POLYMORPHISMS OF APOLIPOPROTEIN E AND ANGIOTENSIN-CONVERTING ENZYME GENES AND CAROTID ATHEROSCLEROSIS IN HEAVY DRINKERS

MALGORZATA BEDNARSKA-MAKARUK*, MARIA RODO, CEZARY MARKUSZEWSKI1, ANNA ROZENFELD2, MALGORZATA ŚWIDERSKA3, BOGUSLAW HABRAT1 and HANNA WEHR

Department of Genetics, 1Department of Substance Dependence Prevention and Treatment, 2Second Department of Neurology and 3Analytical Laboratory Institute of Psychiatry and Neurology, Sobieskiego 9, 02-957 Warsaw, Poland

(First received 23 April 2003; accepted 30 December 2003)

Abstract — Aims: To investigate the influence of apolipoprotein E (APOE) and angiotensin-converting enzyme (ACE) gene polymorphisms on carotid artery atherosclerosis in alcoholism. Methods: Polymorphism of both genes was identified by DNA analysis in 130 male alcohol-dependent patients. Intima–media thickness (IMT) was measured ultrasonographically. Results: Multivariate regression analysis showed that of all the known risk factors the greatest impact on carotid atherosclerosis in alcoholics was exerted by age, hypertension, LDL cholesterol and fasting plasma glucose levels. Subjects carrying the APO E ε4 allele were more liable to develop atherosclerotic changes in carotid arteries compared with subjects with the ε3/ε3 genotype, which showed statistical significance in patients under 50 years of age. No association was shown between ACE I/D polymorphism and carotid atherosclerosis. Conclusions: APO E polymorphism can increase the risk of carotid atherosclerosis development in an alcoholic subject. The association of the APO E ε4 allele with carotid atherosclerosis was significant in younger patients. Since the elevated carotid IMT is considered to be a good marker of increased risk of generalized atherosclerosis the consequences could involve both cardiac and cerebrovascular events.

INTRODUCTION

The relationship between the amount of consumed alcohol and cardiovascular disease is illustrated by a J-shaped curve, which indicates that heavy drinking has a negative influence on the development of atherosclerosis. The more frequent cardiovascular death observed at higher drinking levels was attributed in several publications to non-atherosclerotic causes such as cardiomyopathy or haemorrhagic stroke (Sesso and Gaziano, 1999). However the J-shaped curve was also observed in the prospective population-based Bruneck study, which concerned the influence of alcohol consumption on the incidence and progression of carotid atherosclerosis (Kiechl et al., 1998).

It was also stated that the pattern of drinking had an influence on the development of atherosclerosis. Heavy binge drinking more than regular heavy drinking may cause an enhanced risk of development of atheromatic changes (Kauhanen et al., 1999; Puddey et al., 1999; Britton and McKee, 2000). Heavy binge drinking is common in Eastern and Central European countries, including Poland.

Atherosclerosis is a complicated disease influenced by many genetic and environmental factors. Several candidate genes have been investigated with respect to favouring the development of atherosclerosis. The association of a particular form of the gene with the disease suggests that it contributes to the pathogenesis of the disease.

Apolipoprotein E (Apo E) appears in humans in three different forms named E2, E3 and E4 differing from each other by a single amino acid substitution and coded by three alleles ε2, ε3 and ε4 at a single gene locus. Apo E polymorphism is one of the common genetic factors responsible for inter-individual variations in lipid and lipoprotein levels.

The ε2 allele is associated with the lowest while the ε4 allele with the highest plasma cholesterol levels (Davignon et al., 1988). Several studies in various populations have shown that the ε4 allele was associated with an increased risk of coronary atherosclerosis. The role of the ε2 allele in the development of coronary atherosclerosis remains controversial. It was considered to be protective (Davignon et al., 1988; Wilson et al., 1996), but in a recent Framingham study an association with higher cardiovascular risk in men was observed (Lahoz et al., 2001).

Angiotensin-converting enzyme (ACE) gene polymorphism is based on the presence (insertion - I) or absence (deletion - D) of a 287-bp DNA domain within intron 16. The resulting three genotypes are: DD homozygotes, II homozygotes and ID heterozygotes. An association of the ACE DD genotype with blood pressure and hypertension was observed in men (O’Donnell et al., 1998). It is still uncertain whether ACE I/D polymorphism is associated with an increased risk of atherosclerosis. Some authors observed a positive relationship between the D allele and increased risk of myocardial infarction (Samani et al., 1996) or ischaemic stroke (Sharma, 1998), while the others did not report a significant association between ACE gene polymorphism and the clinical manifestation of ischaemic heart disease (Agerholm-Larsen et al., 2000).

Ultrasound assessment of intima–media thickness (IMT) in carotid arteries is a convenient, non-invasive method to study atherosclerosis in vivo in its early stages and to follow up the progression of atherosclerotic processes (Simon et al., 2002).

In this work, the possibility of an influence of APO E and ACE gene polymorphisms on carotid artery wall thickness was investigated in heavy drinking alcoholics.

