Apolipoprotein E polymorphism in northern Chinese elderly patients with coronary artery disease

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Background and objective Apolipoprotein E is a constituent of lipoproteins with considerable variation due to cysteine-arginine exchanges. We investigated the relationship between apo E gene polymorphism and the occurrence of coronary artery disease (CAD) in the older population of northern China. Methods The distribution of the HhaI polymorphisms of the apolipoprotein E gene was determined among 55 patients with CAD (CAD group), which was compared with that of 36 elderly subjects without CAD (control group). Results Genotype distributions at both sites (apo E gene 112-bp and 158-bp sites) were different between the CAD and control groups. The CAD group had lower apolipoprotein E*ε2*ε2 frequencies than the control group (P<0.05). Conclusion Individuals with apolipoprotein E*ε2*ε2 are likely to have a reduced risk of developing coronary artery disease as demonstrated by elderly subjects in Northern China. (J Geriat Cardiol 2006; 3(2):75-8.)

Key Words apolipoprotein E; DNA polymorphism; elderly; coronary artery disease

Introduction

Identification of genetic factors may help us to study the mechanisms of coronary artery disease (CAD). Variation in one candidate genetic factor, the apolipoprotein (apo) E gene, is known as apo E polymorphism. Apo E is a serum glycoprotein existing in circulating chylomicrons, chylomicron remnants, very low-density lipoprotein, and a subgroup of high-density lipoprotein (HDL). It serves as a ligand of the receptor-mediated uptake of cholesterol-rich particles by hepatocytes and peripheral tissues. The common allele of the apo E gene is the ε3-allele, which encodes for cysteine at amino acid residue 112 and for arginine at residue 158. The ε2-allele encodes for arginine at both residues; ε2-allele also encodes for cysteine at both residues. Several studies have reported that the apo E polymorphism is associated with CAD in different populations.1,5 The goal of this study was to assess the relationship between apo E genotypes and CAD in the elderly population of northern China.

Subjects and methods

Study population

All subjects were elderly Chinese living in northern China and having no blood relationship. 1) The CAD-group consisted of 55 patients (41 males and 14 females) verified by coronary artery angiography (CAG), with an average age of 67 years; CAG showed that there was ≥50% luminal obstruction in at least one branch of the coronary vessel. 2) The Control group consisted of 36 healthy subjects (27 males and 9 females, average age of 68 years) with normal coronary angiogram.

A 10-ml venous blood sample was taken from all subjects and drawn into an EDTA sample tube; this was done before CAG and after at least a 6-h fast. The blood sample was centrifuged within two hours, and plasma and cellular components stored separately at -70°C in aliquot until analysis.

Determination of polymorphisms in the apo E gene

Leucocyte DNA was extracted from these frozen cellular samples through salting.6 Apo E genotype was performed as described by Hixson and Vernier.7 The following primers were used to amplify a 244-bp segment 5'-end of the apo E gene.

P1:5'-ACAGAATTTCGGCCCCGCTGTTACAC-3'
P2:5'-TTAGCTTTGACCGGCTGCTTACCAAGGA-3'

There are two HhaI restriction sites within the region amplified by the PCR; these are located at 112-bp and 158-bp. The controlling marker is pGEM-7zf(+); six pieces were found, and these were 91-bp, 83-bp, 112-bp, 72-bp, 48-bp, 36-bp, and 35-bp. Meanwhile, six genotypes were determined; they were E2/2, E2/3, E2/4, E3/3, E3/4, E4/4, carried by ε2, ε3, ε4.
Biochemical analysis
Total cholesterol, HDL cholesterol, and triglyceride levels were measured by the hospital’s clinical chemistry department using standard enzymatic methods. The LDL cholesterol levels were calculated using the Friedewald formula. Levels of apo A1, apo B, and Lp(a) were measured using immunometric methods. The coefficients of variation between assays were less than 5%.

Statistical analysis
We determined whether or not the distributions of genotypes were in Hardy-Weinberg equilibrium through χ² analysis. The frequencies of the alleles and genotypes among different subgroups were compared using χ² test. Biochemical quantities data were analyzed by Student two-tailed t-test. Population attributable risk was calculated using the odds ratios from the Logistic regression models and the prevalence of apo E genotypes.

Results
Baseline characteristics in the CAD group and control groups
As shown in table 1, there were no differences between the CAD group and control group in age and sex distribution. However, compared with control group, patients in the CAD group had higher serum levels of TG, TC, LDL-C, apoB and Lp(a), lower level of Apo A1.

