Familial apolipoprotein A-I and C-III deficiency, variant II[1]

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Abstract The biochemical, clinical, and genetic features were examined in the proband (homozygote) and heterozygotes (n = 17) affected with familial apolipoprotein A-I and C-III deficiency, variant II (previously described as apolipoprotein A-I absence). The proband was a 45-year-old white female with mild corneal opacification and significant three-vessel coronary artery disease (CAD), who died shortly after bypass surgery. Autopsy findings included significant atherosclerosis in the coronary and pulmonary arteries and the abdominal aorta as well as extracellular stromal lipid deposition in the cornea. No reticuloendothelial lipid deposits in the liver, bone marrow, or spleen were noted (unlike Tangier disease). Laboratory features included marked high density lipoprotein (HDL) deficiency and undetectable plasma apolipoproteins (apo) A-I and C-III. The percentage of plasma cholesterol in the unesterified form was normal at 30%. The activity and mass of lecithin:cholesterol acyltransferase (LCAT) were 42% and 36% of normal, respectively, and the cholesterol esterification rate was 43% of normal. Deficiencies of plasma vitamin E and essential fatty acid (linoleic, C18:2) were also noted. Evaluation of plasma lipoproteins and apolipoproteins in 37 kindred members revealed 17 heterozygotes with HDL cholesterol values below the 10th percentile of normal. Of these, all had apoA-I levels more than one standard deviation below the normal mean, and 37.5% had a similar decrease in apoC-III values. Mean (± SD) plasma HDL cholesterol, apoA-I, and apoC-III values (mg/dl) in heterozygotes were 54.0%, 62.4%, and 79.2% of normal, respectively. No evidence of CAD was observed in 10 heterozygotes 40 years of age or less; however, CAD was detected in 3 of 7 heterozygotes over 40 years of age, one of whom died at age 56 years of complications of myocardial infarction and stroke. The inheritance pattern in this kindred was autosomal codominant. ApoA-I isolated from a heterozygote had an isoelectric focusing pattern and amino acid composition similar to normal. Utilizing DNA isolated from two obligate heterozygotes, no abnormalities in the apoA-I or apoC-III genes were detected by Southern blot analysis utilizing specific probes following restriction enzyme digestion. The data indicate that familial apolipoprotein A-I and C-III deficiency, variant II, is similar to variant I (described by Norum et al. 1982. *N. Engl. J. Med.* **306:** 1513–1519), but differs at the clinical level (lack of xanthomas), the biochemical level (lack of detectable apoA-I, lower apoA-II level), and at the gene level. Our data are consistent with the view that apoA-I is essential for HDL formation but not for LCAT activation, and that apolipoproteins A-I and C-III may be important for vitamin E and essential fatty acid intestinal absorption.**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, Y. Lemeshev, J. P. Segrest, and H. B. Brewer, Jr.** Familal apolipoprotein A-I and C-III deficiency, variant II. *J. Lipid Res.* 1985. **26:** 1089–1101.

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High density lipoproteins (HDL) as isolated from human plasma by ultracentrifugation in the density range of 1.063–1.21 g/ml consist (weight %) of approximately

Abbreviations: apo, apolipoproteins; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; CAD, coronary artery disease.

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50% protein, 25% phospholipid, 20% cholesterol, and 5% triglyceride (1). The major protein constituents of HDL are apolipoproteins (apo) A-I and A-II, which account for over 90% of HDL protein mass (2, 3). ApoA-I has been reported to activate lecithin:cholesterol acyltransferase (LCAT), the enzyme responsible for plasma cholesterol esterification (4). ApoA-II has been noted to enhance the enzymic activity of hepatic lipase, an enzyme that plays a role in hydrolysis of lipoprotein triglyceride and phospholipid (5). Minor protein constituents of HDL include apolipoproteins C-I, C-II, C-III, D, E, and F (6–9). ApoC-I has also been reported to enhance LCAT activity (10), apoC-II to activate lipoprotein lipase activity (11, 12), and apoC-III to inhibit receptor-mediated hepatic chylomicron remnant uptake (13), while apoE facilitates this latter process (13–15).

Decreased plasma levels of HDL have been associated with premature coronary artery disease (CAD), while increased levels (hyperalphalipoproteinemia) have been linked with enhanced longevity (16–18). Familial HDL deficiency is observed in severe hypertriglyceridemia (lipoprotein lipase deficiency, apoC-II deficiency, and type V hyperlipoproteinemia) (19, 20), familial LCAT deficiency (21, 22), Tangier disease (23, 24), apoA-I variants (Milano, Marburg, Giessen, Munster 1–3) (25–29), fish eye disease (30), HDL deficiency with planar xanthomas (31), familial hypoalphalipoproteinemia (32, 33), familial apolipoprotein A-I and C-III deficiency (34), and apolipoprotein A-I absence (35). Data on the various HDL-deficient kindreds have been recently reviewed (36). The purpose of the present study was to more fully define the clinical, biochemical, and genetic features in the apolipoprotein A-I absence kindred (35). The data presented support the concept of a genetic linkage between apoA-I and apoC-III (34, 37) and indicate that the apolipoprotein A-I absence kindred (35) has many of the same biochemical and clinical features as familial deficiency of apolipoproteins A-I and C-III, but differs in other respects including the underlying gene abnormality. We have, therefore, renamed this entity familial apolipoprotein A-I and C-III deficiency, variant II.

METHODS

Clinical data and family studies

The proband was a 45-year-old white female residing in Tuscumbia, AL (northwest region of the state), USA, with a 9-month history of exertional substernal chest pain and a 3-year history of corneal cloudiness (35). Some symptomatic improvement had been noted following treatment with propranolol 80 mg po qid, isosorbine dinitrate 10 mg po qid, and sublingual nitroglycerin 1/150 grain prn. The patient also had a 3-year history of intermittent leg pains not clearly related to exercise. There was no history of cigarette smoking or hypertension. The only known previous surgeries had been for the delivery of two normal children by caesarean section, total hysterectomy and salpingo-oophorectomy at age 26 years for uterine fibroid tumors, and removal of cysts in the left breast.

Physical examination revealed a normally developed female, height 157 cm, weight 47.2 kg, blood pressure 110/70 mm Hg, pulse 65/min, and respirations 20/min. Examination of the eyes revealed arcus and diffuse mild corneal opacification documented by slit lamp examination. Bilateral carotid bruits were noted. The patient had a normal oral cavity. No lymphadenopathy, hepatosplenomegaly, or xanthomas were detected. A grade II/VI systolic ejection murmur was heard at the left sternal border, and neurological examination was within normal limits.

