Influence of variation at the apolipoprotein E locus on lipid and lipoprotein levels in CAPD patients

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Abstract

**Background.** Variation at the apolipoprotein E (apo E) locus influence lipid and lipoprotein levels in the normal population, and is associated with premature coronary artery disease. Patients with end-stage kidney disease or undergoing dialysis treatment are particularly prone to develop accelerated atherosclerosis. 

**Methods.** To evaluate the influence of genetic variation at the apo E locus, apo E genotypes and serum lipid and lipoprotein levels were measured in 51 subjects undergoing continuous ambulatory peritoneal dialysis (CAPD).

**Results.** The distribution of apo E phenotypes and apo E allelic frequency among the CAPD subjects (ε2 0.049; ε3 0.745; ε4 0.206) corresponded to the healthy Swedish population. In the CAPD subjects, total serum and LDL cholesterol levels were high (6.7 ± 1.5 mmol/l and 4.2 ± 1.3 mmol/l respectively) and HDL cholesterol levels were low (1.2 ± 0.5 mmol/l). When directly comparing the two major apo E groups, E 3/3 subjects (n = 30) and E4/3 and 4/4 subjects, ε4-carriers, (n = 16), LDL cholesterol levels were significantly higher among ε4-carriers (4.8 ± 1.1 vs 4.0 ± 1.2 mmol/l, P < 0.03), but total serum cholesterol levels was not higher among the ε4-carriers (7.3 ± 1.3 vs 6.5 ± 1.5 mmol/l, P < 0.08). Serum triglycerides or HDL cholesterol levels did not differ significantly between ε3-homozygotes and ε4-carriers.

**Conclusions.** The results demonstrate a strong effect of variation of the apo E locus on LDL cholesterol levels in CAPD subjects, suggesting that ε4-carriers may be more susceptible to accelerated development of atherosclerosis in this condition.

**Key words:** atherosclerosis; apolipoprotein E; kidney disease; LDL cholesterol; peritoneal dialysis

**Introduction**

Apolipoprotein E (apo E) affect several key pathways in lipoprotein metabolism, and has a profound influence on serum lipid levels. Apo E is transported in several lipoprotein fractions and serves as a ligand for hepatic lipoprotein receptors, thereby influencing clearance of lipoprotein particles [1–4]. Three different alleles (ε2, ε3 and ε4) at the apo E gene locus code for three different protein isoforms, apo E2, E3 and E4, respectively, resulting in six different phenotypes [1,4–6]. It is well known that homozygosity for E2 is a prerequisite for development of type III hyperlipoproteinemia, and in addition, the presence of E4 has been associated with increased low-density lipoprotein (LDL) cholesterol levels [1,7,8]. The exact mechanism for this remains to be determined, but differing interaction between apo E isoforms and the LDL receptor has been suggested to be contributory [9,10]. Several studies have suggested that the apo ε4 allele is a risk factor for development of early coronary heart disease [1,11–14].

In a number of population studies in Caucasians, the relative frequency of the ε alleles have been relatively similar, in general varying between 0.07 and 0.13 for ε2, 0.72 and 0.79 for ε3, and 0.13 and 0.16 for ε4 [1,7,15]. In the Scandinavian population, the ε4 allele is relatively more common [16–18]. In a number of recent studies, a pronounced age dependency of the relative ε allele frequency has been reported, with a decrease in the ε4 allele frequency with age [17,19,20]. The underlying reasons for this decline have not so far been established, although both cardiovascular disease and the recent association of the apo ε4 allele with Alzheimer’s disease [21] could be contributory.