SUBJECTS AND METHODS

The study group consisted of 130 male alcohol-dependent patients, aged 39 to 72 years (mean age 48.2 ± 6.2 years),
entering detoxification treatment after an alcohol abuse period. Diagnosis of alcohol dependence was performed using the ICD-10 criteria. The study was approved by the Ethics Committee of the Institute of Psychiatry and Neurology in Warsaw. The participants gave their informed consent for the investigation. Blood samples were collected in EDTA tubes and lymphocyte DNA was obtained by phenol extraction. APO E genotypes were identified using the Hixson and Vernier (1990) method consisting of amplification of the APO E gene fragment, its enzymatic cleavage by Hha I restriction and identification of DNA fragments after electrophoresis on polyacrylamide gel.

The ACE insertion/deletion (I/D) polymorphism was identified using the PCR method according to Lindpaintner et al. (1995). The reaction products were analysed after agarose gel electrophoresis. The DD genotype was confirmed by a second independent amplification using primers, which recognize only I allele (Lindpaintner et al., 1995).

The blood samples for biochemical analyses were taken after cessation of drinking (up to 7 days). Serum total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-C) (after phosphotungstate-Mg²⁺ precipitation of APO B containing lipoproteins) were determined by enzymatic methods and LDL-cholesterol (LDL-C) concentration was calculated according to the Friedewald formula. Fasting plasma glucose was determined by the enzymatic method.

Measurements of blood pressure were performed after alcohol withdrawal symptoms had resolved. Hypertension was defined as the value of systolic blood pressure (SBP) \( \geq 140 \text{ mm Hg} \) or diastolic blood pressure (DBP) \( \geq 90 \text{ mm Hg} \). The group of hypertensive individuals also included persons with actual normal blood pressure that were treated with antihypertensive drugs. Diabetes mellitus was defined when the fasting plasma glucose level was \( > 7.0 \text{ mmol/l} \) (126 mg/dl).

The ultrasound evaluation of extracranial carotid arteries was performed using an Acuson 128X/10C, a 7.0 MHz colour duplex-type scanner. All the ultrasound imaging examinations were performed by the same person — an experienced sonographer — who was not informed of the participant’s genotype status. A standardized protocol was used: the distance between the lumen–intima interface and the media–adventitia interface, which represents the so-called intima–media thickness (IMT) of the carotid arteries wall was measured bilaterally, on the far wall — at the level of three standardized segments i.e. the distal 10 mm of the common carotid artery (CCA), the bifurcation (BIF) and proximal 10 mm of the internal carotid artery (ICA). CCA IMT value represented an average of 3 measurements at a distance of 10 mm. The IMT values of BIF and ICA represented the greatest intima–media mean thickness in these segments. The single individual largest IMT (Max IMT) was also determined and the mean thicknesses of all six IMT measurements (overall mean IMT) as well as right and left mean IMT (mean of the three segment IMT on both sides) were calculated. The presence of atherosclerotic plaque was defined as IMT \( \geq 1.5 \text{ mm} \) according to Jagiello (2000) and Zanchetti et al. (1992).

**Statistical analysis**

All statistical analyses were performed using STATISTICA version 5.5A. Continuous data were expressed as mean values \( \pm \text{SD} \). Because TG concentration and IMT of the arterial wall were not normally distributed, the values were logarithmically transformed in statistical analysis, but the results were expressed as crude data. Differences in means between the groups were tested using the Mann–Whitney test or analysis of variance (ANOVA) and then a multiple comparison test (Scheffe’s post-hoc test) was used to analyse the differences between pairs of means. Statistical significance of the differences in the frequencies of categorical variables was evaluated using Chi-squared or Fisher exact test. The means of the log transformed carotid IMT values in the studied groups were adjusted for age using the analysis of covariance (ANCOVA) with age as a covariate. Multivariate regression analysis and stepwise multiple regression analysis were performed to assess the combined influence of genetic and non-genetic variables on IMT values. The logistic regression analysis was performed to assess the influence of several variables on the presence of carotid atherosclerosis. The following factors were considered as independent variables: age, SBP and DBP, HDL-C, LDL-C, plasma triglycerides, fasting plasma glucose level, presence of the APO E ε2 allele, presence of the APO E ε4 allele and presence of the ACE D allele. \( P \)-values <0.05 were considered statistically significant.

**RESULTS**

**APO E and ACE genotypes and allele frequencies in alcoholics**

APO E allele frequencies among all 130 male alcoholics were 8.1\% for ε2, 82.3\% for ε3 and 9.6\% for the ε4 allele. This was consistent with the results of the study of the non-selected Polish population sample (Bednarska-Makaruk et al., 2001). The APO E genotypes distribution was in Hardy–Weinberg equilibrium \( (P < 0.9849) \). According to their APO E genotype, patients were divided into three groups: E2 group — carriers of the ε2 allele comprising of 17 persons with ε2/2 and ε2/3 genotypes, E3 group comprising of 89 persons with ε3/3 genotype, and the E4 group — carriers of the ε4 allele comprising of 21 persons with ε4/3 and ε4/4 genotypes. Because of known opposed effects of ε2 and ε4 alleles on plasma lipid concentrations and atherosclerosis (Davignon et al., 1988, Dallongeville et al., 1992) four patients with the ε2/4 genotype were excluded from statistical analyses.

The frequencies of ACE alleles in the investigated group of alcoholics were 46.9\% for the D allele and 53.1\% for the I allele. The ACE genotype distribution was in a Hardy–Weinberg equilibrium \( (P < 0.8926) \). According to ACE I/D polymorphism, patients were divided into three groups: 30 DD homozygotes, 62 ID heterozygotes and 38 II homozygotes.

