Tangier disease: A structural defect in apolipoprotein A-I (apoA-I\textsubscript{Tangier})

(apolipoprotein A-II/dyslipoproteinemia/atherosclerosis/high density lipoproteins/cholesterol)

LINDA L. KAY, ROSEMARY RONAN, ERNST J. SCHAEFFER, AND H. BRYAN BREWER, JR.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

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ABSTRACT  Tangier disease is a familial disorder characterized by orange tonsils, cholesterol ester deposition in reticuloendothelial cells, abnormal chylomicron remnants, and a marked reduction in high density lipoproteins. Plasma concentrations of the apolipoproteins apoA-I and apoA-II in patients with Tangier disease are approximately 1% and 7% of those in normal subjects, respectively. Previous studies have shown that the low plasma concentrations of apoA-I and apoA-II are due to increased fractional catabolism with a relatively normal apoA-I and apoA-II synthesis. Plasma apoA-I and apoA-II were isolated to electrophoretic homogeneity from delipidated plasma lipoproteins from a patient with Tangier disease. apoA-I\textsubscript{Tangier} differed from apoA-I from control subjects in amino acid composition, electrophoretic mobility, apparent molecular weight on sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and heterogeneity of isoforms on iso-electric focusing. apoA-II\textsubscript{Tangier}, however, appeared to be identical to normal apoA-II in amino acid composition and in immunological as well as chemical properties. These results have been interpreted as indicating that apoA-I\textsubscript{Tangier} has a different covalent structure than does normal apoA-I, and apoA-II\textsubscript{Tangier} is identical to normal apoA-II. This structural change in apoA-I\textsubscript{Tangier} is associated with rapid catabolism of apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier} containing plasma lipoproteins, and it leads to the deficiency in high density lipoproteins, abnormal chylomicron remnants, and the intracellular accumulation of cholesterol ester characteristic of Tangier disease.

Tangier disease is a rare familial disorder of lipid transport characterized by hypcholesterolemia, moderate hypertriglyceridemia, abnormal chylomicron remnants, low levels of low density lipoproteins (LDL), and a marked deficiency of high density or α lipoproteins (1–6). Clinically these patients have enlarged orange tonsils, hepatosplenomegaly, lymphadenopathy, and recurrent transient sensory-motor neuropathy. The tissue lipid deposits are composed principally of cholesterol esters and are confined primarily to the macrophages, Schwann cells, and nonvascular smooth muscle cells (7).

In normal subjects, plasma high density lipoproteins (HDL) are composed (wt %) of approximately 25% phospholipids, 20% cholesterol, 5% triglycerides, and 50% protein. Of which 90% is composed of apolipoproteins A-I and A-II (apoA-I and apoA-II). In plasma from Tangier homozygotes, HDL cholesterol concentrations are 5% of normal and apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier} levels are approximately 1% and 7% of normal, respectively (8). Previous kinetic studies in our laboratory have shown that the low plasma concentrations of apoA-I and apoA-II in Tangier disease are due to increased fractional catabolism with a relatively normal apoA-I and apoA-II synthesis (8). One of the major pathways for the biosynthesis of human HDL constituents, particularly apoA-I and apoA-II, is through the catabolism of chy-lomicrons (9). With lipolysis, chyomicron lipid and apoA-I and apoA-II are transferred to HDL. Patients with Tangier disease have fasting chylomicronemia and morphologically abnormal chylomicron remnants as observed by electron microscopy (10, 11). We have previously proposed that there is a metabolic defect in Tangier disease characterized by the failure of the normal conversion of chylomicron lipid and protein constituents into HDL, an enhanced catabolism of apoA-I and apoA-II, and an uptake of abnormal chylomicron remnants by the reticuloendothelial system (12). The purpose of the present study was to purify and characterize apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier} from a patient with homozygous Tangier disease.

MATERIALS AND METHODS

Source and Preparation of Apolipoproteins. Plasma for the isolation of apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier}, was obtained from patients from the original kindred on Tangier Island (1). Plasma from the Tangier and normal donors was collected by plasmapheresis in 0.1% EDTA. Plasma lipoproteins from the patients with Tangier disease were isolated by ultracentrifugation at density 1.21 g/ml (Beckman, 60 Ti rotor, 24 hr) (13). The lipoproteins were initially delipidated by tetramethylurea (14) followed by chlorofrom/methanol, 2:1 (vol/vol) (15), to remove residual lipids. Apolipoprotein HDL was isolated from normal plasma by preparative ultracentrifugation and delipidated with chlorofrom/methanol as reported (16).

