Apolipoprotein B-38.9 does not associate with apo[a] and forms two distinct HDL density particle populations that are larger than HDL

W. Antoinette Groenewegen, Maurizio R. Averna,[1] Judit Pulai, Elaine S. Krul, and Gustav Schonfeld[2]

Department of Medicine, Division of Atherosclerosis and Lipid Research, Washington University School of Medicine, 660 S. Euclid, Box 8046, St. Louis, MO 63110

Abstract We have identified a new truncated apolipoprotein B (apoB) that provides insights into the interaction of apoB with apo[a] and with lipids. Both total and LDL-cholesterol were below the 5th percentile in the proband; Lp[a] was 28 mg/dl. Four other affected individuals were identified in this kindred. Immunoblotting of plasma apoB-containing lipoproteins with an anti-apoB monoclonal antibody revealed a major band for apoB-100 and a minor band with apparent M_r 217 kDa. The apoB truncation is due to a −1 frameshift mutation, consisting of a cytosine deletion at cDNA position 5444, that results in the translation of 22 novel amino acids terminating at residue 1767. The mutation was confirmed in the affected subjects by allele-specific oligonucleotide (ASO) analysis. Gel filtration of whole plasma revealed that the minority of apoB-38.9 eluted with IDL- and LDL-sized particles, while the majority (~60%) eluted between LDL and HDL. Lp[a] eluted between VLDL and LDL. Upon preparative density gradient ultracentrifugation (DGUC), the majority of the plasma apoB-38.9 (~65%) floated at a density of 1.12 g/ml coincident with the major peak of HDL cholesterol. Lp[a] floated at a peak density of 1.08 g/ml between LDL and HDL. Immunoblots of the apoB-38.9-containing HDL density DGUC fractions subjected to nondenaturing gradient gel electrophoresis (GGE) demonstrated two apoB-38.9-containing particle populations with diameters of ~15 nm and ~18 nm, respectively. Lipoproteins of these sizes were also detected when whole plasma was subjected to GGE and immunoblotting. The 15–18 nm lipoproteins correspond to the gel filtration populations eluting between LDL and HDL. Lysine-Sepharose chromatography of plasma yielded retained products that contained apo[a] and apoB-100 but not apoB-38.9. Immunoprecipitation of whole plasma with monospecific polyclonal anti-human apo[a] showed apo[a] and apoB-100, but no apoB-38.9 to be present in precipitates. ApoB-100 and apoB-38.9 were present in supernates. In in vitro incubations, recombinant apo[a] formed complexes with apoB-100 but not with apoB-38.9-containing particles. Our results show that the apoB-38.9 protein can be found in a variety of lipoproteins; however, the majority of apoB-38.9-containing lipoproteins float at a density equivalent to HDL but are larger than HDL, being intermediate in size between apoB-100 LDL and HDL. The heterogeneity of apoB-38.9 lipoproteins may reflect their dual tissue source, i.e., liver and intestine, and the discordance between size and density indicates a disproportionately reduced association of lipids with apoB-38.9. Finally, our data suggest that apoB-38.9 is incapable of forming complexes with apo[a] in plasma.—**Groenewegen, W. A., M. R. Averna, J. Pulai, E. S. Krul, and G. Schonfeld.** Apolipoprotein B-38.9 does not associate with apo[a] and forms two distinct HDL density particle populations that are larger than HDL. *J. Lipid Res.* 1994. **35:** 1012–1025.

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ApoB is the essential structural protein part of apoB-containing lipoproteins (for reviews see refs. 1, 2). In humans, two different forms of apoB are present in plasma. These are classified according to the centile system as apoB-100 and apoB-48. These apoB proteins are essential for the assembly of VLDL in the liver and chylomicrons in the small intestine, respectively. Both apoB-100 and apoB-48 are products of the apoB gene. ApoB-100 results from the translation of the full length apoB mRNA whereas apoB-48 is the translation product of apoB mRNA that has been edited at cDNA position 6666 (C to U) which introduces a stop codon and terminates protein translation at 48% of the full length protein (3).

Detailed structure–function studies of apoB are hampered by the large size of this protein (550,000 Da) and its highly lipophilic nature. Mutant forms of apoB are beginning to provide us with valuable information about structure–function relationships of the apoB protein. In recent years several truncated forms of apoB have been identified

Abbreviations: Lp[a], lipoprotein[a]; apoB, apolipoprotein B; PCR, polymerase chain reaction; ASO, allele-specific oligonucleotide; DGUC, density gradient ultracentrifugation; GGE, nondenaturing gradient gel electrophoresis.

[1]Present address: Istituto di Medicina Interna e Geriatria, Cattedra di Patologia Medica, Via del Vespro 143, 90127 Palermo, Italy.

[2]To whom correspondence should be addressed.

in humans (reviewed in ref. 4). Most of these are due to mutations at the gene level that result in the introduction of premature termination codons. The resultant proteins are truncated at the C-terminal end. Affected individuals heterozygous for truncated apoB species are hypobetalipoproteinemic, with total and LDL-cholesterol concentrations of <5th percentile and very low total apoB concentrations. The size of the truncated apoB protein influences the size and density of the particle that is formed. The physiologic mechanisms responsible for the hypobetalipoproteinemia are not well understood, but decreased rates of production of apoB, increased fractional catabolic rates of VLDL-, IDL-, and LDL-apoB or various combinations of physiologic defects may be involved (5, 6).

We previously described mutations leading to apoB-40 (7, 8), apoB-89 (7, 8), apoB-54.8 (9), apoB-75 (6), and recently apoB-52 (10). Here we describe a new deletion mutation, leading to a truncated apoB species, apoB-38.9. We also report a detailed examination of the physical properties of the apoB-38.9 species by separations based on size and density and on the association of apo[a] with apoB-100 and apoB-38.9.

METHODS

Blood collection and plasma analyses

The proband was identified through screening of individuals with a total plasma cholesterol level below 150 mg/dl as previously described (9). Blood from fasted donors was drawn into tubes containing EDTA (1.0 mg/ml). Small aliquots of whole blood were removed and stored at -70°C for isolation of genomic DNA from the leucocytes (see below). The remaining sample was centrifuged and small aliquots of plasma were stored at -70°C for immunoblotting (see below). For some experiments, blood cells were separated by low speed centrifugation and the leucocytes were immediately isolated from packed blood cells using Histopaque-1077 (Sigma, St. Louis, MO) and DNA was isolated as described below. Total plasma lipids and lipoprotein-lipids were determined in the Washington University Lipid Research Center Core Laboratory using commercial kits (Technicon, Technicon Instruments, Tarrytown, NY) and protocols of the Lipid Research Clinics (11). Two aliquots of 1.5 ml and 14 ml of fresh plasma were used for FPLC and density gradient ultracentrifugation (DGUC), respectively. ApoB and apoA-I plasma concentrations were determined by immunonephelometry (Behring, Somerville, NJ). Lp[a] concentrations were determined by ELISA using a commercial kit (Terumo, Elkton, MD). Lp[a] phenotypes were determined by Dr. Santica Marcovina, at the University of Washington, Seattle, WA (12).

Electrophoresis and immunoblotting

Identification of truncated forms of apoB in plasma was performed as previously described (6). Briefly, apoB was immunoprecipitated from plasma, the immunoprecipitated pellets were washed and then dissolved in SDS-PAGE sample buffer, incubated at 100°C for 5 min and applied to 3–6% gradient SDS-polyacrylamide gels for electrophoresis. Proteins were electrotransferred to Immobilon-P membranes (Millipore Corporation, Bedford, MA) and immunoblotted with an anti-apoB monoclonal antibody (C1.4) as described previously (7). Where indicated, enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham International, Amersham, UK) were used according to the protocols recommended by the manufacturer.

Nondenaturing gradient gel electrophoresis (GGE) was performed essentially as described previously (13) with the exception that whole plasma or specific lipoprotein subfractions were analyzed on gels freshly prepared in the laboratory. Western blots of GGE gels or SDS-PAGE gels of lysine-Sepharose column fractions were performed as previously described using ^{125}I-radiolabeled secondary antibodies (6).

DNA preparation

Genomic DNA was isolated from whole blood as described by Kawasaki (14). Briefly, whole blood was centrifuged and the white cells were washed several times in TE (10 mM Tris-HCl, 1 mM EDTA pH 7.4) until no more red cells remained. The cells were treated with a buffer containing proteinase K at 56°C for 45 min, followed by 95°C for 10 min to inactivate the protease. The DNA was stored at -20°C. When larger amounts of genomic DNA were required, DNA was isolated from a white cell pellet (obtained by Histopaque-1077 separation) (15).

