# Genetic Analysis of a Kindred with Familial Hypobetalipoproteinemia

## Evidence for Two Separate Gene Defects: One Associated with an Abnormal Apolipoprotein B Species, Apolipoprotein B-37; and a Second Associated with Low Plasma Concentrations of Apolipoprotein B-100

**Stephen G. Young,* Sandra J. Bertics,‡ Linda K. Curtiss,§ Brian W. Dubois,‡ and Joseph L. Witztum‡**
**Cardiology Division, and ‡Endocrinology/Metabolism Division, San Diego Veterans Administration Hospital and the University of California, San Diego, San Diego, California 92093; and §Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037*

## Abstract

**In 1979 Steinberg and colleagues recognized a unique kindred with normotriglyceridemic hypobetalipoproteinemia (1979. *J. Clin. Invest.* 64:292–301). We have undertaken an intensive reexamination of this kindred and have studied 41 family members in three generations. In this family we document the presence of two distinct apo B alleles associated with low plasma concentrations of apolipoprotein (apo) B and low density lipoprotein (LDL) cholesterol and we trace the inheritance of these two alleles over three generations. One of the alleles resulted in the production of an abnormal, truncated apo B species, apo B-37. The other apo B allele was associated with reduced plasma concentrations of the normal apo B species, apo B-100.**

**H.J.B., the proband, and two of his siblings had both abnormal apo B alleles and were therefore compound heterozygotes for familial hypobetalipoproteinemia. Their average LDL-cholesterol level was 6±9 mg/dl. All of the offspring of the three compound heterozygotes had hypobetalipoproteinemia, and each had evidence of only one of the abnormal apo B alleles. In the entire kindred, we identified six heterozygotes for familial hypobetalipoproteinemia who had only the abnormal apo B-37 allele and their average LDL cholesterol was 31±12 mg/dl. We identified 10 heterozygotes who had only the allele for reduced plasma concentrations of apo B-100 and their LDL cholesterol level was 31±15 mg/dl. Unaffected family members ($n$ = 22) had LDL cholesterol levels of 110±27 mg/dl.**

**This report describes the first kindred in which two distinct abnormal apo B alleles have been identified, both of which are associated with familial hypobetalipoproteinemia.**

## Introduction

Apolipoprotein (apo)[1] B is a crucial structural protein of chylomicrons, very low density lipoproteins (VLDL), and low density lipoproteins (LDL). Kane and co-workers (1) demonstrated the presence of two different apo B species in plasma. A high molecular weight form, apo B-100, is made by the liver and is found in VLDL and LDL (1, 2). A lower molecular weight form, apo B-48, is made by intestinal mucosal cells (3), and is found in plasma chylomicrons and chylomicron remnants. Recently, our laboratory demonstrated that both apo B species are the product of a single gene (4). The molecular basis whereby one gene yields both apoproteins is not yet understood.

When both forms of apo B are absent, as in recessive (or classical) abetalipoproteinemia, the concentrations of cholesterol and triglycerides in plasma are very low. Normal chylomicrons, VLDL, and LDL are not present in plasma, and the clinical syndrome is usually severe. Affected patients have fat malabsorption, retinitis pigmentosa, neurologic disorders, and acanthocytosis. Heterozygotes for this disorder reportedly have no chemical abnormalities and are asymptomatic (5, 6). Recently, Gregg and co-workers (7) studied liver biopsies of two subjects with abetalipoproteinemia. They found normal to increased levels of apo B messenger RNA (mRNA) and substantial amounts of apo B in hepatocytes, yet virtually no apo B in plasma, suggesting the possibility of a defect in the secretion of apo B-containing lipoproteins.

In contrast to classical abetalipoproteinemia, familial hypobetalipoproteinemia is distinguished by its transmission as an autosomal dominant trait (6, 8, 9). Subjects with the heterozygous form of familial hypobetalipoproteinemia are usually asymptomatic, and their LDL-cholesterol levels are about one-half that of normal. Homozygotes have extremely low LDL-cholesterol levels, and the clinical disorder is usually similar to that seen in patients with classical abetalipoproteinemia (6).

In 1979, Steinberg and co-workers (10) described a unique kindred with familial hypobetalipoproteinemia. The proband, H.J.B., and several of his siblings had extremely low levels of apo B and LDL cholesterol (< 5% that of normal), yet they still had normal concentrations of plasma triglycerides. Although H.J.B. was completely asymptomatic, metabolic ward studies indicated the presence of mild fat malabsorption. LDL isolated from H.J.B.'s plasma had normal uptake when studied in cultured fibroblasts. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of his LDL was said to show a normal apo B size.

Subsequently, Malloy and colleagues (11) reported the existence of a patient who also had extremely low levels of LDL cholesterol, but was normotriglyceridemic. Their patient was able to synthesize apo B-48, but not apo B-100, and, as a result, the patient's plasma contained chylomicrons and chylomicron remnants, but no normal VLDL or LDL. Since then, there have been two other reports of patients who apparently synthesize apo B-48, but not apo B-100 (12, 13). However, there have been no previous reports of any clinical entity associated with an abnormal apo B species in plasma (i.e., an apo B with an abnormal molecular weight).

Recently, our laboratory has undertaken a reexamination of the H.J.B. kindred. In the preceding article (14), we characterized

Address reprints to Stephen G. Young, M.D., Department of Medicine, M-013D, University of California, San Diego, La Jolla, CA 92093.
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1. *Abbreviations used in this paper:* apo, apolipoprotein; NCM, nitrocellulose membrane.

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an abnormal apo B species, apo B-37, which is present in all of H.J.B.'s lipoprotein fractions. Here, we document that apo B-37 is present in eight other members of this kindred, all of whom also have hypobetalipoproteinemia. We analyze the transmission of familial hypobetalipoproteinemia over three generations in the H.J.B. kindred and demonstrate that the proband, H.J.B., and two of his siblings with markedly reduced plasma cholesterol levels, are actually compound heterozygotes for familial hypobetalipoproteinemia. One allele leads to the production of a truncated apo B species, apo B-37. The other abnormal allele results in the production of apo B-48 and apo B-100. However, this allele is associated with markedly reduced plasma concentrations of apo B-100. We trace the transmission of both of these abnormal alleles over three generations. This article and that preceding, document the presence of an abnormal apo B species, apo B-37, thus providing the first example of a structural abnormality in apo B causing a distinct syndrome, in this case familial hypobetalipoproteinemia.

## Methods

*Plasma samples and lipoprotein isolation.* Plasma was obtained from 41 members of the H.J.B. kindred. Nonfasting samples were obtained because we wanted to increase the yield of the triglyceride-rich lipoprotein fractions. One aliquot of each plasma sample was set aside for lipoprotein and apoprotein quantification. To the remainder of the plasma, multiple proteolytic inhibitors were added, as described (14). These studies were approved by the Human Use Committee of the University of California, San Diego.

