

A Truncated Species of Apolipoprotein B (B-38.7) in a Patient With Homozygous Hypobetalipoproteinemia Associated With Diabetes Mellitus

Ken Ohashi, Shun Ishibashi, Michiyo Yamamoto, Jun-ichi Osuga, Yoshio Yazaki, Susumu Yukawa, Nobuhiro Yamada

Abstract—Familial hypobetalipoproteinemia is caused by mutations in the apolipoprotein (apo) B gene. We identified a 57-year-old woman whose plasma total cholesterol and apoB levels were 2.17 mmol/L and 0.03 g/L, respectively. Separation of plasma lipoproteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis revealed the absence of apoB-100 and the presence of a faster-migrating form of apoB with an apparent M_r of 195 kDa. Direct sequencing of a polymerase chain reaction–amplified fragment of the patient’s apoB gene DNA revealed a single C→T transition at nucleotide 5472 that converts glutamine 1755 (CAA) to a stop codon (TAA). We predict this novel nonsense mutation of the apoB gene to produce a truncated protein that contains 1754 amino-terminal amino acid residues of apoB-100. We designated this mutant form of apoB apoB-38.7 by following the centile nomenclature of the apoB species. The same mutation was found in both of her children. The proband revealed clinical findings of retinitis pigmentosa, acanthocytosis, and loss of deep tendon reflexes that are characteristic of severe hypobetalipoproteinemia. In addition, the proband had type II diabetes mellitus with nephropathy, anemia, cholelithiasis, hepatic hemangioma, bronchiectasis, and extensive calcification of major arteries including, the celiac, splenic, and renal. In summary, we have found a novel truncated apoB, apoB-38.7, in a patient with an unusual presentation of hypobetalipoproteinemia that includes diabetes mellitus and extensive arterial calcification. (*Arterioscler Thromb Vasc Biol.* 1998;18:1330-1334.)

Key Words: diabetes mellitus ■ retinitis pigmentosa ■ proteinuria ■ arterial calcification ■ peripheral neuropathy

Familial hypobetalipoproteinemia (HBLP)¹ is a codominant genetic disorder characterized by decreased or absent plasma levels of apolipoprotein (apo) B. (See References 1 and 2 for a review.) Heterozygotes for HBLP have plasma levels of apoB below the fifth percentile and typically are asymptomatic. In Western populations, the frequency of HBLP heterozygotes is estimated between 1 in 500 and 1 in 1000 persons.² Homozygotes and compound heterozygotes have extremely low levels of apoB. The clinical phenotype of HBLP homozygotes is highly variable, with severe cases presenting with fat malabsorption, acanthocytosis, retinitis pigmentosa, and neurological complications resulting from intestinal malabsorption of vitamin E. Symptoms observed in some patients with homozygous HBLP are indistinguishable from those of patients with abetalipoproteinemia, a recessive disease arising from mutations in microsomal triglyceride transfer protein (MTP).^{3,4}

Since Young and his colleagues^{5,6} demonstrated that mutations in the apoB gene cause HBLP, ≈30 mutations have been reported. Most of these mutations are nonsense or frameshift mutations that prevent the translation of the full-length apoB-100 protein. In this report, we describe a

Japanese patient with homozygous HBLP caused by a novel mutation in the apoB gene that gives rise to a truncated apoB peptide, apoB-38.7.

Methods

Clinical Data

The proband (K.H.) is a 57-year-old Japanese woman who was referred to Wakayama Medical College for evaluation of a liver mass. Past medical history was significant for childhood asthma, night blindness, and type II diabetes mellitus diagnosed at age 36 years and managed by diet and insulin. At age 48 she had a retinal hemorrhage due to hemorrhagic glaucoma, resulting in right eye blindness despite photocoagulation therapy, and at age 54 she developed hemoptysis secondary to bronchiectasis. There was no history of diarrhea or steatorrhea. Family history was unremarkable, although there was no reliable information on consanguinity. She had 2 children, a 32-year-old (A.Y.) and a 30-year-old (H.H.) male, who were the only family members accessible to us for medical investigation.

