Characterization of apoA- and apoB-containing lipoprotein particles in a variant of familial apoA-I deficiency with planar xanthoma: the metabolic significance of LP-A-II particles

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Abstract This study describes a variant of familial apoA-I deficiency associated with a moderate risk for premature coronary artery disease. The proband, a 25-year-old man of Philippine origin, and his 62-year-old maternal aunt had peripheral corneal opacification, xanthelasma, and planar xanthoma; the aunt had coronary artery bypass surgery at 61 years of age. Proband's parents and three brothers were asymptomatic and apparently healthy. The characteristic apolipoprotein features of affected patients were the immunochemically and chemically undetectable apoA-I, reduced levels of apoA-II, apoC-II, apoC-III, and apoD, and normal levels of apoB and apoE; except for negligible levels of high density lipoprotein (HDL)-cholesterol (2–3 mg/dl), their plasma lipid profile was normal. The apoA-I levels in all five unaffected relatives were more than one SD below the normal mean values for their age and sex; the HDL-cholesterol levels of proband's unaffected brothers were below the 10th percentile of normal control values. Patient's very low density lipoprotein (VLDL), low density lipoprotein (LDL), and HDL contained 1.4, 80.4, and 18.1%, whereas those of control subjects contained 2.7, 28.8, and 68.1% of the total apolipoprotein mass, respectively. In unaffected relatives, the levels of LP-A-I, but not LP-A-I:A-II, were significantly lower than in controls. Neither of the two patients had detectable concentrations of LP-A-I or LP-A-I:A-II. Their HDL only consisted of LP-A-II particles, the levels of which (7–13 mg/dl) were similar to those of unaffected relatives or controls. There was no difference in the lipid composition of LP-A-II between patients and their relatives. However, LP-A-II from patients contained substantial amounts of apoC-peptides and apoE (0.40–0.98 mg/mg apoA-II), whereas those from unaffected relatives were free of these minor apolipoproteins. In patients, among all four major apoB-containing lipoproteins, only the levels of LP-B and LP-B:C were slightly higher than those in controls. Results of this study suggest a genetic cause for this variant of apoA-I deficiency characterized most probably by autosomal recessive inheritance. It appears that patients are likely to be homozygous for a gene present in single dose in the parents and brothers of the affected proband. Results also show that, in the absence of LP-A-I and LP-A-I:A-II, LP-A-II particles become efficient acceptors and/or donors of apoC-peptides and apoE and suggest that severe deficiency of apoA-I with mild deficiency of other apolipoproteins imparts a moderate risk for premature coronary artery disease. —**Bekaert, E. D., P. Alaupovic, C. S. Knight-Gibson, M. J. Laux, J. M. Pelachyk, and R. A. Norum.** Characterization of apoA- and apoB-containing lipoprotein particles in a variant of familial apoA-I deficiency with planar xanthoma: the metabolic significance of LP-A-II particles. *J. Lipid Res.* 1991. **32:** 1587–1599.

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High density lipoproteins (HDL) of human plasma constitute a mixture of several polydisperse lipoprotein families of particles, each of which is characterized by distinct apolipoprotein composition (1, 2). The presence of lipoprotein particles of similar density properties but distinct chemical composition appears to be the main, if not the sole, reason for the well-documented chemical, immunochemical, and metabolic heterogeneity of this operationally defined class of lipoproteins (1–12). Studies from several laboratories have established that lipoprotein A-I:A-II (LP-A-I:A-II) and lipoprotein A-I (LP-A-I) are the two main HDL families of particles (1, 2, 6, 7, 10–16). There is also some evidence that lipoprotein A-II (LP-A-II) may represent the third minor family of apoA-containing lipoproteins (15, 17, 18). Although a definitive proof for

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; VHDL, very high density lipoproteins; EDTA, ethylenediamine tetraacetate; PWX, patients with xanthomas; RWOX, relatives without xanthomas.

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functional roles of these particles is still lacking, it appears that LP-A-I:A-II particles may be the main acceptors of lipids and apolipoproteins released during the lipolysis of triglyceride-rich lipoproteins (19) and that LP-A-I may function as the acceptor of peripheral cholesterol (5, 10).

The recognition of several genetic deficiencies of HDL particles has provided new means for studying their functional and metabolic properties as well as their potential role in development of coronary artery disease (20, 21). The most common among HDL deficiency syndromes is the familial hypoalphalipoproteinemia (22, 23) accounting for approximately 7% of patients with premature coronary artery disease (24). A number of rare HDL deficiencies characterized by significant reductions in the levels of HDL lipids and apolipoproteins include Tangier disease (25), HDL deficiency with planar xanthoma (26, 27) apoA-I/apoC-III deficiency (28), apoA-I/apoC-III/apoA-IV deficiency (29, 30), apoA-I Milano variant (31), fish eye disease (32), LCAT-deficiency (33, 34), apoA-I deficiency (35, 36), and apoA-II deficiency (37). In some of these deficiency syndromes the molecular defects have been identified (29–31, 35–38), whereas in others they still remain to be elucidated (25–27, 32–34). One of the most interesting aspects of these studies is the observation that decreased concentrations of HDL particles are associated with increased risk of premature coronary artery disease in some but not in all of the recognized HDL deficiency states. With the exception of a study on apoA-I-containing lipoprotein particles in apoA-I Milano variant (39), there have been no attempts to characterize HDL deficiency diseases in terms of their lipoprotein particle spectrum or to identify any possible compositional abnormalities that may or may not be related to increased risk of coronary artery disease.

We have recently identified a case of familial apoA-I deficiency syndrome associated with planar xanthoma, corneal opacity, and a moderate risk for premature coronary artery disease. The purpose of this report is to describe two affected patients and five of their relatives and to present data on the abnormal concentration and composition of their apoA- and apoB-containing lipoprotein particles.

MATERIALS AND METHODS

Patients

The proband (patient III-1, **Fig. 1**) was a 25-year-old man of Philippine origin residing in Ann Arbor, Michigan. During an examination for an inflammatory allergic reaction of the eyes, peripheral corneal opacification and xanthelasma of the inner, lower eyelids of both eyes were noted. The skin of both antecubital fossae had yellowish papules which on biopsy showed foam cells (**Fig. 2**). These lesions were first noted by the patient at age 12. The patient also had a thickened, roughened texture of the skin on his face, the back of his neck and, to a lesser extent, on the rest of the skin surfaces. His tonsils were normal in appearance and there were no tendon xanthomas. His height (175 cm) and weight (63 kg) were normal. The proband's 62-year-old maternal aunt (patient II-7) was the only family member with skin lesions similar to those found in the proband. Clinical symptoms of coronary artery disease were noted when she was 58 years of age and coronary bypass surgery was performed 3 years later. Her menopause began at 52 years of age. There was no evidence of peripheral atherosclerosis. The proband's paternal grandmother died at age 50 from rheumatic heart disease. The proband's paternal grandfather (80 years old), maternal grandparents (83 and 80 years of age), father (II-4), mother (II-5), three brothers (aged 19, 20, and 23 years; III-2, III-3, and III-4), three paternal aunts, two maternal uncles, and two other maternal aunts all had no xanthomas or symptoms of atherosclerosis.