Gel Permeation Chromatography. Forty-two milligrams of delipidated apolipoproteins (ρ < 1.21 g/ml) from a Tangier patient were fractionated at 5°C on a Sephacyrl S-200 superfine column (Pharmacia, 2.5 × 186 cm) in a buffer containing 6 M urea, 0.5 M NaCl, 50 mM glycine, and 2 mM NaOH (pH 8.8). The fractions that contained apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier} were ascertained by NaDodSO\textsubscript{4}/polyacrylamide gel electrophoresis. Eluted fractions containing apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier} were pooled and dialyzed against 0.01 M ammonium bicarbonate (pH 8.2).

Ion-Exchange Chromatography. Samples of partially purified apoA-II\textsubscript{Tangier} from the Sephacryl column or delipidated apoHDL from normal volunteers were applied to a column of DEAE-cellulose (Whatman DE52 microgranular, 1.2 × 23 cm) equilibrated in 0.025 M Tris-Cl/6 M urea (pH 8.0, conductivity 0.79 mS, 25°C). The unadsorbed fraction was eluted with 33 ml of equilibrating buffer and the apoA-II was eluted with a linear gradient (260 ml) of equilibrating buffer and 0.1 M Tris-Cl in 6 M urea (pH 8.0, conductivity 2.75 mS, 25°C). Fractions containing apoA-I and apoA-II were dialyzed against 0.01 M ammonium bicarbonate (pH 8.2), lyophilized, and then

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; apoA-I and apoA-II, apolipoproteins isolated from normal volunteers; apoA-I\textsubscript{Tangier} and apoA-II\textsubscript{Tangier}, apolipoproteins isolated from a patient with homozygous Tangier disease.

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The stacking gel for 18 to 20 cm (pH 8.2). Bicarbonate 0.1% 0.085% bisacrylamide (Bio-Rad), was gel amide, hr. electrophoresis water distilled Samples chromatographed tubes of apoA-Iangier amine the region M 0.023 and ple M 0.01 M against gel slices Tris-HCl (pH 8.8), the gel the protein gel was cut and fractionated by ethanol, 1% ammonium persulfate. The running gel was sliced and stained with chromic conductivity 0.12 M NaDodSO4. After electrophoresis the gel was removed from the tube and a 5-mm vertical gel slice was cut and stained for 15 min in 50% methanol/5% glacial acetic acid/0.1% Coomassie blue (vol/ vol). The stained slice was aligned with the unstained gel to locate the region corresponding to apoA-I. The apoA-I region of the gel was sliced and the protein was eluted from the gel by using the ISCO electroforetic sample concentrator (model 1750) (18). The anode and cathode buffers were 0.21 M Tris and 0.23 M glycine (pH 8.9, conductivity 1.15 mS, 25°C). The sample cup buffer and inner chamber buffer contained 0.021 M Tris and 0.023 M glycine (pH 8.9, conductivity 0.12 mS, 25°C). The gel slices were electrophoresed for 3 hr at 4°C as a power of 1 W and current of 7 mA. The eluted fractions were dia lyzed against 0.01 M ammonium bicarbonate (pH 8.2), lyophilized, and fractionated by gel permeation chromatography on a Sephadex G-15 coarse column (Pharmacia, 0.9 × 71 cm) equilibrated in 0.01 M ammonium bicarbonate (pH 8.2).

For comparison of physicochemical properties of apoA-Iangier, DEAE-cellulose-purified apoA-I was subjected to the identical procedures of preparative NaDodSO4/polyacrylamide gel electrophoresis, electrophoretic elution, dialysis, and gel filtration as outlined above for apoA-Iangier.

Other Procedures. Analytical polyacrylamide gel electrophoresis was performed in 10% acrylamide and 0.2% bisacrylamide at pH 8.9 in the presence of 8 M urea (19). Analytical slab gel electrophoresis was performed with 15% acrylamide containing 0.1% NaDodSO4 (17). Two-dimensional gel electrophoresis was performed with isoelectric focusing as the first dimension followed by NaDodSO4 slab gel electrophoresis. Six
to 10 μg of apolipoprotein were focused at 4°C for 18 hr on 1.5 × 95 mm gels in 7.5% acrylamide/0.2% bisacrylamide/8 M urea containing 3% ampholines (Serva) between pH 5.0 and pH 5.5 (20). After isoelectric focusing the gel was removed from the tube and electrophoresed in a 1.5-mm slab gel containing 18% acrylamide, 0.085% bisacrylamide, and 0.1% NaDodSO4.

