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GENETIC ANALYSIS OF A JAPANESE FAMILY WITH NORMOTRIGLYCERIDEMIC ABETALIPOPROTEINEMIA INDICATES A LACK OF LINKAGE TO THE APOLIPOPROTEIN B GENE

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SUMMARY: Normotriglyceridemic abetalipoproteinemia is a rare familial disorder characterized by an isolated deficiency of apoB-100. We have previously reported a patient with this disease, who had normal apoB-48 but no apoB-100. To elucidate the genetic abnormalities in this family, we studied the linkage of apoB gene using three genetic markers. The proband and her affected brother showed completely different apoB gene alleles, suggesting that the apoB gene itself is not related to this disorder in this family. By contrast, an American case had a point substitution in the apoB gene generating an in-frame stop codon. These results indicate that this disorder can be caused by defect(s) of either an apoB gene or other genes.

Genetic deficiency disorders of apoBs are divided into two types. One is a complete deficiency of apoBs in which patients lack both apoB-100 and apoB-48 in their plasma(1). The other is normotriglyceridemic ABL, characterized by an isolated apoB-100 deficiency (2). Patients with the latter lack plasma LDL, but chylomicrons are present in plasma after feeding fat. What distinguishes this disorder from previously recognized forms of ABL is the presence of apoB-48, which indicates the ability to absorb fat and to form chylomicrons. The pathogenesis of normotriglyceridemic ABL is of particular interest because both apoB-100 and apoB-48 are generated from a single gene on chromosome 2 by a unique

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ABBREVIATIONS:
ABL, abetalipoproteinemia; apoB, apoBs, apoB-100, and apoB-48, apolipoprotein B, Bs, B-100, and B-48, respectively; LDL, low density lipoprotein; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; VNTR, variable number of tandem repeats.

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mechanism, i. e., RNA editing at codon 2153 of apoB mRNA(3-6). This mechanism is specific to apoB mRNA and implies a particular molecular biological significance. So far three cases with this rare disorder have been reported. The first patient was reported by Malloy et al. in 1981 (2), and they demonstrated later that the patient had a single base substitution on codon 2252 in the apoB gene which gives rise to an in-frame stop codon (7). The second patient with normotriglyceridemic ABL was found in Japan by us (8). Recently her sibling was born and he showed a lack of plasma apoB-100 as well as his sister. This is the first sibling case of this rare disorder and this family enables us to perform a genetic linkage study, which leads us to a different conclusion from that of the first reported case (7). We report here that the two sibling patients have an apparently different apoB gene allele, suggesting that this disorder in our patients is caused by a gene or genes other than the apoB gene.

MATERIALS AND METHODS

Materials : The Y family, which is described in the present article, consists of five members. Father, mother and their son T.Y., enjoy good health. The parents are not of consanguineous marriage. T.Y.'s sister, Y.Y., born in 1981, is the case we reported earlier (8). Another patient, K.Y., is her brother born in 1984, and was referred because of extreme malnutrition up to the age of one month. He was examined at Meijo Hospital as was his sister. Fasting peripheral venous blood samples from each family member were collected, and immediately centrifuged and separated into plasma and blood cells. Plasma samples were subjected to immunoblot analysis, and blood cell samples were used to extract DNA as described below.

Immunoblotting of apoB : Monoclonal antibodies against apoB were generated by a fusion of mouse spleen cells immunized with normal human LDL and a mouse myeloma cell line NS-1 (9). Hybridomas were screened by enzyme immunoassay and immunoblotting. The monoclonal antibodies anti-apoB-1, which recognizes only apoB-100 but not apoB-48, and anti-apoB-2, which recognizes both apoB-100 and apoB-48, were isolated from culture medium by an Affigel Protein A affinity column with a MAPS buffer set (Bio-Rad). The patient's lipoprotein fraction of $d<1.063$ was delipidated, electrophoresed on a 3-10% gradient SDS-PAGE (10), electroblotted onto cellulose nitrate, and subjected to immunological detection using these monoclonal antibodies and peroxidase conjugated rabbit anti-mouse IgG (11).