VLDL ($d < 1.006$ g/ml), intermediate density lipoprotein (IDL) ($d = 1.006$–$1.025$ g/ml), LDL ($d = 1.025$–$1.063$ g/ml), high density lipoprotein (HDL) ($d = 1.063$–$1.21$ g/ml), and lipoprotein-depleted plasma ($d > 1.21$ g/ml) were isolated under strictly sterile conditions using standard ultracentrifugation techniques (15). Protein concentration of the lipoprotein fractions was determined with a modification of the Lowry technique, using a BSA standard (16).

*Lipoprotein quantification.* Total cholesterol and triglycerides were measured by enzymatic techniques using an ABA-200 biochromatic analyzer (Abbot Laboratories, Irving, TX), and High Performance Cholesterol Reagent (No. 236691, Boehringer-Mannheim Diagnostics, Indianapolis, IN) and Triglycerides A-gent (No. 6097, Abbott Laboratories). LDL and HDL cholesterol were measured following the removal of the VLDL by ultracentrifugation, according to Lipid Research Clinics procedures (17). Members of the family whose plasma LDL-cholesterol concentration were below the 5% percentile of values for age- and sex-matched populations, as determined by the Lipid Research Clinics prevalence data (18) were classified as having hypobetalipoproteinemia.

*SDS-PAGE.* All lipoprotein fractions from all subjects were analyzed by SDS-PAGE. Apoproteins contained in the different lipoprotein fractions were separated by 3–12% or 3–15% gradient polyacrylamide slab gels containing 0.1% SDS, as previously described (4, 19). As noted in the preceding article, identification of apo B species in gels was facilitated by including the following controls on each gel: (*a*) Sigma Chemical Co. (St. Louis, MO) high molecular weight markers, (*b*) normal LDL isolated in the absence of proteolytic inhibitors (containing apo B-100, B-74, and B-26), (*c*) chylomicrons isolated from the plasma of a normal subject (containing apo B-100 and B-48), and (*d*) H.J.B. HDL (containing apo B-37).

*Antibodies.* Three previously characterized monoclonal antibodies against apo B were utilized in the study: B3 (19, 20), MB19 (4, 19, 20, 21), and MB47 (22). In this study, B3 is designated MB3. MB3 and MB19 both bind to epitopes in the amino-terminal region of apo B-100 (14). Thus, both antibodies bind to the apo B-26 fragment and to the T4 thrombin-cleavage fragment of apo B-100 (14). They also bind apo B-48 of normal chylomicrons and to the abnormal apo B species, apo B-37, contained in H.J.B.'s (and other affected family members') lipoproteins. MB47 binds an epitope in the carboxyl-terminal region of apo B-100 and thus binds to the T2 thrombin-cleavage fragment of apo B-100. It does not bind either apo B-48 of normal chylomicrons or apo B-37 of H.J.B. lipoproteins. Antibody MB19 detects a common form of genetic polymorphism in apo B; MB3 and MB47 do not. For radioimmunoassays (RIA), ascites was used as a source of antibody. For the double-label Western blot assays, we used immunopurified monoclonal antibodies, prepared from whole ascites using LDL- or protein A-Sepharose 4B columns, as previously described (20, 22).

We also utilized a rabbit polyclonal antiserum generated against human LDL-apo B-100 (22).

*Apoprotein quantification.* The total plasma apo B concentration was determined in a solid-phase, competitive RIA using antibody MB3, exactly as described (21). This RIA utilizes 96-well microtiter wells coated with LDL and uses LDL-apo B-100 for the standard curve. Antibody MB3 was chosen for use in the RIA because it bound both normally occurring apo B species, apo B-100 and apo B-48, as well as the abnormal apo B species, apo B-37, which was found in some members of the H.J.B. kindred. Previously, Tsao et al. (20) and Wiklund et al. (23) demonstrated that MB3 binds to a single epitope which is expressed by nearly all LDL particles.

Although the absolute concentration of apo B in H.J.B. plasma was extremely low, we found that > 85% of the total apo B mass was apo B-37 and apo B-48 (14). In the RIA, total apo B mass in plasma (expressed as milligrams per deciliter) was calculated using LDL-apo B-100 to form the standard curve. The protein concentration of the LDL-apo B-100 standard was determined chemically (16). In affected members of the H.J.B. kindred, where a significant fraction of the total apo B is apo B-37, the true apo B mass in plasma will be overestimated by the RIA using MB3, which was calibrated using apo B-100 for the standard curve, (i.e., apo B-37 [203,000 mol wt]) presumably has the same immunoreactivity in the RIA as apo B-100 [550,000 mol wt]). In this report, we do not attempt to correct for this overestimation of the true apo B protein concentration in the subjects with apo B-37 because, in absolute terms, the concentration of apo B-37 in plasma was always quite small (< 12 mg/dl).

In selected members of the kindred, the apo B-100 concentration in plasma was determined in a solid-phase, competitive RIA using antibody MB47, which binds to apo B-100, but not apo B-48 or apo B-37.

Apo A-I concentration was determined in an immunoenzymometric assay, using monoclonal antibodies specific for apo A-I (24). The normal range for plasma apo A-I concentration for this assay is 136–166 mg/ dl.

*Determination of the pattern of MB19 binding to apo B in plasma.* Previously, we demonstrated that monoclonal antibody MB19 binds to apo B in plasma or isolated LDL fractions with one of three distinct patterns of immunoreactivity (strong, intermediate, or weak). These three patterns of immunoreactivity, or "binding patterns," are the result of the codominant inheritance of two common apo B alleles that encode for apo B allotypes $MB19_1$ and $MB19_2$, which have high and low affinity, respectively, for antibody MB19 (21). Thus, subjects with strong and weak binding patterns are homozygous for allotypes $MB19_1$ and $MB19_2$, respectively, whereas those with an intermediate pattern are heterozygotes. The MB19 binding pattern is determined from a plasma RIA (21). Briefly, the apparent plasma apo B concentration determined in a competitive RIA using antibody MB19 is divided by the plasma apo B concentration determined in a competitive RIA using antibody MB47. Plasmas with "apparent MB19 apo B level/MB47 apo B level" ratios less than or equal to 0.1 have a weak pattern of MB19 binding (genotype $MB19_2/MB19_2$); those with ratios between 0.2 and 0.8 have an intermediate pattern (genotype $MB19_1/MB19_2$); and those with ratios greater than or equal to 0.8 have a strong pattern (genotype $MB19_1/MB19_1$). In this study, we used the plasma assay to determine the MB19 binding pattern (or genotype) of family members whose lipoproteins did not contain apo B-37 by analytical SDS-PAGE.

*Simultaneous determination of the pattern of MB19 binding in apo B-100, apo B-48, and apo B-37.* We recently developed a technique that

allowed us to determine simultaneously the MB19 binding pattern of apo B-100 and apo B-48 using a double-label Western blot immunoassay (4). In each case, the MB19 binding pattern of apo B-100 and apo B-48 were the same, indicating that both apo B species are products of a single gene. The MB19 binding pattern obtained with the Western blot assay was always identical to the MB19 binding pattern determined from the MB19 and MB47 plasma RIAs described above. In preliminary experiments, we tested the ability of the Western blot assay to accurately assess the pattern of MB19 binding in the lipoproteins of eight members of the kindred whose lipoproteins did not contain apo B-37. In each case, the MB19 binding pattern of apo B-100 and apo B-48 was identical to that obtained with the plasma assay described above. In this study, we used the Western blot assay only for subjects whose lipoproteins were found to contain apo B-37 on analytical SDS-PAGE. Since the polyacrylamide gels separated apo B-100, apo B-48, and apo B-37, we could then simultaneously determine the MB19 binding pattern for each of the individual apo B species.

