Familial HDL Deficiency Due to Marked Hypercatabolism of Normal ApoA-I

Joseph Emmerich, Bruno Vergès, Igor Tauveron, Daniel Rader, Silvia Santamarina-Fojo, Jürgen Schaefer, Maryse Ayrault-Jarrier, Philippe Thébriot, H. Bryan Brewer, Jr

In this article, we describe a 46-year-old man with severe high-density lipoprotein (HDL) deficiency and his kindred. In the proband, HDL cholesterol and apolipoprotein (apo) A-I levels were 5 and 4.5 mg/dL, respectively. Xanthomata, xanthelasma, arcus corneae, and hepatosplenomegaly were not present. The proband had coronary artery disease, but it was impossible to state whether the HDL deficiency cosegregated with premature coronary artery disease in this kindred. Pedigree analysis was suggestive of a codominant familial disease. Polymerase chain reaction amplification of the apoA-I gene of the proband, followed by subcloning and sequencing, did not reveal any mutation in either the coding regions or intron-exon junctions. A kinetic study using deuterated leucine to endogenously label apoA-I was performed to elucidate the metabolic basis of the apoA-I deficiency. We demonstrated marked hypercatabolism of apoA-I in the proband, with a fractional catabolic rate more than 10 times faster than normal; the plasma residence time of apoA-I in the proband was only 0.38 day compared with 4.10 days in a control subject. The apoA-I production rate was also substantially decreased in the proband. The association of a normal apoA-I gene sequence with marked hypercatabolism of apoA-I is similar to that described in Tangier disease. However, except for the presence of mild, diffuse, corneal deposits, this patient had no evidence of the reticuloendothelial cholesterol deposition characteristic of Tangier disease. This study establishes that a form of severe hypoalphalipoproteinemia distinct from Tangier disease can be caused by marked hypercatabolism of a normal A-I apolipoprotein. (Arterioscler Thromb. 1993;13:1299-1306.)

KEY WORDS • hypoalphalipoproteinemia • Tangier disease • lipoproteins • metabolism • kinetics • stable isotopes
cholesterol levels, and in these cases it was always >15 mg/dL. Tangier disease is a rare codominant familial disease that was originally described in two siblings from Tangier Island. The clinical features of homozygotes for Tangier disease include orange tonsils, hepatosplenomegaly, corneal opacities, and peripheral neuropathies. Homozygotes have elevated plasma triglyceride levels and HDL deficiency (<2% of the normal level). ApoA-I and apoA-II levels in these subjects were measured at 1% and 5% of normal, respectively. Heterozygotes for Tangier disease have HDL cholesterol, apoA-I, and apoA-II levels that are approximately half of normal, without clinical manifestations. A review of the reported cases of Tangier disease revealed a potential increased risk of cardiovascular disease after the age of 40. In contrast to the mutations that cause an absence of apoA-I biosynthesis, markedly premature CAD is not characteristic of Tangier disease. The cause of HDL deficiency in Tangier disease is rapid catabolism of HDL and normal apoA-I. The molecular defect of the disease is unknown but seems to result from abnormal processing of Golgi elements and lysosomes.

We report in this article the underlying kinetic mechanism leading to severe HDL deficiency in a French kindred. The proband had a normal apoA-I gene, marked hypercatabolism of apoA-I, and no clinical features of Tangier disease.