DNA isolation, digestion, and Southern blotting : Genomic DNA was extracted from peripheral white blood cells as described previously (12). The DNA was digested by the restriction enzyme XbaI, and was separated by electrophoresis on 0.7% agarose gel and transferred to a membrane filter by Southern blotting (13). The DNA probe used was pHBC2-1, which was prepared as described elsewhere (14). They were labeled with [α-^{32}P] dCTP using a Random Primed DNA Labeling Kit (Boehringer Mannheim) according to the manufacturer's instructions, hybridized onto the membrane filters, and were autoradiogramed after washing.

Amplification of DNA : We performed amplification of the 5' end signal peptide region of the apoB gene using a modification of the method originally described by Boerwinkle and Chan (15). The "Del" allele lacks 9 basepairs encoding amino acids -16 to -14 (Leu-Ala-Leu) of the apoB signal peptide, while the "Ins" allele does not. This difference is seen as a polymorphism by amplification of the regions flanking this portion. Two PCR primers were synthesized by DNA synthesizer (Applied Biosystems) : 5'-CAGCTGGCGATG GACCCGCCGA-3' and 5'-TTCGGCCCTGGCGCCCGCCAGCA-3'. The reaction was performed at 97℃ for 30 seconds, and 75℃ for 2 minutes, and repeated for 35 cycles. Amplified DNA was electrophoresed on 10% polyacrylamide gel, and was visualized by ethidium bromide stain. Amplification of the 3' VNTR of the apoB gene was performed by the method described by Boerwinkle et al (16). Primers for amplification were synthesized as described above : 5'-ATGGAAACGGAGAAATTATG-3' and 5'-CCTTCTCACTTGGCAAATAC-3'. The reaction was performed at 95℃ for 1 minute, 56℃ for 1 minute, gradually heated to 72℃ over 3 minutes, and 72℃ for 2 minutes, and repeated for 30 cycles. Amplified DNA was electrophoresed on 5% polyacrylamide gel, and was visualized as described above.

RESULTS AND DISCUSSION

ABL is divided into two types; deficiency of both apoB-100 and apoB-48 (classical ABL), and isolated apoB-100 deficiency with intact apoB-48 in plasma (normotriglyceridemic ABL) (2,17). In studies on classical ABL, several reports have concluded that this disorder is not caused by a defect in the apoB gene itself but arises post-translationally. This conclusion is based on linkage analyses (18,19) and/or immunohistochemical examinations (20,21). On the other hand, some investigators have reported that in patients with classical ABL, apoB was not found in the intestine (22,23). The reason for this discrepancy is not known. At present there are three reported cases described as normotriglyceridemic ABL (2,8,24), but the molecular defect of this disorder has not been well investigated. Recently a point substitution producing an in-frame stop codon at the 2252nd amino acid (nucleotide 6963) was reported by Hardman et al. (7), in a case that Malloy et al. documented first as "normotriglyceridemic abetalipoproteinemia" (2).

In the family described in the present report, two siblings were diagnosed as normotriglyceridemic ABL on the bases of clinical features, lipoprotein determination (8), and immunological characterization of the patients' apoB. As shown in Fig.1, d<1.063 lipoprotein of patient Y.Y. (lane 2) contain normal sized apoB-48 but lack apoB-100, in spite of the fact that her parents have both the apoB-48 and apoB-100 (lane 1). Another patient, K.Y., shows the same abnormalities, but an unaffected sibling, T.Y., is free from the de-

