Prevalence of apolipoprotein E genotypes in ischaemic cerebrovascular disease in end-stage renal disease patients

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Abstract

Background. Several studies have demonstrated that atherosclerotic complications are the major cause of morbidity and mortality in end-stage renal disease (ESRD) patients on dialysis. The allele e4 of apolipoprotein E (ApoE) gene has been associated with an increased risk for ischaemic cerebrovascular disease (ICVD) in general populations in recent preliminary cross-sectional studies.

Methods. The aim of our study was to prospectively investigate the risk of ischaemic cerebrovascular disease with respect to the presence of Apo e4 allele in 157 Chinese uraemic patients.

Results. During the 2 year follow-up, the incidence of stroke was 6.4% and the cumulative occurrence of ischaemic cerebrovascular disease was 9.6%. Further analysis showed that the cumulative occurrence of ischaemic cerebrovascular disease was 5.6% in subjects with no ApoE e4 allele and 36.8% in those with one or two ApoE e4 alleles. Univariate analysis showed that the prevalence of ischaemic cerebrovascular disease was significantly higher in the subjects with ApoE e4 allele. The mean ApoA1 plasma concentrations was significantly lower in those with ApoE e4 allele. A stepwise regression analysis showed that the presence of stroke was significantly related to the ApoE e4 genotype (P < 0.001).

Conclusions. The ApoE e4 allele can be regarded as an important risk factor for ischaemic cerebrovascular disease in uraemic patients.

Introduction

Atherosclerotic vascular disease is a major cause of morbidity and mortality in end-stage renal disease (ESRD) patients on dialysis. The uraemic milieu is thought to lead to accelerated atherogenesis but the underlying mechanisms are still poorly understood. Moreover, the risk factors of atherosclerosis identified in the Framingham study [1–2] in non-uraemic populations such as arterial hypertension, lipid dyslipidaemia, and haemostatic abnormalities are very common in these dialysis patients.

Apolipoprotein E is a 299-amino acid protein found in chylomicrons, VLDL, intermediate-density lipoprotein, and HDL [3]. It plays an important role in the metabolism of these lipoproteins by binding to the LDL receptor in hepatic and extrahepatic tissues and a putative ApoE receptor or LDL-receptor related protein [3–5]. The genetic polymorphism of ApoE results from the existence of three common codominant alleles (e2, e3, e4). These three alleles then account for the ApoE polymorphism and determine the six phenotypes E2/2, E2/3, E2/4, E3/3, E4/3, and E4/4. In all populations studied [6], E3 is the most prevalent form.

Until recently, the mechanisms of association were conflicting. In mice made ApoE deficient by genetic targeting, severe dyslipidaemia and vascular lesions developed, whereas transgenic mice who overexpressed ApoE seem to be protected [7–8]. Several recent clinical reports have also demonstrated that ApoE may play a critical role in ischaemic vascular disease such as coronary heart disease [9–12]. However, there are relatively paucity of clinical data concerning the possible role of these triglyceride-rich lipoproteins and ApoE polymorphism in the development of ischaemic cerebrovascular disease (ICVD). Until now, three studies [13–14] have been carried out on the genetic polymorphism of ApoE in non-uraemic populations with ICVD. Two such studies showed a high frequency of the e4 allele along with a low frequency of the e3 allele [14].

The aim of this study was to investigate the impact of ApoE polymorphism on ICVD in the uraemic population. The association between ApoE genotype and lipid profile in uraemic patients on dialysis was also examined.
Materials and methods

All patients from a single dialysis centre were invited to participate in the present study. The baseline examination was conducted between May 1994 and June 1994. Venous blood was taken from 157 ESRD patients (HD, 119 patients; CAPD, 38 patients) aged between 23 and 83 years (mean age 56.5 ± 13.7 years) and from 84 healthy controls aged between 21 and 82 years (mean age 58.6 ± 12.5 years). Informed consent was obtained from each of the studied subjects. The causes of ESRD were chronic glomerulonephritis in 85 patients (54%), diabetic nephropathy in 30 (19%), chronic pyelonephritis in 18 (11%), polycystic kidney disease in three (2%), and other diseases in 11 (7%). The cause was unknown in 10 (7%) patients. The haemodialysis patients received thrice-weekly treatments to maintain a minimum Kt/V urea index of 1.2 per session. On the other hand, the CAPD patients received 4–5 2-l bags to achieve a Kt/V urea index of 1.9 per week.

The follow-up study was conducted between April 1996 and June 1996. The follow-up period for the subjects was 2 years. Among the 157 uraemic patients who participated in the baseline study, 14 died during follow-up. Causes of death and cardiovascular events were recorded.

