A truncated species of apolipoprotein B, B-83, associated with hypobetalipoproteinemia

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Abstract Familial hypobetalipoproteinemia, a syndrome associated with low plasma cholesterol levels, can be caused by apoB gene mutations. We identified a healthy 42-year-old man whose total plasma cholesterol level was 80 mg/dl. His plasma very low density lipoprotein (VLDL) contained a unique truncated apoB species, apoB-83, in addition to the normal B apolipoproteins, apoB-100 and apoB-48. Virtually no apoB-83 was detectable in his low density lipoprotein (LDL). From the subject's kindred, we identified nine other hypocholesterolemic subjects whose VLDL contained apoB-83. A tendency for cholelithiasis was noted in the apoB-83 heterozygotes, particularly in the older individuals. From the apparent size of apoB-83 on SDS-polyacrylamide gels and its reactivity with apoB-specific monoclonal antibodies, we estimated that it would contain approximately 3700–3800 amino acids. DNA sequencing of apoB genomic clones from two affected individuals revealed that apoB-83 was caused by a C→A transversion in exon 26 of the apoB gene (apoB cDNA nucleotide 11458). This mutation converts Ser-3750 (TCA) into a premature stop codon (TAA) and creates a unique *MseI* restriction endonuclease site. Thus, a single nucleotide transversion in the apoB gene results in a unique truncated apoB species, apoB-83, and the clinical syndrome of familial hypobetalipoproteinemia.—**Farese, R. V., Jr., A. Garg, V. R. Pierotti, G. L. Vega, and S. G. Young.** A truncated species of apolipoprotein B, B-83, associated with hypobetalipoproteinemia. *J. Lipid Res.* 1992. **33:** 569–577.

Supplementary key words hypolipidemia • polymerase chain reaction • monoclonal antibody • cholelithiasis • cholesterol

Familial hypobetalipoproteinemia is a syndrome associated with abnormally low plasma concentrations of LDL cholesterol and apoB (1). In the past 4 years, it has been demonstrated that this syndrome can be caused by various apoB gene mutations that interfere with the translation of a full-length apoB-100 molecule (2). These mutations frequently lead to the synthesis of a truncated species of apoB that can be detected in the plasma lipoproteins (2). We recently identified a healthy 42-year-old man with low plasma cholesterol levels; the evaluation of his family revealed a total of ten subjects with the heterozygous form of hypobetalipoproteinemia. The VLDL of these subjects contained a unique truncated apoB species, apoB-83, in addition to the normal B apolipoproteins, apoB-100 and apoB-48. In this study, we characterized the apoB-83 kindred on a clinical and biochemical level, and we determined the molecular basis for the apoB-83 mutation. We also observed a possible association of this mutation with cholelithiasis.

METHODS

Human subjects

A healthy 42-year-old Caucasian man (subject II-7 in Fig. 2) with low plasma cholesterol levels was referred to one of the authors (A. G.) for investigation. Blood samples were obtained from this subject and 16 other family members after they had fasted overnight for 12 h. Each subject gave informed consent, and the study protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas, Dallas, Texas. All family members were interviewed by telephone about their state of health. A detailed history was obtained and a physical examination was performed on three family members (I-3, I-4, and II-7). Two of these three subjects (I-3 and II-7) were familial hypobetalipoproteinemia heterozygotes (see Results).

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein.

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Lipoprotein and apolipoprotein measurements

Fasting plasma samples were analyzed for total cholesterol, triglycerides, and lipoprotein cholesterol according to Lipid Research Clinics procedures (3), except that cholesterol and triglycerides were measured by enzymatic methods (4, 5). The VLDL and intermediate density lipoprotein (IDL) fraction ($d < 1.019$ g/ml) was isolated by ultracentrifugation. Cholesterol was measured in the supernatant and infranatant fractions. Plasma HDL cholesterol was measured after the apoB-containing lipoproteins in the infranatant fraction had been precipitated with heparin-manganese (3). Cholesterol in the LDL fraction was determined from the difference between the cholesterol levels in the $d > 1.019$ g/ml infranatant and the HDL fraction. An electroimmunoassay technique was used to determine apoA-I and apoB concentrations (6).

