Identification of an apoC-II variant (apoC-$II_{Bethesda}$) in a kindred with apoC-II deficiency and type I hyperlipoproteinemia

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Abstract Apolipoprotein (apo) C-II deficiency is characterized by elevated plasma triglycerides, chylomicrons, and very low density lipoproteins, as well as reduced levels of low density and high density lipoproteins. A subject with apoC-II deficiency has been identified with an apoC-II plasma level of less than 0.05 mg/dl. The plasma apoC-II in the proband was immunochemically similar to apoC-II in normal subjects when analyzed by Ouchterlony immunodiffusion, however the apoC-II had an apparently lower molecular weight and higher pI when analyzed by two-dimensional gel electrophoresis. This apoC-II variant, designated apoC-$II_{Bethesda}$, was not affected by neuraminidase treatment or reduction. Two-dimensional gel electrophoresis of the plasma of the mother of the proband revealed both normal apoC-II and apoC-$II_{Bethesda}$, whereas analysis of the father and two siblings revealed apoC-II of normal electrophoretic mobility. These results were interpreted as indicating that the proband was a compound heterozygote with one allele for apoC-$II_{Bethesda}$ inherited from the mother and an allele coding for an abnormality which results in the virtual or complete absence of plasma apoC-II from the father. This proband represents the first example of a compound heterozygote for an apolipoprotein defect associated with a dyslipoproteinemia. —**Sprecher, D. L., L. Taam, R. E. Gregg, S. S. Fojo, D. M. Wilson, M. L. Kashyap, and H. B. Brewer, Jr.** Identification of an apoC-II variant (apoC-$II_{Bethesda}$) in a kindred with apoC-II deficiency and type I hyperlipoproteinemia. *J. Lipid Res.* 1988. **29:** 273–278.

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ApoC-II deficiency is a rare genetic disease characterized by marked elevations of plasma triglycerides, chylomicrons, and very low density lipoproteins, as well as reduced levels of LDL and HDL (1). The characteristic apolipoprotein abnormality in this disease is a deficiency of apoC-II, the cofactor for lipoprotein lipase, the enzyme attached to the capillary endothelium which catalyzes the hydrolysis of plasma triglycerides to fatty acids and monoglycerides. In virtually all of the reported kindreds, apoC-II has not been detected in plasma (2–11). The deficiency of apoC-II is associated with elevated levels of plasma triglycerides and chylomicrons. Postheparin lipoprotein lipase activity is normal when assayed in the presence of plasma from normal subjects or purified apoC-II.

Recently the amino acid sequence of apoC-II isolated from normal subjects was determined (12), and the cDNA for apoC-II was cloned (13–15). ApoC-II is synthesized as a 101 amino acid protein, preproapoC-II, which undergoes co-translational cleavage of a 24 amino acid prepeptide, and post-translational cleavage in plasma to the 73 amino acid mature apoC-II (16). ApoC-II is located on chromosome 19 (15, 17), in tandem array with apoC-I and apoE (18, 19). The LDL receptor is also located on this chromosome (20). The complete solid phase synthesis of biologically active apoC-II has been reported (21).

We have recently reported the analysis of the apoC-II gene in two kindreds with apoC-II deficiency (22). The identification of apoC-II in the plasma of one of the patients with apoC-II deficiency prompted an analysis of the plasma apoC-II isoforms and is the subject of this report.

MATERIALS AND METHODS

The proband is a 12-yr-old white female with a history of hypertriglyceridemia in the range of 500–1200 mg/dl first documented at the age of 6. She is asymptomatic with a normal physical examination and no history of pancreatitis. She has been treated with a low fat diet, and her triglycerides currently range from 500 to 700 mg/dl. The proband has two siblings, ages 14 and 18. There is no history of consanguinity in the kindred.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo, apolipoprotein; $NaDodSO_4$, sodium dodecylsulfate.

TABLE 1. Plasma lipid and lipoprotein cholesterol concentrations

Subject	Total Cholesterol	Triglyceride	Cholesterol		
			Chylomicrons + VLDL	LDL	HDL
			mg/dl		
Normal (n = 1088)	189 ± 40[a]	87 ± 43	16 ± 11	123 ± 35	50 ± 14
Proband[b]	144 ± 26	729 ± 296	87 ± 20	36 ± 6	19 ± 6
Mother	260 ± 14	138 ± 36	19	171	82 ± 7
Father	197	140	31	118	49
Sister	163	99	15	89	59
Brother	183	229	28	106	49

[a]Values are means ± SD. SD is presented only when there are at least three values. Other results are the mean of two values.
[b]Data on the proband represents the mean of nine values over the 4-yr diet treatment period.

