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Apolipoprotein E Gene Polymorphisms and Risk for Coronary Artery Disease in Chinese Xinjiang Uygur and Han Population

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Aim The study was designed to examine the relationship between polymorphism at the apolipoproteinlipoprotein E gene and the risk of coronary artery disease (CAD). Furthermore, the association of the polymorphism with the classical risk factors was analyzed.

Methods A total of 124 patients with angiographically verified CAD or myocardial infarction were prospectively evaluated. Data referring to hypertension, diabetes and tobacco consumption were recorded. The plasma levels of total cholesterol, HDL cholesterol, apolipoproteinlipoprotein AI and B and triglycerides were determined. DNA was obtained from the 124 patients and from 70 controls. In order to determine the apolipoproteinlipoprotein E genotypes, DNA was PCR amplified and digested with HhaI.

Results The frequency of the ε2, ε3 and ε4 are 0.155 ±0.300, 0.648 ±0.342, and 0.197 ±0.246 in Uygur population and 0.081 ±0.196, 0.772 ±0.315, and 0.146 ±0.237 in Han population respectively. The frequency of the ε2, ε3 and ε4 are 0.060 ±0.198, 0.758 ±0.302, and 0.182 ±0.250 in the patient group and 0.193 ±0.286, 0.671 ±0.370, and 0.136 ±0.224 in the control group respectively. ε2 frequency of Uygur' patient and control are 0.050 ±0.221 and 0.290 ±0.336. Serum LDL cholesterol, TC and TG values tended to decrease from the apolipoproteinlipoprotein E-4 phenotypes to apolipoprotein E-2 phenotypes. Deletion polymorphism of ε2 compared with common risk factors for CAD, its risk ratio (RR) is 4.38.

Conclusion These studies showed that the apolipoprotein E phenotype distribution in Uygur population differed significantly from Han population in Xinjiang. CAD patients have significantly lower ε2 allele than controls especially in Uygur population. Deletion of ε2 may be a risk factor for CAD. (***Chin J Arterioscler***, 2003, 11(5): 0429-0434)

1 INTRODUCTION

Coronary artery disease (CAD) is a complex trait in which inherited and environmental risk factors interact to drive the disease process[1]. The fact that classical cardiovascular risk factors, such as total cholesterol (TC), hypertension, tobacco consumption and diabetes, are not present in all patients suffering from early coronary disease and that reduction in morbidity and mortality derived from the treatment of these factors is about 30% prompted several groups to explore other factors which may be involved in the pathogenesis of the atherosclerosis and thrombosis. Recent research has been directed towards the study of new factors implicated in the development of the disease, such as procoagulant factors, homocysteinemia and genetic polymorphisms[2].

Genetic studies have identified polymorphisms and mutations in the apolipoprotein E gene and other genes associated with high concentration of plasma total and low density lipoprotein(LDL)-cholesterol and CAD. To evaluate the significance of the apolipoprotein E polymorphism as a risk factor for CAD, we determined apolipoprotein E phenotypes in 124 CAD patients (including 45 angina pectoris and 79 MI) and 70 controls and examined the relationship between polymorphism at the apolipoprotein E gene and the risk for CAD. We also analyzed the association between these polymorphisms and several biochemical parameters.

2 OBJECT AND METHODS

2.1 Object of study

Prospectively, 124 patients with an average age of 56 years (range 27-71) were evaluated. All patients had suffered a first episode of coronary disease with angiographically verified CAD, defined according to the guidelines of the WHO MONICA protocol as stable angina and unstable angina. They complained of chest discomfort, precipitated by exertion or emotion, with ST-T depression on the electrocardiogram at rest or during an exercise test and relieved by rest or nitrates, and this occurred at rest and was of recent onset or progressive. Other cases were diagnosed as myocardial infarction according to two of the

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following criteria: chest discomfort longer than 30 min, enzymatic criteria and serial electrocardiographic changes.

Patients with a documented history of hypertension or with systolic blood pressure values equal to or greater than 140 mm Hg were defined as hypertensives. Those with a history of hypercholesterolemia or showing TC concentrations equal to or greater than 2 g/L were diagnosed as hypercholesterolemia. Patients with a history of diabetes or basal plasma glucose level greater than 12 g/dL were defined as diabetics. In addition, a smoking history was collected by means of a structured questionnaire from all subjects.

