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Genetics

A major insertion accounts for a significant proportion of mutations underlying human lipoprotein lipase deficiency

(human genetic disease/structural rearrangements)

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ABSTRACT **Lipoprotein lipase (LPL; triacylglycero-protein acylhydrolase, EC 3.1.1.34) is an important enzyme involved in triacylglycerol metabolism. Primary LPL deficiency is a genetic disorder that is usually manifested by a severe elevation in triacylglycerol levels. We have used a recently isolated LPL cDNA clone to study 15 probands from 11 families with this inherited disorder. Surprisingly, 7 of the probands from 4 families, of different ancestries, had a similar insertion in their LPL gene. In contrast to other human genetic disorders, where insertions are rare causes of mutation, this insertion accounts for a significant proportion of the alleles causing LPL deficiency. Detailed restriction mapping of the insertion revealed that it was unlikely to be a duplication of neighboring DNA and that it was not similar to the consensus sequence of human L1 repetitive elements. This suggests that there must be other mechanisms of insertional mutagenesis in human genetic disease besides transposition of mobile L1 repetitive elements.**

Lipoprotein lipase (LPL; triacylglycero-protein acylhydrolase, EC 3.1.1.34) plays a crucial role in triacylglycerol metabolism. It regulates the distribution of energy in the form of free fatty acids and the transport of cholesterol from triacylglycerol-rich particles to low and high density lipoproteins (LDL and HDL). The clinical syndrome of primary LPL deficiency may be detected in early infancy and presents with colicky pain, hepatosplenomegaly, or failure to thrive. Often it is not recognized until later in childhood with the occurrence of abdominal pain, the development of eruptive xanthomas, the presence of lactescent plasma, or the development of other symptoms and signs of the chylomicronemia syndrome.

This disorder was first described in 1932 in a young male with xanthomata and hepatosplenomegaly (1). In 1960 Havel and Gordon (2) demonstrated defective clearance of triacylglycerol-rich lipoproteins in this disorder related to diminished lipolytic activity in plasma after intravenous administration of heparin. Harlan *et al.* (3) found decreased LPL activity in adipose tissue to be associated with the decrease in post-heparin plasma lipolytic activity in two patients. In 1974 the post-heparin plasma lipolytic activity was separated into LPL and hepatic lipase action (4). Until recently, biochemical studies of LPL deficiency have been solely dependent on measurement of enzyme activity. Recently, however, it has been possible to quantify enzyme mass by using an enzyme-linked immunoabsorbent assay (ELISA) with monoclonal antibodies for LPL (5).

LPL has been purified and characterized with regard to structure. It is a glycoprotein with a molecular mass of about 54 kilodaltons and a carbohydrate content of 3–10% by weight (6). LPL cDNA clones have been obtained from human adipose tissue (7), bovine mammary gland (8), and mouse macrophages (9). The human and mouse clones were obtained by screening cDNA expression libraries with antibodies to bovine milk LPL. The bovine clone was identified by hybridization to a synthetic oligonucleotide whose sequence was based on amino acid sequence data from purified bovine LPL. We recently isolated (S.D.) a human LPL cDNA (HLPL26) by screening a human adipose tissue cDNA library with a bovine LPL cDNA clone (10). This cDNA is 1.36 kilobases (kb) long and corresponds to base pairs (bp) 271–1630 of the LPL cDNA sequence (7). The basic defect underlying human LPL deficiency is not known. The isolation of this and other cDNA clones has allowed studies of the molecular pathology underlying this disorder.

We now report the molecular defects in some patients with severe hypertriglyceridemia caused by LPL deficiency. We have studied 15 probands from 11 unrelated families. The initial proband was found to have inherited two different major structural rearrangements at the LPL locus. It appears that one rearrangement is the result of a deletion and one, the result of an insertion. Surprisingly, 6 of the additional patients from 3 families had a similar insertion in their LPL gene.