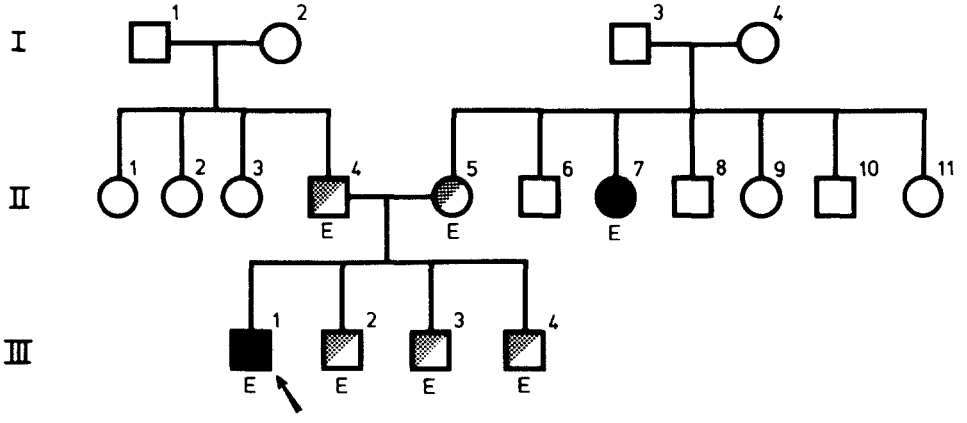

Fig. 1. Pedigree of the family with the apoA-I deficiency. The proband is designated by the arrow, patients with xanthoma (PWX) are designated by a closed symbol, and sampled family members without xanthoma (RWOX) by a half-filled symbol. Subjects who have not been sampled are designated by an open symbol.

Plasma samples

Venous blood was drawn into EDTA-containing Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) from patients and relatives after an overnight fast of 12–14 h. Plasma was recovered by low-speed centrifugation (1000 *g*) for 10 min at 4°C. Preservatives were added to final concentrations of 500 units/ml penicillin-G, 50 μg/ml streptomycin sulfate, 1.3 mg/ml ϵ-amino caproic acid, and 0.5 mg/ml reduced glutathione (40).

Isolation of lipoprotein density classes

Lipoprotein density classes were isolated by sequential preparative ultracentrifugation using Quick-Seal tubes (Beckman, Palo Alto, CA) according to a previously described procedure (41). Very low density lipoproteins (VLDL), LDL, and high density lipoprotein subclasses (HDL_2 and HDL_3) were isolated at solution densities 1.006 g/ml, 1.063 g/ml, 1.125 g/ml, and 1.21 g/ml, respectively. The infranatant fraction at $d > 1.21$ g/ml was considered as very high density lipoproteins (VHDL).

Preparation of immunosorbers

Monoclonal antibodies F59 4A12 2F4 (anti-apoA-I), CdB_5 (anti-apoA-II), and D_6 (anti-apoB) were produced, purified, and characterized as previously described (42–44). The coupling of monoclonal antibodies to the cross-linked agarose activated with N-hydroxysuccinimide (Affi-Gel 10, Bio-Rad Laboratories, Richmond, CA) and the preparation of immunosorbers were previously described (45, 46). The binding capacities of the anti-apoA-I, anti-apoA-II, and anti-apoB immunosorbers were 0.018 mg of apoA-I, 0.027 mg of apoA-II, and 0.052 mg of apoB per ml of gel, respectively. The immunosorbers were stable for more than 6 months at 4°C without loss of binding capacity.

Isolation of apoA-I- and apoA-II-containing lipoprotein particles

Apolipoprotein A-I is present in LP-A-I:A-II and LP-A-I, two major HDL lipoprotein families of particles. They were isolated from whole plasma by immunoaffinity chromatography on anti-apoA-I and anti-apoA-II immunosorbers as previously described (15, 16).

Apolipoprotein A-II occurs in the form of three distinct lipoprotein families of particles including LP-A-I:A-II, LP-A-II, and LP-A-II:B:C:D:E (abbreviated as LP-A-II:B complex) (15, 17, 18, 43, 47). Thus, the fractionation of apoA-II-containing lipoproteins involves a three-step immunoaffinity chromatographic procedure. To isolate these lipoprotein families, plasma (4 ml) was first applied on the anti-apoA-II immunosorber and incubated overnight at 4°C. The unretained lipoproteins and proteins were eluted with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 g/ml EDTA. The retained lipoproteins (apoA-II-containing lipoproteins) were desorbed with 3 M NaSCN and eluted at a flow rate of 30 ml/h. The retained fraction was concentrated in dialysis bags using polyvinyl-pyrrolidone PVP 40 (Sigma, St. Louis, MO) outside the bags and applied to the anti-apoB immunosorber without overloading the binding capacity of the immunosorber. After an overnight incubation at 4°C, the retained and unretained fractions were eluted as described in the previous step. The retained fraction contained only LP-A-II:B complex particles, whereas the unretained fraction consisted of LP-A-I:A-II and LP-A-II particles. The concentrated unretained fraction was applied to the anti-apoA-I immunosorber and the retained (LP-A-I:A-II) and unretained (LP-A-II) fractions were eluted and concentrated as described above. This last step was only required for the fractionation of apoA-II-containing lipoproteins from relatives without xanthomas (RWOX).

Sequential immunoprecipitation of apoB-containing lipoprotein particles

Distribution of apoB among recognized apoB-containing lipoprotein families LP-B, LP-B:C, LP-B:C:E, and LP-A-II:B complex (43, 47–49) was estimated by a procedure based on the separation of LP-A-II:B complex by immunoaffinity chromatography on an anti-apoA-II immunosorber and fractionation of remaining apoB-containing lipoproteins by sequential immunoprecipitation with polyclonal antisera to apoE and apoC-III (43, 47, 48). This procedure was applied to LDL fractions (d 1.006–1.063 g/ml) isolated from both patients. The LP-A-II:B complex was separated from other apoB-containing lipoproteins by immunoaffinity chromatography on an anti-apoA-II immunosorber as described in the previous paragraph. The unretained fraction was concentrated to approximately one-third of its volume and used in the next step for the sequential immunoprecipitation of apoB-containing lipoproteins according to a previously described procedure (48, 50). Concentrations of apoB-containing lipoprotein families are expressed only in terms of apoB (mg/dl). The percentage content of apoB in LP-A-II:B complex, LP-B:C:E + LP-B:E, LP-B:C, and LP-B was calculated from the sum of apoB concentrations of these lipoprotein families. ApoB was measured by electroimmunoassay.

Lipid and apolipoprotein analyses

Total cholesterol and triglycerides in the plasma were determined by enzymic procedures as previously described (51). HDL-cholesterol was estimated according to a modified heparin-manganese precipitation procedure (52). These three assays were standardized with serum calibrators and control samples supplied by the Centers for Disease Control, Atlanta, GA. Neutral lipids of the isolated lipoprotein families were estimated by the gas-liquid chromato-