Isolation and characterization of plasma lipoproteins

For each subject (except subject I-2), the VLDL ($d < 1.006$ g/ml) were isolated from fresh plasma by ultracentrifugation (7). (For subject I-2, blood was obtained for lipoprotein and apolipoprotein measurements, but additional plasma was not available for lipoprotein fractionation.) The VLDL, IDL (d 1.006–1.025 g/ml), LDL (d 1.025–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) fractions were isolated by sequential ultracentrifugation from the fasting plasma of three affected subjects (I-3, II-7, and II-9) and three unaffected subjects (I-4, II-8, III-9). For these six subjects, the lipoprotein fractions were also isolated from blood obtained 2 h after fat-rich meal. The protein content of the lipoprotein fractions was analyzed by electrophoresis on 3–12% gradient SDS-polyacrylamide gels (8) that were stained with silver. In some instances, the SDS-polyacrylamide gel proteins were electrophoretically transferred to nitrocellulose membranes for Western blotting (8). In several Western blot experiments, we analyzed the binding of a ^{131}I-labeled monoclonal antibody (MB3) to the apoB species in the VLDL fractions of three affected and three unaffected subjects. Antibody MB3 binds to the amino-terminal region of apoB (9) and therefore detects apoB-48, apoB-83, and apoB-100. Immunoblots were quantitively analyzed using a gel scanner (AMBIS, San Diego, CA). To assess LDL particle size, we used nondenaturing gradient polyacrylamide gel electrophoresis, as described (10, 11).

Enzymatic amplification of DNA and DNA sequencing

To determine the mutation in the apoB gene that results in apoB-83, a segment of exon 26 of the apoB gene corresponding to amino acids 3523–3850 was enzymatically amplified from the genomic DNA of two apoB-83 heterozygotes (subjects I-3 and II-7). The apoB DNA segment was amplified from 0.5 μg of white blood cell DNA using *Thermus aquatics* DNA polymerase (12) and oligonucleotides B83-1 (5′ TTTTGCTGcAGAAGCCACACTCCAACGCAT 3′; apoB cDNA nucleotides 10775–10804) and B83-2 (5′ AATGAgAATTCCAGCAGGTACAGAGAACTT 3′; complementary to apoB cDNA nucleotides 11759–11730). Single base mismatches (indicated by lower case letters) were included in the oligonucleotide sequences to create *Pst*I (B83-1) and *Eco*RI (B83-2) restriction enzyme sites. DNA amplification was performed for 30 cycles at denaturation, annealing, and extension temperatures of 96° (1 min), 60° (2 min), and 74°C (2 min), respectively. The amplified DNA was cloned into M13mp18 and M13mp19 and sequenced according to the method of Sanger, Nicklen, and Coulson (13).

*Mse*I restriction enzyme digests

*Mse*I restriction enzyme digests were performed on a 554-bp fragment of the apoB gene that was amplified from the genomic DNA of family members using oligonucleotides B83-2 and B83-3 (5′ CCACCAGCAT TGGTAGGAGAC 3′; apoB cDNA nucleotides 11206–11226). *Mse*I was obtained from New England BioLabs (Beverly, MA), and the restriction enzyme digests were performed according to the conditions suggested by the manufacturer. The *Mse*I-digested DNA fragments were electrophoresed on an 8% polyacrylamide gel and stained with ethidium bromide (14).

Statistical analysis

To compare the concentrations of lipids, lipoproteins, and apolipoproteins in the apoB-83 heterozygotes with those of the unaffected family members, an unpaired two-tailed *t*-test was performed. The frequency of gallstones in affected and unaffected family members was compared using the Fisher exact test.

RESULTS

A 42-year-old man of Danish descent, the proband of the kindred, was referred to the University of Texas Southwestern Medical Center for the evaluation of a total plasma cholesterol concentration of 80 mg/dl. Subsequent evaluation revealed that the total plasma cholesterol levels of the proband's 70-year-old father and 64-year-old mother were 159 mg/dl and 245 mg/dl, respectively. We examined the VLDL of these three subjects by SDS-polyacrylamide gel electrophoresis and found an abnormal species of apoB, and apoB-83 (named according to the centile system described by Kane, Hardman, and Paulus (15)), in the

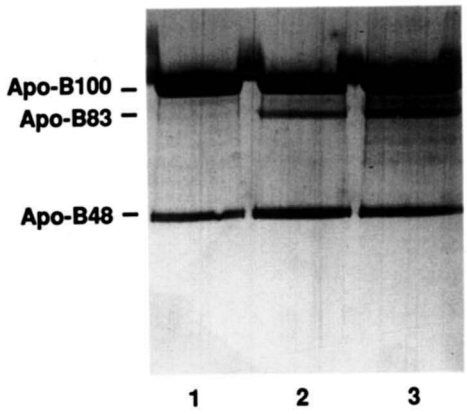

Fig. 1. An SDS-polyacrylamide gel demonstrating the presence of apoB-83 in the VLDL of the proband and his father. The delipidated VLDL proteins (5 μg/lane) were electrophoresed on a 3–12% SDS-polyacrylamide slab gel, and the gel was stained with silver. Very low density lipoproteins of the proband's mother (lane 1), the proband (lane 2), and the proband's father (lane 3) are shown.

