Irvine H. Page Young Investigator Award

Plasma Apolipoprotein A-I Absence Associated with a Marked Reduction of High Density Lipoproteins and Premature Coronary Artery Disease

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A 45-year-old woman with corneal opacification and severe coronary artery disease was noted to have the following plasma lipid levels (mg/dl, ± sd): total cholesterol 111 ± 13, triglyceride 62 ± 6, very low density lipoprotein cholesterol 4 ± 1, low density lipoprotein cholesterol 106 ± 14, and high density lipoprotein (HDL) cholesterol 1 ± 1 (normal, 50 ± 14). Her two offspring and one brother were found to have HDL cholesterol values (mg/dl) of 23, 20, and 20, respectively. The percentage of cholesterol in the esterified form in the patient's plasma was normal at 70%. Lipoprotein electrophoresis showed no alpha lipoprotein band, and no HDL was detectable when plasma was subjected to analytic ultracentrifugation. Only trace amounts of lipids were noted within the HDL density region following preparative ultracentrifugation. Mean plasma apolipoprotein (apo) A-II, apo B, and apo C-II plasma levels were 13.8%, 130.8%, and 26.6% of normal, respectively. The ratio of apo B to cholesterol within LDL was elevated. Apo A-I, the major HDL protein constituent, was immunologically undetectable in this patient's plasma. A decreased HDL cholesterol concentration has been associated with premature coronary artery disease. These data indicate that plasma apo A-I absence results in a striking reduction in HDL, is associated with premature coronary artery disease, and represents a new distinct disease entity.

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High density lipoproteins (HDL) are found in human plasma in the density range 1.063 to 1.21 g/ml, and consist by weight of approximately 50% protein, 25% phospholipid, 20% cholesterol, and 5% triglyceride.1,2 Decreased plasma HDL cholesterol and increased low density lipoprotein (LDL) cholesterol concentrations have been independently associated with premature coronary artery disease (CAD).3-6 Apolipoproteins (apo) A-I and A-II are the major protein constituents of HDL, comprising approximately 90% of total HDL protein mass.7,8 Over 90% of plasma apo A-I and A-II mass is normally present within the HDL density region, with the remainder being found mainly in the 1.21 g/ml infranate, and a small fraction in the 1.063 g/ml supernate.9-11 Other apolipoproteins within HDL include apo C-I, C-II, C-III, E, and D.1,2,7,8,12-15

Apolipoprotein A-I is a single polypeptide of 243 amino acid residues, contains no carbohydrate, and has a molecular weight of approximately 28,000.16-18 Apo A-I has been reported to activate lecithin:cholesterol acyltransferase (LCAT),19 the enzyme that appears to be responsible for cholesterol esterification in plasma.20 The other major protein within HDL, apo A-II, has a molecular weight of approximately 17,000, contains no carbohydrate, and consists of two
identical peptide chains of 77 amino acids attached by a single disulfide bond.\textsuperscript{21} Apo A-II has been reported to activate the enzyme hepatic lipase, which causes hydrolysis of triglyceride and phospholipid.\textsuperscript{23} Both apo A-I and A-II can combine with lecthin to form protein-phospholipid complexes with a hydrated density of HDL.\textsuperscript{23}

Decreased levels of HDL cholesterol are seen in obese individuals,\textsuperscript{24} hypertriglyceridemic subjects,\textsuperscript{25} and patients with LCAT deficiency.\textsuperscript{26,27} Fish eye disease,\textsuperscript{28} apo A-I,\textsuperscript{29,30} and Tangier disease.\textsuperscript{31-34} The most striking HDL deficiency is observed in Tangier homozygotes.\textsuperscript{33} These latter patients may develop enlarged orange tonsils, hepatosplenomegaly, lymphadenopathy, transient mild peripheral neuropathy, corneal infiltration, and somewhat premature CAD.\textsuperscript{31-34} Cholesterol-ester laden reticuloendothelial cells have been noted in the bone marrow, tonsils, skin, rectal mucosa, liver, spleen, lymph nodes, and conjunctiva of these patients.\textsuperscript{35-38} Tangier homozygotes have hypertriglyceridemia, hypocholesterolemia, fasting chylomicronemia, increased VLDL levels, decreased levels of LDL, and a striking deficiency of HDL and its constitutive proteins, apo A-I and A-II.\textsuperscript{31-38} It has been suggested that the lipid deposition in macrophages seen in Tangier homozygotes is due to the uptake of abnormal triglyceride-rich lipoproteins.\textsuperscript{39,40} Human lymph chyomicrons contain significant quantities of apo A-I and A-II, which can serve as precursors for these constituents within plasma HDL.\textsuperscript{42} This conversion process appears to be defective in Tangier homozygotes,\textsuperscript{43} resulting in rapid and altered apo A-I and A-II fractional catabolism, both under steady state baseline conditions,\textsuperscript{41} and following HDL infusion.\textsuperscript{44,45}