Laboratory data were within normal limits except for a reduced plasma cholesterol level of 111 mg/dl, an HDL cholesterol concentration of 1 mg/dl (normal, 50), a decreased vitamin E level of 186 μg/dl (normal, 500–1200), and a slightly prolonged prothrombin time of 13.6 seconds (control, 11.7). Vitamin A levels in plasma were normal. A resting electrocardiogram (ECG) showed frequent premature ventricular contractions, and T wave inversions in leads II, III, and AVF. A graded exercise test was positive for ischemic changes on ECG. Coronary artery catheterization revealed a high grade narrowing of the right (95%) and circumflex coronary arteries (75%), as well as the first diagonal branch of the left anterior descending coronary artery (50%). Some narrowing of the left main and left anterior descending coronary arteries was also noted. The patient had normal left ventricular function.

The patient was referred for bypass surgery to the University of Alabama Medical Center, Birmingham, AL; and four saphenous vein grafts were placed to her right, circumflex, left anterior descending, and first diagonal coronary arteries, respectively. Approximately 12 hr after surgery, while in the intensive care unit, the patient suddenly developed rapid supraventricular tachycardia, became hypotensive, and then asystolic. She could not be resuscitated.

Autopsy examination revealed diffuse corneal opacification, no xanthomas, and a normal oral cavity and tonsils. Gross and microscopic examination of the brain, thyroid gland, liver, gallbladder, spleen, pancreas, bone marrow, kidneys, adrenals, and gastrointestinal tract were within normal limits. No lipid deposition in reticuloendothelial cells was noted in any of these tissues. Examination of the cornea revealed mild diffuse granular lipid deposition in the stroma demonstrated by oil red 0 stain on light microscopy. This deposition was in extracellular locations when viewed by electron microscopy. Examination of the lungs demonstrated a few fibrotic adhesions of the left and right pleural surfaces. The heart weight was 325 grams.

Four saphenous vein grafts were identified starting 3–5 cm above the aortic valve, and attaching to the marginal branch of the circumflex artery, the left anterior descending artery, the first diagonal branch, and the right coronary artery, respectively. All grafts were patent. An 80% narrowing of the right coronary artery of 0.5 cm in length was noted approximately 2 cm from the origin, and an additional 50% narrowing of 1.5 cm in length was noted 6 cm from the origin of this artery. There was no occlusion distal to the graft insertion in this vessel. A 50% narrowing of the left main coronary artery and a 75% narrowing of the left anterior descending coronary artery were observed at its origin. Diffuse severe contraction necrosis of the left ventricle was noted. The descending abdominal aorta had moderate atherosclerosis with significant calcification and ulceration, as did the pulmonary and carotid arteries. Uterus, tubes, and ovaries were absent secondary to previous salpingo-oophorectomy.

A complete family tree is shown in **Fig. 1.** Criteria for designating family members as heterozygotes included being a true relative of the homozygous proband, having a plasma HDL cholesterol of less than the 10th percentile, based on Lipid Research Clinics criteria by age and sex (38), and having an apoA-I concentration more than one standard deviation below the normal mean (see laboratory methods). Information was derived from interviews with family members, their personal physicians, as well as medical record review. The proband's father died at age 76 years of complications relating to surgery for prostate carcinoma, and the proband's mother died at age 72 years of congestive heart failure felt to be secondary to coronary artery disease (CAD). The proband's father had nine siblings, seven of whom were deceased. One sister died at age 86 years of unknown causes. One brother died at age 72 years of a documented myocardial infarction. One sister died at age 65 years of suspected heart disease (sudden death). One brother expired at age 68 years secondary to benign prostatic hypertrophy, bladder obstruction, and septicemia. Another sister died at age 61 years due to cancer (primary site unknown) metastatic to bone. One brother expired at age 32 years following an electrical accident. Another brother died at age 54 years of a suspected myocardial infarction. Autopsies were performed on none of these cases. Two sisters of the proband's father were still alive at age 75 and 78 years, respectively. One of these, age 75, was a heterozygote (see Fig. 1, Tables 1 and 2) with a history of angina. Both parents of the proband's father died of unknown causes at ages 84 and 62 years, respectively.

The proband's mother had eight siblings, six of whom were deceased. One sister died suddenly at age 60 years of a suspected myocardial infarction, and two brothers died at ages 58 and 61 years of age of documented myocardial infarction. One brother and one sister were alive and well at ages 71 and 69 years, respectively, with no history of premature CAD. One brother died at the age of 63 years of emphysema secondary to smoking. One sister died in early childhood of unknown causes. One sister was noted to be a heterozygote, and at age 66 years had a history of angina and palpitations. Both parents of the proband's mother died of heart trouble, with the father expiring at age 53 years of probable myocardial

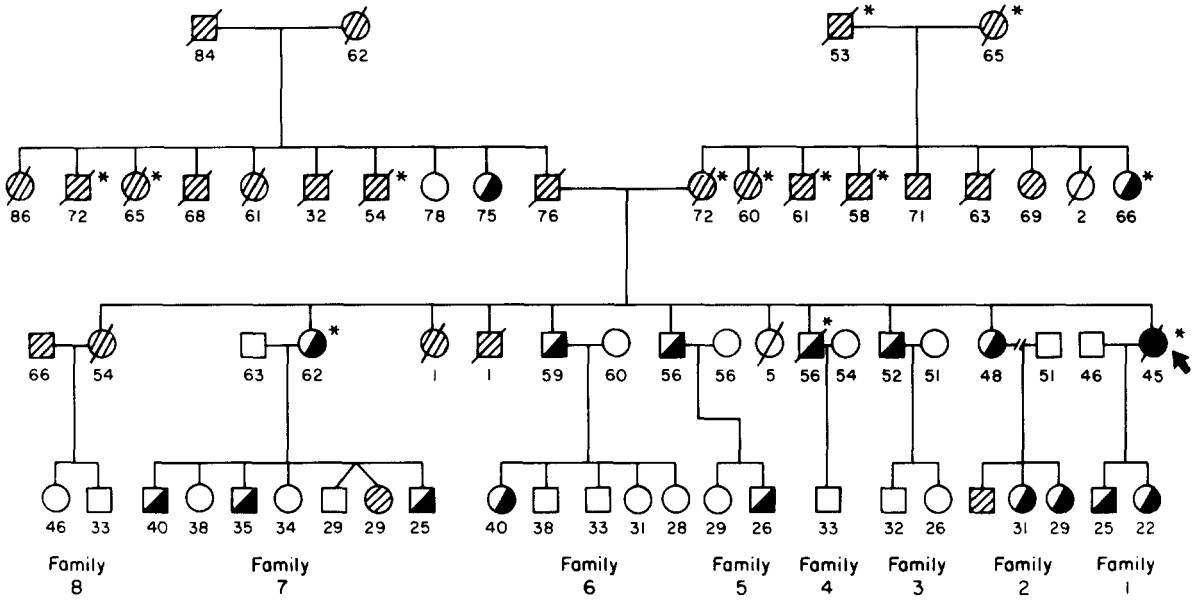

Fig. 1. The complete kindred is shown, with the proband being designated by the arrow, homozygotes designated by a closed symbol (●), heterozygotes by a half closed symbol (◒), and normal subjects by an open symbol (○). Subjects who were not sampled are marked with stripes (⊘), deceased individuals are designated by a large diagonal line, and subjects with documented or possible coronary artery disease by *. The ages of subjects at time of sampling, study, or at death are listed.

infarction, and the mother at age 65 years of hypertension and congestive heart failure.