The apoproteins of delipidated VLDL were separated on 3–12% or 3–15% polyacrylamide slab gels. For these studies, reducing agents were not included in the sample buffer as prior studies indicated that these interfered with the ability of antibody MB19 to detect the polymorphism (4). The separated apoproteins were then transferred to a nitrocellulose membrane (NCM) at 150 V for 24 h at 4°C. After blocking remaining binding sites on the NCM in a phosphate-buffered saline (PBS) buffer containing 4% nonfat milk for 30 min, the NCM was incubated in a PBS buffer containing 1% BSA and 0.1% Tween 20 and $10^7$ cpm/ml of $^{125}$I-immunopurified MB19 (to detect the polymorphism) and $10^7$ cpm/ml of $^{131}$I-immunopurified MB3 (to quantify relative amount of apo B on the NCM) for 18 h at 4°C. Both antibodies were radioiodinated by the lactoperoxidase technique (Enzymobead, Bio-Rad Laboratories, Richmond, CA) according to manufacturer's instructions. Specific activities of the antibodies ranged from 5,000 to 10,000 cpm/ng.

Antibody MB3 was chosen to quantify the relative amount of apo B on the NCM because (*a*) it does not detect the MB19 polymorphism in apo B, (*b*) it binds to apo B-100, apo B-48, and apo B-37 (14), (*c*) unlike other antibodies that bound all apo B species (i.e., MB11, MB24), preliminary solid-phase RIA studies demonstrated that MB3 competed relatively poorly with MB19 for binding to apo B-100 of LDL, and (*d*) our preliminary studies indicated that the $^{131}$I-MB3 provided an accurate indication of the relative amount of each of the apo B species that had been transferred from the gel and immobilized on the nitrocellulose membrane.

After incubation of the NCM with radiolabeled antibodies, the NCM was extensively washed with a PBS buffer containing 1% nonfat milk and 0.2% Tween 20, and autoradiography was performed. Then, the apo B-100, apo B-48, and apo B-37 bands, as well as background samples from each lane, were removed and counted in a double-channel gamma counter. In each experiment, chylomicrons isolated from plasma of normal subjects having each of the three different MB19 binding patterns were included as controls. The MB19 binding pattern of the control subjects had been independently established by plasma RIA (21). From 5,000 to 100,000 cpm of $^{125}$I-MB19 and $^{131}$I-MB3 bound to each apo B species; background radioactivity averaged < 10% of the radioactivity present in the apo B bands. The MB19 binding pattern of the apo B-100 and apo B-48 bands of H.J.B. family members was determined by comparing the $^{125}$I-MB19/$^{131}$I-MB3 ratios in the apo B-100 and B-48 bands with the respective ratios obtained in control subjects. The MB19 binding pattern of the apo B-37 band was determined by comparing the $^{125}$I-MB19/$^{131}$I-MB3 ratio in the apo B-37 band with the ratio obtained in the apo B-48 band of control subjects. This latter comparison was justified because our preliminary studies indicated that the epitope recognized by MB3 was expressed nearly equally in apo B-37 and apo B-48 immobilized on NCMs. Thus, the $^{125}$I-MB19/$^{131}$I-MB3 ratio obtained in the apo B-37 and apo B-48 bands depends only on the affinity of MB19 for the apo B species. In preliminary experiments, we also utilized the double-label Western blot assay to assess the MB19 binding pattern of apo B-37 contained in HDL preparations (where apo B-37 was the only apo B species present). The $^{125}$I/$^{131}$I ratio in the apo B-37 band of HDL was the same as the $^{125}$I/$^{131}$I ratio determined for the apo B-37 band in VLDL.

## Results

### *Lipoprotein and apoprotein concentrations in the H.J.B. kindred*

The clinical characteristics of H.J.B., the proband, and lipoprotein concentrations of his immediate family were previously reported by Steinberg et al. (10). H.J.B. and 40 other family members included in this study were asymptomatic with respect to neurological disorders, retinal disease, or clinical manifestations of fat malabsorption. None had clinical manifestations of atherosclerotic disease. In the preceding article, we document the existence and characterize an abnormal apo B species, apo B-37, in the plasma lipoproteins of H.J.B. In this study, we characterize the genetics of hypobetalipoproteinemia in the H.J.B. kindred. A family tree, which includes all 41 members of the kindred studied, is presented in Fig. 1. Nonfasting plasma lipoprotein concentrations as well as apo A-I and apo B concentrations for all family members studied are shown in Table I. H.J.B. and other affected members with hypobetalipoproteinemia are remarkable for the fact that despite extraordinarily low concentrations of cholesterol, they have relatively normal triglyceride levels (10). Members of the family who had hypobetalipoproteinemia (an LDL-cholesterol concentration < 5% that of normal) are identified by a dark-highlighted circle or square in Fig. 1.

### *Identification of apo B-37 in the H.J.B. kindred*

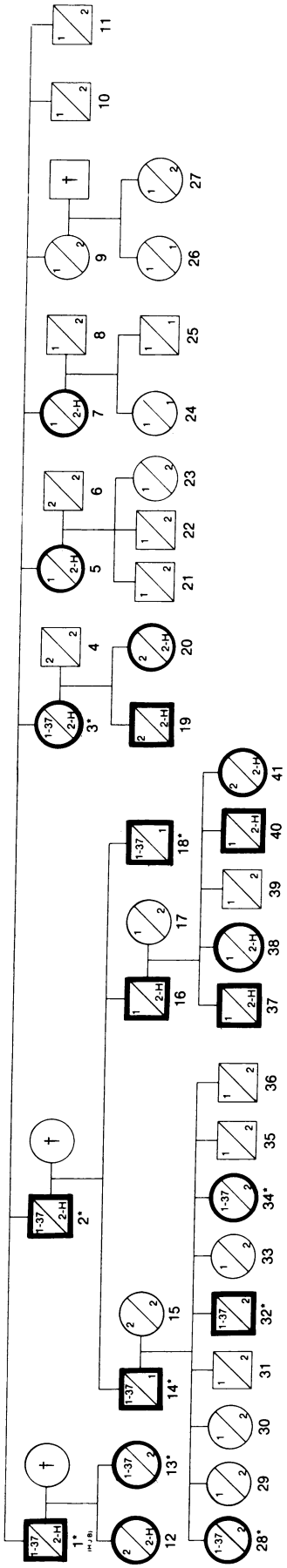
All lipoprotein fractions of all family members were studied by analytical SDS-PAGE. Apo B-37 was detected in the lipoproteins of H.J.B. and eight other family members spanning three generations. Family members whose lipoproteins contained apo B-37 are designated by an asterisk in Table I and in Fig. 1. All subjects whose lipoproteins contained apo B-37 had hypobetalipoproteinemia.