Physical examination revealed a lean, pale woman 145 cm tall and weighing 37.5 kg. Pulse was 96 bpm and regular; blood pressure was 142/62 mm Hg. Visual acuity was lost in the right eye and 0.06/1.0 in the left with a narrow visual field. Intraocular pressure was 40 mm Hg in the right eye and 15 mm Hg in the left. Retinal pigmentation, hard exudates, and extensive photocoagulation scars

Received September 17, 1997; revision accepted March 17, 1998.

From the Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo (K.O., S.I., J.i.-O., Y.Y., N.Y.), and the Third Department of Internal Medicine, Wakayama Medical College, Wakayama (M.Y., S.Y.), Japan.

Correspondence to Shun Ishibashi, MD, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. E-mail ishishash-ty@umin.u-tokyo.ac.jp

© 1998 American Heart Association, Inc.

TABLE 1. Plasma Concentrations of Lipids, Apolipoproteins, and Vitamin E in the Kindred of K.H.

	K.H.	A.Y.	H.H.
TC, mmol/L	2.17	3.21	3.28
TG, mmol/L	0.64	0.49	0.70
HDL-C, mmol/L	1.99	1.76	1.84
apoA-I, g/L	1.33	1.66	1.75
apoA-II, mg/L	200	370	420
apoB, mg/L	30	274	268
apoC-II, mg/L	32	16	21
apoC-III, mg/L	75	62	67
apoE, mg/L	35	34	36
Lp(a), mg/L	56	129	134
Vitamin E, mg/L	7	7	8

Lp(a) indicates lipoprotein(a). Apolipoprotein concentrations were measured by the single radial immunodiffusion method.⁷ Lp(a) concentrations were determined by ELISA. Vitamin E concentrations were determined by high-performance liquid chromatography.

were noted in both optic fundi. Gross hearing loss was noted in the right ear. Examination of the chest and abdomen was unremarkable. Neurological examination revealed paresthesia in both hands, "stocking-glove" type hypesthesia, absent deep tendon reflexes in the lower extremities, and positive Romberg's sign. Examination revealed no abnormal pyramidal, cerebellar, or posterior column abnormalities. Laboratory tests revealed anemia (hemoglobin, 7 g/dL) with acanthocytosis, proteinuria (0.6 to 2.3 g/d), mild hyperglycemia (fasting plasma glucose, 120 to 160 mg/dL; stable HbA1c, 6.9%), and reduced creatinine clearance (40 mL/min). Plasma lipid analysis showed that she was moderately hypocholesterolemic, with total cholesterol (TC) levels of 2.17 mmol/L, plasma triglycerides (TGs) of 0.64 mmol/L, HDL cholesterol of 1.99 mmol/L, and plasma apoB of 0.03 g/L.⁷ Prothrombin time was 10.7 seconds. Plasma concentrations of vitamins K₁ and K₂ were normal: 0.27 and <0.05 ng/mL, respectively. Plasma cholesteryl ester transfer activity was 3.98 nmol · mL⁻¹ · min⁻¹ (normal value, 2.39 ± 0.32).⁸ A.Y. and H.H. were also hypocholesterolemic (3.2 to 3.3 mmol/L). The results of lipid analysis of K.H., A.Y., and H.H. are summarized in Table 1. The electroretinogram was flat in both eyes. Abdominal CT scan revealed extensive calcification of the major arteries, including celiac, splenic, common hepatic, renal, and superior mesenteric and a segment of the abdominal aorta (Figure 1). Hepatic hemangioma and cholelithiasis were found on ultrasonography and MRI. Liver biopsy revealed mild fatty changes. After a 12-hour fast, the

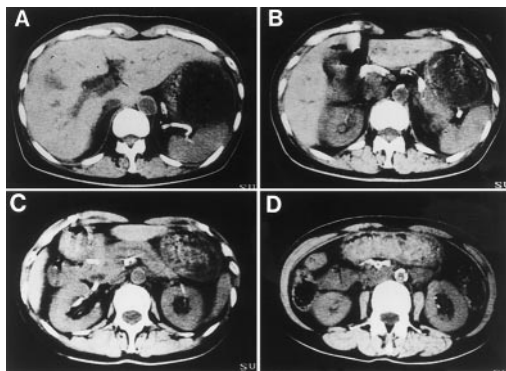


Figure 1. Abdominal CT scan. Four cross sections are shown (A to D, rostral to caudal). Hepatic hemangioma in the right lobe (A) and calcification of splenic (A and B), celiac (B), common hepatic (B), renal (C), and superior mesenteric (D) arteries and a segment of abdominal aorta (D) are noted.