METHODS

Subjects. Our initial proband was a 20-year-old man who was diagnosed with LPL deficiency at age 18 months with triacylglycerol levels of 18,000 mg/dl. He was started on a low-fat diet (10% of caloric intake, <20 g of fat per day) with a significant reduction in his triacylglycerol level. He has not had any significant abdominal pain or pancreatitis. He has no eruptive xanthomas or hepatosplenomegaly. His parents are nonconsanguineous, and both are healthy and free of atherosclerotic disease. An additional 14 patients from 10 unrelated families of different ancestries diagnosed with classical LPL deficiency were subsequently studied. These families were ascertained in the Pacific Northwest as well as from colleagues in Canada, the United States, and Europe. Consanguinity was present in three families. We assume that the mutation on both chromosomes in the consanguineous families is identical. Thus, a maximum of 19 mutant alleles for LPL deficiency were available for study.

Measurement of LPL Activity and Mass. Plasma post-heparin LPL activity in all patients was measured 10 min following a heparin bolus (60 units/kg) after an overnight fast, by using a triacylglycerol/phosphatidylcholine emulsion as reported (11). LPL activity was calculated as the activity in whole plasma inhibited by a specific monoclonal antibody added to plasma and is expressed as nmol of fatty acids released per min per ml of plasma. LPL mass released into plasma post-heparin was measured by ELISA (5).

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Abbreviations: LPL, lipoprotein lipase; RFLP, restriction fragment length polymorphism.

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DNA Analysis. DNA was prepared from fresh EDTA-treated blood by a published technique (12). Aliquots (5 μg) of DNA from 12 control subjects, the initial proband, and his mother and father were digested with 12 restriction endonucleases. DNA fragments were electrophoresed through a 0.7% agarose gel and transferred to nylon filters by means of Southern blotting as described (12–14). The human LPL cDNA clone HLPL26 (10) was used for hybridization. The 1.36-kb insert isolated by *Eco*RI digestion of HLPL26 corresponds to bp 271–1630 of the sequence reported by Wion *et al.* (7). More detailed restriction mapping was carried out on DNA from the proband, his parents, and one control by using the restriction endonucleases *Xba* I, *Nco* I, *Eco*RI, *Pst* I, and *Stu* I and subclones of HLPL26.

The 1.36-kb *Eco*RI insert of HLPL26 was divided into a 5′ fragment of 485 bp and a 3′ fragment of 875 bp by digestion with *Ava* I, for which there is a single restriction site at position 756 of the human cDNA sequence (Fig. 1). Both *Eco*RI–*Ava* I fragments were subcloned into the *Eco*RI and *Ava* I sites of the vector pUC19. These two subclones were used separately as probes (probe 485 and probe 875). The 875-bp fragment corresponds to bp 756–1630 of the human cDNA sequence. There are two *Stu* I sites at positions 1034 and 1253 of this cDNA. This restriction enzyme was used to digest the 875-bp fragment and the two most 3′ fragments were used as probes (probe 219 and probe 536). Probe 536 corresponds to the most 3′ end of the coding sequence (Fig. 1).

DNA analysis on 14 additional patients was carried out with the restriction enzymes *Eco*RI, *Pst* I, *Xba* I, *Stu* I, *Bgl* I, *Hin*dIII, *Pvu* II, and *Bam*HI by the method described above. In three families, segregation analysis of the *Hin*dIII, *Bam*HI, and *Pvu* II restriction fragment length polymorphisms (RFLPs) was done to determine which haplotypes segregated with the mutant alleles.

A genomic clone of human LPL was isolated by screening a cosmid library with the LPL cDNA clone. Single and double digestions of this clone with the restriction enzymes *Eco*RI, *Pst* I, *Stu* I, *Xba* I, *Hin*dIII, and *Hin*cII allowed the construction of a partial genomic map.

RESULTS

The initial proband from family 1 had no identifiable activity or mass in either pre-heparin or post-heparin plasma (Fig. 2). Blot hybridization of genomic DNA from the affected person revealed two abnormal restriction fragments when the DNA was digested with *Bgl* I, *Bgl* II, *Bam*HI, *Eco*RI, *Hin*cII, *Hin*dIII, *Pst* I, *Pvu* II, *Nco* I, *Stu* I, *Xmn* I, or *Xba* I and probed with a cDNA probe derived from HLPL26. Blot hybridization of DNA from 12 controls, digested with the same enzymes, detected RFLPs with *Hin*dIII, *Pvu* II, and *Bam*HI as previously reported (15, 16) and confirmed that the abnormal fragments seen in the proband were the result of two distinct rearrangements and not RFLPs. This proves that LPL deficiency is inherited as an autosomal recessive trait.