Growing evidence indicate that subjects with end-stage kidney disease or undergoing dialysis treatment have an increased cardiovascular morbidity and frequently have dyslipidaemias [22,23]. The underlying mechanisms for these lipid and lipoprotein disturbances remains largely to be determined. So far, only limited information on the impact of variation at the apo E locus on serum lipids in renal disease is available [24,25]. In the present study we have therefore investi-
Experimental procedure

Subjects

Fifty-one adult patients with end-stage renal disease undergoing CAPD at the Renal Medicine Clinic at Huddinge University Hospital, were recruited to participate in the study. The cause of renal failure was in 19 cases glomerulonephritis, in 11 cases diabetic nephropathy, in nine cases interstitial nephritis, in six cases other diseases, and six subjects suffered from end-stage renal disease of unknown cause. On average the duration of CAPD treatment was 11 ± 10 months (range 1–54 months). Nine of the subjects had previously been treated with haemodialysis prior to CAPD treatment, and of these subjects, none had been on CAPD treatment for less than 6 months. The CAPD regimen had a prescribed dialysate volume of 6 litres in seven subjects, 8 litres in 25 subjects, 10 litres in eight subjects, and 12.5 litres in two subjects. Nine patients were treated with automated peritoneal dialysis with an average prescribed daily dialysate volume of 21 litres (range 12.5–25 litres). Four of the patients were treated with hypolipidaemic drugs, in three cases simvastatin (10–20 mg/day) and in one case bezafibrate (200 mg/day). The presence of cardiovascular disease was defined as previous verified myocardial infarction, or angina pectoris, with a typical clinical presentation or verified through a positive stress test. The clinical characteristics of the patient population is summarized in Table 1. Results were compared with a previously characterized healthy control group, consisting of 244 men and 163 women, aged 17–86 years [17].

Analytical procedures

All blood samples were drawn between 0800 hours and 0900 hours after an overnight fast. Samples were drawn into tubes with or without EDTA. Plasma or serum was separated immediately by centrifugation in the cold at 2000 r.p.m. for 20 min. Phenylmethylsulphonyl fluoride was added to all samples at a final concentration of 0.01 mmol/l. Unless immediately analysed, the plasma or serum aliquots were frozen and stored at −70 °C until analysis. For genotyping, CAPD patients and healthy controls was performed using parallel samples of venous blood were collected in EDTA-containing tubes and frozen at −70 °C.

Plasma lipid and lipoprotein levels were determined in fresh plasma samples. Triglycerides and cholesterol were determined using standard enzymatic techniques (Boehringer Mannheim, Mannheim, Germany). HDL cholesterol levels were analysed after precipitation of apo B-containing proteins with phosphotungstic acid [26]. LDL cholesterol levels were calculated using the Friedewald formula. In some cases where fasting triglyceride levels were >4 mmol/l, plasma lipoproteins levels were assayed using a combination of ultracentrifugation and precipitation as outlined in detail elsewhere [27]. The remaining plasma and serum samples were immediately frozen and kept at −70 °C.

Apo E genotyping was carried out on genomic DNA originating from leukocytes in venous blood samples drawn into EDTA-containing tubes. The genomic DNA was prepared using Qiagen Blood & Cell Culture DNA Midi Kits (Qiagen GmbH, Hilden, Germany). The procedure utilized the minisequencing technique as adapted for apo E genotyping [29], modified to allow easy detection and rapid handling of multiple samples without the use of radioactivity [30]. In the present study, the method was modified to allow the simultaneous evaluation of all three apo e allelic variants (AffiGene Apo E Kit, Sangtec Medical, Stockholm, Sweden). Briefly, the minisequencing procedure was carried out in streptavidin-coated microtitre wells. A 203-bp fragment of the apo E gene, including the sites for codons 112 and 158 (ApoE Primer Kit, Sangtec Medical, Stockholm, Sweden) was amplified. The downstream primer was biotinylated, enabling the anticoding strand of the PCR product to attach to the coated wells. Two 20-mer ‘detection primers’ were annealed to the attached strands, and the DNA polymerase extension reaction was carried out with dithiophenyl(DNP)-labelled mononucleotides (DNP-T or DNP-C). For each polymorphic site, two wells with the amplified strand were used, adding DNP-T to one well, and DNP-C to the other well. The wells were rinsed, and anti-DNP-antibodies, conjugated with alkaline phosphatase, were added. The bound antibodies were detected using an ELISA procedure. Apo E genotypes were determined based on the patterns obtained with the different DNP-antibody reactions. The automated procedure allowed 22 samples to be processed in 4 h.