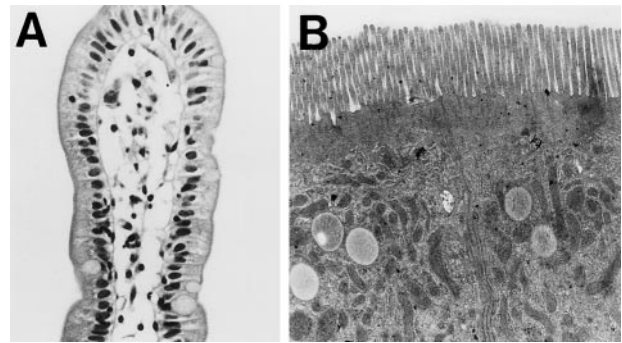


Figure 2. Microscopic examination of biopsied specimen from duodenal mucosa. A, Hematoxylin-eosin staining of microvilli. Enterocytes are apparently normal and do not contain vacuoles, a hallmark of lipid accumulation. B, Electron microscopy of the luminal side of duodenal enterocytes. Electron-lucent lipid droplets are seen in the apical cytoplasm in some sections (magnification ×10 000).

duodenal mucosa was biopsied endoscopically. Hematoxylin-eosin staining of the biopsied specimen showed no evidence of lipid accumulation in the enterocytes (Figure 2A). Transmission electron microscopy revealed moderate accumulation of fat droplets in the apical cytoplasm of the enterocytes in some sections (Figure 2B).

Plasma Lipoprotein and Apolipoprotein Analyses

Blood samples were collected in EDTA-containing tubes. After separation of blood cells for isolation of genomic DNA, the plasma was mixed immediately with a protease inhibitor cocktail containing EDTA, benzamidine, NaN₃, and PMSF. For determination of the lipid contents in each lipoprotein fraction, VLDL ($d < 1.006$ g/mL), IDL ($d = 1.006$ to 1.019 g/mL), LDL ($d = 1.019$ to 1.063 g/mL), HDL₂ ($d = 1.063$ to 1.125 g/mL), and HDL₃ ($d = 1.125$ to 1.21 g/mL) were isolated by sequential ultracentrifugation as previously described.⁹ TC, free cholesterol, and TGs were measured enzymatically. For the apolipoprotein analyses, VLDL+IDL ($d < 1.019$ g/mL), LDL, and HDL ($d = 1.063$ to 1.21 g/mL) were isolated.

After dialysis against a saline solution containing 10 mmol/L phosphate buffer, pH 7.4, 0.15 mol/L NaCl, 1 mmol/L EDTA, and 1 mmol/L PMSF, each lipoprotein fraction was delipidated and subjected to SDS-polyacrylamide gel electrophoresis (PAGE, 3% to 15%). Proteins were visualized by staining with Coomassie brilliant blue R-250.

Haplotype Analyses and DNA Sequences of ApoB

Genomic DNA was prepared from blood cells with the use of a QIAamp blood kit (Qiagen). The DNA fragments of a variable number of tandemly repeated short DNA sequences (VNTR) in the 3' end of the apoB gene and CA repeats in intron 10 of the MTP gene were prepared as described.^{10,11} A 581-nucleotide (nt) DNA (nts 5271 to 5852) of the apoB gene was prepared by polymerase chain reaction (PCR) with the following primers: primer A (sense nts 5271 to 5294), 5'-CAGGCCATGATTCTGGGTGTGCGAC-3' and primer B (antisense nts 5873 to 5852), 5'-CCCATTGCCATTGTATGTGCATC-3' according to Collins et al.¹² The amplified DNAs were directly sequenced by the dideoxy chain-termination method with the use of an ABI 373 DNA sequencer (Perkin-Elmer Cetus, Applied Biosystems).

