Frequency of ApoB and ApoE Gene Mutations as Causes of Hypobetalipoproteinemia in the Framingham Offspring Population

Francine K. Welty, Carlos Lahoz, Katherine L. Tucker, Jose M. Ordovas, Peter W.F. Wilson, Ernst J. Schaefer

Abstract—Hypobetalipoproteinemia (HBLP) is characterized by plasma concentrations of apolipoprotein B (apoB) and low density lipoprotein cholesterol (LDL-C) below the fifth percentile. Some forms of HBLP have been shown to be due to truncated forms of apoB-100. A total of 3873 subjects participating in the Framingham Offspring Study had LDL-C levels measured every 4 to 5 years throughout a 25-year period. Seventy-five subjects were identified with persistent HBLP, defined as an LDL-C ≤ 70 mg/dL on at least 2 observations, for a prevalence of 1.9% in this population. Compared with subjects with LDL-C ≥ 70 mg/dL, subjects with HBLP had significantly lower mean levels of total cholesterol, LDL-C, triglyceride, and apoB; higher levels of high density lipoprotein cholesterol; and a higher prevalence of the E2/E3 genotype: 38.7% versus 10.9% (P < 0.001). Men with HBLP had a larger mean LDL particle size than did men with an LDL-C ≥ 70 mg/dL. One individual had a truncated apoB as a cause of HBLP, for a prevalence of 0.03%. Medical causes of HBLP included 2 cases of Crohn’s disease, 1 of hemochromatosis, and 1 of hepatitis. Three subjects with HBLP developed coronary heart disease, for an incidence of 4% compared with 5% in those with an LDL-C ≥ 70 mg/dL (P = NS). The incidence of cancer was 8% in those with HBLP compared with 4% in those with an LDL-C ≥ 70 mg/dL (P = 0.21). In conclusion, a truncated apoB was a rare cause of HBLP, whereas the E2/E3 genotype was a much more common cause. A large prospective study is needed to evaluate the incidence of cancer and atherosclerosis in subjects with HBLP. (Arterioscler Thromb Vasc Biol. 1998;18:1745-1751.)

Key Words: hypobetalipoproteinemia ■ apolipoproteins ■ coronary heart disease ■ LDL cholesterol ■ cancer

ApoB plays a central role in lipoprotein metabolism and exists in 2 isoforms in plasma, apoB-100 and apoB-48.1 ApoB-100 is synthesized by the liver and is secreted in the form of VLDL, which is metabolized in plasma to form LDL. ApoB-100 contains the LDL receptor–binding domain; therefore, VLDL remnants (IDL) and LDL are removed from the circulation by binding to hepatic LDL receptors.1 High levels of apoB and LDL cholesterol (LDL-C) have been found to be predictive of coronary artery disease (CHD).2

ApoE is a major protein component of VLDL and functions as a ligand in the receptor-mediated clearance of chylomicron and VLDL remnants in the liver.3 Three commonly occurring apoE isoforms have been identified: apoE2, apoE3, and apoE4. ApoE3 is the most common isoform. ApoE4 differs from apoE3 by a cysteine-to-arginine amino acid substitution at residue 112; apoE2 differs from apoE3 by an arginine-to-cysteine substitution at amino acid residue 158.4 ApoE isoforms have been found to influence cholesterol variability in the general population.4,5

Hypobetalipoproteinemia (HBLP) is characterized by plasma concentrations of apoB and LDL-C that are lower than the fifth percentile for age and sex.6 Some forms of HBLP have been shown to be due to mutations in the apoB gene, causing truncated forms of apoB-100.6 The low levels of apoB-containing lipoproteins and LDL-C would be predicted to protect subjects with HBLP from coronary artery disease; however, no study has systematically evaluated kindreds with HBLP for atherosclerosis. The prevalence of HBLP and its causes have not been evaluated in a longitudinal, population-based study. The consequences of a naturally occurring very low LDL-C present throughout an entire lifespan are not known. Meta-analyses of the cholesterol-lowering studies have shown higher mortality rates from accidental deaths, cancer, and noncardiovascular causes in subjects with total cholesterol levels (TC) < 160 mg/dL.2–10 However, these studies are limited in their design, and the low cholesterol may be secondary to undetected cancer, gastrointestinal disease, liver disease, weight loss, and alcoholism.