VLDL of the proband and his father (**Fig. 1**). Because their VLDL also contained apoB-48 and apoB-100, we assumed that the proband and his father were heterozygous for an apoB gene mutation causing the synthesis of apoB-83.

By extending the family study, we identified a total of ten living family members with hypobetalipoproteinemia (**Table 1** and **Fig. 2**). Apolipoprotein B-83 was detectable in the VLDL of nine of these subjects; for the remaining subject (I-2), no plasma was available for analysis, but because she had very low plasma apoB and LDL cholesterol levels (Table 1), we are confident that she also was heterozygous for hypobetalipoproteinemia. The mean levels of plasma apoB and LDL cholesterol in the ten apoB-83 heterozygotes were 24.5 mg/dl and 49.7 mg/dl, respectively, values that were far lower than those of the unaffected subjects ($P < 0.0001$). Mean HDL cholesterol levels tended to be higher in affected subjects, but the difference was not statistically significant ($P = 0.107$).

No family members, affected, or unaffected, reported symptoms of intestinal fat malabsorption, neurologic disease, or retinopathy. However, four of the eleven affected subjects, as opposed to none of the seven unaffected subjects, had documented gallstone disease ($P = 0.12$). Subjects I-2 and I-3 had cholecystectomies for gallstones at age 55 and 58, respectively. Subject II-2 had gallstones detected at age 48 by an abdominal ultrasound examination. Subject I-1, an obligate heterozygote who died at age 68 of renal failure and rheumatoid arthritis, had a cholecystectomy for cholelithiasis at age 45. The oldest living family members, I-2 and I-3, were 74 and 70 years old, respectively, and had no history of coronary heart disease.

From the migration of apoB-83 on SDS-polyacrylamide gels (as compared with apoB-100, apoB-86 (16), apoB-74 (15), and apoB-48), we estimated that apoB-83 would contain ~3700–3800 amino acids. Western

TABLE 1. Lipoprotein and apolipoprotein concentrations in the apolipoprotein B83 kindred

Subject No.	Age/Gender	Total Chol	Tg	LDL Chol[a]	HDL Chol	ApoB	ApoA-I
				mg/dl			
ApoB-83 heterozygotes							
I-2	74/F	148	320	33(< 5)	51	38	92
I-3	70/M	159	142	NA	NA	44	77
II-2	50/M	108	209	51(< 5)	27	35	71
II-3	45/F	170	80	62(< 5)	88	26	138
II-7	42/M	80	15	23(< 5)	47	13	82
II-9	31/M	83	14	26(< 5)	55	7	102
III-4	27/F	132	42	79(~ 15)	47	18	109
III-5	27/F	134	28	58(< 5)	74	16	105
III-6	26/F	136	83	62(< 5)	56	31	104
III-7	23/M	114	172	53(< 5)	41	17	88
Mean ± SD	41.5 ± 18.5	126.4 ± 30.1 ($P = 0.0002$)	110.5 ± 100.0 ($P = 0.897$)	49.7 ± 18.7 ($P < 0.0001$)	54.0 ± 17.4 ($P = 0.107$)	24.5 ± 12.1 ($P < 0.0001$)	96.8 ± 19.3 ($P = 0.912$)
Unaffected subjects							
I-4	64/F	245	234	NA	NA	NA	NA
II-1	55/F	200	83	139(~ 45)	45	70	84
II-4	37/M	248	67	197(> 95)	45	86	98
II-8	39/M	286	171	171(~ 85)	49	129	107
III-1	32/F	168	82	118(~ 65)	36	83	102
III-2	29/M	182	101	123(~ 55)	35	66	82
III-9	13/M	175	75	117(~ 80)	34	53	102
Mean ± SD	38.4 ± 16.9	214.9 ± 45.0	116.1 ± 62.6	144.2 ± 32.9	40.7 ± 6.4	81.2 ± 26.3	95.8 ± 10.4

Abbreviations: Total Chol, total plasma cholesterol; Tg, total plasma triglycerides; LDL Chol, LDL cholesterol; HDL Chol, HDL cholesterol; ApoB, apolipoprotein B; ApoA-I, apolipoprotein A-I; NA, not available.