The proband had ten siblings, three of whom died in early life of probable gastroenteritis, and one of whom died at age 54 years following an automobile accident. One sibling died at age 56 years of coronary artery disease and stroke. He was a diabetic requiring insulin therapy, had a history of hypertension, and was a heterozygote. His first myocardial infarction occurred at age 46 years, resulting in congestive heart failure and a left ventricular aneurysm. Following resection of the aneurysm, the patient had a stroke resulting in a moderate right hemiparesis. At age 56 years, the patient expired following a second myocardial infarction. One additional heterozygous sibling, a sister, had a history of angina and dyspnea on exertion and was 62 years of age. She was also diabetic, hypertensive, and a cigarette smoker. All other siblings and offspring of the siblings as well as the offspring of the proband were in good health. No xanthomas or corneal opacification were noted in any of the heterozygotes or unrelated relatives.

Lipoprotein analysis

Blood was obtained in 0.1% EDTA in the fasting state and shipped on ice by overnight air freight to Bethesda, MD. Plasma was separated by a brief centrifugation (4°C, 30 min, at 2500 rpm). Plasma was subjected to ultracentrifugation at density 1.006 g/ml for 18 hr at 4°C at 39,000 rpm in a Beckman 40.3 rotors and Beckman L265B ultracentrifuges (Beckman Instruments, Palo Alto, CA). The 1.006 g/ml supernatant and infranate were separated utilizing a tube slicer (Nuclear Supply Corp., Washington, DC). Plasma was also subjected to dextran magnesium sulfate precipitation for the determination of HDL cholesterol (39). Cholesterol and triglyceride were determined in plasma, the 1.006 g/ml infranate, and the dextran magnesium sulfate supernate by enzymatic methods utilizing a Gilford 3500 analyzer (Gilford Diagnostics, Oberlin, OH) (35, 39). Very low density lipoproteins (VLDL) cholesterol and LDL cholesterol were calculated by difference. Enzymatic assays were standardized utilizing serum calibrators obtained from the Centers for Communicable Disease Control, Atlanta, GA, as well as from Gilford Diagnostics. Results obtained for enzymatic analysis correlated very highly and gave absolute values very similar to those obtained by standard Lipid Research Clinics methods (39, 40). Total and free cholesterol values in plasma were measured by enzymatic methods with a Beckman cholesterol analyzer utilizing both cholesterol esterase and cholesterol oxidase, and omitting the cholesterol esterase for determination of free cholesterol.

Analytical ultracentrifugation

Analytical ultracentrifugation of plasma samples shipped on ice by overnight air freight to Berkeley, CA was performed and the computer-derived schlieren patterns were obtained by procedures that corrected for the concentration dependence of flotation rate and the Johnston-Ogston effect. HDL_{2b}, HDL_{2a}, and HDL_3 concentrations were determined as previously described (41).

Apolipoprotein analysis

Plasma apolipoprotein A-I, A-II, B, and C-II concentrations as well as apoB values in the 1.006 g/ml infranate were measured by radial immunodiffusion as previously described (35, 42) utilizing plasma that had been stored for 3–7 days at 4°C. All other apolipoprotein analysis was performed on separate aliquots of plasma stored at −70°C. Group 1 (mean age 21 ± 2 years) served as normal controls for subjects under 30 years of age, while group 2 (mean age 37 ± 5 years) served as controls for subjects 30 years of age or older. ApoB values are 73.5% of those previously reported because of a change in the apoB standard (35). The plasma of the proband was tested for the presence of apolipoproteins A-I, A-II, A-IV, B, C-I, C-II, apoC-III, D, E, and F by the Ouchterlony technique utilizing monospecific antibody. ApoC-III plasma levels were determined by radioimmunoassay in the laboratory of Drs. Kashyap and Srivastava, as previously described (43). ApoA-I was measured in the proband's plasma by radioimmunoassay and apoD was measured by both radial immunodiffusion and enzyme-linked immunoassay in the laboratory of Dr. Albers (44). Plasma samples had been stored for up to 2 years at −70°C prior to use in determination of apoD and apoC-III. Such long-term storage at −70°C has not significantly altered values obtained on plasma previously assayed in the fresh state. All apoD values reported for heterozygotes are based on radial immunodiffusion.

High density lipoprotein subfractions HDL_2 (d 1.063–1.125 g/ml) and HDL_3 (d 1.125–1.21 g/ml) were isolated from the plasma of the proband, her two offspring, one heterozygous sibling, and normal controls by sequential ultracentrifugation. HDL was delipidated and subjected to monodimensional isoelectric focusing (pH 4–6, 7.5% polyacrylamide) for the determination of apoA-I isoforms (45). In addition, apoA-I (10 mg) was isolated by Sephacryl S-200 column chromatography in 6 M guanidine (2.5 × 150 cm column) from the plasma of the proband's daughter. This purified apoA-I did not react against other apolipoprotein antisera, and yielded a single band when subjected to 12% acrylamide SDS polyacrylamide gel electrophoresis. This apoA-I was subjected to amino acid analysis as previously described (46).

Lecithin:cholesterol acyltransferase activity and mass

The activity and mass of LCAT as well as the cholesterol esterification rate were determined in the proband's plasma (stored at −70°C and shipped on dry ice) in the

laboratory of Dr. John J. Albers as previously reported (47–49).

Fatty acid analysis

Fatty acid analysis of the proband's plasma (stored at −70°C and shipped on dry ice) was performed in the laboratory of Dr. William E. Connor. The lipid classes were fractionated by thin-layer chromatography. Fatty acids of different lipid classes were analyzed by gas–liquid chromatography equipped with a capillary column as described previously (50).

Apolipoprotein A-I and C-III gene analysis

DNA was isolated from blood cell nuclei of both the offspring of the proband by the method of Bell et al. (51). DNA (10 μg) was digested with restriction enzymes EcoRI, PstI, BamH1, HindIII, SacI, and SmaI using a ratio of two units of enzyme per μg of DNA at 37°C respectively. Digested DNA preparations were subjected to 1.0% agarose gel electrophoresis (45 volts, 16 hr). DNA was then transferred to nitrocellulose filter paper by the method of Southern (52). ^{32}P-labeled nick-translated insert cDNA for apoA-I from two different laboratories (53, 54) and for apoC-III (37) were utilized as probes for the identification of apoA-I and apoC-III specific bands by radioautography.