Methods

Clinical Data

The proband is a 46-year-old man who was hospitalized for angina pectoris. The clinical examination was normal, without tonsillar abnormality or neuropathy. Xanthoma, xanthelasma, and arcus cornea were not found. The absence of hepatosplenomegaly was confirmed by ultrasound. Slit-lamp examination revealed mild corneal deposits (Fig 1). The patient had normal thyroid, kidney, and liver function. Plasma lipid values were cholesterol, 92 mg/dL; triglycerides, 229 mg/dL; HDL cholesterol, 5 mg/dL; and apoA-I, 4.5 mg/dL. Cardiac catheterization demonstrated a 90% stenosis of the proximal left anterior descending coronary artery (LAD) and several atheromatous plaques in the circumflex and right coronary arteries. Because of the failure of angioplasty to restore a normal diameter to the LAD, coronary artery bypass surgery was performed. During this procedure many calcified atheromatous plaques were noted in the aortic root, an unusual finding in a patient of this young age. The parents of the proband were deceased at the time this study was conducted, and their deaths were not related. His father had died of coronary heart disease (CHD) at age 77 and his mother of cancer at age 66. He has three brothers, 54, 49, and 48 years old, a 43-year-old sister, a 20-year-old daughter, and an 18-year-old son (Fig 2). One brother of the patient (II3) has decreased levels of HDL cholesterol and apoA-I, as do the two children (III1 and III2) of the proband (15 to 23 mg/dL). All immediate relatives of the patient except one brother (II3) participated in the family study.

Plasma Lipid and Apolipoprotein Analyses

After a 12-hour fast blood was collected in EDTA (0.1 mg/mL) from the proband, three of his four sib-


FIG 2. Pedigree of the family. Filled symbol (arrow) denotes the proband and semifilled symbols denote individuals with decreased levels of high-density lipoprotein cholesterol. /, Deceased; □, males; ○, females.

DNA Preparation and Oligonucleotide Synthesis

Genomic DNA was isolated from leukocytes as described.34 Synthetic oligonucleotides were synthesized by the phosphoramidite method in a DNA synthesizer (Applied Biosystems). The sequence and positions of the primers, according to the numbering system of Shoulders et al,3 are shown in Table 1. Primer sequences contained restriction sites that allowed subcloning of the polymerase chain reaction (PCR) products; restriction sites for EcoRI, HindIII, and SstI were introduced into the primers for subcloning.

PCR, Cloning, and DNA Sequencing

Three genomic DNA fragments of the apoA-I gene were amplified from 1 μg of DNA from the patient by the PCR technique. The buffer and deoxynucleoside triphosphates used for the reaction were prepared with the GeneAmp DNA amplification reagent kit (Perkin-Elmer Cetus, Norwalk, Conn) as recommended, using 50 pmol of each primer. PCR was performed for 30 cycles using Thermus aquaticus DNA polymerase (Perkin-Elmer Cetus), with a 2-minute extension at 72°C, 1-minute denaturation at 95°C, and 1-minute primer annealing at 60°C for primers P1 and P2, at 55°C for primers P3 and P4, and at 60°C for primers P5 and P6. The amplified regions, the 453-bp fragment I (bases 214 to 667), 559-bp fragment II (bases 614 to 1173), and 766-bp fragment III (bases 1578 to 2344), include all the coding exons and splice junctions of the apoA-I gene.34 For cloning and sequencing, an aliquot of each amplified product from the patient was purified from an agarose gel, digested with restriction enzymes, ligated to the pGEM -3Zf(+) vector (Promega, Madison, Wis), and cloned using Escherichia coli DH5α as the host.35 Double-stranded DNA sequencing of the cloned apoA-I gene fragments was done by the deoxyribonucleotide chain-termination method of Sanger et al.36

Endogenous Labeling Study Protocol

Endogenous labeling of apoA-I was performed using a stable isotopically labeled amino acid as previously reported.37 Trideuterated L-leucine, with the three deuterium atoms (D) positioned in one of the methyl groups of the amino acid (MSD Isotopes, St Louis, Mo), was used for the kinetic study. Multiple labeling of the amino acid shifts the labeled leucine peak well out of the range of the peak because of the natural abundance of heavy isotopes on mass spectrometric analysis. The deuterated leucine was dissolved in a 0.9% NaCl solution, sterile filtered, and tested for pyrogenicity and sterility before use.