Blood for lipid and lipoprotein analysis was collected in 0.1% EDTA after a 12–14 hr fast. Cholesterol and triglyceride concentrations in plasma and lipoprotein fractions were determined in an AutoAnalyzer II (Technicon, Tarrytown, NY) by the techniques standardized by the Lipid Research Clinic (23, 24). Lipids were also quantitated by enzymic procedures utilizing the Gilford System 3500 analyzer (Gilford Instruments, Oberlin, OH) (25, 26). Values for lipids, lipoproteins, and apolipoproteins determined in normal controls have been previously reported (27).

Plasma concentrations of apolipoproteins A-I, A-II, and B (27) were determined by radial immunodiffusion, and apoE (28) as well as apoC-II (29) were quantitated by radioimmunoassay. The immunochemical properties of apoC-II in plasma were also evaluated by Ouchterlony immunodiffusion (30). Lipoprotein lipase activity was determined on postheparin plasma following fractionation by heparin affinity chromatography by the procedure of Nakaya, Schaefer, and Brewer (31) as previously reported.

Plasma two-dimensional gel electrophoresis was performed as previously described (32). Briefly, in the first dimension, 3–4 μl of plasma was isoelectrofocused (pH 4 to 7) in tube gels and $NaDodSO_4$ slab gel electrophoresis was performed in the second dimension with 15% (w/v) acrylamide. Following electrophoresis the proteins were transferred to nitrocellulose paper and immunoblotted (33). The monospecific antibody to apoC-II utilized in the immunoblot as well as the Ouchterlony immunodiffusion analyses were prepared against rabbit apoC-II purified by high pressure liquid chromatography (34).

RESULTS

Plasma lipids and lipoproteins in the proband and immediate family are included in **Table 1.** The values for the proband were obtained while on a 20 g/day fat diet. Plasma triglycerides, chylomicrons, and VLDL were elevated, and the LDL as well as HDL cholesterol levels were reduced in the proband. The lipid and lipoprotein values in the parents and two siblings were normal.

The plasma concentrations of apolipoproteins A-I, A-II, B, E, and C-II are shown in **Table 2.** In the proband apoA-I and apoB were reduced, apoE was elevated, and apoC-II was markedly reduced to less than 0.05 mg/dl. The apoC-II levels in the mother, father, and two siblings of the proband were reduced and the apoA-I level in the mother was elevated (Table 2).

Lipoprotein lipase activity in the proband was virtually zero but increased to 5.8 μmol/ml per min when assayed in the presence of normal plasma or purified apoC-II (control lipoprotein lipase activity = 6.9 ± 1.1 μmol/ml per min).

TABLE 2. Plasma apolipoprotein concentrations

Subjects	ApoA-I	ApoA-II	ApoB	ApoC-II	ApoE
			mg/dl		
Normal (n = 50)	117 ± 17[a]	27 ± 4	85 ± 19	5.2 ± 0.3[b]	5.7 ± 1.4
Proband	80	29	53	< 0.05	15.2
Mother	214	36	143	2.5	4.3
Father	138	32	117	3.6	3.1
Sister	125	26	67	1.7	4.2
Brother	125	32	108	2.8	4.3

[a]Values are means ± SD.
[b]Normal range is included in reference 29.

Analysis of plasma apoC-II in the proband by Ouchterlony immunodiffusion is illustrated in **Fig. 1.** A line of identity was present between normal plasma (well 1), the apoC-II standard (well 2), and the plasma of the proband (well 3). These results indicated that the apoC-II present in the plasma of the proband was immunochemically similar to the purified apoC-II standard and the apoC-II present in normal plasma. Precipitant bands were also observed in the mother, father, and two siblings of the proband when plasma was analyzed by immunodiffusion with the apoC-II antibody (data not shown).