Quantitation of apo-A-I and apo-A-II was performed by radial immunodiffusion as described (21). Ouchterlony double immunodiffusion was performed in 1% agarose (22). Acid hydrolyses of protein samples were performed for 24 hr in 6 M HCl (Pierce) containing 2-mercaptoethanol (1:2,000, vol/vol) at 110°C. Amino acid analyses were performed in Beckman model 121 and 121 MB amino acid analyzers equipped with M126 and System AA data processors.

RESULTS

Plasma lipoproteins from patients with Tangier disease were isolated by ultracentrifugation at ρ < 1.21 g/ml. Approximately 50% of plasma apo-A-I(Tangier) and 80% of apo-A-II(Tangier) were isolated within the ρ < 1.21 g/ml fraction. The isolated lipoproteins were delipidated with tetramethylurea/chloroform/methanol, solubilized in urea, and chromatographed on Sephacryl S-200 (Fig. 1 and Fig. 2A). The column eluate was monitored by analytical slab NaDodSO4/polyacrylamide electrophoresis. Three fractions, designated 1, 2, and 3 (Fig. 1), containing partially purified apo-A-I(Tangier), apo-A-II(Tangier), and the C apolipoproteins, respectively, were eluted from the column.

Isolation and Characterization of apo-A-I(Tangier). Apo-A-I(Tangier) was purified to homogeneity from Sephacryl S-200 fraction 1 (Fig. 1) by preparative NaDodSO4/polyacrylamide gel electrophoresis (Fig. 2B). Six milligrams of fraction 1 containing partially purified apo-A-I(Tangier) was applied to preparative NaDodSO4 gels and electrophoresed, and the band corresponding to the electrophoretic position of apo-A-I was eluted from the mincd gels by electrophoresis. Purified apo-A-I(Tangier) (400 μg) was dialyzed, lyophilized, and freed from low molecular weight constituents by chromatography on Sephadex G-15. Apo-A-I(Tangier) gave a single band on NaDodSO4 gel electrophoresis (1.5-mm gel, 1.5 μg of protein; Fig. 2B).

Table 1. Amino acid compositions of apolipoproteins

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>ApoA-I(Tangier) (n = 4)</th>
<th>ApoA-I (n = 4)</th>
<th>ApoA-II(Tangier) (n = 3)</th>
<th>ApoA-II (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>8.48 ± 0.1</td>
<td>8.63 ± 0.1</td>
<td>8.47 ± 0.1</td>
<td>4.02 ± 0.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.53 ± 0.1</td>
<td>4.08 ± 0.1</td>
<td>7.80 ± 0.1</td>
<td>7.86 ± 0.1</td>
</tr>
<tr>
<td>Serine</td>
<td>8.13 ± 0.1</td>
<td>6.20 ± 0.1</td>
<td>7.60 ± 0.1</td>
<td>7.76 ± 0.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>17.13 ± 0.1</td>
<td>19.23 ± 0.2</td>
<td>20.57 ± 0.1</td>
<td>20.78 ± 0.2</td>
</tr>
<tr>
<td>Proline</td>
<td>5.05 ± 0.1</td>
<td>4.40 ± 0.2</td>
<td>6.10 ± 0.1</td>
<td>5.97 ± 0.1</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.53 ± 0.2</td>
<td>4.83 ± 0.1</td>
<td>4.13 ± 0.1</td>
<td>4.13 ± 0.1</td>
</tr>
<tr>
<td>Alanine</td>
<td>7.78 ± 0.1</td>
<td>8.10 ± 0.1</td>
<td>6.70 ± 0.1</td>
<td>6.85 ± 0.1</td>
</tr>
<tr>
<td>Valine</td>
<td>5.30 ± 0.1</td>
<td>4.90 ± 0.1</td>
<td>7.57 ± 0.1</td>
<td>7.41 ± 0.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.23 ± 0.1</td>
<td>1.28 ± 0.2</td>
<td>1.17 ± 0.1</td>
<td>1.11 ± 0.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.20 ± 0.1</td>
<td>0.10 ± 0.1</td>
<td>1.10 ± 0.1</td>
<td>1.04 ± 0.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>12.95 ± 0.1</td>
<td>15.68 ± 0.2</td>
<td>10.67 ± 0.1</td>
<td>10.70 ± 0.1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.83 ± 0.1</td>
<td>2.73 ± 0.1</td>
<td>5.20 ± 0.1</td>
<td>5.30 ± 0.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.88 ± 0.1</td>
<td>2.58 ± 0.1</td>
<td>5.21 ± 0.1</td>
<td>5.18 ± 0.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.20 ± 0.1</td>
<td>2.33 ± 0.1</td>
<td>0.09 ± 0.1</td>
<td>0.10 ± 0.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.20 ± 0.3</td>
<td>8.55 ± 0.2</td>
<td>11.67 ± 0.1</td>
<td>11.82 ± 0.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.83 ± 0.2</td>
<td>6.50 ± 0.1</td>
<td>0.29 ± 0.1</td>
<td>0.10 ± 0.1</td>
</tr>
</tbody>
</table>