Polymerase chain reaction and DNA sequencing

Two PCR primers, B38.9-1 and B38.9-2, encompassing the region of the apoB gene predicted to contain the mutation were synthesized by the Protein Chemistry Core Facility at the Washington University Medical School. The forward primer, B38.9-1, started at cDNA position 4412 (5'-CAAATTTCTAGATTCGAATATCAAAT-3') and the reverse primer, B38.9-2, was the complement to the cDNA sequence between cDNA position 5847 and 5864 (5'-gggaattcATTTGTATGTGCATCGAT-3') according to the sequence of Knott et al. (16). The small type at the 5'-end of the oligonucleotide encodes an added *Eco*RI site. The PCR-amplified gene fragment encompasses a region of the apoB gene which includes the sites of the previously described apoB-31 (17) and apoB-40 mutations (8). The 1460 bp PCR product contains a unique internal *Xba*I site near the 5'-end and a *Cla*I site near the 3'-end in addition to the engineered *Eco*RI site. Genomic DNA (2.5 μg) and

50 pmol of each forward and reverse primer were amplified in 5 × 100 μl reactions with recombinant *Taq* polymerase as reported previously (18). The DNA was denatured at 94°C for 4 min and 30 cycles of PCR at 92°C for 1 min and 58°C for 5 min were performed in an automated temperature cycler (CoyTempCycler, Ann Arbor, MI). After checking a small aliquot (3 μl) of each reaction for the formation of the correct PCR product, the five reactions were combined, gel-purified by Magic PCR Preps (Promega, Madison, WI), and digested with *Xba*I and *Cla*I (both from Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's instructions. The DNA was recovered by precipitation and ligated into pGEM7Zf that had been digested with the same restriction enzymes as the PCR product and gel-purified as above. *E. coli* JM 109 were transformed with the recombinant plasmid. Clones were expanded by standard methods and plasmid DNA was prepared by the Magic Miniprep procedure (Promega, Madison, WI). Clones containing the correct insert were identified by digesting 0.5 μg plasmid DNA with *Xba*I and *Cla*I. Two μg of plasmid DNA was sequenced by the Sequenase dideoxy sequencing procedure (United States Biochemical Corp., Cleveland, OH) (19) using primer B38.9-3 which started at cDNA position 5276 (5'-CATGATTCTGGGTGTCGACA-3').

Allele-specific oligonucleotide analyses

A pair of allele-specific oligonucleotides (ASO) was synthesized with the following sequences: B38.9-wt: 5'-TTGACAACATTTACAGCT-3' and B38.9-mt: 5'-CTTGACAAATTTACAGCT-3'. The oligonucleotides were labeled at the 5'-end with 5'-γ-^{32}P]ATP (Amersham, Arlington Heights, IL) using T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN). The 1460 bp PCR product was generated from genomic DNA as described above. Forty μl of each PCR product was denatured in NaOH (final concentration 0.2 N). The mixture was heated to 95°C for 5 min and quenched with 800 μl of ice-cold 15 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M trisodium citrate). For each sample two aliquots of 400 μl were applied to a nitrocellulose membrane (Schleicher & Schuell Inc., Keene, NH) using a slot-blot apparatus. The membrane was baked at 80°C for 2 h under vacuum. The membrane was cut into two strips, each containing an identical set of samples. The strips were placed in separate bags containing a prehybridization solution of 6 × SSC, 0.1% SDS, and 10 × Denhardt's (0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin). The strips were incubated for 6 h at 42°C after which the solution was replaced with fresh hybridization solution (as above) containing either the wildtype or mutant ASO. The membranes were hybridized for 15 h at 44°C. The membranes were initially washed twice at room temperature in 6 × SSC for 5 min. The membranes were then washed in decreasing salt concentrations (i.e. increasing stringency) and the final wash was at 44°C in 2 × SSC for 30 min. The membranes were exposed for 24 h at −70°C using Kodak XAR-5 film and intensifying screens.

3' VNTR analysis

Genomic DNA was isolated from all the members of the kindred as described above. The polymorphic region in the 3' untranslated region of the apoB gene was amplified by PCR using primers and conditions as described previously (10). The various alleles were assigned by comparison with known alleles, kindly provided by Lawrence Chan.

FPLC separation of plasma

Plasma (1.5 ml) was chromatographed on two 25-ml Superose 6 columns connected in series at room temperature as described previously (7). The column eluent fractions were analyzed enzymatically for cholesterol (Wako Pure Chemicals, Richmond, VA) and Lp[a] as described above. Equal aliquots (35 μl) were removed from each column fraction and applied to 3-6% gradient SDS-PAGE gels for electrophoresis, immunoblotting, and radiochemical detection of apoB as described previously (6). Bands corresponding to apoB-100 and apoB-38.9 on the resulting autoradiograph were scanned using a laser densitometer. Areas under the peaks were determined using SigmaScan (Jandel Scientific, Corte Madera, CA). The densitometric areas corresponding to either apoB-100 or apoB-38.9 were summed and the values representing percents of the total densitometric area determined for apoB-100 and apoB-38.9 in any particular elution fraction were used to generate the distribution curve for each apoB species.

Density gradient ultracentrifugation of plasma (DGUC)

The DGUC method was performed as described elsewhere (20) with some modifications. Briefly, venous blood was collected into tubes containing EDTA (1 mg/ml) after a 12-h fast and plasma was centrifuged for 15 min at 1500 *g* at 4°C. D-Phe-L-Phe-L-Arg chloromethylketone (20 μM; PPACK) and D-Phe-L-Pro-L-Arg chloromethylketone (20 μM; both from Calbiochem, La Jolla, CA) were added promptly to prevent cleavage of apoB by kallikrein and thrombin, respectively. Plasma was adjusted to a density of 1.040 g/ml with solid potassium bromide (KBr). The density gradient was prepared in 40 ml QuickSeal tubes (Beckman Instruments, Fullerton, CA) as follows. Six ml solution A (0.195 M NaCl; 1 mM EDTA-Na_2, d 1.006 g/ml) was loaded into the tube using a syringe equipped with a 3.5-in long 18-gauge needle with the end bevel removed. This solution was carefully underlayered with 10 ml of solution A adjusted to d 1.020 g/ml with KBr. Fourteen ml of plasma (d 1.040 g/

ml) was subsequently underlayered. Finally, 10 ml of solution A adjusted to d 1.210 g/ml with KBr was underlayered into the same tube. A blank gradient containing 14 ml of solution A adjusted to d 1.040 g/ml instead of plasma was used as balance. The tubes were centrifuged in a Beckman 50.2 Ti rotor for 24 h at 45,000 rpm at 12°C. The rotor was stopped without the brake and the gradient was pumped off from the top by pumping solution A, adjusted to d 1.300 g/ml with NaBr, into the bottom of the tube with a peristaltic pump set at 1 ml/min. Fifty fractions of approximately 1 ml (34 drops) were collected using a fraction collector (Gilson, Middleton, WI). The fractions were analyzed enzymatically for cholesterol and triglycerides (Wako Pure Chemicals, Richmond, VA), protein (21) and Lp[a] as described above. Equal aliquots (35 μl) were removed from each DGUC fraction and applied to 3–6% gradient SDS-PAGE gel for electrophoresis and immunoblotted for apoB as described above for the FPLC fractions. The resulting autoradiograph was scanned and the bands for apoB-100 and apoB-38.9 were quantified as described above for FPLC fractionation. Fifty fractions were collected on the blank gradient as above. Consecutive pairs of tubes were pooled and the density was measured on the 25 2-ml fractions using a DMA 35 densitometer (PAAR, Graz, Austria). The DGUC was performed twice in 5 months on fresh plasma from the proband and the results were highly reproducible.

Lysine-Sepharose chromatography

One-ml aliquots of plasma were applied to a 4-ml column of lysine-Sepharose 4B (Pharmacia/LKB, Uppsala, Sweden) equilibrated with 50 mM phosphate, 0.15 M NaCl, 3 mM EDTA and 0.02% sodium azide, pH 7.4 (22). The column was washed after the application of plasma with the same buffer until the absorbance at 280 nm was zero. Nonspecific protein was eluted from the column with the same buffer containing 0.5 M NaCl. Lp[a] was eluted with 200 mM ϵ-aminocaproic acid (EACA) in the equilibration buffer. Columns were washed with buffer containing 400 mM ϵ-aminocaproic acid (1–2 bed volumes) before re-equilibrating the columns with the starting buffer.

Reconstitution of r-apo[a] with apoB

Reconstitution experiments were carried out essentially as described by Chiesa et al. (23). Dr. Richard Lawn, Stanford, CA (24) kindly provided the r-apo[a]. Fifteen μg of the lipoprotein under study was incubated at 37°C with 0.3 μg of r-apo[a] in 0.9% NaCl in a final volume of 40 μl for different lengths of time. At the end of the incubation time, a 20-μl aliquot was removed and electrophoresed on a 4% nondenaturing gel as described (13). The gel was immunoblotted using an ^{125}I-labeled monoclonal anti-apo[a] antibody (generously provided by Dr. Santica Marcovina, Seattle, WA) as described (6).