RESULTS

Plasma lipoprotein and apolipoprotein analysis

Plasma lipid and lipoprotein cholesterol concentrations are given in **Table 1.** The homozygous proband had plasma cholesterol and HDL cholesterol values that were both below the 5th percentile of normal control values for her age and sex (38). Her plasma triglyceride, VLDL cholesterol, and LDL cholesterol levels were decreased as well, but were still within the 10–90th percentiles. Apolipoproteins A-I and C-III could not be detected in the proband's plasma by Ouchterlony plate analysis, while immunoprecipitation bands for apolipoproteins A-II, A-IV, B, C-I, C-II, E, and F were clearly evident. Apolipoproteins A-I and C-III were also not detected by radioimmunoassay in the proband's plasma. The sensitivity of the apoA-I assay was in the range of 28 ng/ml, and for the apoC-III assay, 1 ng/ml. Values for plasma apoA-II and apoC-II were markedly decreased (see Table 2). In addition, apoD levels in the homozygote could not be detected in the proband's plasma by radial immunodiffusion, but values of 0.5 ± 0.1 mg/dl were obtained by enzyme-linked immunoassay.

Criteria for designating kindred members as heterozygotes were: having a plasma HDL cholesterol below the 10 percentile of normal (38) and apoA-I concentration more than one standard deviation below the normal mean. Seventeen of 31 kindred members (true relatives rather than spouses) sampled met these criteria, and most of these individuals had values for these parameters that were below the 5th percentile for HDL cholesterol and more than two standard deviations below the normal mean for apoA-I (Table 1 and **Table 2**). Two sets of normal control groups were used for apolipoprotein A-I, A-II, B, and C-II determinations as previously mentioned (Tables 1 and 2). Seven of 16 heterozygotes tested had plasma apoC-III concentrations more than one standard deviation below the normal mean (Table 2). Mean HDL cholesterol, apoA-I, and apoC-III concentrations in plasma were significantly ($P < 0.05$) lower in heterozygotes than in normal controls in unaffected relatives. The mean plasma apoD concentration (± SD) in eight heterozygotes tested (cases 3–7, 8, 12, 15) was 4.2 ± 1.0 mg/dl (range 3.2–6.3, normal, 6.2 ± 1.0 mg/dl).

Clinical and genetic data

A complete pedigree for the kindred is shown in **Fig. 1.** The mode of inheritance of the biochemical abnormality (decreased plasma HDL cholesterol and apoA-I concentrations) is most consistent with an autosomal codominant pattern. Both offspring of the homozygous proband were heterozygotes, and approximately 50% of the offspring of heterozygotes were also heterozygotes. The proband was documented at autopsy to have significant premature atherosclerosis of the coronary, pulmonary, and carotid arteries, as well as the aorta. One heterozygous sibling died at age 56 years of premature coronary and cerebrovascular disease, and one other sibling had angina at age 62. Based on history, there appears to have been some premature coronary artery disease in previous generations as previously mentioned.

Analytic ultracentrifugation

Analytic ultracentrifugation analyses of plasma lipoproteins were performed on the proband, her two offspring, and one heterozygous sibling (see **Fig. 2**). In all heterozygotes, HDL_3 was the predominant HDL species and no HDL_{2b} was detectable. The proband had no detectable HDL by this method.

Apolipoprotein A-I isoelectric focusing and amino acid composition

Isoelectric focusing patterns of HDL protein in the two offspring of the proband and one heterozygous sibling indicated that the heterozygotes had a normal apoA-I isoform pattern with proapoA-I (apoA-I_1) and the two major plasma apoA-I isoforms (apoA-I_3 and apoA-I_4) in the normal position (see **Fig. 3**). Moreover the relative dis-

TABLE 1. Plasma lipid and lipoprotein cholesterol values (mg/dl)

Subject	Age	Sex	Cholesterol	Triglyceride	VLDL	LDL	HDL
Family 1							
1** Proband	45	F	111[a]	62	4	106	1[a]
2 Spouse	46	M	247	221	47[b]	174	26[a]
3* Offspring 1	25	M	189	108	26	143	20[a]
4* Offspring 2	22	F	240[c]	124	34[c]	183[c]	23[a]
Family 2							
5* Sibling 1	48	F	253	100	14	199[c]	40[d]
6* Offspring 1	31	F	200	51	12	160	28[a]
7* Offspring 2	29	F	144	33[d]	3[d]	116	25[a]
Family 3							
8* Sibling 2	52	M	146[d]	70	16	99	30[d]
9 Spouse	51	F	208	80	16	133	59
10 Offspring 1	32	M	184	157	29	118	37
11 Offspring 2	26	F	129[d]	37[d]	8	65[a]	56
Family 4							
12* Sibling 3	56	M	219	181	28	171	20[a]
13 Spouse	54	F	358[c]	670[c]	160[c]	159	39
14 Offspring 1	33	M	215	119	19	159	37
Family 5							
15* Sibling 4	56	M	163[d]	86	11	119	30[d]
16 Spouse	56	F	244	108	10	150	84
17 Offspring 1	29	F	187	123	7	140	40[a]
18* Offspring 2	26	M	130[a]	70	3[d]	99	28[a]
Family 6							
19* Sibling 5	59	M	160[a]	84	6	128	26[a]
20 Spouse	60	F	202	91	4	141	57
21* Offspring 1	40	F	189	78	13	158	18[a]
22 Offspring 2	38	M	226	281[b]	32	158	36
23 Offspring 3	33	M	182	139	14	111	57
24 Offspring 4	31	F	179	77	4	113	62
25 Offspring 5	28	F	170	181	18	105	47
Family 7							
26* Sibling 6	62	F	215	208	51[b]	129	35[a]
27 Spouse	63	M	227	163	9	171	47
28* Offspring 1	40	M	232	123	35	166	31[a]
29 Offspring 2	38	F	243[b]	411[c]	66[c]	144	33[a]
30* Offspring 3	35	M	212	187	31	161	20[a]
31 Offspring 4	34	M	196	208	25	113	58
32 Offspring 5	29	M	205	222	47[c]	121	37
33* Offspring 6	25	M	155	225	30	99	26[a]
Family 8							
34 Offspring 1	46	F	181	52[d]	8	126	47
35 Offspring 2	33	M	205	216	27	142	35
Paternal siblings							
36 Sibling 1	78	F	243	469[c]	111	200[b]	43
37* Sibling 2	75	F	110[a]	58[a]	11	68[a]	31[a]
Maternal sibling							
38* Sibling 1	66	F	203	444[c]	59	122	22[a]
Homozygote (n = 1)	45	F	111 ± 5[e]	62 ± 2	4 ± 1	106 ± 6	1 ± 1[a,e]
Heterozygote (n = 17)	44 ± 17	9M, 8F	186 ± 41	131 ± 99	23 ± 16	136 ± 35	27 ± 6[a,e]
Unaffected relatives (n = 14)	34 ± 15	6M, 8F	193 ± 30	192 ± 125	20 ± 29	130 ± 32	45 ± 10
Spouses (n = 6)	55 ± 6	2M, 4F	248 ± 57	222 ± 192	41 ± 39	155 ± 16	52 ± 20
Control subjects							
Normals (n = 1088)			189 ± 40	87 ± 43	16 ± 11	123 ± 35	50 ± 14
Normals (Group 1) (n = 50)	21 ± 2		181 ± 21	79 ± 28	10 ± 5	121 ± 20	50 ± 14
Normals (Group 2) (n = 31)	37 ± 5		192 ± 38	116 ± 40	23 ± 11	116 ± 33	53 ± 14