For example, *Pst* I-digested DNA from a normal control subject revealed fragments of 11, 6, and 2.5 kb, whereas the

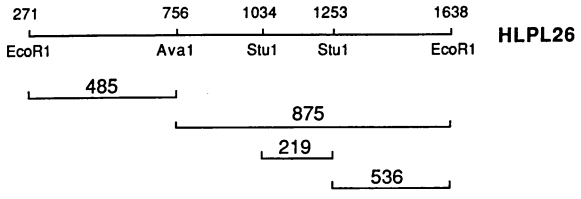

FIG. 1. LPL cDNA clone and subclones used as probes. Numbers above the top line correspond to the position of the restriction enzyme recognition sites in the published LPL cDNA sequence (7); 485, 875, 219, and 536 are the names assigned to the subclones.

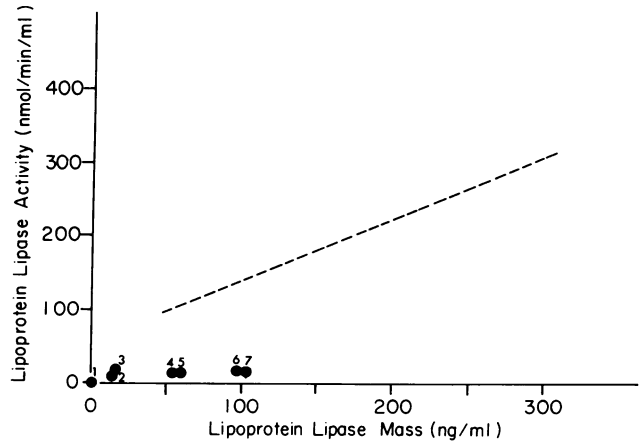

FIG. 2. Post-heparin plasma LPL activity was essentially absent in proband 1 (13 nmol of fatty acids released per min per ml) from family 1, probands 2 and 3 from family 2, probands 4 and 5 from family 3, and probands 6 and 7 from family 4. Values in 34 normolipidemic control subjects are shown by the dashed line. A similar lack of LPL mass was seen in proband 1 (0 ng/ml). In contrast, in probands 2–7 from families 2, 3, and 4 there was identifiable LPL protein in post-heparin plasma but it was catalytically defective.

proband DNA was missing the 6-kb fragment and had, in addition to the 11- and 2.5-kb fragments, fragments of 8 and 12 kb. The proband's parents had the 3 bands seen in the control, and in addition a 12-kb band was seen in the mother's DNA and an 8-kb band in the father's (Fig. 3). The absence of the 6-kb band in the proband implied that this DNA fragment was involved in both rearrangements.

Further mapping using a 5′ (probe 485) and a 3′ (probe 875) subclone of HLPL26 revealed that the normal 11-kb fragment hybridized only with the 5′ subclone and the normal 6-kb fragment hybridized very weakly to the 5′ probe and strongly to the 3′ probe. The detection of the mutant 12-kb band with both probe 485 and probe 875 indicates that this mutant gene is the result of a deletion with loss of at least one *Pst* I site. The mutant 8-kb band hybridized well with probe 875 but gave a very faint signal after hybridization with probe 485 (Fig. 3). The intensity of the 8-kb band after hybridization with probe 485 or 875 was similar to the intensity of the 6-kb band after hybridization with the same probe. This suggests that this mutant gene is likely the result of an insertion in the 6-kb fragment.

To test these hypotheses, restriction mapping using different enzymes, including *Nco*I (which has only one recognition site within the LPL cDNA) and *Stu* I, was performed (Fig. 4). *Nco* I-digested control DNA gave a signal at ≈26 kb when hybridized with the HLPL26 cDNA probe. This band was replaced in the proband by two mutant bands at ≈20 and ≈28 kb. The mother and father had the normal 26-kb band, and in addition the 20-kb band was seen in the mother as a result of a 6-kb deletion and the 28-kb band was seen in the father as the result of a 2-kb insertion (Fig. 4). *Stu* I-digested DNA from control subjects gave bands at 5.6, 3.8, 2.9, 2.2, and 1.6 kb when hybridized with the HLPL26 cDNA probe. DNA from the proband and his father had an additional 4.2-kb band. The mother's DNA gave bands identical to the control (Fig. 5*A*). When the same filter was stripped and rehybridized with probe 485, the 5.6-kb and 2.9-kb bands were seen in the control and the father and an additional 2.2-kb band was seen in the proband and his mother (Fig. 5*A*). Restripping and hybridization of the filter with probe 875 revealed bands at 3.8, 2.2, and 1.6 kb as well as a faint band at 2.9 kb. An additional 4.2-kb band was detected in the proband and his father (Fig. 5*A*). Restripping and hybridization with probe 219 revealed a band at 2.2 kb in the control and the proband's

