Apolipoprotein E polymorphism influences lipid phenotypic expression, but not the low density lipoprotein subfraction distribution in familial combined hyperlipidemia

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Received 3 January 1996; revised 19 April 1996; accepted 10 June 1996

Abstract

The impact of apo E polymorphism on interindividual variation in plasma lipid, lipoprotein concentrations, and LDL subfraction profiles was studied in 201 well-defined patients (88 men and 103 women) with familial combined hyperlipidemia (FCH). When corrected for the concomitant influences of age, gender and obesity, the allelic variation in the apo E gene was shown to explain a statistically significant portion of the variability in lipid and (apo)lipoprotein concentrations. Carriers of the apo ε2 allele exhibited a substantially higher plasma triglyceride concentration and a lower low density lipoprotein (LDL) cholesterol level, while subjects with the apo ε4 allele had significant higher total plasma cholesterol and LDL cholesterol levels. In line with this observation, our FCH population was characterized by an over-representation of the apo E4 allele as compared with a Dutch standard population ($\chi^2 = 55.2$, $P < 0.0001$). The contribution of apo E polymorphism to trait variability was different between sexes for plasma triglyceride, VLDL cholesterol, VLDL triglycerides, and high density lipoprotein (HDL) cholesterol levels. Apo E polymorphism had no impact on chemical composition of VLDL; for LDL particles the apo ε2 allele was associated with a lower cholesterol to protein (C/P) ratio, whereas the opposite was true for the apo ε4 allele. Despite the demonstrated impact of apo E polymorphism on plasma lipids and LDL chemical composition, in all phenotypic groups a dense LDL subfraction profile predominated. Thus, apo E polymorphism contributes to the lipid phenotypic expression in FCH, whereas further evidence was obtained that a dense LDL subfraction profile is an integral feature of FCH.

Keywords: Familial combined hyperlipidemia; Apolipoprotein E polymorphism; Phenotypic expression; Genetics

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PII S0021-9150(96)05924-2
1. Introduction

Familial combined hyperlipidemia (FCH) is a common heritable lipid disorder, in which affected individuals show elevations of plasma cholesterol, total triglycerides or both, with differing lipid phenotypes occurring within affected first-degree relatives, together with a high prevalence of premature cardiovascular disease [1-3]. Originally, it was supposed that FCH was caused by the variable expression of a single autosomal dominant gene with primary action on plasma triglyceride levels and secondary on cholesterol levels [1]. Nowadays, FCH is considered to be more heterogeneous, with on the one hand hepatic VLDL overproduction [4,5], possibly amplified by additional defects as insulin resistance, and on the other hand a deficiency in the clearance of circulating triglyceride-rich lipoproteins [6]. To the latter molecular defects in the LpL gene may contribute [7].

Apolipoprotein (apo) E, consisting of 3 common isoforms E2, E3 and E4, is one of the major protein constituents of chylomicrons and VLDL remnants, and is involved in the subsequent catabolism of these particles by allowing specific binding to hepatic receptors. Population studies have shown that the apo E polymorphism could explain a significant part of the inter-individual variability in plasma cholesterol and LDL cholesterol [8-10]. In these studies, the apo ¿2 allele was frequently associated with lower levels of total plasma cholesterol, LDL cholesterol and apo B, whereas for the apo ¿4 allele the opposite was observed, inherent to the different binding activities of apo E isoproteins to the hepatic receptor [11-13]. Similar effects on lipid and lipoprotein concentrations due to apo E polymorphism were documented in a homogeneous sample of patients with familial hypercholesterolemia exhibiting the same LDL receptor mutation [14]. These effects appeared to be dependent of age, gender, and body mass index (BMI), similar to reported epidemiological observations [15,16]. Consequently, the effects of apo E polymorphism, age, gender and BMI may, at least in part, also explain the great interindividual variability in lipid phenotypic expression in FCH, despite the probability of a common defect assumed by its inherited nature.

