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# GENETIC EXCLUSION OF APO-B GENE IN RECESSIVE ABETALIPOPROTEINEMIA

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**Summary:** Abetalipoproteinemia is a recessive genetic disorder of unknown origin, which is characterized by absence of circulating apo-B-containing lipoproteins, malabsorption of intestinal fat, and degenerative neurological and retinal lesions. In this study, four families were analysed for genetic linkage between the abetalipoproteinemia phenotype and the apo-B genotype determined from polymorphisms of XbaI, MspI, EcoRI and PvuII restriction sites and that of the 3'-minisatellite of the apo-B gene. The results definitively exclude mutation of the apo-B gene as a causal factor of abetalipoproteinemia in three families. Consanguinity of the parents in the fourth family made genotyping less conclusive. 

Abetalipoproteinemia (ABL) and hypobetalipoproteinemia (HBL) form a heterogeneous group of genetic disorders in which plasma apolipoprotein B (apo-B) or apo-B containing lipoproteins (chylomicrons, VLDL, and LDL) are either absent or markedly reduced (reviews ref 1-5). Soon after birth, homozygous patients present a syndrome of diarrhea, fat and vitamin malabsorption and failure to thrive. Thereafter, tocopherol and retinol deficiencies result in progressive spinocerebelar degeneration and retinitis pigmentosa. Differential diagnosis is based on the plasma lipid levels of

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**Abbreviations**
ABL, abetalipoproteinemia; ACAT, acylCoA-cholesterol-acyltransferase; HBL, hypobetalipoproteinemia; LDL, low density lipoprotein; PCR, polymerase chain reaction, RFLP, restriction fragment length polymorphism; VLDL, very low density lipoprotein; VNTR, variable number of tandem repeats.

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heterozygous relatives who, in the case of ABL, have normal apo-B levels, but, of HBL, have less than 50% of normal levels. It is now established (5) that HBL results from mutations of the apo-B gene. The abnormal allele expresses an mRNA with a premature stop codon which is translated into a truncated apo-B. Genetic linkage analyses of two families with ABL published by Talmud et al (6) however show that the ABL phenotype fails to segregate with the apo-B genotype. Huang et al (7) have definitively excluded an abnormal apo-B gene in three of eight families with ABL, whereas in the remaining five linkage analysis was not totally incompatible with (undefined) abnormalities in the apo-B allele. Biochemical and cell biology studies of patients with ABL have in fact suggested that this phenotype could arise from a variety of molecular defects in lipoprotein synthesis and secretion (8-14). In this study, we report the results of linkage analyses in four families with recessive ABL, using restriction fragment length polymorphisms (RFLP) and the polymorphism of the variable number of tandem repeats (VNTR) in the 3' minisatellite of the apo-B gene. In three families, a defect of the apo-B gene could convincingly be excluded as the cause of the disease, but in the fourth, consanguinity of the parents made the results less conclusive.

## Patients and Methods

**PATIENTS**

In the four families studied, there were either one (Pr. and Pe.) or two (L. and B.) affected members. In family B, the parents were first cousins. All patients initially presented the typical ABL picture of severe diarrhea and steatorrhea, malnutrition, acanthocytosis and absence of immunodetectable apo-B in plasma. The oldest patients B.E. (41 yr) and B.C. (35 yr) had developed neurological disorders (ataxia, dysarthria, etc) and retinitis pigmentosa whereas I.Pe. (18 yr) and O.Pr. (21 yr) presented only retinal pigmentation. The two siblings of family L., L.C. (5 yr) and L.L. (2 yr), diagnosed a few weeks after birth and treated since then, exhibited neither neurological nor retinal abnormalities.

**METHODS**

Lipid were determined using automated enzymatic techniques, apolipoproteins AI was essayed by nefelometry and apo-B by ELISA (15,16). The lipoproteins were separated by agarose electrophoresis and fractionated by ultracentrifugation (17). Absence of normal or truncated forms of apo-B was checked by immunoblotting of lipoprotein fractions with polyclonal and monoclonal antibodies.

