Donor splice mutation generates a lipid-associated apolipoprotein B-27.6 in a patient with homozygous hypobetalipoproteinemia

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Abstract We report the characterization of a new truncated apolipoprotein (apo) B, originally identified in the plasma of a homozygous proband and three heterozygous family members with hypobetalipoproteinemia. Using Western blotting, the truncated apoB species was estimated to be 27.5% the size of apoB-100. After fast protein liquid chromatography of plasma from the proband (CD) and mother (OS), the truncated apoB was eluted with particles whose sizes were between normal low and high density lipoproteins. Sequencing of exons 21–24, including the intron–exon boundaries, revealed a T→C transition at +2 of intron 24, homozygous in CD and heterozygous in OS, thus disrupting the 5' donor splice site and interrupting the translation of serine$_{1254}$. On the basis of this, the truncated protein was estimated to be approximately apoB-27.6. The reason for this approximation is that splice-junction mutations can generate different mRNA transcripts, and the truncated protein might represent a mixture of novel carboxy-terminal peptides, terminated by in-frame STOP codons. To date, apoB-27.6 is the smallest truncated species identified in plasma and associated with lipid. An explanation for this could be the hydrophobic nature of the novel carboxy-terminal peptides, which might enable stabilization of the particles by solubilization of sufficient lipid.—**Talmud, P. J., E. S. Krul, M. Pessah, G. Gay, G. Schonfeld, S. E. Humphries, and R. Infante.** Donor splice mutation generates a lipid-associated apolipoprotein B-27.6 in a patient with homozygous hypobetalipoproteinemia. *J. Lipid Res.* 1994. **35:** 468–477.

Supplementary key words splicing defect • low density lipoprotein • truncated apoB

Familial hypobetalipoproteinemia (HBL) is a codominant disorder arising from mutations in the apolipoprotein (apo) B gene. Homozygotes for the disorder have trace amounts of plasma apoB and often display the symptoms of fat malabsorption and neuropathies associated with the deficiency of fat-soluble vitamins, while heterozygotes have apoB levels below the 5th percentile and usually are free of symptoms (reviewed in ref. 1). HBL is caused by a range of mutations in the apoB gene that result in the truncation of apoB to different sizes, ranging from apoB-2 to apoB-89, that have either been predicted from DNA mutation analysis (2–4) or identified in the plasma of HBL patients by protein analysis (reviewed in ref. 1). To date, the smallest truncated species associated with lipid and detected in the plasma of the HBL heterozygote is apoB-31 (5). This variant is 1525 amino acids (aa) in length, and was detected in the high density lipoprotein (HDL) density range, as well as in the infranatant, but was absent from the very low density lipoprotein (VLDL) and low density lipoprotein (LDL) density ranges of the HBL patient. By contrast, apoB-29 which is 1305 aa in length (2) was undetected in the plasma, and was neither associated with lipid nor present in the lipoprotein-deficient fraction, despite precautions to minimize protein degradation and the use of sensitive immunoblotting techniques. In in vitro studies, constructs expressing apoB-29 and apoB-28 (6, 7) were associated with lipid and secreted into the medium, whereas apoB-23 was found only in the infranatant. However, in the HBL patient with the mutation generating apoB-29, the truncated protein appeared to be unstable and prone to degradation. Thus, the two truncated forms identified in patients with HBL, apoB-31 and apoB-29, have helped to define a region of apoB, between residues 1305 and 1525, sufficient for the stable formation and secretion of lipoproteins with a similar density as HDL but not able to stabilize large lipid-rich particles of the size and density of VLDL or LDL.

Abbreviations: apo, apolipoprotein; HBL, hypobetalipoproteinemia; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; aa, amino acids; VNTR, variable number of tandem repeats; FPLC, fast protein liquid chromatography.

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In this study we have identified traces of a truncated apoB species, estimated as apoB-27.6, in the plasma of a patient with homozygous HBL and that of her heterozygous mother. Thus apoB-27.6 is the smallest truncated species detected in vivo circulating in plasma and associated with lipid. The truncation is the result of a unique splice mutation in the apoB gene that is predicted to result in the production of one or more novel hydrophobic carboxy-peptides. This may explain the ability of this small truncated apoB species to associate with sufficient lipid to avoid degradation.

METHODS

Family

The D family has been described elsewhere (8). Briefly, the proband CD is a young Caucasian woman (born 1965) who at the age of 21 was referred to the Center Hôpitalier de Verdun suffering from chronic fatty diarrhoea. Acanthocytes were present in the blood but the patient showed no signs of neuropathies or retinopathies. Lipid analysis showed that she was severely hypocholesterolemic, with plasma total cholesterol measurements of 19 mg/dl, plasma triglycerides of 6 mg/dl, and trace amounts of apoB. Jejunal and ileal biopsies showed an accumulation of fat vacuoles in enterocytes. Histochemical and immunoenzymatic stains of enterocytes and intestinal organ culture showed defective apoB synthesis. The mother, brother, and sister on clinical examination were normal, but had less than half the expected plasma concentration of cholesterol, triglycerides, and apoB. The father was not identified but from the HLA typing it was concluded that the children were from a consanguineous relationship. The diagnosis of homozygous HBL was given, with the mother, sister, and brother being heterozygous for the disorder. Available family members had been previously genotyped for the XbaI, EcoRI, and BamHI polymorphisms of the apoB gene (8). Only the XbaI polymorphism was informative and the inheritance of this polymorphism was in agreement with the homozygous inheritance of the apoB gene in the proband. Genotyping of the apoB 3' variable number of tandem repeats (VNTR) and XbaI polymorphism together with lipid, apoB, and apoA-I concentrations are presented with the family pedigree in **Fig. 1.**

Electrophoresis and immunoblotting

ApoB was immunoprecipitated from plasma samples prior to electrophoresis as previously described (9). Immunoprecipitation 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) (10).