[a]The LDL cholesterol concentration percentile according to age- and sex-matched controls in the Lipid Research Clinics Prevalence Study (32) is shown in parentheses.

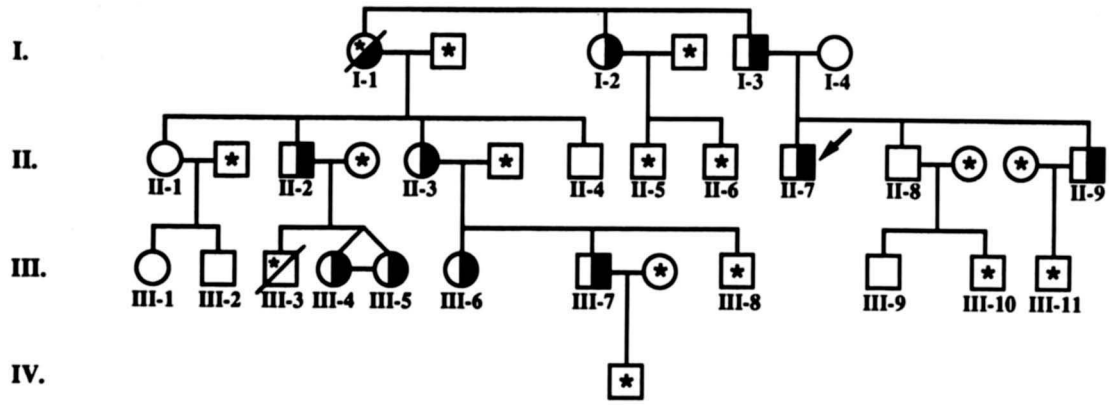

Fig. 2. The apoB-83 kindred. Subjects who are heterozygous for the apoB-83 defect are indicated by a half-shaded circle (female) or square (male). A slash through a circle or a square indicates that the individual is deceased. Subject I-1, who is deceased, was presumed to be a hypobetalipoproteinemia heterozygote based on inference from the pedigree. The proband is indicated by an arrow. Subjects from whom a blood sample was not obtained are indicated by an asterisk.

blots with several apoB-specific monoclonal antibodies provided support for this estimate and demonstrated that apoB-83 did not contain the carboxyl-terminal portion of apoB-100 (**Fig. 3**). To determine the mutation that produces apoB-83, a segment of the apoB gene spanning apoB-100 amino acids 3523–3850 was enzymatically amplified from the DNA of two affected subjects, cloned into M13, and sequenced. We found that one-half of the subclones from each subject contained a C→A transversion at apoB cDNA nucleotide 11458 (**Fig. 4**). This mutation converts Ser-3750 (TCA) into a premature stop codon (TAA) and explains the synthesis of apoB-83. This mutation also creates a unique *Mse*I restriction endonuclease site, which was detectable in the apoB gene of each of the apoB-83 heterozygotes that we examined (**Fig. 5**).

In the VLDL of the affected subjects, apoB-83 was invariably present in low concentrations compared with apoB-100, as judged by SDS-polyacrylamide gels (Fig. 1). When immunoblots of VLDL samples were probed with ^{131}I-labeled MB3 antibody (17, 18) and then scanned, we found that the radioactivity in the apoB-83 band was only 10–15% of that in the apoB-100 band. Although small amounts of apoB-83 were observed in the IDL fractions (data not shown), apoB-83 was virtually undetectable in the LDL fractions from fasting or postprandial plasma. As shown in **Fig. 6A**, there were no detectable differences in the protein content of the LDL fractions from affected and unaffected subjects. Consistent with these results, apoB-83-containing LDL particles were not observed on nondenaturing gradient polyacrylamide gels, even when the gels were heavily overloaded and silver-stained (data not shown). One affected subject (I-3) had a large amount of lipoprotein[a] (Lp[a]) in the HDL fraction (Fig. 6B). When the HDL sample was reduced with 2-mercaptoethanol, the Lp[a] dissociated into apo[a] and apoB-100 (Fig. 6B). Apolipoprotein B-83 was not detectable in the Lp[a] of this subject.