Apo E gene 112-bp and 158-bp sites polymorphisms genotype frequency distribution characteristics in CAD group and control group
Genotype distributions at 112-bp and 158-bp sites of the 2 groups were shown in table 2. The CAD group had lower apolipoprotein E “ε2” frequencies than the control group (P<0.05).

Relation between blood lipid levels and apoE genotypes and risk factors of CAD
Table 3 shows blood lipid levels in subjects of different Apo E genotypes.

We also performed a Logistic regression analysis on blood lipids, apo E gene site genotype frequencies, and occurrence of CAD. The logistic analysis of maximum likelihood estimates suggested that the ε2 allele frequencies were significantly correlated with occurrence of CAD (RR =3.4487, 95%CI 1.2613-6.6592, P<0.01, Table 4).

Discussion
Multiple risk factors for CAD have been identified in previous studies. Recently, pathogenesis studies of CAD included a continued search for risk factors for CAD and identification of susceptible genetic mechanisms in pathogenesis. A number of studies have shown that coronary artery disease severity is associated with the epsilon 2/ epsilon 3/ epsilon 4 polymorphism in the coding region of the apolipoprotein E gene. Apolipoprotein E (apoE) has important functions in systemic and local lipid transport. Apolipoprotein E is a protein constituent of both triglyceride-rich lipoproteins (TRL) as well as HDL, which play an important role in liver uptake of TRL remnants. Apolipoprotein E has three common alleles known as ε2, ε3, and ε4. The receptor-binding function of apo E is allele-specific. The various apo E isoforms differ in binding affinity for the LDL-receptor and the LDL-receptor related protein, for HDL cholesterol, and for triglyceride-rich lipoprotein particles. Apo E2 and E3 preferentially bind to the smaller of the known HDL fractions, while apo E4 more often bind to the larger, triglyceride-rich LDL fractions. The relationship between apolipoprotein (apo) E and coronary atherosclerotic disease has been the subject of a considerable amount of research. However, this relationship is far from being clearly defined, as results were still controversial, subjectable to further debate.

The present study investigated blood lipids levels in 91 geriatric subjects (55 patients with CAD and 36 healthy persons), and analyzed the apo E gene 112-bp and 158bp sites polymorphism features. The demographic information for 91 geriatric subjects showed that those with CAD tended to have more unfavorable lipoprotein variables. Genotype distributions at both sites differed from the CAD group and control group. In addition, the control group demonstrated higher apo E 2 frequencies than the CAD group (P <0.05). The variation at
Table 2. The genotype frequency of apo E gene in two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>E22 (%)</th>
<th>E23 (%)</th>
<th>E24 (%)</th>
<th>E33 (%)</th>
<th>E34 (%)</th>
<th>E44 (%)</th>
<th>e2 (%)</th>
<th>e3 (%)</th>
<th>e4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (%)</td>
<td>5.56</td>
<td>19.44</td>
<td>5.56</td>
<td>44.44</td>
<td>22.22</td>
<td>2.73</td>
<td>21.0%</td>
<td>65.1%</td>
<td>13.9%</td>
</tr>
<tr>
<td>CAD group (%)</td>
<td>3.64</td>
<td>14.55</td>
<td>5.45</td>
<td>54.55</td>
<td>16.36</td>
<td>16.36</td>
<td>13.6%</td>
<td>70.0%</td>
<td>16.4%</td>
</tr>
</tbody>
</table>