Three days before the start of the study, subjects were placed on a controlled isoweight diet containing 47% of calories as carbohydrate, 37% as fat, and 16% as protein, with 200 mg of cholesterol per 1000 kcal and a polyunsaturated to saturated fat ratio of 0.3. The study was begun after a 12-hour fast. Subjects were given a priming bolus injection of 1.2 mg/kg of D3-leucine, immediately followed by a constant infusion of 24 μg/kg-min of D3-leucine for 16 hours. During the constant infusion, the controlled isoweight diet was divided into small equal meals given every 2 hours. Blood samples were drawn from the opposite arm into tubes containing 0.1% EDTA 10 minutes before the priming bolus and then at 10 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 14, and 16 hours and kept at 4°C. Plasma was separated by centrifugation (2300 rpm) for 30 minutes at 4°C. Inhibitors of protease and bacterial growth (aprotinin, 200 U/ml; 0.05% NaCl) were added to all samples.

<table>
<thead>
<tr>
<th>Table 1. Oligonucleotide Sequences Used as Primers</th>
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Free plasma amino acids were isolated from 0.5 mL plasma at selected time points by cation-exchange column chromatography. One milliliter of 30% acetic acid was added to 0.5 mL plasma and applied to prefilled Poly-Prep columns (Bio-Rad, Richmond, Calif). The columns were washed with 8 mL distilled water and eluted directly with 4 mL of 4N NH₄OH into 5-mL reaction vials (Reacti-Vial, Pierce, Rockford, Ill). After lyophilization to dryness in a speed-vac concentrator (Savant, Farmingdale, NY), the samples were derivatized to the N-heptfluorobutyryl isobutyl esters. Derivatization was performed by adding 500 μL of isobutyl hydrochloride (HCl) (Altech Associates, Inc, Deerfield, Ill) to each sample. Samples were heated to 110°C for 1 hour, cooled to room temperature, and again lyophilized to dryness. The residue was dissolved in 200 μL high-performance liquid chromatography-grade ethyl acetate and 150 μL heptfluorobutyric anhydride (Altech Associates) and then heated to 150°C for 10 minutes. After cooling to room temperature and lyophilizing to dryness, the derivatized product was dissolved in ethyl acetate and subjected to gas chromatographic-mass spectrometric (GC/MS) analysis.

Isolation of lipoproteins and apolipoproteins. To isolate total apoA-I, the total lipoprotein fraction was isolated from plasma at selected time points by ultracentrifugation at a density of 1.25 g/mL. Separate 4-mL aliquots of plasma were used for isolation of very-low-density lipoproteins (VLDLs; d < 1.006 g/mL). All samples were dialyzed against 10 mmol/L NH₄HCO₃ (pH 8.2) with 0.01% EDTA and 0.013% NaN₃, lyophilized, and delipidated with ethanol/ether (2:1, vol/vol). Samples were solubilized for electrophoresis in 4% sodium dodecyl sulfate (SDS) containing 0.5 mol/L Tris HCl (pH 6.8), 8% sucrose, and 1% dithiothreitol. Total apoA-I and VLDL apoB-100 were isolated by preparative discontinuous sodium dodecyl sulfate–polyacrylamide gradient (5%/15%) gel electrophoresis as previously described. Apolipoproteins from the samples were identified by comparing their migration into the gel with the migration of apolipoprotein standards and known molecular-weight standards and were confirmed by immunoblotting with specific antibodies. The stained apolipoproteins were cut from the gels and the gel slices were dried in a 90°C oven.

Preparation for MS analysis. The dried protein-containing gel slices were hydrolyzed in 6N HCl (Sequanal Grade, Pierce) for 24 hours at 110°C under nitrogen vacuum. After removal of HCl by speed-vac lyophilization, samples were dissolved in 50% acetic acid and applied to cation-exchange columns (prefilled Poly-Prep columns, Bio-Rad Laboratories). Amino acids were recovered by elution with 4N NH₄OH and then derivatized to the N-heptfluorobutyryl isobutyl esters as described above for free plasma amino acids.