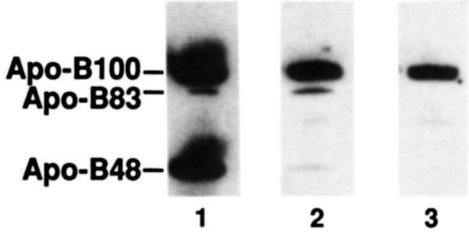

Fig. 3. A Western blot demonstrating the binding of several apoB-specific monoclonal antibodies to apoB-83. Twenty micrograms of the delipidated VLDL proteins from subject I-3 was electrophoresed on a 3–12% gradient SDS-polyacrylamide gel, and the separated apolipoproteins were then electrophoretically transferred to a nitrocellulose membrane for Western blots. Lane 1 shows the binding of antibody MB24; lane 2, antibody MB47; lane 3, antibody MB43. Antibody MB24 binds within apoB-100 amino acids 186–607; antibody MB47 binds within apoB-100 amino acids 3441–3569; and antibody MB43 binds within apoB-100 amino acids 4027–4081 (9, 29).

When we analyzed VLDL samples from apoB-83 heterozygotes and unaffected subjects on silver-stained SDS-polyacrylamide gels, we observed an increased amount of apoB-48, relative to apoB-100, in the affected subjects (**Fig. 7**). Immunoblots of VLDL samples (probed with ^{131}I-labeled antibody MB3) were scanned and demonstrated a two- to threefold increase in apoB-48, relative to apoB-100, in three apoB-83 heterozygotes when compared with three unaffected subjects.

DISCUSSION

In this study, we describe a new apoB gene mutation that causes hypobetalipoproteinemia. A C→A transversion in exon 26 of the apoB gene (cDNA nucleotide

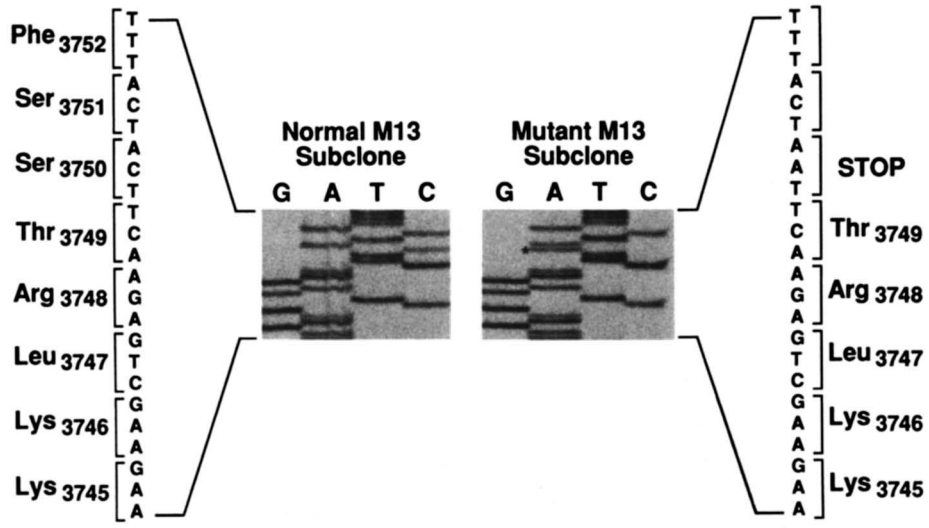

Fig. 4. Autoradiograms of DNA sequencing ladders demonstrating the apoB-83 mutation. The appropriate portion of the apoB gene (see Methods) was enzymatically amplified from the DNA of two apoB-83 heterozygotes (subjects I-3 and II-7). The amplified DNA was then subcloned into M13 and sequenced. For each subject, one-half of the M13 clones contained the normal apoB sequence (left), and one-half contained a C→A transversion at cDNA nucleotide 11458 (right; mutation indicated by an asterisk). No other changes from the consensus apoB sequence (30, 31) were noted in the mutant subclones.

11458) produces a premature stop codon and results in the synthesis of a truncated species of apoB, apoB-83. This mutation, like the previously described mutations causing hypobetalipoproteinemia, is thus far unique, occurring only within a single family. It seems likely, however, that future studies may reveal that an individual mutation may account for multiple cases of hypobetalipoproteinemia within a particular geographical or ethnic group. The apoB-83 mutation is easy to identify because a truncated apoB protein is present in the subject's VLDL (Fig. 1) and because the mutation creates a unique *Msp*I restriction endonuclease site in the apoB gene (Fig. 5).