70 controls from the same population (Urumqi, Northern-west China), who were matched with patients for ethnicity, recruited from hospital staff and blood donors. These controls, who were not clinically evaluated, were representative of the diet and smoking habits and genetic factors involved in cardiovascular risk[3].

2.2 Biochemical Analysis

During hospitalization and after fasting for 12 h, a lipid profile study which included determination of TC, HDL cholesterol (HDLC), apolipoprotein A1 and B and triglycerides (TG) was carried out. The lipid profiles were determined enzymatically using USA Beckerman Synchron CX7 Clinical Systems automated biochemical analyzer. Cholesterol was determined through an enzymatic colorimetric test (cholesterol CHOD-PAP method).

Lp(a) in plasma was measured using a commercially available ELISA [Tint Elize Lp(a) strip-well format]. HDLC was determined after precipitation of the remaining lipoproteins by the addition of phosphotungatic acid and magnesium ions to the sample; subsequent centrifugation leaves HDL available for enzymatic assay (cholesterol CHOD-PAP method). TG were measured using a colorimetric end-point method (GPO-PAP).

Apolipoproteins were determined by photometric measurement of the antigen-antibody reaction (ovine anti-human apolipoproteins) by the end-point method (immunoturbidimetric test).

2.3 DNA Genotyping

DNA was obtained following an extracted method[4] from the 124 patients and 70 controls. The apolipoprotein E genotypes were determined after PCR amplification of a 244-bp fragment, followed by digestion with the restriction enzyme HhaI. Briefly, DNA were amplified with primers F4 (5'-ACA GAA TTC GCC CCG GCC TGG TAC AC-3') and F6 (5'TTA GCT TGG CAC GGC TGT CCA AGG A-3') described by Emi et al[5]. The PCR was performed in a volume of 30 μL containing 200 ng genomic DNA. The amount of [Mg^{2+}], dNTP (Promega, Madison, WI, USA) and Taq polymerase (Promega) used in each reaction were 1.67 mmol/L, 25 μmol/L, and 0.75 unit, respectively. Thermal cycles (DNA Thermal Cycler 480, Perkin Elmer Cetus, Norwalk, CT, USA) started with 94 ℃ for 5 min and were followed by 30 cycles of 94 ℃ for 45 s, 62 ℃ for 45 s, and 72 ℃ for 1 min.

A total volume of 10 μL containing 5 units of HhaI (Promega) diluted in manufacturer recommended buffer was added directly to the PCR product (8.0 μL) and incubated at 37 ℃ for 4 h. Each reaction mixture was loaded onto an 8% polyacrylamide nondenaturing gel (1.5 mm thick × 25 cm long, Promega) and electrophoresed for 3 h under constant current (45 mA). After electrophoresis, the gel was treated with ethidium bromide (0.2 mg/L) for 30 min and DNA fragments were visualized by Image Master(r) VDS (Pharmacia Biotech, Switzerland). The sizes of HhaI fragments were estimated by comparison with known size markers [pGEM-7zf (+)].

Consent was obtained from patients and controls before participation and investigation conforms with the principles outlined in the Declaration of Helsinki[6].

2.4 Statistical Analysis

Using SPSS (Statistics Package for Social Science) for Windows. Differences in values were compared using the χ^2 test. To analyze differences between parametric variables, the T-test or ANOVA test was used. For nonparametric variables, we used the Kruskal-Wallis test.

Odds ratios were also calculated using Logistic Regression.

3 RESULTS

3.1 Anthropometric characteristics and lipid values of the patients and control groups

124 patients with AP (45 cases) and MI (79 cases) were analyzed (Table 1). It showed that age, smoking, hypertension, diabetes, F. history, TG, HDL, LDL, apolipoprotein B, apolipoprotein B/A and lipoprotein(a) in patients groups were different from those in control groups.

3.2 Prevalence of apolipoprotein E phenotypes and ε allele frequencies in controls and in patients with coronary artery disease

The CAD phenotype frequencies were significantly different from the control distribution as judged from the χ^2 test of the goodness-of-fit of CAD data to the control frequencies based on the Xinjiang allele frequencies (χ^2 = 21.178, P = 0.001, 6 × 2 comparison) (Table 2).