Diagnosis of previous and new ischaemic stroke

All medical records of subjects with symptoms suggestive of ischaemic stroke before the baseline examination and during follow-up were reviewed by one of the authors. The verified ischaemic stroke was defined as a clinical syndrome consisting of neurological deficits persisting over 24 h and observed by at least one neurologist, in the absence of other diseases accounting for the symptoms. Patients with subarachnoid haemorrhage and haemorrhagic strokes were excluded from the study. A computed tomograph was performed in all cases but was required only as supportive evidence for the diagnosis of ischaemic stroke. In the following analyses, non-fatal and fatal strokes were combined because of a limited number of stroke end-points. If a subject had more than one stroke during the follow-up period, only one stroke event was included in the statistical analyses.

Risk factors evaluation

The atherosclerotic risk factors such as hypertension and diabetes mellitus were evaluated based on multiple laboratory measurements. Patients were classified as hypertensive when the blood pressure was > 95 mmHg diastolic and/or > 160 mmHg systolic and/or treated with antihypertensive medications. Diagnosis of diabetes mellitus were considered if the subjects were taking oral hypoglycaemic agents or insulin and/or fasting blood glucose level was > 140 mg/dl.

Lipoprotein analysis

Blood samples were obtained in the morning after an overnight fast. The serum was separated and stored at 4 °C for analysis of cholesterol, triglyceride, and high-density lipoprotein (HDL)-containing cholesterol. Cholesterol and triglyceride were analysed enzymatically. HDL cholesterol was measured after phosphotungstate precipitation and centrifugation of LDL at the isoelectric point of LDL (pI = 7.5). LDL-containing cholesterol was all measured directly.

Whole blood from control and studied patients (in a fasted state) was drawn into tubes containing ethylelediaminetetra-acetic acid (1.0 mg/ml). The separated blood cells were used for determining ApoE genotype.

Amplification of genomic DNA by PCR

Leucocyte DNA was extracted from the whole blood by using the method of Higuchi [15]. We PCR-amplified the desired DNA fragment by utilizing a DNA thermal cycler (MJ) along with an upstream primer:

5’-TCCAAGGAGCTGCAGGCGGCA-3’ and a downstream primer:

5’-ACAGAAATTCGCCCGCTCTGTA-3’

Each amplification reaction contained 250 ng of genomic DNA, 20 pmol of each primer, 10% dimethylsulfoxide, 220 μM of each dNTP, and 0.3 μl of Dynazyme DNA polymerase (2 units/μl) in a final volume of 25 μl. Thermal profile of the PCR was denaturation for 60 s at 95 °C, annealing for 60 s at 55 °C, and extension for 2 min at 72 °C. A total of 30 cycles was performed. The PCR products were digested with EcoRI. The DNA fragments were separated by electrophoresis on an ethidium bromide-containing 4% agarose gel. DNA fragments were visualized by UV transillumination (Fig. 1). ApoE genotypes for the case and control groups were determined in a blinded fashion by scoring for a unique combination of fragment sizes as described by Hixon and Vernier [16].

Statistical analysis

Statistical analysis were performed using the SPSS/PC program. Data are given as mean ± SEM or percentages. For comparisons of the parameters between the control and the patients’ groups as shown in Table 1, the non-parametric Mann-Whitney two-sample test for unpaired data was performed. χ² test was used to assess the differences between groups when appropriate. The stepwise logistic regression was used to describe the strength of the prognostic factors selected which included: hypertension, ApoAl level, ApoB level, ApoE genotype, diabetes mellitus, age, and sex.

Results

The observed allele frequencies of the ApoE gene in normal controls were 6% for ε2, 82% for ε3, and 12% for ε4, whereas those for uraemic patients were 5.7% for ε2, 86.3% for ε3, and 8% for ε4, respectively. As in Table 1. Absolute and relative frequency distribution of the ApoE gene polymorphism in uraemic subjects with ischaemic cerebrovascular diseases, uraemic controls, and healthy subjects

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>%</th>
<th>No. of subjects</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uraemic with ICVD</td>
<td>7</td>
<td>36.8*</td>
<td>8</td>
</tr>
<tr>
<td>Uraemic subjects</td>
<td>19</td>
<td>12</td>
<td>130</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>12</td>
<td>14.3</td>
<td>72</td>
</tr>
</tbody>
</table>

*P < 0.001, significant difference between uraemic control and healthy subjects by χ² test.
most populations, e3 is the most common genotype in both normal controls and uraemic patients. In the present study, there was no significant difference in ApoE e4 allelic frequencies between uraemic subjects and controls. To investigate the effect of the ApoE e4 on the risk of stroke, we divided the subjects into those with one or two e4 alleles (E4) and those without the allele (non-E4).