In this study the plasma lipid and apolipoprotein content of a patient with premature CAD and a striking deficiency of HDL was investigated. The clinical features and the plasma lipid and apolipoprotein abnormalities of this patient differed significantly from those of patients with homozygous Tangier disease, and appear to represent a heretofore undescribed genetically-determined disease entity.

Case Report

Patient’s History

The patient was a 45-year-old white woman with a 9-month history of increasing exertional substernal chest pain and a 3-year history of corneal opacification. A resting electrocardiogram showed frequent premature ventricular contractions, and T wave inversions in leads II, III, and AVF. A graded exercise test was positive for ischemic changes on electrocardiography. The patient was treated with propranolol 80 mg by mouth four times a day, isosorbide dinitrate 10 mg by mouth four times a day, and sublingual nitroglycerin 1/150 grain as necessary, with some improvement in symptoms.

The patient had no history of hypertension or smoking. She had not had a tonsillectomy. She delivered two normal children by caesarean section, had one miscarriage, underwent a hysterectomy at age 26 years for uterine fibroid tumors, and left breast surgery for fibrocystic disease at age 32 years. Corneal cloudiness had been noted by her family members for the past 3 years. She had a history of emotional liability for which she was treated with diazepam.

The patient’s two children were alive and well at ages 21 and 25 years. Her father died at age 76 years of prostate cancer, and her mother at age 72 years of arteriosclerosis, heart failure, and aortic valve disease. Two of the patient’s siblings died at ages 1 and 5 years of gastroenteritis, one died at age 4 years of polio, and one died as a result of a car accident at age 54 years. Three brothers and one sister were alive and well at ages 52, 57, 60, and 48 years, respectively. One brother, age 56 years, had a long history of hypertension, sustained a myocardial infarction at age 45 years, and underwent an aortic valve replacement and a ventricular arrhythmia resection at age 46 years. One year later he sustained a stroke which resulted in a permanent left hemiparesis. A sister, age 62 years, had a history of angina.

Examination and Course

Physical examination revealed a normally developed white woman whose height was 157 cm, weight 47.2 kg, blood pressure 110/70 mm Hg, pulse 65/min, and respirations 20/min. Examination of the eyes revealed arcus, and corneal opacification visible without slit lamp examination. Her visual acuity was only slightly impaired. Bilateral carotid artery bruits were heard by auscultation. Examination of the oral cavity was normal, except that the patient’s serum vitamin A and E levels as measured by high
pressure liquid chromatography were 54.6 μg/dl (normal, 30–80 μg/dl) and 186 μg/dl (normal, 500–1200 μg/dl), respectively. The prothrombin time was slightly prolonged at 13.6 seconds (control, 11.7 sec).

Coronary artery catheterization revealed a 95% narrowing of the right coronary artery, a 75% narrowing of the circumflex coronary artery, a 50% narrowing of both the left main and left anterior descending coronary arteries, and an 80% narrowing of the first diagonal branch. Normal left ventricular contractility was noted. The patient was referred for coronary artery bypass surgery to another institution, and four saphenous vein grafts were placed. Approximately 12 hours after surgery her cardiac rhythm changed from a normal sinus rhythm to a rapid supraventricular arrhythmia, which caused the patient to become hypotensive. She subsequently had a cardiac arrest and could not be resuscitated. An autopsy confirmed the severe coronary artery disease and corneal opacification. All grafts were patent, no other significant abnormalities were noted. Examination of the liver, spleen, lymph nodes, and tonsils were within normal limits.