*p<0.05, compared with control group

Table 3. Blood lipid levels of different genotypes at apoE gene (mean±SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>E22 (n=4)</th>
<th>E23 (n=15)</th>
<th>E24 (n=5)</th>
<th>E33 (n=46)</th>
<th>E34 (n=17)</th>
<th>E44 (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mmol/L)</td>
<td>1.88±0.56</td>
<td>1.92±0.65</td>
<td>2.11±0.70</td>
<td>2.17±0.69</td>
<td>2.21±0.71</td>
<td>2.20±0.56</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.69±0.84</td>
<td>4.95±0.92</td>
<td>5.24±0.99</td>
<td>5.33±0.87</td>
<td>5.31±0.94</td>
<td>5.29±0.78</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.12±0.25</td>
<td>1.05±0.17</td>
<td>0.96±0.20</td>
<td>1.00±0.21</td>
<td>0.90±0.18</td>
<td>0.91±0.20</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.06±0.88</td>
<td>3.24±0.85</td>
<td>3.36±0.85</td>
<td>3.38±0.83</td>
<td>3.45±0.92</td>
<td>3.43±0.91</td>
</tr>
<tr>
<td>apoA1 (g/L)</td>
<td>1.12±0.18</td>
<td>1.10±0.22</td>
<td>1.06±0.18</td>
<td>0.93±0.20</td>
<td>0.96±0.23</td>
<td>1.00±0.22</td>
</tr>
<tr>
<td>apoB (g/L)</td>
<td>0.87±0.15</td>
<td>0.94±0.18</td>
<td>0.99±0.21</td>
<td>1.06±0.19</td>
<td>1.27±0.27</td>
<td>1.09±0.25</td>
</tr>
<tr>
<td>Lp(a) (g/L)</td>
<td>0.23±0.14</td>
<td>0.25±0.17</td>
<td>0.28±0.22</td>
<td>0.27±0.19</td>
<td>0.28±0.16</td>
<td>0.26±0.19</td>
</tr>
</tbody>
</table>

Table 4. The Logistic analysis of maximum likelihood estimates in the population in the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>B (parameter estimate)</th>
<th>SE (standard error)</th>
<th>WALD (Chi-Square)</th>
<th>Sig (significance, Pr)</th>
<th>Exp(B) (Risk Ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.6864</td>
<td>.7280</td>
<td>5.3659</td>
<td>.0205</td>
<td>3.2215</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.9143</td>
<td>.4795</td>
<td>3.6366</td>
<td>.0565</td>
<td>2.4952</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.8491</td>
<td>.6130</td>
<td>1.9187</td>
<td>.1660</td>
<td>2.3376</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.9375</td>
<td>.9643</td>
<td>4.0366</td>
<td>.0445</td>
<td>2.9413</td>
</tr>
<tr>
<td>Family history</td>
<td>0.1646</td>
<td>.7667</td>
<td>0.0461</td>
<td>.8301</td>
<td>1.1789</td>
</tr>
<tr>
<td>TC</td>
<td>0.1465</td>
<td>.5048</td>
<td>0.0842</td>
<td>.7717</td>
<td>2.1578</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-1.3882</td>
<td>.4387</td>
<td>10.0152</td>
<td>.0016</td>
<td>1.2495</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.2686</td>
<td>.5820</td>
<td>4.9028</td>
<td>.0268</td>
<td>2.3756</td>
</tr>
<tr>
<td>e2 absence</td>
<td>1.2380</td>
<td>.5568</td>
<td>4.9437</td>
<td>.0262</td>
<td>3.4487</td>
</tr>
</tbody>
</table>

The apo E gene locus may affect levels of total cholesterol and LDL cholesterol in the general geriatric population. The presence of the ε2 allele may result in decreased LDL cholesterol due to delayed clearance of chylomicron remnants by the liver and upregulation of LDL receptor activity. These associations affecting well-known lipid-related CAD risk factors suggest that variation at this locus could be a major determinant of CAD risk in the general geriatric population. Variability in the reported associations between apo E genotype and CAD risk may be due to different environmental exposures affecting the association between apo E alleles and plasma lipid levels. Several diseases and their treatment may also influence this relationship. A recent study showed that the therapeutic effects of HMG-CoA reductase inhibitors (statins) may depend on the presence of a functional apolipoprotein E. The variability in drug response originates partly from genetics, with possible consequences for drug efficacy, adverse effects, and toxicity.
Until now, pharmacogenetics mainly indicated the best known source of variability, that is, the variability caused by drug metabolism. From the risk concept emphasizing impaired metabolism and adverse effects, we now moved to an approach which is a personalized, genotype-dependent adaptation of therapy. Similar gene-environment interactions have been reported in several other populations. 22, 23

These data provide evidence suggesting that apo E gene is a prototypical susceptibility gene, and they also suggest that apo E gene polymorphisms play an important role in the pathophysiology of atherosclerosis and occurrence of coronary atherosclerotic disease, with a decreased burden of disease being observed in CAD patients with the ε4 allele, compared to subjects with ε3 and ε2 alleles; the ε2 allele has a potential protective effect on developing CAD. 24-26 More research is required to define the role of apo E genotyping in the management of coronary atherosclerotic disease in its various forms.

References