**, Denotes homozygote; * denotes heterozygote.
[a]Less than 5th percentile for age and sex;
[b]greater than 90th percentile;
[c]greater than 95th percentile;
[d]less than 10th percentile based on LRC population data (55).
[e]Significantly different ($P < 0.05$) from normal by t test analysis; values in the homozygote represent triplicate analyses on two plasma samples.

TABLE 2. Apolipoprotein concentrations (mg/dl)

Subject	ApoA-I	ApoA-II	ApoB	ApoB Infranate	ApoC-II	ApoC-III
Family 1						
1** Proband	UD[a]	3.4[a]	104.5	102.7	0.8[a]	UD[a]
2 Spouse	100.7[b]	29.5	149.6[c]	143.3[d]	3.7	ND
3* Offspring 1	69.4[a]	24.9	101.9	97.2	3.2	9.4
4* Offspring 2	77.6[a]	27.2	129.7[c]	125.8[d]	4.2	5.2[b]
Family 2						
5* Sibling 1	91.7[a]	55.8[d]	106.2	85.3	5.2	10.1
6* Offspring 1	66.7[a]	42.8[d]	80.5	73.4	2.0[b]	6.7
7* Offspring 2	72.4[a]	30.2	62.2[b]	55.7[b]	2.1[b]	4.8[b]
Family 3						
8* Sibling 2	84.4[a]	27.6[b]	69.9	69.0	1.4	4.7[b]
9 Spouse	149.8	36.8[c]	100.3	96.6	3.4	4.6[b]
10 Offspring 1	112.3	33.7	93.7	87.7	3.8	10.5
11 Offspring 2	145.6[c]	28.8	67.0[b]	64.9	0.5[a]	14.8
Family 4						
12* Sibling 3	64.6[a]	24.9[b]	117.0	116.1	3.2	12.3
13 Spouse	167.2[c]	42.2[d]	144.2[c]	123.0	7.2[c]	21.9[d]
14 Offspring 1	101.7[b]	31.6	85.3	80.3	3.0	12.2
Family 5						
15* Sibling 4	98.0[b]	28.4	110.1	108.8	2.2[b]	12.3
16 Spouse	169.6[c]	42.6[d]	115.5	112.8	4.2	14.7
17 Offspring 1	107.5	25.4	118.1	107.6	3.4	13.1
18* Offspring 2	74.9[a]	23.7	78.4	77.1	4.8	3.7[b]
Family 6						
19* Sibling 5	90.6[a]	28.9	102.5	102.5	1.7[b]	5.2[b]
20 Spouse	133.8	32.6	107.7	95.0	1.8[b]	7.0
21* Offspring 1	58.9[a]	13.0[a]	145.9[c]	115.3	1.6[b]	6.1[b]
22 Offspring 2	144.6	38.4[c]	154.5[d]	131.7[c]	4.6	16.8[c]
23 Offspring 3	151.2	35.0	83.3	73.7	6.0[c]	14.3[c]
24 Offspring 4	160.3[c]	29.4	73.7	62.5[b]	2.4[b]	14.9[c]
25 Offspring 5	144.6	30.5	92.5	66.9	3.7	13.1
Family 7						
26* Sibling 6	117.0[b]	29.8	117.8	108.1	3.6	12.0
27 Spouse	133.4	34.9	116.3	101.0	5.8[c]	14.0
28* Offspring 1	94.5[a]	27.6[b]	124.9[c]	115.2	4.6	16.1[c]
29 Offspring 2	117.2	29.2	140.1[c]	117.7	11.9[d]	23.1[d]
30* Offspring 3	69.5[a]	28.8	134.9[c]	117.6	4.9	14.7
31 Offspring 4	151.8	44.9[d]	96.6	79.1	4.6	13.7
32 Offspring 5	135.4[c]	34.2[c]	93.2	83.4	6.6[d]	14.7
33* Offspring 6	86.2	29.2	78.1	74.9	5.0[c]	6.0[b]
Family 8						
34 Offspring 1	134.6	26.4[b]	73.0	69.7	2.3[b]	ND
35 Offspring 2	131.7	32.2	75.8	68.8	3.4	13.6
Paternal siblings						
36 Sibling 1	61.5[a]	22.2[a]	99.2	89.4	11.9[d]	11.5
37* Sibling 2	72.4[a]	18.2[a]	65.8[b]	63.4	3.1	ND
Maternal sibling						
38* Sibling 1	61.5[a]	22.2[a]	99.2	89.4	11.9[d]	11.5
Homozygote (n = 1)	UD	3.4 ± 0.2	104.5 ± 0.4	102.7 ± 0.3	0.8 ± 0.1[a]	UD
Heterozygote (n = 17)	79.4 ± 15.4[a,e]	28.4 ± 9.3	101.5 ± 25.4	93.8 ± 21.9	3.8 ± 2.5	8.8 ± 4.0[a]
Unaffected relatives (n = 14)	128.6 ± 26.3	31.6 ± 5.7	96.1 ± 25.5	84.5 ± 21.0	4.9 ± 3.3	14.4 ± 3.0
Spouses (n = 6)	142.4 ± 25.6	36.4 ± 5.2	122.3 ± 20.0	112.0 ± 18.7	4.4 ± 1.9	12.4 ± 6.9
Normals (Group 1) (n = 50)	117.2 ± 17.0	27.1 ± 4.2	82.2 ± 25.6	75.1 ± 10.7	3.1 ± 1.1	
Normals (Group 2) (n = 31)	137.0 ± 20.0	32.1 ± 3.9	96.0 ± 28.8	88.0 ± 25.0	4.1 ± 1.6	11.1 ± 4.8

**, Denotes homozygote; * denotes heterozygote. UD, undetectable; ND, not determined.
[a]More than two SD below normal mean.
[b]More than one SD below normal mean.
[c]More than one SD above normal mean.
[d]More than two SD above normal mean.
[e]Significantly different ($P < 0.05$) from normal by t test analysis.
ApoC-II normal values are based on 29 normolipemic subjects; values in the homozygote represent triplicate analyses on two plasma samples.