In affected family members, apo B-37 was found in every density class, including HDL. In VLDL and IDL, apo B-100 and apo B-48 were also present. In subjects 1, 2, and 3, apo B-100, apo B-48, and apo B-37 were present in LDL. In subjects 13, 14, 18, 28, 32, and 34, apo B-100 and a small amount of apo B-37 (but not apo B-48) were present in LDL. Fig. 2 in the preceding article demonstrates the presence of apo B-37 in the VLDL of members of the kindred (subjects 2 and 18). In HDL, apo B-37 was the only apo B species. Fig. 2 of this article demonstrates the presence of apo B-37 in the HDL of eight affected members of the kindred and the absence of apo B-37 in the HDL of two unaffected subjects. Fig. 3 demonstrates that apo B-37 was not found in the VLDL fractions of unaffected family members.

Apo B-37 was confined to the lipoprotein fraction of plasma. In competitive RIAs using antibodies MB3, MB47, and the polyclonal antiserum, no apo B was detectable in the lipoprotein-depleted plasma ($d$ = 1.21 g/ml bottom fraction) of any of the 41 members of the H.J.B. kindred.

### *Analysis of hypobetalipoproteinemia in the H.J.B. kindred*

*Subjects 1, 2, and 3: the compound heterozygotes.* In addition to H.J.B., two siblings, subjects 2 and 3, had extremely low LDL concentrations (Table I). Although subject 3 had a nonfasting LDL concentration of 17 mg/ml, a prior fasting measurement was 4 mg/ml (13), similar to those of H.J.B. and subject 2. Each



of these three subjects had total plasma apo B concentrations < 10% of normal. All three had apo B-37 in their lipoproteins.

Whereas both children of H.J.B. (subjects 12 and 13) had hypobetalipoproteinemia, only one (subject 13) had apo B-37. All three children of subject 2 (subjects 14, 16, and 18) had hypobetalipoproteinemia; yet only two (subjects 14 and 18) had apo B-37. Subject 3 had two children (subjects 19 and 20); both had hypobetalipoproteinemia, but neither had apo B-37. Thus, all of the children of subjects 1, 2, and 3 had hypobetalipoproteinemia: three had apo B-37. Despite being younger, the LDL-cholesterol and apo B levels in the seven hypobetalipoproteinemic children were actually higher than the extremely low concentrations found in their affected parents.

From an examination of these data, we postulated that the three siblings (subjects 1, 2, and 3) were actually compound heterozygotes for hypobetalipoproteinemia, whereas each of their children were heterozygotes. We hypothesized that subjects 1, 2, and 3 had one apo B allele, which resulted in reduced plasma concentrations of apo B-100, and a second apo B allele that yielded the abnormal apo B species, apo B-37 (which was also present in plasma in greatly reduced concentrations). Each of the offspring inherited one or the other abnormal allele and each had hypobetalipoproteinemia.

In order to further understand the inheritance of the apo B alleles, we utilized a common genetic polymorphism in apo B, the MB19 polymorphism, as a genetic marker. Antibody MB19 binds apo B allotypes $MB19_1$ and $MB19_2$ with high and low affinity, respectively. We have shown that the apo B genotype (with respect to the MB19 polymorphism) can be determined from the plasma MB19 binding ratio, which is calculated by dividing the apparent apo B concentration determined in an RIA that utilizes antibody MB19 by the apo B concentration determined in an RIA that uses antibody MB47. The MB19 apo B level/MB47 apo B level ratio reliably discriminates subjects homozygous or heterozygous for the two MB19 apo B alleles. Subjects homozygous for the $MB19_2$ allele have ratios < 0.1; homozygotes for the $MB19_1$ allele have ratios near 1.0. Genetic and immunochemical studies performed to date have indicated

*Figure 1.* The H.J.B. kindred. Only members of the kindred whose blood samples were obtained are pictured; family members whose blood was not obtained are not shown. Members of the kindred who are deceased are shown by a *dagger*. H.J.B. is subject *1*. All other subjects are referred to by number. Lipoprotein concentrations of all subjects are included in Table I. (*Squares*) male subjects; (*circles*) females. Subjects whose LDL-cholesterol concentration is less than the fifth percentile of age- and sex-matched controls (18) are designated by dark-highlighting. Subjects whose lipoproteins contain apo B-37 have an *asterisk* next to their number. The apo B genotype (consisting of two apo B alleles) is shown for each subject. See Results for a complete analysis of the apo B genotypes of the family members. The normal apo B alleles $MB19_1$ and $MB19_2$ are designated *1* and *2*, respectively. The $MB19_1$ and $MB19_2$ alleles result in normal plasma concentrations of the apo B allotypes $MB19_1$ and $MB19_2$, respectively. The $MB19_1$-37 allele is designated *1-37*. This allele encodes for the truncated apo B species, apo B-37. In nine subjects with apo B-37, we did not observe any examples of genetic recombination disrupting the linkage between the $MB19_1$ marker and the apo B-37 species (see Results and Table III). The $MB19_2$-H allele is designated *2-H*. This hypobeta (H) allele results in reduced plasma concentrations of apo B-100. We did not observe any examples of genetic recombination disrupting the linkage of the $MB19_2$ marker and hypobeta.

*Table I. Lipoprotein and Apoprotein Concentrations and MB19 Binding Ratios in the H.J.B. Kindred*