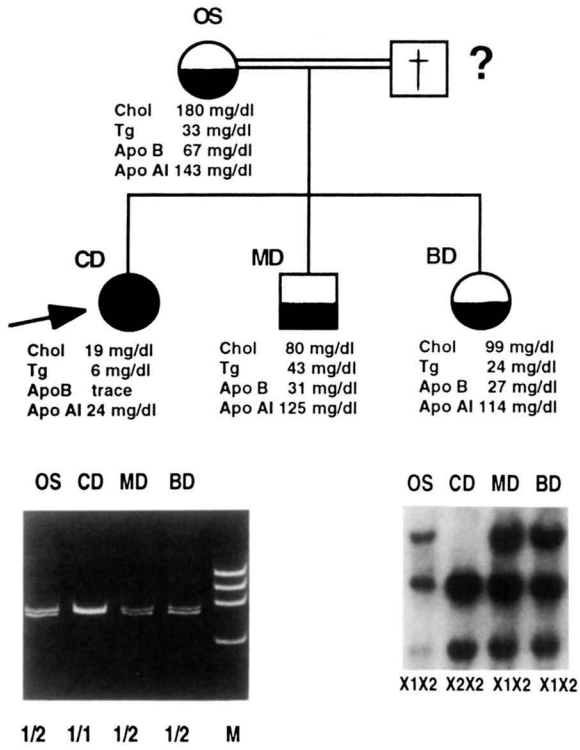


Fig. 1. Pedigree of the D family The proband is identified by an arrow. Total cholesterol, triglycerides, apoB, and apoA-I levels as reported previously (8) are given below. In the lower part of the figure, the VNTR and XbaI genotyping is presented. M, molecular weight markers from ϕX 174-HaeIII digest. Top to bottom 1350, 1058, 872, and 603 bp.

FPLC separation of plasma

Plasma (1.5 ml) was chromatographed on two 25-ml Superose 6 columns at room temperature as described previously (10). The column eluent was analyzed enzymatically for cholesterol (Wako Pure Chemicals, Richmond, VA). Equal aliquots (35 μl) of each column fraction were applied to 3–6% gradient SDS-PAGE gels for electrophoresis and immunoblotting was carried out as described above. Bands corresponding to apoB-100 or apoB-27.6 on the resulting autoradiographs were scanned using a laser densitometer. Areas under the peaks were determined using Sigma Scan (Jandel Scientific, San Raphael, CA).

DNA analysis

DNA was extracted from leucocytes (11). DNA was digested with XbaI, and Southern blotting and hybridization were performed as described (8). The 3' hypervariable region of the apoB gene (VNTR) was amplified by polymerase chain reaction (PCR). Oligonucleotides used as primers were 5'-AACGGAGAAATTATGGAGGG-3'

and 5'-AGGTTGTTCCTCAGGATCAA-3'. Amplification was obtained by 25 cycles of PCR in an automated thermal cycler (Perkin-Elmer Cetus) using Taq polymerase (Perkin-Elmer Cetus) following the manufacturer's instructions. Amplified DNA was separated by 4.4% PAGE at 30 mA for 25 min and visualized by ethidium bromide (12, 13).

Direct sequencing

Direct sequencing was carried out on exons 21–24 including the flanking intron sequences. Genomic DNA from CD, OS, and an unrelated control was amplified by PCR in a total volume of 50 μl reaction mixture, 250 ng of each primer, 200 ng genomic DNA, 1U Taq polymerase (BRL) in a buffer recommended by the manufacturers with a magnesium concentration of 0.1–0.15 mM, using a Cambio Intelligent Heating Block (Cambio, Cambridge, UK). The primers used for the amplification were as follows.

Exon 21 5' oligo 5'-TCCACAAACAAGCTAAGTG-3'
3' oligo 5'-CCTGCAGTGCAGGTCAGAT-3'
22 5' oligo 5'-CTGAACCATCCTTGTATCT-3'
3' oligo 5'-TAGTTCAGCCTGTAACCAC-3'
23 5' oligo 5'-ACTTCCAAAGTAATGCAGG-3'
3' oligo 5'-ACCTAGCTCAGAGTTGAG-3'
24 5' oligo 5'-GTGATCAGTGACTGGCAACGAAGA-3' (biotinylated primer)
3' oligo 5'-CTTAGGGTGAAGTACAGATCTAATGAC-3'

Direct sequencing of exons 21–23 was carried out as before (14) using PCR priming oligos as primers for sequencing. DNA was sequenced from both directions. Exon 24 was sequenced using one biotinylated oligo in the PCR amplification followed by purification and separation of the double-stranded DNA using streptavidin-coated beads (Dynal, Oslo, Norway). A third oligo internal to the PCR fragment, 5'-TCGCCACAGTGTTTGTGCTATAG-3' was used as a sequencing primer. In all cases sequencing was carried out following the Sequenase version 2 protocol (United States Biochemical Corporation, Cleveland, OH).