**Plasma Lipid and Lipoprotein Analysis**

Blood was collected in 0.1% EDTA after a 12 to 14-hour overnight fast, and plasma was separated by centrifugation. The patient was sampled 6 weeks and 1 week prior to her open heart surgery. Cholesterol and triglyceride concentrations in plasma and lipoprotein fractions were determined in an Autoanalyzer II (Technicon, Tarrytown, New York) by established Lipid Research Clinics methodology. These assays were standardized utilizing standards obtained from the Center for Communicable Disease Control, Atlanta, Georgia. Lipids were also assayed by enzymatic procedures utilizing the Gilford System 3500 Analyzer (Gilford Instruments, Oberlin, Ohio). Total and free cholesterol were quantitated in plasma utilizing the Beckman cholesterol analyzer as previously described. Normal control subjects for standardization of this analyzer were included.

Electron Microscopy of Plasma Lipoproteins

Lipoprotein fractions were dialyzed against 0.1 M ammonium bicarbonate, pH 8.5, 0.01% EDTA, 0.002 M sodium azide solution, and diluted to an appropriate concentration (usually 100 to 250 μg protein/ml). Samples were negatively stained by mixing 10 μl of the diluted lipoprotein fraction with an equal amount of 2% aqueous sodium phosphotungstate, pH 7.4, sprayed with an Effa spray mounter (Ernest F. Fullman Inc., Schenectady, New York) onto a formvar-coated grid, and allowed to air dry. The grids were examined with a Phillips EM300 electron microscope.

Apolipoprotein Analysis

Apo A-I, A-II, and C-II were isolated from human HDL and VLDL as previously described. Apo E was isolated from plasma lipoproteins of density < 1.019 g/ml by heparin affinity chromatography. Apolipoproteins migrated as discrete bands on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) utilizing a modification with 10% acrylamide of the method of Weber and Osborn as previously described. Antiserum against these apolipoproteins as well as LDL (density 1.030 to 1.050 g/ml) were raised in either goats, sheep, or rabbits. Apolipoproteins formed a line of immunoprecipitation only against their own respective antisera when tested with antisera for apo A-I, A-II, B (LDL), C-II, E, and albumin.

Concentrations of apo A-I, A-II, B, and C-II in plasma and lipoprotein fractions were determined by radial immunodiffusion, following delipidation with methanol/diethyl ether (3:1) (except for apo B), and resolubilization in 0.05 M sodium barbital, 9 mM sodium azide, pH 8.3 for immunochromatographic measurement. Apolipoprotein standards were obtained as previously de-
Table 1. Plasma Lipid and Lipoprotein Cholesterol Concentrations (mg/dl)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 1088)</td>
<td>189 ± 40</td>
<td>87 ± 43</td>
<td>16 ± 11</td>
<td>123 ± 35</td>
<td>50 ± 14</td>
</tr>
<tr>
<td>Normal women (aged 45-50 years, n = 53)</td>
<td>225 ± 43</td>
<td>107 ± 49</td>
<td>19 ± 12</td>
<td>144 ± 43</td>
<td>62 ± 13</td>
</tr>
<tr>
<td>Normal controls* (n = 6)</td>
<td>150 ± 6</td>
<td>104 ± 18</td>
<td>16 ± 3</td>
<td>85 ± 7</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>Normal controls† (n = 50)</td>
<td>181 ± 21</td>
<td>79 ± 28</td>
<td>10 ± 5</td>
<td>121 ± 20</td>
<td>50 ± 14</td>
</tr>
<tr>
<td>Patient</td>
<td>111 ± 13</td>
<td>62 ± 6</td>
<td>4 ± 1</td>
<td>106 ± 14</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Patient’s daughter</td>
<td>240 ± 5</td>
<td>124 ± 2</td>
<td>34 ± 2</td>
<td>183 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Patient’s son</td>
<td>189 ± 6</td>
<td>108 ± 3</td>
<td>26 ± 3</td>
<td>143 ± 3</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Patient’s brother</td>
<td>219 ± 3</td>
<td>181 ± 5</td>
<td>28 ± 2</td>
<td>171 ± 4</td>
<td>20 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± sd, triplicate analyses were done on the study subject’s plasma. In the patient’s case, two separate plasma samples were analyzed.