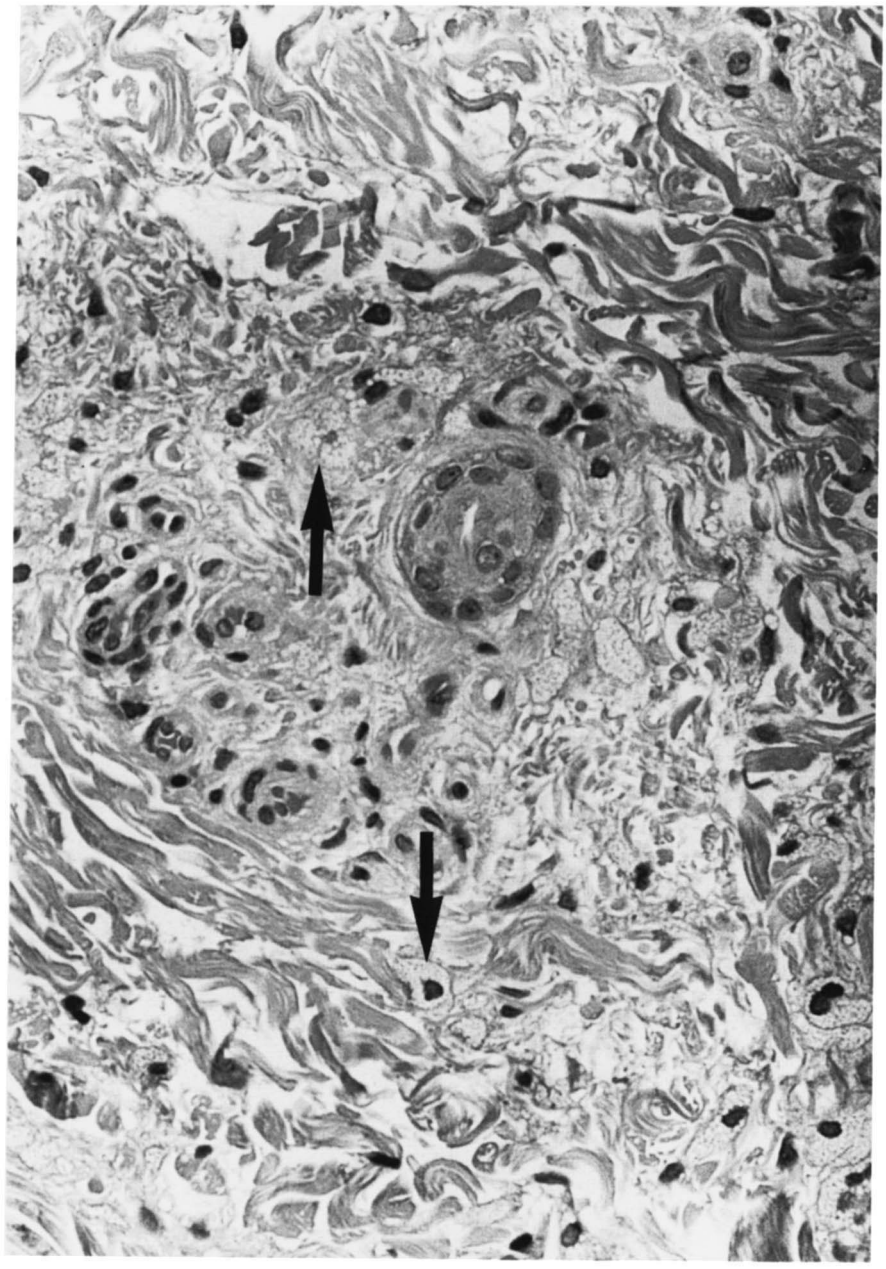

Fig. 2. Cutaneous xanthoma in the proband (×400). The arrows indicate vacuolated histiocytes in the dermis. Formalin-fixed tissue was stained with hematoxylin and eosin.

graphic procedure of Kuksis et al. (53) and phospholipids by the method of Gerlach and Deuticke (54).

Apolipoproteins A-I, A-II, B, C-II, C-III, D, and E were quantified by previously described electroimmunoassays developed in this laboratory (50, 51).

Immunoelectrophoresis of whole plasma was performed in 1% agarose gels as previously described (41). Isoelectric focusing of delipidized whole plasma was carried out in agarose–urea–Pharmalyte (pH 4.0–6.5) system as described by McDowell, Wisdom, and Trimble (55). Protein bands were stained with Coomassie Brilliant Blue-R dissolved in Universal solvent mixture (methanol–acetic acid–water 40:10:50, v/v/v).

RESULTS

Plasma lipids and apolipoproteins

Concentrations of plasma lipids and apolipoproteins in members of this kindred are presented in **Table 1.** Two patients with xanthomas (PWX) had plasma total cholesterol values that were 50th (patient 1) and 10th (patient 2)

TABLE 1. *Concentrations of plasma lipids in patients with familial apoA-I deficiency*

Subjects	Total Cholesterol	Triglycerides	Phospholipids	HDL-Cholesterol
		mg/dl		
Patients with xanthomas				
Patient 1 (III-1)	198.0	34.5	327.0	2.0
Patient 2 (II-7)	184.0	73.9	303.0	NM[a]
Family members without xanthomas				
Father of patient 1 (II-4)	293.0	181.0	369.0	NM
Mother of patient 1 (II-5)	300.0	53.0	396.0	NM
Brother of patient 1 (III-2)	174.0	75.0	NM	29
Brother of patient 1 (III-3)	222.0	90.0	NM	34
Brother of patient 1 (III-4)	225.0	110.0	NM	33
Mean ± SD	243.0 ± 53	101.8 ± 49.0		32.0 ± 2.6
Control subjects				
Normolipidemic men (n = 27)[b]	204 ± 34.0	97.8 ± 33.3	305 ± 43.8	46.4 ± 6.9
Normolipidemic women (n = 34)	191 ± 32.1	82.9 ± 33.5	300 ± 24.2	61.1 ± 13.9

[a]NM, not measured due to insufficient amount of plasma.
[b]Normolipidemic men and women, 30–60 years of age, were employees of the Oklahoma Medical Research Foundation. All subjects were healthy, asymptomatic Caucasians with no history of familial dyslipoproteinemias or diabetes; mean ± SD.

percentiles of normal control values for their age and sex (56). Their relatively low plasma triglyceride levels were within the 5–10th percentiles of normal control levels (56), whereas their plasma phospholipid concentrations were slightly higher (10–15%) than those considered to be within the normal range for their age and sex (57). The concentration of proband's HDL-cholesterol was negligible. Although the HDL-cholesterol of patient 2 was not measured due to insufficient amount of plasma available for lipid and apolipoprotein analyses, the fact that her apoA-I was undetectable would suggest an equally low value for HDL-cholesterol. Plasma lipid profile of proband's parents was characterized by increased concentrations of total cholesterol (above 95th percentile of normal control values for their age and sex), increased concentrations of phospholipids, and normal to slightly elevated levels of triglycerides. Plasma cholesterol levels of proband's brothers were between the 90th and 95th percentiles of normal control values for their age. Their triglyceride concentrations were within the normal range. However, all three brothers had HDL-cholesterol levels below the 10th percentile of normal control values for their age and sex.

The percentage of total cholesterol in the esterified form was slightly decreased in patients 1 and 2 (65.2 ± 1.0%, mean ± SD) but within the normal range in other family members (70.5 ± 1.2%).

Apolipoprotein A-I was undetectable by electroimmunoassay in plasma from either patient 1 or 2 (**Table 2**). They also had markedly reduced levels of apolipoproteins A-II, C-III, and D; the concentration of apoA-II was 10–20% and those of apoC-II, apoC-III, and apoD were 30–50% of the mean of concentrations found in normolipidemic controls (49). In contrast, the levels of apoE

TABLE 2. Concentrations of plasma apolipoproteins in patients with familial apoA-I deficiency

Subjects	Apolipoproteins						
	A-I	A-II	B	C-II	C-III	D	E
				mg/dl			
Patients with xanthomas							
Patient 1 (III-1)	<0.05	14.0	94.6	1.4	3.3	6.7	11.7
Patient 2 (II-7)	<0.05	7.8	138.6	1.1	2.2	6.7	11.2
Family members without xanthomas							
Father of patient 1 (II-4)	106.1	73.9	110.1	2.8	10.1	15.1	14.9
Mother of patient 1 (II-5)	118.1	80.5	151.0	1.8	8.3	16.4	17.5
Brother of patient 1 (III-2)	85.1	75.6	95.7	2.1	9.0	11.1	14.5
Brother of patient 1 (III-3)	86.3	86.8	93.1	2.2	10.6	17.4	20.2
Brother of patient 1 (III-4)	96.5	84.3	115.4	3.2	12.9	14.4	18.3
Mean ± SD	98.4 ± 13.9	80.2 ± 5.5	113.0 ± 23.2	2.4 ± 0.5	10.2 ± 1.7	14.8 ± 2.4	17.0 ± 2.4
Control subjects							
Normolipidemic men (n = 27)[a]	122.7 ± 16.4	60.9 ± 11.7	115.8 ± 29.7	3.1 ± 0.8	7.8 ± 1.8	9.5 ± 2.3	11.4 ± 3.8
Normolipidemic women (n = 34)	149.5 ± 21.8	74.3 ± 15.5	93.8 ± 22.0	3.6 ± 1.3	8.2 ± 2.1	8.4 ± 1.6	12.9 ± 4.9

[a]Control subjects are described in the footnote to Table 1; mean ± SD.