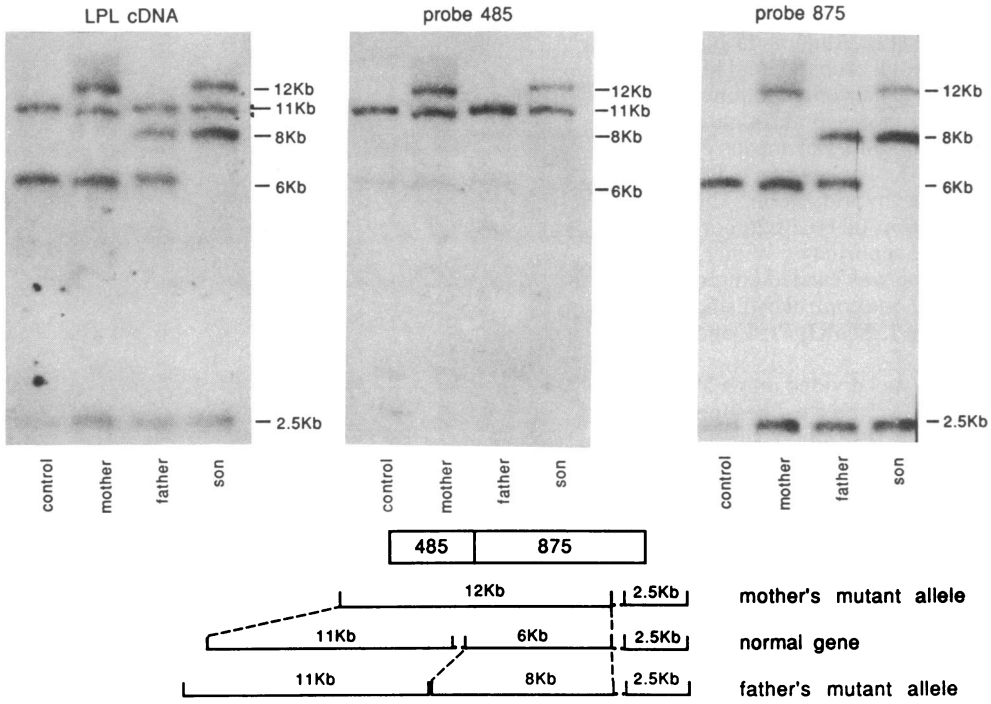

FIG. 3. Southern blot hybridization of *Pst* I digest of control DNA and DNA from mother, father, and proband (son) with the 1.36-kb LPL cDNA insert of HLPL26. After stripping, the filter was hybridized with probe 485 (bp 271–756 of the LPL cDNA) and then with probe 875 (bp 756–1630).

mother, whereas the proband and his father had bands at 2.2 and 4.2 kb. Probe 536 hybridized only with a 3.8-kb band in all four subjects (Fig. 5*B*). Since probes 219 and 536 were isolated by *Stu* I digestion of the 875 insert, these findings show that no *Stu* I sites were lost in the father's mutation, and the abnormal fragment can only be explained by a 2-kb insertion involving the 2.2-kb fragment. These experiments show that the mother's mutation is probably the result of a 6-kb deletion and the father's mutation, the result of a 2-kb insertion. This conclusion is supported by the fact that with five additional enzymes (*Bgl* I, *Eco*RI, *Pvu* II, *Xba* I, and *Xmn* I), the father's mutant band was always 2 kb bigger than the normal band that hybridizes with probe 219.