There was a decrease in the number of CAD patients

with the phenotypes E2/3 and E2/4 as compared with the distribution of the E phenotypes in the control population.

The ε2 allele frequency in the CAD patients (0.060 ± 0.198) was lower than in the control population (0.193 ±0.286) ($P < 0.01$), with a corresponding increase in the ε3 and ε4 allele frequency (Table 3).

Table 1. Anthropometric characteristics and average lipoproteins values in the patients and control groups

Index	Control	CAD
n	70	124
Age(years)	43 ±10	56 ±10[b]
Men(%)	58(84%)	112(90%)
Nationality(Uygur)	31(44%)	40(32%)
Smoker(%)	26(37%)	76(61%)[b]
Hypertension(%)	7(10%)	41(33%)[b]
Diabetes(%)	3(4%)	21(17%)[b]
F. History	4(6%)	19(15%)[a]
TG(mmol/L)	1.91 ±1.60	2.38 ±0.99[a]
TC(mmol/L)	5.37 ±1.08	5.52 ±1.35
HDL(mmol/L)	1.01 ±0.23	0.89 ±0.11[b]
LDL(mmol/L)	3.36 ±0.98	4.14 ±1.26[b]
Apo A(g/L)	1.29 ±0.21	1.14 ±0.76
Apo B(g/L)	1.27 ±0.47	0.99 ±0.25[b]
Apo B/A	0.67 ±0.14	0.94 ±0.28[b]
LP(a)(mg/L)	113 ±75	215 ±171[a]

Data presented are $\bar{x} \pm s$ or percent of patients. a: $P < 0.05$, b: $P < 0.01$ compared with controls.

3.3 Relationship between risk factors for coronary artery disease and apoprotein E phenotypes

After PCR amplification and HhaI digestion, the six genotypes of apolipoprotein E were found in controls and patients. Genotype frequencies were in the Hardy-Weinberg equilibrium in the control population. Table 4 summarizes the presence of the common CAD risk factors in the different apolipoprotein E phenotypes. Of the six common apolipoprotein E phenotypes, six were observed in the CAD patients and five with absence of E4/4 were observed in the controls. Serum LDL cholesterol, TC and TG values tended to decrease from the apolipoprotein E-4 phenotypes to apolipoprotein E-2 phenotypes, while the serum apolipoprotein A, apolipoprotein B, Lp(a) and HDL cholesterol levels were similar in different apolipoprotein E phenotypes as judged on the basis of one-way analysis of variance (Table 4).

Table 2. Prevalence of apolipoprotein E phenotypes in controls and patients with CAD

Apo E phenotype	Controls(n= 70)	CAD(n= 124)
E2/2	4.3%(3)	2.4%(3)
E2/3	18.6%(13)	4.8%(6)
E2/4	11.4%(8)	2.4%(3)
E3/3	50.0%(35)	57.3%(71)
E3/4	15.7%(11)	32.3%(40)
E4/4	0%(0)	0.8%(1)

$P < 0.01$(χ^2= 21.178, P= 0.001, 6×2 comparison)

Table 3. ε allele frequencies in controls and patients with CAD

Population (n)	ε2	ε3	ε4
Controls(70)	0.193 ±0.286	0.671 ±0.370	0.136 ±0.224
CAD(124)	0.060 ±0.198[a]	0.758 ±0.302	0.182 ±0.250

a: $P < 0.01$, compared with controls

Table 4. Presence of risk factors for coronary artery disease in subjects with different apoprotein E phenotypes