RESULTS

In a previous study of this family it had been observed that the proband had a single truncated apoB species present in small amounts in the plasma and sized as 25.8% the size of apoB-100 (15). Plasma from the mother, brother, and sister had normal apoB-100 as well as the truncated apoB species, both in reduced amounts. In this present study the inheritance of the apoB gene in the family was determined using the 3' VNTR and XbaI polymorphism and this showed that while the mother, sister, and brother were heterozygous for both polymorphisms with a genotype classified as VNTR 1/2, XbaI 1/2, the proband was homozygous for VNTR allele 1 and the presence of the XbaI cutting site (2/2) (Fig. 1).

Plasma from the proband (CD) and mother (OS) were further investigated and the molecular weight was estimated by electrophoresis and immunoprecipitation. Plasma from seven subjects with previously sized apoB truncations (apoB-31, B-37, B-40, B-54.8, B-55, B-75, and B-89), as well as apoB-48, apoB-100 and fragments apoB-26 and apoB-74, from thrombin-digested LDL, were used as molecular weight markers (**Fig. 2A**). In the proband, CD, only a single apoB fragment was detected with the total absence of apoB-100 or any other apoB fragment, which clarified that the detectable apoB fragment was not an apoB degradation product. The novel truncated apoB was seen in the plasma of CD and OS, while OS had, in addition, a normal apoB-100 immunoreactive band. No additional apoB species were present. The novel truncated apoB migrated on the polyacrylamide gel with a mobility slightly slower than apoB-26, the amino-terminal proteolytic cleavage product of LDL, and was estimated from the regression curve to have a molecular weight equivalent to 27.5% the size of apoB-100 (Fig. 2B). On the basis of this protein sizing, the mutation was predicted to be at or near residue 1247 encoded by exon 24 of the gene. Direct sequencing of exon 24 and flanking intron sequence was carried out on DNA from the proband, mother, and an unrelated control. Sequencing revealed a single T→C transition at nucleotide +2 of intron 24 (**Fig. 3**). CD was homozygous for this mutation while OS was heterozygous at this site. The normal 5' (donor) splice site of intron 24 has the sequence AAAG/**gt**aaa. The donor splice dinucleotide GT is highly conserved and forms part of the recognition sequence for correct splicing.

To confirm that this was the only nucleotide change in CD and OS, exons 21–23 and flanking intron sequences were sequenced and no other sequence changes were identified (results not shown). However, a sequence difference was observed in intron 24 in CD, OS, and the unrelated control compared to the reported intron 24 sequence (16). All three samples were homozygous for a single G nucleotide at position +10 of intron 24, whereas in the published sequence there is a GG dinucleotide at positions +10 and +11.

Characterization of lipoproteins

Plasma lipoprotein profiles from CD and OS were obtained by gel filtration chromatography on Superose 6 columns. For CD (**Fig. 4A**) cholesterol peaked in the HDL range with only trace amounts in VLDL and a shoulder in the LDL range, suggesting that HDL was the major cholesterol carrier. Immunoblot assay of apoB