**DNA extraction and analysis :**

DNA was prepared from leukocytes (18) and Southern blotting and hybridization analysis of genomic DNA performed essentially as previously described (12). Ten µg of DNA were digested with restriction endonucleases (XbaI, MspI, EcoRI, PvuII) under conditions recommended by the manufacturers. The DNA fragments were separated according to size on a 1% agarose gel and then transferred to Gene Screen-Plus sheets. Prehybridization and hybridization of filters with $^{32}$P-labelled apo-B probes

were performed as previously described (12). The apo-B cDNA or genomic probes used from Southern blotting were ABF, SB9 and AB1, which span the entire apo-B gene (19). For analysis of the 3'-hypervariable region of apo-B gene(VNTR), the DNA sequences located within the targeted region were amplified by polymerase chain reaction (PCR), the sequence of the 5' oligonucleotide used to prime the PCR being 5' AACGGAGAAATTATGGAGGG 3', and that of the 3' primer, 5' AGGTTGTTCCTCAGGATCAA 3'. Amplification was attained by 25 cycles of PCR in an automated thermal cycler (Perkin Elmer/Cetus) using *thermus aquaticus* DNA polymerase following the manufacturer instructions. Amplified DNA was submitted to electrophoresis on 4.4% acrylamide gels at 30 mA for 25 min and visualized using ethidium bromide (20,21).

## Results

All patients had remarkably low levels of plasma cholesterol and triglycerides (Table 1), immunologically undetectable apo-B and apo-AI below 30% of control levels. Chylomicrons were absent in postprandial plasma and agarose electrophoresis revealed traces of beta and prebeta-lipoproteins and a decreased HDL band.

In family L, RFLPs were non-informative, except for XbaI. The father was heterozygous for the X1 and X2 alleles and the mother, homozygous for X2. One affected child was homozygous for the X2 allele and the other, heterozygous for X1 and X2 . Lack of cosegregation with the apo-B gene was confirmed by the VNTR polymorphism. As shown in Fig 1, the siblings had inherited different alleles (1/3 and 2/3, respectively) from their parents.

Table 1. PLASMA LIPIDS AND APOPROTEINS COMPOSITION (mg/dl)

| Family L. | CHOL | TG | AI | B |
|---|---|---|---|---|
| L. S. (father) | 203 | 70 | 132 | 67 |
| L. M.T. (mother) | 190 | 30 | 175 | 62 |
| L. C. (patient) | 22 | 4 | 35 | 0 |
| L. L. (patient) | 3 | 23 | 45 | 0 |

| Family Pr. | CHOL | TG | AI | B |
|---|---|---|---|---|
| Pr. J.C. (father) | 137 | 84 | 130 | 46 |
| Pr. P. (mother) | 359 | 90 | 257 | 155 |
| Pr. S. (brother) | 250 | 93 | 149 | 98 |
| Pr. O. (patient) | 23 | 3 | 46 | 0 |

| Family Pe. | CHOL | TG | AI | B |
|---|---|---|---|---|
| Pe. S. (father) | 226 | 217 | 135 | 150 |
| Pe. B. (mother) | 180 | 134 | 135 | 110 |
| Pe. S. (sister) | 230 | 67 | 150 | 110 |
| Pe. G. (sister) | 205 | 62 | 165 | 100 |
| Pe. I. (patient) | 30 | 2 | 50 | 0 |

| Family B. | CHOL | TG | AI | B |
|---|---|---|---|---|
| B. J. (father) | 175 | 65 | 115 | 79 |
| B. R. (mother) | 310 | 64 | 224 | 144 |
| B. E. (patient) | 39 | 5 | 6 | 0 |
| B. C. (patient) | 35 | 3 | 57 | 0 |

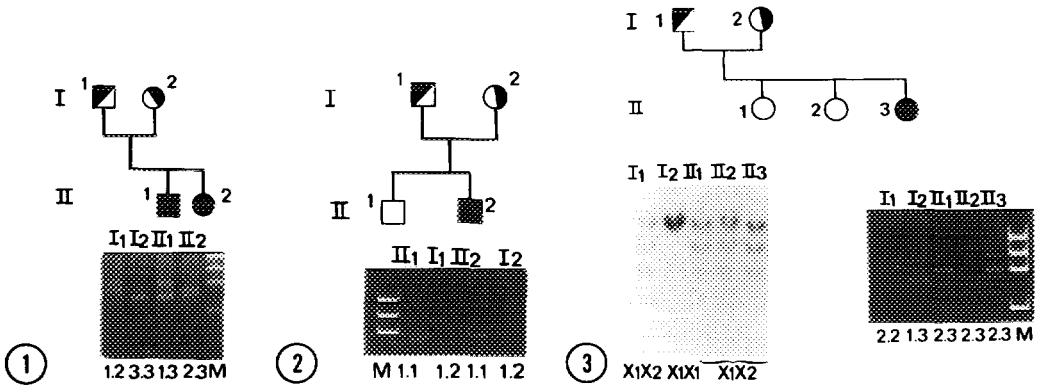


**Figure 1** . Genotyping with the apo-B minisatellite in Family L.
M. Molecular weight standards obtained from phage $\phi$ x 174 DNA-Hae III digest (top to bottom 1350, 1058, 872, 603 bp).