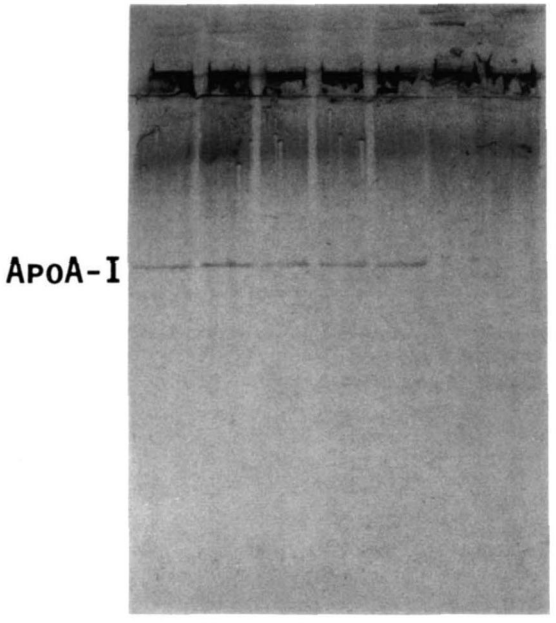

Fig. 3. Isoelectric focusing of delipidized whole plasma. From left to right, plasma from proband's brothers (lanes 1, 2, and 3), plasma of a normolipidemic subject (lane 4), apoA-I standard (lane 5), and proband's plasma (normal concentration in lane 6 and a threefold concentrated sample in lane 7). Protein bands were stained with Coomassie Brilliant Blue-R.

were normal; the concentration of apoB was within the normal range in patient 1 but elevated in patient 2. The levels of apoA-I in all five RWOX were more than one standard deviation below the normal mean values for their age and sex (49). The levels of apoE were increased in all five RWOX, whereas the concentrations of apoB were in the upper normal range for male subjects but markedly increased in proband's mother. The concentrations of apoC-II and apoC-III were normal, but the concentrations of apoA-II and apoD were slightly to moderately elevated in all five RWOX.

To confirm the immunochemically determined absence of apoA-I, proband's plasma samples were tested by agarose immunoelectrophoresis (41) and isoelectric focusing (55). Proband's immunoelectrophoretic pattern (not shown) revealed no immunoprecipitin lines against anti-apoA-I serum either in the cathodal or anodal regions; a positive precipitin line of proband's plasma against anti-apoA-II was only present in the anodal region. In contrast, plasma sample from one of proband's brothers (III-4) gave positive precipitin lines both with anti-apoA-I and anti-apoA-II sera. As shown in **Fig. 3,** isoelectric focusing of proband's plasma followed by staining with Coomassie Brilliant Blue-R showed the absence of an apoA-I protein band. On the other hand, the apoA-I protein band was detected in plasma of all three proband's brothers.

Distribution of apolipoproteins in lipoprotein density classes of PWX

There were several marked differences in the apolipoprotein distributions between the proband and previously described normolipidemic, asymptomatic subjects (58). Although 94% of the total apoA-II resided in lipoproteins with d > 1.063 g/ml, one-third of that amount was present in the VHDL fraction (d > 1.21 g/ml). The most unusual feature of apolipoprotein distribution pattern in PWX was the high accumulation of apolipoproteins C-II, C-III, and E in LDL (53–73% of the total apolipoprotein contents) in comparison with normal controls (7–22% of the total apolipoprotein contents of C-II, C-III, and E). As a consequence, very small percentages of apoC-II and apoC-III were detected in VLDL (6–14%) and HDL (21–22%) of PWX; the percentage of apoE was very small in VLDL but normal in HDL. ApoB was detected almost quantitatively in LDL with minimal amounts occurring in VLDL and HDL_2. Whereas in normal subjects almost 95% of apoD was present in HDL and VHDL (58), in PWX more than two-thirds of apoD was detected in LDL. Another way to illustrate the abnormal distribution of apolipoproteins in major lipoprotein density classes of PWX is to compare the percentage distribution of total apolipoprotein mass in density classes of patients and controls; such calculation shows that patients' VLDL, LDL, and HDL contained 1.4, 80.4, and 18.1%, whereas those of control subjects contained 2.7, 28.9, and 68.1% of the total apolipoprotein mass, respectively.

Characterization of apoA-II-containing lipoprotein particles in PWX

In plasma of PWX, approximately 5% of the total apoA-II was present in LP-A-II:B complex and 95% in the form of LP-A-II particles devoid of apoB. The concentration of apoA-II in LP-A-II particles of PWX was similar to those of RWOX and normal controls (**Table 3**). The protein moiety (expressed as the sum of all separately measured apolipoproteins) accounted for 49.3 and 35.5% of the total lipoprotein mass of LP-A-II particles in patients 1 and 2, respectively. The lipid composition of LP-A-II particles differed from that of normal LP-A-II particles by a significantly higher percentage of triglycerides and a lower percentage of phospholipids (**Table 4**); it was similar to the lipid composition of LP-A-II particles from RWOX except for a slightly higher ratio of cholesterol esters/free cholesterol. However, the cholesteryl ester/free cholesterol ratio in LP-A-II particles of patients 1 and 2 was lower than those of LP-A-I and, especially, LP-A-I:A-II particles of both the RWOX and normal controls.

Apolipoprotein A-II was the main apolipoprotein constituent of LP-A-II particles from both patients (**Table 5**). However, in sharp contrast to the apolipoprotein composi-

TABLE 3. Concentrations of apoA-I and apoA-II associated with LP-A-I, LP-A-II, and LP-A-I:A-II particles in patients with familial apoA-I deficiency

	ApoA-I		ApoA-II	
	LP-A-I	LP-A-I:A-II	LP-A-II	LP-A-I:A-II
	mg/dl (%)		*mg/dl (%)*	
Patients with xanthomas				
III-1			13.0	
II-7			7.0	
Relatives without xanthomas[a] (n = 3)				
Mean ± SD	25.2 ± 4.2 (23.6 ± 4.5)	82.3 ± 10.9 (76.4 ± 4.5)	10.7 ± 5.5 (13.9 ± 7.7)	67.3 ± 8.8 (86.1 ± 7.7)
Control subjects[b] (n = 15)				
Mean ± SD	47.2 ± 13.4[c] (31.9 ± 8.3)	95.7 ± 6.4 (68.1 ± 8.3)	8.9 ± 4.9 (10.5 ± 5.5)	76.1 ± 10.8 (89.5 ± 5.5)

[a]Relatives without xanthomas include proband's father (II-4), mother (II-5), and one of his brothers (III-2).
[b]Controls consisted of seven men and eight women volunteers randomly selected from a pool of normolipidemic employees of the Oklahoma Medical Research Foundation. All subjects were asymptomatic and healthy Caucasians with no history of familial hyperlipoproteinemias or diabetes. They were all nonsmokers and only two men were regular alcohol drinkers, but their alcohol consumption did not exceed 50 g/week. All women were premenopausal and none was using contraceptive agents. With the exception of one man, age 64 years, the mean age of men (34.8 ± 6.7 years) was very similar to that of women (35.6 ± 8.5 years).
[c]Mean significantly different ($P < 0.05$) from those of relatives without xanthomas by Student's *t*-test.

tion of LP-A-II particles from RWOX and normal controls, approximately 50% of the protein moiety consisted of apolipoproteins C-II, C-III, D, and E; among these apolipoproteins, apoC-peptides were the minor and apoD and apoE the major constituents.