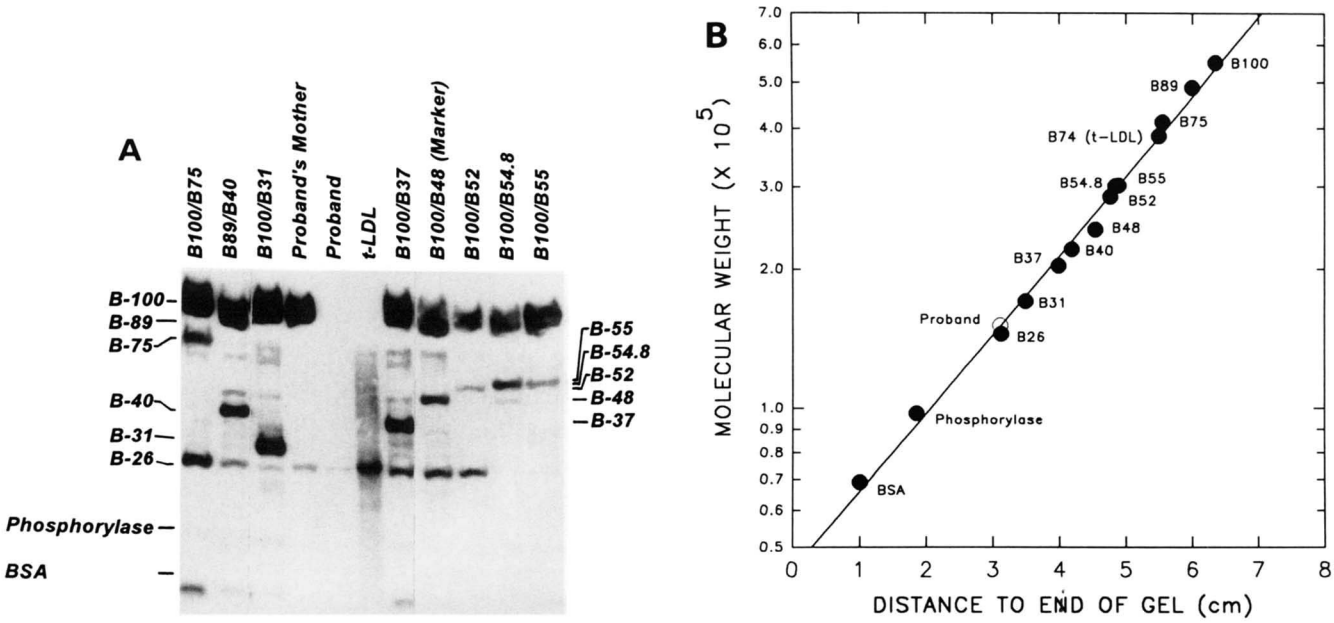


Fig. 2. A: Estimation of the molecular weight of the proband's truncated apoB by comparison with other known apoB truncations. Immunoprecipitated apoB from the plasma of the proband, proband's mother, seven unrelated subjects having truncated apoBs previously reported, and one control subject (Marker) were electrophoresed on a 3–6% SDS-PAGE gel. Thrombin-digested LDL (t-LDL) was also run. Immunoblotting with an anti-apoB monoclonal antibody (C1.4) was performed as described in Methods and the resultant autoradiograms are shown. Plasma from subjects with previously described apoB phenotypes are identified at the top of each lane and the truncated apoB species is identified at each side of the gel by the nomenclature (i.e., B-89, B-75, etc). B: Plot of the mobility of truncated apoB species and molecular weight markers on 3–6% gradient SDS-PAGE. The plot is derived from the autoradiogram shown in Fig. 1. Molecular weights for the previously characterized truncated apoB species were based on the percent length of apoB-100 × 550,000. B-74 and B-26 molecular weights were taken as 385,000 and 145,000 according to Cardin et al. (26). Mobilities for B-74 and B-26 were averaged using the thrombin-derived fragments (t-LDL) and corresponding fragments detected in the apoB immunoprecipitates. Molecular weights for pre-stained phosphorylase and bovine serum albumin were 97,400 and 69,000, respectively. Mobilities for these two markers were measured directly on the Immobilon transfers. The migration of the proband's truncated apoB species is indicated by the open circle. The molecular weight of the proband apoB (151,030) was estimated from the regression curve shown: slope = 0.1697, y-intercept = 44,627, r = 0.9972.

showed that while small amounts of apoB were present in the LDL and HDL size range, the peak of truncated apoB eluted at fraction 37, that is at a size range between LDL and HDL. This suggests that apoB-27.6 forms a smaller than normal LDL particle. This is seen more clearly in the profile for OS (Fig. 4B). While there is clearly a major cholesterol peak in the LDL range, the second peak is in the HDL range. Immunoblotting of the FPLC fractions from OS showed a large peak of apoB-100 peaking at tube 32, which was slightly smaller than average normolipidemic LDL (fractions 25–27). Similar observations have been made in individuals heterozygous for apoB-52 and apoB-54.8, in that these subjects' apoB-100-LDL tended to have some smaller LDL particles eluting at fractions 30–32 (17). This was not observed in subjects heterozygous for apoB-75, suggesting that truncations shorter than apoB-75 may influence apoB-100 metabolism and particle size distribution. At present we do not have an explanation for this. Two peaks of apoB-27.6 were detected, the major peak at fraction 38, with a shoulder peaking at fraction 31. Density gradient ultracentrifugation of CD and OS plasma, followed by immunoblotting, revealed that apoB-27.6 floated with a peak density of 1.105 g/ml and 1.145 g/ml in each subject, respectively (data not shown). FPLC fractions 26–44 (the major peak for apoB-27.6) and fractions 45–58 (the major peak for HDL cholesterol) from CD, were pooled and the lipid composition (% by weight) was estimated. The ratio for fractions 22–44 for unesterified/esterified cholesterol was 0.14/0.43 (0.32), and that of phospholipid/total cholesterol was 0.81/0.55 (1.42), approximately that expected for an HDL_2 type particle. The ratio of unesterified/esterified cholesterol for fractions 45–58 of 0.5 (0.06/0.12) and the phospholipid/total cholesterol ratio of 2.2 (0.40/0.18) were higher in CD than what would be expected for HDL. The problems of fat malabsorption and defective triglyceride metabolism may account for this unusual HDL lipid profile.