In this study, 75 individuals of 3873 Framingham Offspring were identified with persistent HBLP over a period of
25 years. Sixty-five of these subjects were evaluated for truncated forms of apoB, apoE genotypes, and other medical causes for low levels of cholesterol. The prevalence of CHD and cancer was also noted.

Methods

Study Population

The original Framingham cohort was enrolled in 1948; they had TC levels measured every 2 years and LDL-C measured in cycles 10 to 12. In 1972, the offspring of the original cohort of the Framingham Heart Study were enrolled and have been followed up with regular clinic exams and coronary disease surveillance at regular (typically every 4 to 5 years) intervals for each of 5 cycles. Those subjects with an LDL-C <70 mg/dL at 2 or more of the 5 exams and who were not receiving lipid-lowering medications were considered to have persistent HBLP. Subjects were also included if LDL-C was between 70 and 90 mg/dL at only 1 examination and <70 mg/dL at all other exams tested. The LDL-C was used to define the phenotype, since LDL-C was measured at all 5 cycles and apoB at only cycle 3 (1983 to 1987). An LDL-C of 70 mg/dL was chosen because it is lower than the fifth percentile for all ages of both sexes. In addition, 92% of 134 subjects with truncated apoBs had an LDL-C <70 mg/dL [Reference 6 and F.K.W. et al, unpublished data, 1998].

Individuals who regularly smoked at least 1 cigarette per day during the year before each cycle examination were classified as current smokers. Body mass index (BMI) was computed by dividing weight (kilograms) by the square of the height (meters). Diabetes mellitus was considered present if fasting blood glucose exceeded 140 mg/dL or if insulin or oral hypoglycemic agents were used. Hypertension was defined as a blood pressure level >140/90 mm Hg or if a subject used antihypertensive agents.

CHD was determined by a panel of physician-investigators using heart study examination data and hospital records. The clinical categories for CHD included myocardial infarction, angina pectoris, coronary insufficiency, and coronary death. A review panel of neurologists determined the presence of definite cerebrovascular disease, including either stroke or transient ischemic attacks. Cancer was determined to be present by obtaining clinical pathology reports; only verified cancer records were used.

Diet intake was estimated with the Willett 126 semiquantitative food-frequency questionnaire among members of the Offspring Study Cohort during 1991 to 1994, the fifth cycle of data collection. The food-frequency questionnaire is designed to obtain the individual’s usual intake over the past year by asking about the frequency of consumption of a list of major food items and food groups. The Willett questionnaire has been validated for intake of several nutrients by comparison with multiple diet records among women17 and men18 and against blood nutrient levels.19

Lipid Analysis

Fasting lipoprotein profiles were measured as described previously.20,21 The level of LDL-C was calculated with the Friedewald equation22 in all cases when triglyceride levels were <400 mg/dL. In those subjects with triglyceride levels >400 mg/dL, analytical ultracentrifugation was used to calculate the LDL-C level. All LDL-C levels were confirmed by analytical ultracentrifugation for the offspring at cycle 3. The laboratory participates in the Centers for Disease Control and Prevention (Atlanta, Ga) lipid standardization program.

Apolipoprotein, Lipoprotein(a) [Lp(a)], and LDL Particle Size Measurements

Lp(a) was determined by using a commercially available ELISA and a monoclonal antibody against apo(a) that does not cross-react against plasminogen and a second polyclonal antibody directed against the apo(a) portion of Lp(a) (Terumo Medical Corp).23 The assay was standardized with respect to the mass of the Lp(a) particle and expressed in milligrams of total mass of Lp(a). Coefficients of variance were 2.5% and 3.4% for intrarun and interrun variability, respectively.21 ApoB was assayed in plasma and lipoprotein fractions with a noncompetitive ELISA and immunopurified polyclonal antibodies.24 The coefficient of variation for the apoB assay was <5% within runs and <10% between runs.24 ApoA-I was assayed by a noncompetitive ELISA.25