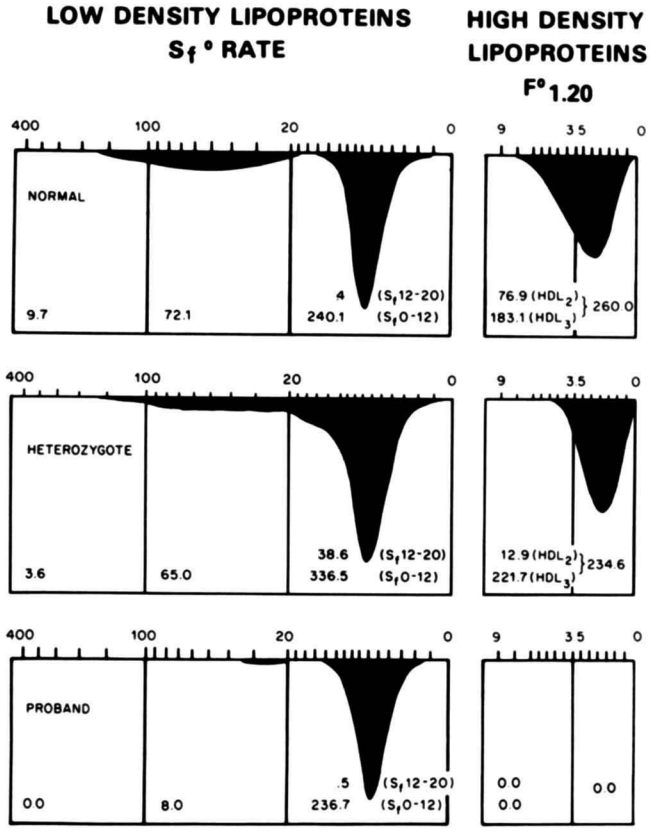

Fig. 2. Analytic ultracentrifugation pattern for a normal subject (top panel), a heterozygote (middle panel), and the proband (bottom panel) are shown. Note the undetectable HDL in the proband, and the reduced HDL_2 in the heterozygote.

tribution of these isoforms was similar to normal. ApoA-I isolated by column chromatography from the proband's daughter was subjected to amino acid composition analysis. The results of these analyses are shown in **Table 3** and indicate that this heterozygote's apoA-I amino acid composition was similar to normal.

Fatty acid analysis

The percentage of total cholesterol in the unesterified form in the proband's plasma was normal at 30% as determined by enzymatic methods as well as by thin-layer chromatography. All heterozygotes also had a normal percentage of free cholesterol in their plasma (28 ± 3%). Results of fatty acid analysis of phospholipid, free fatty acid, triglyceride, and cholesteryl ester constituents of the proband's plasma are given in **Table 4.** Of note is the marked decrease in 18:2 that was observed. In the cholesteryl ester fraction, the ratio of 18:2/18:1 was 1.13 (normal, 2.51 ± 0.62). The content of 18:2 in phospholipids, free fatty acids, triglycerides, and cholesteryl esters (given as percent total fatty acids) was 12.4%, 12.6%, 6.8%, and 33.3%, respectively. These values are substantially lower than that from a normal plasma analyzed at the same time (30.4%, 30.5%, 34.3%, and 64.1%, respectively). Similar normal values have been previously reported (55). In addition, the eicosatrienoic acid [C20:3 (n–9)] level, a biochemical indicator of essential fatty acid deficiency in different plasma lipid fractions, was also elevated. Moreover, the ratio of eicosatrienoic acid:arachidonic acid, which is elevated in essential fatty acid deficiency, was 0.05 in plasma (normal 0.01 ± 0.01). These data indicate that the proband had a moderate deficiency of linoleic acid in all lipid fractions.

Lecithin:cholesterol acyltransferase activity and mass, and cholesterol esterification

Measurement of LCAT activity and mass in the proband's plasma yielded values of 39.7 nmol/hr per ml and 2.14 μg/ml, respectively (normal, 95 nmol/hr per ml and 5.9 μg/ml, respectively) (46, 47). Plasma cholesterol esterification rate was 46 nmol/hr per ml (normal, 106 nmol/hr per ml). Therefore, the proband had an LCAT activity and mass that were 37.7% and 30.5% of normal, respectively, and a cholesterol esterification rate that was 43% of normal.

Gene analysis

DNA obtained from the white cells of the proband's two offspring was subjected to restriction enzyme digestion, and Southern blot analysis was carried out using radiolabeled nick-translated apoA-I and apoC-III insert DNA

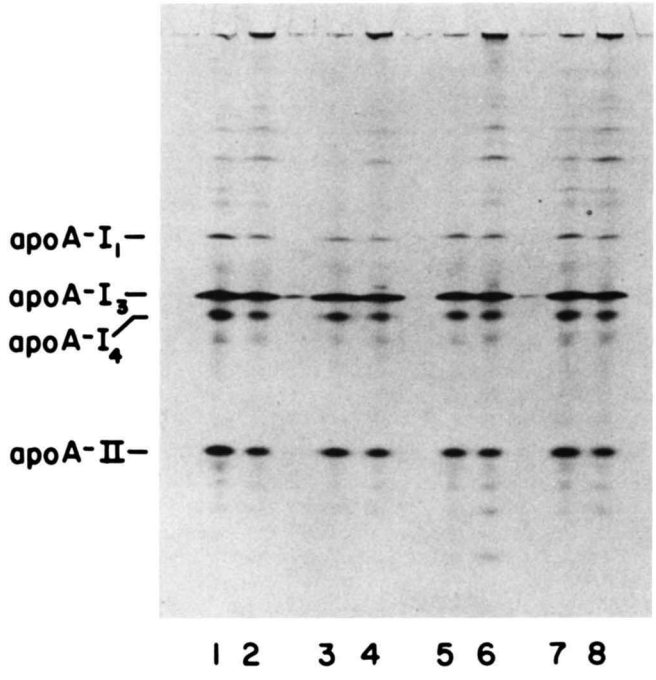

Fig. 3. Isoelectric focusing of delipidated HDL_3 (lanes 1, 3, and 5) and HDL_2 (lanes 2, 4, 6) as isolated from the plasma of the proband's male offspring (lanes, 1, 2), female offspring (lanes 3, 4), and a normal subject (lanes 7, 8).