RESULTS

Proband and his kindred

The proband is a 71-year-old man (subject I-1 in **Table 1** and **Fig. 1** and **Fig. 2**) with non-insulin-dependent diabetes treated with Glipizide (one 5 mg tablet/day). His blood urea nitrogen (BUN) and creatinine levels were within the normal range (15 mg/dl and

TABLE 1.

Subject	Gender	Age	BMI	TChol	TG	LDL-C	HDL-C	ApoB	ApoA-I	Lp[a]
		yr	*kg/m²*				mg/dl			
Affected apoB-100/apoB-38.9										
I-1[a]	M	71	23.3	74[b]	67	27[b]	32	31	118	28
I-3	M	70	28.1	103[b]	124	51[b]	30[b]	55	105	45
II-3	M	37	25.3	115[b]	32[b]	47[b]	60	41	149	5
II-5	F	45	36.1	123[b]	53	62[b]	53	38	131	138
II-7	F	39	29.8	163	34	89	70	52	166	173
III-6	F	19	20.9	116[b]	40	33[b]	75	26	150	48
III-7	M	20	24	81[b]	39	34[b]	43	26	110	65
Unaffected apoB-100/apoB-100										
II-1	M	38	35	242	171	159	46	141	141	24
II-6	M	46	23.4	206	223	128	38	106	120	15
II-8	M	42	26.5	218	151	137	49	119	136	9
III-5	F	26	26.4	156	228	102	25	94	80	39

Plasma lipid profiles were determined using protocols of the Lipid Research Clinics and plasma apoB, apoA-I, and Lp[a] concentrations were determined as described in Methods. ApoB phenotypes were assigned on the basis of Western blot analysis. BMI, body mass index; TChol, total plasma cholesterol; TG, total plasma triglyceride; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; ApoB, apolipoprotein B; ApoA-I, apolipoprotein A-I; Lp[a], lipoprotein[a].

[a]The Lp[a] phenotype for the proband (I-1) was determined as 17/6 (see Methods).

[b]< 5th percentile.

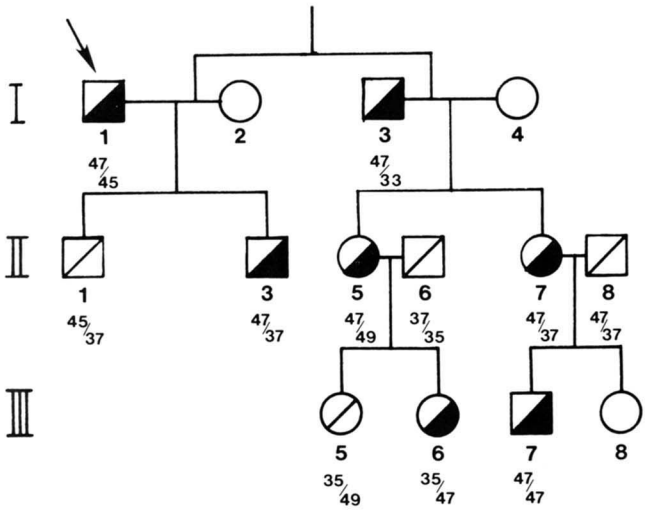

Fig. 1. Pedigree of the apoB-38.9 kindred. ApoB phenotypes were determined by Western blot analysis (Fig. 2). ApoB-100/apoB-38.9 phenotype: ◪, ◪; apoB-100/apoB-100: ▨, ⊘; not tested: ○. The proband is identified by the arrow. The plasma lipid data are given in Table 1. The 3' VNTR results are given below each appropriate member of the kindred.

1.2 mg/dl, respectively) and he has microalbuminuria (269 mg/24 h, normal range 2–21 mg/24 h). His plasma total and LDL-cholesterol concentrations were <5th percentile for his age, race, and sex. The immunoblot of the proband's plasma revealed apoB-100 as the major form of apoB (Fig. 2, lefthand panel). In addition, a second apoB immunoreactive band was present which had an apparent molecular size between the previously identified truncated apoB proteins apoB-37 (25) and apoB-40 (8). This protein accounted for approximately 5% of the total apoB protein as estimated from scans of the immunoblots. The molecular weight of this new protein estimated from this blot was 217,200 Daltons (or apoB-39.5). A protein of the same size was also present in the plasma of his brother, his brother's two daughters (Fig. 2, righthand panel) and three other members of this kindred (results not shown). Plasma total and LDL-cholesterol concentrations in the affected relatives were below the 5th percentile except for individual II-7. However, her plasma Lp[a] levels were very high (173 mg/dl) and this may account for her slightly higher total and LDL-cholesterol levels. The unaffected relatives had normal plasma lipid concentrations. The Lp[a] levels in this kindred are shown in Table 1. The proband's plasma Lp[a] concentration was 28 mg/dl.

ApoB-38.9 mutation

Initially, it was important to exclude the possibility that the truncated protein in this kindred was due to the same mutation that gives rise to one of three previously identified mutations, apoB-37, apoB-39, or apoB-40, because the protein bands of these mutant proteins migrated quite close to the newly identified band (Fig. 2). To exclude the apoB-37 and apoB-40 mutations, two separate allele-specific oligonucleotide (ASO) analyses were performed using primers and conditions as reported previously (8, 25). For both mutations, under conditions where the wildtype ASOs hybridized to the proband's DNA, there was no detectable hybridization of the mutant apoB-37 or apoB-40 ASOs (results not shown). We therefore concluded that the mutation leading to the truncated protein in this newly identified kindred was neither an apoB-37 nor an apoB-40 mutation.

Genomic DNA, isolated from the proband's white blood cells, was used to amplify the region of the apoB gene that includes the potential mutation sites from apoB-31 (17) to apoB-40 (8). The fragment was amplified by polymerase chain reaction (PCR) and cloned into pGEM7Zf as described in the Methods section. Fifteen clones were sequenced in the 5' to 3' direction using a primer directed 5' to the site of the previously identified apoB-37. Three clones (**Fig. 3**) had the wildtype sequence according to Knott et al. (16). The remaining twelve clones had a deletion of a cytosine-residue at cDNA position 5444 (Fig. 3). The expected frequency of clones containing the mutant versus the wildtype sequence is 50%. The reason for the discrepancy in our experiment (12

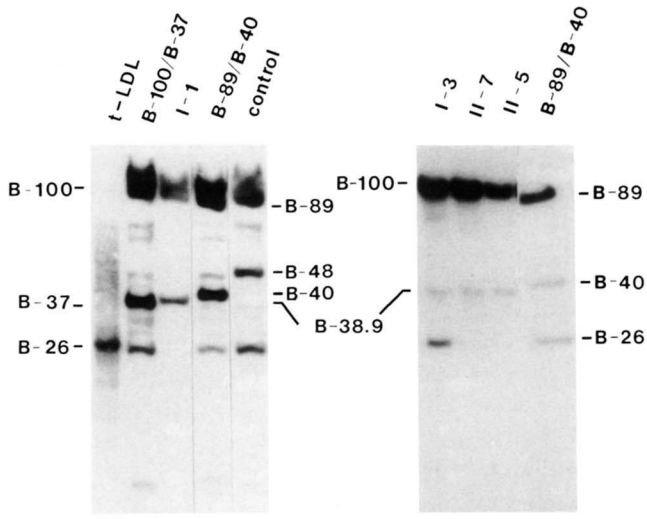

Fig. 2. Identification of the apoB-38.9 protein. Immunoprecipitated apoB from the plasma of the proband (I-1) was run on a 3–6% SDS-PAGE gel. For comparison, samples containing previously identified apoB truncations B-37, B-40, and B-89 were run along side the proband's. A control sample identified the positions of apoB-100 and apoB-48. Thrombin-digested LDL apoB-100 (t-LDL) was used to identify the position of apoB-26. On a separate 3–6% SDS-PAGE gel, immunoprecipitated apoB from individuals I-3, II-7, and II-5 were run alongside apoB from the previously identified apoB-40/apoB-89 individual. Immunoblotting with an anti-apoB monoclonal antibody (C1.4) was performed as described in the Methods Section. ECL detection was used for the lefthand blot. The resultant fluoro- and autoradiograms are shown with the apoB species as determined by molecular weights indicated. ApoB-37 plasma kindly provided by Dr. Steve Young, Gladstone Institute, San Francisco, CA.