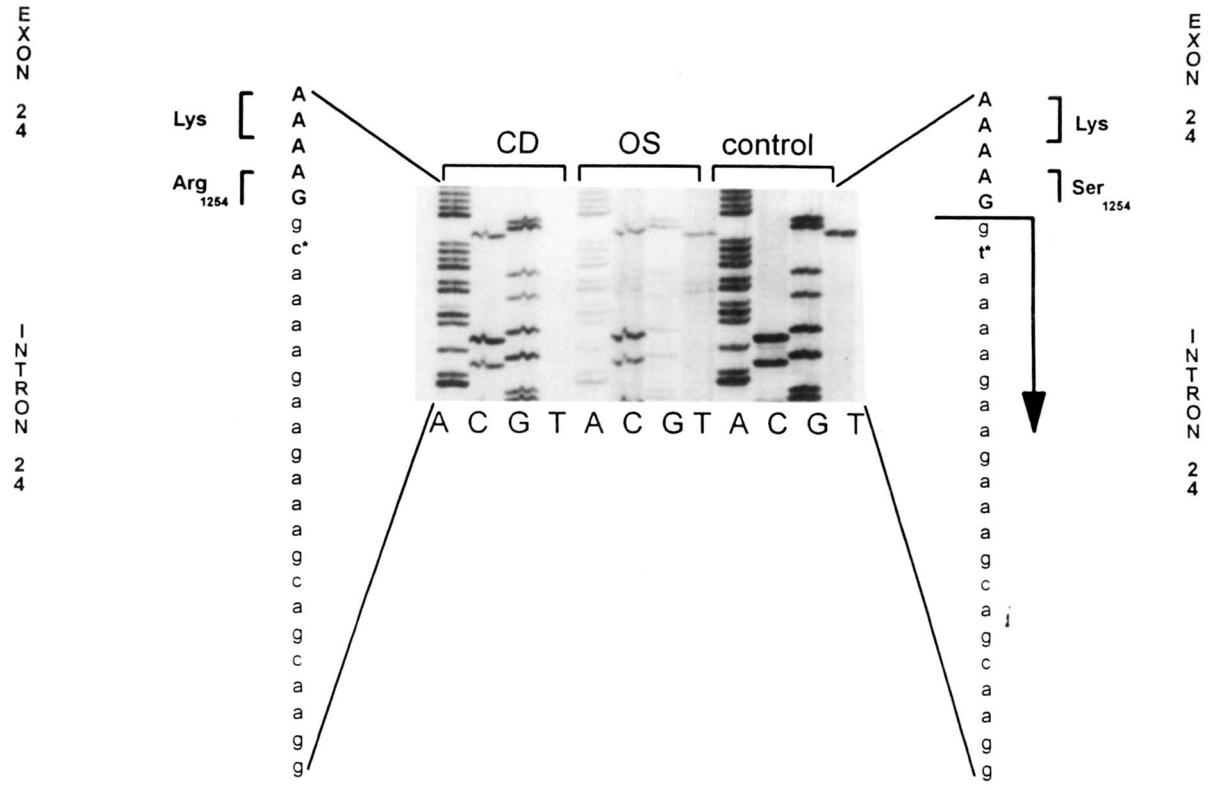


Fig. 3. Direct sequencing of exon 24/intron 24 boundary from CD, OS, and control. The T→C transition at +2 of intron 24 is marked by an asterisk.