TABLE 3. Amino acid composition

Amino Acid	Composition, Mol % ApoA-I[a] (n = 4)	ApoA-I[b] (#1)	(#2)
Aspartic acid	8.63 ± 0.2	8.57	8.57
Threonine	4.08 ± 0.2	4.25	4.25
Serine	6.20 ± 0.2	6.17	6.17
Glutamic acid	19.23 ± 0.4	19.33	19.28
Proline	4.40 ± 0.4	4.41	4.49
Glycine	4.83 ± 0.2	4.39	4.38
Alanine	8.10 ± 0.2	8.10	8.06
Valine	4.90 ± 0.2	5.06	5.02
Methionine	1.28 ± 0.4	0.91	0.90
Isoleucine	0.10 ± 0.2	0.00	0.00
Leucine	15.68 ± 0.4	15.67	15.66
Tyrosine	2.73 ± 0.2	2.99	2.99
Phenylalanine	2.58 ± 0.2	2.68	2.66
Histidine	2.33 ± 0.2	2.06	2.08
Lysine	8.55 ± 0.4	8.70	8.75
Arginine	6.50 ± 0.2	6.71	6.75

[a]Mean values ± SD for normal apoA-I.
[b]ApoA-I isolated from the proband's daughter; results of two analyses are given.

(36, 52, 53). ApoA-I and apoC-III specific bands, as identified by radioautography for the two heterozygotes, were similar to normal. Data for apoA-I are shown in **Fig. 4.**

DISCUSSION

Familial HDL deficiency in the absence of severe hypertriglyceridemia or an increased plasma free:total cholesterol ratio (as in LCAT deficiency) has been noted in hypoalphalipoproteinemia (32, 33), apoA-I variants (25–29), Tangier disease (23, 24), fish eye disease (30), HDL deficiency with planar xanthomas (31), deficiency of apolipoproteins A-I and C-III (34), and apolipoprotein A-I absence (35). Only modest apoA-I deficiency (plasma levels of about 25% of normal) are observed in hypoalphalipoproteinemia, fish eye disease, and apoA-I variants (25–30, 32, 33). The striking corneal opacification noted in fish eye disease, as well as familial LCAT deficiency, was not observed in our kindred (21, 22, 30). The defects in hypoalphalipoproteinemia and fish eye disease are unknown, while specific amino acid substitutions in apoA-I have recently been reported in subjects who are heterozygous for apoA-I_{Milano}, apoA-$I_{Marburg}$, apoA-$I_{Giessen}$, and apoA-$I_{Munster\ 1-3}$ (25–29). The apoA-I abnormality in these latter kindreds was initially detected by isoelectric focusing. No such abnormality was observed in the kindred described in this report.

In homozygous Tangier disease, marked HDL deficiency exists, and plasma apoA-I and apoC-III levels are approximately 1% and 50% of normal, respectively (56–58). An abnormal apoA-II-rich HDL particle has been reported in these patients (56, 59). ApoA-$I_{Tangier}$ has been shown to differ from normal apoA-I in its amino acid composition (46), isoelectric focusing pattern (60), and in vivo metabolic characteristics (61). The precise defect remains to be defined. The kindred described here differs from Tangier disease because of undetectable plasma apoA-I and apoC-III and lack of cholesteryl ester deposition in reticuloendothelial cells.

Premature CAD, mild diffuse corneal opacification, and planar xanthomas have been noted in homozygotes for both HDL deficiency with planar xanthomas (31) and familial deficiency of apolipoproteins A-I and C-III (34). In the proband for the former kindred, the plasma apoA-I concentration was markedly reduced at 2 mg/dl, and

TABLE 4. Fatty acid content (percent of total)

Fatty Acids	Phospholipid	Free Fatty Acid	Triglyceride	Cholesteryl Ester
13:0	–	–	–	0.03
14:0	0.58	0.26	1.46	0.79
14:1	–	0.28	0.38	0.10
15:0	0.16	–	0.31	0.22
16:0	31.24	23.00	28.66	14.64
16:1 (n-7)	1.37	3.59	7.92	10.44
17:0	0.38	0.43	0.28	0.16
17:1 (n-9)	0.20	0.48	0.53	0.53
18:0	16.57	11.13	3.81	0.89
18:1 *trans*	0.34	1.08	0.47	0.14
18:1 (n-9)	18.09	29.71	45.83	29.40
19:0	–	0.11	–	0.18
18:2 (n-6)	12.36	12.57	6.84	33.32
18:3 (n-6)	0.25	0.58	0.33	1.22
20:0	–	–	–	–
18:3 (n-3)	0.13	0.41	0.15	0.27
20:1 (n-9)	0.31	0.38	0.51	0.17
18:4 (n-3)	–	–	–	0.02
21:0	–	–	–	0.06
20:2 (n-6)	0.40	–	0.12	0.05
20:3 (n-9)	0.60	0.29	0.29	0.18
20:3 (n-6)	4.64	1.92	0.21	0.83
22:0	–	–	–	–
20:3 (n-3)	–	–	–	–
20:4 (n-6)	8.06	9.05	0.73	5.11
22:1 (n-11)	–	–	–	–
22:1 (n-9)	–	–	–	–
20:4 (n-3)	0.09	0.29	0.13	0.05
20:5 (n-3)	0.26	0.62	–	0.22
24:0	–	0.40	0.09	0.16
22:4 (n-6)	0.62	–	0.10	–
24:1 (n-9)	0.22	–	–	0.05
22:5 (n-6)	0.46	0.42	–	–
22:5 (n-3)	0.30	0.67	–	–
22:6 (n-3)	1.41	2.02	0.10	0.15
Unknown	0.96	0.31	0.75	0.62
Total fatty acids				
Saturated	48.93	35.33	34.61	17.13
Monounsat.	20.53	35.52	55.64	40.83
(n-6)	26.79	24.54	8.33	40.53
(n-3)	2.19	4.01	0.38	0.71

[a]Analysis performed on plasma of proband; proband's ratio of 18:2/18:1 fatty acids in the cholesteryl ester fraction was 1.13 (normal 2.51 ± 0.62). Normal percentages for 18:2 fatty acids in phospholipids, free fatty acids, triglyceride, and cholesteryl ester are 23.0%, 9.0%, 22.5%, and 45.6%; –, none detected.