GC/MS analysis. The isotope ratios of D₃-leucine were quantitated by GC/MS on a Finnigan MAT 4500 (Finnigan MAT, San Jose, Calif) in the chemical ionization mode. The selected positively charged ions with an m/z of 384 for leucine and an m/z of 387 for D₃-leucine were monitored. At least three measurements were made of each apolipoprotein at each time point. The isotope ratio (IR) was defined as the ratio of D₃-leucine to unlabeled leucine.

The enrichment was determined by the formula (IRt - IR0) x 100, where IRt is the isotope ratio at time t and IR0 is the isotope ratio at time zero.

The enrichment was then converted to the tracer to tracee ratio by using the formula e(t)/[e₀ - e(t)], where e(t) is the enrichment of each sample at time t and e₀ is the enrichment of the infusate (0.99 for the D₃-leucine used in this study).

Data analysis. The tracer to tracee ratio data of VLDL apoB-100, apoA-I, and apoA-II were simultaneously fitted to monoeponential functions using SAAAM 30 programs. The function was defined as the tracer to tracee ratio, Aₜ[1 - e⁻kt], where Aₜ is the precursor pool tracer to tracee ratio for the apolipoprotein, k is the fractional synthesis rate, and d is the delay. Tracer to tracee ratios from VLDL apoB-100, apoA-I, and apoA-II were simultaneously analyzed using the VLDL apoB-100 plateau tracer to tracer ratio as the estimate of the precursor pool isotopic enrichment for VLDL apoB-100 and plasma apoA-I and A-II. We have found that the apoA-I fractional synthesis rates as
determined by monoexponential analysis of endogenous labeling data using the VLDL apoB-100 plateau tracer to tracee ratio as an estimate of the precursor pool tracer to tracee ratio for apoA-I synthesis are in excellent agreement with apoA-I fractional catabolic rates obtained simultaneously using the exogenously radiolabeled apoA-I. The residence time is the reciprocal of the fractional synthesis rate. The production rate (PR) of the apolipoprotein, expressed in milligrams per kilogram-day, is determined from the formula

\[ PR = (FSR) \times \text{Apolipoprotein Pool Size/Body Weight} \]

where apolipoprotein pool size is estimated by taking the plasma apolipoprotein concentration times the plasma volume (0.04 L/kg body weight) and FSR is the fractional synthesis rate.

The study protocol was approved by the ethics committee of the Clermont-Ferrand faculty of medicine and the Institutional Review Board of the National Heart, Lung, and Blood Institute. Informed consent was obtained from the patients and control subjects.

Results
Plasma Apolipoprotein and Lipid Analyses
Table 2 shows the lipid and apolipoprotein levels of the proband, his brother, and the two children of the proband, all of whom have decreased levels of HDL cholesterol, as well as lipid levels of the other members of the family who had normal HDL cholesterol concentrations. In the proband, levels of total cholesterol were low, triglycerides moderately increased, and HDL cholesterol only 5 mg/dL. By ultracentrifugation, values of HDL cholesterol, low-density lipoprotein cholesterol, and VLDL cholesterol were 3, 53, and 36 mg/dL, respectively. ApoB, E, and C-II values were within the normal range, and apoC-III was slightly decreased to 68% of normal. ApoA-II, A-TV, C-I, and D were decreased to 17%, 21%, 20%, and 37% of normal, respectively. The apoA-I level was 4.5 mg/dL, only 3% of normal. Measurement of fasting plasma apoA-I levels was performed on five occasions, and values were always <5 mg/dL. One-dimensional isoelectric focusing of plasma apoA-I levels was performed on five occasions, and values were always <5 mg/dL. One-dimensional isoelectric focusing of plasma followed by anti-apoA-I immunoblotting (Fig 3) confirmed very low levels of apoA-I and a normal apoA-I charge, except for an increased proapoA-I to apoA-I ratio compared with normal. Plasma LCAT activity and cholesterol esterification rate were 60% of normal, whereas the fractional esterification rate was normal. CETP mass was 71% of normal.