| Group | Age-sex | Triglycerides‡ | Total Cholesterol | LDL Cholesterol | HDL Cholesterol | Apo A-I | Apo B | MB19/MB47 ratio§ |
|---|---|---|---|---|---|---|---|---|
| | *yr* | *mg/dl* | *mg/dl* | *mg/dl* | *mg/dl* | *mg/dl* | *mg/dl* | |
| Hypobetalipoproteinemic subjects | | | | | | | | |
| Compound heterozygotes | | | | | | | | |
| *1 (H.J.B.) | 75-M | 163 | 31 | 1 | 20 | 75 | 3 | |
| *2 | 71-M | 37 | 42 | 1 | 35 | 79 | 8 | |
| *3 | 56-F | 27 | 98 | 17 | 77 | 178 | 9 | |
| Heterozygotes | | | | | | | | |
| 5 | 68-F | 45 | 130 | 62 | 59 | 141 | 50 | 0.9–1.5 |
| 7 | 60-F | 107 | 107 | 41 | 48 | 163 | 37 | 1.0–1.2 |
| 12 | 43-F | 31 | 109 | 27 | 76 | 141 | 26 | 0.02 |
| *13 | 38-F | 35 | 127 | 52 | 69 | 144 | 42 | |
| *14 | 45-M | 146 | 74 | 31 | 27 | 107 | 31 | |
| 16 | 41-M | 108 | 70 | 24 | 29 | 144 | 21 | 0.9–1.5 |
| *18 | 38-M | 48 | 69 | 21 | 41 | 121 | 27 | |
| 19 | 34-M | 359 | 90 | 33 | 32 | 113 | 34 | 0.02 |
| 20 | 33-F | 39 | 120 | 43 | 68 | 161 | 27 | 0.01 |
| *28 | 22-F | 95 | 98 | 31 | 53 | 224 | 39 | |
| *32 | 15-M | 120 | 62 | 17 | 19 | 78 | 27 | |
| *34 | 10-F | 19 | 78 | 31 | 46 | 128 | 29 | |
| 37 | 19-M | 88 | 80 | 25 | 37 | 133 | 15 | 0.9–1.5 |
| 38 | 17-F | 130 | 69 | 26 | 29 | 112 | 18 | 0.9–1.2 |
| 40 | 12-M | 64 | 79 | 14 | 57 | 206 | 14 | 0.8–1.2 |
| 41 | 12-F | 205 | 72 | 13 | 35 | 163 | 15 | 0.01–0.03 |
| Unaffected subjects | | | | | | | | |
| 4 | 59-M | 734 | 230 | 95 | 27 | 138 | 185 | 0.01 |
| 6 | 69-M | 141 | 207 | 141 | 39 | 154 | 132 | 0.01 |
| 8 | 66-M | 341 | 205 | 100 | 31 | 117 | 102 | 0.60 |
| 9 | 73-F | 247 | 233 | 144 | 42 | 182 | 110 | 0.3–0.7 |
| 10 | 63-M | 79 | 157 | 106 | 38 | 173 | 92 | 0.4–0.7 |
| 11 | 77-M | 162 | 194 | 116 | 51 | 140 | 89 | 0.5 |
| 15 | 44-F | 110 | 207 | 137 | 51 | 166 | 119 | 0.05 |
| 17 | 40-F | 162 | 150 | 94 | 28 | 171 | 78 | 0.4 |
| 21 | 44-M | 100 | 173 | 110 | 43 | 147 | 96 | 0.6 |
| 22 | 41-M | 393 | 279 | 171 | 46 | 154 | 140 | 0.5 |
| 23 | 40-F | 37 | 180 | 94 | 76 | 258 | 84 | 0.5 |
| 24 | 34-F | 125 | 151 | 87 | 42 | 126 | 74 | 1.2 |
| 25 | 28-M | 72 | 150 | 91 | 41 | 84 | 77 | 1.1 |
| 26 | 45-F | 49 | 183 | 113 | 62 | 202 | 86 | 1.0 |
| 27 | 32-F | 88 | 178 | 119 | 37 | 74 | 90 | 0.6 |
| 29 | 21-F | 107 | 189 | 113 | 56 | 253 | 78 | 0.6 |
| 30 | 18-F | 113 | 212 | 139 | 49 | 176 | 114 | 0.6 |
| 31 | 16-M | 87 | 117 | 73 | 31 | 126 | 85 | |
| 33 | 12-F | 188 | 124 | 71 | 30 | 122 | 88 | 0.5 |
| 35 | 7-M | 39 | 208 | 137 | 61 | 255 | 78 | 0.5 |
| 36 | 5-M | 53 | 162 | 111 | 44 | 120 | 70 | 0.5 |
| 39 | 16-M | 147 | 106 | 57 | 31 | 121 | 48 | 0.4–0.7 |

* Designates subjects with apo B-37 in their plasma lipoproteins. ‡ Plasma samples were not fasting samples. Differences in triglyceride (TG) concentrations should therefore be interpreted with caution. § MB19 binding ratios determined from plasma samples as described in Methods. This assay was used for subjects whose lipoproteins did not contain apo B-37. For subjects whose lipoproteins contained apo B-37, the binding of antibody MB19 to apo B-100, apo B-48, and apo B-37 was studied using the double-label Western blot assay, as described in the Methods.

that the apo B in plasma is normally produced in roughly equal amounts from both apo B alleles (21, 25). Consequently, in normo- or hyperlipidemic heterozygotes for the MB19 polymorphism, equal amounts of apo B from each allele are present in plasma and hence the MB19/MB47 ratios are near 0.5 (25). The MB19/MB47 ratios were determined with the plasma assay

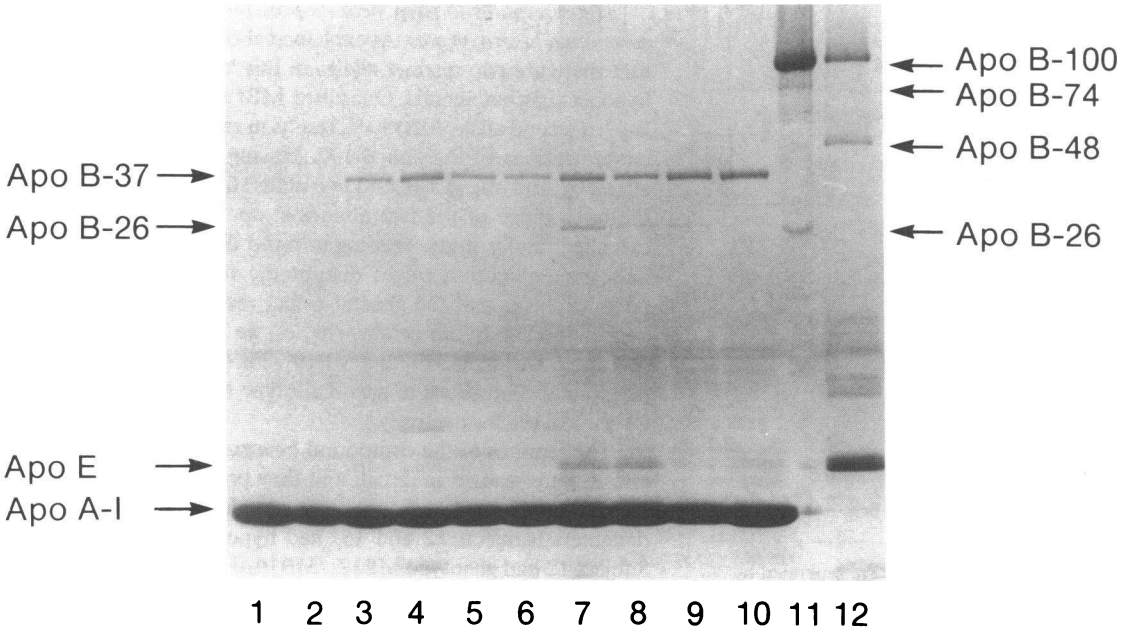



*Figure 2.* HDL preparations from members of the H.J.B. kindred analyzed by SDS-PAGE. 60 μg of delipidated lipoprotein samples were loaded onto each lane of the 3–15% gradient gel. The first 10 lanes show HDL preparations from members of the H.J.B. kindred. Lane *1*, subject 30; lane *2*, subject 31; lane *3*, subject 34; lane *4*, subject 32; lane *5*, subject 18; lane *6*, subject 14; lane *7*, subject 13; lane *8*, subject 3; lane *9*, subject 2; lane *10*, subject 1. HDL from subject 13 (lane *7*) was isolated in the absence of proteolytic inhibitors. As a result, there is a prominent apo B-26 band (see preceding article). Lane *11* shows LDL from a control subject which was isolated in the absence of proteolytic inhibitors. Lane *12* shows chylomicrons isolated from the plasma of a normal subject.

in all family members, except those whose lipoproteins contained apo B-37. The ratios are given in Table I, and the corresponding genotypes are indicated in Fig. 1.