Risk factor	E2/2 (n= 6)	E2/3 (n= 19)	E2/4 (n= 11)	E3/3 (n= 106)	E3/4 (n= 51)	E4/4 (n= 1)
Sex (Men)*	100 (6)	100 (19)	81.8 (9)	84.9 (90)	90.2 (46)	100 (1)
Age (> 45 yr)*	83.3 (5)	73.7 (14)	63.6 (7)	71.7 (76)	64.7 (33)	100 (1)
Smoking*	50.0 (3)	21.1 (4)	45.5 (5)	49.1 (52)	72.5 (37)	100 (1)
Hypertension*	0 (0)	10.5 (2)	9.1 (1)	34.0 (36)	17.6 (9)	0 (0)
Diabetes*	0 (0)	15.8 (3)	9.1 (1)	17.0 (18)	3.9 (2)	0 (0)
F. History*	0 (0)	0 (0)	0 (0)	15.1 (16)	13.7 (7)	0 (0)
TG (mmol/L)	1.53 ±1.07	1.68 ±0.75	2.11 ±1.70	2.28 ±1.36	2.35 ±1.11	2.64 ±0.00
TC (mmol/L)	4.71 ±0.65	5.48 ±0.93	5.38 ±1.52	5.37 ±1.20	5.63 ±1.22	5.97 ±0.00
HDL (mmol/L)	0.84 ±0.22	0.92 ±0.11	0.85 ±0.07	0.97 ±0.21	1.02 ±0.23	1.03 ±0.00
LDL (mmol/L)	3.29 ±1.09	3.98 ±0.96	3.84 ±1.47	3.56 ±1.15	3.72 ±1.17	3.88 ±0.00
ApoA (g/L)	1.29 ±0.20	1.22 ±0.19	1.16 ±0.24	1.14 ±0.28	1.31 ±1.13	1.14 ±0.00
ApoB (g/L)	1.20 ±0.30	1.14 ±0.31	1.11 ±0.65	1.08 ±0.40	1.08 ±0.26	1.29 ±0.00
Lp (a) (mg/L)	300 ±171	210 ±111	132 ±193	200 ±169	216 ±165	203 ±0.00

3.4 Differences of apolipoprotein E phenotypes and ε allele frequencies in controls and patients with different nationality

The apolipoprotein E phenotype distribution in Uygur population differed significantly from Han population in Xinjiang (Table 5).

Table 5. Prevalence of apolipoprotein E phenotypes in Xinjiang Uygur and Han population

Apo E phenotype	Uygur population			Han population		
	CAD (n= 40)	Control (n= 31)	total (n= 71)	CAD (n= 84)	Control (n= 39)	total (n= 123)
E2/2	5.0% (2)	9.7% (3)	7.0% (5)	1.2% (1)	0% (0)	0.8% (1)
E2/3	0% (0)	25.8% (8)	11.3% (8)	7.1% (6)	12.8% (5)	8.9% (11)
E2/4	0% (0)	12.9% (4)	5.6% (4)	3.6% (3)	10.3% (4)	5.7% (7)
E3/3	42.5% (17)	41.9% (13)	42.3% (30)	64.3% (54)	56.4% (22)	61.8% (76)
E3/4	52.5% (21)	9.7% (3)	33.8% (24)	22.6% (19)	20.5% (8)	22.0% (27)
E4/4	0% (0)	0% (0)	0% (0)	1.2% (1)	0% (0)	0.8% (1)

Uygur are compared with. Han: $P< 0.05$($\chi^2= 12.023$, $P= 0.034$, 6×2 comparison); Patients are compared with control in Uygur: $P< 0.01$ ($\chi^2= 25.502$, $P= 0.0001$, 6×2 comparison); Patients are compared with control in Han: $P> 0.05$($\chi^2= 4.301$, P= 0.507, 6×2 comparison)

CAD patients have significantly lower ε2 allele and slightly higher ε3 or ε4 allele frequency than controls especially in Uygur population (Table 6).

Table 6. εAllele frequencies in controls and in patients with different nationality

Groups (n)	ε2	ε3	ε4
Uygur population			
Controls (31)	0.290±0.336	0.597±0.392	0.113±0.213
CAD (40)	0.050±0.221*	0.688±0.293	0.263±0.253
Total(71)	0.155±0.300#	0.648±0.342	0.197±0.246
Han population			
Controls (39)	0.115±0.213	0.731±0.341	0.154±0.234
CAD (84)	0.065±0.187	0.791±0.301	0.143±0.240
Total(123)	0.081±0.196	0.772±0.315	0.146±0.237

v. control: * $P< 0.05$(t test); v. Han: # $P< 0.05$(t' test)

3.5 Decrease of ε2 allele frequency as a risk factor for CAD

Deletion polymorphism of ε2 compared with common risk factors [gender (man), age (> 45), smoking, hypertension, diabetes, family history, TC, HDL, LDL] for CAD by using Logistic Regression with Analysis of maximum likelihood estimates in patients with CAD and control subjects, it's risk ratio (RR) is 4.38, indicating decrease of ε2 allele frequency as a risk factor for CAD.