Direct Cloning and DNA Sequencing of Amplified DNA
The three amplified fragments were cloned as described in "Methods." At least five clones of each apoA-I gene fragment were selected at random and analyzed by sequencing. No mutation was found in the coding regions as well as in exon-intron junctions of the proband's apoA-I gene (data not shown).

Kinetic Study
The tracer to tracee ratios of free plasma leucine remained in the steady state throughout the infusion period. The tracer to tracee ratios of VLDL apoB-100 reached a plateau by 10 hours in the patient and control subject (Fig 4). The tracer to tracee ratio curves of apoA-I are shown in Fig 5. The apoA-I tracer to tracee ratios in the patient rapidly increased and reached a plateau by 12 hours, whereas those in the control subjects increased much more slowly and did not reach plateau by 16 hours. The apoA-I kinetic parameters derived from the monoexponential function analysis of these data are presented in Table 3. The fractional synthesis rate of apoA-I was 2.61 d⁻¹ in the patient compared with 0.24 d⁻¹ in the control subject, indicating that the turnover of apoA-I in the patient is more than 10 times faster than in the control subject. The production rate of apoA-I was 4.7 mg/kg-d in the patient compared with 13.4 mg/kg-d in the control subject.
TABLE 3. Apolipoprotein A-I Kinetic Parameters

<table>
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<tr>
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<th>Concentration (mg/dL)</th>
<th>FSR (d⁻¹)</th>
<th>RT (d)</th>
<th>PR (mg/kg-d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>4.5</td>
<td>2.61</td>
<td>0.38</td>
<td>4.7</td>
</tr>
<tr>
<td>Control</td>
<td>140</td>
<td>0.24</td>
<td>4.10</td>
<td>13.4</td>
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FSR, fractional synthesis rate; RT, residence time; PR, production rate.

Discussion

In the present study, we describe a proband and his kindred with severe HDL deficiency. The proband has HDL cholesterol and apoA-I levels of only 5 and 4.5 mg/dL, respectively. Pedigree analysis determined that one brother and the two children of the proband had HDL cholesterol levels approximately half of normal (Table 2). The parents of the proband were deceased, so it was not possible to confirm the vertical transmission of the disease through three generations. However, the transmission of HDL deficiency is suggestive of a codominant familial disease with a putative homozygosity for the proband. There does not appear to be a link between HDL deficiency and premature CAD in the kindred. The proband presented at age 45 with severe CHD and unusual calcified atheromatous plaques of the aortic root despite the absence of other risk factors for CAD. However, another brother with normal levels of HDL and apoA-I (45 and 138 mg/dL, respectively) developed angina pectoris at age 47 and was found to have documented CAD requiring percutaneous transluminal coronary angioplasty. One other brother with decreased levels of HDL was free of clinical CHD at age 49. For these reasons it is impossible to state that HDL deficiency cosegregates with premature CAD in this kindred.

Because of the very low level of apoA-I with the absence of signs of Tangier disease, the patient was initially suspected to have a mutation in the apoA-I gene. PCR amplification of the apoA-I gene of the patient, followed by subcloning and sequencing, did not reveal any mutation in the coding regions of the gene or in its intron-exon junctions. To understand the metabolic basis of the apoA-I deficiency, we performed a kinetic study using deuterated leucine to endogenously label apoA-I. Total plasma apoA-I was isolated from the d<1.25 g/mL lipoprotein fraction and compared with a normal subject studied in an identical fashion. We demonstrated marked hypercatabolism of apoA-I, with a turnover rate nearly 11 times faster than in normal subjects. This provides the major metabolic explanation for the hypoalphalipoproteinemia in this patient. This apoA-I catabolic rate approaches but is not as rapid as the apoA-I catabolic rate in Tangier disease.²² The apoA-I production rate in our patient was also somewhat decreased, similar to that seen in Tangier disease;²² the explanation for this finding could be underestimation of the mass of the total apoA-I pool (if a significant portion is extravascular) or catabolism of a large amount of proapoA-I before its conversion to mature apoA-I. However, we cannot rule out an additional metabolic defect in apoA-I production contributing to the low level of apoA-I in this patient.