The apoC-II present in the proband was evaluated by two-dimensional gel electrophoresis (**Fig. 2**). No apoC-II was detected in the electrophoretic position of normal apoC-II in the electrophoretogram by silver staining, however, a new very light spot was detected of lower apparent molecular weight and more basic pI (Fig. 2A). The electrophoretogram from the proband was further analyzed by immunoblot utilizing a high titer monospecific antibody to apoC-II. In the proband a major single apoC-II isoform designated apoC-$II_{Bethesda}$ was observed at the position of the new isoform identified by silver staining (Fig. 2B). To

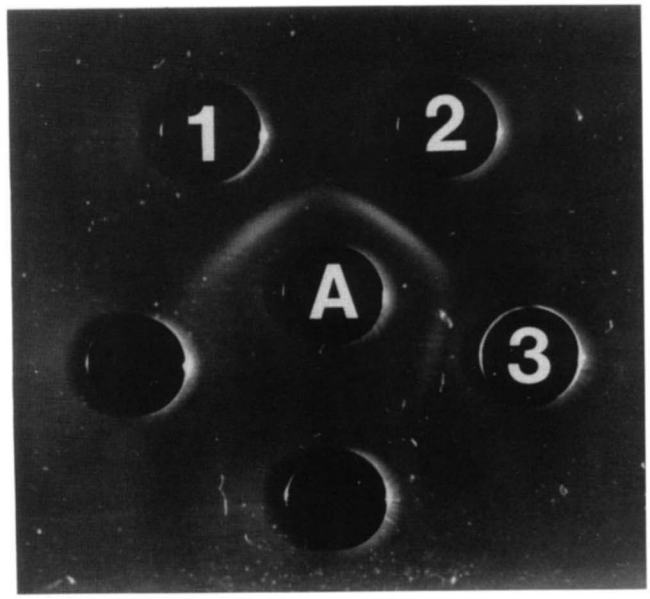

Fig. 1. Ouchterlony immunodiffusion of plasma from the proband with apoC-II deficiency. Well A contains 15 μl of the apoC-II antibody; wells 1, 2, and 3 contain 15 μl of normal plasma, 0.75 μg apoC-II standard, and 15 μl of plasma from the proband, respectively.