Other Mutations

The common cholesteryl ester transfer protein (CETP) gene mutations, an intron 14 splicing defect and an exon 15 missense mutation, Asp442Gly, were evaluated as described.^{13,14}

Results

Lipid contents in each lipoprotein fraction from K.H. are shown in Table 2. Note that LDL contained only 0.12 mmol/L TC. VLDL contained more TC than did LDL.

TABLE 2. Lipid Contents of Plasma Lipoproteins From K.H.

	VLDL	IDL	LDL	HDL ₂	HDL ₃
TC, mmol/L	0.30	0.02	0.12	1.48	0.44
FC, mmol/L	0.12	0.01	0.05	0.54	0.08
TG, mmol/L	0.41	0.01	0.01	0.1	0.04

FC indicates free cholesterol. VLDL, IDL, LDL, HDL₂, and HDL₃ were isolated by sequential ultracentrifugation.

Most of the TC, 81%, was present in HDL. The HDL₂-C/HDL₃-C ratio was increased to 3.3.

We sought to identify the cause of K.H.'s hypocholesterolemia. We genotyped the 3' VNTR of the patient's apoB gene and the CA repeats in intron 10 of the MTP locus. The patient was homozygous for the apoB locus ($\beta 37/\beta 37$) and heterozygous for the MTP locus. This finding is consistent with the level of plasma TC found in homozygous HBLP and the presence of immunoreactive apoB in plasma (Table 1).

Lipoproteins subjected to SDS-PAGE and Coomassie staining revealed a protein of apparent M_r of 195 kDa in VLDL, LDL, and HDL fractions but not in the $d > 1.21$ g/mL fraction (Figure 3). Neither apoB-100 nor apoB-48 was detectable. Based on the apparent molecular weight of the apoB moiety, we estimated a protein size of 1700 to 1800 amino acids. We prepared DNAs by PCR of the apoB gene flanking the site of the predicted mutation. Sequencing of the prepared DNAs revealed a single C→T transition at position 5472 that converts glutamine 1755 (CAA) to a stop codon (TAA) (Figure 4). The calculated centile fraction of the mutant apoB is 38.7%, ie, 1754 of 4536 amino acids. We designated this truncated apoB species apoB38.7, by following the centile nomenclature of the apoB species classification.¹⁵ We confirmed that both of the proband's sons were heterozygous for the identical mutation.

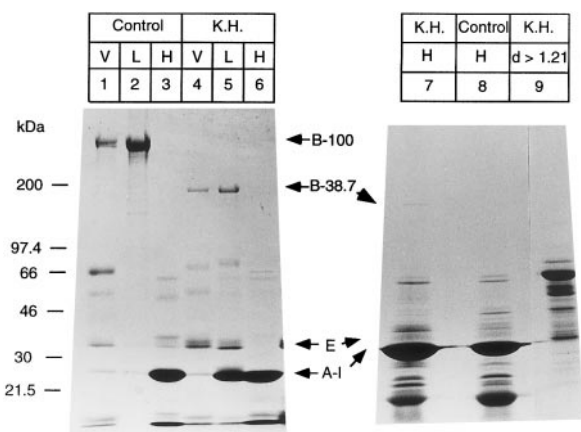
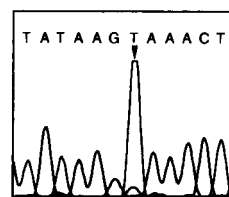


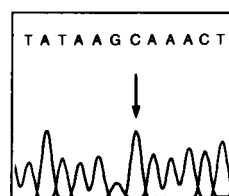
Figure 3. SDS-PAGE analyses of plasma lipoproteins from K.H. and a normal control subject. VLDL+IDL (V), LDL (L), and HDL (H) were isolated by sequential ultracentrifugation at densities of 1.019, 1.063, and 1.21 g/mL, respectively. After dialysis and delipidation, 20 μ g protein of each lipoprotein fraction (lanes 1 through 6) and 50 μ g protein of HDL and the lipoprotein-depleted fraction ($d > 1.21$) (lanes 7 through 9) were subjected to SDS-3% to 15% PAGE. Proteins were visualized by staining with Coomassie brilliant blue R-250. ApoB38.7 was visible in lanes 4, 5, and 7 but not in lane 9, indicating that this truncated apoB is distributed from VLDL through HDL but not in the $d > 1.21$ g/mL fraction.