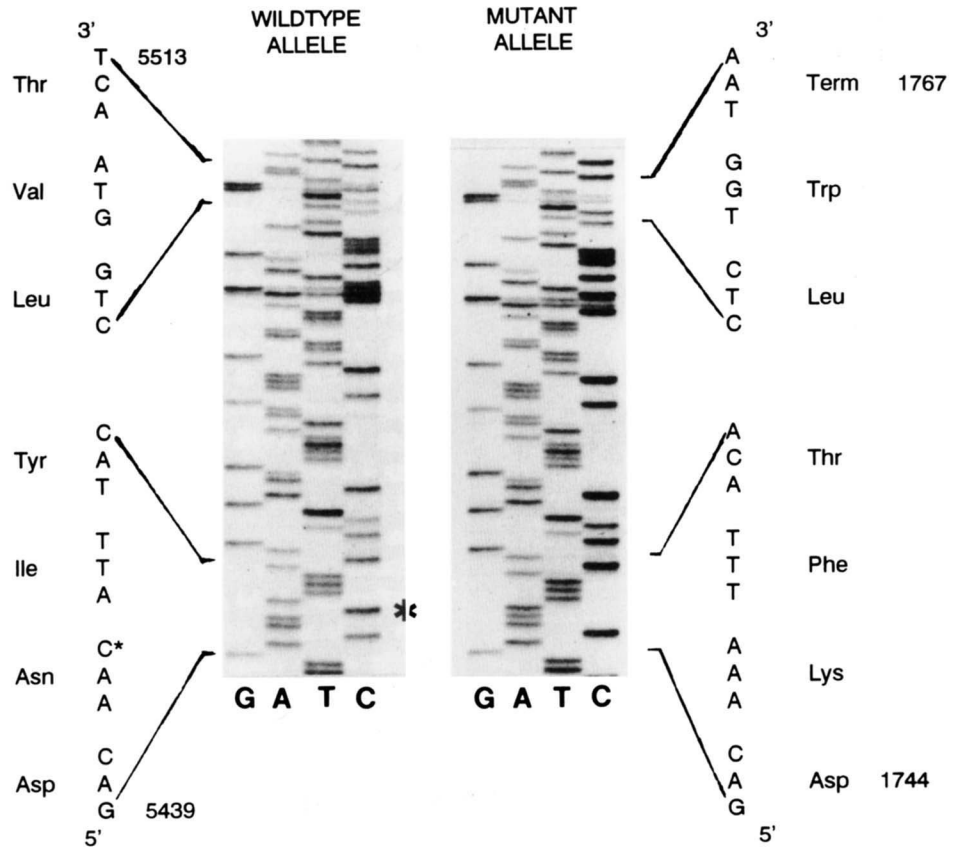

Fig. 3. Identification of the apoB-38.9 mutation. The region of the apoB gene predicted to contain the mutation, as determined by the size of the protein on the Western blot, was amplified by PCR using primers and conditions described in the Methods section. The fragment was cloned into pGEM7Zf and clones containing the correct insert were identified as described in the Methods section. Clones were sequenced by standard dideoxysequencing procedure. Fifteen clones were sequenced and 12 yielded the mutant sequence shown. The * in the wildtype sequence indicates the base that is deleted in the mutant clones. The numbers beside the wildtype sequence (left) indicate cDNA position and the numbers beside the mutant sequence (right) indicate the amino acid residue. The predicted new 22-amino acid sequence is given in the Results section.

versus 3) is unclear. The deleted cytosine results in a frameshift leading to 22 new amino acid residues (lys-phe-thr-ala-leu-thr-ser-phe-ile-ser-lys-leu-leu-ile-tyr-ser-tyr-ser-pro-ile-leu-trp) and a termination codon at amino acid residue 1767. This predicts a protein containing 1766 amino acids which is 38.9% of the full length protein (4536 amino acids) according to the centile nomenclature (26). This protein was thus assigned the name apoB-38.9.

In addition to identifying the apoB-38.9 mutation, the sequencing results also confirmed that for all the clones that were sequenced, the apoB-39 (27) mutation sites (and also apoB-37) were normal.

Allele-specific oligonucleotide and 3' VNTR analyses for the apoB-38.9 mutation

To confirm the deletion of the cytosine-residue in the proband and his son, allele-specific oligonucleotides were designed and an ASO assay was carried out as described in the Methods section. Plasmid DNA from clones containing wildtype (lane marked B-100) and mutant sequences (lane marked B-38.9) were applied to the membrane to confirm selective binding of the ASOs. DNA amplified from the proband (I-1) and his affected son (II-3) hybridized to both wildtype and mutant ASOs, confirming that both alleles are present in their genomes (**Fig. 4**). Conversely, the DNA from the unaffected son (II-1) and the previously described apoB-40/apoB-89 individual (8) only hybridized to the wildtype ASO, confirming that their genomes do not contain the apoB-38.9 mutation. In a separate ASO analysis, the presence of the mutation in individuals I-3, II-5, and II-7 was also confirmed (results not shown). The 3' VNTR alleles of the apoB gene were determined as described in Methods. The results (Fig. 1) indicate that the allele carrying the 3' β 47 repeat co-segregates with the mutant allele in all the affected members of the kindred.

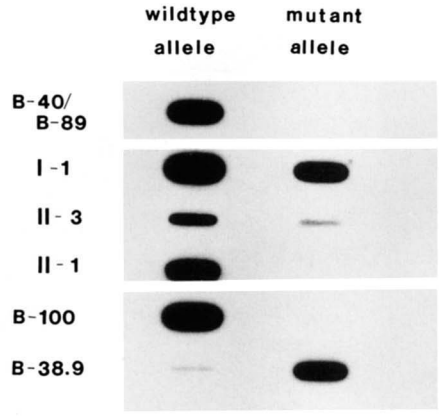

Fig. 4. Confirmation of the apoB-38.9 mutation by allele-specific oligonucleotide analysis. The region of the apoB gene predicted to contain the apoB-38.9 mutation was amplified by PCR and the reaction products were blotted onto a nitrocellulose membrane as described in Methods. Each identical half of the membrane was hybridized with one of the ^{32}P-end labeled ASOs, selectively washed and exposed to X-ray film as described in Methods. B-89/B-40 indicates PCR-amplified DNA from an individual previously identified with these truncated apoB species. The members of the kindred can be identified from the data in Table 1 and Fig. 1: I-1 and II-3 have apoB-100/apoB-38.9 phenotypes and II-1 has the apoB-100/apoB-100 phenotype. B-100 and B-38.9 indicate DNA from plasmids containing the wildtype B-100 or mutant B-38.9 sequences, respectively, as controls.

Physical characterization of apoB-38.9-containing particles

Gel filtration. **Fig. 5** shows the size-separation profile of plasma from the proband. The cholesterol elution profile indicated a normal VLDL particle size and broad peaks in the LDL and HDL size ranges. The shoulder visible before the leading edge of the LDL-associated cholesterol peak is likely due to the presence of IDL- and Lp[a]-sized particles. The major peak of LDL eluted with a peak at fraction 28, slightly smaller than normolipidemic plasma LDL which peaks between fractions 25 and 27. ApoB-100 was associated with particles of VLDL, IDL, Lp[a], and LDL-size. Thirty-nine percent of apoB-38.9 eluted as peaks corresponding in size to IDL and LDL (fractions 16–34) with the major peak (60% of total) eluting between LDL- and HDL-sized particles (fractions 35–50). Approx. 1% of total apoB-38.9 eluted as VLDL-sized particles. Lp[a] eluted between fractions 18–26 in the IDL size range. Both apoB-100 and apoB-38.9 were detectable in these fractions; approximately 40% of the total apoB-100 and 15% of the total apoB-38.9 were found in these fractions.