In the nine family members whose lipoproteins contained apo B-37, the MB19 binding pattern was determined using a double-label Western blot assay recently developed in our laboratory (4). The advantage of this assay is that it allows a direct determination of the MB19 binding pattern of each apo B species, as described in Methods. Thus, we could determine which MB19 allele was associated with each of the apo B species, apo B-100, apo B-48, and apo B-37. In subjects 1, 2, and 3, antibody MB19 bound weakly to apo B-100 of VLDL. In all three individuals, antibody MB19 also bound weakly to apo B-48. Therefore, the normal apo B species, apo B-100 and B-48, were the products of an apo B allele encoding for allotype $MB19_2$. In contrast, in all three subjects, antibody MB19 bound very strongly to apo B-37, indicating that apo B-37 was the product of an apo B allele encoding for allotype $MB19_1$. The results of a representative double-label Western blot immunoassay for genetic analysis of subjects 1 and 2 are shown in Table II. Also included in the experiment shown in Table II was subject 18, one of the offspring of subject 2. The apo B-37 of subject 18 was also a product of a $MB19_1$ allele. The apo B-100 and apo B-48 were the products of a $MB19_1$ allele inherited from the mother of subject 18. Results of the assays performed in all nine subjects whose lipoproteins contained apo B-37 are summarized in Table III.

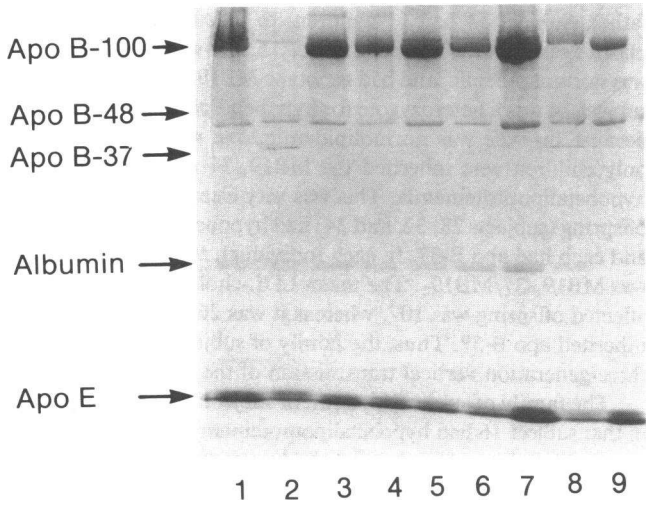



*Figure 3.* VLDL preparations from members of the H.J.B. kindred analyzed by SDS-PAGE. 10–75 mg of delipidated lipoprotein samples were loaded onto lanes of a 3–15% gradient gel. Lanes *1–8* show VLDL preparations from members of the H.J.B. kindred. Lane *1* shows VLDL from subject 12; lane *2*, subject 1; lane *3*, subject 9; lane *4*, subject 11; lane *5*, subject 10; lane *6*, subject 5; lane *7*, subject 7; lane *8*, subject 3; lane *9*, chylomicrons from a normal control subject.

Subjects 1, 2, and 3 were therefore compound heterozygotes for hypobetalipoproteinemia and were heterozygotes for the MB19 polymorphism. One allele produced apo B-37, which reacted with antibody MB19 with high affinity. In these individuals, the plasma concentration of apo B-37 was < 8 mg/dl. We termed this allele "$MB19_1$-37" to indicate that apo B-37 was always linked to the $MB19_1$ polymorphism. The other apo B allele produced the apo B-100 and apo B-48, each of which reacted with antibody MB19 weakly. The apo B-100 concentrations in the plasma of subjects 1, 2, and 3 (determined by an RIA using the apo B-100 specific monoclonal antibody MB47) were 0.375, 1.7, and 2.7 mg/dl, respectively. Thus, this allele resulted in a mark-

*Table II. Western Blot Assay for Determination of the Pattern of MB19 Binding for Apo B-100, Apo B-48, and Apo B-37 in Subjects 1, 2, and 18*

| Group | $^{125}$I-MB19/$^{131}$I-MB3 ratio* | | |
|---|---|---|---|
| | Apo B-100 | Apo B-48 | Apo B-37 |
| Controls | | | |
| MB19 binding pattern and (apo B genotype) | | | |
| 1 Strong ($MB19_1/MB19_1$) | 3.5 | 2.3 | |
| 2 Intermediate ($MB19_1/MB19_2$) | 1.9 | 1.4 | |
| 3 Weak ($MB19_2/MB19_2$) | 0.6 | 0.3 | |
| Subjects with apo B-37‡ | | | |
| 1 | 0.4 | 0.3 | 2.3 |
| 2 | 0.6 | 0.5 | 2.5 |
| 18 | 3.3 | 2.3 | 2.6 |

* Apoproteins of delipidated VLDL preparations were separated by SDS-PAGE on 3–12% slab gels, then electrophoretically transferred to a NCM. The NCM was then incubated with $^{125}$I-MB19 (to detect the polymorphism) and $^{131}$I-MB3 (to quantitate apo B binding to the NCM). The apo B bands were then sliced from the NCM and counted, and the ratio of $^{125}$I-MB19 counts divided by $^{131}$I-MB3 counts was calculated. The absolute ratio of the $^{125}$I/$^{131}$I counts is shown for each of the apo B species in control subjects and subjects with apo B-37. Controls were three normolipidemic subjects whose MB19 binding patterns had been independently determined by plasma RIA (21). The control subject with the strong binding pattern had a plasma MB19/MB47 ratio of 1.1; the subject with an intermediate pattern, 0.55; and the subject with a weak pattern, 0.01.
‡ Subjects 1 and 2 are compound heterozygotes for hypobetalipoproteinemia. Subject 18, the son of subject 2, is a heterozygote for hypobetalipoproteinemia (see Fig. 1).

edly reduced concentration of the normally occurring apo B species, apo B-100, resulting in hypobetalipoproteinemia (H). We have termed this hypobeta (H) allele "$MB19_2$-H" to indicate that it was always linked to the $MB19_2$ allele.

*Table III. Patterns of MB19 Binding for Apo B Species in the Nine Members of the Kindred Whose Lipoproteins Contained Apo B-37*

| Subject | Apo B-100 | Apo B-48 | Apo B-37 |
|---|---|---|---|
| 1 | $MB19_2$ | $MB19_2$ | $MB19_1$ |
| 2 | $MB19_2$ | $MB19_2$ | $MB19_1$ |
| 3 | $MB19_2$ | $MB19_2$ | $MB19_1$ |
| 13 | $MB19_2$ | $MB19_2$ | $MB19_1$ |
| 14 | $MB19_1$ | $MB19_1$ | $MB19_1$ |
| 18 | $MB19_1$ | $MB19_1$ | $MB19_1$ |
| 28 | $MB19_2$ | $MB19_2$ | $MB19_1$ |
| 32 | $MB19_2$ | $MB19_2$ | $MB19_1$ |
| 34 | $MB19_2$ | $MB19_2$ | $MB19_1$ |

Determination of the MB19 binding pattern for each apo B species was made by the double-label Western blot immunoassay as explained in Methods. An example of one of the double-label assays is shown in Table II. In all nine family members with apo B-37, the apo B-100 and apo B-48 species were both products of a $MB19_2$ allele or both products of the $MB19_1$ allele. In all nine subjects, the apo B-37 species was a product of a $MB19_1$ allele.