Therefore, the objective of this study was to estimate the specific impact of apo E polymorphism on plasma lipids, lipoproteins and LDL subfraction profiles in well-defined FCH patients, taking concomitant influences of age, gender and BMI into account.

2. Subjects and methods

2.1. Families

As a part of an ongoing study aiming at the identification of biochemical and genetical aspects underlying FCH, extended FCH kindreds were recruited by family studies of probands attending the out-patient clinic of the Academic Hospital of Nijmegen. These probands repeatedly exhibited a combined hyperlipidemia with both plasma cholesterol and triglyceride concentrations above the 90th percentile for age and gender. Families were only included when they satisfied the following criteria: the presence of a multiple type hyperlipidemia in first-degree relatives with elevated levels of total plasma cholesterol and/or triglycerides using the age- and sex-related 90th percentile upper levels of the prospective cardiovascular Münster (PROCAM) study [17]. Thus, besides a proband presenting a combined hyperlipidemia, the presence of at least one first-degree relative with hypertriglyceridemia or hypercholesterolemia was obligatory. Furthermore, at least one of the first-degree relatives should have cardiovascular disease before the age of 60 years.

Families were excluded when first-degree family members had tendon xanthomata. All individuals were Caucasian with an age of 12 years or older. Information on medical status (i.e., diabetes, renal impairment and liver disease), medication use, alcohol intake, smoking habits, and hormonal status in women was collected by questionnaire. The study protocol was approved by the ethical committee of our Institute. None of the probands was homozygous for the apo ¿2 allele or had hyperlipidemia due to a secondary cause (i.e., diabetes mellitus, hypothyroidism and hepatic or...
renal impairment), which was excluded by standard laboratory tests. Hypolipidemic drugs were discontinued at least 4 weeks prior to drawing of the blood.

2.2. Plasma

Overnight fasted venous blood samples were drawn by vein puncture into K$_3$EDTA containing vacutainer tubes. Non-local participants were visited at their homes, and blood was transported directly to the laboratory. On arrival at the laboratory within 2 h, plasma was isolated for determination of the lipid and lipoprotein levels, and the LDL subfraction profile.

2.3. Plasma lipid and lipoprotein assays and apo E phenotype determination

VLDL was isolated from whole plasma by ultracentrifugation at density 1.006 g/ml for 16 h at 36,000 rpm in a fixed angle rotor (TFT 45.6 rotor, Kontron, Zürich), in a Beckman L7-55 ultracentrifuge (Beckman, Palo Alto, CA). High density lipoprotein (HDL) cholesterol was determined by the polyethylene glycol 6000 method [18]. LDL cholesterol was calculated by subtraction of VLDL cholesterol and HDL cholesterol from total plasma cholesterol. For determination of its chemical composition LDL was isolated by sequential ultracentrifugation of the VLDL infranatant for another 20 h. Total cholesterol and triglyceride concentrations were determined by enzymatic, commercially available reagents (Boehringer-Mannheim, FRG, cat. no. 237574 and Sera Pak, Miles, Italy cat. no. 6639, respectively). To obtain the chemical composition data of VLDL and LDL additional unesterified cholesterol and phospholipids were determined using commercially available reagents (Boehringer-Mannheim, FRG, cat. no. 310328, 691844). The protein content of VLDL and LDL was determined using the Lowry method [19] with chloroform extraction to remove turbidity due to lipids. From these data the mean ratio of cholesterol (unesterified cholesterol plus cholesterol moiety of cholesterol ester [≈ 0.59*weight of cholesterol ester]) to protein was calculated. The chemical composition of each VLDL or LDL particle is expressed in percentage of dry mass of each contributing component (i.e., free cholesterol, cholesterol esters, triglycerides, phospholipids and proteins). Total plasma apo B concentrations were determined by immunonephelometry [20]. To achieve accurate results in relation to the Center for Disease Control Standardisation Program, the obtained values were recalculated on the basis of an exchange of sera with Dr. S Marcovina (Northwest Lipid Research Laboratory, Seattle, WA). Apo E phenotypes were determined after iso-electric focusing of VLDL lipoproteins, as described previously [21]. Results obtained for 534 samples agreed, with two exceptions, well in comparison to apo E genotyping according to Hixson et al. [22] (performed by Dr. M.J.V. Hoffer, MGC-Department of Human Genetics, Leiden University, Leiden).