**Figure 2** . Genotyping of the apo-B minisatellite in Family Pr.

**Figure 3** . Genotyping of Family Pe, with XbaI (left) and VNTR (right) polymorphisms of apo-B gene.

In family Pr, the RFLPs obtained with XbaI, EcoRI, MspI and PvuII were inconclusive. However, the VNTR polymorphism (Fig 2) showed that both the proband and his normal brother were homozygous for the allele 1 inherited from their mother and father.

In family Pe, the proband (II 3, Fig 3) and two unaffected siblings had inherited the same XbaI haplotype (X1/ X2). VNTR polymorphism haplotyping showed the proband and his two normal brothers all to have inherited the same 2/3 haplotype. In these three families therefore haplotyping excluded that ABL phenotype could result from a defect in the apo-B gene.

In the fourth family, B, the parents were first cousins and clinically normal, but their two daughters were affected. Both patients and their parents had the same XbaI and MspI RFLP haplotypes. The VNTR haplotype was identical for the mother and the two children (2/2), but not for their the father (2/4). Alhough the findings for this family do suggest that the apo-B gene and the ABL phenotype are not linked, an unambiguous conclusion was impossible in view of the consanguinity of the parents.

## Discussion

ABL is a rare autosomal recessive disorder which is characterized by malabsorption of fat soluble vitamins, resulting from defective assembly or secretion of chylomicrons. Lipoprotein secretion by hepatocytes is also impaired, producing liver steatosis. Apo-B containing lipoproteins (VLDL,

LDL) are absent in the plasma of homozygous patients, but their heterozygous relatives have normal levels. The absence of circulating chylomicrons, VLDL and LDL prevents normal transfer of lipid and fat-soluble vitamins (particularly tocopherol) from the intestine to the liver and extrahepatic tissues. Tocopherol deficiency plays a major role in the development of the crippling syndrome of spinocerebellar degeneration and retinitis pigmentosa observed in untreated ABL patients (22). The molecular mechanism (s) responsible for the ABL phenotype were formerly considered to involve defects in apoprotein B synthesis, but recent studies have indicated that the ABL phenotype is heterogenous. Apo-B has been found to be undetectable in the intestinal cells of some patients (8,11,12), but in others, there were either decreased or normal amounts present (9,10,12-14), with the corresponding mRNAs either depressed or enhanced. In contrast with the report of Levy et al (11), we have found that intestinal explant cultures from ABL patients with ABL synthesize apo-B48 which, compared with that of controls, seems to be normally glycosylated (12). These findings suggest that the ABL phenotype may be produced by more than one molecular defect. In the analysis done by Talmud et al (6) of linkage between the ABL phenotype and the apo-B haplotypes determined using several RFLPs, two affected children in each of two families had inherited different alleles from one or both parents. This effectively excludes the possibility that a mutation in the apo-B gene transmitted as a recessive trait could produce the ABL syndrome. In a more extensive study, Huang et al (7) analysed eight ABL families with one or more affected children. Apo-B haplotyping was established both by RFLP with eight endonucleases and by determination of the 3'-minisatellite (VNTR) polymorphism. Here, an abnormal apo-B gene was unequivocally excluded in three of the families, which agrees with the findings of Talmud et al (6), but in the remaining five, the absence of linkage was not conclusive.

In our own series of four families, which one or two ABL patients, probands were analysed by apo-B haplotyping. RFLPs using the endonucleases, XbaI, MspI, EcoRI and PvuII proved to be unequally informative. Nevertheless, XbaI and VNTR haplotyping conclusively excluded the possibility of the apo-B gene being linked to the ABL phenotype in three families. The fourth family (B) was complicated by the consanguinity of the parents, but both affected children and their normal mother were homozygous for three different alleles (X1/X1, M2/M2, V2/V2) whereas the father had X1/X1, M2/M2 and V2/V4 haplotypes, suggesting that the apo-B gene also fails to segregate with the disease in this family.

Our linkage analyses of four families with abetalipoproteinemia strongly suggest, that, like in the five out of ten families described by other authors (6,7), ABL is not attributable to an abnormality of the apo-B gene.

These results, however, do not exclude the possibility of the ABL phenotype's arising from other subtle but functionally essential defects of apo-B expression in other patients. It does however seem likely that in most cases ABL is caused by defects in other proteins involved in the assembly or secretion of lipoproteins. Putative candidates include triglyceride, phospholipid or cholesterol transfer proteins, glycosyltransferases, ACAT and the poorly known macromolecules participating in the sorting and secretion of exportable proteins.

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