

## Genetic Evidence from Two Families that the Apolipoprotein B Gene Is Not Involved in Abetalipoproteinemia

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### Abstract

Abetalipoproteinemia (ABL) is a recessive disorder in which affected individuals have extremely low or undetectable levels of serum apo B-containing lipoproteins. Using restriction fragment length polymorphisms, we have studied two families, each with two children with classical ABL born of normal parents. In each of these families, the two affected children have inherited different apo B alleles from at least one parent, whereas the siblings would be anticipated to share common alleles if this disorder were due to an apo B gene mutation. This linkage study shows that in these families, the apo B gene is discordant with ABL and therefore the disorder is caused by a defect in another gene, which is important for the normal synthesis or secretion of apo B-containing lipoproteins from both the liver and intestine.

### Introduction

Inherited inability to secrete apo B-containing lipoproteins is exemplified by abetalipoproteinemia (ABL)<sup>1</sup> and homozygous hypobetalipoproteinemia (HBL). Patients with classical ABL are characterized by extremely low or undetectable plasma concentrations of apo B and of the apo B-containing lipoproteins (1, 2). The clinical features are fat malabsorption and acanthocytosis, both of which are present from birth, and a progressive spinocerebellar degeneration and retinopathy, which typically develop in the second decade of life. The latter features can be prevented by the administration of large oral

doses of vitamin E (100 mg/kg per d) (3). Obligatory heterozygotes are phenotypically normal and have normal concentrations of the serum lipoproteins and apo B. Family studies and a high degree of consanguinity indicate the autosomal recessive inheritance of a rare gene (2). The molecular defect causing ABL remains unknown.

Conversely, HBL is an autosomal codominant disorder; obligate heterozygotes have half the normal plasma apo B and apo B-containing lipoproteins and may be asymptomatic or show some of the features of ABL. Homozygous HBL is, however, indistinguishable both biochemically and clinically from ABL. The difference in the mode of inheritance in ABL and HBL has aided in the differential diagnosis of these two disorders (4). There is good evidence from molecular genetic studies that HBL is caused by a defect in the apo B gene itself. Low levels of apo B mRNA in liver biopsies from patients with HBL have been reported, suggesting a defect in production in these individuals (5). Young et al. have described an individual, from a large kindred, with compound heterozygous HBL. In this case one apo B allele produced a truncated apo B, apo B-37, and the other allele caused low levels of apo B-100 in the plasma. In this kindred, both alleles cosegregate with HBL (6, 7). We have also studied two patients with HBL and documented the presence of premature termination codons in the coding sequence of the apo B gene, resulting in the production of short apo B proteins (7a).

As raised apo B levels are associated with hyperlipidemia and risk of myocardial infarction (8), factors controlling apo B synthesis in the liver and intestine are important to our understanding of the development of dyslipoproteinemia. It may therefore be possible to improve our understanding of the control of apo B synthesis by studying patients with rare inborn errors in the synthesis and secretion of apo B-containing lipoproteins. In this study, we have used restriction fragment length polymorphisms (RFLP) to perform linkage analysis on two families, each with two children with ABL. Our results provide the first clear genetic evidence that in these two families, ABL is not caused by a defect in the gene for apo B.

### Methods

**Patients.** Two families, each with two affected children, were studied. These patients had the classical recessive form of ABL. The J family has been previously reported (case 1 and 2 of references 3, 9, and 10). Family M has not been previously reported. C.M. presented in infancy with fat malabsorption and acanthocytosis. Her brother was diagnosed at birth by examination of cord blood.

These results were presented in preliminary form at the North Atlantic Treaty Organization Advanced Research Workshop, Limone, Italy, March 1988, and the International Symposium on Intestinal Lipid Metabolism, Hamburg, Federal Republic of Germany, May 1988.

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1. *Abbreviations used in this paper:* ABL, abetalipoproteinemia; HBL, hypobetalipoproteinemia; RFLP, restriction fragment length polymorphism.

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**Lipid determinations.** Whole blood was collected into EDTA and the plasma was stored at  $-20^{\circ}\text{C}$  before estimating the lipid, lipoprotein, and apo B concentrations. For samples within the normal range, apo B was assayed immunochemically using a polyclonal antiserum (Orion Diagnostics, Espoo, Finland) that correlates well with the results obtained with radioimmunoassay. For samples with apo B levels  $< 50$  mg/dl, an ELISA assay was used (11). Samples of standards (LDL-apoB) ranged from 0 to 0.5 mg/dl; this accounts for the small amounts of apo B detected in the patients using this method. Total cholesterol and total triglyceride concentrations were estimated by routine enzymatic methods using commercial kits supplied by Boehringer-Mannheim Corp. (London, UK) and Metachem Diagnostics (Rugby, UK), respectively.