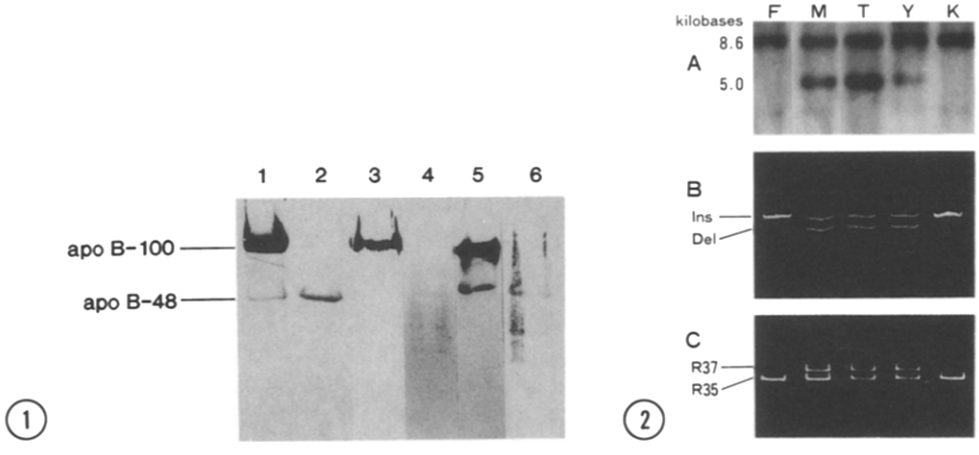

Fig.1. SDS-PAGE and immunoblotting analysis of patient's apoB. Lipoproteins of d <1.063 from the father (lanes 1,3,5) and the patient Y.Y. (lanes 2,4,6) were delipidated and applied to SDS-PAGE (3-10% acrylamide gradient) gel (10). The apolipoproteins were electroblotted onto cellulose nitrate and were subjected to immunological detection using a monoclonal antibody which recognizes only apoB-100 (lanes 3 and 4) or another monoclonal antibody which recognizes both apoB-100 and apoB-48 (lanes 5 and 6).

Fig.2. Polymorphic studies of the apoB gene in the Y family. The genomic DNAs were subjected to endonuclease digestion or to amplification by PCR. Lanes are of the father (F), the mother (M), the unaffected sibling T.Y. (T), the patient Y.Y. (Y), and the other patient K.Y. (K). (A): XbaI RFLP. X1 and X2 genotypes correspond to 8.6 and 5.0 kilobases fragments, respectively (25). Y.Y. had an X1X2 genotype, while K.Y. had an X1X1 genotype. (B) : Polymorphism of the apoB gene signal peptide region. Y.Y. had a Del/Ins genotype and K.Y. had an Ins/Ins genotype. (C) : Polymorphism of the 3' VNTR region. R35 and R37 correspond to DNA containing 35 and 37 tandem repeats, respectively (16). Y.Y. showed an R37/R35 genotype and K.Y. had an R35/R35 genotype.

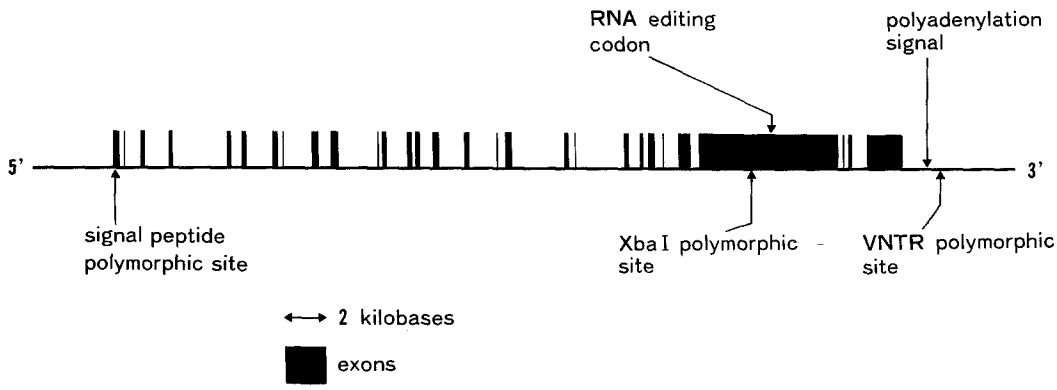

Fig.3. The structure of the apoB gene and the polymorphic sites examined in this study. The apoB gene consists of 29 exons (indicated as vertical bars and squares). Three polymorphic sites used in this study are shown. The apoB-48 RNA editing codon and the polyadenylation signal are also indicated.

fect (data not shown). Monoclonal antibody anti-apoB-1 recognized the father's apoB-100 (lane 3) but did not bind to the patient's apoB-100 (lane 4). Another monoclonal antibody, anti-apoB-2, recognized the father's apoB-100 and apoB-48 (lane 5) and also recognized patient Y.Y.'s apoB-48 (lane 6). These results indicate that the patients' apoB has the same apparent molecular weight and antigenic epitopes as normal apoB-48.