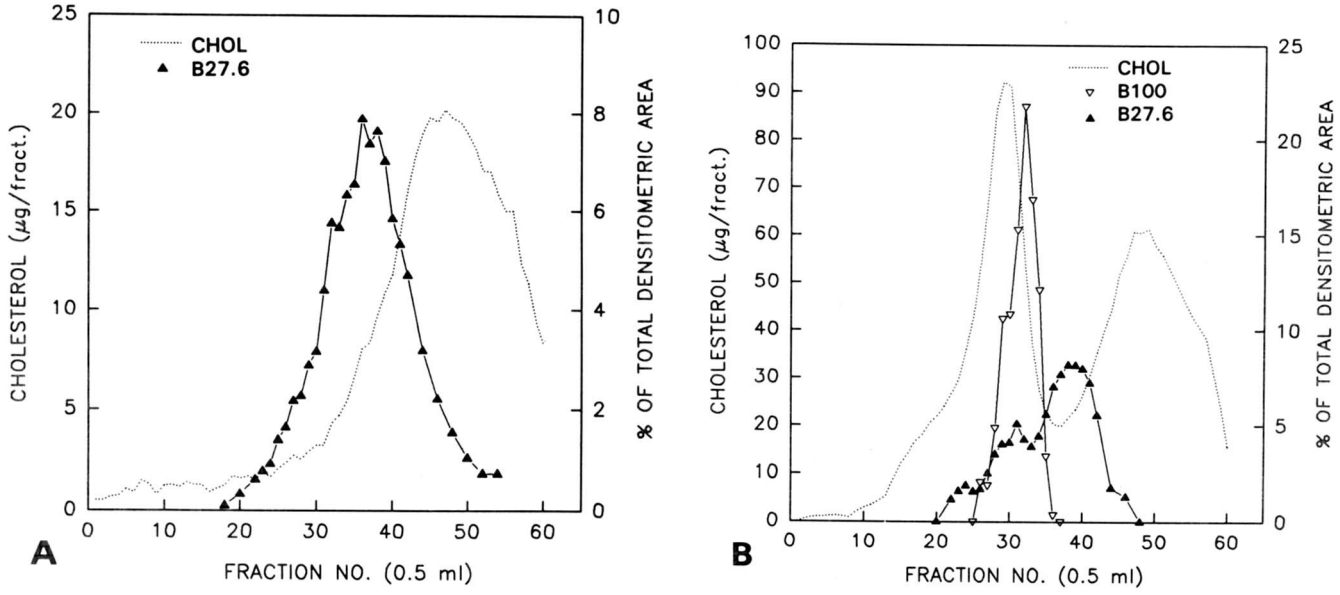


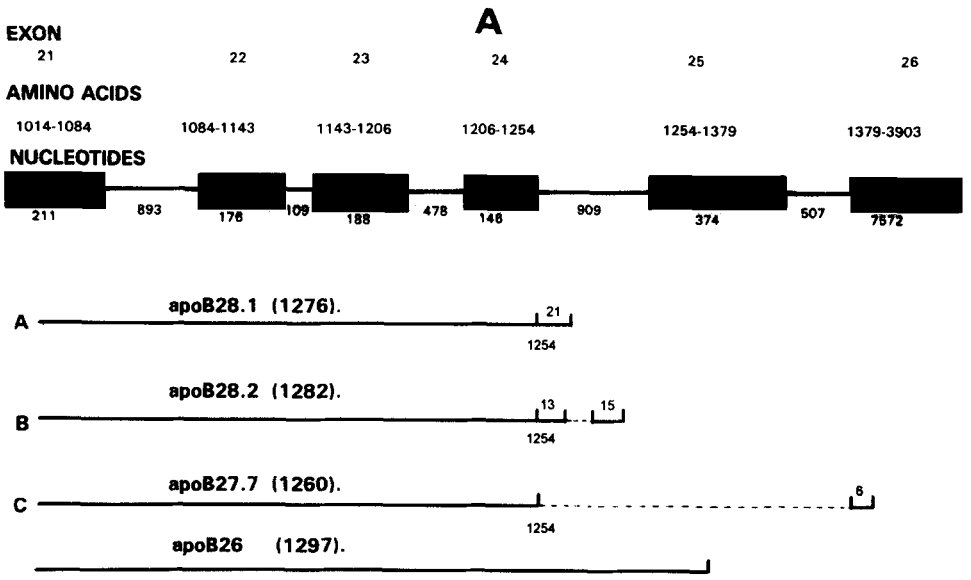
Fig. 4. Superose 6 gel filtration of plasma from the proband homozygous for apoB-27.6 (A) and from the proband's mother who is heterozygous for apoB-27.6 (B). Plasma (1.5 ml) from each subject was chromatographed on two Superose 6 columns as described in Methods. For reference, peak fractions from normolipidemic plasma elute between fraction 5–9 for VLDL, 25–27 for LDL, and 44–47 for HDL. Total cholesterol was determined in each fraction and is represented by a dotted line (μg/fraction). Aliquots from each fraction were electrophoresed on 3–6% gradient SDS-PAGE gels and immunoblotted with an anti-apoB monoclonal antibody as described in Methods. The resultant autoradiographs were scanned to determine the areas under the peaks corresponding to either apoB-100 or apoB-27.6 in each gel lane (each lane representing a single column fraction). The total area in all lanes for apoB-100 or apoB-27.6 were summed separately, and the results are expressed as the percent of the total densitometric area for each apoB species (equivalent to the percent of total plasma apoB-100 or apoB-27.6 in each column fraction across the FPLC profile). Note that in (B) apoB-27.6 accounted for less than 1% of the total apoB in the plasma.

DISCUSSION

We have identified a mutation in the apoB gene in a patient with homozygous HBL that results in a truncation estimated as 27.5% of the full length apoB-100. The patient is homozygous for a T→C transition at position +2 in the GT dinucleotide that forms the 5' donor splice in intron 24. The mother of the proband is heterozygous at this site and although DNA from the brother and sister were not sequenced, their lipoprotein profiles (8), the presence of the truncated apoB in the plasma (15), and VNTR and XbaI genotyping suggest that they were also heterozygous at this site. No other sequence changes were seen in the sequence of exons 21–24 in CD and OS suggesting that the truncated protein resulted from this splicing defect. The truncated apoB was assigned the name apoB-27.6, which reflects the last amino acid of the reported apoB sequence, Ser_{1254}, but may underestimate the size of the true protein product which can only be predicted. As apoB is synthesized in the liver and small intestine, it was not possible to obtain biopsy material for mRNA analysis as a means of confirming the splicing defect. Additionally, we noticed in all three sequenced samples the absence of a single G nucleotide at position +10 or +11 of intron 24 which probably reflects an error in the original published sequence and, under normal circumstances, would not affect the apoB coding sequence. However, in the case of CD and OS, this single G, compared to the published GG, would alter the predicted frame of translation from intron sequence.