Statistical analysis

Data are presented as means ± SD. For analysis of apo E genotypes, the number of a given allele, e.g. e3, in the pair of alleles can be 0, 1 or 2, corresponding to individual relative frequencies of 0, 50 or 100% respectively. A carrier of a specific e allele was defined as a subject carrying the E4, E3 or E2 phenotype in homo- or heterozygous form. Comparisons between groups were carried out with a two-tailed Student’s t test. Apo e allele frequency distribution in CAPD patients and healthy controls was performed using the chi-square test.

Results

Among the CAPD subjects, total serum and LDL cholesterol levels were increased and HDL cholesterol levels decreased compared to healthy controls (Table 2). Also, fasting triglyceride levels were higher among the patients. The distribution of apo E phenotypes and alleles are given in Table 3. The dominating genotypes were E3/E3 and E4/E3 and only a few subjects were found with E 3/2, 4/2 or 4/4 genotypes. There was a relatively high relative frequency of the e4-allele (0.206) and this frequency was similar to the distribution among healthy Swedes.

As seen in Table 4, serum total and LDL cholesterol levels were higher in the E 4/3 and E 4/4-groups.

Table 1. Clinical baseline data on the CAPD subjects (n = 51)

| Age (years) | 55 ± 13 (range 30–79) |
| Sex (M/F) | 25/26 |
| Cardiovascular disease | 12/51 |
| Body weight (kg) | 70 ± 16 |
| BMI (kg/m²) | 23.9 ± 4.2 |
| S-Albumin (g/l) | 29.9 ± 4.7 |
| S-creatinine (mmol/l) | 653 ± 167 |
| Haemoglobin (g/l) | 110 ± 23 |
Serum lipid and lipoprotein levels in CAPD patients

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Serum cholesterol (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>LDL cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 2/2</td>
<td>6.7 ± 1.5†</td>
<td>1.2 ± 0.5†</td>
<td>2.8 ± 1.7†</td>
</tr>
<tr>
<td>E 3/2</td>
<td>5.5 ± 1.2</td>
<td>1.5 ± 0.5</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>E 4/2</td>
<td>1.2 ± 0.5†</td>
<td>1.5 ± 0.5</td>
<td>2.8 ± 1.7†</td>
</tr>
<tr>
<td>E 4/3</td>
<td>1.2 ± 0.5†</td>
<td>1.5 ± 0.5</td>
<td>2.8 ± 1.7†</td>
</tr>
<tr>
<td>E 4/4</td>
<td>1.2 ± 0.5†</td>
<td>1.5 ± 0.5</td>
<td>2.8 ± 1.7†</td>
</tr>
</tbody>
</table>

Table 3. Apo E genotypes and alleles in CAPD subjects

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>E 2/2</th>
<th>E 3/2</th>
<th>E 4/2</th>
<th>E 4/3</th>
<th>E 4/4</th>
<th>Apo e allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 51</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>14</td>
<td>2</td>
<td>e 2 0.049, e 3 0.745, e 4 0.206</td>
</tr>
<tr>
<td>n = 407</td>
<td>4</td>
<td>42</td>
<td>14</td>
<td>65</td>
<td>43</td>
<td>e 2 0.078, e 3 0.719, e 4 0.203</td>
</tr>
</tbody>
</table>

Apo e allele frequency was higher among the non-CAD subjects than among the CAD subjects (0.782 vs 0.625).

Discussion

In the present study we demonstrate a significant impact of variation at the apo e locus on serum lipid levels in CAPD-patients. As treatment regimens are continuously improved among subjects undergoing dialysis treatment for end-stage kidney disease with an increased survival time, the possibilities for follow-up are also increasing. It has now become apparent that under these conditions, hyperlipidaemia and accelerated atherosclerosis is common [22,23]. Although clearly several different factors might contribute to the dyslipidaemic conditions in CAPD, very little is currently known about the underlying mechanisms. The impact of genetic susceptibility to development of atherosclerosis is presently under intense scrutiny, and in particular, interest has focused on variation at the apo e locus. Among healthy individuals it has been well established in a large number of population studies, that carriers of the apo e4-allele have higher serum total and LDL cholesterol levels [1,7]. This has led to the notion that e4-carriers have an increased risk for premature atherosclerosis, and homozygosity for apo e4 has indeed been found to be more prevalent in subjects with early coronary disease [12]. Another interesting finding is that the apo e4 allele is associated with decreased longevity, with a decrease in the apo e4 allele frequency among the elderly [17,19,20]. Recently, the apo e4 allele was also associated with Alzheimer’s disease [21]. The role of variation at the apo e locus in renal disease is however largely unknown.

