Plasma Apolipoproteins in Tangier Disease, as Studied with Two-Dimensional Electrophoresis

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Tangier disease is characterized by a deficiency of high-density lipoproteins and of their major protein constituent, apolipoprotein (apo) A-I. We used high-resolution two-dimensional electrophoresis to examine the principal plasma apolipoproteins (A-I, A-II, A-IV, E, C-II, and C-III) of three persons with Tangier disease, one homozygous patient and his two heterozygous children, comparing the patterns with those for healthy subjects. Characteristic abnormalities were found in the distribution of the isoproteins of apo A-I, there being a normal concentration of pro apo A-I but dramatically decreased concentrations of the other apo A-I isoproteins. We also found hitherto-undescribed polypeptide abnormalities in apo C-III: sialylated and nonsialylated forms of apo C-III appear as double spots having the same isoelectric points but different molecular masses. No other substantial difference was detected in the polypeptide distribution of the other plasma apolipoproteins.

Additional Keyphrases: heritable disorders · isoproteins · apolipoproteins A-I and C-III

Tangier disease, an autosomal recessive disorder affecting lipoprotein metabolism, is characterized by a deficiency or absence of high-density lipoproteins (HDLs) and moderate hypertriglyceridemia; low-density lipoproteins are decreased, and chylomicrons are increased.6 Clinically, patients with Tangier disease show hepatomegaly, hyperlipidemia, cholesterol deposits in the tissues, and neuropathy. Apolipoprotein A-I (apo A-I), the major HDL apolipoprotein in a healthy subject, is greatly decreased in Tangier disease. In homozygotes, it is present at only 2% of the normal concentration, in heterozygotes at 62% (7). Furthermore, the apo A-I in affected subjects differs functionally and metabolically from normal apo A-I. Abnormalities in the pattern of the isomers of apo A-I have been demonstrated (2), but the other main apolipoproteins (A-II, C-II, C-III, E, and A-IV) have not generally been studied with regard to this disease.

Here we present two-dimensional electrophoresis (2DE) maps of the main apolipoproteins from three persons with Tangier disease. This method, which separates proteins according to their isoelectric point (pI) and their relative molecular mass (Mr), gives very high resolution (3), and thus is particularly useful for the study of plasma apolipoproteins (4, 5). Although most of the apolipoproteins (A-IV, E, A-II, C-II) do not seem to be altered in the Tangier patients, there are very evident changes in apo A-I as well as in apo C-III.

Materials and Methods

Our subjects with Tangier disease, a homozygous father and his two heterozygous children, came from the Bordeaux region. Results of laboratory and clinical observations of these individuals have already been reported (6). We compared the 2DE results for them with those for healthy subjects who had come to the Center for Preventive Medicine at Vandoeuvre-Les-Nancy for health screening (7).

Blood collected after 12 h of fasting into tubes containing lithium heparin was promptly centrifuged and the plasma was stored at -20 °C until analysis.

2DE was performed as described by Anderson and Anderson (3), slightly modified to provide a map of all of the principal apolipoproteins on a single gel (5). Plasma proteins were denatured by heating at 95 °C for 5 min after having been diluted fivefold with a solution containing 20 g of sodium dodecyl sulfate, 50 mL of 2-mercaptoethanol, and 100 mL of glycerol per liter. We applied 15 μL of samples and electrofocused them in 1 mm (i.d.) × 18 cm tubes. The pH gradient was made with LKB ampholytes (LKB, Uppsala, Sweden; pH 3.5–9.5 = 2%; pH 2.5–4 = 0.16%; pH 9–11 = 0.40%), and electrophoresis in the second dimension was performed on a 18 × 18 cm polyacrylamide slab gel with a gradient range from 10 to 20%, i.e., 100 to 200 g of polyacrylamide per liter. We also used another gradient, from 16 to 20%, to study low-Mr apolipoproteins (apo A-II, C-II, C-III).

After electrophoresis, the peptides were silver stained by the method of Oakley et al. (8), as modified in our laboratory (9). We identified apo A-I, A-II, A-IV, and C-II by comparing their M₉ and pI values with those found in other studies (4). Apo D and C-III were identified after electrotransfer and immunochromic staining (10) with goat antibodies (provided by Prof. J. C. Fruchtart, Institut Pasteur, Lille).

Results

Figure 1a shows the principal apolipoproteins seen on 2DE of the plasma of a normolipemic control subject. Figure 1b depicts the electrophoresis of plasma from the patient homozygous for Tangier disease, who has an apo E 3/2 phenotype. In the normolipemic subject, apo A-I is present as three spots, with pI from 5.4 to 5.9. Isoprotein 2 has a slightly higher M₉ than isoprotein 4 (Figure 2a). The spots corresponding to isoproteins 4, 5, and 6 all have the same M₉.

In the patient with Tangier disease, apo A-I is decreased to four spots, corresponding to isoproteins 2, 3, 4, and 5 (Figure 2b). Isoprotein 3 shows the same M₉ as isoprotein 2. Isoproteins 4 and 5 are present, but in very small amounts. A second abnormality appears in the forms of apo C-III in the patient with Tangier disease. Apo C-III in the control normolipemic subjects (Figure 3a) is always present as the
The sialylated forms C-III-1 and C-III-2 (plus the nonsialylated form C-III-O, when its concentration in plasma is great enough to be detected by silver staining), but in the Tangier patient, each of these forms, the C-III-O as well as two dialylated ones, appears as a double spot (Figure 3b). These double spots have the same pI but different Mr, the heavier protein appearing to be present in the greater quantity.