Detailed restriction mapping of the genomic clone of human LPL revealed that within the 2.2-kb *Stu* I fragment, there are no *Pst* I or *Eco*RI sites but there is one *Hin*dIII and one *Hin*cII site. Southern blot hybridization of *Hin*dIII/*Hin*cII double digests of DNA from the proband showed that the mutant band inherited from the father is 1.4 kb long. If the duplication involved 2 kb of DNA normally found between the two *Stu* I sites, then, in addition to the 1.4-kb mutant band, one would expect to see another mutant band at ≈0.6 kb to account for 2 kb of additional DNA. The absence of such a band indicates that there is no faithful replication of the restriction map of neighboring DNA in the insertion and that this is more likely due to transposition of DNA from a distinct location.

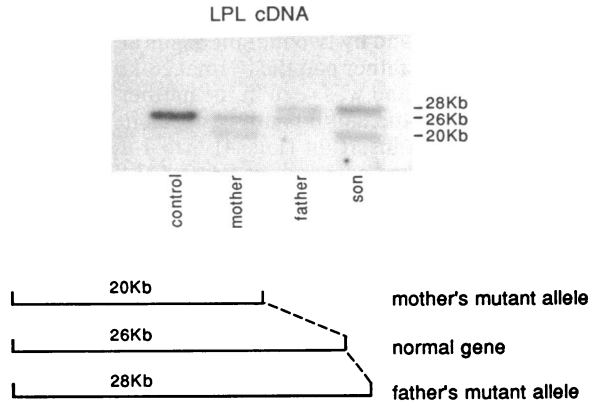

FIG. 4. Southern blot hybridization of *Nco* I digest of control DNA and DNA from mother, father, and proband with the 1.36-kb LPL cDNA insert of HLPL26.

A detailed restriction map of the published L1 consensus sequence (17) was obtained and analyzed with the computer program SEQUENCE (courtesy of A. Delaney, University of British Columbia, Vancouver). Within the 6161 bp there is no sequence of 2 kb free of *Stu* I, *Eco*RI, and *Pst* I recognition sites that has at least one *Hin*dIII and one *Hin*cII site. Therefore an unmodified L1 sequence is unlikely to be the inserted sequence.

DNA analysis of an additional 14 patients from 10 families revealed that three pairs of affected siblings had an insertion similar to that described in the initial proband based on detailed restriction mapping with eight different enzymes. Two examples of this analysis are shown in Fig. 6. Hybridization of *Xba* I-digested DNA from a control subject, from the proband of family 1, and from probands 2, 4, and 6 (of families 2, 3, and 4) with HLPL26 revealed that the proband from family 1 is missing the 10-kb band seen in the control but has, in addition to the normal 13.5-kb band, bands at 12 and 17 kb. The mutant 12-kb band was also seen in probands 2, 4, and 6 in addition to the normal 10- and 13.5-kb bands. When DNA from the same individuals was digested with *Eco*RI, the mutant 5.7-kb band was seen in all affected patients. A similar pattern was seen in all seven probands when DNA was digested with six other enzymes. To examine whether this insertion represented a normal variant, an additional 300 normolipidemic controls and 75 hypertriglyceridemic patients were analyzed; these samples failed to reveal the band pattern seen in these probands.

The initial proband with the insertion in one LPL allele and deletion in the other has no identifiable LPL protein. Studies of post-heparin plasma from the 6 patients of families 2, 3, and 4 revealed that the insertion in one gene, which is likely to have resulted in a null allele with a grossly normal homologous gene, has resulted in identifiable LPL that is catalytically defective (Fig. 2).

Segregation analysis of three of the four families showed that the mutant allele with the insertion cosegregates with the 6-kb *Hin*dIII, 4.9- and 2.7-kb *Pvu* II, and 33-kb *Bam*HI