LDL particle size was determined as previously described.26 In brief, plasma was subjected to 2% to 16% nondenaturing gradient gel electrophoresis and stained with Sudan black. Seven LDL subgroups have previously been identified on nondenaturing gradient gel electrophoresis and have been shown to be correlated with specific LDL subclass density ranges.26–28 Based on migration distance and volume of protein in each band determined by laser scanning densitometry, an LDL score was calculated as a measure of LDL particle size. A smaller score denotes a larger LDL particle size.29 LDL particles of 1, 2, and 3 comprise large LDL and LDL 4 through 7 comprise small (diameter <255 Å), dense (d >1.038 g/mL).26

Isolation and Characterization of Lipoproteins for Truncated ApoB

Sixty-five subjects with LDL-C <70 mg/dL over the 25-year period returned for phlebotomy between 1992 and 1995. The following proteolytic inhibitors were added to plasma before sequential ultracentrifugation: 0.1% prococol (Sigma), 0.1 mmol/L NaN3, and aprotinin (Trasylol FBA; 2000 kallikrein units per 10 mL of plasma). The VLDL (d <1.006 g/mL), IDL (d =1.006 to 1.019 g/mL), and LDL (d =1.019 to 1.063 g/mL) fractions were isolated from fresh plasma by sequential ultracentrifugation.26 The apoB content of each lipoprotein fraction was visually assessed by 4% to 12% gradient SDS gel electrophoresis on gels stained with 0.1% Coomassie Brilliant Blue R-250 and silver stain as previously described.30,31

ApoE Genotyping

ApoE genotypes were determined by polymerase chain reaction amplification as previously described.32

Statistical Analysis

Each subject with HBLP was matched to at least 2 subjects with LDL-C levels ≥70 mg/dL by age (within 1 year), sex, and BMI. Characteristics were compared by using the paired t test and conditional logistic regression for continuous and discrete variables, respectively. The distribution of apoE genotypes in those with HBLP was compared with those with LDL-C ≥70 mg/dL by Pearson’s χ2 test. In the comparison of male subjects with LDL-C ≥70 mg/dL with female subjects with LDL-C ≥70 mg/dL and male HBLP subjects with female HBLP subjects, multivariate linear regression was performed to adjust for age and BMI. All data are presented as mean±SD. Two-tailed P values <0.05 were considered statistically significant.

Results

Prevalence of HBLP

Seventy-five of 3873 individuals (2555 kindreds) had persistently low LDL-C, for a prevalence of 1.9% for persistent HBLP. Of the 75 subjects, 5 had died by the end of cycle 3, 1 had Crohn’s disease and refused further evaluation, and 3 lived out of state and 1 out of the country and were not available for blood draw. After exclusion of these 10 subjects, 65 subjects were available for evaluation for truncated apoBs and apoE genotype.

Clinical Characteristics, Lipoproteins, and Apolipoproteins

Compared with those with LDL-C ≥70 mg/dL, the subjects with HBLP were significantly younger (mean±SD
TABLE 1. Characteristics of Subjects With HBLP Matched to Subjects With an LDL-C ≥70 mg/dL