2.4. LDL subfractionation

LDL subfractions were detected by single spin density gradient ultracentrifugation [23]. After ultracentrifugation up to five LDL subfractions could be distinguished as distinct bands in the middle of the tube concentrated in the following density ranges: LDL1 (1.030–1.033 g/ml), LDL2 (1.033–1.040 g/ml), LDL3 (1.040–1.045 g/ml), LDL4 (1.045–1.049 g/ml), and LDL5 (1.049–1.054 g/ml). The ultracentrifugation tubes, containing the LDL subfractions stained with Coomassie Brilliant Blue R, were placed in a specially designed rack and photographed. Accurate documentation of the different LDL subfraction patterns was obtained by scanning the obtained slides in triplicate on a LKB 2202 ultrascan laser densitometer (Pharmacia, Uppsala, Sweden). The mean peak heights (h1–h5) of the LDL subfractions (LDL1–LDL5) on the three scans were used to calculate the variable $K$ as a continuous variable, that best describes each individual LDL subfraction pattern [24]. The contribution of each LDL subfraction, expressed by its peak height (%h1–%h5) relative to the total LDL subfraction profile, (total LDL [100%] = %h1 + %h2 + %h3 + %h4 + %h5), was calculated. The relative peak heights of LDL3 and the less fre-
sequently occurring LDL4 and/or LDL5 were added to give %h3' = (%h3 + %h4 + %h5), where LDL[100%] = LDL1[%h1] + LDL2[%h2] + LDL3 [%h3']. When a subfraction profile was characterized by a predominance of buoyant LDL particles (h1−h3 > 0), variable K was calculated by K = (%h1−%h3')/(%h2−%h3' + 1). In case of a predominance of heavy, dense LDL subfractions (h1−h3 < 0), variable K was calculated by: K = (%h1−%h3')/(%h2−%h1 + 1). A negative value (−1 < K < 0) reflects a dense LDL subfraction profile and a positive K value (0 ≤ K < 1) a buoyant profile.

2.5. Statistical methods

All statistical analyses were performed for affected FCH relatives as a group and because of different frequency distributions in men and women for plasma lipids, lipoproteins, and concomitant effects (i.e. age and BMI), shown by epidemiological studies [15,25], also an additional analysis was performed for men and women separately. Differences between men and women for the quantitative trait means depicted in Table 1 were evaluated by Student’s t test. Allele frequencies were determined by the gene-counting method under the hypothesis of a Hardy-Weinberg equilibrium. Differences in apo E phenotype frequencies between FCH relatives and either spouses or a representative sample of the Dutch population [8] were evaluated by the χ2 test. Multiple regression analysis was used on the parameters to estimate the possible concomitant effects of age, gender, BMI, smoking habits, and hormonal status in women. Initially, affected FCH subjects were distinguished from unafflicted relatives on the basis of elevated lipid concentrations (total plasma cholesterol ≥ 6.5 mmol/l and/or plasma triglycerides ≥ 2.0 mmol/l). By this classification age, gender and BMI significantly contributed to the variation of a majority of the parameters, whereas smoking habits and hormonal status in women did not. When percentile data (total plasma cholesterol and/or total plasma triglycerides > 90th percentile for age and gender) were used to identify affected relatives, age and BMI did not contribute anymore to the variation in the parameters, since these concomitant effects appeared to be incorporated in the percentile data itself. Therefore, the actually presented analyses were performed with data of relatives classified by using the percentile data. A two-way ANOVA with independent variables ‘apo E phenotype’ and ‘sex’ towards dependent lipid parameters followed by Scheffe’s post-hoc multiple comparison test was performed to determine the effects of apo E polymorphism on plasma lipids, (apo)lipoproteins, the chemical composition of VLDL and LDL and the LDL subfraction profile, for each sex separately. Statistical analyses were performed by the Department of Medical Statistics using procedures available in the Statistical Analysis System software package (SAS Institute, Cary, N.C.). A trait difference with P < 0.05 was considered to be significant.