Characterization of apoB-containing lipoprotein particles in PWX

The study on the distribution of apolipoproteins in lipoprotein density classes of patients 1 and 2 showed that LDL contained, in addition to apoB, the greatest proportions of total plasma apoC-peptides, apoD, and apoE. The application of sequential immunoprecipitation to the fractionation of apoB-containing lipoprotein particles in LDL from patients 1 and 2 indicated that the previously identified apoB-containing lipoproteins including LP-A-II:B complex, LP-B:C:E, LP-B:C, and LP-B (43, 47–49) also occurred in patients with apoA-I deficiency (**Table 6**). Among these lipoprotein families, LP-A-II:B complex accounted for less than 5% of the total apoB content of LDL. The major apoB-containing lipoprotein was LP-B accounting for almost 80% of the total apoB; the relative-

TABLE 4. Percent lipid composition of apoA-containing lipoprotein particles from patients with familial apoA-I deficiency

Lipoproteins	Triglycerides	Cholesterol Esters	Free Cholesterol	Phospholipids
		%		
LP-A-I				
Relatives without xanthomas (n = 3)	13.3 ± 6.4*	25.0 ± 5.5	6.0 ± 0.6	55.7 ± 9.6
Controls[a] (n = 14)	2.8 ± 1.6	24.2 ± 6.7	6.1 ± 1.9	66.7 ± 8.4
LP-A-I:A-II				
Relatives without xanthomas (n = 3)	10.2 ± 1.7*	33.4 ± 2.2	4.6 ± 0.5	51.8 ± 1.7
Controls (n = 14)	3.4 ± 1.5	32.3 ± 4.5	5.9 ± 1.1	58.2 ± 5.2
LP-A-II				
Patients with xanthomas				
III-1	5.3	21.3	7.2	66.2
II-7	19.0	15.8	5.0	60.2
Relatives without xanthomas	12.7 ± 8.5*	14.0 ± 2.6	5.9 ± 1.4	67.1 ± 7.8
Controls[b] (n = 5)	0.3 ± 0.05	12.3 ± 3.9	4.3 ± 2.3	82.4 ± 4.8

*Mean significantly different ($P < 0.001$) from those of control subjects by Student's *t*-test.
[a]Control subjects are described in the footnote to Table 3.
[b]Control subjects consisted of three men and two women randomly selected from the pool of normolipidemic employees of the Oklahoma Medical Research Foundation described in the footnote to Table 3.

TABLE 5. Percent apolipoprotein composition of apoA-containing lipoprotein particles from patients with familial apoA-I deficiency

Lipoproteins	Apolipoproteins					
	A-I	A-II	C-II	C-III	D	E
	%					
LP-A-I						
Relatives without xanthomas (n = 3)	80.1 ± 5.5[a]	ND[b]	1.4 ± 1.3	3.1 ± 2.9	2.6 ± 2.3	12.8 ± 2.8
Controls[c] (n = 14)	88.7 ± 6.5	ND	0.6 ± 0.6	2.3 ± 2.5	5.7 ± 3.4	2.3 ± 2.5
LP-A-I:A-II						
Relatives without xanthomas (n = 3)	52.7 ± 1.3	37.2 ± 1.1	0.4 ± 0.2	2.1 ± 2.9	3.9 ± 2.8	3.7 ± 1.6
Controls (n = 14)	51.1 ± 3.7	35.1 ± 4.7	0.9 ± 0.4	2.4 ± 1.7	6.2 ± 1.7	4.2 ± 2.8
LP-A-II						
Patients with xanthomas						
III-1	ND	54.6	0.8	3.5	19.3	21.8
II-7	ND	47.0	ND	2.0	12.3	38.6
Relatives without xanthomas (n = 3)	ND	95.8 ± 2.0	ND	ND	4.1 ± 2.0	ND
Controls (n = 5)	ND	77.0 ± 6.8	ND	ND	22.8 ± 6.8	ND

[a]Mean ± SD.
[b]ND, not detected by electroimmunoassay.
[c]All control subjects are described in the footnote to Table 3.

ly triglyceride-rich LP-B:C:E and LP-B:C particles accounted for 5 and 13% of the total apoB content of LDL, respectively. It appears that the moderately increased levels of plasma apoB in PWX were due to higher concentrations of LP-B and LP-B:C particles in comparison with those of normolipidemic subjects (49). The concentrations of LP-A-II:B complex and LP-B:C:E particles were within the normal range.

Characterization of apoA-containing lipoprotein particles in RWOX

The apoA-I concentrations associated with LP-A-I and LP-A-I:A-II particles in RWOX were found to be lower than those of normolipidemic controls (Table 3). However, a statistically significant difference ($P < 0.05$) was only reached in the case of LP-A-I particles. Consequently, the percentage distribution of apoA-I in LP-A-I:A-II particles was slightly higher in RWOX (76–77%) than in normal controls (68–69%). There was no significant difference between RWOX and control subjects with respect to the concentration and percentage distribution of apoA-II in LP-A-II and LP-A-I:A-II particles (Table 3).

The lipid profiles of LP-A-I, LP-A-II, and LP-A-I:A-II particles from RWOX were characterized by significantly higher percentage contents of triglycerides ($P < 0.001$) and lower relative contents of phospholipids in comparison with those from normal controls (Table 4). The cholesteryl ester/free cholesterol ratio of LP-A-I:A-II particles from both RWOX and normal subjects was higher than those of LP-A-I and LP-A-II particles, reflecting decreasing relative contents of cholesteryl esters rather than free cholesterol in these latter particles. The lipid composition of LP-A-II particles from RWOX was similar to that of PWX.

The apolipoprotein composition of LP-A-I particles from RWOX was characterized by a lower percentage of apoA-I and a higher percentage of apoE in comparison with that of normal controls (Table 6). In contrast, there was no difference in the apolipoprotein composition of LP-A-I:A-II particles between RWOX and normolipidemic subjects. The protein moiety of LP-A-II particles from RWOX consisted of apoA-II as the predominant (96%) and apoD as a minor (4%) apolipoprotein; the LP-A-II family consisted mainly of particles with apoA-II as the sole apolipoprotein and a small amount of particles which contained both apoA-II and apoE. The apolipoprotein profile of LP-A-II family from RWOX was very similar to that of normal controls except for a smaller percentage of apoD. However, its apolipoprotein composition was quite different from the apolipoprotein composition of LP-A-II

TABLE 6. Plasma concentrations and percent distribution of apoB associated with apoB-containing lipoprotein families isolated from LDL of patients with familial apoA-I deficiency

Lipoprotein Particles	LP-A-II:B Complex	LP-B:C:E	LP-B:C	LP-B
	mg/dl (%)			
Patient 1 (III-1)	3.7 (4.1%)	5.2 (5.7%)	12.0 (13.1%)	70.3 (77.1%)
Patient 2 (II-7)	4.2 (3.2%)	6.3 (4.8%)	16.9 (13.0%)	102.9 (79.0%)

TABLE 7. Association of apolipoproteins C and E with apoA-containing lipoprotein particles in patients with familial apoA-I deficiency

Subjects	LP-A-I: ApoC + ApoE[a] / ApoA-I	LP-A-I:A-II: ApoC + ApoE / ApoA-I	LP-A-II: ApoC + ApoE / ApoA-II
		mg/1 mg	
Patients with xanthomas			
Patient 1 (III-1)	0	0	0.49
Patient 2 (II-7)	0	0	0.98
Relatives without xanthomas			
Father of patient 1 (II-4)	0.17	0.08	0.03
Mother of patient 1 (II-5)	0.29	0.12	0.02
Brother of patient 1 (III-2)	0.18	0.04	0
Control subjects[b]			
Normolipidemic men (n = 4)	0.13 ± 0.09[c]	0.10 ± 0.02	0
Normolipidemic women (n = 7)	0.06 ± 0.03	0.15 ± 0.06	0
All subjects (n = 11)	0.08 ± 0.06	0.13 ± 0.06	0

[a]Results are expressed as milligrams of apoC-II, apoC-II, and apoE associated with 1 mg of apoA-I in LP-A-I and LP-A-I:A-II particles and 1 mg of apoA-II in LP-A-II particles.
[b]All control subjects are described in the footnote to Table 3.
[c]Mean ± SD.

particles from PWX; it was characterized by a smaller percentage of apoD and by the absence of apolipoproteins C-II, C-III, and E.