**APO E and ACE genotypes and clinical and biochemical characteristics**

Table 1 shows the clinical characteristics of alcoholic patients divided according to their APO E genotype. Mean age was slightly higher in the E2 group in comparison with the E3 and E4 groups, but the differences did not reach statistical significance. SBP and DBP and fasting glucose levels were similar in all three genotype groups.

The E2 group tended to have the highest mean TG and HDL-C values, whereas in the E4 group the highest mean
LDL-C and the lowest HDL-C were observed; however, the differences were not statistically significant. When the smaller group of those patients in whom lipids were determined within 3 days after alcohol withdrawal was taken into account (data not shown), the results were similar — in the E4 group the highest mean TC and LDL-C were observed and the difference concerning TC reached statistical significance ($P = 0.025$).

Table 2 shows the clinical characteristics of alcoholic patients depending on ACE genotype. All investigated parameters i.e. mean age, blood pressure, fasting glucose and lipid concentrations were similar in the three genotype groups. Only II homozygotes tended to have the lowest TG levels in comparison with D allele carriers.

**Table 2. Characteristics of alcoholics according to ACE genotype (Means ± SD)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Variable</th>
<th>Group</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>ID</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.2 ± 5.0</td>
<td>48.0 ± 6.1</td>
<td>49.5 ± 7.2</td>
<td>0.279</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>123.7 ± 16.5</td>
<td>125.4 ± 13.9</td>
<td>125.9 ± 11.7</td>
<td>0.800</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>73.8 ± 10.6</td>
<td>80.4 ± 9.2</td>
<td>78.9 ± 8.7</td>
<td>0.591</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>93.4 ± 17.8</td>
<td>95.9 ± 28.5</td>
<td>97.5 ± 18.6</td>
<td>0.666</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>198.6 ± 39.1</td>
<td>202.2 ± 42.4</td>
<td>201.1 ± 48.1</td>
<td>0.940</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>130.7 ± 80.8</td>
<td>140.3 ± 84.1</td>
<td>116.1 ± 62.0</td>
<td>0.389</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>67.2 ± 32.5</td>
<td>64.6 ± 25.6</td>
<td>68.8 ± 27.2</td>
<td>0.802</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>105.3 ± 33.6</td>
<td>109.9 ± 37.6</td>
<td>111.6 ± 37.6</td>
<td>0.787</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>3.50 ± 1.30</td>
<td>3.50 ± 1.18</td>
<td>3.39 ± 1.34</td>
<td>0.920</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

LDL-C and the lowest HDL-C were observed; however, the differences were not statistically significant. When the smaller group of those patients in whom lipids were determined within 3 days after alcohol withdrawal was taken into account (data not shown), the results were similar — in the E4 group the highest mean TC and LDL-C were observed and the difference concerning TC reached statistical significance ($P = 0.025$).

Table 2 shows the clinical characteristics of alcoholic patients depending on ACE genotype. All investigated parameters i.e. mean age, blood pressure, fasting glucose and lipid concentrations were similar in the three genotype groups. Only II homozygotes tended to have the lowest TG levels in comparison with D allele carriers.

**Table 2. Characteristics of alcoholics according to ACE genotype (Means ± SD)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Variable</th>
<th>Group</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>ID</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.1 ± 8.3</td>
<td>47.7 ± 5.5</td>
<td>49.0 ± 7.0</td>
<td>0.067</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>126.0 ± 14.4</td>
<td>126.0 ± 13.5</td>
<td>120.5 ± 14.7</td>
<td>0.261</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>78.5 ± 9.1</td>
<td>80.2 ± 9.0</td>
<td>77.3 ± 10.9</td>
<td>0.418</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>86.3 ± 23.6</td>
<td>96.3 ± 23.6</td>
<td>96.1 ± 17.8</td>
<td>0.306</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>215.7 ± 53.3</td>
<td>195.8 ± 39.5</td>
<td>209.3 ± 47.8</td>
<td>0.191</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>144.6 ± 87.0</td>
<td>129.0 ± 78.5</td>
<td>121.2 ± 58.6</td>
<td>0.712</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>78.4 ± 32.4</td>
<td>65.3 ± 27.2</td>
<td>63.9 ± 27.5</td>
<td>0.289</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>113.1 ± 42.4</td>
<td>104.8 ± 35.7</td>
<td>121.1 ± 33.9</td>
<td>0.201</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>3.34 ± 1.61</td>
<td>3.43 ± 1.20</td>
<td>3.66 ± 1.20</td>
<td>0.732</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PLT, platelet count; SBP, systolic blood pressure; DBP, diastolic blood pressure.