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBLP (n=20)</td>
<td>LDL-C &gt;70 mg/dL</td>
<td>LDL C ≥70 mg/dL</td>
<td>P for Male HBLP and LDL ≥70 mg/dL</td>
<td>HBLP (n=49)</td>
<td>LDL-C &gt;70 mg/dL</td>
<td>LDL C ≥70 mg/dL</td>
<td>P for Female HBLP and LDL ≥70 mg/dL</td>
</tr>
<tr>
<td>Age, y</td>
<td>41.9±10.9</td>
<td>41.0±9.3</td>
<td>0.54</td>
<td>41.8±7.4</td>
<td>42.6±7.6</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6±5.1</td>
<td>26.1±4.0</td>
<td>0.66</td>
<td>23.2±3.9</td>
<td>23.1±3.8</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>128±20</td>
<td>202±39</td>
<td>&lt;0.0001</td>
<td>141±20</td>
<td>198±38</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>63±30</td>
<td>145±119</td>
<td>&lt;0.0001</td>
<td>59±30</td>
<td>83±41</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C, mg/dL</td>
<td>13±6</td>
<td>27±22</td>
<td>0.05</td>
<td>12±6</td>
<td>20±12</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>63±17</td>
<td>129±31†</td>
<td>&lt;0.0001</td>
<td>65±22</td>
<td>122±32</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>48±15‡</td>
<td>43±12</td>
<td>0.11</td>
<td>62±15</td>
<td>58±14</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA-1, mg/dL</td>
<td>127±48</td>
<td>134±35</td>
<td>0.66</td>
<td>160±36</td>
<td>153±31</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>54±18</td>
<td>91±21</td>
<td>&lt;0.0001</td>
<td>56±24</td>
<td>75±26</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>63±5.5</td>
<td>11.7±12.3</td>
<td>0.39</td>
<td>12.6±14.8</td>
<td>12.7±14.9</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL particle size, score</td>
<td>2.41±1.04</td>
<td>3.38±1.34</td>
<td>&lt;0.0001</td>
<td>2.25±0.85</td>
<td>2.27±0.85</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.0001 for male subjects with LDL-C ≥70 mg/dL compared with female subjects with LDL-C ≥70 mg/dL.
†P=0.009 for male subjects with LDL-C ≥70 mg/dL compared with female subjects with LDL-C ≥70 mg/dL.
‡P=0.01 for male HBLP compared with female HBLP.
§P=0.02 for male HBLP compared with female HBLP.

ApoE genotypes were available for 62 of the 65 subjects with HBLP and were compared with apoE genotypes in subjects with LDL-C ≥70 mg/dL matched to the HBLP subjects by age, sex, and BMI. Subjects with HBLP were more likely to have at least 1 E2 allele (43.5%) compared with subjects with an LDL-C ≥70 mg/dL (14.5%, P<0.001). As shown in Table 2, the distribution of apoE genotypes was significantly different in subjects with HBLP compared with subjects with an LDL-C ≥70 mg/dL (P<0.001). The subjects with HBLP had a higher prevalence of the apoE2/E3 genotype, 38.7%, compared with 10.9% in subjects with an LDL-C ≥70 mg/dL (P<0.001) and a trend toward a lower prevalence of the apoE3/E4 genotype, 8.1% versus 16.7% (P=0.11).

LDL Particle Size
Eight-seven percent of subjects with HBLP had a particle size of 1, 2, or 3, whereas only 54.4% of subjects with LDL-C ≥70 had a particle size of 1, 2, or 3. In the subjects with LDL-C ≥70 mg/dL, men had significantly smaller LDL particle sizes than did women (P<0.0001, Table 1). In contrast, there were no sex differences in LDL particle sizes in the subjects with HBLP (Table 1). Thus, men with HBLP had significantly larger LDL particle sizes than did men with LDL-C levels ≥70 mg/dL (48±15 versus 43±12 mg/dL, respectively, P=0.11).

Causes of HBLP
One subject had a truncated apoB, an apoB-55. Therefore, the frequency of a detectable truncated apoB as a cause of HBLP was 1.5%, and the frequency of a truncated apoB in this population was 0.03%. The mutation in the apoB gene causing apoB-55 has been described.38

ApoE genotypes were available for 62 of the 65 subjects with HBLP and were compared with apoE genotypes in subjects with LDL-C ≥70 mg/dL matched to the HBLP subjects by age, sex, and BMI. Subjects with HBLP were more likely to have at least 1 E2 allele (43.5%) compared with subjects with an LDL-C ≥70 mg/dL (14.5%, P<0.001). As shown in Table 2, the distribution of apoE genotypes was significantly different in subjects with HBLP compared with subjects with an LDL-C ≥70 mg/dL (P<0.001). The subjects with HBLP had a higher prevalence of the apoE2/E3 genotype, 38.7%, compared with 10.9% in subjects with an LDL-C ≥70 mg/dL (P<0.001) and a trend toward a lower prevalence of the apoE3/E4 genotype, 8.1% versus 16.7% (P=0.11).