4 DISCUSSION

Our observation of a significantly decreased frequency of the ε2 allele ($P< 0.01$) and slightly increased ε3 and ε4 allele ($P> 0.05$) in patients with angiographically verified CAD or MI is in accordance with Menzel et al[7].

Furthermore, several clinical studies have reported that the frequency of the ε4 allele is higher in individuals with cardiovascular disease, eg, myocardial infarction survivors or subjects with angiographic evidence of atherosclerosis, than in control subjects[8-11]. However, Utermann G et al[12] were not able to confirm this finding. Menzel et al[7] have suggested that the ε2 allele may have a protective effect on the development of coronary atherosclerosis. Sheehan D et al[13] found that healthy Irish adults with the apolipoprotein E4 genotype have higher serum total and LDL-cholesterol levels than those with E2 or E3 apolipoprotein E genotypes and therefore may have a higher risk of atherosclerotic coronary artery disease and coronary heart disease in later life. Kuusi et al[14] observed a significantly increased frequency of the ε4 allele in patients with angiographically verified CAD and suggested that the high ε4 allele frequency in the Finnish population may be one factor contributing to Finns' increased susceptibility to CAD. The ε4 (epsilon 4) allele of the gene coding for apolipoprotein E is associated with an atherogenic lipid profile that has been linked to increased risk of coronary artery disease (CAD). They noted although ε2 allele frequency was lower in the patients with CAD than in the general population, no statistical difference was found between two groups. But our findings suggested the impact of ε2 allele on the CAD risk seems to be of more importance than ε4 in Xinjiang Uygur and Han population, unlike in Finns. We also find that the apolipoprotein E phenotype distribution in Uygur population differed significantly from Han population in Xinjiang.

Apolipoprotein E, a normal constituent of plasma chylomicrons, very low density lipoproteins (VLDL), and high density lipoproteins (HDL)[15], binds with high affinity to receptors in liver and extrahepatic cells, thereby mediating the uptake of apolipoprotein E-containing lipoproteins, especially the cholesterol-enriched remnants of triglyceride-rich lipoproteins. In addition, it is considered that an HDL subfraction containing apolipoprotein E plays an important role in the reverse transport of choles-

terol from peripheral cells to the liver[16].

As we find, several studies have demonstrated associations between the ε4 allele and increased LDL cholesterol levels and the ε2 allele and low LDL cholesterol in normal subjects[17]; a similar association has been found in patients with myocardial infarction[18].

The genetic polymorphism of apolipoprotein E is due to three common alleles, ε2, ε3, and ε4, at a single autosomal gene locus[19]. These alleles determine the six phenotypes E2/2, E3/3, E4/4, E4/3, E4/2, E3/2. The isoprotein apolipoprotein E-2, a product of theε2 allele, is a mutant differing from the most common isoprotein apolipoprotein E-3 by a single amino acid interchange (arg158 → cys158). This mutation leads to a protein that has a much lower affinity for the cellular apolipoprotein E receptor[20]. The other mutant, apolipoprotein E-4, differs from the parent apolipoprotein E-3 by a single cys112 → arg112 interchange[21]. Low density lipoprotein(LDL) metabolism is associated with the apolipoprotein E polymorphism, and as much as 16% of the genetic variance in LDL concentration can be accounted for by allelic differences at the apolipoprotein E gene locus. Since LDL metabolism is closely associated with atherogenesis, it is plausible to presume that the apolipoprotein E genotype may convey a susceptibility to atherosclerosis.

Corbo RM[22] suggested that the apolipoprotein ε4, based on some functional properties of it and on its distribution among human populations, could be identified as a 'thrifty' allele. The exposure of apolipoprotein ε4 to the contemporary environmental conditions (Western diet, longer lifespans) could have rendered it a susceptibility allele for CAD and Alzheimer (AD). The absence of the association of apolipoprotein ε4 with CAD and AD in Sub-Saharan Africans, and its presence in African Americans, seems to confirm this hypothesis. Apart from the analytical problems, one should be aware of ethnic variation of the apolipoprotein E genotype, which is highly variable among different populations. apolipoprotein ε4 allele frequency in Europe is distributed along a decreasing north/south gradient. ε4 allele frequency in black African and in Papua New Guineans is high; in contrast, its frequency in Asian populations is low[23].