Table 1
Description of independent and dependent measures in the affected FCH subjects

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 113)</th>
<th>Men (n = 88)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.3 ± 16.8</td>
<td>45.1 ± 15.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 3.9</td>
<td>26.7 ± 3.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Dependent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol a</td>
<td>6.91 ± 1.35</td>
<td>7.12 ± 1.25</td>
<td>N.S.</td>
</tr>
<tr>
<td>Triglycerides a</td>
<td>2.75 ± 1.75</td>
<td>4.03 ± 3.16</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL-cholesterol a</td>
<td>1.19 ± 0.93</td>
<td>1.85 ± 1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-triglycerides a</td>
<td>1.86 ± 1.46</td>
<td>3.04 ± 2.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol a</td>
<td>1.14 ± 0.34</td>
<td>0.90 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol a</td>
<td>4.58 ± 1.26</td>
<td>4.39 ± 1.33</td>
<td>N.S.</td>
</tr>
<tr>
<td>Apolipoprotein in-B (mg/dl)</td>
<td>168.8 ± 35.3</td>
<td>181.1 ± 38.7</td>
<td>0.04</td>
</tr>
<tr>
<td>K value</td>
<td>-0.41 ± 0.27</td>
<td>-0.51 ± 0.24</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are presented as mean ± S.D.; N.S., not significant.

a in mmol/l.
Table 2
Apo E phenotype and allele frequencies in our FCH population (affected and unaffected subjects), compared to two control populations

<table>
<thead>
<tr>
<th>Apo E phenotypes</th>
<th>Standard Dutch control population</th>
<th>All FCH relatives</th>
<th>Affected (n = 201)</th>
<th>Unaffected relatives (n = 357)</th>
<th>Spousesb (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 558)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/2</td>
<td>13 (0.01)</td>
<td>7 (0.01)</td>
<td>0 (0.00)</td>
<td>6 (0.02)</td>
<td>1 (0.01)</td>
</tr>
<tr>
<td>E3/2</td>
<td>261 (0.13)</td>
<td>66 (0.12)</td>
<td>27 (0.13)</td>
<td>39 (0.11)</td>
<td>13 (0.13)</td>
</tr>
<tr>
<td>E3/3</td>
<td>1128 (0.56)</td>
<td>257 (0.46)</td>
<td>75 (0.37)</td>
<td>180 (0.50)</td>
<td>57 (0.56)</td>
</tr>
<tr>
<td>E4/2</td>
<td>45 (0.02)</td>
<td>22 (0.04)</td>
<td>10 (0.05)</td>
<td>13 (0.04)</td>
<td>4 (0.04)</td>
</tr>
<tr>
<td>E4/3</td>
<td>512 (0.25)</td>
<td>165 (0.30)</td>
<td>67 (0.33)</td>
<td>100 (0.28)</td>
<td>24 (0.23)</td>
</tr>
<tr>
<td>E4/4</td>
<td>59 (0.03)</td>
<td>41 (0.07)</td>
<td>22 (0.11)</td>
<td>19 (0.05)</td>
<td>3 (0.03)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>e2</td>
<td>332 (0.08)</td>
<td>102 (0.09)</td>
<td>37 (0.09)</td>
<td>64 (0.09)</td>
<td>19 (0.09)</td>
</tr>
<tr>
<td>e3</td>
<td>3029 (0.75)</td>
<td>745 (0.67)</td>
<td>244 (0.61)</td>
<td>499 (0.70)</td>
<td>109 (0.74)</td>
</tr>
<tr>
<td>e4</td>
<td>675 (0.17)</td>
<td>269 (0.24)</td>
<td>121 (0.30)</td>
<td>151 (0.21)</td>
<td>27 (0.17)</td>
</tr>
</tbody>
</table>

χ² = 35.1, df = 2, P < 0.0001

χ² = 47.1, df = 2, P < 0.0001

χ² = 9.4, df = 2, P = 0.009

N.S.