Density gradient ultracentrifugation (DGUC). The density distribution of the proband's plasma is shown in **Fig. 6.** The cholesterol distribution along the density gradient profile shows three major peaks, the first in the density region of VLDL and IDL ($d < 1.020$ g/ml, fractions 1–5). The second and third cholesterol peaks eluted between the density of LDL ($1.032 < d < 1.064$ g/ml, fractions 11–24) and HDL ($1.069 < d < 1.194$ g/ml, fractions 26–44). A shoulder was present in the HDL density region between d 1.069 g/ml and d 1.098 g/ml. Most of the triglycerides were found in the VLDL and IDL region (results not shown). ApoB-100 was associated primarily with particles in the LDL density range, however, ≈10%

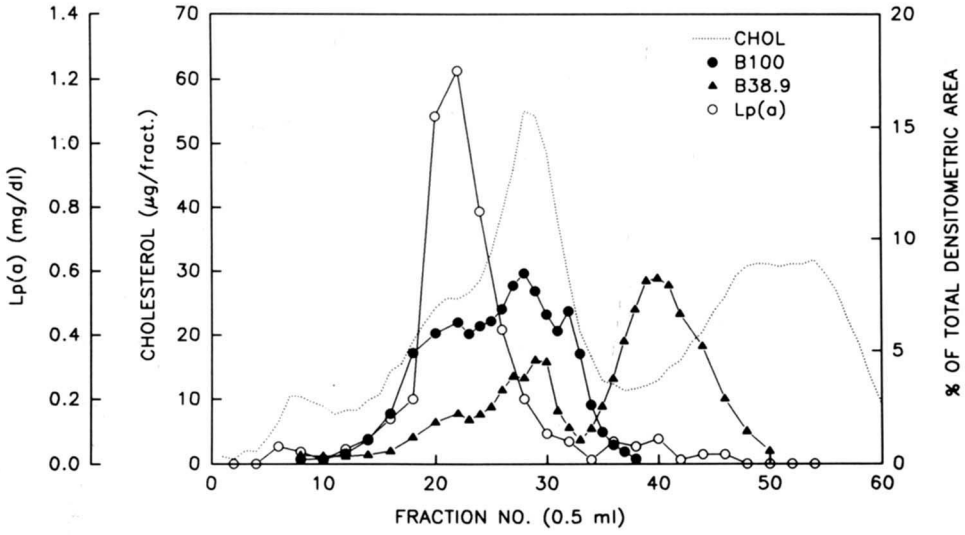

Fig. 5. Separation of plasma apoB-100 from apoB-38.9 by FPLC. Plasma (1.5 ml) was subjected to gel permeation chromatography on two Superose 6 columns as described in Methods. The dotted line represents μg/fraction total cholesterol determined in each fraction. The open and closed triangles represent percents of the total summed densitometric areas for apoB-100 and truncated apoB, respectively, determined in each column fraction. Normal plasma VLDL, LDL, and HDL elute between fractions 5–9, 25–27, and 44–47, respectively. The open circles represent Lp[a] concentration determined in each fraction as described in Methods.

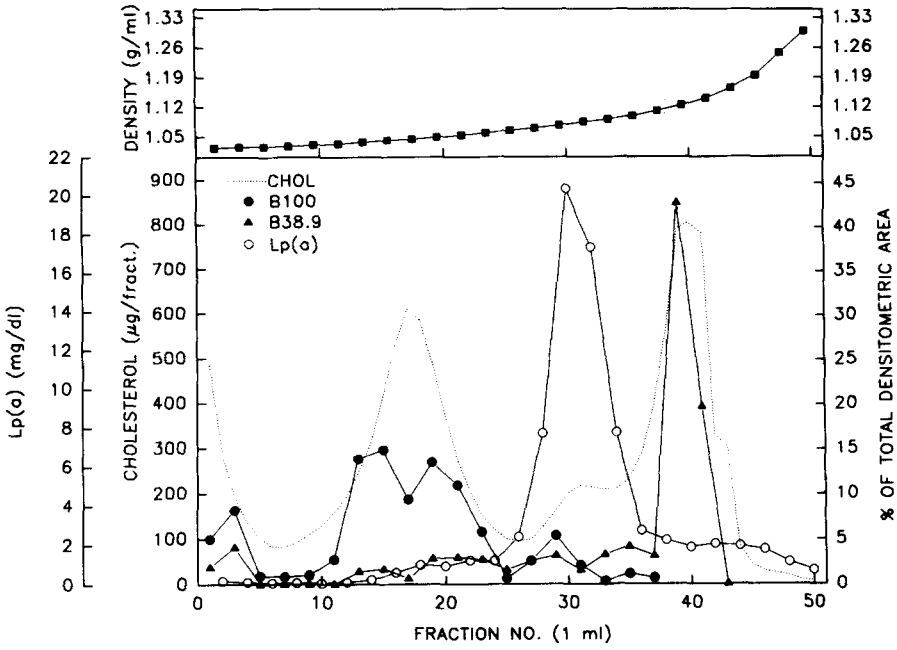

Fig. 6. Separation of plasma apoB-100 from apoB-38.9 by density gradient ultracentrifugation. Fourteen ml of plasma from subject I-1 was separated on a density gradient as described in Methods. Eluent fractions were analyzed for total cholesterol (dotted line) and for apoB-100 (solid circles) and apoB-38.9 (solid triangles) as described for Fig. 5. The profile of the density gradient is shown at the top of the graph. VLDL floats at the very top of the tube in fractions 1-3. The open circles represent Lp[a] concentration determined in each fraction as described in Methods.

of apoB-100 was found in the VLDL and IDL density area and ≈10% in the density range d 1.069–1.084 g/ml (fractions 26–32). No apoB-100 was detected in the HDL density range (d>1.10 g/ml). The largest proportion of apoB-38.9 (≈65% of total) eluted in the HDL density range (1.10<d<1.194 g/ml, fractions 37–43) with a small peak (≈5% of total) associated with VLDL and IDL density. The remainder was present between densities 1.036 to 1.10 g/ml (fractions 12–36). A major peak for Lp[a] eluted at a density range of 1.069–1.098 g/ml (fractions 26–35). Both apoB-100 and apoB-38.9 were present at the peak density of Lp[a], however, apoB-100 accounted for approximately 95% of the total apoB protein in this fraction (with the remainder being apoB-38.9) as estimated from the scans of the immunoblots.

Nondenaturing gradient gel electrophoresis. Consistent with the findings on FPLC (Fig. 5), nondenaturing gradient electrophoresis of whole plasma on 2–16% gels followed by immunoblotting with region-specific monoclonal anti-apoB antibodies confirmed the presence of apoB-38.9-containing particles intermediate in size between LDL and HDL. In fact, two subpopulations of small apoB-38.9-containing particles were seen, one larger and one smaller than 17 nm (**Fig. 7,** lane 2). We hypothesized that these two populations corresponded to the apoB-38.9-containing lipoproteins that floated at HDL density in the DGUC experiment (Fig. 6). To test this hypothesis, the following experiment was performed. Fractions 38–42 from a DGUC profile of the proband were pooled and applied onto a 4–30% nondenaturing gradient gel for electrophoresis and immunoblotting (**Fig. 8,** lane 5). Normal LDL (lane 2), HDL_2 (lane 3), HDL_3 (lane 4) from a B100 homozygote, and lipoproteins isolated between the densities of 1.063–1.21 g/ml from a B100/B31 simple heterozygote and a B89/B40 compound heterozygote were run for comparison. Replicate gels were subjected to Coomassie blue staining (left panel), or immunoblotted with monoclonal antibodies to apoB (middle panel) and apoA-I (righthand panel). The apoB-containing lipoproteins were clearly separated from the apoA-I-containing particles. The apoB-38.9-containing lipoproteins seen in lane 5 (Fig. 8) migrated as two distinguishable bands of approximately 15 nm and 18 nm in size, consistent with findings on the 2–16% gels using whole plasma. This experiment demonstrated that the DGUC fractions 38–42 (Fig. 6) did contain two populations. These probably correspond to the apoB-38.9 particles seen in FPLC fractions 35–50 (Fig. 5). It was of interest to note that two populations of apoB-31 particles having HDL density also were noted (lane 6, Fig. 8) and this was especially evident on shorter exposures of the immunoblot (not shown). The apoB-38.9 particles were on average of smaller size than the two populations of apoB-31 HDL density particles. Thus, apoB-38.9 is found in a variety of discrete particle