In the plasma lipoprotein fractions, apoB-83 was detectable in the VLDL fraction on SDS-polyacrylamide gels, although it was invariably present in low amounts as compared with apoB-100 (see Fig. 1). Apolipoprotein B-83 was virtually undetectable, however, in the LDL fraction. This is in contrast to the apoB-67 (19) and apoB-89 (20) mutations, in which the truncated species is found in the LDL. In one affected subject, a large amount of Lp[a] was found in the HDL fraction; apoB-83 was not detectable in the Lp[a] of this subject (Fig. 6). This result is consistent with the hypothesis that apoB-83 lacks the region of the apoB molecule needed for apo[a] binding. It is possible, however, that apo[a] does bind a apoB-83, but that apoB-83-containing Lp[a] was not present in sufficient quantities to be detected.

We estimate that apoB-83 accounts for only 1–2% of the total plasma apoB in apoB-83 heterozygotes. Why are the apoB-83 levels so low? Clearly, either decreased secretion of apoB-83-containing lipoproteins from hepatocytes, or enhanced removal of these lipoproteins from the plasma, or a combination of both factors must be involved. Recently, studies have shown that lipoproteins containing apoB-87 (3978 amino acids) and apoB-89 (4039 amino acids), truncated apoB species that contain the putative LDL receptor-binding region of apoB-100 (amino acids 3000–3700) (9), bind with increased affinity to LDL receptors (21, 22) and are removed more rapidly from the plasma than lipoproteins containing the full-length apoB-100 (20). These data suggest that the carboxyl-terminal ~500 amino acids of apoB-100 (residues 4000–4536) might actually have the effect of decreasing the affinity of apoB-100 for the LDL receptor. It is tempting to speculate that apoB-83, 3749 amino acids in length, also has a high affinity for the LDL receptor and is cleared rapidly from the plasma. The virtual absence of apoB-83 in the LDL fractions of the affected subjects would be consistent with this theory (Fig. 6A). On the other hand, shorted truncated apoB species that lack the putative LDL receptor-binding region (such as apoB-67 (19), apoB-46 (23), apoB-37 (24), or apoB-31 (25)) are also present in very low concentrations in the plasma. For these shorter truncated forms of apoB, it seems likely that decreased synthesis and secretion from cells may, at least in part, explain their low plasma (1, 2). Diminished secretion remains a possible mechanism for the low plasma levels of apoB-83 as well. To answer these questions, more lipoprotein turnover studies of human subjects with hypobetalipoproteinemia will be required.