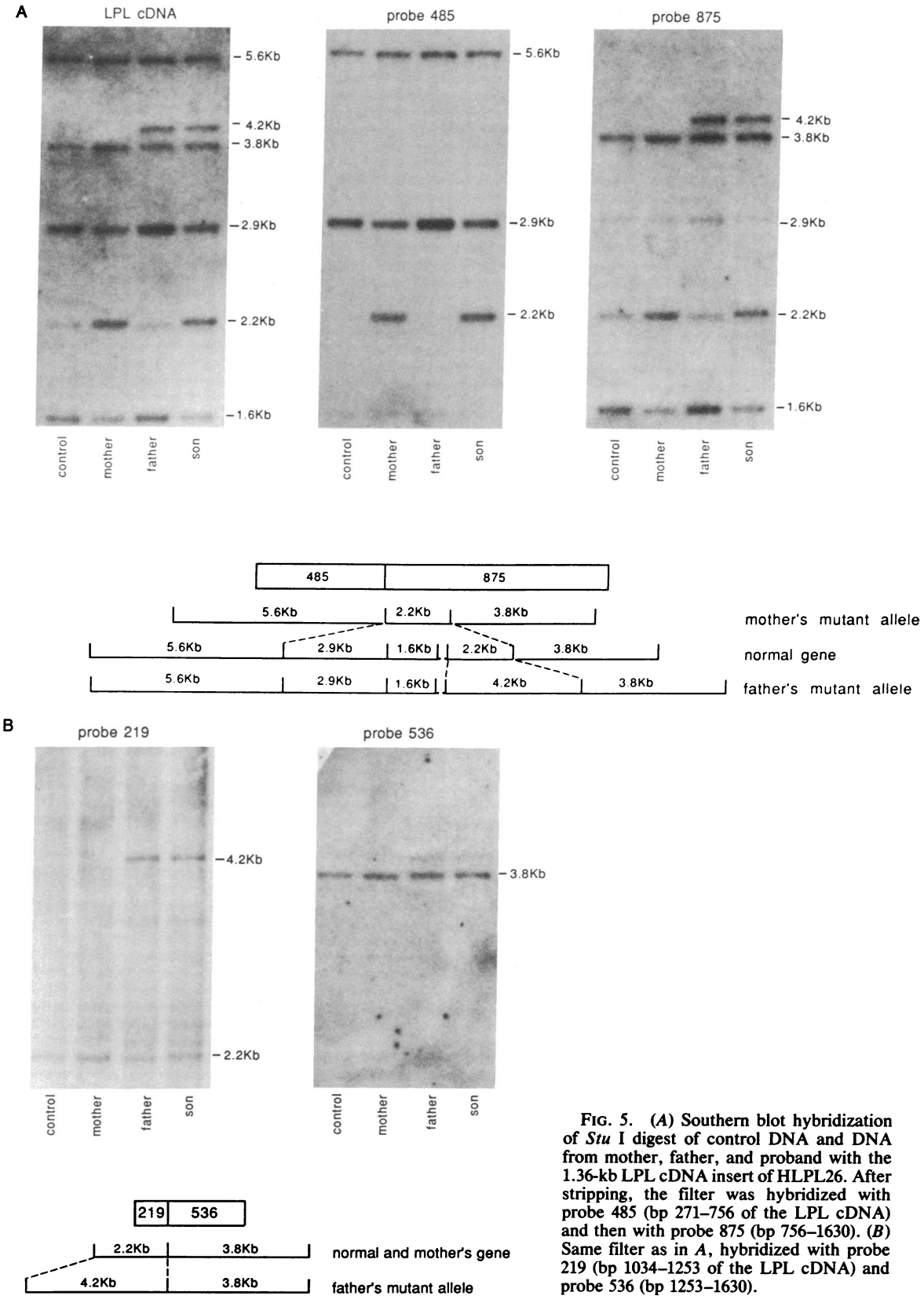

FIG. 5. (*A*) Southern blot hybridization of *Stu* I digest of control DNA and DNA from mother, father, and proband with the 1.36-kb LPL cDNA insert of HLPL26. After stripping, the filter was hybridized with probe 485 (bp 271–756 of the LPL cDNA) and then with probe 875 (bp 756–1630). (*B*) Same filter as in *A*, hybridized with probe 219 (bp 1034–1253 of the LPL cDNA) and probe 536 (bp 1253–1630).

polymorphic fragments in two of the three families. This haplotype is also present in the affected siblings of the third family studied, but the origin of each haplotype cannot be definitively established due to the unavailability of a crucial relative for studies. This is suggestive of a common single origin for this mutation.