cDNA 5472
 TAT AAG TAA ACT
 Tyr Lys Stop Thr
 1753 1754 1755 1756

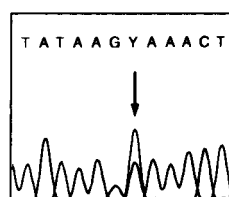
Patient:
K.H.



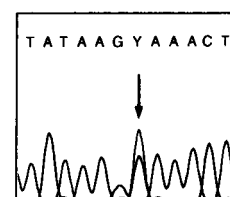
Normal:



1753 1754 1755 1756
 Tyr Lys Gln Thr
 TAT AAG CAA ACT



A.Y.



H.H.

Figure 4. DNA sequences of apoB in the kindred of K.H. Genomic DNA was prepared from blood cells. A 581-nt DNA (nts 5271 to 5852) of the apoB gene was prepared by PCR. The amplified DNAs were directly sequenced by the dideoxy chain-termination method with an ABI 373 DNA sequencer (Perkin-Elmer Cetus, Applied Biosystems). K.H. had a single C→T transition at position 5472 that converts glutamine 1755 (CAA) to a stop codon (TAA). Both of the proband's sons (A.Y. and H.H.) were heterozygous for the identical mutation.

We suspected that the proband had a CETP deficiency based on the elevated plasma HDL-C level (1.99 mmol/L) and the increased HDL₂-C/HDL₃-C ratio (3.3).¹⁶ We genotyped K.H.'s CETP gene for the common CETP gene mutations, an intron 14 splicing defect and an exon 15 missense mutation, Asp442Gly, but neither mutation was detected, supporting the finding of normal plasma CETP activity in K.H.

Discussion

The results demonstrate a novel truncation mutation of apoB, apoB-38.7, in the plasma from a patient with homozygous HBLP. ApoB-38.7 is the result of a nonsense mutation at Gln1755 that yields a protein 38.7% the size of apoB-100. Homozygosity for the apoB38.7 allele is supported by homozygosity of the 3' VNTR allele of apoB. Both of the proband's sons were found to be heterozygous for the mutant allele.

Approximately 30 different mutations have been reported to cause HBLP (see Reference 1 for a review and References 17 through 19), and only 6 were found to be present in the homozygous state.^{12,18-22} Patients with the most severe phenotype were siblings who were compound heterozygotes for apoB-2 and apoB-9.²³ The siblings' LDL-C levels were undetectable, and they presented with steatorrhea, neurological deficits, and retinitis pigmentosa, a complex of symptoms clinically indistinguishable from that of abetalipoproteinemia. An 8-year-old patient homozygous for apoB-50 also exhibited neurological abnormalities resulting from a nearly complete absence of vitamin E in the plasma.²¹ Neither neurological symptoms nor retinal degeneration was reported in patients homozygous for apoB-25,²⁰ apoB-27.6,¹⁸ apoB-29,¹² apoB-39,¹² and apoB-45.2¹⁹ or compound heterozygous for apoB-40/apoB-89.²⁴ In particular, a 48-year-old patient homozygous for apoB-45.2 had a normal plasma level of vitamin E.¹⁹ In this context, it is noteworthy that K.H. is homozygous for apoB-38.7 and has neurological deficits, retinal pigmentation, and a flat electroretinogram, the latter 2 of which are indicative of retinitis pigmentosa, despite a normal plasma level of vitamin E (Table 1). Other confounding factors, such as long-standing diabetes mellitus and advancing age, may account for the relatively severe clinical presentation of HBLP in K.H.