LDL Particle Size
Eight-seven percent of subjects with HBLP had a particle size of 1, 2, or 3, whereas only 54.4% of subjects with LDL-C ≥70 had a particle size of 1, 2, or 3. In the subjects with LDL-C ≥70 mg/dL, men had significantly smaller LDL particle sizes than did women (P<0.0001, Table 1). In contrast, there were no sex differences in LDL particle sizes in the subjects with HBLP (Table 1). Thus, men with HBLP had significantly larger LDL particle sizes than did men with LDL-C levels ≥70 mg/dL (48±15 versus 43±12 mg/dL, respectively, P=0.11).

Causes of HBLP
One subject had a truncated apoB, an apoB-55. Therefore, the frequency of a detectable truncated apoB as a cause of HBLP was 1.5%, and the frequency of a truncated apoB in this population was 0.03%. The mutation in the apoB gene causing apoB-55 has been described.38

ApoE genotypes were available for 62 of the 65 subjects with HBLP and were compared with apoE genotypes in subjects with LDL-C ≥70 mg/dL matched to the HBLP subjects by age, sex, and BMI. Subjects with HBLP were more likely to have at least 1 E2 allele (43.5%) compared with subjects with an LDL-C ≥70 mg/dL (14.5%, P<0.001). As shown in Table 2, the distribution of apoE genotypes was significantly different in subjects with HBLP compared with subjects with an LDL-C ≥70 mg/dL (P<0.001). The subjects with HBLP had a higher prevalence of the apoE2/E3 genotype, 38.7%, compared with 10.9% in subjects with an LDL-C ≥70 mg/dL (P<0.001) and a trend toward a lower prevalence of the apoE3/E4 genotype, 8.1% versus 16.7% (P=0.11).
Compared with those with an LDL-C ≥70 mg/dL, subjects with HBLP had no significant differences in dietary intake of saturated fat (22±11 versus 24±11 g, respectively, \( P = 0.09 \)) and cholesterol (227±112 versus 245±110 mg, respectively, \( P = 0.19 \)). Therefore, diet is an unlikely cause for the HBLP.

Medical causes of HBLP were investigated. Two subjects have Crohn’s disease, and 1 has hemochromatosis. As mentioned previously, 5 subjects have died: 1 from hepatitis, 1 from a myocardial infarction, and 3 from cancer. One subject with leiomyosarcoma died 7 years after the first LDL-C measurement of 78 mg/dL in cycle 1; the second LDL-C was 27 mg/dL. The second subject had colon cancer metastatic to the liver and died 10 years after the first LDL-C of 64 mg/dL; the second LDL-C was 54 mg/dL. The third subject had laryngeal cancer, Laennec’s cirrhosis, and CHD. He died from the laryngeal cancer 11 years after the first LDL-C measurement of 79 mg/dL; the second LDL-C was 30 mg/dL and the third, 26 mg/dL. A decreasing LDL-C was present in all 3 of these patients.

Prevalence of CHD
Two of the 5 deceased subjects had CHD. One of these 2 died from an anterior myocardial infarction at the age of 48. The second also had cancer and is described above. One living subject has documented coronary artery disease. The lipid levels and levels of Lp(a) and homocysteine for these 3 individuals are shown in Table 3. The parents of the individual with the truncated apoB-55 were both members of the original cohort. Analysis of frozen plasma from the mother revealed that she did not carry the mutation. The finding of very low cholesterol levels over a period of 16 years (range, 110 to 143 mg/dL) in the father strongly suggested that he carried the mutation. An autopsy performed at his death in 1965 revealed that he had severe, diffuse atherosclerosis of the coronary arteries, aorta, and iliac arteries. His only atherosclerotic risk factor was borderline diastolic hypertension, with diastolic readings of 88 to 96 mm Hg over a 16-year period. He did not smoke, have diabetes mellitus, or have a family history of premature atherosclerotic heart disease.

