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Characterization of Apolipoprotein E Genetic Variations in Taiwanese: Association with Coronary Heart Disease and Plasma Lipid Levels

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Abstract Apolipoprotein E (apoE, protein; APOE, gene) is important in lipoprotein metabolism. Three isoforms, apoE2 (Cys112 Cys158), apoE3 (Cys112 Arg158), and apoE4 (Arg112 Arg158), are present in the general population. This report investigates the frequency distribution of apoE isoforms and the association of APOE genotypes with plasma lipid profile and coronary heart disease (CHD) in a population of Taiwan. ApoE isoforms were determined genetically by polymerase chain reaction and Hinf restriction enzyme digestion in control and coronary heart disease (CHD) patients. Plasma lipid and lipoprotein concentrations were also determined. The control group exhibited frequencies of 84.6% APOE3, 7.9% APOE4, 7.5% APOE2, 7.0% APOE3E3, 14.4% APOE3E4, 13.6% APOE2E3, and 14% APOE2E4. Comparable frequencies were observed in the CHD group. In both APOE2 carrier and APOE3E3 groups, the CHD patients expressed abnormal lipid profiles while the control group expressed normal lipid profiles. The APOE4 carriers, however, expressed abnormal lipid profiles in both normal control and CHD groups. Extremely high apoE levels in the hypertriglyceridemic group (TG > 400 mg/dL) seemed to be undesirable and were often observed in CHD patients.

Multiple genes are involved in the cause of coronary heart disease, which is closely related to lipoprotein metabolism. Racial differences in gene composition influence the morbidity of the disease (for reviews see Breslow 2000; Fazio et al. 2000). One of the genes involved in lipoprotein metabolism is apolipoprotein E (APOE). ApoE is found in several classes of lipoprotein except for low-density lipoprotein (LDL). Besides mediating the cellular uptake of lipoproteins, apoE also mediates the movement of cholesterol from peripheral tissues for catabolism.

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The affinity of apoE to the apoB.E receptor (LDL receptor) is far greater than the apoB100 which is present on the LDL particle. The apoE isoforms subclassify their binding affinities to various levels, and these characteristics are determined genetically (see review Mahley 1988).

ApoE is a 299-amino-acid protein with its gene residing on human chromosome 19 (Olaisen et al. 1982; Das et al. 1985). Different populations exhibit various frequency distribution of three apoE isoforms; apoE2 (Cys112 Cys158), apoE3 (Cys112 Arg158), and apoE4 (Arg112 Arg158). The majority of every population examined so far shows an APOE3E3 genotype with variable APOE2 and APOE4 allele frequencies (Davignon et al. 1988).

Some investigators report that the APOE4 allele is a risk factor for CHD (Corbo et al. 1999; Dzimiri et al. 1999). However, several reports have found no correlation between APOE genotypes and CHD (de Andrade et al. 1995; Lehtinen et al. 1995; Malle et al. 1996). In order to investigate whether different apoE isoforms contribute to CHD prevalence in our population, we determined the APOE genotypes in normal controls and CHD patients and analyzed their lipid parameters and their correlation with APOE genotypes.

Materials and Methods

Study Population. Random population of control subjects (n = 286) included 156 males and 130 females aged between 20 and 84 years. Among the controls, 122 subjects were undergoing annual physical checkups and showed a normal electrocardiogram with no history of or risk factors for CHD (Wu et al. 1993a). Another 164 subjects were randomly chosen from the general public, and all showed a normal electrocardiogram according to the Whitehall criteria (Rose et al. 1977) and no angina or claudication based on the Rose criteria (Rose et al. 1982). The 172 CHD survivors included 111 males and 61 females. They all had a past history of cardiac infarction (Wu et al. 1993b). All subjects were Taiwanese.

Plasma Lipid and Lipoprotein Measurement. Blood samples were collected from overnight-fasted subjects in a seated position into heparin-containing tubes. In the case of CHD patients, blood samples were collected three months after the episode of cardiac infarction. Plasma was separated, aliquoted, and stored at -70°C until assayed. The buffy coat was used for DNA extraction.

Plasma total cholesterol (TC), triglyceride (TG), LDL cholesterol (LDLC), HDL cholesterol (HDLc), and lipoprotein(a) [Lp(a)] concentrations were determined as described (Wu et al. 1993a). Plasma concentrations of apoA1 and apoB were analyzed by immuno-turbidometry and electroimmunodiffusion (Wu et al. 1996). ApoE concentrations were determined by ELISA using rabbit antihuman apoE antibody (DAKO, Denmark), goat antirabbit antibody conjugated to alkaline phosphatase (Cappel, Organon Teknika, Belgium) and substrate p-nitrophenyl phosphate (Sigma 104, St. Louis, MO).
Genetic Analysis of ApoE Isoforms. DNA isolation followed the established procedure (Wu et al. 1993b). Oligonucleotides 5'-GGGCACGGGTGCTGCAAGGAG-3' and 5'-CACGGGCCTGTTCCACCA-3' (nucleotides 3681–3699 and 3962–3981, respectively, in Paik et al. [1985] DNA sequence) were used for polymerase chain reaction. Amplification was carried out at 94°C for 1 min and at 72°C for 3 min for 30 cycles after the initial denaturation at 94°C for 3 min. The main DNA bands used for differentiating ApoE genotypes after HhaI digestion are the cleavage of a 91 base pair (bp) (Cys112) into 71 bp and 20 bp (Arg112) and an 83 bp (Cys158) into 48 bp and 35 bp (Arg158). These patterns were visualized on 10% nondenaturing polyacrylamide gel and SYBR green I staining (Molecular Probes, Eugene, OR).