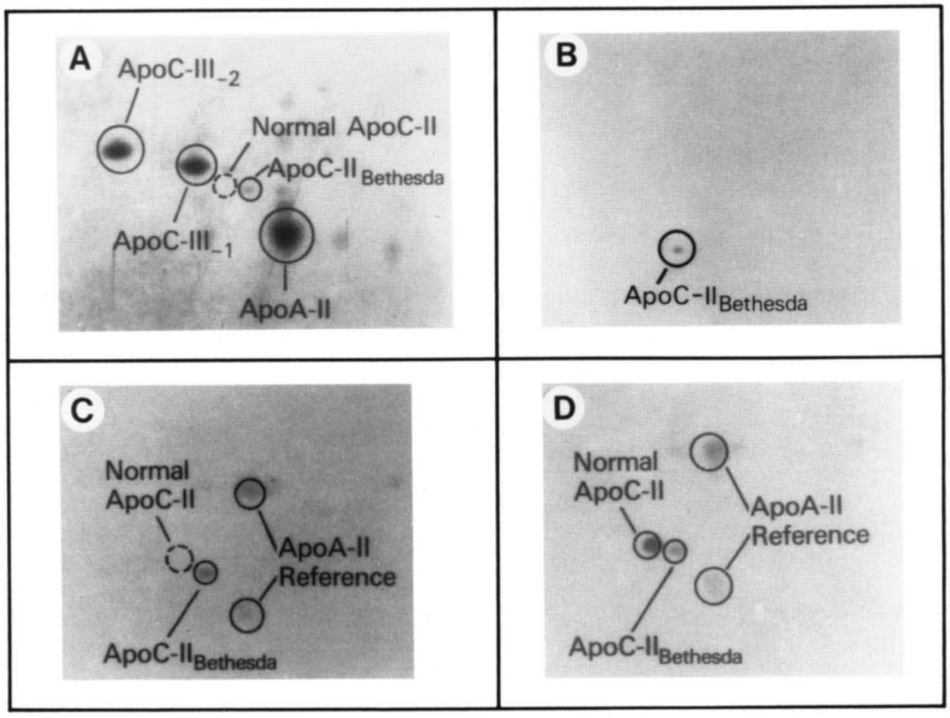

Fig. 2. Two-dimensional gel electrophoresis of plasma apoC-II from the proband with apoC-$II_{Bethesda}$. Plasma apolipoproteins were separated by two-dimensional gel electrophoresis and visualized by silver staining or immunoblot. For immunoblot, apolipoproteins were transferred following electrophoresis to nitrocellulose paper and exposed to high and low titers of antibodies to apoC-II and apoA-II, respectively. ApoA-II isoforms (monomer and dimer) provided internal coordinates for localization of apoC-II isoforms. Panel A: Plasma electrophoretogram from the proband stained with silver revealed an apoC-II isoform, designated apoC-$II_{Bethesda}$, with lower apparent molecular weight and more basic pI ($\sim$ 4.75) than the electrophoretic position of normal apoC-II (broken circle, pI $\sim$ 4.70). Panel B: Immunoblot of the plasma apolipoproteins with an apoC-II antibody confirmed the identity of the apoC-$II_{Bethesda}$ variant. Panel C: Plasma immunoblot utilizing both apoC-II and apoA-II antibodies to confirm the electrophoretic position of apoC-$II_{Bethesda}$. Panel D: Immunoblot of an electrophoretogram of a mixture of plasma from the proband with apoC-$II_{Bethesda}$ and normal plasma.

illustrate this difference in electrophoretic mobility, a low concentration of a monospecific antibody to apoA-II was also included in a separate electrophoretogram to provide a reference for the position of the apolipoproteins (Fig. 2C). To confirm the electrophoretic position of apoC-II$_{Bethesda}$, plasmas from both the proband and a normolipidemic subject were mixed and the combined sample was analyzed by two-dimensional gel electrophoresis (Fig. 2D). Normal apoC-II and apoC-II$_{Bethesda}$ were clearly separated on the electrophoretogram with the apoC-II$_{Bethesda}$ being of lower apparent molecular weight and a more basic pI. The pI values of normal apoC-II and apoC-II$_{Bethesda}$ were 4.70 and 4.75, respectively. Treatment of the plasma of the proband with neuraminidase or reduction had no effect on the electrophoretic position of the apoC-II$_{Bethesda}$ variant.

The C-II apolipoproteins present in the father, mother, and two siblings of the proband were also analyzed by two-dimensional gel electrophoresis (**Fig. 3**). The C-II apolipoproteins present in the father and two siblings were identical to apoC-II of normal subjects on the two-dimensional electrophoretograms (Fig. 3A, C, and D). The electrophoretogram of the mother, however, contained both normal apoC-II and apoC-II$_{Bethesda}$ (Fig. 3B).

DISCUSSION

The clinical features of the proband in the present study are characteristic of the reported cases of apoC-II deficiency with elevated levels of plasma triglycerides, chylomicrons, and VLDL, as well as reduced levels of LDL, HDL, apoA-I and apoB (2–11). In general, the hypertriglyceridemia and the severity of the clinical symptoms in patients with apoC-II deficiency appear to be somewhat ameliorated when compared to patients with lipoprotein lipase deficiency and severe hypertriglyceridemia. Other apolipoproteins [e.g., apoH (31)] may serve as cofactors for lipoprotein lipase in the absence of apoC-II, thereby reducing the severity of the hypertriglyceridemia.

ApoC-II was identified in the plasma of the patient with apoC-II deficiency by immunoblot and Ouchterlony analysis. The apoC-II identified has been designated apoC-II$_{Bethesda}$. Electrophoretically normal apoC-II, as well as apoC-II$_{Bethesda}$ were present in the plasma of the mother, whereas reduced levels of apparently normal apoC-II were present in the father and two siblings. These results are interpreted as indicating that the proband contains an apoC-II variant which has been inherited from