The GT dinucleotide at the 5' donor splice site is involved in binding of U1 small nuclear ribonucleoprotein particles and forms the cleavage site in the spliceosome for correct lariat formation (18). Mutations at this conserved GT dinucleotide diminish correct splicing and promote aberrant joining of exons (19). Splice-site mutations may generate a number of different products either by activation of cryptic splice sites which may lack a portion of the coding sequence, or contain additional sequence of intronic origin, or exon skipping. In some cases full length mRNA can be transcribed at reduced amounts. Thus, the mRNA transcript may represent a mixture of all these alternatives and, in addition, the efficiency of transcription may be greatly reduced, compatible with the low levels of apoB protein detected in the patient. Mutations in the guanine residue of the GT dinucleotide donor splice site are crucial for correct splicing (20) and there are many examples of this in the literature (reviewed in ref. 21). In the field of lipid research, for example, a patient has been reported where a G→A transition at +1 of intron 3 results in apoA-II deficiency. No apoA-II was detected in the plasma of the patient using immunological methods and it was suggested that the effect of the mutation would be the activation of a potential cryptic site in intron 3 resulting in an in-frame termination in the 4th exon and a protein of only 30 amino acids (22). $ApoC\text{-}II_{Hamburg}$ is the result of G→C transversion at +1 of intron II of the apoC-II gene. A small amount of correctly spliced message was produced and detected on a Northern blot, but the majority of the mRNA production was incorrectly spliced leading to premature termination and apoC-II deficiency (23). In one family, familial apoE deficiency has been shown to be the result of a splice mutation in the apoE gene, the result of an A to G substitution in the penultimate 3' nucleotide of the 3rd intron of the gene. This abolishes the correct 3' splice site and creates a STOP codon within the intronic sequence resulting in a truncated apoE peptide (24). A splice mutation in the apoB gene at +1 of intron 5 (G→T) in a patient with HBL appeared to interfere with proper splicing and no plasma apoB was detected (4). There are examples in the literature of T→C transitions at +2 of the 5' donor splice site resulting in abnormal splicing and the production of aberrant proteins; for example, in the ColIA gene giving rise to Ehlers Danlos VIIB syndrome (25), in the β hemoglobin gene in β thalassaemia (26), and in the RBI gene leading to retinoblastoma (27).

In the present study, a T→C transition at +2 of intron 24 was the only mutation detected from our sequencing of exons 21–24 and flanking intron sequences. It is, therefore, likely that this is the mutation causing HBL, but without mRNA analysis it is not possible to predict the precise consequence of the mutation. Some of the possible outcomes are presented in **Fig. 5A and B.** The translation could continue into intron 24, Ser_{1254} would be substituted by Arg_{1254} followed by 21 novel amino acids until an in-frame stop codon was reached. This would generate an apoB-28.1 (1276 aa). Alternatively, within intron 24 there is a consensus cryptic splice site aaa**gt**aaa similar to the splice sequence at the exon 24/intron 24 junction AAG**gt**aaa. It is possible that this cryptic site is activated and that splicing progresses onto exon 25 resulting in the translation of 13 amino acids from intron 24 before the cryptic splice site, and an additional 15 amino acids into exon 25 before an in-frame STOP is reached, generating an apoB-28.2 (1282 aa). Finally, exon skipping could occur and exon 24 could be spliced to exon 26 directly, thus excluding exon 25. This would result in a frame shift and 6 novel amino acids would be translated until an in-frame STOP codon was reached, giving rise to an apoB-27.7 (1260 aa). It is likely that, as in the case of other splicing mutations, several options are possible, with a mixture of mRNA species being generated and translated. In the absence of biopsy material, ectopic mRNA transcripts could resolve this. This approach has been successful for some genes, (reviewed in ref. 21), but to date ectopic transcript analysis from cultured fibroblasts or keratinocytes has not been achieved for the apoB gene (R. Frants, personal communication). The presence of a small amount of truncated protein, sized as 27.5% apoB-100 by SDS-PAGE



B

A.Translation continues into intron 24 until an inframe STOP. - Apo B28.1 (1276)

```
EXON 24⌈INTRON 24
AAA  AG⌋g  caa  aag  aag  aaa  gca  gca  agg  ctt  ctt
Lys  Arg1254 Gln  Lys Lys  Lys  Ala  Ala  Arg  Leu  Leu

gaa  cca  tgc  aaa  gta  aat  gaa  aga  ttt  tac ata  gca
Glu  Pro  Cys  Lys  Val  Asn  Glu  Arg  Phe  Tyr Ile  Ala

tga
STOP
```

B.Translation continues into intron until cryptic splice, splices onto exon 25 and translates until an inframe STOP - Apo B28.2 (1282)

```
EXON 24 ⌈INTRON 24
AAA   AG⌋g  caa  aag  aag  aaa  gca  gca  agg  ctt  ctt
Lys  Arg1254 Gln  Lys  Lys  Lys  Ala  Ala  Arg  Leu  Leu

                          ⌈EXON 25
gaa  cca  tgc  aaa  gtaaa ⌈CGA  TGG  CCG  GGT  CAA
Glu  Pro  Cys  Lys        ⌊Arg  Trp  Pro  Gly  Gln

ATA  TAC  CTT  GAA  CAA  GAA  CAG  TTT  GAA  AAT  tga
Ile  Tyr  Leu  Glu  Gln  Glu  Pro  Phe  Glu  Asn  STOP
```

C.Skips exon 25, exon 24 spliced to exon 26 - Apo B27.7 (1260).

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EXON 24⌋EXON 26
AAA  AG⌋G  ATC  TGG  AGA  AAC  AAC  ATA  TGA
Lys  Arg   Ile  Trp  Arg  Asn  Asn  Ile  STOP
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Fig. 5. A: Schematic diagram showing the predicted alternative products resulting from the splicing mutation based on similar mutations reported in the literature (21). The proteolytically derived apoB fragment, apoB-26, is also shown for comparison. The apparent molecular weight for apoB-26 is 145,000 ± 3000 as determined by Cardin et al. (28). Knott et al. (29) identified the proteolytic cleavage site for this fragment to reside at amino acid 1297, which, based on the centile system of apoB nomenclature, would yield apoB-28.6. B: Possible alternative translation products resulting from the splicing mutant in intron 24.

in the plasma of CD and OS, supports the existence of a truncated protein, but if one or more alternative transcripts exist these would not be distinguishable by size using the electrophoresis methods described here. Nonetheless, the detected mutation gives rise to a truncated apoB species as we have clearly shown.