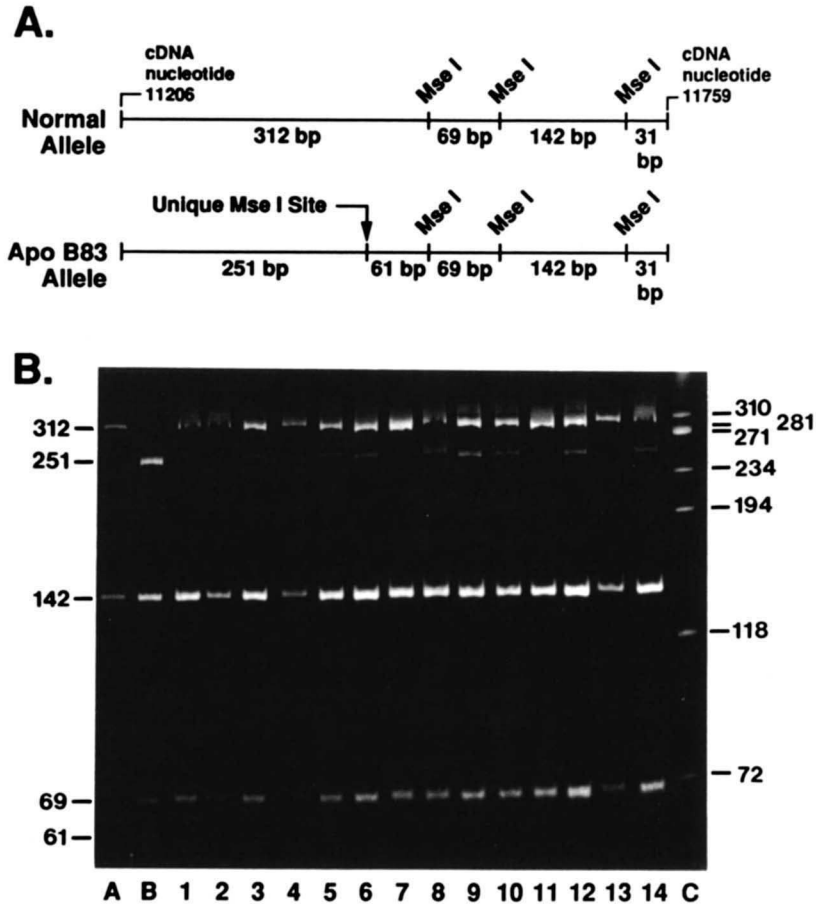

Fig. 5. An *Mse*I digest of a 554-bp fragment of the apoB gene. A 554-bp segment of the apoB gene (cDNA nucleotides 11206–11759) containing the region of the apoB-83 mutation was enzymatically amplified from the genomic DNA of apoB-83 family members. Panel A shows the predicted *Mse*I digest pattern for a normal apoB allele and a mutant apoB-83 allele. Panel B shows an ethidium bromide-stained gel demonstrating the *Mse*I digests from various family members. Lane A shows the digest of the 554-bp DNA fragment amplified from a normal M13 clone; lane B, a mutant M13 clone; lane C, ϕX174/*Hae*III markers. *Mse*I digests from affected family members are shown in lanes 1 (II-2), 3 (I-3), 5 (II-9), 6 (II-3), 8 (III-4), 9 (III-5), 10 (II-7), 12 (III-7), and 14 (III-6); unaffected members are shown in lanes 2 (I-4), 4 (III-9), 7 (II-4), 11 (III-2), and 13 (III-1).

It would be reasonable to hypothesize that the apoB-83 mutation, which is located about 4.8 kb downstream from the mRNA apoB-48 editing site, would not interfere with apoB-48 synthesis in the intestine. If this is true, apoB-83 heterozygotes would have two apoB alleles making apoB-48, but only one allele making apoB-100, and one might predict that their VLDL would contain a "double dose" of apoB-48, relative to apoB-100, compared with the VLDL of unaffected subjects. Our data showing a two- to threefold increase in apoB-48, relative to B-100, in the plasma VLDL of apoB-83 heterozygotes as compared with unaffected subjects support this hypothesis (Fig. 7). A similar observation for the VLDL of apoB-67 heterozygotes was recently made by Welty et al. (19).

The development of cholelithiasis in four apoB-83 heterozygotes (the subjects heterozygous for the mutation causing apoB-83) suggests the possibility that hypobetalipoproteinemia may be associated with gallstones (most likely cholesterol gallstones), particularly in older individuals. A number of anecdotal reports of cholelithiasis or gallbladder disease occurring in subjects with hypobetalipoproteinemia provide support for this association. Mars et al. (26) reported that a 37-year-old woman with familial hypobetalipoproteinemia and a demyelinating disorder had cholelithiasis. In that kindred, two other family members with hypobetalipoproteinemia (a 64-year-old woman and a 39-year-old man) were reported to have had mild cholecystitis and gallbladder dysfunction, although the presence of gallstones was not specified. All three subjects appeared to have the heterozygous form of hypobetalipoproteinemia. Kahn and Glueck (27) reported that a 76-year-old woman with heter-