**Table 1. Characteristics of alcoholics according to APO E genotype (Means ± SD)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>Age (years)</td>
<td>51.1 ± 8.3</td>
<td>47.7 ± 5.5</td>
<td>49.0 ± 7.0</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>126.0 ± 14.4</td>
<td>126.0 ± 13.5</td>
<td>120.5 ± 14.7</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DBP (mm Hg)</td>
<td>78.5 ± 9.1</td>
<td>80.2 ± 9.0</td>
<td>77.3 ± 10.9</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fasting glucose (mg/dl)</td>
<td>86.3 ± 23.6</td>
<td>96.3 ± 23.6</td>
<td>96.1 ± 17.8</td>
<td>0.306</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC (mg/dl)</td>
<td>215.7 ± 53.3</td>
<td>195.8 ± 39.5</td>
<td>209.3 ± 47.8</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>144.6 ± 87.0</td>
<td>129.0 ± 78.5</td>
<td>121.2 ± 58.6</td>
<td>0.712</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL-C (mg/dl)</td>
<td>78.4 ± 32.4</td>
<td>65.3 ± 27.2</td>
<td>63.9 ± 27.5</td>
<td>0.289</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL-C (mg/dl)</td>
<td>113.1 ± 42.4</td>
<td>104.8 ± 35.7</td>
<td>121.1 ± 33.9</td>
<td>0.201</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC/HDL-C</td>
<td>3.34 ± 1.61</td>
<td>3.43 ± 1.20</td>
<td>3.66 ± 1.20</td>
<td>0.732</td>
<td></td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PLT, platelet count; SBP, systolic blood pressure; DBP, diastolic blood pressure.

**APO E and ACE genotypes in alcoholics and carotid IMT**

An increased IMT (≥1 mm) was detected in 71.5% of our alcoholic male patients, whereas the presence of an atherosclerotic plaque (defined as max IMT ≥1.5 mm) was observed in 34.6% of them. The overall mean IMT ≥1 mm (another marker of increased risk of atherosclerosis) was observed in 31.5% of alcoholics. These frequencies were high in comparison with the overall Polish population as will be discussed later.

The results of IMT carotid measurements in various APO E groups are shown in Table 3. Alcoholics — carriers of the ε4 allele (E4 group) had a significantly higher mean right CCA and right BIF IMT, as well as right mean IMT in comparison with the ε3/3 homozygotes (E3 group), whereas in the case of max IMT value the difference was borderline significant ($P = 0.052$). Carriers of ε2 alleles (E2 group) also tended to have higher IMT parameters, except right mean ICA in comparison with the E3 group. The left mean IMT values tended to be higher both in the E4 and in E2 groups in comparison with the E3 group. The differences in IMT between APO E groups were still observed after adjustment of IMT means for age. They were statistically significant only for
The prevalence of carotid atherosclerotic plaques (defined as IMT ≥1.5 mm in any investigated segment) was higher in the carriers of ε2 (47.1%) and ε4 (47.6%) alleles than in the E3 group (29.2%). The difference was statistically significant when ε2 and ε4 were pooled together (P = 0.049) (data not shown).

No significant differences depending on the ACE genotype were observed in carotid IMT measurements or in the prevalence of carotid atherosclerosis (Table 4).

Multivariate regression analysis was performed to assess the independent effects of the different risk factors on IMT values. It indicated that age, SBP, fasting plasma glucose...
levels and the APO E ε4 allele were significantly associated with increased right carotid mean IMT, whereas age, SBP and DBP and LDL-C levels were associated with increased left carotid mean IMT, as well as with overall mean IMT and max IMT (Table 5). Stepwise multiple regression analysis showed that age, APO E ε4, SBP and fasting glucose levels explained 12.2, 4.1, 3.6 and 2.5% of the variance of the right carotid IMT, respectively, whereas SBP, age, DBP and LDL-C explained 10.1, 4.6, 3.8 and 3.5% of the variance of left carotid IMT, respectively. Age, SBP, DBP and LDL-C also explained 11.6, 5.7, 3.9 and 3.4% of the variance of overall mean IMT and 12.7, 2.5, 6.1 and 5.6% of the variance of max IMT, respectively (data not shown).

Stepwise multiple regression analysis performed in two subgroups according to age (Table 6), showed that in younger alcoholics (<50 years) the APO E ε4 allele and SBP were significantly associated with increased investigated IMT values, whereas in older subjects (>50 years) age and plasma fasting glucose (borderline) were significantly associated with increased right carotid mean IMT, SBP and DBP and LDL-C (in different combinations) with increased left carotid mean IMT, overall mean IMT and max IMT.

Table 7 shows the clinical, biochemical and genetic characteristics of the study group according to the presence or absence of carotid plaques. Mean age, LDL-C and SBP were significantly higher in alcoholics with carotid plaques (max IMT ≥1.5 mm) than in those with normal carotid arteries (max IMT < 1 mm). Logistic regression analysis selected hypertension, age >50 years, APO E ε4 allele and LDL-C > 100 mg/dl as significant predictors of the presence of the carotid plaque.

**DISCUSSION**

Carotid atherosclerosis as measured by IMT seems to be more frequent and advanced in our group of male alcoholics in comparison with the sample of the Polish population group of 256 men aged 39–69 years. Mean IMT of BIF and CCA was 0.91 ± 0.27 mm in alcoholics and 0.76 ± 0.01 mm in the above mentioned population group. The 'average-normal' range of mean BIF and CCA IMT (0.6–0.8 mm) was observed in 44 and 57% men from both groups, respectively (Zieliński, 2001). In the present study, the prevalence of early