ApoA-containing lipoprotein particles as acceptors of apoC-peptides and apoE

This study has provided the opportunity to further explore one of the previously proposed functions of HDL as the acceptors and/or donors of apoC-peptides and apoE (59–61). More specifically, it has been suggested that LP-A-I:A-II particles might be the acceptors of apoC-peptides and apoE released during the lipolysis of triglyceride-rich lipoproteins (19) and that LP-A-I particles may participate in the reverse cholesterol transport as acceptors of peripheral cholesterol (5, 10). The determination of apoC-II, apoC-III, and apoE levels associated with a given amount of apoA-I in LP-A-I and LP-A-I:A-II particles of normolipidemic subjects (**Table 7**) showed that LP-A-I particles contain greater amounts of minor apolipoproteins in men than in women, but that LP-A-I:A-II particles carry greater amounts of these apolipoproteins in women than in men. However, LP-A-II particles isolated from normolipidemic subjects of either sex were found to be free of apoC-peptides and apoE. In RWOX, minor apolipoproteins were also carried on LP-A-I and LP-A-I:A-II particles with only minute amounts being present on LP-A-II particles. In contrast, the LP-A-II particles from patients 1 and 2 carried relatively large amounts of apoC-II, apoC-III, and apoE (Table 7), suggesting that LP-A-II particles may replace LP-A-I and LP-A-I:A-II particles in one of their normal functions as the acceptors of minor apolipoproteins. To what extent LP-A-II particles may replace LP-A-I particles as acceptors of peripheral cholesterol is less clear, although the relative content of free cholesterol in LP-A-II particles was somewhat higher than in either LP-A-I or LP-A-II particles from RWOX and normal controls (Table 4).

DISCUSSION

The growing number of reported familial HDL disorders may now be classified into two categories of deficiency syndromes characterized either by the partial or complete deficiency of immunoreactive apoA-I. Under this classification system, the former category encompasses five recognized HDL deficiencies including Tangier disease (25), HDL deficiency with planar xanthoma (26, 27), fish-eye disease (32), apoA-I Milano variant (31), hypoalphalipoproteinemia (22, 23), and apoA-I deficiency with corneal opacity (36). The latter category includes apoA-I/apoC-III deficiency (28), apoA-I/apoC-III/apoA-IV deficiency (29, 30), and a case of apoA-I deficiency (35). The presently reported case of familial HDL deficiency characterized by the absence of apoA-I also belongs to the latter category. On the basis of this criterion, the present variant of apoA-I deficiency clearly differs from all cases of partial apoA-I deficiency as well as from apoA-I/apoC-III and apoA-I/apoC-III/apoA-IV deficiencies. Clinically, two members of this kindred are characterized by corneal opacity, planar xanthoma, and xanthelasma. The finding of coronary artery disease in one of the two subjects at the age of 58 years may indicate the promotion of coronary atherosclerosis in the apolipoprotein deficiency. In comparison, the other reported case of apoA-I deficiency (35) was also characterized by the absence of apoA-I, reduced

levels of apoA-II, planar xanthoma, and an increased risk of premature coronary artery disease. However, in contrast to the present case, the apoC-III levels were reported to be normal and corneal opacity and xanthelasma were not detected.

Both patient 1 and patient 2 have two unusual abnormalities, planar xanthoma and undetectable apoA-I protein. The finding of an unusual trait in relatives suggests a genetic cause for the trait, especially when the trait involves severe deficiency of a protein. Several modes of inheritance could explain the occurrence of a rare trait in the proband and his unaffected mother's sister, including autosomal dominant with variable penetrance, and polygenic inheritance. The finding of lipoprotein abnormalities, especially a large reduction of the fraction of apoA-I associated with LP-A-I particles, in both parents and the brother of the man with xanthoma suggests to us that autosomal recessive inheritance is the most likely. Both patients 1 and 2 are likely to be homozygous for a gene present in single dose in the parents and brothers of the affected man. Analysis of the gene for apoA-I may indicate whether this interpretation is correct.

Although low concentrations of HDL and partial or total absence of apoA-I are the most characteristic biochemical features of all known HDL deficiencies, there are considerable differences rather than uniformity in the clinical symptoms characteristic of these deficiency states. In general, a complete deficiency of apoA-I seems to predispose affected patients to an earlier and more severe onset of coronary artery disease than the partial deficiency of apoA-I. The severity of premature coronary artery disease is further enhanced by a simultaneous complete deficiency of apoC-III as exemplified by both apoA-I/apoC-III and apoA-I/apoC-III/apoA-IV deficiencies. The presence of apoC-III in the affected patients of the kindred presented here and the other reported case of apoA-I deficiency may be one of the possible reasons for a delayed onset of coronary artery disease in these patients.

This study represents the first attempt at characterizing a case of familial HDL deficiency by measuring the concentration and composition of apoA- and apoB-containing lipoprotein particles as defined by their apolipoprotein composition. The major finding of this investigation was that HDL of both patients with planar xanthoma consist of LP-A-II particles of normal concentration but abnormal lipid and apolipoprotein composition in comparison with those of RWOX and normal controls. This finding also confirmed the previously reported presence of LP-A-II in Tangier disease (17) and normolipidemic subjects (18). The LP-A-II particles from PWX had high percentages of apoC-peptides, apoD, and apoE, accounting for almost 50% of the total protein content of these particles in contrast with the absence of apoC-peptides and apoE and low percentage of apoD in LP-A-II particles from RWOX and normal controls. The main abnormality of apoA-containing lipoproteins in RWOX was the reduced concentration of LP-A-I rather than LP-A-I:A-II particles.

The levels of potentially atherogenic apoB-containing lipoproteins in LDL of patients with planar xanthoma accounted for more than 95% of the total plasma apoB. The percentage contents of cholesterol-rich LP-B and triglyceride-rich LP-B:C, LP-B:C:E, and LP-A-II:B complex particles were similar in both patients. However, the concentrations of all these particles were higher in patient 2 than in patient 1. The main feature of this lipoprotein particle profile was an almost twofold increase in the levels of LP-B:C particles and decreased levels of LP-B:C:E and LP-A-II:B complex particles in comparison with normolipidemic subjects (49). Although the levels of LP-B are almost 50% lower than those of patients with familial hypercholesterolemia (49), their atherogenic potential may be enhanced in the absence of apoA-I-containing lipoproteins. There is some indication that increased ratios of LP-B:C/LP-A-II:B + LP-B:C:E may be associated with an increased risk of vascular disease (62). The relative contribution of individual apoB-containing lipoproteins to the development of premature coronary artery disease remains to be determined.

It has frequently been emphasized that one of the important aspects of studying rare genetic disorders of lipid transport is the information they provide about the normal metabolic processes (20). What has been learned so far from such studies about the function and metabolism of HDL? Some of the more pertinent findings suggest that apoA-I appears to be essential for the formation of normal HDL, that apoA-I is not essential for LCAT activation, and that apoA-I and/or apoC-III may be important for the intestinal absorption and transport of vitamin E and essential fatty acids (20, 30). The present study confirms the first conclusion, and, in view of normal cholesteryl ester/free cholesterol ratio in patients 1 and 2, the second conclusion. Furthermore, this study has provided additional information pertaining to the previously proposed role of HDL particles as the acceptors and/or donors of apoC-peptides and apoE. It appears from the available data that, both in normolipidemic subjects and RWOX, apoC-peptides and apoE bind preferentially, if not exclusively, to LP-A-I and LP-A-I:A-II particles. However, in the absence of apoA-I-containing lipoproteins, this role seems to be assumed by LP-A-II particles which become efficient acceptors and/or donors of these minor apolipoproteins. In contrast to the effective role of LP-A-II particles as acceptors of apoC-peptides and apoE, their capacity to accept free cholesterol from peripheral cells remains to be established. If, in analogy with LP-A-I:A-II particles, they cannot fulfill this latter function, it is possible that a moderately increased risk of coronary artery disease seen in these patients but not in patients with apoA-II deficiency (37) may be related to the absence of LP-A-I rather than LP-A-I:A-II particles. If substantiated in fur-

ther experiments, this may give further credence to the suggestion that LP-A-I particles represent the protective, anti-atherogenic component of apoA-containing lipoproteins (10, 63).