In addition to this complex of classic symptoms typical of severe HBLP, K.H. had a wide variety of conditions, such as type II diabetes mellitus, hepatic hemangioma, cholelithiasis, proteinuria, and a history of retinal hemorrhage, hemoptysis, and arterial calcification. Except for cholelithiasis, which has been reported to be prevalent in the affected members of an apoB-83 kindred,²⁵ the significance of the constellation of these diseases within this patient is currently unknown. These florid complications may be attributable to other recessive mutations. Because HBLP is thought to be protective against atherosclerosis, it is important to note that K.H. had severe calcification of major arteries. One report indicates a relative paucity of coronary artery morbidity and mortality among first-degree relatives of patients with heterozygous HBLP²⁶; furthermore, the virtual absence of atherosclerosis was reported in a 76-year-old subject with HBLP.²⁷

According to her medical history, K.H. had received photocoagulation therapy for "hemorrhagic glaucoma," which presumably resulted from proliferative diabetic retinopathy. Her renal disease and peripheral neuropathy are also compatible with the clinical picture of diabetic microangiopathy. Because hyperlipidemia is a risk factor for diabetic retinopathy²⁸ and cholesterol-lowering therapy retards the progression of diabetic nephropathy,²⁹ it is noteworthy that she had advanced complications due to long-standing diabetes.

Another remarkable finding is the unusually high plasma TC and HDL-C levels of K.H. when compared with other HBLP homozygotes. The high HDL₂-C/HDL₃-C ratio (Table 2)¹⁶ and the association with atherosclerosis³⁰ in K.H. are consistent with the clinical features of CETP deficiency, which is a common cause of hyperhighdensitylipoproteinemia in the Japanese.¹³ However, CETP activity was not decreased, and mutations in either intron 14 or exon 15 of the

CETP gene, the most common mutations causing CETP deficiency in the Japanese,¹³ were not found in the patient. Therefore, it is unlikely that K.H. had CETP deficiency. The associated proteinuria may, at least in part, account for the exceptionally high TC levels in K.H.

We detected apoB-38.7 from VLDL through HDL but not in the $d < 1.21$ g/mL fraction (Figure 3). Previous studies have shown that the buoyant density of the apoB-containing lipoproteins is largely proportional to the size of apoB between apoB-31 and apoB-37. It has been reported that apoB-31,³¹ apoB-32,³² and apoB-32.5³³ are present in the $d > 1.21$ g/mL fraction, but apoB-37 is not,⁵ suggesting that a portion of the apoB proteins between apoB-32.5 and apoB-37 is critical for the distribution to the $d > 1.21$ g/mL fraction. This finding is in agreement with our observation that apoB-38.7 was not present in the $d > 1.21$ g/mL fraction. ApoB-37,⁵ apoB-38.9,¹⁷ apoB-40,³⁴ and apoB-46³⁵ were reported to be present in HDL, but apoB-39,¹² apoB-50,²¹ and the other species larger than apoB-50 were not, indicating that a portion of the apoB between apoB-37 and apoB-46 is essential for distribution in HDL. This finding is consistent with our observation that apoB-38.7 was distributed in HDL.

In summary, we have found a novel, truncated apoB, apoB-38.7, in a patient with an unusual presentation of HBLP, including diabetes mellitus and extensive arterial calcification.

Acknowledgments

We thank Dr R. Takatsuki for the ophthalmological evaluation of K.H., S. Fukuda for electron microscopy, Drs S.G. Young and M. Kinoshita for helpful comments, and Dr P.K. Frykman for critical reading of the manuscript.