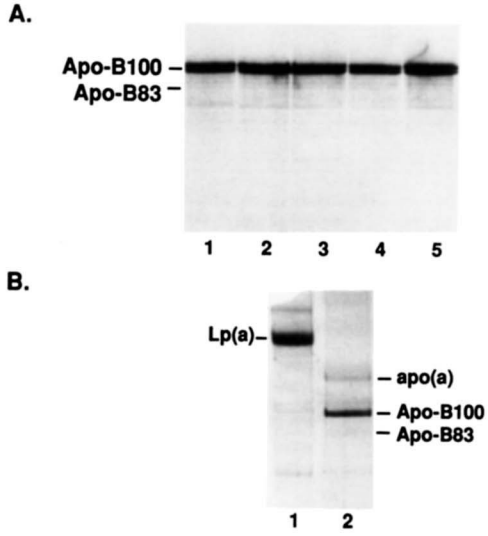

Fig. 6. Apolipoprotein B-83 was not detectable in the LDL or Lp[a] fractions. Panel A shows an SDS-polyacrylamide gel of the LDL fractions of affected and unaffected family members. Three micrograms of delipidated LDL protein was electrophoresed on a 3–12% SDS-polyacrylamide gel, which was stained with silver. Lanes 1 and 4 show the LDL of two affected subjects (I-3 and II-7, respectively). Lanes 2, 3, and 5 show the LDL from three unaffected family members (III-9, II-8, and I-4). Panel B shows an SDS-polyacrylamide gel of the HDL fraction (d 1.075–1.21 g/ml) of subject I-3. Lane 1 shows the HDL fraction electrophoresed under nonreducing conditions; lane 2 shows the same HDL fraction after reduction with 2% 2-mercaptoethanol.

ozygous familial hypobetalipoproteinemia had an edematous gallbladder, with acute and chronic cholecystitis found at laparotomy, although there was no mention of gallstones. Krul et al. (22) described a 46-year-old woman with familial hypobetalipoproteinemia (a compound heterozygote) who had undergone a cholecystectomy. The proband of the kindred reported by Steinberg et al. (28), a 64-year-old man with familial hypobetalipoproteinemia (a compound heterozygote), had gallstones. The proband's father, an obligate heterozygote, had gallstone disease in his 50's, and the proband's nephew, an apoB-37 heterozygote (subject 18 in ref. 24), had gallstone disease at the age of 33. Finally, a female apoB-46 heterozygote (subject 13 in ref. 23) had a cholecystectomy in her 50's. From these cases and the findings in our report, it appears that hypobetalipoproteinemia certainly does not prevent gallstone formation and may, in fact, increase the likelihood of developing gallstones.

The mechanism for the possible association of cholelithiasis and hypobetalipoproteinemia is unclear. It is possible that subjects with hypobetalipoproteinemia might secrete excess neutral sterols into their bile. Or perhaps the cholesterol derived from the hepatic uptake of remnant lipoproteins is preferentially secreted into bile when a defect in apoB synthesis is present. One could also speculate that the inability to make normal amounts of apoB in the liver and the intestine leads indirectly to decreased hepatic bile acid secretion, thereby increasing the cholesterol saturation of bile. Although intestinal malabsorption of bile acids is a predisposing factor for gallbladder disease, there is currently no evidence to suggest that malabsorption of bile acids occurs in heterozygous hypobetalipoproteinemia. To our knowledge, the bile composition of subjects heterozygous for an apoB gene mutation has not been studied. Vega et al. (6) reported that a subject with low cholesterol levels had slightly increased bile acid secretion and decreased cholesterol saturation of bile; however, it was not known whether that subject's low cholesterol levels were due to an apoB gene defect or to other factors. We believe that more studies are required, both to determine the frequency of gallstones in hypobetalipoproteinemia and to examine the chemical composition of bile in well-documented hypobetalipoproteinemia heterozygotes.

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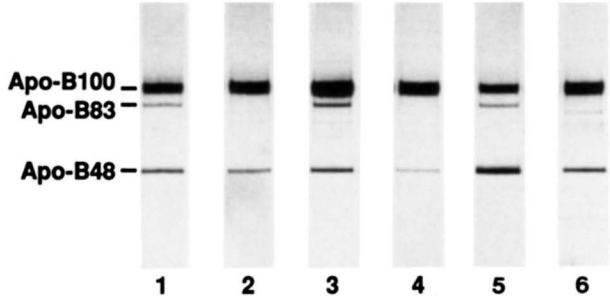

Fig. 7. An SDS-polyacrylamide gel of the VLDL fractions of four family members. The VLDL were isolated from plasma of four family members; 5 μg of delipidated VLDL protein was electrophoresed on a 3–12% SDS-polyacrylamide gel. The gel was stained with silver. Lanes 1 and 3 show the fasting VLDL of two apoB-83 heterozygotes (II-7 and I-3, respectively), and lane 5 shows the postprandial VLDL from one apoB-83 heterozygote (II-7). Lanes 2 and 4 show the fasting VLDL of two unaffected subjects (III-9 and II-8, respectively), and lane 6 shows the postprandial VLDL from another unaffected subject (I-4). The apoB-48/apoB-100 ratios quantified by immunoblot scanning for the apoB-83 heterozygotes and for the unaffected subjects were 0.13 ± 0.04 (SD) and 0.05 ± 0.1, respectively.

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