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REFERENCES

1. Albers, J. J., and F. Aladjem. 1971. Precipitation of ^{125}I-labeled lipoproteins with specific polypeptide antisera. Evidence for two populations with differing polypeptide compositions in human high density lipoproteins. *Biochemistry.* **10:** 3436–3442.
2. Alaupovic, P. 1984. The physiochemical and immunological heterogeneity of human plasma high-density lipoproteins. *In* Clinical and Metabolic Aspects of High-Density Lipoproteins. N. E. Miller and G. J. Miller, editors. Elsevier, Amsterdam. 1–44.
3. Kostner, G. M., and A. Holasek. 1977. The separation of human serum high density lipoproteins by hydroxyapatite column chromatography. Evidence for the presence of discrete subfractions. *Biochim. Biophys. Acta.* **488:** 417–431.
4. Weisgraber, K. H., and R. W. Mahley. 1980. Subfractionation of human high density lipoproteins by heparin-Sepharose affinity chromatography. *J. Lipid Res.* **21:** 316–325.
5. Fielding, C. J., and P. E. Fielding. 1981. Evidence for a lipoprotein carrier in human plasma catalyzing sterol efflux from cultured fibroblasts and its relationship to lecithin: cholesterol acyltransferase. *Proc. Natl. Acad. Sci. USA.* **78:** 3911–3914.
6. Atmeh, R. F., J. Shepherd, and C. J. Packard. 1983. Subpopulations of apolipoprotein A-I in human high-density lipoproteins. Their metabolic profiles and response to drug therapy. *Biochim. Biophys. Acta.* **751:** 175–188.
7. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
8. McVicar, J. P., S. T. Kunitake, R. L. Hamilton, and J. P. Kane. 1984. Characteristics of human lipoproteins isolated by selected-affinity immunosorption of apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA.* **81:** 1356–1360.
9. Nestel, P. J. 1987. High-density lipoprotein turnover. *Am. Heart J.* **113:** 518–521.
10. Barbaras, R., P. Puchois, J. C. Fruchart, and G. Ailhaud. 1987. Cholesterol efflux from cultured adipose cells is mediated by LpA_I particles but not by LpA_I:A_{II} particles. *Biochem. Biophys. Res. Commun.* **142:** 63–69.
11. Ohta, T., S. Hattori, S. Nishiyama, and I. Matsuda. 1988. Studies on the lipid and apolipoprotein compositions of two species of apoA-I-containing lipoproteins in normolipidemic males and females. *J. Lipid Res.* **29:** 721–728.
12. James, R. W., A. Proudfoot, and D. Pometta. 1989. Immunoaffinity fractionation of high-density lipoprotein subclasses 2 and 3 using anti-apolipoprotein A-I and A-II immunosorbent gels. *Biochim. Biophys. Acta.* **1002:** 292–301.
13. Norfeldt, P. I. P., S. O. Olofsson, G. Fager, and G. Bondjers. 1981. Isolation and partial characterization of the lipoprotein families A and A-I from high-density lipoproteins of human serum. *Eur. J. Biochem.* **118:** 1–8.
14. Nestruck, A. C., P. D. Niedmann, H. Wieland, and D. Seidel. 1983. Chromatofocusing of human high density lipoproteins and isolation of lipoproteins A and A-I. *Biochim. Biophys. Acta.* **753:** 65–73.
15. Alaupovic, P., E. D. Bekaert, and E. Koren. 1990. Isolation, characterization and quantitation of subclasses of plasma lipoproteins defined by their apolipoprotein composition. *In* Biotechnology of Dyslipoproteinemias: Applications in Diagnosis and Control. C. Lenfant, R. Paoletti, A. Albertini, and A. L. Catapano, editors. Raven Press, New York. 179–188.
16. Bekaert, E. D., P. Alaupovic, C. Knight-Gibson, P. Blackett, and M. Ayrault-Jarrier. 1991. Composition of plasma apoA-I-containing lipoprotein particles in children and adults. *Pediatr. Res.* **29:** 315–321.
17. Assmann, G., P. N. Herbert, D. S. Frederickson, and T. Forte. 1977. Isolation and characterization of an abnormal high density lipoprotein in Tangier disease. *J. Clin. Invest.* **60:** 242–252.
18. März, W., and W. Gross. 1988. Immunochemical evidence for the presence in human plasma of lipoproteins with apolipoprotein A-II as the major protein constituent. *Biochim. Biophys. Acta.* **962:** 155–158.
19. Alaupovic, P. 1981. David Rubenstein Memorial Lecture: the biochemical and clinical significance of the interrelationship between very low density and high density lipoproteins. *Can. J. Biochem.* **59:** 565–579.
20. Schaefer, E. J. 1984. Clinical, biochemical, and genetic features in familial disorders of high density lipoprotein deficiency. *Arteriosclerosis.* **4:** 303–322.
21. Lusis, A. J. 1988. Genetic factors affecting blood lipoproteins: the candidate gene approach. *J. Lipid Res.* **29:** 397–429.
22. Vergani, C., and A. Bettale. 1981. Familial hypoalphalipoproteinemia. *Clin. Chim. Acta.* **114:** 45–52.
23. Third, J. L. H. C., J. Montag, M. Flynn, J. Freidel, P. M. Laskarzewski, and C. Glueck. 1984. Primary and familial hypoalphalipoproteinemia. *Metabolism.* **33:** 136–146.
24. Ordovas, J. M., F. Civeira, J. Genest, and E. J. Schaefer. 1990. Genetic high density lipoprotein deficiency states. *In* Biotechnology of Dyslipoproteinemias: Applications in Diagnosis and Control. C. Lenfant, R. Paoletti, A. Albertini and A. L. Catapano, editors. Raven Press, New York. 261–273.
25. Fredrickson, D. S. 1964. The inheritance of high density lipoprotein deficiency (Tangier disease). *J. Clin. Invest.* **43:** 228–236.
26. Lindeskog, G. R., A. Gustafson, and L. Enerback. 1972. Serum lipoprotein deficiency in diffuse "normolipidemic" plane xanthoma. *Arch. Dermatol.* **106:** 529–532.
27. Gustafson, A., W. J. McConathy, P. Alaupovic, M. D. Curry, and B. Persson. 1979. Identification of lipoprotein families in a variant of human plasma apolipoprotein A deficiency. *Scand. J. Clin. Lab. Invest.* **39:** 377–387.
28. Norum, R. A., J. B. Lakier, S. Goldstein, A. Angel, R. B. Goldberg, W. D. Block, D. K. Noffze, P. J. Dolphin, J. Edelglass, D. D. Bogorad, and P. Alaupovic. 1982. Familial deficiency of apolipoproteins A-I and C-III and precocious coronary-artery disease. *N. Engl. J. Med.* **306:** 1513–1519.
29. Ordovas, J. M., D. K. Cassidy, F. Civeira, C. L. Bisgaier, and E. J. Schaefer. 1989. Familial apolipoprotein A-I, apo-