---

**Table 5. Risk factors affecting carotid atherosclerosis in alcoholics based on multivariate regression analysis**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Right mean IMT</th>
<th>Left mean IMT</th>
<th>Overall mean IMT</th>
<th>Max IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>β 0.261</td>
<td>P 0.010</td>
<td>β 0.220</td>
<td>P 0.035</td>
</tr>
<tr>
<td>SBP</td>
<td>β 0.365</td>
<td>P 0.010</td>
<td>β 0.502</td>
<td>P 0.0008</td>
</tr>
<tr>
<td>DBP</td>
<td>β −0.217</td>
<td>P 0.128</td>
<td>β −0.305</td>
<td>P 0.040</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>β 0.195</td>
<td>P 0.047</td>
<td>β −0.004</td>
<td>P 0.972</td>
</tr>
<tr>
<td>HDL-C</td>
<td>β 0.086</td>
<td>P 0.404</td>
<td>β 0.024</td>
<td>P 0.820</td>
</tr>
<tr>
<td>LDL-C</td>
<td>β 0.090</td>
<td>P 0.360</td>
<td>β 0.222</td>
<td>P 0.031</td>
</tr>
<tr>
<td>E2</td>
<td>β 0.073</td>
<td>P 0.466</td>
<td>β −0.028</td>
<td>P 0.788</td>
</tr>
<tr>
<td>E4</td>
<td>β 0.237</td>
<td>P 0.015</td>
<td>β 0.049</td>
<td>P 0.623</td>
</tr>
<tr>
<td>ACE-D</td>
<td>β 0.070</td>
<td>P 0.456</td>
<td>β 0.057</td>
<td>P 0.556</td>
</tr>
</tbody>
</table>

β, the standard regression coefficient; R², the multiple coefficient of determination; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; E2, presence of APO E ε2 allele (ε2/2 and ε2/3 genotype = 1; ε3/3, ε4/3 and ε4/4 genotype = 0); E4, presence APO E ε4 allele (ε4/4 and ε4/3 genotype = 1; ε3/3, ε2/3 and ε2/2 genotype = 0); ACE-D, presence of ACE D allele (II genotype = 0, ID genotype = 1, DD genotype = 2).

---

**Table 6. Risk factors affecting carotid atherosclerosis in alcoholics based on multivariate stepwise regression analysis in relation to age**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Right mean IMT</th>
<th>Left mean IMT</th>
<th>Overall mean IMT</th>
<th>Max IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≤50 years</td>
<td>R² change</td>
<td>β 0.156</td>
<td>P 0.002</td>
<td>β 0.067</td>
</tr>
<tr>
<td>E4</td>
<td>β 0.060</td>
<td>P 0.038</td>
<td>β 0.066</td>
<td>P 0.043</td>
</tr>
<tr>
<td>SBP</td>
<td>β 0.111</td>
<td>P 0.047</td>
<td>β 0.191</td>
<td>P 0.008</td>
</tr>
<tr>
<td>Age ≥50 years</td>
<td>R² change</td>
<td>β 0.077</td>
<td>P 0.086</td>
<td>β 0.068</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>β 0.163</td>
<td>P 0.005</td>
<td>β 0.163</td>
<td>P 0.005</td>
</tr>
</tbody>
</table>

R², the multiple coefficient of determination; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, LDL cholesterol; E4, presence APO E ε4 allele (ε4/4 and ε4/3 genotype = 1; ε3/3, ε2/3 and ε2/2 genotype = 0).

---

β, the standard regression coefficient; R², the multiple coefficient of determination; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; E2, presence of APO E ε2 allele (ε2/2 and ε2/3 genotype = 1; ε3/3, ε4/3 and ε4/4 genotype = 0); E4, presence APO E ε4 allele (ε4/4 and ε4/3 genotype = 1; ε3/3, ε2/3 and ε2/2 genotype = 0); ACE-D, presence of ACE D allele (II genotype = 0, ID genotype = 1, DD genotype = 2).
TABLE 7. Comparison of alcoholics with normal carotid arteries (IMT < 1 mm) vs alcoholics with carotid atherosclerotic plaques (IMT ≥1.5 mm)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal IMT n = 37</th>
<th>Carotid plaque n = 45</th>
<th>P-value*</th>
<th>Odds ratio (95% CI)</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.3 ± 4.9</td>
<td>50.8 ± 6.9</td>
<td>0.003</td>
<td>1.15 (1.05–1.27)</td>
<td>0.005</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>122.6 ± 13.3</td>
<td>129.5 ± 13.5</td>
<td>0.046</td>
<td>1.04 (1.00–1.08)</td>
<td>0.041</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>79.2 ± 8.7</td>
<td>80.1 ± 9.1</td>
<td>0.460</td>
<td>1.01 (0.96–1.07)</td>
<td>0.678</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>96.1 ± 19.1</td>
<td>101.8 ± 26.6</td>
<td>0.357</td>
<td>1.01 (0.99–1.03)</td>
<td>0.305</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>193.0 ± 34.6</td>
<td>203.9 ± 49.5</td>
<td>0.693</td>
<td>1.01 (0.99–1.02)</td>
<td>0.295</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>135.7 ± 81.6</td>
<td>124.3 ± 80.6</td>
<td>0.320</td>
<td>0.39 (0.04–3.10)</td>
<td>0.370</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>67.7 ± 31.3</td>
<td>64.8 ± 28.9</td>
<td>0.706</td>
<td>1.00 (0.98–1.01)</td>
<td>0.691</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>98.8 ± 30.5</td>
<td>116.3 ± 38.1</td>
<td>0.049</td>
<td>1.02 (1.00–1.03)</td>
<td>0.053</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>3.35 ± 1.21</td>
<td>3.68 ± 1.36</td>
<td>0.248</td>
<td>1.24 (0.83–1.81)</td>
<td>0.294</td>
</tr>
<tr>
<td>Age &gt;50 years (%)</td>
<td>21.6</td>
<td>53.3</td>
<td>0.003</td>
<td>4.14 (1.54–11.18)</td>
<td>0.006</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>23.5</td>
<td>57.1</td>
<td>0.003</td>
<td>4.33 (1.57–11.99)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>8.8</td>
<td>14.6</td>
<td>0.679</td>
<td>1.24 (0.31–4.96)</td>
<td>0.759</td>
</tr>
<tr>
<td>LDL-C &gt;100 mg/dl (%)</td>
<td>43.8</td>
<td>68.6</td>
<td>0.047</td>
<td>2.81 (1.01–7.76)</td>
<td>0.047</td>
</tr>
<tr>
<td>APO E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2 allele-carrier (%)</td>
<td>11.4</td>
<td>18.2</td>
<td>0.406</td>
<td>1.72 (0.46–6.40)</td>
<td>0.412</td>
</tr>
<tr>
<td>(ε2/2 + ε2/3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4 allele-carrier (%)</td>
<td>2.9</td>
<td>22.7</td>
<td>0.027</td>
<td>10.00 (1.17–85.31)</td>
<td>0.036</td>
</tr>
<tr>
<td>(ε4/4 + ε4/3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε3/3 genotype (%)</td>
<td>87.5</td>
<td>59.1</td>
<td>0.010</td>
<td>0.24 (0.08–0.75)</td>
<td>0.015</td>
</tr>
<tr>
<td>ACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD genotype (%)</td>
<td>24.3</td>
<td>17.9</td>
<td>0.467</td>
<td>0.67 (0.22–2.00)</td>
<td>0.470</td>
</tr>
<tr>
<td>II genotype (%)</td>
<td>21.6</td>
<td>37.8</td>
<td>0.114</td>
<td>2.20 (0.81–6.00)</td>
<td>0.121</td>
</tr>
<tr>
<td>ID genotype (%)</td>
<td>54.1</td>
<td>44.4</td>
<td>0.386</td>
<td>0.68 (0.28–1.65)</td>
<td>0.389</td>
</tr>
<tr>
<td>D allele-carrier (%)</td>
<td>78.4</td>
<td>62.2</td>
<td>0.114</td>
<td>0.45 (0.17–1.24)</td>
<td>0.121</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; CI, confidence interval.