Two mutations of apoA-I have been associated with an increased catabolic rate of apoA-I and hypoalphalipoproteinemia. ApoA-I<sub>Mn</sub> (Arg<sub>251</sub>→His) was found to have a mean residence time of 2.77 days compared with 4.70 days in control subjects.⁴⁷ ApoA-I<sub<s>1</sub></s> (Gly<sub>29</sub>→Arg) had a mean residence time of 1.79 days compared with 4.93 days in control subjects.⁴⁸ The much shorter residence time of apoA-I in our patient (0.38 day) is consistent with the much lower HDL cholesterol and apoA-I than found in patients with either of these apoA-I mutants.

The association of a normal apoA-I gene sequence with hypercatabolism of apoA-I is similar to the situation in Tangier disease, in which the fractional catabolic rate of apoA-I is even greater and the hypoalphalipoproteinemia more severe.²⁰²² No kinetic data from a stable-isotope constant-infusion study are available for total apoA-I in Tangier disease. Nevertheless, our results can be compared with those obtained with radiotracers in Tangier disease.²² The residence times of mature apoA-I in two Tangier disease patients were 0.5 and 1.5 days. The production rate of mature apoA-I in Tangier disease was decreased as well, to 2.0 and 1.5 mg/kg-d (compared with 10.2 and 7.5 mg/kg-d in control subjects). This is not due to defective conversion of proapoA-I to mature apoA-I⁴⁹,⁵⁰ but rather a result of marked hypercatabolism of proapoA-I before its conversion to the mature form.⁵⁰ The decreased production rate of mature apoA-I in our patient may be caused by a similar process. This is supported by the increased ratio of proapoA-I to mature apoA-I on apoA-I isoelectric focusing, similar to that seen in Tangier disease patients. Nevertheless, a primary defect in the conversion of proapoA-I to mature apoA-I or a secondary effect on apoA-I biosynthesis cannot be ruled out.

Familial LCAT deficiency syndromes (classic LCAT deficiency and fish-eye disease) cause severe hypoalphalipoproteinemia.⁵¹ The decrease in LCAT activity and of the cholesterol esterification rate to 60% of normal in this patient is not consistent with a primary LCAT deficiency syndrome and is probably secondary to hypercatabolism of LCAT in association with HDL. The same range of LCAT activity has been observed in obligate heterozygotes for LCAT deficiency,⁵¹ apoA-I<sub>Mn</sub>, heterozygotes,⁵² and apoA-I frameshift-202-mutation heterozygotes,⁴⁶ compared with the virtually absent LCAT activity in homozygous LCAT deficiency and fish-eye disease. In Tangier disease, LCAT mass, LCAT activity, and cholesterol esterification rate are decreased to 22%, 29%, and 33%, respectively, of normal,⁵³ consistent with the very high rate of HDL catabolism. Therefore, our patient is unlikely to have a primary defect in LCAT as the cause of his HDL deficiency.

The proband in this study also has a low level of low-density lipoprotein cholesterol, as is also seen in Tangier disease,⁹,²⁰ although the plasma apoB level is normal. The mechanism for the low level of low-density lipoprotein cholesterol in Tangier disease is thought to be impaired lipolysis of triglyceride-rich lipoprotein particles; the mechanism in this patient is unknown, but his moderate hypertriglyceridemia could be consistent with this mechanism.

The clinical features of our proband are different from those observed in Tangier disease, which is characterized by cholesterol ester deposition in the tonsils, spleen, liver, lymph nodes, thymus, intestinal mucosa,
Schwann cells, and cornea. Except for the presence of mild, diffuse, corneal deposits, our patient had no evidence of cholesterol deposition in the reticuloendothelial system. Hence, it is likely that the defect leading to hypercatabolism in this kindred is different from that in Tangier disease. Elucidation of the cause of hypercatabolism in this kindred will provide new insights in the regulation and metabolism of HDL.

References


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