Statistical Analysis. Differences in genotype and allele frequencies between the control and patient groups were tested using the chi-square test, and analysis of covariance (ANCOVA) was carried out to compare the lipid levels of various genotypes with sex, age, and body mass index (BMI) adjustment. A 5% significance level was used.

Results and Discussion

Our results indicated that in Taiwanese, the ApoE3E3 genotype (Cys112 Arg158) was the most common in the control and the CHD groups. ApoE2E3 (Cys112 Cys158 for apoE2) and ApoE4E4 (Arg112 Arg158 for apoE4) were the next most common genotypes with a frequency not exceeding 0.15 in either group (Table 1). Other genotypes such as ApoE2E2, ApoE4E4, or ApoE2E4 were rare, with frequencies ranging from 0 to 0.014. No statistical difference in genotype or allelic distribution was found between the patients and the control groups.

Different populations exhibited slightly different distribution frequencies of ApoE alleles. The allelic frequency for ApoE4 was highest (0.368) for Papua

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control (n = 286)</th>
<th>CHD (n = 172)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE3E3</td>
<td>0.706</td>
<td>0.762</td>
<td>0.250</td>
</tr>
<tr>
<td>ApoE2E3</td>
<td>0.136</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>ApoE3E4</td>
<td>0.144</td>
<td>0.139</td>
<td></td>
</tr>
<tr>
<td>ApoE2E4</td>
<td>0.014</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoE2</td>
<td>0.075</td>
<td>0.040</td>
<td>0.299</td>
</tr>
<tr>
<td>ApoE3</td>
<td>0.846</td>
<td>0.881</td>
<td></td>
</tr>
<tr>
<td>ApoE4</td>
<td>0.079</td>
<td>0.070</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Genotype and Allele Frequency of ApoE in the Control and the CHD Groups
New Guineans (Kamboh et al. 1990), followed by Nigerian blacks (frequency 0.31, Kamboh et al. 1989), US black and Australian aborigines (both with a frequency of 0.26, Kamboh et al. 1989, 1991) and Greenland Inuit (frequency 0.23, Gerdès et al. 1996). In Amerindian, Greenland Inuit, and Australian aborigines, no APOE2 allele was found (Gamboa et al. 2000; Gerdès et al. 1996; Kamboh et al. 1991). Alaska natives displayed 0.02 APOE2 and 0.193 APOE4 (Scheer et al. 1995). Caucasians displayed a high frequency of APOE2 allele (0.09) and a relatively low frequency (0.15) of APOE4 allele (Sanghera et al. 1996). In a survey of European and African populations, Seixas et al. (1999) found that the strength of linkage disequilibrium was highest for the APOE2 allele and lowest for the APOE4 allele. This finding suggests that the origin of the APOE gene has followed a 4→3→2 pathway. This concept has been suggested earlier by Gerdès et al. (1996).

It is interesting to note that the ancestral APOE4 allele has maintained its high frequency in some populations. Genetic composition may be involved in regulating plasma lipid concentration in response to diet (Or dovás 1999) and APOE may be one of those genes (Weintraub et al. 1987). Perhaps the maintenance of the APOE4 allele is related to the dietary habits of the population. The advantage of APOE4 in our ancestors was an efficient utilization of nutrients in a period of food scarcity, while the disadvantage of APOE4 in the modern world is its association with coronary heart disease. Therefore the evolution of apoE isoforms has been necessary.

Affinity for LDL receptor is lower for apoE2 protein and higher for the apoE4 variant, while apoE3 shows normal binding activity. Therefore APOE2 and APOE4 alleles are associated with high TG and high TC, respectively. Our results showed that compared with the major APOE3 allele, subjects with the APOE4 allele displayed significantly decreased HDL-C and increased apoB and TG levels in the control group (p < 0.025) (Table 2); no significant difference was detected in the patient group. We did not observe a correlation of APOE genotypes and CHD, yet we observed an abnormal lipid profile that is comparable with that in the CHD cases in the control APOE4 carrier group. This finding indicates that the APOE4 carriers inherently display an abnormal plasma lipid profile.