*Mann–Whitney or Chi-squared test.

**Logistic regression analysis.

Atherosclerotic lesions in carotid arteries defined as max IMT ≥1 mm in the group of male alcoholics was also relatively high (71.5%) compared with this parameter found in the Italian population when taking into account the group of the same age and sex (37.7%) (Prati et al., 1992). Overall mean IMT ≥1 mm was observed in 31.5% of our alcoholics in comparison to 7.2% observed in white American men of the same age (5552 persons from the whole population-based ARIC study) (Chambless et al., 1997).

There is a general association between carotid atherosclerosis and the prevalence of coronary atherosclerosis and cerebral stroke (Bots et al., 1997; O’Leary et al., 1999). The epidemiological data indicate that the mean IMT ≥1 mm at any age is associated with a significantly increased risk of myocardial infarction and/or cerebrovascular disease (Simon et al., 2002). As carotid atherosclerosis is often the direct cause of ischemic stroke, the risk investigated in the present work concerns cerebrovascular consequences, which can depend on the genetic characteristics of an alcoholic subject.

There are several reports of the relationship between carotid atherosclerosis and the APO E genotype. Most demonstrated that carriers of the ε4 allele had a higher risk of carotid atherosclerosis (Terry et al., 1996; Cattin et al., 1997; Ilveskoski et al., 2000; Haraki et al., 2002). Studies concerning the relationship of the ε2 allele with carotid atherosclerosis yielded conflicting results — some authors demonstrated that the ε2 allele was protective (Ilveskoski et al., 2000; Slooter et al., 2001), while others found that the ε2/3 was a risk factor (de Andrade et al., 1995).

The allele relationship of APO E gene polymorphism and carotid atherosclerosis in alcoholic patients has not been reported previously. We have found that in our group of alcoholics, carriers of the ε4 allele had a higher risk of carotid atherosclerosis. Carriers of the ε2 allele also tended to have higher carotid IMT values in comparison to individuals with a ε3/3 genotype. Logistic regression analysis indicated the APO E ε4 allele as a significant predictor of the presence of carotid plaque. The multivariate analysis performed in the whole group showed an association of the ε4 allele only with right mean carotid IMT. This association could explain ~4% of variance of IMT of the right carotid artery. The multivariate analysis performed in different groups of age showed a significant association of the ε4 allele with all investigated carotid IMT values only before age 50 years — this association could explain 15.6% of variance of IMT of the right carotid artery and 6.7% of variance of IMT of the left carotid artery in younger alcoholics. We did not find any association of the ε4 allele with carotid IMT in older alcoholics. The differences in influence of the ε4 allele on right and left carotid IMT could be explained by the observation that right and left carotid arteries are differently exposed to shear forces because of their anatomical localization on the arterial tree. The same observation was noticed by Zannad et al. (1998).