The apo E phenotype and allele frequencies in 2018 randomly selected 35-year-old Dutch males (8).

bRepresents the apo E phenotype and allele frequencies in the spouses of FCH relatives.

Differences between different population samples were analyzed by χ² analysis; N.S., not significant.

3. Results

3.1. Subjects

In total, 40 multi-generational FCH kindreds including 660 individuals (558 FCH relatives and 102 spouses) were used in this study. Based on percentile data, 201 individuals were found to be affected by exhibiting a total plasma cholesterol and/or a plasma triglyceride concentration above the 90th percentile for age and gender. The anthropometric measurements of the affected subjects, as well as the plasma lipid and lipoprotein levels (88 men and 113 women) are shown in Table 1. Both in men as well as in women mean age and BMI were similar. Men exhibited higher concentrations of plasma triglycerides, VLDL cholesterol, VLDL triglycerides and apo B, and lower concentrations of HDL cholesterol. Total plasma cholesterol and LDL cholesterol concentrations were not different between sexes. Despite lower triglyceride levels in women, both sexes had, on the average, a substantial negative K value, which represents a LDL subfraction profile in which small dense LDL particles predominate. This feature was more pronounced in men.

3.2. Phenotype and allele frequencies

Table 2 depicts the phenotype and allele frequencies in our sample of Dutch FCH patients and unaffected relatives compared to a standard Dutch control population (8). Apo E phenotype and apo e allele frequencies in spouses (n = 102) were similar compared to the Dutch control population, but differed significantly from those in affected and unaffected FCH relatives (Table 2). The apo e2 allele frequency was comparable in both FCH relatives and controls, but the apo e4 allele frequency in FCH relatives, especially in FCH patients, was higher.

3.3. Effects of apo E polymorphism on lipids and (apo)lipoproteins

The impact of apo E polymorphism on trait means in the 88 male and 113 female affected FCH relatives is summarized in Table 3. Reported
3.4. Effects of apo E polymorphism on VLDL and LDL composition

Apo E polymorphism had no detectable effect on VLDL chemical composition (data not shown), except that men exhibited an overall lower protein content, and therefore a higher C/P ratio in all phenotype groups (data not shown), in agreement with the slightly lower K values in the males. On the contrary, apo E polymorphism had...
3.5. Effects of apo E polymorphism on LDL subfraction distribution

The distribution of LDL subfractions in a LDL subfraction profile were both described by the amount of cholesterol measured in each separated LDL subfraction (Fig. 1), and by the continuous variable $K$, taking the relative contribution of each LDL subfraction into account (Table 5). Data of 12 affected subjects were missing due to technical errors or lack of sufficient amount of plasma. As a consequence of the effect of apo E polymorphism on total plasma LDL cholesterol, highest individual LDL subfraction cholesterol concentrations were observed in patients with the apo E4/4 phenotype and lowest with apo E3/2 phenotype (Fig. 1). However, no effect of apo E polymorphism on the distribution of LDL subfractions pattern was observed, despite the established qualitative effect on lipid and apolipoprotein concentrations. Affected FCH relatives exhibited a predominance of heavy LDL particles as described by negative $K$ values in all apo E phenotype groups (Table 5). Men showed more dense LDL subfraction profiles (i.e. more negative $K$ values) than women. Additionally,
Fig. 1. Cholesterol content of 5 isolated LDL subfractions contributing to the total LDL subfraction profile according to the apolipoprotein E phenotypes of 189 affected FCH patients. The black and the hatched bars represent men and women, respectively. Mean sex-difference if all apo E phenotypes are considered together for LDL1 $P = 0.006$, for LDL2 $P = 0.016$, for LDL3 $P = 0.026$, for LDL4 n.s., and for LDL5 n.s.