We confirmed these findings by 2DE of plasma from the patient's two heterozygous children. Both children show the same abnormality of apo C-III-1 and C-III-2; the concentration of apo C-III-O is insufficient for this protein to be seen (Figures 3c, d).

The other apolipoproteins (apo C-II and A-II) appear normal in comparison with the pattern for control subjects.

**Discussion**

Various abnormalities affecting the HDL composition have been described in recent years: fish-eye disease (11), familial lecithin–cholesterol acyltransferase (EC 2.3.1.43; phosphatidylcholine–sterol acyltransferase) deficiency (12), apo A-I Milano deficiency (13), familial apo A-I + C-III deficiency (14), and Tangier disease. All these abnormalities are associated with alterations in certain apolipoproteins.

In Tangier disease, the deficiency of HDL is associated with a quantitatively significant deficiency of apo A-I. The principal circulating forms of apo A-I in normal subjects are isoproteins 4 and 5, which constitute 95% of the total apo A-I; isoproteins 2 and 3 constitute only 2% (2). Isoprotein 2, or "pro-apo A-I," synthesized in the liver or the intestine, is transformed into the mature isoform in the plasma compartment or in the lymph by a protease (15).

Ghiselli et al. (16) suggest that the mature plasma isoproteins of apo A-I in plasma are derived from the precursor isoprotein 3 by deamidation. A deficiency of conversion of pro-apo A-I into mature protein would account for the abnormal HDL and apo A-I metabolism in Tangier disease (17). Indeed, in this case (2) the proportion of isoprotein 2 reached 50%, and that of isoproteins 4 and 5 reached 46%.

**Fig. 1.** Two-dimensional electrophoretic profile of human plasma apolipoproteins: (a) control subject; (b) homozygous patient with Tangier disease

**Fig. 2.** Isoforms of plasma apo A-I: (a) control subject; (b) homozygous patient with Tangier disease

Orientation of 2DE gels as in Fig. 1

**Fig. 3.** Two-dimensional electrophoretic pattern of plasma apo C-III in: (a) control subject; (b) homozygous patient, (c, d) heterozygous children of patient with Tangier disease

Orientation of 2DE gels as in Fig. 1
However, the electric charge and the molecular mass of apo A-I is the same in Tangier disease as in normal subjects (18), and the amino acid sequence of pro apo A-I is also the same (19). Weech et al. (20) recently showed that, for this apolipoprotein, the electrophoretic properties and the immunological reactions with four monoclonal antibodies are the same in patients with Tangier disease as in healthy controls.

Law and Brewer (21) confirmed that the relative increase of pro-apo A-I is the consequence of accelerated catabolism of the A-I isoproteins, owing to a post-translational defect. 2DE, which characterizes proteins by two properties (Mr, and pl), is particularly useful for investigating molecular abnormalities and variants, both in unselected subjects and in patients (4).

Our results for a homozygous patient and two heterozygous subjects confirm the large decrease in isoproteins 4 and 5 of apo A-I. The amount of isofrom 2 appears to be about the same as that of the control subject. Isoform 3 is present just as a single spot.

In our general, presumably healthy population, 20.6% of the subjects have the same 3/2 phenotype as our homozygous patient. Their mean plasma cholesterol concentration is 5.36 mmol/L, significantly lower than the overall mean (5.94 mmol/L). The patient’s cholesterol value was 1.34 mmol/L, and this decreased cholesterol concentration may be related to the presence of allele 2, which could in turn be related to the decreased conversion of very-low-density lipoproteins to intermediate-density lipoproteins catalyzed by lipoprotein lipase (EC 3.1.1.34) (21). On the other hand, the 3/3 phenotype seen in the heterozygous Tangier patients is present in 55.5% of our population (Boerwinkle E, et al., ms. submitted for publication, Ann Hum Genet).

Zannis et al. (2) reported that the concentration of apo C in plasma of a Tangier-disease patient was only half the most prevalent concentration in normal persons, but they observed no modification of charge or Mr. In the three subjects that we studied, apo C-III-1 and C-III-2 were detected as two subunits with the same pl but different Mr. The presence of these abnormal C-III apolipoproteins may account for the increased very-low-density lipoproteins and chylomicrons that are characteristic of Tangier disease, probably by increased inhibition of lipoprotein lipase (22). Furthermore, nascent chylomicrons might contain the pro-apolipoprotein such that conversion of pro-apo A-I to apo A-I occurs only after the protein is released from chylomicrons (13). Apo A-I is rapidly freed from the chylomicrons when the lipoprotein interacts with lipoprotein lipase, which is regulated by apo C-III. Thus there may be a relationship between an abnormal apo C-III and an abnormal metabolism of apo A-I in Tangier disease. One can compare these results with those of Heinen et al. (24), who found that all classes of lipoproteins in Tangier disease present abnormalities. Study of the amino acid sequences of these proteins should reveal any changes in primary structure.

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References