The 2-year incidence of ICVD was 6.4% (10/157) and the cumulative occurrence of ICVD in the uraemic subjects was 9.6% (19/197). Further analysis showed that the cumulative occurrence of ICVD was 5.6% in subjects with no e4 allele and 36.8% in those with one or two e4 alleles (Table 1). Univariate analysis showed that the prevalence of ICVD was significantly higher in the subjects with ApoE e4 allele ($P < 0.001$).

Serum lipid and lipoprotein concentrations of the uraemic controls as well as uraemic patients with ICVD are shown in Table 2. The ApoA1 concentration, serum cholesterol, as well as LDL-cholesterol concentrations were significantly lower in uraemic patients with ApoE e4 allele than those without the ApoE e4 allele. There was no significant difference in plasma triglyceride and ApoB concentrations between these two groups, although the mean serum triglyceride levels were higher in the uraemic patients with ApoE e4 allele.

The stepwise logistic regression analysis indicated that only ApoE genotype (e4) was significant ($P < 0.05$) and may serve as an independent predictor of the occurrence of the ischaemic cerebrovascular disease in dialysis patients (Table 3).

**Discussion**

The distribution of the different genotypes in our control group were similar to those previously reported in the other populations [6,17–21]. Studies involving Caucasians have so far shown average relative allele frequencies of 0.77–0.78 for e3, 0.08 for e2, and 0.14 for e4 [6,18–20]. In some of these ethnic groups such as Finnish people who are known to have increased

**Table 2.** Baseline characteristics and prevalence of cardiovascular risk factors in uraemic patients

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>E4 ($n = 19$)</th>
<th>non-E4 ($n = 138$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.5 ± 2.9</td>
<td>57.5 ± 1.2</td>
<td>ns*</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>9/10</td>
<td>88/50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>42</td>
<td>35.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>26.3</td>
<td>26.9</td>
<td>ns</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>186.4 ± 10.9</td>
<td>193.3 ± 4.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL</td>
<td>31.4 ± 3.3</td>
<td>35.7 ± 1.4</td>
<td>ns</td>
</tr>
<tr>
<td>LDL</td>
<td>106.5 ± 8.2</td>
<td>119.4 ± 3.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>256.9 ± 66.0</td>
<td>233.0 ± 21.9</td>
<td>ns</td>
</tr>
<tr>
<td>ApoA1 (mg/dl)</td>
<td>96.9 ± 5.5</td>
<td>108.7 ± 2.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>92.4 ± 8.7</td>
<td>95.8 ± 3.6</td>
<td>ns</td>
</tr>
</tbody>
</table>

*ns: not significant.

**Table 3.** Results of the logistic regression model

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Coefficient</th>
<th>S.E.</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE genotype</td>
<td>−1.3378</td>
<td>0.3823</td>
<td>12.25</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.2881</td>
<td>0.3665</td>
<td>0.62</td>
<td>0.9513</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>−0.1238</td>
<td>0.3902</td>
<td>0.13</td>
<td>0.7237</td>
</tr>
<tr>
<td>ApoA1 level</td>
<td>−0.0180</td>
<td>0.0161</td>
<td>1.37</td>
<td>0.2629</td>
</tr>
<tr>
<td>Sex</td>
<td>0.5473</td>
<td>0.6328</td>
<td>0.75</td>
<td>0.0860</td>
</tr>
<tr>
<td>Age</td>
<td>0.0598</td>
<td>0.0332</td>
<td>3.24</td>
<td>0.0720</td>
</tr>
<tr>
<td>ApoB level</td>
<td>0.0034</td>
<td>0.0082</td>
<td>0.17</td>
<td>0.6773</td>
</tr>
</tbody>
</table>

*Statistically significant.
incidence of atherosclerosis, the relative allele frequencies were found to be 0.73 for e3, 0.04 for e2 and 0.23 for e4, respectively. Although the e4 allele frequency in our uraemic population was found to be 0.08 which was lower than the subjects in the normal control group viz. e4 0.12, the difference did not reach statistical significance.