Prevalence of Cancer and Overall Mortality
As noted above, of the 5 subjects who have died, 3 died from cancer. Of the remaining living subjects with HBLP, 3 have cancer. Therefore, of the original 75 subjects with HBLP, 6 have developed cancer, for an incidence of 8% over a 25-year period. The cancer cases were diagnosed and the deaths occurred after the first 5 years of the study. The cancer incidence in those with an LDL-C ≥70 mg/dL is 4% and not significantly different from that of the HBLP group (\( P = 0.21 \)).

Discussion
The current study, which shows that persistent HBLP occurs at a frequency of 1.9%, or ≈1/50, is the first longitudinal

---

### Table 2. Distribution of ApoE Genotypes in Subjects With HBLP Compared With Subjects With an LDL-C ≥70 mg/dL Matched by Age, Sex, and BMI

<table>
<thead>
<tr>
<th>ApoE Genotype</th>
<th>HBLP</th>
<th>LDL-C ≥70 mg/dL</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E2</td>
<td>2</td>
<td>3.2</td>
<td>1</td>
</tr>
<tr>
<td>E2/E3</td>
<td>24</td>
<td>38.7</td>
<td>15</td>
</tr>
<tr>
<td>E3/E3</td>
<td>28</td>
<td>45.2</td>
<td>92</td>
</tr>
<tr>
<td>E3/E4</td>
<td>5</td>
<td>8.1</td>
<td>23</td>
</tr>
<tr>
<td>E2/E4</td>
<td>1</td>
<td>1.6</td>
<td>4</td>
</tr>
<tr>
<td>E4/E4</td>
<td>2</td>
<td>3.2</td>
<td>3</td>
</tr>
</tbody>
</table>

---

### Table 3. Levels of Lipids, Lp(a), and Homocysteine in HBLP Subjects With CHD

<table>
<thead>
<tr>
<th>Subject 1 (male)</th>
<th>TC</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>Triglyceride</th>
<th>Lp(a)</th>
<th>Homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>127</td>
<td>32</td>
<td>79</td>
<td>79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle 2*</td>
<td>93</td>
<td>40</td>
<td>30</td>
<td>112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle 3</td>
<td>71</td>
<td>27</td>
<td>26</td>
<td>89</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Mean for all men</td>
<td>219</td>
<td>44</td>
<td>142</td>
<td>126</td>
<td>15.7</td>
<td></td>
</tr>
</tbody>
</table>

% denotes percentile; CI, confidence interval. All lipid and lipoprotein values are in mg/dL except for homocysteine, which is in nmol/L.

*Diagnosed with cancer.
†Heavy smoker (3 packs per day).
study to evaluate the frequency of persistent HBLP in a population over 25 years. One other population-based study was cross sectional. In a Danish study of 10,440 newborn infants, 266 had VLDL-LDL concentrations lower than the 2.5 percentile. Analysis of the families of 176 infants revealed that the frequency of 3-generation mendelian inheritance of HBLP was 0.09%.

During random screening of 125 families, Laskarzewski et al identified 1 kindred with HBLP (prevalence of 0.8%). Glueck et al identified 13 hypocholesterolemic kindreds in a population sample of <6000 kindreds (prevalence of 0.2%). Cottrill et al found 3 kindreds with HBLP among 1200 subjects (prevalence of 0.25%). These data suggest that familial HBLP might occur at a frequency of 1/100 to 1/500, which is less frequent than observed in our study. In contrast to the current study, these studies performed a single TC measurement and were unable to determine whether the HBLP resulted from an isolated cause or was persistent.