*Normal control subjects for ultracentrifugation studies (see table 2).
†Normal control subjects for apolipoprotein data (see table 3).

Table 2. Lipoprotein Composition (mg/dl)

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Protein</th>
<th>Phospholipid</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>Normal</td>
<td>15.3 ± 1.3</td>
<td>34.8 ± 6.1</td>
<td>11 ± 2</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>5.9 ± 0.4</td>
<td>3.5 ± 1.0</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>IDL</td>
<td>Normal</td>
<td>4.2 ± 0.7</td>
<td>16.2 ± 2.3</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>LDL</td>
<td>Normal</td>
<td>78.5 ± 2.7</td>
<td>60.0 ± 6.0</td>
<td>81 ± 6</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>116.2 ± 1.0</td>
<td>52.8 ± 1.7</td>
<td>105 ± 2</td>
</tr>
<tr>
<td>HDL₆₉</td>
<td>Normal</td>
<td>22.5 ± 6.6</td>
<td>15.2 ± 1.5</td>
<td>14 ± 2</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>4.8 ± 0.5</td>
<td>2.0 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td>HDL₂₃₋₄₂₂</td>
<td>Normal</td>
<td>83.2 ± 7.3</td>
<td>40.2 ± 1.8</td>
<td>26 ± 2</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>5.0 ± 0.4</td>
<td>2.5 ± 0.6</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

VLDL (d < 1.006 g/ml), IDL (d = 1.006 to 1.019 g/ml), LDL (d = 1.019 to 1.063 g/ml), HDL₆₉ (d = 1.063 to 1.10 g/ml), and HDL₂₃₋₄₂₂ (d = 1.10 to 1.21 g/ml) were isolated by sequential ultracentrifugation from six normal control subjects and the patient. In the patient’s case, two separate plasma samples were analyzed. All values are given as means ± sd. Triplicate analyses were done on all samples.

Table 3. Apolipoprotein Concentrations (mg/dl)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
<th>Apo C-II</th>
<th>Apo B</th>
<th>Apo B in 1.006B*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 50)</td>
<td>117.2 ± 17.0</td>
<td>27.1 ± 4.2</td>
<td>3.1 ± 1.1</td>
<td>117.2 ± 25.6</td>
<td>109.4 ± 27.1</td>
</tr>
<tr>
<td>Normal controls (n = 6)</td>
<td>104.5 ± 6.5</td>
<td>28.7 ± 1.2</td>
<td>3.0 ± 0.5</td>
<td>102.2 ± 0.5</td>
<td>93.7 ± 7.8</td>
</tr>
<tr>
<td>Patient</td>
<td>ND</td>
<td>3.4 ± 0.5</td>
<td>0.8 ± 0.2</td>
<td>142.1 ± 1.0</td>
<td>139.6 ± 1.3</td>
</tr>
<tr>
<td>Patient’s daughter</td>
<td>77.6 ± 1.2</td>
<td>27.2 ± 0.6</td>
<td>4.2 ± 0.4</td>
<td>176.3 ± 1.9</td>
<td>171.0 ± 1.4</td>
</tr>
<tr>
<td>Patient’s son</td>
<td>69.4 ± 0.4</td>
<td>24.9 ± 0.7</td>
<td>3.2 ± 0.2</td>
<td>138.5 ± 2.2</td>
<td>132.2 ± 2.4</td>
</tr>
<tr>
<td>Patient’s brother</td>
<td>64.6 ± 1.7</td>
<td>21.7 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>159.1 ± 1.2</td>
<td>157.9 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± sd; normal controls (n = 6) were used for comparison to patient data given in table 2. Triplicate analyses were done on study subjects' plasma. In the patient's case, two separate plasma samples were analyzed. ND is not detectable.