**DNA extraction and digestion.** DNA was extracted by the Triton X-100 method (12) and digested with Hinc II, Pvu II, Xba I, Eco RI and Msp I (2–10 U/ $\mu\text{g}$  DNA) under conditions recommended by the manufacturers (Anglian Biotech. Ltd., Colchester, UK). The DNA fragments were separated by size on a 1% (Pvu II, Hinc II, Xba I, and Msp I) or 0.7% (Eco RI) agarose gel and transferred to Hybond-N (Amersham International, Amersham, UK) by Southern blotting as previously described (13).

**DNA probes.** The probes used were a 3.5-kb Eco RI unique fragment (pAB3.5C) of the apo B gene to detect the Xba I RFLP (14), a 2-kb Hind III unique fragment (BH2) to detect the Msp I and Eco RI RFLP (15). The labeling of probes, hybridization, and washings were carried out as previously described (16). A map of the apo B gene showing the position of the variable sites and fragment sizes of the Pvu II, Hinc II, Xba I, and Eco RI RFLP appears in Fig. 1. The hypervariable region identified at the 3' untranslated end of the gene was detected using the enzyme Msp I.

## Results

Two pairs of siblings with the classical, recessively inherited form of ABL and their parents (in both cases unrelated) were studied. In Family J, there is an unaffected older daughter. The relevant biochemical findings are given in Table I. The diagnosis of the classical form of ABL was made on the basis of the plasma lipid and apo B concentrations of the parents, which were within the normal range and the absence of clinical and hematological abnormalities in the parents. The relatively low cholesterol and apo B concentrations observed in the parents in the M family are at the lower end of the normal range and may result from the low-fat diet adopted by this family.

Figs. 2 and 3 show the pedigrees of the two families studied. In both cases, it is possible to distinguish unambiguously all four parental chromosomes. In the J family (Fig. 2) we have used five RFLP of the apo B gene to deduce the parental haplotypes. The affected son, M.J., is homozygous for all five polymorphisms. This allows us to deduce the four parental

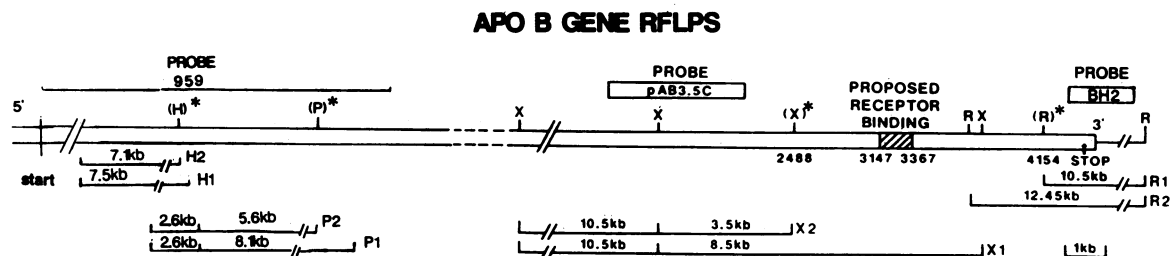
haplotypes and hence determine the phase of the RFLP variable sites of the parental chromosomes. Both affected children have inherited the apo B gene defined by haplotype IV from their father, whereas one child (M.J.) has inherited haplotype II and his sibling (S.J.) haplotype I from their mother. The unaffected sister N.J. has inherited haplotypes II and III.

In the M family (Fig. 3) we have made use of the length polymorphism identified by Msp I to distinguish all four parental chromosomes. The mother has alleles *M2* and *M3* and the father has alleles *M1* and *M2*. The two Msp I sites span a hypervariable region in the 3' untranslated region of the gene, which consists of a varying number of repeats of a AT-rich, 30-bp consensus sequence (17). P.M. has inherited allele *M2* from the father and allele *M2* from the mother; C.M. has inherited allele *M1* from the father and allele *M3* from the mother.

Thus, in both families, the children have inherited different alleles of the apo B gene from one or both parents. These observations are incompatible with the hypothesis that a mutation in, or close to, the gene for apo B causes classical recessive ABL in these families.

## Discussion

Previous studies in patients with ABL provide no clear evidence as to whether this condition is caused by a mutation of the apo B gene itself or whether it results from an abnormality in a gene or genes necessary for the biosynthesis and secretion of apo B-containing lipoproteins. Two groups have failed to detect apo B epitopes in intestine (18, 19), which suggests a defect in apo B synthesis, whereas others, using similar techniques, have reported the presence of apo B protein using antibodies in both intestine (20) and liver (20, 21). Lackner et al. have also detected increased amounts of normal-sized apo B mRNA in liver biopsies of ABL patients in addition to the absence of gross deletions or insertions in the apo B gene of these patients (21). We have also examined the gross structure of the apo B gene in the four patients reported here and a further five unrelated ABL patients. We have hybridized Southern blots of DNA from these patients, digested with a number of restriction enzymes, with DNA probes that span the apo B gene. No gross alterations of gene structure could be detected in any of the samples (not shown). Together, these data suggest that ABL may not be caused by a defect in the apo B gene and that it may be a heterogeneous disorder caused by defects of genes that are separately involved in regulating the synthesis, assembly, or secretion of apo B-containing lipoproteins.