The present linkage study reveals that there is no linkage between the apoB gene and the occurrence of normotriglyceridemic ABL in this Japanese family. We utilized the restriction fragment length polymorphism (RFLP) by XbaI. Fig.2 (A) shows the sizes of the fragments of the apoB gene produced by digestion with XbaI in the family members. The father had only a 8.6 kilobases fragment, indicating an X1X1 genotype, according to the terminology of Hegele et al (25). The mother had both 8.6 and 5.0 kilobase fragments, indicating an X1X2 genotype. Patient Y.Y. showed a X1X2 genotype, while the other patient, K.Y., showed a X1X1 genotype. We also examined two other polymorphic sites of the apoB gene. In the signal peptide coding region which is located in the upstream domain of the apoB gene, patient Y.Y. showed an Ins/Del genotype, while another affected sibling K.Y. showed a homozygous pattern for the Ins allele (Fig.2 (B)). In the polymorphic study of the 3' VNTR region which is located at the most downstream portion of the apoB gene, as shown in Fig.2 (C), patient Y.Y. has the R37 allele from her mother and R35 from her father, while patient K.Y. shows a R35/R35 genotype. Taken together, in all of these three polymorphic studies, the two affected siblings showed different combinations of the inherited apoB gene. As shown in Fig.3, the distance between the signal peptide polymorphic site and the XbaI polymorphic site is less than 36 kilobases, and that between the XbaI polymorphic site and the 3' VNTR region is about only 6 kilobases. The occurrence of genetic recombinations between these marker positions is highly unlikely, based on the frequency of the genetic recombination in human beings (26).

Deduced from these results, we can summarize the apoB gene haplotypes of the family members in the pedigree tree as shown in Fig.4. The two affected siblings, Y.Y. and K.Y., have inherited at least one different allele of the apoB gene from their mother; the alleles designated IV and III, respectively. This result indicates that the occurrence of an isolated

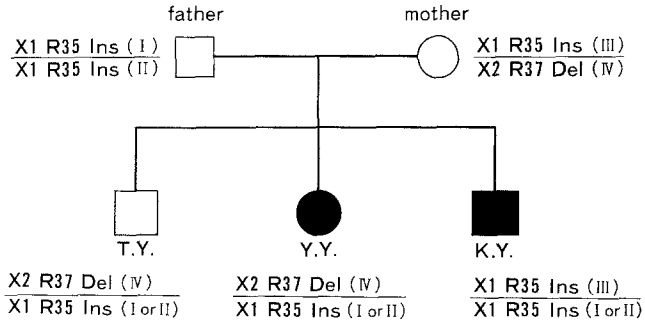

Fig.4. The haplotype analysis of the apoB gene among the members of the Y family. Genotypes of the apoB gene were studied by the combination of XbaI RFLP, polymorphism of the 3' VNTR region and polymorphism of the signal peptide. The father had only an X1/R35/Ins haplotype (I or II). Among the siblings, the X2 allele was always associated with alleles R37 and Del, indicating that the mother had an X1/R35/Ins haplotype (III) and an X2/R37/Del haplotype (IV). Square : male, Circle : female. The solid square and circle indicate the patients.

apoB-100 deficiency in this kindred is not linked to the apoB gene, i.e., the molecular cause of this disorder is not due to an apoB gene mutation.

With regard to patients who have a normal apoB gene, the possible mechanism of the apoB-100 deficiency could be defects in transcription, RNA processing, or translational and/or posttranslational processing. We failed to find evidence of any other protein deficiency in the Y family, suggesting that the general capacity for protein secretion from the liver is intact in this family. We detected a small amount of apoB-100-like protein in these patients' plasma in the very early period of their lives, but it disappeared completely after they were one year old (8). Genetic switching mechanisms in apoB synthesis have been reported in experimental animals (27) and in human beings (28), and another possible explanation could be defects in mechanisms of this sort. Further studies concerning the genetic defect in this family should provide us with important insights into understanding the mechanism of apoB synthesis.

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