*1.006B represents the 1.006 g/ml infranate.
scribed, and their concentrations determined by amino acid analysis on a Beckman Model 121 Automatic Amino Acid Analyzer. Standards were utilized for each radial immunodiffusion plate. The limit of sensitivity for the apo A-I assay was 0.01 mg/dl. The apolipoprotein content of plasma and lipoprotein fractions was also assayed utilizing antisera against apo A-I, A-II, B, C-II, and E on Ouchterlony plates with varying amounts of sample and antisera. In addition, the apolipoprotein composition of isolated lipoprotein fractions was assessed by SDS PAGE.58

*Figure 1.* Agarose lipoprotein electrophoresis of normal plasma (right panel) and the patient's plasma (left panel) demonstrating the absence of alpha lipoproteins in the patient's plasma.

*Figure 2.* Analytic ultracentrifugation patterns of normal plasma (upper panel) and the patient's plasma (lower panel), showing no detectable HDL in the patient's plasma. Values represent total lipoprotein concentration in mg/dl, and flotation rates are listed on the abscissa.
Results

Plasma Lipid and Lipoprotein Analysis

Analysis of plasma lipid and lipoprotein cholesterol values revealed that the patient had significant hypocholesterolemia, mild hypotriglyceridemia, slightly reduced VLDL and LDL cholesterol levels, and strikingly low HDL cholesterol concentrations, as shown in table 1. Her two offspring and one brother, age 56 years, were sampled in the fasting state, and their lipid data are also included in table 1. The percentage of unesterified cholesterol in the patient’s plasma was 30.1% ± 4.0% (normal, 25% to 35%), and in her LDL fraction (d = 1.019 to 1.063 g/ml) was 28.3% ± 1.1%. Agarose lipoprotein electrophoresis revealed an absence of alpha lipoproteins, with the major lipoprotein species in the patient’s plasma having an electrophoretic mobility between slow pre-beta lipoproteins and beta lipoproteins (LDL) (figure 1). A faint pre-beta band was also detected by electrophoresis. Analytic ultracentrifugation of the patient’s plasma revealed an absence of HDL (figure 2).

Data from preparative ultracentrifugation of plasma from six normal control subjects and the patient are shown in table 2. The mean recovery of cholesterol and triglyceride following ultracentrifugation in lipoprotein fractions was 90.0% ± 3.2% and 99.0% ± 4.9%, respectively, for normal subjects, and 99.0% ± 1.0% and 96.8% ± 1.3%, respectively, for the patient. Reduced amounts of VLDL and IDL and only trace amounts of HDL constituents were present in the patients’ plasma as compared to normal plasma (table 2). The patient’s LDL differed from normal LDL in that it was somewhat enriched in protein, triglyceride, and poor in cholesterol, presumably accounting for its abnormal electrophoretic mobility.

Electron Microscopy

Lipoprotein fractions isolated from the plasma of the fasted (over 12 hours) patient and normal controls were examined by electron microscopy to determine whether morphological abnormalities of the plasma lipoproteins could be detected.

Figure 3. Electron micrographs of lipoprotein fractions from a normal subject (N) and the patient (P) are shown. The VLDL (d < 1.006 g/ml) of the patient contained no large particles as seen in normal VLDL (×107,000). The LDL (d = 1.019 to 1.063 g/ml) of the patient contained no stacked discs and appeared similar in size and shape to normal LDL (×200,000). HDL (d = 1.10 to 1.21 g/ml) from the patient contained filamentous material, with a wide range of particle sizes, different from normal HDL (×200,000). Bar markers represent 100 nm in all figures.
The VLDL (d > 1.006 g/ml) fraction from the patient (figure 3) contained very few large particles resembling the 300 to 400 Å diameter particles observed in normal plasma (figure 3). Smaller particles ranging from 100 to 250 Å in diameter were observed in this fraction from the patient (figure 3). Interconnecting filaments between particles were observed in the patient's VLDL and not in normal VLDL. The LDL particles (d = 1.019 to 1.063 g/ml) of the patient appeared to be similar to normal LDL in size and shape, and contained no stacked discs. Lipoproteins from the patient's IDL fraction (d = 1.006 to 1.019 g/ml) were similar to her VLDL in size and shape, and the patient's HDL2b particles (d = 1.063 to 1.10 g/ml) appeared similar to those in her LDL (micrographs not shown). The most striking morphological difference was found in the HDL2a+3 (d = 1.10 to 1.21 g/ml) fraction from the patient (figure 3). In this fraction, particles ranging from 50 to 500 Å in diameter, but primarily in the range of 100 to 200 Å in diameter, were noted, with interconnecting filaments or strands emanating from the surface of the particles. No particles 50 to 70 Å in diameter typical of normal HDL2a+3 (figure 3) were noted in the patient. Particles observed in normal HDL fractions did not exhibit the filamentous projections noted in this patient.