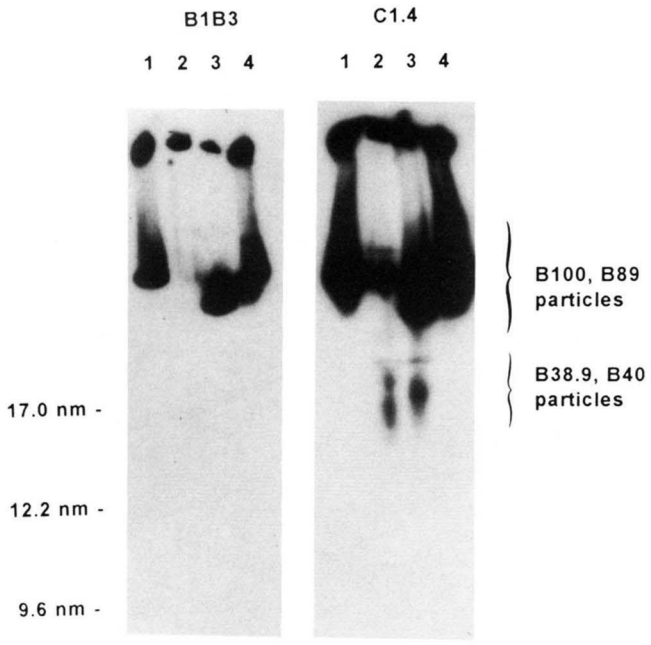

Fig. 7. Nondenaturing gradient gel electrophoresis (2–16%) (GGE) of whole plasma from the proband, an apoB-89/apoB-40 compound heterozygote, and two normolipidemic control subjects. Twenty μl of plasma was applied per lane of the GGE gel and run as described in Methods. Reference proteins were run as size calibrators and the migrations of thyroglobulin (17.0 nm), apoferritin (12.2 nm), and catalase (9.6 nm) deduced from a Coomassie blue-stained portion of the gel are shown for reference purposes. Plasma samples applied to each lane were as follows: lanes 1 and 4, two unrelated normolipidemic controls (apoB-100/apoB-100); lane 2, proband; and lane 3, apoB-89/apoB-40 hypobetalipoproteinemic subject. Replicate portions of the gel were electrotransferred to Immobilon-P and immunoblotted with a monoclonal antibody directed towards either the carboxyl terminal region of apoB (B1B3, apoB amino acids 3506–3635) or the amino terminal region of apoB (C1.4, apoB amino acids 470–526) as indicated. Lipoprotein particles immunoreactive to antibody B1B3 contained full-length apoB-100 or apoB-89 as indicated. Particles immunoreactive exclusively with antibody C1.4 contained only apoB-38.9 or apoB-40 and these migrated further than the apoB-100- or apoB-89-containing particles. This experiment could not distinguish between the apoB-38.9- or apoB-40-containing particles co-migrating with the larger sized apoB-100- or apoB-89-containing particles seen by FPLC (Fig. 5).

populations ranging in size from IDL through small LDL and in density from IDL through HDL.

Characterization of the interaction of apoB-38.9 with apo[a]

Lysine-Sepharose chromatography. As the density gradient ultracentrifugation profiles of the proband's plasma seemed to suggest that apoB-38.9 did not form complexes with apo[a], lysine-Sepharose chromatography was used further to investigate the form of apoB associated with the apo[a] of Lp[a]. Whole plasma from the proband, a B100 homozygous subject matched for Lp[a] concentrations and a B100 homozygous subject deficient in Lp[a] were applied onto a lysine-Sepharose column. The Lp[a] bound to the lysine-Sepharose was eluted with 200 mM EACA and this fraction was applied to 3–6% SDS-PAGE (**Fig. 9**). After Coomassie staining (left panel), the material eluting with 200 mM EACA appeared to be plasminogen (PA) and barely detectable amounts of apoB or apo[a] at the top end of the gel. After immunoblotting with anti-apoB (MAb C1.4, middle panel) the proband (lane 2) and subject 1 (lane 1) had detectable apoB in the 200 mM EACA eluting fraction. The third subject (lane 3) had no detectable apoB, probably because he had barely detectable plasma concentrations of Lp[a]. Only apoB-100 was seen in the EACA elutable fraction from the proband (lane 2, middle panel). No apoB-38.9 nor any degradation products were detectable by immunoblotting, even after longer exposures of the autoradiographs, providing evidence that apoB-38.9 did not associate with apo[a]. ApoB-100 degradation products were seen in the eluted Lp[a] from subject 1.

To confirm that Lp[a] was, in fact, eluted from the lysine-column with EACA, a replicate gel piece was electrotransferred and immunoblotted with a rabbit polyclonal antibody (R136) directed against human Lp[a] (Fig. 9, righthand panel). Preliminary experiments with this antibody had shown that human LDL pre-adsorbed with a commercially available monoclonal antibody directed against apo[a] did not react on Western blots with the rabbit antibody (data not shown). Subject 1 and the proband had detectable apo[a], whereas no immunoreactive material was detected in the 200 mM EACA wash from the Lp[a]-deficient subject 3. The doublet band of apo[a] in the fraction from the proband most likely reflects the apo[a] phenotype of 17/6, whereas the isoforms (26/16) in subject 1 are not as well resolved. There appear to be degradation products of apo[a] in the fraction from subject 1.

Immunoprecipitation of Lp[a] from the proband's plasma. A second approach to confirm that apoB-38.9 does not associate with apo[a] was carried out by immunoprecipitation using a monospecific polyclonal antibody directed against the Lp[a] particle (generously provided by Dr. Santica Marcovina). Two 10-μl aliquots of the proband's plasma were immunoprecipitated with 30 μl each of polyclonal anti-Lp[a] antibody (24 mg/ml) using the standard immunoprecipitation method described (6). Aliquots of both the pellets and the supernatants were subjected to a 3–6% SDS-PAGE and electrotransferred to Immobilon-P as described in the Methods section. One set of samples was blotted with the anti-apoB monoclonal antibody C1.4 and a replicate set with a monoclonal antibody directed against kringle 4 of apo[a] (generously provided by Dr. Santica Marcovina). The results are shown in **Fig. 10.** Panel A shows that nearly all of apo[a] was precipitated by the polyclonal anti-Lp[a] antibody. Panel B shows that while apoB-100 was precipitated with the Lp[a] particles, no apoB-38.9 was found in the immunoprecipitate. Longer exposure of the C1.4 immunoblot (panel B) did not reveal any apoB-38.9 protein in the pellet of the im-

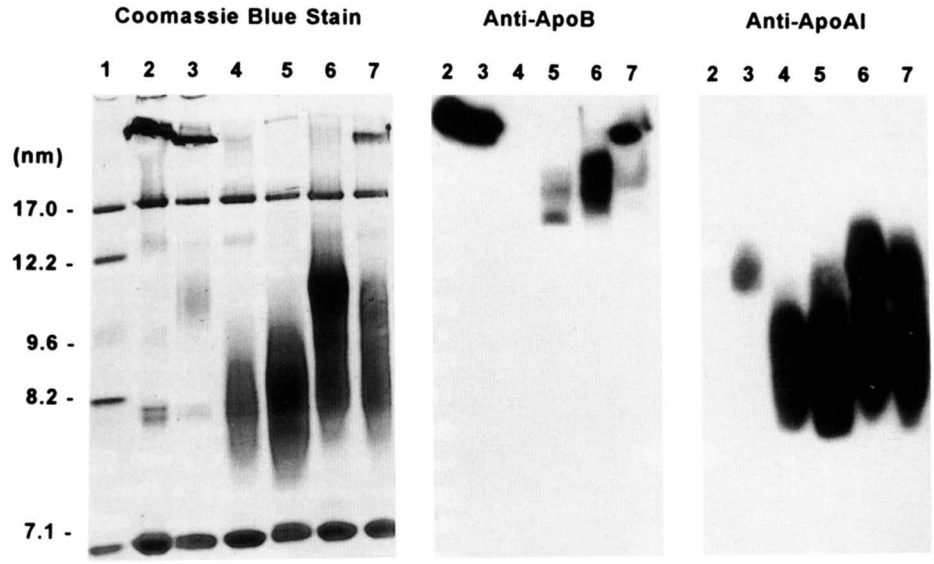

Fig. 8. Nondenaturing gradient gel electrophoretic (GGE) analysis of apoB-containing lipoprotein populations. Twenty-five μg of each lipoprotein fraction was applied to the GGE (4–30%) gels and run as described in Methods. Reference proteins were run as size calibrators (lane 1): thyroglobulin (17.0 nm), apoferritin (12.2 nm), catalase (9.6 nm), LDH (8.2 nm), and BSA (7.1 nm). Thyroglobulin and BSA were added to each lipoprotein sample as internal standard markers. Samples applied to each lane were as follows: lane 2, B100/B100 LDL; lanes 3 and 4, HDL_2 (d 1.063–1.125 g/ml) and HDL_3 (d 1.125–1.21 g/ml), respectively, isolated by sequential ultracentrifugation from a B100/B100 subject for comparison; lane 5, apoB-38.9-containing fraction from I-1 plasma (from pooled fractions 38–42 of density gradient shown in Fig. 6, $1.10 < d < 1.094$ g/ml); lanes 6 and 7, d 1.063–1.21 g/ml fractions from a B100/B31 (kindly provided by Dr. Stephen Young) and B89/B40 subject, respectively. The lefthand panel depicts the Coomassie Blue-stained gel. The middle and righthand panels depict the autoradiograms of immunoblots of lanes 2–7 run on replicate gel portions and probed with an anti-apoB (MAb C1.4) (described in legend to Fig. 7) or anti-apoA-I (MAb A5.6) monoclonal antibody as indicated.

munoprecipitate (results not shown) indicating that no apoB-38.9 was associated with Lp[a]. The polyclonal anti-Lp[a] antibody used in these experiments did not precipitate LDL when an Lp[a]-free LDL fraction was used (results not shown). In addition, the monoclonal anti-apo[a] antibody did not crossreact with apoB precipitated with a polyclonal anti-apoB antibody (R197-4). We conclude that this alternative approach confirms the lysine-Sepharose data of a lack of association of apoB-38.9 with apo[a].