*Heterozygotes for hypobetalipoproteinemia: offspring of the compound heterozygotes.* As explained above, we have proposed that there are two distinct alleles in this kindred that result in hypobetalipoproteinemia. One allele, $MB19_1$-37, encodes for apo B-37; a second allele, $MB19_2$-H, results in greatly reduced plasma concentrations of the apo B-100. Having identified these two alleles by studying H.J.B. and two of his siblings, we next traced the inheritance of the two abnormal apo B alleles in a more extended family study, keeping in mind the possibility that genetic recombination might disrupt the linkage of the MB19 polymorphism and the genetic defect resulting in hypobetalipoproteinemia. In our genetic studies, we have termed the normal apo B alleles, $MB19_1$ and $MB19_2$. These alleles yield normal plasma concentrations of apo B allotype $MB19_1$ and apo B allotype $MB19_2$, respectively.

The families of the compound heterozygotes (subjects 1, 2, and 3) were studied in detail, and they proved to be highly informative. The wife of subject 1 was deceased. Both of his daughters (subjects 12 and 13) had hypobetalipoproteinemia. Subject 12 had genotype $MB19_2/MB19_2$-H. The lipoproteins of subject 13 contained apo B-37 and her genotype was $MB19_1$-37/$MB19_2$. Thus, each child was a heterozygote for familial hypobetalipoproteinemia, having inherited one or the other of the father's abnormal apo B alleles. Despite the fact that the children were younger and female, their LDL levels were much higher than their father, a compound heterozygote.

Like subject 1, the genotype of subject 3 was $MB19_1$-37/$MB19_2$-H. Her spouse's genotype was $MB19_2/MB19_2$. Both children of subject 3 (subjects 19 and 20) had hypobetalipoproteinemia, but lacked apo B-37, and had genotypes $MB19_2$/$MB19_2$-H.

The family of subject 2 was most revealing because it contained numerous family members in three generations. Subject 2 had three children, all of whom had hypobetalipoproteinemia. Two had apo B-37 (subjects 14 and 18); their genotypes were identical: $MB19_1$-37/$MB19_1$. Subject 16, whose lipoproteins did not contain apo B-37, had genotype $MB19_1/MB19_2$-H. All three children had LDL-cholesterol levels higher than that of their father. Subject 14 had 11 children; to date we have obtained plasma samples from nine. Subject 15, the wife of subject 14, was normolipidemic, and had genotype $MB19_2/MB19_2$. Because subject 14 was a heterozygote for hypobetalipoproteinemia, and because his wife was normolipidemic, one would predict that only children who inherited the $MB19_1$-37 allele should have hypobetalipoproteinemia. This was very clearly the case. Three offspring (subjects 28, 32, and 34) had hypobetalipoproteinemia and each had apo B-37. In each individual, the apo B genotype was $MB19_1$-37/$MB19_2$. The mean LDL-cholesterol level of unaffected offspring was 107, whereas it was 26 in those who had inherited apo B-37. Thus, the family of subject 2 demonstrates three-generation vertical transmission of the $MB19_1$-37 allele.

The family of subject 16, a son of subject 2, was informative in that subject 16 had hypobetalipoproteinemia but did not have apo B-37. Subject 16 inherited the hypobeta allele ($MB19_2$-H) from his father. When the plasma of subject 16 was analyzed to determine the MB19/MB47 ratio, values of 0.9–1.5 were found in multiple assays. In normolipidemic individuals, a ratio of 0.9–1.5 would indicate a genotype of $MB19_1/MB19_1$. However, in view of the fact that subject 16 had hypobetalipoproteinemia, and in light of his father's genotype, we presumed that the genotype of subject 16 was $MB19_1/MB19_2$-H. The reason that the plasma RIA yielded a high MB19/MB47 ratio in subject 16 is

that the $MB19_2$-H allele is responsible for only a very small fraction of the apo B in plasma. Almost all of the apo B in his plasma is a product of the normal allele, $MB19_1$ (inherited from his mother). Thus, the plasma immunoassay cannot detect the heterozygous state for the MB19 polymorphism when the product of one allele is present in markedly reduced amounts in plasma. (In this individual, the Western blot assay would also give misleading results because it cannot separate apo B-100 and apo B-48 produced by different apo B alleles.)

The proposed $MB19_1/MB19_2$-H genotype for subject 16 was strongly supported by examining the lipoprotein concentrations and MB19 genotypes of his spouse and his offspring. The MB19 genotype of his normolipidemic spouse, subject 17, was $MB19_1/MB19_2$ (MB19/MB47 ratio of 0.4 on repeated assays). The MB19/MB47 ratio determined from the plasma of subject 41, a daughter with hypobetalipoproteinemia, was 0.01–0.03 on repeated assays. Thus, she was homozygous for the $MB19_2$ polymorphism. Her genotype was $MB19_2/MB19_2$-H, having inherited the $MB19_2$ allele from her mother and the $MB19_2$-H allele from her father. Three other children with hypobetalipoproteinemia, subjects 37, 38, and 40, had plasma MB19/MB47 ratios of near 1.0. They, like their father, subject 16, had genotypes $MB19_1/MB19_2$-H.

The concentration of LDL cholesterol in subject 39 was slightly less than the fifth percentile, according to the Lipid Research Clinics prevalence study (18). However, we did not classify him as having hypobetalipoproteinemia in this study for two reasons. First, although low, his plasma concentrations of total and LDL cholesterol and apo B were at least twice as high as those of his siblings with hypobetalipoproteinemia. Secondly, his MB19/MB47 ratio was between 0.4 and 0.7 on repeated assays. Thus, his genotype was $MB19_1/MB19_2$. (If he had inherited the $MB19_2$-H allele, the plasma MB19/MB47 ratio would have been either high (near 1.0) or low ($< 0.1$), depending on the other apo B allele.) The relatively low LDL-cholesterol level in subject 39 may have been due to environmental or other genetic factors, inasmuch as his mother (genotype $MB19_1/MB19_2$) had a low-normal LDL-cholesterol concentration (near the 15% percentile).

*Analysis of other siblings of the compound heterozygotes.* Three of H.J.B.'s siblings had normal lipoprotein concentrations (subjects 9, 10, and 11). All three had MB19/MB47 ratios between 0.4 and 0.6, and therefore had genotypes $MB19_1/MB19_2$. Plasmas from two children of subject 9 were examined. As expected neither subject had hypobetalipoproteinemia. Two other siblings of H.J.B. had hypobetalipoproteinemia but did not have apo B-37 (subjects 5 and 7); their MB19/MB47 ratios were 0.9–1.5. Like subjects 16, 37, 38, and 40, their proposed genotypes are $MB19_1/MB19_2$-H. Plasmas from several children of these subjects were examined. None had inherited the $MB19_2$-H allele.