Interestingly, in alcoholics, the percentage of the variance of carotid IMT (determined by stepwise multiple regression analysis) explained by the APO E ε4 allele was of the same degree as that explained by SBP and DBP or LDL cholesterol — the well known risk factors of atherosclerosis.
It was stated that in a general population (Davignon et al., 1988; Dallongville et al., 1992; Bednarska-Makaruk et al., 2001) subjects with APO E ε4 had higher lipid levels, particularly their LDL-C levels. Additionally, in a recent Framingham study (Corella et al., 2001) a positive significant association was noted between alcohol and LDL-C concentration in non-alcoholic men carrying the ε4 allele. One could expect the same in our group of alcoholics. We have stated only a tendency of LDL-C increase in APO E ε4 allele carriers, possibly because a small sample size was used.

LDL-C is usually low in heavy drinkers. (Taskinen et al., 1987). It was suggested (Naruszewicz et al., 1990) that modification by acetaldehyde caused its fast removal from the bloodstream — mainly to the liver (Nagelkerke et al., 1984). However, being an APO E ε4 carrier can cause an increase of the LDL-C level in an alcoholic. It may have an effect on several situations in his life. First — during a period of non-drinking there is the above mentioned lipid increasing tendency. Second, an enhanced hyperlipaemic response after drinking there is the above mentioned lipid increasing mechanisms of APO E ε4 allele. Wang et al. (1995) suggested that Apo E was involved directly in atherogenesis not necessarily in connection with lipoprotein levels through its direct effect on the vessel wall — it was stated that apo E can be involved in cellular proliferation or the adhesion process (Vogel et al., 1994; Riddel and Owen, 1995). Other possible mechanisms could also exist.

Other studies on the influence of ACE I/D polymorphism on carotid IMT reported various findings. Some stated an association between the D allele and increased carotid IMT (Castellano et al., 1995; Hosoi et al., 1996; Kauma et al., 1996), others did not find any correlation (Dessi-Fulgheri et al., 1995; Arnett et al., 1998; Huang et al., 1999, Hung et al., 1999; Mannami et al., 2001). No study could demonstrate a relationship between the D allele and carotid plaque or stenosis. A recent meta-analysis that had included 23 studies containing 9833 subjects revealed a significant, although weak, positive association between the D allele and common carotid IMT. The association was stronger among high-risk populations (Sayed-Tabatabaei et al., 2003).

There are no data in the literature concerning the relationship of ACE gene polymorphism and carotid atherosclerosis in alcoholic patients. We did not observe any relationship between ACE polymorphism and carotid IMT in our group of alcoholics. It has been suggested that multiple genes and non-genetic risk factors are involved in the development of atherosclerosis. Only some of them were recognized so far. Even less is known about particular gene–gene and gene–environmental interactions. This could explain the difficulty to evaluate the predictive values of separated genetic risk factors (Doevendans et al., 2001). Recently, evidence was shown for multiple interactions of the APO E and ACE polymorphisms with different genetic and environmental factors: eNOS gene polymorphism*APO E polymorphism (Asakimori et al., 2003) and age*ACE genotype, body mass index*APO E genotype (Tabara et al., 2001), SBP*ACE genotype (Kawamoto et al., 2002), the smoking*APO E genotype (Karvonen et al., 2002), the smoking*ACE genotype (Sayed-Tabatabaei et al., 2004) and diabetes*APO E genotype (Elousa et al., 2004).

Our study demonstrated that the influence of different risk factors on carotid IMT values in alcoholics was age-dependent. The APO E ε4 allele as a genetic risk factor was significant in younger patients. High DBP, high LDL-C and high plasma fasting glucose were significantly associated with increased carotid IMT values in alcoholics >50 years. SBP was an age-independent risk factor. Other possible genetic factors, as well as the gene–gene and gene–environmental interactions could influence IMT values.

CONCLUSIONS

The APO E ε4 allele and other risk factors such as age, hypertension, high LDL-C and high glucose levels can increase the risk of carotid atherosclerosis development in an alcoholic subject. The influence of different risk factors on carotid IMT values in alcoholics was age-dependent. Since the elevated carotid IMT is considered to be a good marker of increased risk of generalized atherosclerosis, the consequences could involve both cardiac and cerebrovascular events.

Acknowledgements — The study was supported by a grant from the Polish State Agency for the Prevention of Alcohol Problems.

REFERENCES


Jagiello, T. (2000) Ultrasonografia Dopplerowska tętnic domóz-

Hung, J., McQuillan, M., Nidorf, M. et al. (1999) Angiotensin-

Converting enzyme I/D polymorphism and arterial wall thickness

in a general population. The Vobarno Study. Circulation 91,

2721–2724.

Cattin, L., Fiscaro, M., Tonizzo, M. et al. (1997) Polymorphism of

apolipoprotein E gene and early carotid atherosclerosis defined by

ultrasonography in asymptomatic adults. Arteriosclerosis

Thrombosis and Vascular Biology 17, 91–94.


of coronary heart disease incidence with carotid arterial wall thickness

and major risk factors: the Atherosclerosis Risk in Communities


483–484.

determines the effect of the APOE locus on IDL-cholesterol

concentrations in men: the Framingham Offspring Study. American

Journal of Human Nutrition 73, 736–745.


relationship and public health implications. Clinica Chimica Acta

246, 51–57.


of plasma triglyceride levels by ApoE phenotype: a meta-analysis.


Dessi-Fulgheri, P., Catalini, R., Sarzani, R. et al. (1995) Angiotensin-

converting enzyme gene polymorphism and carotid atherosclerosis

in a low-risk population. Journal of Hypertension 13, 1593–1596.