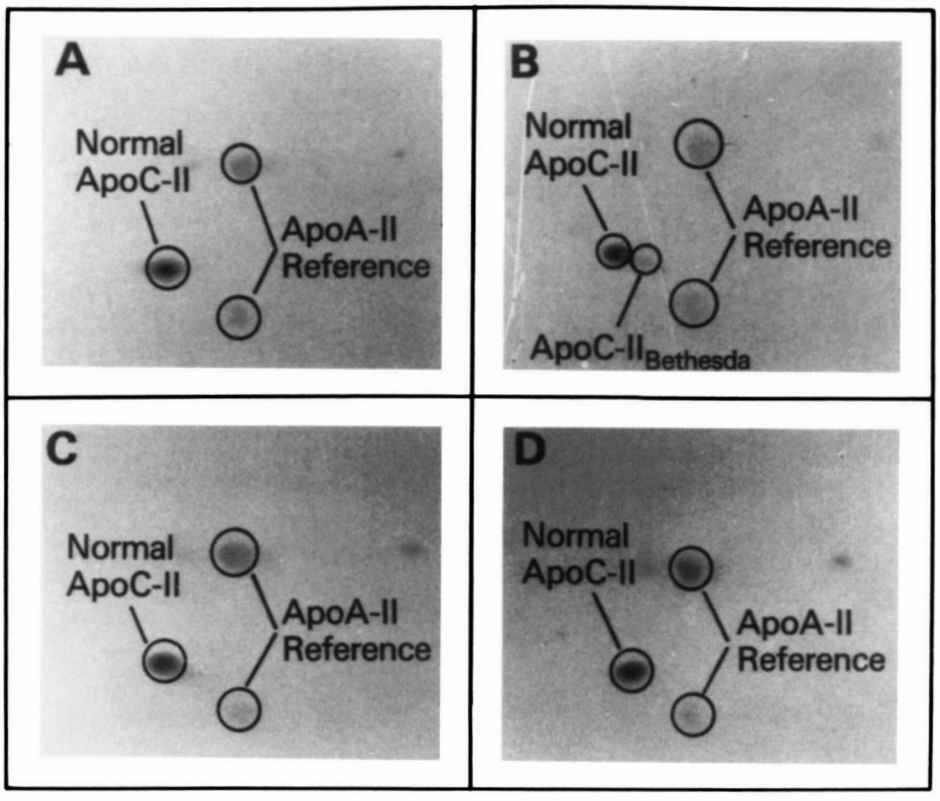

Fig. 3. Immunoblots of plasma apoC-II from the kindred of the proband with apoC-II$_{Bethesda}$. Panels include A, father; B, mother; C, brother; D, sister. The immunoblots of plasma apoC-II of the father, brother, and sister contained apoC-II with normal apparent molecular weight and pI. The immunoblot of plasma apoC-II from the mother (panel B) contains apparently normal apoC-II and the apoC-II$_{Bethesda}$ variant.

the mother. Little, if any, normal apoC-II is present in her plasma, suggesting that the allele from her father may be a null allele or an allele coding for an abnormality which results in the virtual absence of plasma apoC-II. The proband therefore is a compound heterozygote.

Recently, Maguire et al. (35) reported the identification of two mutant forms of apoC-II in a kindred of patients originally described with apoC-II deficiency (2, 4). The apoC-II variants, codified apoC-II-X and apoC-II-Y, differed in pI but were similar in molecular weight to normal apoC-II. The plasma concentration of the apoC-II variants, based on polyacrylamide gel electrophoresis, appeared to be only moderately reduced when compared to apoC-II in normal subjects (35). A unique feature of the apoC-II mutant in this kindred is the failure of these variants to form insoluble antigen-antibody complexes with antibodies to apoC-II when analyzed by immunodiffusion and electroimmunoassay. The mutant C-II apolipoproteins were nonfunctional and unable to activate lipase. This interesting kindred, originally described with a deficiency of apoC-II, now appears to have mutant forms of apoC-II present in plasma at presumably near normal concentration; these variants are nonfunctional and have unusual immunological properties.

The kindred reported by Maguire et al. (35) is significantly different from the kindred described in the present report. In this proband the level of apoC-II is extremely low, consistent with the designation of the dyslipoproteinemia as apoC-II deficiency, and there is no marked abnormality in the immunological properties of the apoC-$II_{Bethesda}$variant. In addition, the proband is a compound heterozygote and represents the first case of an apolipoprotein defect with this type of genetic abnormality. A genetic compound has been previously reported in patients with familial hypercholesterolemia and a defect in the LDL receptor (36).

The discovery of a compound heterozygous type of apolipoprotein defect gives additional insights into the heterogeneity of the molecular defects present in patients with similar biochemical syndromes. The nature of the defect in apoC-$II_{Bethesda}$ is as yet not known, however the recognition that structural defects in apoC-II may be associated with defective cofactor function for lipoprotein lipase heralds a new era in the biochemical study of patients with hypertriglyceridemia and the type I as well as type V phenotypes.

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