DISCUSSION

This study demonstrates defects in the LPL gene as a cause of hypertriglyceridemia and LPL deficiency. Of the 19 LPL alleles studied, 4 represented a similar insertion, whereas 1 constituted a deletion. Therefore in this series of patients

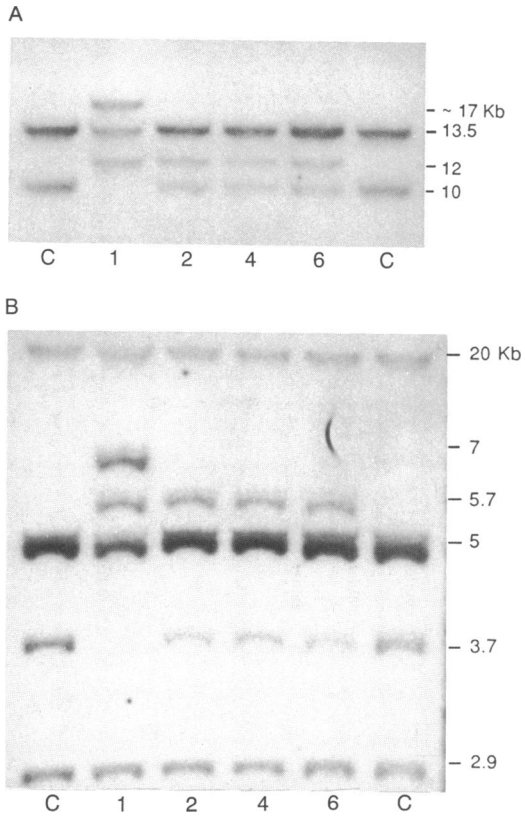

FIG. 6. Southern blot hybridization of *Xba* I (*A*) and *Eco*RI (*B*) digests of control DNA (lanes c) and DNA from proband 1 (family 1), proband 2 (family 2), proband 4 (family 3), and proband 6 (family 4) with the 1.36-kb LPL cDNA probe.

with LPL deficiency, major rearrangements account for ≈25% of the mutations in the LPL gene, with a single unique insertion being the most common. These results clearly illustrate that molecular heterogeneity underlies the biochemical heterogeneity of LPL deficiency.

Major structural rearrangements are a frequent cause of mutagenesis in the human genome and account for a significant number of the mutations in the low density lipoprotein receptor (18), hypoxanthine (guanine) phosphoribosyltransferase (19), factor VIII (20), and Duchenne muscular dystrophy (21, 22) genes. In almost all instances these major rearrangements are caused by deletion of DNA.

Genomic insertions produced by mobile elements account for a significant proportion of spontaneous mutations in yeast (23) and *Drosophila* (24). However, major insertional events are rare causes of mutagenesis in the human genome. Recently, two different sporadic insertions in the hemophilia A (factor VIII) gene have been described (25). However, in contrast to those described in the factor VIII gene, the insertions we detected in the LPL gene are inherited, appear similar to one another, and in this series of patients account for ≈25% of the mutations studied that cause LPL deficiency.

The ancestral backgrounds of these four families are different, representing Irish, English, French, German, and Polish descent for many generations. There is certainly no apparent genetic relationship among these families. Whether this insertional event can be explained by a single origin that occurred in the distant past or whether it relates to multicentric origins has been examined by analysis of RFLPs within the LPL gene in three of the four families. The results clearly show a similar DNA haplotype around the insertion, which is compatible with a single origin for this mutation. The disparate ancestries of these patients suggest that this must be a mutation that occurred many centuries ago. The classic example of a single origin of a mutation accounting for the vast majority of cases of a genetic disease is α_1-antitrypsin deficiency. However, no common insertional event has yet been described that accounts for a significant proportion of the mutations underlying any human genetic disorder. The restriction map of the additional DNA is not similar to neighboring DNA, suggesting that duplication is an unlikely cause of this rearrangement.

Why this gene was involved in a single insertional event while other genes are more likely to have deletions as a cause for mutagenesis is uncertain. It would appear that mechanisms for both these mutational events differ. Deletions in the low density lipoprotein receptor gene are often due to *Alu–Alu* recombination events (26), whereas the insertions characterized at the molecular level are due to insertions of L1 sequences (25). Detailed restriction mapping of the LPL insertion with seven enzymes has revealed that it does not bear similarity with the consensus sequence of human L1 elements (17). Significant modification of a mobile transposed L1 sequence cannot be excluded. The absence of similarity between the restriction maps of the inserted fragment and the L1 consensus sequence strongly suggests that there are other mechanisms of insertional mutagenesis in human genetic disease besides transposition of mobile L1 elements.

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