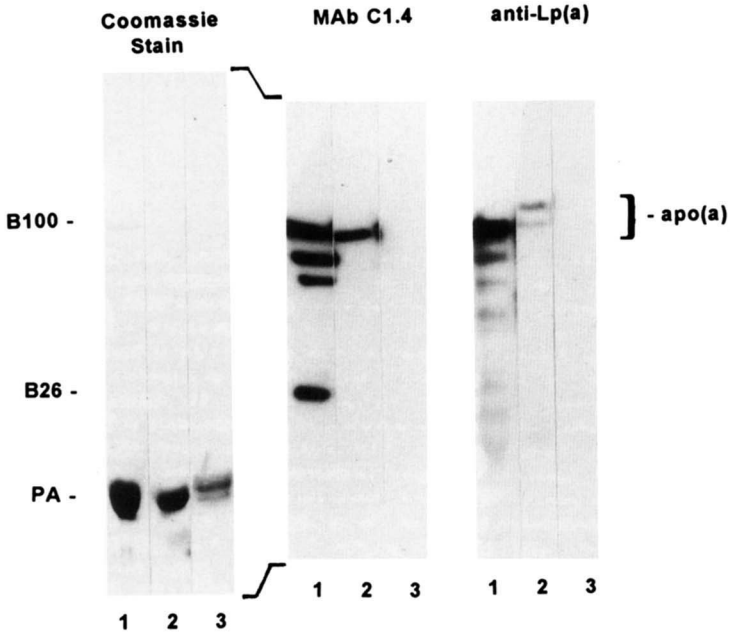

Fig. 9. SDS-PAGE (3–6%) analysis of the Lp[a]-containing fractions eluted from the lysine-Sepharose columns with 200 mM ϵ-aminocaproic acid (EACA). The proband is shown in lane 2 and two unrelated normal individuals lanes 1 and 3. Subjects whose fractions are shown in lanes 1 and 3 had plasma Lp[a] concentrations of 25 and 1.6 mg/dl, respectively. Each lane contained 0.035 A_{280} units of protein. The first panel represents the Coomassie blue-stained gel with the bands corresponding to apo[a] and apoB barely visible. Under the conditions described in the text, plasminogen (PA) eluted with Lp[a] in the 200 mM EACA wash, and can be seen in all three subjects. The second panel represents the autoradiograph of the Western blot using MAb C1.4 which is directed to the amino terminus of apoB. The additional bands below apoB-100 in lane 1 correspond to apoB degradation products. The third panel represents the autoradiograph of the Western blot using a rabbit polyclonal antibody (R136) directed against Lp[a]. (Preliminary experiments failed to show any reactivity of this antibody with apoB devoid of apo[a] on Western blots.) The Lp[a] phenotypes were determined by Dr. Santica Marcovina (12) and corresponded to 26/16 and 17/6 for subject 1 and the proband, respectively.

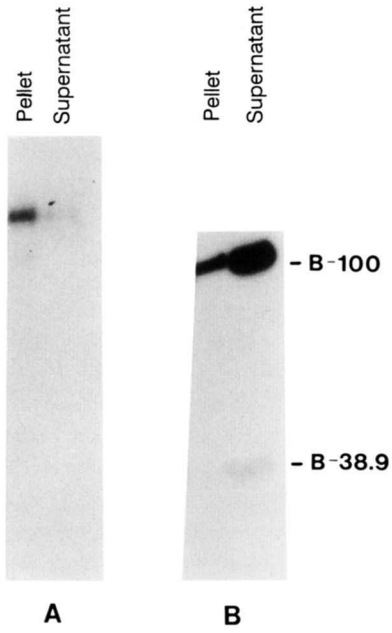

Fig. 10. SDS-PAGE analysis of the pellet and supernatant from immunoprecipitates with a purified polyclonal anti-Lp[a] antibody. Two 10-μl aliquots of the proband's plasma were immunoprecipitated with 30 μl each of purified polyclonal anti-Lp[a] antibody (24 mg/ml) using a standard immunoprecipitation method described (6). The immunoprecipitate was pelleted, the supernatant was removed, and an equal volume of SDS-PAGE sample loading buffer was added to the supernatant. The pellets were washed twice as described and resuspended in 50 μl SDS-PAGE sample loading buffer. Aliquots of each of the resuspended pellets (40 μl) and supernatants (70 μl) were separated on 3–6% SDS-PAGE and immunoblotted as described in Methods. Panel A represents the resulting autoradiogram using the anti-apo[a] monoclonal antibody (kindly provided by Dr. Santica Marcovina). The lanes show apo[a] reactivity. Panel B shows the replicate samples blotted with the anti-apoB MAb C1.4. The positions to which apoB-100 and apoB-38.9 migrated in I-1 plasma immunoprecipitated with anti-apoB (R197-4) are indicated alongside the C1.4 blot.

Reconstitution of recombinant apo[a] with apoB-38.9. A third approach to demonstrate the lack of association of apo[a] with apoB-38.9 was performed by incubating apoB-38.9- and apoB-100-containing lipoproteins with recombinant apo[a] protein (r-apo[a]) and analyzing the resulting particle populations on a non-denaturing gel. A DGUC profile of the proband's plasma was prepared as described in the Methods section. Fractions 14–18 were combined to give an Lp[a]-free "LDL-pool." Similarly, fractions 38–42 were combined to give an "HDL-pool" which contained apoB-38.9 without any apoB-100 or Lp[a]. The "HDL-pool" was incubated with r-apo[a] as described in the Methods section. As a positive control for reconstitution, the Lp[a]-free "LDL-pool" was incubated with r-apo[a] under the same conditions; r-apo[a], and the "LDL-" and "HDL-"pools, incubated for 6 h without r-apo[a], were used as negative controls. Incubation mixtures were analyzed on a 4% non-denaturing gel and immunoblotted with the monoclonal apo[a] antibody as described in the Methods section. LDL ($1.019 > d > 1.063$) from a normal control was used as a sizemarker for LDL particles. **Fig. 11** shows the resulting autoradiogram. "Free" apo[a] migrated to the position indicated. When the "LDL-pool" was incubated with r-apo[a] (lane 1), there was association as indicated by the broad band corresponding to LDL size (lane 8). Lane 2 shows that apo[a] was absent in the "LDL-pool" incubated in the absence of r-apo[a]. No evidence of association between apoB-38.9-containing particles and r-apo[a] was seen as shown by the absence of any larger particles after incubation of r-apo[a] with the "HDL-pool" (lanes 3–5) at any of the incubation times. The absence of any apoB-38.9 or HDL-sized particles demonstrates that apo[a] did not associate nonspecifically with either apoB-38.9 or with apoA-I, A-II or other components of HDL. Lane 6 shows the absence of apo[a] in the "HDL-pool" incubated without r-apo[a]. Thus we conclude that while r-apo[a] was able to associate with apoB-100 particles, apoB-38.9 did not associate with r-apo[a] and this provides further evidence that the association point of apoB-100 with apo[a] lies beyond the N-terminal 38.9% of the apoB protein.

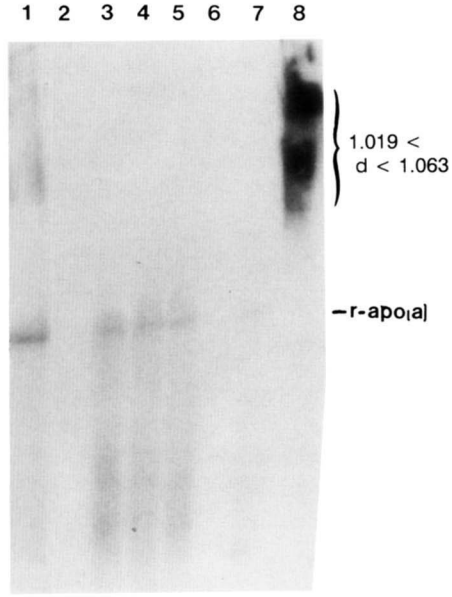

Fig. 11. Analysis of lipoproteins reconstituted with recombinant apo[a] (r-apo[a]) by 4% nondenaturing gel electrophoresis. An aliquot of r-apo[a] (0.3 μg) was incubated with an apoB-38.9 "HDL-pool" (15 μg of fractions 38–42, Fig. 6) for 0, 1, or 6 h as described in the Methods section. Similarly, an apoB-100 "LDL-pool" (15 μg of fractions 14–18, Fig. 6) was incubated with r-apo[a] (0.3 μg) as a positive control for reconstitution of apo[a] with apoB-100 LDL. The resulting particles were analyzed on a 4% nondenaturing gel as described in the Methods section. Lane 1: Lp[a]-free "LDL-pool" incubated with r-apo[a] for 6 h; lane 2: Lp[a] free "LDL-pool" incubated for 6 h without r-apo[a]; lane 3: 0-h incubation: "HDL-pool" containing apoB-38.9 was added to r-apo[a] and immediately loaded onto the gel; lane 4: as lane 3 but incubated for 1 h before applying to the gel; lane 5: as lane 4 but incubated for 6 h; lane 6: "HDL-pool" incubated for 6 h without r-apo[a]; lane 7: r-apo[a] alone; and lane 8: LDL density fraction d 1.019–1.063 from normal control.