References

1. Kane JP, Havel RJ. Disorders of the biogenesis and secretion of lipoprotein containing the B apolipoproteins. In: Scriber CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Basis of Inherited Disease*. 7th ed. New York, NY: McGraw-Hill; 1995:1853-1885.
2. Linton MF, Farese RV Jr, Young SG. Familial hypobetalipoproteinemia. *J Lipid Res*. 1993;34:521-541.
3. Sharp D, Blinderman L, Combs KA, Kienzle B, Rocci B, Wager-Smith K, Gil CM, Turck CW, Bouma M-E, Rader DJ, Aggerbeck LP, Gregg RE, Gordon DA, Wetterau JR. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinemia. *Nature*. 1993;365:65-69.
4. Shoulders CC, Brett DJ, Bayliss JD, Narcisi TME, Jarmuz A, Grantham TT, Leoni PRD, Bhattacharya S, Pease RJ, Cullen PM, Levi S, Byfield PGH, Purkiss P, Scott J. Abetalipoproteinemia is caused by defects of the gene encoding the 97 kDa subunit of a microsomal triglyceride transfer protein. *Hum Mol Genet*. 1993;2:2109-2116.
5. Young SG, Bertics SJ, Curtiss LK, Witztum JL. Characterization of an abnormal species of apolipoprotein B, apolipoprotein B-37, associated with familial hypobetalipoproteinemia. *J Clin Invest*. 1987;79:1831-1841.
6. Young SG, Northey ST, McCarthy BJ. Low plasma cholesterol levels caused by a short deletion in the apolipoprotein B gene. *Science*. 1988;241:591-593.
7. Noma A, Yokosuka T, Kitamura K. Plasma lipids and apolipoproteins as discriminators for presence and severity of angiographically defined coronary artery disease. *Atherosclerosis*. 1983;49:1-7.
8. Kato H, Nakanishi T, Arai H, Nishida H, Nishida T. Purification, microheterogeneity, and stability of human lipid transfer protein. *J Biol Chem*. 1989;264:4082-4087.
9. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest*. 1955;34:1345-1353.