**Figure 1.** A map of the apo B gene illustrating the DNA probes used in the identification of the RFLP and the variable restriction enzyme sites (\*). P, Pvu II; H, Hinc II; X, Xba I, and R, Eco RI. The fragment sizes of the alleles for each RFLP are shown.

Table I. Serum Lipid, Apo, and Vitamin E Concentrations in Patients with ABL and their First-Degree Relatives

	Cholesterol	Triglyceride	Apo B	Vitamin E
	mmol/liter		mg/dl	μmol/liter
Mr. J.	6.6	1.4	112	23.6
Mrs. J.	4.8	0.6	74	24.3
N.J.	3.5	1.1	83	17.4
M.J.	0.9	<0.1	0.24	1.5*
S.J.	0.6	<0.1	0.09	0.6*
Mr. M	4.0	2.0	84	25.1
Mrs. M.	3.7	0.7	61	23.0
C.M.	0.9	<0.1	0.57	4.5*
P.M.	0.9	<0.1	0.31	3.4*
Normal range	3.8–6.8	0.4–1.8	60–140	11.5–35.0

\* Patients received vitamin E therapy in addition to other fat-soluble vitamins and a low-fat diet.

This study demonstrates for the first time that ABL can be caused by defects of a gene or genes that do not code for apo B and are not closely linked to it. In a recessive disorder like ABL, affected siblings should inherit the same defective gene from both the mother and father. Analysis of RFLP haplotypes in the affected siblings from both of the families studied here rules out a mutation in or near the apo B gene as the cause of ABL in these kindreds.

Many of the steps in the biosynthesis of apo B-containing lipoproteins in the liver and intestine have yet to be explained. Pulse-chase experiments in a hepatoma cell line (HepG2) show that the time taken from the start of apo B mRNA translation on the ribosome to the secretion of apo B from the cell in a lipoprotein particle is ~ 30 min (22). During this time, protein folding, disulfide bond formation, and lipoprotein assembly must take place. Apo B is O- and N-linked glycosylated and other posttranslational events including acylation and phosphorylation take place (23–25). There are therefore several potential points in this process at which defects in proteins that carry out these steps may block or alter the rate of production of apo B-containing lipoproteins. Such defects would result in the features of recessively inherited ABL found in our patients. It remains to be seen whether in different patients with ABL the defect is in different genes, resulting in the same phenotype. The possibility remains that defects in the coding

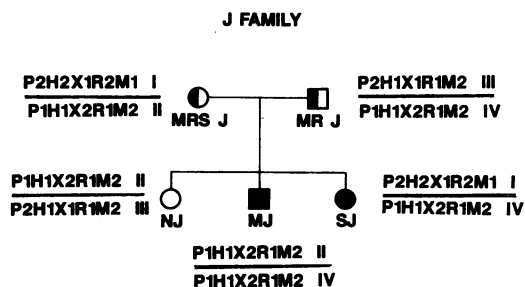


Figure 2. Pedigree of the J family. DNA was digested with the enzymes Pvu II (P), Xba I (X), Hinc II (H), Eco RI (R), and Msp I (M). The unambiguously deduced haplotypes are designated I–IV. □, male; ○, female.

## M FAMILY

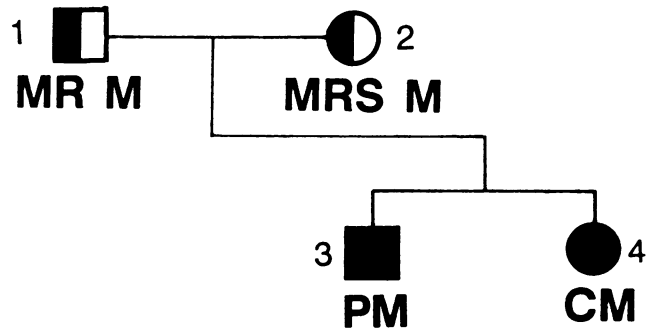


Figure 3. Pedigree of the M family. DNA was digested with the enzyme Msp I and hybridized with the genomic probe BH2. Msp I alleles in this family are numbered 1–3. There is an invariant band of 2.6 kb. Alleles *M2* and *M3* are not easy to distinguish. The broad doublet in the second track, from Mrs. M., indicates that she is *M2M3*. P.M. is homozygous for the *M2* allele, Mr. M. is *M1M2*, and C.M. is *M1M3*. Family members are numbered 1–4. □, male; ○, female.

region of apo B or upstream promoter regions of apo B may account for this clinical syndrome.

An efficient strategy has recently been proposed to map defects causing recessive disorders, by studying the affected offspring of consanguineous marriages (26). This approach may be applicable to determine the gene defect in ABL. It is possible that common variations in the gene or genes whose functions appear to be vital for the normal secretion of apo B containing lipoproteins, may also be involved in the abnormal secretion of apo B-containing lipoproteins in patients with some common forms of dyslipoproteinemia.

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