Inconsistency about the risk effect of ApoE polymorphism on coronary heart disease (CHD) had been reported for more than a decade [22–26]. Context-dependency of ApoE polymorphism-associated effects has also been reported recently in long-term prospective studies [27–29]. Ethnic origin, gender, and life style including diet have been recognized as factors which can influence a specific ApoE phenotype. In addition, several recent pilot studies [13–14] have demonstrated that ApoE e4 is associated with ischaemic cerebrovascular disease in the general population. In two earlier reports, there was higher prevalence of ApoE e4 polymorphism in ICVD patients than in control subjects. However, in the study conducted by Coudere et al. [14] ApoE e2 allele was found to be a risk factor for cerebrovascular disease. The discrepancies between these studies were most probably due to differences in patient selection criteria. In our uraemic population, we found that the incidence and cumulative occurrence of stroke increased significantly in those with the ApoE e4 allele. This preliminary observation probably suggests that e4 allele could be a predisposing genetic marker for ischaemic cerebrovascular disease in these uraemic patients.

Cardiovascular diseases account for >50% of overall mortality and morbidity in uraemic patients, according to the EDTA Registry [30]. What has become clear is that these uraemic patients have a high incidence and prevalence of atherosclerotic diseases which may be related to numerous traditional atherogenic risk factors such as hypertension, diabetes mellitus, abnormalities of calcium and phosphate metabolism, as well as uraemic dyslipidemia. However, these classic factors do not explain all cerebrovascular events in uraemic patients, and new risk factors have to be sought. In recent years, apolipoproteins such as ApoA1 and ApoE have been one of the main targets of interest. In this study a stepwise logistic regression analysis was performed to remove possible bias due to unmatched factors and to appreciate the influence of covariates. In addition to the matching variables of age and sex, each of the variables strongly associated with the presence of ischaemic cerebrovascular disease was included in the model. The results clearly show that in these uraemic subjects, ApoE e4 was independently associated with ICVD.

The mechanisms by which ApoE influences vascular atherogenesis are far from being understood. Over the past few years, the role that ApoE plays in lipoprotein metabolism and atherogenesis has been extensively studied using genetically modified mice. Some authors found that severe dyslipidaemia and atherosclerotic lesions develop in ApoE-deficient mice [6,31]. Others [32–33] have also demonstrated that the introduction of an ApoE gene into ApoE deficient mice leads to normalization of plasma lipid levels, protection from diet-induced atherosclerosis, and regression of pre-existing plaques. The results of these studies suggest a direct effect of ApoE on lipoprotein metabolism and atherosclerosis on at least two levels. First, it inhibits the formation of atherosclerotic plaques via its reducing effect of plasma remnant lipoprotein levels. Secondly, the regression of plaques is enhanced via its stimulatory effect on cellular cholesterol efflux.

In contrast to most previous studies [6,34–35], the total cholesterol and LDL cholesterol in our uraemic subjects with ApoE e4 allele were significantly lower than in those without. From previous experimental studies, it was found that E4-containing lipoproteins such as VLDL and chylomicron remnants are catabolized at a higher rate, with an efficient delivery of cholesterol and triglycerides to the liver [36–38]. This in turn leads to a down-regulation of LDL receptors and a higher plasma LDL concentration. In addition, differences in hepatic cholesterol synthesis could also affect the observed cholesterol level [39]. As the hepatic cholesterol homeostasis is directly related to the individual’s nutritional status, and the nutritional status of uraemic subjects are generally poorer, this probably may negate the effect of ApoE apolipoproteins on plasma cholesterol levels. In subjects with e4 allele, there is also a higher rate of ApoE-mediated conversion of VLDL remnants to LDL. However, in uraemic patients, due to a reduced activity of lipoprotein lipase, such conversion could be defective resulting in an accumulation of VLDL remnants. In fact, our present study observed higher triglyceride levels in uraemic subjects with Apo e4 allele although the results did not reach a significant level.

Results from the present study also demonstrated that ApoA1 concentrations were significantly lower in subjects with ApoE e4 than those without e4. Such an association between ApoA1 and stroke has been described recently by several authors [15,29–30]. These authors found that a low ApoA1 concentration increases the risk for cerebral and lacunar infarct. The protective effect of ApoA1 may be related to its action against the accumulation of platelet thrombi at sites of vascular damage. The hypothesis that ApoE polymorphism and/or ApoA1 could be a marker of atherosclerosis suggests that apolipoproteins may be more important in atherogenesis than the lipid component. Since genetic factors may influence the concentration of the protein moiety, more than environmental factors, genetic factors may have significant role in determining the predisposition of the subjects to stroke. On the basis of our findings, we can speculate that it is very likely that the ApoE e4 allele may form an increased risk for ICVD in uraemic subjects.

In conclusion, our study has demonstrated that at least for Chinese uraemic patients, carrying ApoE e4 allele is a risk factor for ischaemic cerebrovascular disease.
References


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