HBLP has been shown to be due to mutations in the apoB gene causing truncated forms of apoB-100. In the current population-based study, the frequency of a truncated apoB was 1.5% among the subjects with HBLP and 0.03% in the total Framingham Offspring population. Mutations in the 5’ portion of the apoB gene (shorter than 30% of the length of apoB-100) may not yield a truncated protein in the plasma; therefore, our data may underestimate the frequency of a truncated apoB as a cause of HBLP. However, under the assumption that mutations are distributed randomly throughout the apoB-100 gene, we would predict that at most, only 1 additional truncated apoB in the first third of the gene may exist in the Framingham population.

Two other studies have estimated the frequency of apoB gene mutations causing truncated apoBs in selected groups of people. In the first, among SDS polyacrylamide gel screening of 75 healthy adults of the Kaiser Foundation Health Plan with plasma TC levels <120 mg/dL, 1 kindred was identified with a truncated apoB, for a prevalence of 1.3%. This study screened only healthy, selected adults and therefore differed from ours, which screened an entire population. In a second study, screening for apoB truncations was performed in 525 subjects with TC levels lower than the 10th percentile by using immunoblots of plasma. Four truncated apoBs were identified, for a prevalence of 0.76%. This study was also not population based.

The results of our study suggest that the E2/E3 genotype is 3 times as prevalent and the E3/E4 genotype is 50% less prevalent among subjects with HBLP compared with subjects with higher LDL-C levels. Persons with a single apoE2 allele are thought to have TC levels that are 10 mg/dL lower and persons with a single apoE4 allele are thought to have TC levels 10 mg/dL higher than persons with other apoE allele combinations. Postulated mechanisms for this observation include more efficient absorption of cholesterol with an apoE4 isofrom than with an apoE2 or apoE3 isofrom and an increased rate of clearance of remnant lipoproteins with apoE4; this increased clearance may lead to decreased hepatic LDL receptor activity and elevated plasma LDL-C levels. ApoE2 is associated with decreased clearance of remnant particles; this decreased clearance may lead to increased hepatic LDL receptor activity and decreased plasma LDL-C levels.

Of the subjects with HBLP in the current study, 2 have Crohn’s disease, 1 has hemochromatosis, and 1 died from hepatitis. In contrast, there were no cases of liver or gastrointestinal disease in the healthy members of the Kaiser study; however, the Kaiser study selected healthy subjects and was not population based.

Diet is not a factor in the etiology of the low cholesterol in the current study, since the hypocholesterolemic subjects had similar caloric, fat, and cholesterol intakes compared with the subjects with LDL-C levels ≥70 mg/dL. The causes for the remaining cases of HBLP in the current study are unknown.

The characterization of this group of subjects with persistently low LDL-C levels over a 25-year period has allowed us to study other characteristics of their lipids and disease associations. In the current study, women with LDL-C levels ≥70 mg/dL have significantly lower levels of triglyceride, LDL-C, and VLDL-C and higher levels of HDL-C than do men with an LDL-C ≥70 mg/dL. These observations have been observed previously in the general population. In contrast, the subjects with HBLP have no significant sex differences in lipid levels, with the exception that HDL-C was significantly higher in women than in men. Of note, HDL-C levels were higher in both men and women with HBLP compared with those with LDL-C levels ≥70 mg/dL. Thus, HBLP may confer protection against atherosclerosis not only by lowering LDL-C levels but also by raising HDL-C levels in both sexes. In addition, HBLP may confer additional protection in men by lowering LDL-C and triglyceride to levels comparable to those in women.