Apolipoprotein Analysis

Analysis of lipoprotein fractions by SDS PAGE are shown in figure 4. No apo A-I band was detected in any of the patient's lipoprotein fractions. In addition, no apo A-I was noted in the

Figure 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% acrylamide) of molecular weight standards, apo A-IV, apo E, apo A-I, VLDL (d < 1.006 g/ml), IDL (d = 1.006 to 1.21 g/ml), LDL (d = 1.019 to 1.063 g/ml), and HDL (d = 1.063 to 1.21 g/ml) are shown. Lipoprotein fractions from a normal subject are in positions 1, 3, 5, and 7, and from the patient in positions 2, 4, 6, and 8. No apo A-I was detectable in the patient's lipoprotein fractions. Apolipoproteins of molecular weight less than 20,000 can be seen as a single band at the bottom of the gels (includes apo A-II and the C apolipoproteins).
Figure 5. Immunodiffusion plate with apo A-I antisera in the center well, the patient's plasma at the number 1 and 4 positions, normal plasma at the number 2 and 5 positions, and apo A-I standard at the number 3 position, demonstrating no detectable apo A-I in the patient's plasma.

The patient's two children and one brother had reduced apo A-I levels, normal apo A-II values, and slightly elevated apo B values (table 3); however, their LDL apo B:LDL cholesterol ratios were normal at 0.93, 0.92, and 0.92, respectively.

Discussion

Abnormalities of plasma apolipoproteins appear to be associated with a number of dyslipidemic disease states. Patients with abetalipoproteinemia have no detectable apo B in their plasma, a striking deficiency of VLDL and LDL plasma constituents, malabsorption of fat and fat-soluble vitamins, and may develop spino- cerebellar ataxia, acanthocytosis, and retinitis pigmentosa. Patients with no detectable plasma apo C-II, the activator protein for lipoprotein lipase, may develop marked hypertriglyceridemia and pancreatitis. Most patients with Type III hyperlipoproteinemia appear to have an abnormal apo E on isoelectric focusing, and the presence of this abnormality appears to predispose to the development of this form of hyperlipoproteinemia. Type III apo E on lipoproteins is catabolized at a slower fractional catabolic rate than normal apo E in both normal and Type III hyperlipoproteinemic subjects. Recently, there was an abnormal cysteine-containing apo A-I (apo A-I{\text{MM}}(\text{ino})) described in a patient with decreased HDL levels and mild hypertriglyceridemia. Patients with homozygous Tangier disease have a striking deficiency of plasma apo A-I, A-II, and HDL lipid constituents, marked cholesterol ester deposition in reticuloendothelial cells, and a striking and aberrant hypercatabolism of apo A-I and A-II. Fish eye disease, characterized by low HDL levels, corneal opacification, and a normal ratio of unesterified:esterified cholesterol in plasma, may also be due to an abnormal apolipoprotein.

