.873-fold higher for apo E4 carriers than for individuals with other genotypes. Because we adjusted the OR by known risk factors, these findings suggest that carrying the apo E4 allele is an independent genetic risk factor for serious CAD. Obesity, smoking, hypertension, high TC, LDL-C levels, a family history of CAD, and other risk factors can strengthen the allele's role in the progression of CAD. Single-factor logistic regression analysis revealed a significant association between the apo E4 allele and the severity of stenosis ($F = 39.99$, $P < 0.001$). This result strongly suggests that the apo E4 allele directly controls the progression of CAD; its role is unrelated to lipid levels or other risk factors. Therefore, the apo E4 allele may serve as a genetic marker for susceptibility to serious CAD.

The correlation between the apo E2 allele and CAD is still debated. Yang et alfound that the apo E2 allele has a protective effect on the cardiovascular system.[15] However, another study has linked the presence of the apo E2 allele to a greater risk of CAD.[16] Our study showed that the apo E2 allele was associated with a decreased severity of stenosis and shorter vessel disease. The apo E2 allele had a protective effect on the coronary arteries. An earlier autopsy study showed that the apo E2 allele was protective against fibrous cap atheromas[17]; our results are consistent with these findings. Additionally, BMI was associated with the characteristics of diseased vessels in male Chinese CAD patients. Clearly, further studies with larger samples are needed to fully understand this relationship.

The precise mechanism of interaction between apo E polymorphism and CAD is not yet clear. Apolipoprotein E4 allele may lead to increased TC and LDL-C levels; this increase is the result of chylomicron remnants that lead to the downregulation of the LDL receptor.[11] Elevated TC levels lead to atherosclerosis, which is why antioxidant treatments can slow the progression of atherosclerosis[18];

and healthy diets and lifestyles may therefore slow the progression of CAD.[19] A population-based autopsy study had proved that the apo E4 genetic variant was associated with pathological intimal thickening and atherosclerotic burden in the carotid arteries.[20] But elevated lipid levels were not associated with characteristics of angiographically-defined CAD in our study. They seem to have a minimal effect on the progression of CAD; and some studies have shown that there is no correlation between lipid levels and severity of CAD.[13] So we speculate that the essential cause in apo E4 carries is weaker antioxidant function than apo E2 and apo E3. It appears that the association of the apo E4 allele with CAD could be due to its default action in protecting lipoproteins and the endothelial cells of coronary arteries from oxidative damage. And based on genetic deficiency, atherosclerosis appears to be accelerated by the interactions among the apo E4 allele and other CAD risk factors such as elevated lipid levels, smoking, obesity, and hypertension. Therefore, the apo E4 allele is not only an independent risk factor for CAD, but also an important genetic marker predicting severity of CAD. Compared to apo E4 allele carriers, apo E2 carriers have low TC levels which slows atherosclerosis because few foam cells are formed. Perhaps most importantly, apo E2 has 2 free sulphydryl groups, making it a stronger antioxidant than either apo E3 or apo E4. The allele's protective role is independent of lipid levels and other risk factors.

Conclusions

Male Chinese CAG-defined CAD patients have a high distribution of the apo E4 allele. The apo E4 allele is associated with the presence of serious CAD. It is an independent genetic factor predicting severity of CAD. Coronary artery disease is accelerated by the interactions between the apo E4 allele and other CAD risk factors. The apo E2 allele may play a protective role in the development of CAD.

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