* The data are presented as mean ± SEM for the indicated number of analyses.
In order to compare the chemical and metabolic properties of apoA-I\textsubscript{Tangier}, purified apoA-I, isolated from normal donors, was fractionated by preparative NaDodSO\textsubscript{4} gel electrophoresis. On Ouchterlony double immunodiffusion apoA-I\textsubscript{Tangier} and apoA-I showed a line of complete immunological identity (Fig. 3A). ApoA-I\textsubscript{Tangier} appeared to have a slightly greater molecular weight than did apoA-I from normal subjects on analytical NaDodSO\textsubscript{4} gel electrophoresis with 15% acrylamide (Fig. 3B). On analytical polyacrylamide gel electrophoresis (pH 8.9) in urea, apoA-I\textsubscript{Tangier} appeared as a doublet, whereas normal apoA-I migrated in the same position as principally a single band (Fig. 3C). On two-dimensional electrophoresis apoA-I\textsubscript{Tangier} had greater heterogeneity of the isoforms than did apoA-I (Fig. 4). The amino acid composition of apoA-I\textsubscript{Tangier} differed from that of apoA-I isolated from normal subjects (Table 1). In summary, apoA-I\textsubscript{Tangier} differed from apoA-I isolated from normal subjects in amino acid composition, apparent molecular weight on NaDodSO\textsubscript{4} gel electrophoresis, migration as a doublet on polyacrylamide disc gel electrophoresis, and heterogeneity of isoforms on two-dimensional polyacrylamide gel electrophoresis.

**Isolation and Characterization of apoA-II\textsubscript{Tangier}**

ApoA-II\textsubscript{Tangier} was purified to homogeneity from Sephacryl S-200 fraction 2 (Fig. 1) by ion-exchange chromatography on DEAE-cellulose (Fig. 5). Sixty milligrams of Sephacryl S-200-purified apoA-II\textsubscript{Tangier} was chromatographed on DEAE-cellulose and the fractions identified by NaDodSO\textsubscript{4} slab electrophoresis as containing apoA-II\textsubscript{Tangier} were pooled, dialyzed, lyophilized, and freed from low molecular weight constituents by chromatography on Sephadex G-15. The yield of apoA-II\textsubscript{Tangier} was 3.9 mg; purified apoA-II\textsubscript{Tangier} gave a single band on NaDodSO\textsubscript{4} gel electrophoresis (Fig. 2C).

For comparison apoA-II was purified to homogeneity from HDL of normal donors (16). ApoA-II\textsubscript{Tangier} and apoA-II showed complete immunochemical identity when analyzed by Ouchterlony immunodiffusion (Fig. 6A). ApoA-II\textsubscript{Tangier} and normal apoA-II had similar electrophoretic positions on NaDodSO\textsubscript{4} gel electrophoresis (Fig. 6B) and polyacrylamide electrophoresis (pH 8.9) in 8 M urea (Fig. 6C). Two-dimensional electrophoresis of apoA-II\textsubscript{Tangier} and apoA-II revealed identical electrophoretic patterns (Fig. 7). The amino acid compositions of apoA-II\textsubscript{Tangier} and apoA-II were virtually identical (Table 1). ApoA-II\textsubscript{Tangier}, therefore, was similar to normal apoA-II in electrophoretic mobility, apparent molecular weight on NaDodSO\textsubscript{4} gel electrophoresis, isoelectric point, and amino acid composition.

**DISCUSSION**

The results presented in this report indicate that apoA-I\textsubscript{Tangier} differed from apoA-I isolated from normal subjects. Subtle diff-

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**Fig. 5.** DEAE-cellulose chromatography of Sephacryl S-200 fraction 2 (Fig. 1). Sixty milligrams of sample was applied to a column (1.2 × 23 cm, 4°C) equilibrated in 0.025 M Tris-HCl/6 M urea (pH 8.0) and eluted with 33 ml of equilibrating buffer. ApoA-II\textsubscript{Tangier} (indicated by bar) was eluted from the column with a 280-ml gradient of equilibrating buffer and 0.1 M Tris-HCl/6 M urea (pH 8.0).