The apo e allele distribution in the CAPD subjects was very similar to the distribution in the healthy population. The absence of overrepresentation of the apo e4 allele suggest that it was not intrinsically associated with renal disease. However, as the CAPD subjects had high mean total and LDL cholesterol levels, the impact of additional risk factors on serum lipid levels, such as presence of the e4 allele may be particularly pronounced in this population. Indeed, in spite of the heterogeneity of the CAPD patients and the somewhat limited number of subjects in the present study compared to population studies, we could dem-

Table 4. Effect of apo E genotypes on serum lipid levels in CAPD subjects

<table>
<thead>
<tr>
<th>Apo E phenotypes</th>
<th>n</th>
<th>Serum cholesterol (mmol/l)</th>
<th>Serum TG (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>LDL cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 2/2</td>
<td>2</td>
<td>6.90 ± 0.99</td>
<td>2.65 ± 1.34</td>
<td>1.10 ± 0.57</td>
<td>4.61 ± 0.95</td>
</tr>
<tr>
<td>E 3/2</td>
<td>30</td>
<td>6.49 ± 1.50</td>
<td>2.83 ± 2.09</td>
<td>1.24 ± 0.61</td>
<td>3.98 ± 1.16</td>
</tr>
<tr>
<td>E 4/2</td>
<td>3</td>
<td>5.43 ± 2.67</td>
<td>3.13 ± 0.57</td>
<td>1.33 ± 0.67</td>
<td>2.68 ± 1.90</td>
</tr>
<tr>
<td>E 4/3</td>
<td>14</td>
<td>7.36 ± 1.21</td>
<td>2.66 ± 0.83</td>
<td>1.26 ± 0.40</td>
<td>4.91 ± 1.02</td>
</tr>
<tr>
<td>E 4/4</td>
<td>2</td>
<td>6.70 ± 1.98</td>
<td>3.50 ± 1.70</td>
<td>0.95 ± 0.35</td>
<td>4.17 ± 1.57</td>
</tr>
</tbody>
</table>

Serum lipid levels are given as mmol/l and expressed as means ± SD. To convert to mg/dl, multiply by 38.7 for cholesterol and 88.5 for triglycerides.
onstrate a clearly significant increase in the levels of the atherogenic LDL cholesterol fraction among the \( e4 \) carriers. This may be of particular importance in the present CAPD subjects, as the HDL cholesterol levels were lower compared to normals, thus implying an increased susceptibility to development of atherosclerosis. Under these conditions, with an unfavourable ratio between LDL- and HDL-cholesterol levels, the additional increase in LDL cholesterol levels in the \( e4 \)-carriers compared to apo \( e3 \) homozygotes might be of importance in the development of accelerated atherosclerosis. Although the number of subjects with established cardiovascular disease was relatively small and the results have to be interpreted with caution, it was nevertheless of interest that the apo \( e4 \) allele frequency was even higher in this group. Further studies are, however, needed to verify this finding.

In conclusion, our findings underscore previous results that genetic factors may contribute to conditions compatible with accelerated atherosclerosis among CAPD subjects [31], and demonstrate for the first time an impact of variation at the apo E gene locus in CAPD patients. The results demonstrate further the need for prospective studies in these subjects aimed at elucidating the impact of genetic variation at the apo E locus on development of atherosclerosis. In addition, the results suggest that genotyping of apo E may be of importance for the optimal evaluation and treatment of dyslipidaemias in CAPD subjects.

Acknowledgements. This study was supported by a Baxter Extramural Grant as well as grants from the Trygg-Hansa Foundation, and the Karolinska Institute. The excellent technical assistance of Ms. Eva Rystedt and Ms. Ewa Malmberg is gratefully acknowledged. Dr Berglund is a Florence Irving Associate Professor of Medicine, and an Established Scientist of the American Heart Association, New York City Affiliate.

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Received for publication: 8.7.96
Accepted in revised form: 13.9.96