Corbo RM et al[24] suggested that quantitative data are consistent with the hypothesis that apolipoproteinE has an anti-atherosclerotic role and the apolipoprotein E quantitation could be a useful parameter for defining cardiovascular risk. Apolipoprotein ε4 allele appears to be a risk factor for CAD in the Italian population and could act by its association with low apolipoprotein ε4 levels.

A common assumption underlying most genetic studies is that individuals with different genotypes respond similarly to exposure to internal (epigenetic and background genotype effects) and external (ecological) environments. Stengard JH[25] studied that their observations were consistent with a complex pathobiology of CAD involving biochemical and physiological agents that are under the influence of interactions between genetic and environmental factors. Information about these interactions is necessary for developing a more precise risk assessment and ultimately to improve public health and clinical strategies to prevent this devastating disease both at the individual and population levels. Our studies confirm and extend, the apolipoprotein E phenotype distribution in Uygur population differed significantly from Han population that observed in Xinjiang Uygur and Han population. CAD patients have significantly lower ε2 allele and slightly higher ε3 or ε4 allele frequency than controls especially in Uygur population. Deletion of ε2 may be a risk factor for CAD.

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新疆维汉两民族冠心病患者载脂蛋白 E 基因的多态性

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[关键词] 载脂蛋白 E; DNA 多态性; 危险因子; 冠心病

目的 探讨新疆乌鲁木齐地区维汉两民族冠心病患者载脂蛋白 E 基因多态性与冠心病的关系。**方法** 用酚氯仿抽提核酸法从凝血块中分离 DNA, 用聚合酶链反应- 限制片长多态性方法对新疆乌鲁木齐地区维汉两民族 124 例冠心病患者和 70 例对照组人群进行载脂蛋白 E 基因多态性(由 ε2、ε3 和 ε4 决定的 E2/2、E3/3、E4/4、E4/2、E4/3 和 E3/2) HhaI 酶切研究。**结果** (1) 维吾尔族中载脂蛋白 E ε2、ε3 和 ε4 等位基因频率分别为 0.155 ±0.300、0.648 ±0.342 和 0.197 ±0.246, 与汉族(0.081 ±0.196、0.772 ±0.315 和 0.146 ±0.237) 比较, ε2 明显增高($P< 0.05$), ε3 和 ε4 虽有减低但无显著差别($P> 0.05$)。(2) 载脂蛋白 E ε2、ε3 和 ε4 等位基因频率在冠心病组的分布分别为 0.060 ±0.198、0.758 ±0.302 和 0.182 ±0.250, 与对照组(0.193 ±0.286、0.671 ±0.370 和 0.136 ±0.224) 比较, ε2 明显减低($P< 0.01$), 维吾尔族中更明显(0.050 ±0.221 对 0.290 ±0.336, $P< 0.05$), ε3 和 ε4 虽有升高但无显著差别($P> 0.05$)。(3) 由 ε2 到 ε4 低密度脂蛋白胆固醇、总胆固醇和甘油三酯逐渐升高。将等位基因与冠心病其它危险因子一起作 Logistic 回归分析发现, ε2 缺失(危险比 RR= 4.38, $P< 0.05$) 为冠心病的危险因子之一。**结论** 新疆乌鲁木齐地区维汉两民族人群中(1) 维族和汉族人群中载脂蛋白 E 基因型分布有非常显著性差异($P< 0.01$); 维族人群中 ε2 等位基因频率明显高于汉族。(2) 冠心病患者载脂蛋白 E ε2 等位基因频率明显降低, 其中维族更明显, ε3 和 ε4 有所升高; 从 ε2 到 ε4, LDL、TG 和 TC 有升高趋势。(3) 载脂蛋白 E 基因多态性(ε2 等位基因缺失) 为冠心病的危险因子之一, 亦即 ε2 与冠心病呈负相关。

(此文编辑 胡必利)