**Fig. 6.** (A) Ouchterlony immunodiffusion of purified apoA-II in well 1 and apoA-II\textsubscript{Tangier} in well 2. Well AB contains goat antibody prepared against apoA-II isolated from normal donors. (B) Polyacrylamide slab gel electrophoresis of purified apoA-II and apoA-II\textsubscript{Tangier} in 0.1% NaDodSO\textsubscript{4}/15% acrylamide. Lane 1, apoA-II\textsubscript{Tangier}; lane 2, apoA-II. (C) Polyacrylamide disc gel electrophoresis (pH 8.9) in 8 M urea. Lane 1, apoA-II\textsubscript{Tangier}; lane 2, apoA-II.

**Fig. 7.** Comparison of the isoforms of apoA-II\textsubscript{Tangier} and apoA-II on two-dimensional electrophoresis. Isoelectric focusing (pH 5.0–5.5) was performed on 10 μg of each protein in the horizontal direction followed by slab gel electrophoresis (18% acrylamide) in 0.1% NaDodSO\textsubscript{4} in the descending direction. An internal standard (I.S.) was included in each sample. To facilitate comparison of the isoforms of apoA-II\textsubscript{Tangier} and apoA-II, the two slab gels were overlayed, the internal standards of each slab gel were aligned, and the apoA-II\textsubscript{Tangier} slab gel was moved vertically 1 cm. (Displacement of the apoA-II\textsubscript{Tangier} slab gel is indicated by the vertical arrow.)
ferences were observed in apparent molecular weight on NaDodSO4 gel electrophoresis, electrophoretic mobility, and heterogeneity of isoforms on isoelectric focusing. The amino acid composition of apoA-ITangier was clearly different from that of normal apoA-I. ApoA-II_Tangier, however, appeared to be identical to normal apoA-II in amino acid composition and physical-chemical and immunological properties. These combined data were interpreted as indicating that apoA-ITangier had a different structural entity from apoA-I; however, apoA-II_Tangier and apoA-II were identical.

To ascertain if the structural change present in apoA-ITangier were of functional significance, a number of in vitro and in vivo studies comparing apoA-ITangier and apoA-I were undertaken. In previous studies from our laboratory we have demonstrated an increased catabolism and relatively normal synthetic rate of both apoA-I and apoA-II in homozygous patients with Tangier disease (8). The increased rate of catabolism of apoA-I and apoA-II persisted despite an increase in the pool size of HDL to near normal plasma levels by HDL infusion (23, 24). These studies on HDL metabolism in patients with Tangier disease were all performed with HDL, apoA-I, and apoA-II isolated from normal subjects. The kinetic studies have now been extended to an analysis of the metabolism of radiolabeled apoA-ITangier and apoA-II_Tangier in normal volunteers and patients with Tangier disease (unpublished data). ApoA-ITangier had a plasma residence time half that of apoA-I in normal subjects; however, similar residence times were observed in Tangier homozygotes. In contrast, the residence times of apoA-II_Tangier and apoA-II were very similar in both normal subjects and Tangier homozygotes (unpublished data). An in vitro analysis of apoA-ITangier was performed with radiolabeled apoA-ITangier, and apoA-I associated with normal or Tangier lipoproteins and rat liver hepatocytes and Kupffer cells. ApoA-ITangier lipoproteins were more rapidly taken up by Kupffer cells and hepatocytes than were apoA-I lipoproteins (25). No significant difference between the uptake of apoA-II and apoA-II_Tangier lipoproteins was observed with either hepatocytes or Kupffer cells.

The combined results from these structural and metabolic studies are interpreted as indicating that apoA-ITangier is structurally and functionally abnormal. We proposed that this structural change in apoA-ITangier is the molecular defect in Tangier disease. ApoA-ITangier may represent a precursor form of circulating apoA-I or an apoA-I resulting from a mutation that was associated with a change in the covalent structure of the protein. The structural change in apoA-ITangier may result in significant alterations in the normal molecular properties and functions of apoA-I. These may include a deficiency in cofactor function (e.g., lecithin cholesterol acyltransferase), a defect in the role of apoA-I as a structural component of HDL (26), or a decrease in protein–protein association with apoA-II and other apolipoproteins (27). On the basis of the results presented in this report we propose that the structural defect in apoA-ITangier ultimately leads to an abnormal catabolism of apoA-ITangier- and apoA-II_Tangier-containing lipoproteins from the plasma. This defect in metabolism produces a delayed clearance of chylomicrons, with the generation of abnormal remnants, which do not generate HDL constituents and are cleared from the plasma by the reticuloendothelial system. This accelerated catabolism as well as the deficiency of normal HDL results in the intracellular accumulation of cholesterol esters, the characteristic lipid deposit in Tangier disease.

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