The patient described in this report exhibited hypcholesterolemia, decreased triglyceride and VLDL levels, and an apo B-rich, triglyceride-rich LDL particle with abnormal electrophoretic mobility. Recently, Kwiterovich et al. have reported five kindred members with phytosterolemia, tendinous and tuberous xanthomas, premature CAD, and increased LDL apo B:LDL cholesterol ratios of 1.21 (not quite as elevated as the ratio in our patient of 1.32). Avogaro et al. have suggested that a decreased total cholesterol:apo B ratio is associated with an increased risk of CAD. In our normal subjects, this ratio was 1.54, whereas in this patient it was 0.78. Recently, Sniderman et al. have reported that subjects with familial hyperapobetalipoproteinemia have premature coronary artery disease with normal LDL cholesterol levels, but increased LDL apo B concentrations. The proband had an elevated...
Apo A-I was not detected immunochemically in this patient's plasma, and the deficiency of this apolipoprotein was presumably the cause for the marked deficiency of HDL constituents, plasma apo A-II and C-II, and the somewhat abnormal apolipoprotein was presumably the cause for the decreased plasma vitamin E levels and the markedly reduced plasma apo A-I levels that were approximately 50% of normal, suggesting that they were heterozygotes and the patient was a homozygote for apo A-I absence. Detailed family studies are in progress to delineate the mode of inheritance of this disease.

The small amounts of HDL detected in our patient's plasma contained no apo A-I but did contain apo A-II, predominantly in the density range 1.10 to 1.21 g/ml. The patient's HDL contained phospholipid, cholesterol, and triglyceride, and when observed ultrastructurally, consisted mainly of particles 100 to 200 Å in diameter with interconnecting filaments or strands, which appeared to emanate from the surface of these particles. Small HDL particles, in the HDL₃ (d = 1.125 to 1.21 g/ml) region, containing only apo A-II have been isolated from the plasma of Tangier homozygotes; however, these particles were not noted to have interconnecting filaments. Presumably, the abnormal HDL observed in our patient was produced as a result of the apo A-I deficiency, and was due to the presence of apo A-II and phospholipid in HDL₃. In addition, the patient was found to have very small VLDL particles of low flotation rate.

Corneal opacification as noted in our patient has been associated with a number of HDL deficiency states: fish eye disease, homozgyous Tangier disease, and familial LCAT deficiency. While Tangier homozygotes develop cholesterol ester deposition in their reticuloendothelial cells at an early age, presumably due to uptake of abnormal lipoproteins, corneal infiltration does not generally develop until these patients are over 40 years of age, suggesting that the mechanisms for these pathological processes are different. Our patient's corneal opacification was not noted until she was 42 years old.

Early work by Barr et al. pointed to a association between CAD and decreased HDL levels. Miller and Miller first stressed the importance of HDL cholesterol as an independent risk factor for CAD, and their work was confirmed by a number of large population studies. Patients with homozgyous Tangier disease appear to be at risk for premature CAD after the age of 40 years, but their CAD prevalence is not nearly as striking and as premature as that seen in homozgyous familial hypercholesterolemia. The patient described in this report was noted to have severe CAD at age 45 years, which was confirmed at autopsy in the absence of known risk factors, other than the striking deficiency of HDL constituents and the elevated LDL apo B:LDL cholesterol ratio. Clinically and biochemically, her disease state was distinguishable from homozgyous Tangier disease in several respects. The patient did not have enlarged tonsils or a history of tonsillectomy, nor hepatosplenomegaly, lymphadenopathy, or peripheral neuropathy on physical examination. Histologically, the liver, spleen, and lymph nodes were normal at autopsy. In addition, the patient was not hypertri glyceridemic, and her plasma contained no detectable apo A-I. The available data suggest that this patient's disease was genetic, since her two offspring and one brother had HDL cholesterol and apo A-I levels that were approximately 50% of normal, suggesting that they were heterozygotes and the patient was a homozygote for apo A-I absence. Detailed family studies are in progress to delineate the mode of inheritance of this disease.

The combined data may be summarized as follows: plasma apo A-I absence is associated with a striking reduction in HDL constituents, markedly reduced plasma apo A-II and C-II levels, an abnormal apo B-rich LDL particle, decreased and small VLDL particles, corneal opacification, and premature CAD. Apolipoprotein A-I absence appears to represent a new distinct disease entity.

Acknowledgments

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References


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