lipoprotein C-III, and apolipoprotein A-IV deficiency and premature atherosclerosis due to deletion of a gene complex on chromosome 11. *J. Biol. Chem.* **264:** 16339–16342.
30. Schaefer, E. J., J. M. Ordovas, S. W. Law, G. Ghiselli, M. L. Kashyap, L. S. Srivastava, W. H. Heaton, J. J. Albers, W. E. Connor, F. T. Lindgren, I. Lemeshev, J. P. Segrest, and H. B. Brewer, Jr. 1985. Familial apolipoprotein A-I and C-III deficiency, variant II. *J. Lipid Res.* **26:** 1089–1101.
31. Francheschini, G., C. R. Sirtori, A. Capurso, K. H. Weisgraber, and R. W. Mahley. 1980. A-I_{Milano} apoprotein. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J. Clin. Invest.* **66:** 892–900.
32. Carlson, L. A., and B. Philipson. 1979. Fish-eye disease. A new familial conditions with massive corneal opacities and dyslipoproteinemia. *Lancet.* **II:** 921–924.
33. Norum, K. R., and E. Gjone. 1967. Familial plasma lecithin:cholesterol acyltransferase deficiency. Biochemical study of a new inborn error of metabolism. *Scand. J. Clin. Lab. Invest.* **20:** 231–243.
34. Alaupovic, P., W. J. McConathy, M. D. Curry, H. N. Magnani, H. Torsvik, K. Berg, and E. Gjone. 1974. Apolipoproteins and lipoprotein families in familial lecithin:cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab. Invest.* **33, Suppl. 37:** 83–87.
35. Schmitz, G., and K. Lackner. 1989. High density lipoprotein deficiency with xanthomas: a defect in apoA-I synthesis? *In* Atherosclerosis. VIII. G. Crepaldi, A. M. Gotto, E. Manzato, and G. Baggio, editors. Excerpta Medica, Amsterdam. 399–403.
36. Funke, H., A. von Eckardstein, P. H. Pritchard, M. Karos, J. J. Alvers, and G. Assmann. 1991. A frameshift mutation in the human apolipoprotein A-I gene causes high density lipoprotein deficiency, partial lecithin:cholesterol acyltransferase deficiency, and corneal opacities. *J. Clin. Invest.* **87:** 371–376.
37. Deeb, S. S., K. Takata, R. Peng, G. Kajiyama, and J. J. Albers. 1990. A splice-junction mutation responsible for familial apolipoprotein A-II deficiency. *Am. J. Hum. Genet.* **46:** 822–827.
38. Karathanasis, S. K., E. Ferris, and I. A. Haddad. 1987. DNA inversion within the apolipoproteins AI/CIII/AIV-encoding gene cluster of certain patients with premature atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **84:** 7198–7202.
39. Cheung, M. C., A. V. Nichols, P. J. Blanche, E. L. Gong, G. Franceschini, and C. R. Sirtori. 1988. Characterization of apoA-I-containing lipoproteins in subjects with A-I Milano variant. *Biochim. Biophys. Acta.* **960:** 73–82.
40. Lee, D. M., A. J. Valente, W. H. Kuo, and H. Maeda. 1981. Properties of apolipoprotein B in urea and in aqueous buffers. The use of glutathione and nitrogen in its solubilization. *Biochim. Biophys. Acta.* **666:** 133–146.
41. Alaupovic, P., D. M. Lee, and W. J. McConathy. 1972. Studies on the composition and structure of plasma lipoproteins. Distribution of lipoprotein families in major density classes of normal human plasma lipoproteins. *Biochim. Biophys. Acta.* **260:** 689–707.
42. Petit, E., M. Ayrault-Jarrier, D. Pastier, H. Robin, J. Polonovski, I. Aragon, E. Hervaud, and B. Pau. 1987. Monoclonal antibodies to human apolipoprotein A-I: characterization and application as structural probes for apolipoprotein A-I and high density lipoprotein. *Biochim. Biophys. Acta.* **919:** 287–296.
43. Alaupovic, P., C. Knight-Gibson, C. S. Wang, D. Downs, E. Koren, H. B. Brewer, Jr., and R. E. Gregg. 1991. Isolation and characterization of an apoA-II-containing lipoprotein (LP-A-II:B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia. *J. Lipid Res.* **32:** 9–19.
44. Koren, E., D. Solter, D. M. Lee, Z. Reiner, W. J. McConathy, N. Dashti, and P. Alaupovic. 1986. Characterization of a monoclonal antibody that binds equally to all apolipoprotein and lipoprotein forms of human plasma apolipoprotein B. I. Specificity and binding studies. *Biochim. Biophys. Acta.* **876:** 91–100.
45. Dashti, N., E. Koren, and P. Alaupovic. 1989. Identification and partial characterization of discrete apolipoprotein A-containing lipoprotein particles secreted by human hepatoma cell line HepG2. *Biochem. Biophys. Res. Commun.* **163:** 574–580.
46. Koren, E., C. Knight-Gibson, G. Wen, L. E. DeBault, and P. Alaupovic. 1986. Characterization of a monoclonal antibody that binds equally to all apolipoproteins and lipoprotein forms of human plasma apolipoprotein B. II. Isolation of apolipoprotein B-containing lipoproteins from human plasma. *Biochim. Biophys. Acta.* **876:** 101–107.
47. Alaupovic, P., M. Tavella, J. M. Bard, C. S. Wang, P. O. Attman, E. Koren, C. Corder, C. Knight-Gibson, and D. Downs. 1988. Lipoprotein particles in hypertriglyceridemic states. *Adv. Exp. Med. Biol.* **243:** 289–297.
48. Alaupovic, P., M. Tavella, and J. Fesmire. 1987. Separation and identification of apoB-containing lipoprotein particles in normolipidemic subjects and patients with hyperlipoproteinemias. *Adv. Exp. Med. Biol.* **210:** 7–14.
49. Alaupovic, P., W. J. McConathy, J. Fesmire, M. Tavella, and J. M. Bard. 1988. Profiles of apolipoproteins and apolipoprotein B-containing lipoprotein particles in dyslipoproteinemias. *Clin. Chem.* **34:** B13–B27.
50. Alaupovic, P., C. S. Wang, W. J. McConathy, D. Weiser, and D. Downs. 1986. Lipolytic degradation of human very low density lipoproteins by human milk lipoprotein lipase: the identification of lipoprotein B as the main lipoprotein degradation product. *Arch. Biochem. Biophys.* **244:** 226.
51. Wang, C. S., P. Alaupovic, R. E. Gregg, and H. B. Brewer, Jr. 1987. Studies on the mechanism of hypertriglyceridemia in Tangier disease. Determination of plasma lipolytic activities, k_1 values and apolipoprotein composition of the major lipoprotein density classes. *Biochim. Biophys. Acta.* **920:** 9–19.
52. Warnick, G. R., and J. J. Albers. 1978. A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J. Lipid Res.* **19:** 65–76.
53. Kuksis, A., J. J. Myher, L. Marai, and K. Geher. 1981. Determination of plasma lipid profiles by automated gas chromatography and computerized data analysis. *J. Chromatogr. Sci.* **13:** 423–430.
54. Gerlach, E., and B. Deuticke. 1963. Methode zur Microbestimmung von Phosphat in der Papierchromatographie. *Biochem. Z.* **337:** 477–479.
55. McDowell, I. F. W., G. B. Wisdom, and E. R. Trimble. 1989. Apolipoprotein E phenotype determined by agarose gel electrofocusing and immunoblotting. *Clin. Chem.* **35:** 2070–2073.
56. Heiss, G., I. Tamir, C. D. Davis, H. A. Tyroler, B. M. Rifkind, G. Schonfeld, D. Jacobs, and I. D. Frantz, Jr. 1980. Lipoprotein-cholesterol distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. *Circulation.* **61:** 302–315.
57. Furman, R. H., R. P. Howard, K. Lakshmi, and L. N.

Norcia. 1961. The serum lipids and lipoproteins in normal and hyperlipidemic subjects as determined by preparative ultracentrifugation. Effects of dietary and therapeutic measures. Changes induced by in vitro exposure of serum to sonic forces. *Am. J. Clin. Nutr.* **9:** 73–102.
58. Puchois, P., C. Luley, and P. Alaupovic. 1987. Comparison of four procedures for separating apolipoprotein A- and apolipoprotein B-containing lipoproteins in plasma. *Clin. Chem.* **33:** 1597–1602.
59. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* **25:** 1017–1058.
60. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
61. Blum, C. B. 1982. Dynamics of apolipoprotein E metabolism in humans. *J. Lipid Res.* **23:** 1308–1316.
62. Alaupovic, P., D. H. Blankenhorn, C. Knight-Gibson, M. Tavella, J. M. Bard, D. Shafer, E. T. Lee, and J. Brasuell. 1991. ApoB-containing lipoprotein particles as risk factors for coronary artery disease. *Adv. Exp. Med. Biol.* **285:** 299–309.
63. Puchois, P., A. Kandoussi, P. Fievet, J. L. Fourner, M. Bertrand, E. Koren, and J. C. Fruchart. 1987. Apolipoprotein A-I-containing lipoproteins in coronary artery disease. *Atherosclerosis.* **68:** 35–40.