*Apo B concentrations in the heterozygotes for hypobetalipoproteinemia with apo B-37.* In the six heterozygotes for the hypobetalipoproteinemia who had apo B-37 (subjects 13, 14, 18, 28, 32, and 34), apo B-100 concentrations measured in the competitive RIA that used antibody MB47 averaged 22.8 mg/dl, significantly higher than the apo B-100 concentrations measured in the compound heterozygotes (subjects 1, 2, and 3). In the compound heterozygotes, the apo B-100 concentrations were all $< 3.0$ mg/dl. The higher plasma concentrations of apo B-100 in these heterozygotes are consistent with the presence of one normal apo B allele. In these same heterozygotes, the total apo B concentration in plasma, as measured by an RIA using antibody MB3 (which binds apo B-100, apo B-48, and apo B-37) averaged 32.5 mg/dl, higher than the measurement obtained in the RIA using MB47. This result is consistent with the presence of a small amount of apo B-37 (and apo B-48) in the plasma of these subjects.

## Discussion

In the original report of Steinberg and co-workers (10), it was recognized that the H.J.B. kindred was unique in that some family members had markedly reduced LDL-cholesterol concentrations yet had normal triglyceride concentrations. However, the mechanism leading to the profound hypocholesterolemia in affected subjects was not elucidated. We have reexamined this unique kindred and in this article demonstrate that the hypobetalipoproteinemia can be accounted for by two distinct apo B alleles that can be traced over three generations. One of these alleles encodes for an abnormal apo B species, apo B-37. The apo B-37 allele was always associated with the $MB19_1$ polymorphism, and the allele was designated $MB19_1$-37. The other allele results in reduced plasma concentrations of the normal apo B species, apo B-100. This allele was always associated with the $MB19_2$ polymorphism, and it has been designated $MB19_2$-H. In the first generation that we studied (H.J.B.'s generation), there were eight siblings. Three siblings (subjects 1, 2, and 3) were compound heterozygotes for familial hypobetalipoproteinemia; two (subjects 5 and 7) were heterozygotes for familial hypobetalipoproteinemia; and three (subjects 9, 10, and 11) had normal genotypes. The parents of the eight siblings are deceased. However, the mother is known to have had a fasting LDL-cholesterol concentration of 46 mg/dl (13), a level typical of that found in the heterozygotes for hypobetalipoproteinemia. In that all eight siblings were heterozygous for the MB19 polymorphism, we propose that one parent had genotype $MB19_1$-37/$MB19_1$, and the other had genotype $MB19_2/MB19_2$-H.

Several investigators have reported that subjects with a heterozygous form of familial hypobetalipoproteinemia have a reduced risk for cardiovascular disease (8, 26). Although our kindred is too small to address that question systematically, it is of interest that members of the H.J.B. kindred with hypobetalipoproteinemia reported no symptoms of atherosclerotic disease. The proband, H.J.B., one of the compound heterozygotes, is extremely healthy at the age of 75. His mother, who was almost certainly a heterozygote for hypobetalipoproteinemia, died at the age of 95, and one of the mother's siblings is known to have lived to be 105!

In analyzing the genetics of hypobetalipoproteinemia in the H.J.B. kindred, we were fortunate to be able to utilize another polymorphism in apo B, the MB19 polymorphism, as a genetic marker. The immunochemical basis of this extremely common polymorphism has been documented by our laboratory (21). Recently, we demonstrated that the polymorphism is expressed in parallel in apo B-100 and apo B-48 (4). The MB19 polymorphism itself has no apparent influence on plasma apo B or lipoprotein concentrations, at least in a Southern California population (25). Our ability to detect the MB19 polymorphism enabled us to show that, in the three compound heterozygotes, apo B-37 was a product of an allele different from that of apo B-100 and apo B-48. In these three individuals, antibody MB19 bound to apo B-37 strongly, but bound to apo B-100 and apo

B-48 weakly. In three generations of hypobetalipoproteinemic subjects with apo B-37, antibody MB19 invariably bound apo B-37 with high affinity. Thus, in the limited number of family members studied, we observed no evidence for genetic recombination disrupting the linkage of these two markers on the apo B allele.

Equally intriguing, our data suggest that in this family the other allele resulting in hypobetalipoproteinemia is linked to the $MB19_2$ polymorphism. This allele, which we termed hypobeta allele (H), is associated with reduced plasma concentrations of the normal apo B species, apo B-100. Data obtained in three generations of the H.J.B. kindred strongly support the idea that this hypobeta allele is linked to the $MB19_2$ marker. We found no example of genetic recombination resulting in a disruption of the linkage between the $MB19_2$ marker and hypobeta.

In the preceding article, we document that apo B-37 is not an artifact produced during lipoprotein isolation, and that apo B-37 is an amino-terminal fragment of apo B-100. It is present in all lipoprotein classes, including HDL. Of particular interest, apo B-37 is the only major apoprotein in a unique lipoprotein (Lp-B37) found in the HDL density class. Why an apo B allele that encodes for a truncated apo B species leads to hypobetalipoproteinemia is at present unknown. It is possible that apo B-37 is synthesized at a reduced rate. Alternatively its catabolism may be greatly accelerated, either before its secretion from hepatocytes (or intestinal cells), or intravascularly after its secretion. In any case, the existence of an abnormally short apo B species that retains the capacity to bind lipids should prove valuable in understanding of the structural features of apo B required for its various functions, such as lipid binding and interaction with cellular lipoprotein receptors. For example, studies are currently underway to determine whether apo B-37 can mediate the uptake of Lp-B37 particles by the LDL receptor.

Although the apo B-37 allele was of immediate interest because its product, a truncated apo B species, had not been previously described, the other apo B allele in the H.J.B. kindred leading to hypobetalipoproteinemia, the hypobeta allele, is also quite interesting. This allele resulted in low plasma concentrations of the normal apo B species, apo B-100. Metabolic studies will be required to determine whether the low concentrations of apo B-100 are the result of enhanced degradation or reduced synthesis. If the latter turns out to be the explanation, it is tempting to speculate that the defect in the hypobeta allele is in the promoter region of the apo B gene. Recently Myers et al. (27) have demonstrated that point mutations in the promoter region of a B-globin gene can lead to such reduced gene transcription. There are, of course, many other possibilities.

We are now beginning to study the molecular basis for the two abnormal apo B alleles in the H.J.B. kindred. These studies will be aided by the knowledge emerging from other labs on the complementary DNA (cDNA) sequence and the deduced amino acid sequence of apo B (28–34). Studies to elucidate the structure and organization of the promoter region of apo B may also be quite helpful in the analysis of this family. We believe that careful biochemical and genetic analysis of this and other kindreds with hypobetalipoproteinemia will enhance our understanding of the structure and function of apo B as well as factors affecting regulation of its synthesis. Such investigations may even provide clues that will improve our understanding of the normal variations in plasma cholesterol concentrations in the general population.

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