An interesting aspect of the sizing of this truncated apoB species is its size in relation to apoB-26. The molecular weight of apoB-26 was estimated originally by Cardin et al. (28) as 145,000 ± 3,000 based on a total molecular weight of 550,000 for glycosylated apoB-100. On the basis of the amino acid sequence, the size of the

proteolytic product, apoB-26, would be 28.4% the size of apoB-100 (145,223/512,000). DNA sequence data and direct peptide sequencing have revealed the proteolytic cleavage site and identified $lysine_{1297}$ as the carboxyl-terminus of apoB-26 (29, 30). This agrees with the latter size estimate and predicts that the protein represents an apoB-28.6 (1297/4536 amino acid). Why this newly identified truncated apoB of predicted length 1260–1282 migrates slightly slower than apoB-26 (1297 residues) on SDS-PAGE gels is not clear, but may be due to the possible introduction of a glycosylation site or the result of a novel sequence of carboxyl-terminal amino acids.

CD has homozygous HBL, yet apart from fat malabsorption, identified at the age of 21, she suffered none of the neurological symptoms associated with abetalipoproteinemia or homozygous HBL (1). Liver fibrosis was identified in CD after 2 years of vitamin A and E supplementation and was thought to be partly due to the adverse effect of vitamin A supplementation (31). This lack of neurological symptoms is in contrast to the apoB-25 homozygous HBL patient who was identified at 1 year of age suffering from fat malabsorption and failure to thrive (3), and the apoB-2/B-9 compound heterozygous siblings who suffered severe liver disease and neuropathies associated with vitamin E deficiency (4). The homozygous HBL patient reported by Ross et al. (32) suffered from retinitis pigmentosa and other neuropathies with no circulating plasma apoB although low levels of a normal length mRNA were detected. In contrast, the apoB-39 homozygous HBL patient showed no retinopathies or neuropathies (2) nor did the apoB-50 HBL homozygous patient, although this child on diagnosis was obese and showed signs of mental retardation that might have been associated with another disorder (33). Other compound heterozygous HBL patients, for example apoB-37/B-86 (34), and the patients with apoB-61 and low expression of apoB-100 (35) and the apoB-40/B-89, had detectable circulating plasma apoB and were asymptomatic (10). As the apoB-27.6 identified in plasma of CD was found to be associated with lipid, this suggests that it is able to support some fat-soluble vitamin transport. In HBL homozygous patients who have no detectable circulating apoB-containing particles, fat-soluble vitamin transport is impaired and this is the major cause of the neurological symptoms. This suggests that there is a relationship between the clinical severity of the homozygous HBL and the length and physico-chemical properties of the truncated apoB.

Electron micrographs of delipidated LDL have shown that apoB is composed of multiple domains arranged in an elongated structure on the surface of LDL, cross-linked at positions of affinity (36). Furthermore, in studies of transiently expressed C-terminal truncated apoB fragments in HepG2 cells, it has been shown that the lipoprotein core circumference is proportional to apoB length (37). This helps explain results from in vitro expression studies of truncated apoB cDNA in McArdle 7777 cells that show that while the smallest constructs, apoB-18 and apoB-23, were secreted into the medium they did not associate with lipid and appeared solely in the bottom of the salt gradient, indicating a minimal size necessary for lipid association, while constructs expressing apoB-28–apoB-53 show progressive decrease in particle density which directly correlated with the length of the apoB species (7). In HepG2 cell expression studies, some apoB-23 was found associated with lipid and secreted into the medium (6). Secretion of this protein was stimulated by culture of the cells with oleate, as was the secretion of constructs of larger truncated species, indicating that the control of apoB was not at the level of mRNA synthesis but the rate of degradation (38). In contrast to apoB-28 (7), in vitro studies of the apoB-29 construct showed association with lipid but a very low level of overall expression (6). It could be that in the HBL patient with the apoB-29 truncation, as with the in vitro studies, expression of this truncated protein is low.

All three of the possible novel peptides resulting from the splicing mutation are predicted to be rich in hydrophobic amino acids. The apoB-28.2 has 11 hydrophobic amino acids out of a total of 28 novel residues, 8/21 of the novel amino acids of apoB-28.1 are hydrophobic, and 3/6 amino acids of the predicted apoB-27.7 are hydrophobic. Molecular modelling of these novel peptides showed no helical structure but, interestingly, the apoB-28.1 and apoB-28.2 have a region rich in Arg and Lys residues that shows strong homology with the receptor-binding domain of apoE. The hydrophobic nature of these peptides could provide an explanation why the apoB-27.6 was detectable in the plasma while apoB-29 was not (2) as these additional hydrophobic amino acids might enable additional lipid binding. If the apoB-27.6 is glycosylated, this might add stability to the protein and although it would be possible to de-glycosylate apoB-27.6 it is unlikely that the difference (approximately 2000 mol wt) would be detectable on PAGE. It will be interesting to see whether other truncations, smaller than apoB-27.6, are detected in the plasma of HBL patients.

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