Doevendans, P. A., Jukema, W., Piepering, W. et al. (2001) Molecular

genetics and gene expression in atherosclerosis. International

Journal of Cardiology 80, 161–172.


APOE genotype with carotid atherosclerosis in men and women:

the Framingham Heart Study. Journal of Lipid Research 45,

1868–1875.


apolipoprotein and lipoprotein profile after alcohol withdrawal:

effect of apolipoprotein E polymorphism. Alcoholism: Clinical and

Experimental Research 26, 501–508.

Haraki, T., Takegoshi, T., Kitoh, C. et al. (2002) Carotid artery intima-

media thickness and brachial artery flow-mediated vasodilatation in

asymptomatic Japanese male subjects amongst apolipoprotein E


apolipoprotein E by gene amplification and cleavage with HhaI.


Hosoi, M., Nishizawa, Y., Kogawa, K. et al. (1996) Angiotensin-

converting enzyme gene polymorphism is associated with carotid

arterial wall thickness in non-insulin-dependent diabetic patients.


Huang, X. H., Loimaal a, A., Nen enen, A. et al. (1999) Relationship of

angiotensin-converting enzyme gene polymorphism to carotid

arterial wall thickness in middle-aged men. Journal of Molecular

Medicine 77, 853–858.

Hun g, J., McQuillan, M., N idorf, M. et al. (1999) Angiotensin-

converting enzyme gene polymorphism and carotid wall thickening

in a community population. Arteriosclerosis Thrombosis and


Iveskoski, E., Loimaal a, A., Mercuri, M. F. et al. (2000)

Apolipoprotein E polymorphism and carotid artery intima-media

thickness in a random sample of middle aged men. Atherosclerosis

153, 147–153.

Jagiello, T. (2000) Ultrasonografi a Dopplerowska tętnic domóz-
gowych. (Doppler ultrasound measurements of extracerebral


Uwopyszczewic h Nauki – Os wiata “UN-O”, Warszawa.


polymorphism affects carotid atherosclerosis in smoking


alcohol drinking and progression of atherosclerosis. Arteriosclerosis,

Thrombosis and Vascular Biology 19, 3001–3006.

Kauma, H., Päivänsalo, M., Savolainen, M. J. et al. (1996) Association

between angiotensin converting enzyme gene polymorphism and


Kawamoto, R., Kohara, K., Tabara, Y. et al. (2002) An interaction

between systolic blood pressure and angiotensin-converting

enzyme gene polymorphism on carotid atherosclerosis.

Hypertension Research 25, 785–789.

Kiechl, S., Willeit, J., Rungger, G. et al. (1998) Alcohol consumption

and atherosclerosis: what is the relation? Prospective results from

the Bruneck Study. Stroke 29, 900–907.


phenotype and cardiovascular disease in the Framingham Heart

Study. Atherosclerosis 154, 529–537.

Lamisse, F., Schellenberg, F., Bouyou, E. et al. (1994) Plasma lipid

and alcohol consumption in alcoholic men: effect of withdrawal.


evaluation of angiotensin-converting-enzyme gene polymorphism

and the risk of ischemic heart disease. The New England Journal

of Medicine 332, 706–712.

Mannami, T., Katsuya, T., Baba, S. et al. (2001) Low potentiality of

angiotensin-converting enzyme gene insertion/deletion polymorphism

as a useful predictive marker for carotid atherogenesis in a large
general population of a Japanese city. The Suita Study. Stroke 32,

1250–1256.


(1984) In vivo catabolism of biologically modified LDL. Arteriosclerosis

4, 256–264.


density lipoprotein composition in some chronic alcohols: a possible

mechanism. Alcohol and Alcoholism 25, 533–538.


Evidence for association and genetic linkage of the angiotensin-

converting enzyme locus with hypertension and blood pressure in

men but not women in the Framingham Heart Study. Circulation 97,

1766–1772.

O’Leary, D. H., Polak, J. F., Kronmal, R. A. et al. the Cardiovascular

Health Study Collaborative Research Group. (1999) Carotid-artery

intima and media thickness as a risk factor for myocardial infarction

and stroke in older adults. The New England Journal of Medicine

340, 14–22.

Prati, P., Vanuzzo, D., Casaroli, M. et al. (1992) Prevalence and
determinants of carotid atherosclerosis in a general population.

Stroke 23, 1705–1711.

Puddey, I. B., Rakic, V., Dimmitt, S. B. et al. (1999) Influence of pattern

of drinking on cardiovascular disease and cardiovascular risk factors —

a review. Addiction 94, 649–663.


platelet aggregation by apo E is not mediated by membrane

cholesterol depletion. Thrombosis Research 80, 499–508.

Samani, N. J., Thompson, J. R., O’Toole, L. et al. (1996) A meta-

analysis of the association of the deletion allele of the angiotensin-

converting enzyme gene with myocardial infarction. Circulation


Sayed-Tabatabaei, F. A., Houwing-Duistermaat, J. J., van Duijn, C. M.

et al. (2003) Angiotensin-converting enzyme gene polymorphism

and atherosclerosis: what is the relation? Prospective results from


154, 529–537.


stroke. Journal of Neurology Neurosurgery and Psychiatry 64,

227–230.


thickness: a new tool for diagnosis and treatment of cardiovascular