DISCUSSION

In this report we describe a new truncated form of apoB, apoB-38.9, associated with the hypobetalipoproteinemic phenotype. The truncated protein was identified by immunoblotting of the proband's plasma, revealing an apoB immunoreactive band close to the previously identified apoB-37 and apoB-40 proteins and having an estimated size of apoB-39.5 (Fig. 2). ASO analyses performed on DNA from the proband identified in this report demonstrated that his apoB truncation was not due to either of the mutations giving rise to apoB-40 or apoB-37. A fragment of the apoB gene encompassing the apoB-31 to apoB-40 mutations' sites was then cloned. Sequencing of the apoB-39 mutation site reported by Collins et al. (27) showed a wildtype sequence in all clones and thus ruled out this mutation. Further sequencing of this region of the apoB gene revealed a deletion of a cytosine nucleotide at cDNA position 5444 (Fig. 3) resulting in a frameshift that extends for 22 amino acid residues before a termination codon is generated at amino acid residue 1767. The calculated centile fraction of the translation product is 38.9% or 1766/4536 amino acids. We therefore named this truncated protein apoB-38.9. The presence of this deletion was confirmed by ASO analysis (Fig. 4) and by noting the segregation of the 3' VNTR allele 3' β 47 with the apoB-38.9 mutation.

Collins et al. (27) described a truncated form of apoB due to a deletion of a guanine nucleotide at cDNA position 5591 which predicted a truncated protein 1799 amino acids long. Calculation of the centile fraction of that translation product is 39.66%. At the time of their report, few truncated forms of apoB had been described and the authors designated their truncated protein apoB-39. However, apoB-38.9 identified in this report differs from apoB-39 by 33 amino acids or 0.7% of the wildtype apoB protein and not 0.1% as the assigned names may suggest.

Recently, we described a 5 bp deletion mutation of the apoB gene which results in apoB-52 (10). In that report we examined the incidence of deletion mutations in the apoB gene and noted that a high proportion of the mutations leading to truncated forms of apoB are due to deletions of a small number of base pairs. Deletion of a single nucleotide was the most common deletion mutation documented for the apoB gene. The mutation described here adds to this list and confirms that the apoB gene appears to be particularly prone to small deletion mutations.

The FPLC plasma profile is a reliable method to study the size spectrum of lipoproteins (28). In previous studies, we showed that apoB-75- (6), apoB-54.8-, and apoB-52- (10) containing lipoproteins are present in small amounts in VLDL and IDL. However, the vast majority of these truncated apoBs is in LDL, which are smaller than apoB-100-LDL on gel filtration and/or GGE. Thus, LDL containing these longer truncations comprise single truncated LDL populations of fairly narrow size distribution. No apoB-containing lipoproteins are present in the HDL density range. By contrast, the FPLC profile for the newly identified apoB truncation shows that apoB-38.9-containing particles are more heterogeneous in size. In addition to small amounts in the VLDL–IDL size ranges, there are particles close to apoB-100 LDL in size (Fig. 5). However, the majority (65%) of the apoB-38.9 particles are much smaller than LDL (Figs. 5, 7, and 8) and float well within the HDL density range (Figs. 6 and 8). It is interesting that the HDL-like apoB-38.9 comprises two particle populations of clearly distinct sizes. Plasma from apoB-27.6/apoB-100 (29), apoB-31/apoB-100 (30), and apoB-40/apoB-89 heterozygotes (M. R. Averna, unpublished observations) separated on FLPC Superose 6 columns also demonstrated IDL, LDL, and small LDL subpopulations associated with the truncated apoBs similar to the sizes of apoB-38.9 particles described here. On DGUC of apoB-31 plasma, the small LDL floated well within the HDL density range (30). The increased density in relation to size noted previously for apoB-37-, apoB-31-, and apoB-32-containing particles is probably attributable to the relatively high protein/lipid ratio for these particles (30) and this report extends these observations to apoB-38.9-containing particles.

Western blots of GGE gels of HDL fractions from heterozygotes with apoB-31, apoB-37, or apoB-46 have also suggested the presence of at least two subpopulations of particles containing these short truncated forms of apoB (17). In this report, HDL fractions from an apoB-38.9 heterozygote, apoB-31 heterozygote, and an apoB-89/apoB-40 compound heterozygote show at least two subpopulations of HDL density particles containing these truncations (Fig. 8).

Why truncated forms of apoB shorter than apoB-48 tend to distribute in greater numbers of distinct subpopulations than lipoproteins containing truncated apoBs larger than apoB-48 is an intriguing question. One possible factor may be the different tissue sources of truncated forms of apoB smaller than apoB-48. Krul et al. (31) demonstrated that the intestine was capable of synthesizing truncations smaller than apoB-48 whereas the mRNA for truncations larger than apoB-48 was edited normally and therefore the intestine did not express truncations > apoB-48. Consequently, apoB truncations < apoB-48 probably are secreted by the intestine and the liver, whereas apoB truncations > apoB-48 are secreted by the liver but not by the intestine. The two organ sources for truncations shorter than apoB-48 may contribute the greater heterogeneity of populations of apoB-38.9 particles seen in this report. The presence of apoB-100 may also affect the size distribution of apoB truncation-containing lipoproteins as suggested by a recent study. ApoB-27.6 was found to be associated with several particle size populations in an apoB-27.6/apoB-100 hetero-

zygote, while in an apoB-27.6 homozygote (29) a more uniform distribution was observed. This suggests that the presence of apoB-100 may influence the assembly or catabolism of the smaller apoB truncations, so that heterozygotes for a truncation may exhibit more heterogeneity of particles containing the truncated forms of apoB than homozygotes.

Finally, we examined whether apo[a] associated with apoB-38.9. The apoB-38.9 proband had relatively high plasma concentrations of Lp[a] (28 mg/dl). Upon gel filtration on FPLC, the Lp[a] eluted in the IDL size region which is consistent with particles that are larger than LDL (32). ApoB-100- and apoB-38.9-containing particles eluted in the same fractions as apo[a] (Fig. 5) which could imply co-elution of several different populations of the same particle size, e.g., apoB-100-IDL, apoB-38.9-IDL and two populations of Lp[a] particles, one associated with apoB-100 and the other with apoB-38.9. The latter possibility was considered as the precise region of apoB that is involved in the binding of apo[a] has not conclusively been determined. However, on the DGUC profile, the majority (65%) of apoB-38.9 was present at an HDL density, clearly separated from the Lp[a] particles. Less than 5% of the apoB floating with apo[a] in the Lp[a] fraction consisted of apoB-38.9. This implied, but did not prove conclusively, that apoB-38.9 does not associate with apo[a] to form an Lp[a] particle. To investigate this further, three complementary experiments were carried out. In the first, plasma Lp[a] was isolated by lysine-Sepharose chromatography (Fig. 9). Under conditions where Lp[a] is eluted from the lysine column, apoB-100 and apo[a] bands were detected in the eluate but no band of apoB-38.9 size was detected. ApoB-100 and apo[a] were also detected in a normolipidemic control sample but not in a subject with barely detectable levels of Lp[a] (Fig. 9). In the second approach, apoB-38.9 was not detected in an immunoprecipitate of Lp[a] under conditions where apoB-100 was clearly immunoprecipitated (Fig. 10). In the final approach, reconstitution of r-apo[a] with apoB-38.9 particles was unsuccessful while apoB-100 clearly did associate with r-apo[a] in this experiment (Fig. 11). Taken together, our results demonstrate that the site of attachment of apo[a] lies beyond the N-terminal 38.9% of apoB. Earlier unpublished studies suggested that apoB-46 did not form complexes with apo[a] (33) and more recently, Farese et al. (34) provided some evidence to suggest that truncations as long as apoB-83 were incapable of forming Lp[a] complexes. Further, Guevara et al. (35) concluded, based on analyses of accessible cysteine sulfydryl groups and computer modeling predictions of the structures of apoB-100 and apo[a], that Cys3734 of apoB interacts with Cys4057 of apo[a]. Our data are in accord with these observations.

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