* $P < 0.05$, ** $P < 0.01$ when equivalent LDL subfractions are compared with E4/4, # $P < 0.05$ E3/2 vs. E3/3 or E4/3, all differences are observed both for men and women.

Each apo E phenotype showed a predominance of dense LDL3 particles, as measured by the cholesterol content in the subfractions both for men and women (Fig. 1). In the general picture there was one exception in women with apo E4/4. Only in this group did the distribution of LDL subfractions show a buoyant profile with highest concentration in LDL1 (Fig. 1).

4. Discussion

Although the genetic nature of FCH seems obvious, it remains to be established whether different affected subjects represent the same metabolic defect or whether additional genetic factors (e.g. gender and apo E polymorphism), or concomitant influences of age and BMI could amplify the lipid phenotypic expression of FCH. Population based studies have documented the genetic variance of plasma lipids both in normolipidemic subjects [8–10,12] and in hypercholesterolemic subjects [14], associated with the apo E phenotype. In the present study, we could demonstrate that the apo E polymorphism also contributes to the magnitude of lipid and lipo-
Table 5
Distribution of LDL subfraction profiles, described by a continuous variable $K$ according to apo E phenotypes in 189 affected subjects of a sample of Dutch FCH families

<table>
<thead>
<tr>
<th>Trait</th>
<th>Sex</th>
<th>Apo E phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E3/2</td>
</tr>
<tr>
<td>Number</td>
<td>♀</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>12</td>
</tr>
<tr>
<td>$K$-value</td>
<td>♀</td>
<td>−0.34 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>−0.45 ± 0.07</td>
</tr>
</tbody>
</table>

Values are presented as mean ± S.E.M.; *Differences between 5 phenotypes if both men and women are considered together; b sex difference $P = 0.004$.

protein concentrations among affected subjects of 40 well-defined FCH families.

Like familial dysbetalipoproteinemia (FD) in which other factors next to the apo E2/2 phenotype contribute to the expression of the hyperlipidemia [26], a similar coincidence of factors may appear in FCH. Clearly, the presumed overproduction of VLDL particles as a primary cause of FCH, would be exaggerated by the differential effect of the apo $\varepsilon 2$ allele on VLDL remnants and of the apo $\varepsilon 4$ allele on LDL cholesterol. The effect of the apo $\varepsilon 2$ allele was confirmed by the observed accumulation of beta-VLDL in FCH subjects with extensive premature atherosclerosis related to a heterozygote apo E3/2 pattern [27], which was independent of the LDL receptor status as reported by others [28]. In our FCH sample, indeed, higher VLDL cholesterol and triglycerides concentrations were observed in subjects with apo E3/2. The observed elevated LDL levels in subjects with the possession of an apo E4 allele hypothetically results from the more efficient uptake of chylomicrons and VLDL remnants by the liver increasing intracellular cholesterol, thereby reducing LDL receptor activity and thus elevating plasma LDL cholesterol levels [11].