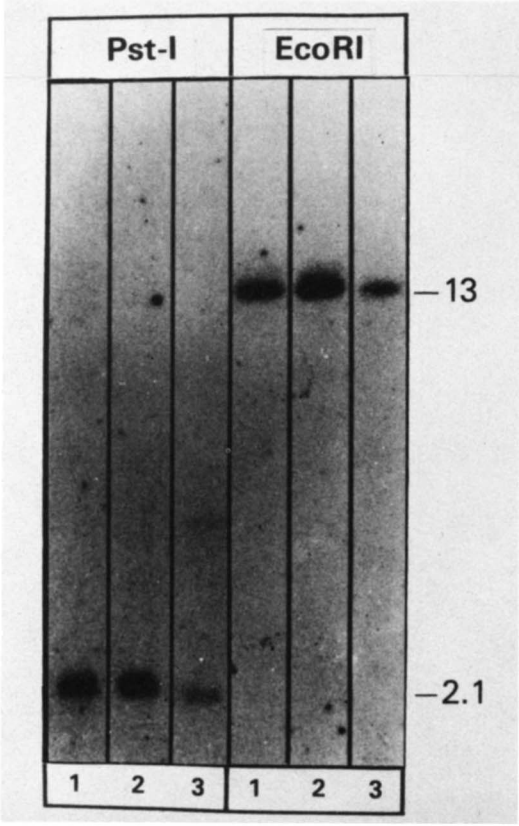

Fig. 4. DNA obtained from the white cells of the proband's male offspring (lane 1), female offspring (lane 2), and a normal subject (lane 3) were subjected to restriction enzyme digestion (EcoRI or Pst-I); and Southern blots as shown were obtained utilizing radiolabeled nick-translated apoA-I insert DNA. No detectable difference in these patterns could be discerned between the two heterozygotes and the normal subject. The numbers 2.1 and 13 refer to the size of the gene fragment in kilobases.

apoC-III levels were normal (31). In the latter kindred the mean apoA-I level in the two probands was 0.0059 mg/dl by radioimmunoassay, and apoC-III was not detectable by electroimmunoassay (34). When the DNA isolated from the white cells of homozygotes for this latter disease entity were cleaved by the restriction endonuclease EcoRI and analyzed by Southern blot analysis utilizing a radiolabeled apoA-I gene probe, the probe hybridized to a unique 6.5 kb band in contrast to a normal individual where only a 13 kb band was present (54). Similar experiments in heterozygotes demonstrated the presence of both the normal 13 kb band and the abnormal 6.5 kb band (54). Detailed analysis has indicated the presence of a 7.5 kb insert into an exon of the apoA-I gene. This insert contains the new EcoRI site which results in the new 6.5 kb band (54). Recent data have documented that the apoC-III gene is approximately 2.6 kilobases downstream of the 3′ end of the apoA-I gene, accounting for the observed genetic linkage of apoA-I and apoC-III (36).

In our kindred no abnormality in the apoA-I, apoC-III gene complex was detected when DNA from heterozygotes was analyzed by Southern blot analysis following restriction enzyme digestion (Fig. 4). These data indicate that our kindred does not share the same genetic defect as the kindred described by Norum et al. (34, 37). The nature of the gene defect in our kindred remains to be elucidated. In addition, the homozygote in our kindred lacked planar xanthomas, her plasma apoA-I and apoC-III concentrations were undetectable, and her apoA-II levels were 10.6% of normal, instead of 50.9% of normal as observed in homozygous familial deficiency of apolipoproteins A-I and C-III (34). Therefore, our kindred, originally named apolipoprotein A-I absence (35), was affected with a disease entity somewhat different though very similar to familial deficiency of apolipoproteins A-I and C-III, and after discussions with Dr. Robert Norum of Detroit we have renamed this new disease entity familial apolipoprotein A-I and C-III deficiency, variant II. The linkage of apoA-I and apoC-III in our kindred is based primarily on the undetectable levels of these apolipoproteins in the homozygous proband, since heterozygotes had only moderate reductions (especially for apoC-III). We do not feel that primary defects in other apolipoproteins are present in this disorder because no other apolipoproteins, including apoA-IV, were lacking in the homozygote or were reduced in heterozygotes.

Clinical and autopsy data in the kindred described in this report lend further support to the concept that decreased HDL is associated with premature CAD (16, 17). The proband's death following bypass surgery despite patent grafts may have been due to decreased coronary perfusion intraoperatively since diffuse left ventricular contraction band necrosis was observed at autopsy (62). The proband and only homozygote in the kindred had severe aortic, carotid, pulmonary and coronary artery atherosclerosis. Her oophorectomy may have also been a contributing risk factor (63, 64). In addition, one heterozygous sibling died of documented CAD and cerebrovascular disease at age 56 years. Why certain HDL-deficient kindreds develop premature CAD (31–35, 65) but not others (25–30), remains to be determined. Differences may relate to variability in LDL and HDL levels, other risk factors, as well as alterations in total HDL flux.

Plasma lipoprotein data on kindred members as shown in Table 1 indicate that a reduction in HDL was the major abnormality observed in both the homozygous proband and heterozygotes. Mean HDL cholesterol values were 2% of normal for the proband, and 54% of normal for heterozygotes. Analytic ultracentrifugation data indicate that reductions in HDL observed in heterozygotes are due primarily to decreases in HDL_2, consistent with previous observations that fluctuations in HDL levels are largely due to changes in HDL_2 (41). Apolipoprotein analysis indicated undetectable plasma levels of apolipoprotein A-I and C-III in the proband, and decreased levels in heterozygotes (especially for apoA-I). These data, espe-

cially the findings in the proband, support the concept of a genetic linkage between apoA-I and apoC-III (34, 37). The increased apoB:cholesterol ratio within LDL observed in the homozygote (0.97, ratio in young controls, 0.62; ratio in middle-aged controls, 0.76), was not observed in heterozygotes (ratio, 0.67). An elevated apoB:cholesterol ratio within LDL may also predispose to premature CAD (66, 67). The data suggest that the kindred was not affected with hyperapobetalipoproteinemia, but that the apoB enrichment of LDL in the proband may have been secondary to HDL or apolipoprotein deficiency.

Pedigree analysis as shown in Fig. 1 is consistent with an autosomal mode of inheritance in which all the offspring of the homozygous proband were heterozygotes, and approximately 50% of the offspring of heterozygotes were themselves heterozygotes. Since the severe biochemical and clinical abnormalities observed in the homozygote are only partially expressed in heterozygotes, the best term for this form of inheritance is autosomal codominant.

Data from the proband in this kindred indicate that apoA-I is not essential for activation of LCAT, since LCAT activity and cholesterol esterification rate were proportional to the LCAT mass present in plasma. ApoC-I has also been reported to activate LCAT (10), and this presumably accounts for the relatively normal degree of cholesterol esterification in the proband since apoC-I was present in her plasma. Other apolipoproteins may also activate LCAT. ApoA-I and apoC-III are not only protein constituents of plasma HDL, but also of lymph chylomicrons. Deficiencies of linoleic acid (C18:2) (an essential fatty acid) and alpha tocopherol in the plasma of the proband suggest that apoA-I and/or apoC-III may be important for normal intestinal absorption of these constituents.

The data are consistent with the following concepts: *1*) familial apolipoprotein A-I and C-III deficiency, variant II, is a distinct disease entity; *2*) apoA-I and apoC-III are genetically linked; *3*) apoA-I is not essential for LCAT activation and cholesterol esterification in plasma; *4*) apoA-I and/or apoC-III may play a role in intestinal vitamin E and essential fatty acid absorption; and *5*) decreased HDL is associated with premature CAD.

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