A study among Caucasians by de Knijff et al. (1991) showed that the APOE2 allele decreased the mean plasma Lp(a) level by 24.8%, while the APOE4 allele increased the mean Lp(a) level by 25.7%. The EARS study (Tiret et al. 1994) found the decreasing effect of APOE2 on Lp(a) level, but no effect was found for the APOE4 on Lp(a) level. We have analyzed the effect of APOE gene on Lp(a) level by a general linear model (GLM) (Dobson 1990). Our results agreed with those of several other reports (Feussner et al. 1993; Ferrieres et al. 1994; Heng et al. 1995) that there was no correlation of APOE genotype with plasma Lp(a) level.

The APOE2 carriers displayed higher apoE concentrations, while the APOE4 carriers displayed lower apoE concentrations than the APOE3E3 subjects.
Table 2. The Mean Plasma Lipid Concentration, mg/dL (SEM), of Different APOE Genotypes in Control and CHD Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>CHD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 39</td>
<td>n = 106</td>
<td>n = 39</td>
<td>n = 15</td>
</tr>
<tr>
<td>M/F</td>
<td>18.9(2.1)</td>
<td>109.8(7)</td>
<td>24.1(5)</td>
<td>11.4(4)</td>
</tr>
<tr>
<td>Age</td>
<td>54.9(2.1)</td>
<td>50.2(1.1)</td>
<td>47.4(2.1)</td>
<td>59.7(2.4)</td>
</tr>
<tr>
<td>BMI</td>
<td>24.5(0.5)</td>
<td>23.6(0.3)</td>
<td>24.3(0.5)</td>
<td>25.2(1.0)</td>
</tr>
<tr>
<td>TC</td>
<td>193(5.2)</td>
<td>191(2.6)</td>
<td>195(5.5)</td>
<td>177(7.4)</td>
</tr>
<tr>
<td>TG</td>
<td>124(14.9)</td>
<td>100(5.6)</td>
<td>130(20.8)*</td>
<td>191(27.3)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>104(5.0)</td>
<td>115(2.5)</td>
<td>124(5.6)</td>
<td>101(5.5)</td>
</tr>
<tr>
<td>ApoB</td>
<td>78(4.0)</td>
<td>84(2.0)</td>
<td>93(4.8)*</td>
<td>75(3.8)</td>
</tr>
<tr>
<td>HDLC</td>
<td>53(2.9)</td>
<td>55(1.3)</td>
<td>46(2.1)*</td>
<td>32(3.1)</td>
</tr>
<tr>
<td>ApoAl</td>
<td>177(4.8)</td>
<td>124(1.9)</td>
<td>113(3.1)</td>
<td>107(5.4)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>13.6(2.3)</td>
<td>13.0(1.0)</td>
<td>12.1(1.8)</td>
<td>18.8(3.3)</td>
</tr>
<tr>
<td>ApoE</td>
<td>3.7(0.3)*</td>
<td>3.0(0.1)</td>
<td>2.7(0.1)*</td>
<td>3.9(0.4)*</td>
</tr>
</tbody>
</table>

Notes: *p < 0.05 denotes comparison between APOE2/E3 versus APOE3/E3, APOE3/E4 versus APOE3/E3. All comparisons were adjusted for sex, age, and body mass index. Abbreviations: SEM, standard error of the mean; n, number of specimens; M/F, male/female; BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; ApoB, apolipoprotein B; HDLC, high-density lipoprotein cholesterol; ApoAl, apolipoprotein A1; Lp(a), lipoprotein (a); ApoE, apolipoprotein E.

in both control and CHD groups. The apoE and TG levels are positively correlated. Our data revealed that a continued rise of apoE level was observed in hypertriglyceridemic (TG > 400 mg/dL) CHD patients, while in the hypertriglyceridemic control subjects the apoE level actually decreased as the TG level continued to rise (unpublished results). An increase in the apoE level could increase the VLDL secretion, and in an abnormally high apoE condition, lipase-mediated VLDL lipolysis might be masked (Huang et al. 1999).

In a separate observation, two subjects (both APOE4 carriers) with extremely high TG levels of 1021 and 1253 mg/dL displayed apoE levels of 8.8 and 43.5 mg/dL, respectively. The former (female, age 80), with a TG level of 1021 mg/dL and an apoE level of 8.8 mg/dL, was in the control group and the latter (female, age 66), with a TG level of 1253 mg/dL and an apoE level of 43.5 mg/dL, was a CHD patient. Whether this association of TG and apoE concentration is important in CHD needs further investigation.

In summary, the Taiwanese population exhibited a high frequency (0.846) of the APOE3 allele and comparable frequencies for APOE2 (0.075) and APOE4 (0.079) alleles. The APOE4 carriers inherently expressed abnormal lipid profiles even in the normal control group. The association of APOE genotype or allele frequencies with CHD, however, was not apparent in this population.
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Literature Cited


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