When apo E alleles effects are primarily related to FCH, the allele frequency would deviate from normal. In a comparative study between hypercholesterolemic patients and non-obese patients with combined hyperlipidemia (serum cholesterol $\geq 7.5$ mmol/l and serum triglyceride $\geq 2.0$ mmol/l) the apo $\varepsilon 2$ allele, together with smoking habits and fasting insulin concentrations explained 30% of the hypertriglyceridemia and the low HDL-cholesterol levels [29]. Besides specific apo $\varepsilon 2$ related differences on plasma lipids, also the frequency of the apo $\varepsilon 2$ allele was higher in the combined hyperlipidemic group, which confirms that the apo $\varepsilon 2$ allele has a plasma triglyceride raising effect in primary hypercholesterolemic patients. We observed, however, a similar allele frequency of apo $\varepsilon 2$, but a significant increase in the presence of apo $\varepsilon 4$ in our sample of FCH patient as compared with a representative Dutch control population [8] and the spouses in this study. The apo $\varepsilon 2$ frequency similar to the representation in the control population is in agreement with the report of Lussier-Cacan et al. confirming the presence of a normal apo E2 frequency in FCH [30]. Consequently, this may imply that apo E2 isoform has only secondary modulating effects on the expression of FCH. The over-representation of apo E4 in our sample corresponds with results in strictly unrelated patients with primary combined hyperlipidemia [31], and also with the frequency found in patients with coronary heart disease [32,33]. Moreover, the normal apo E allele distribution in the spouses of our FCH families suggests that the over-representation of apo $\varepsilon 4$ is not simply explained by a high apo $\varepsilon 4$ allele frequency in spouses. Therefore, Houlston et al. suggested that the possession of the apo E4 allele is one of the predisposing genetic factors to the development of the combined hyperlipidemic phenotype of FCH [31].
Although affected subjects with hypertriglyceridemia and the apo E3/2 phenotype may exhibit a relative increase of triglyceride in VLDL particles, on average, VLDL particles were not affected in their composition by qualitative differences in the apo E iso protein content. Furthermore, no increase of cholesterol content as seen in so called VLDL remnants could be observed with the apo E 3/2 phenotype. This apparent homogeneity of VLDL particles in FCH comparable with VLDL of normolipidemic subjects has been reported several times [4,34,35]. Our results affirm that the produced VLDL particles maintain their normal composition irrespective of the apo E iso protein component. So, the accumulating effects of apo E2 on VLDL particles may be subordinate to the overproduction. Furthermore, diminished catalytic LpL activity in FCH [6] may prevent increased VLDL remnant formation. The effects of apo E phenotypes on LDL particle composition reflect the effects as observed on plasma lipids. These compositional changes are potentially associated with alterations in LDL heterogeneity [36]. A high cholesterol ester content and high C/P ratio is usually associated with large buoyant LDL particles, whereas a high triglyceride content and low C/P ratio coincides with small dense LDL particles [36,37]. Only in women with apo E4, exhibiting elevated LDL cholesterol and low plasma triglyceride concentrations the buoyant LDL1 and LDL2 subfractions predominated. Surprisingly, no significant differences in the overall dense LDL subfraction profiles between the various apo E phenotype groups were observed. Thus, the affected FCH subjects in this sample could be distinguished by the presence of a small dense LDL subfraction profile, as reported previously [38–41], despite the documented variation in plasma lipids. We considered several explanations for this apparent contradiction: (i) the compositional differences in LDL due to apo E polymorphism may be too small to cause identifiable changes in LDL subfraction profiles; (ii) it has to be noted that all LDL C/P ratios were below 1.45, which is usually associated with dense particles [36]; and (iii) pharmacological studies in FCH patients showed the persistence of small dense LDL particles in FCH patients despite substantial reduction of plasma triglyceride concentrations by gemfibrozil administration [39,40]. This is in line with the identification of a major gene effect on the small dense LDL subfraction distribution in these FCH families [41].

In conclusion, in the genetically and metabolically heterogeneous lipid disorder FCH we demonstrated that the apo E polymorphism by itself contributes significantly to the lipid and lipoprotein concentrations of affected subjects, independent of its allele frequency. It is likely that in FCH populations the apo e4 allele is slightly over-represented. However, apart from this, apo E polymorphism itself has no distinct effect on the distribution of LDL subfractions. This finding provides further evidence that a LDL subfraction profile consisting of small dense LDL particles is a characteristic feature in FCH patients, irrespective of apo E related impact on plasma lipid and lipoprotein concentrations.

Acknowledgements

The authors thank Mrs D. Kampschreur, L. van Mourik, and M. Demacker for their help in collecting the blood samples of participating family members, Mrs A. Hijmans for the excellent performance of the isoelectric focusing of apo proteins, Dr. J.J.P. Kastelein (University Hospital of Amsterdam) for the permission to study 4 out-clinic FCH patients and their relatives, and Dr. R. de Graaf (Department of Medical Statistics of the University of Nijmegen) for performing the statistical analyses. This work was supported by research grant no 92.056 of the Dutch Heart Foundation.

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