Properties of LDL-C that may confer resistance to the development of atherosclerosis include LDL particle size. Small, dense LDL particles have been associated with the presence of myocardial infarction and coronary artery disease. Sex differences have been noted in regard to LDL particle size. In the Framingham Study, LDL size distribution was skewed toward larger LDL particles in women (prevalence of LDL-1, 30% and of LDL-2, 31%), whereas men exhibited a more symmetric distribution (prevalence of LDL-3, 42%). The prevalence of small, dense LDL particles 4 to 7 was 33% in men, 5% in premenopausal women, and 14% in postmenopausal women. McNamara et al suggested that estrogen levels may account for some of the male-female differences observed in LDL particle size. In the current study, men with HBLP had significantly larger LDL particle sizes than did men with LDL-C levels ≥70 mg/dL. In fact, LDL particle size in men with HBLP was similar to that of women. These larger LDL particles in men with HBLP may be less susceptible to oxidation and therefore, may provide protection against the development of CHD. Because HBLP abolishes the sex differences in levels of apoB-containing lipoproteins while increasing levels of HDL-C and LDL particle size in men, HBLP may be especially beneficial for men.
No study has prospectively examined subjects with HBLP for atherosclerosis; however, the low LDL-C levels would be predicted to prevent the development of CHD. A surprising finding in the current study was the occurrence of coronary artery disease in 3 subjects with HBLP and severe coronary, aortic, and iliac atherosclerosis at autopsy in the apoB-55 proband’s father who carried the mutation.\(^ {35}\) Low HDL-C levels were noted in 1 of the 3 subjects and may be responsible for the development of CHD in this subject. At least 4 other subjects with truncated apoBs have documented CHD. The proband of the apoB-40/89 kindred is a compound heterozygote with both mutations.\(^ {32}\) Her mother, who was heterozygous for 1 of the 2 mutations, and her brother, who was either a compound heterozygote with both mutations or heterozygous for 1 of the other mutation, both died from myocardial infarctions in their fifth decades.\(^ {32}\) A woman with an apoB-46 truncation had 30% lesions in her coronary arteries at catheterization.\(^ {35}\) A man with an apoB-31 truncation also has CHD (personal communication, A Goto, 1997). Therefore, subjects with HBLP can develop coronary artery disease.

The incidence of cancer in those with HBLP was twice as high as for those with an LDL-C \(\geq 70\) mg/dL; however, the sample size for the low LDL-C group is small, and this difference is not significantly different. Low cholesterol levels have been associated with increased mortality from hemorrhagic stroke, cancer, other noncardiovascular diseases, injury, and suicide in several observational studies and clinical trials.\(^ {7,16–16}\) A causal relation between low cholesterol levels and mortality has not been demonstrated. Other possible reasons for this association include a fall in cholesterol levels with the onset of disease and the presence of occult diseases in persons with low cholesterol levels on entry to these studies. A decreasing LDL-C was present in all 3 of the cancer deaths in the current study; therefore, a slowly progressive cancer may have been the cause of the low LDL-C in these subjects. Further research is needed to specifically define the association between low cholesterol, cancer, and mortality. Subjects with HBLP due to truncated apoBs have a very low cholesterol for their entire life. A prospective analysis of these kindreds for association with cancer and atherosclerosis would be very informative.

In summary, we have identified a group of subjects with persistent HBLP over a 25-year period and defined the prevalence of HBLP in a population. We have shown that a truncated apoB is a rare cause and that the E2/E3 genotype is a more common cause of the low LDL-C. In addition to low levels of LDL-C, these subjects have other cardioprotective properties of lipids, including larger LDL particle size in men and higher levels of HDL-C. A few of these subjects have coronary artery disease. After exclusion of cancer cases and deaths during the first 5 years, the incidence of cancer is 8%. Continued surveillance of these subjects over their lifetime should provide important information on the association of a naturally occurring low cholesterol with disease associations.

Acknowledgments

Dr Welty is the Irving and Charlotte Rabb Harvard Scholar in Medicine in memory of Dr Grete Bribing and in honor of the 50th anniversary of admission of women to Harvard Medical School. This study was supported by National Heart, Lung, and Blood Institute grant HL02626 (to F.K.W.), National Institutes of Health subcontract No. HV83-03 and contract 53-3K06-5-10 from the US Department of Agriculture Research Service.

References


Frequency of ApoB and ApoE Gene Mutations as Causes of Hypobetalipoproteinemia in the Framingham Offspring Population
Francine K. Welty, Carlos Lahoz, Katherine L. Tucker, Jose M. Ordovas, Peter W. F. Wilson and Ernst J. Schaefer

doi: 10.1161/01.ATV.18.11.1745

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/11/1745

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/