10. Boerwinkle E, Fourest E, Chan L, Xiong W. Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: applications to the apolipoprotein B 3' hypervariable region. *Proc Natl Acad Sci U S A*. 1989;86:212–216.
11. Sharp D, Ricci B, Kienzle B, Lin MCM, Wetterau JR. Human microsomal triglyceride transfer protein large subunit gene structure. *Biochemistry*. 1994;33:9057–9061.
12. Collins DR, Knott TJ, Pease RJ, Powell LM, Wallis SC, Robertson S, Pullinger CR, Milne RW, Marcel YL, Humphries SE, Talmud PJ, Lloyd JK, Miller NE, Muller D, Scott J. Truncated variants of apolipoprotein B cause hypobetalipoproteinemia. *Nucleic Acids Res*. 1988;16:8361–8375.
13. Inazu A, Jiang XC, Haraki T, Kamon N, Koizumi J, Mabuchi H, Takeda R, Takata K, Moriyama Y, Doi M, Tall A. Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high-density lipoprotein cholesterol. *J Clin Invest*. 1994;94:1872–1882.
14. Gotoda T, Kinoshita M, Ishibashi S, Inaba T, Harada K, Shimada M, Osuga J, Teramoto T, Yazaki Y, Yamada N. Skipping of exon 14 and possible instability of both the mRNA and the resultant truncated protein underlie a common cholesteryl ester transfer protein deficiency in Japan. *Arterioscler Thromb Vasc Biol*. 1997;17:1376–1381.
15. Kane JP, Hardman DA, Paulus HE. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc Natl Acad Sci U S A*. 1980;77:2465–2469.
16. Yamashita S, Hui DY, Wetterau JR, Sprecher DL, Harmony JAK, Sakai N, Matsuzawa Y, Tarui S. Characterization of plasma lipoproteins in patients heterozygous for human plasma cholesteryl ester transfer protein (CETP) deficiency: plasma CETP regulates high-density lipoprotein concentration and composition. *Metabolism*. 1991;40:756–763.
17. Groenewegen WA, Averna MR, Pulai J, Krul ES, Schonfeld G. 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.
18. Talmud PJ, Krul ES, Pessah M, Gay G, Schonfeld G, Humphries SE, Infante R. Donor splice mutation generates a lipid-associated apolipoprotein B-27.6 in a patient with homozygous hypobetalipoproteinemia. *J Lipid Res*. 1994;35:468–477.
19. Young SG, Bihain B, Flynn LM, Sanan DA, Ayrault-Jarrier M, Jacotot B. Asymptomatic homozygous hypobetalipoproteinemia associated with apolipoprotein B45.2. *Hum Mol Genet*. 1994;3:741–744.
20. Huang L-S, Ripps ME, Korman SH, Deckelbaum RJ, Breslow JL. Hypobetalipoproteinemia due to an apolipoprotein B gene exon 21 deletion derived by Alu-Alu recombination. *J Biol Chem*. 1989;264:11394–11400.
21. Hardman DA, Pullinger CR, Hamilton RL, Kane JP, Malloy MJ. Molecular and metabolic basis for the metabolic disorder of normotriglyceridemic abetalipoproteinemia. *J Clin Invest*. 1991;88:1722–1729.
22. Tennyson GE, Gabelli C, Baggio G, Bilato C, Brewer HB Jr. Molecular defect in the apolipoprotein B gene in a patient with hypobetalipoproteinemia and three distinct apo B species. *Clin Res*. 1990;38:482A. Abstract.
23. Huang L-S, Kayden H, Sokol RJ, Breslow JL. Apo B gene nonsense and splicing mutations in a compound heterozygote for familial hypobetalipoproteinemia. *J Lipid Res*. 1991;32:1341–1348.
24. Talmud P, King-Underwood L, Krul E, Schonfeld G, Humphries S. The molecular basis of truncated forms of apolipoprotein B in a kindred with compound heterozygous hypobetalipoproteinemia. *J Lipid Res*. 1989;30:1773–1779.
25. Farese RV Jr, Garg A, Pierotti VR, Vega GL, Young SG. A truncated species of apolipoprotein B, B-83, associated with hypobetalipoproteinemia. *J Lipid Res*. 1992;33:569–577.
26. Glueck CJ, Gartside PS, Mellies MJ, Steiner PM. Familial hypobetalipoproteinemia: studies in 13 kindreds. *Trans Assoc Am Physicians*. 1977;90:184–203.
27. Kahn JA, Glueck CJ. Familial hypobetalipoproteinemia: absence of atherosclerosis in a postmortem study. *JAMA*. 1978;240:47–48.
28. Chew EY, Klein ML, Ferris FL III, Remaley NA, Murphy RP, Chantry K, Hoogwerf BJ, Miller D. Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy: Early Treatment Diabetic Retinopathy Study (ETDRS) report 22. *Arch Ophthalmol*. 1996;114:1079–1084.
29. Lam KS, Cheng IK, Janus ED, Pang RW. Cholesterol-lowering therapy may retard the progression of diabetic nephropathy. *Diabetologia*. 1995;38:604–609.
30. Ramsbottom D, O'Neill A, Sexton DM, Gafoor RA, Bouchier-Hayes D, Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest*. 1996;97:2917–2923.
31. Young SG, Hubl ST, Smith RS, Snyder SM, Terdiman JF. Familial hypobetalipoproteinemia caused by a mutation in the apolipoprotein B gene that results in a truncated species of apolipoprotein B (B-31). *J Clin Invest*. 1990;85:933–942.
32. McCormick SPA, Fellowes AP, Walmsley TA, George PM. Apolipoprotein B-32: a new truncated mutant of human apolipoprotein B capable of forming particles in the low density lipoprotein range. *Biochim Biophys Acta*. 1992;1138:290–296.
33. Young SG, Pullinger CR, Zysow BR, Hofmann-Radvani H, Linton MF, Farese RV Jr, Terdiman JF, Snyder SM, Grundy SM, Vega GL, Malloy MJ, Kane JP. Four new mutations in the apolipoprotein B gene causing hypobetalipoproteinemia, including two different frameshift mutations that yield truncated apolipoprotein B proteins of identical length. *J Lipid Res*. 1993;34:501–507.
34. Krul ES, Kinoshita M, Talmud P, Humphries SE, Turner S, Goldberg AC, Cook K, Boerwinkle E, Schonfeld G. Two distinct truncated apolipoprotein B species in a kindred with hypobetalipoproteinemia. *Arteriosclerosis*. 1989;9:856–868.
35. Young SG, Hubl ST, Chappell DA, Smith RS, Claiborne F, Snyder SM, Terdiman JF. Familial hypobetalipoproteinemia associated with a mutant species of apolipoprotein B (B-46). *N Engl J Med*. 1989;320:1604–1610.