Homozygous deletion of exon 9 causes lipoprotein lipase deficiency: possible intron-Alu recombination

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Abstract We studied a homozygous deletion in the lipoprotein lipase gene at the molecular level. Comprising the end of intron 8, the whole of exon 9, and about two-thirds of intron 9, this 2.136-kb deletion caused complete lipoprotein lipase deficiency and severe hypertriglyceridemia (type I hyperlipoproteinemia). Intron 9 of a normal control subject was also sequenced in order to define the exact borders of the deletion. Up to now, only the first 0.721 kb of intron 9 had been sequenced. Thus the complete sequence of intron 9 (3.090 kb) is now available. Three Alu sequences were characterized in the normal intron 9, while the proband had only the third complete Alu sequence. The first Alu sequence was located in the deleted region, and only the left arm of the second was present, as the deletion began near its center. A stem-loop structure involving a 14-nt region towards the end of intron 8 and an Alu sequence in intron 9 might have led to the deletion. Sequence analysis showed that the three Alu sequences belonged to the 40-million-year-old Alu-Sa subclass. **—Benlian, P., J. Etienne, J-L. de Gennes, L. Noé, D. Brault, A. Raisonnier, F. Arnault, J. Hamelin, L. Foubert, J-C. Chuat, C. Tsé, and G. Galibert.** Homozygous deletion of exon 9 causes lipoprotein lipase deficiency: possible intron-Alu recombination. *J. Lipid Res.* 1995. **36:** 356–366.

Supplementary key words type I hyperlipoproteinemia • Alu sequence • stem-loop • polymorphism • normal intron 9 sequence

Lipoprotein lipase (LPL) is an enzyme that plays a major role in the metabolism of circulating triglyceride-rich lipoprotein. It hydrolyzes chylomicron and VLDL triglycerides, thereby delivering fatty acids to tissues for storage or oxidation. The first nine exons of the LPL gene encode the LPL protein, while exon 10 corresponds to the entire 3' noncoding sequence (1, 2). Point mutations, leading to amino acid substitutions in essential domains of the protein, are the principal known alterations of this gene.

We report the molecular characterization of a novel defect (3) in the human LPL gene. It involves a 2.136-kb deletion spanning the 3' end of intron 8, the whole of exon 9, and about two-thirds of intron 9. This homozygous mutation caused LPL deficiency and severe hypertriglyceridemia (type I hyperlipoproteinemia). Moreover, as intron 9 had only been partially sequenced (4), we determined its entire nucleotide sequence in order to define the exact borders of the deletion.

MATERIALS AND METHODS

PCR

Genomic DNA was isolated by standard methods from peripheral blood leukocytes. PCR amplification of proband DNA was carried out in a 100-μl reaction mixture containing 30 pM primer, 500 ng of template, and 1.5 mM $MgCl_2$. The sample was denatured at 94°C (5–7 min), followed by 35 cycles at 94°C (1 min denaturation), 50°C (1 min annealing), 72°C (1 min extension), and a final extension step of 7 min.

PCR of normal control DNA was carried out in a 100-μl reaction mix containing 50 pM primer, 125 ng of template, and 2.5 mM $MgCl_2$. The sample was denatured at 94°C (10 min), followed by 30 cycles at 94°C (1 min denaturation), 52°C (1 min annealing), 72°C (2 min extension), and a final extension step of 8 min.

Sequencing

PCR products from the proband ($P1_A$, $P1_B$) and from two normal control subjects (C1, C2) were isolated in a 1% agarose LMP gel, eluted, purified by resins (Promega kit), precipitated with ethanol, and restricted with appropriate enzymes. After final purification, the DNA was

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; VLDL, very low density lipoprotein; nt, nucleotide; aa, amino acid; PCR, polymerase chain reaction; kb, kilobase; bp, base pair.

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inserted into the pUC18 vector at the Bam HI and PstI sites (proband) or the Bam HI and Hind III sites (control). Sequencing was carried out in both directions by means of the technique of Sanger, Nicklen, and Coulson (5). The oligonucleotides used for sequencing are listed in **Table 1**, and the sequencing strategy is given in Fig. 3.

RESULTS

Exon 9 deletion of the LPL gene

Analysis of genomic DNA from the propositus, based on the LPL restriction map (2), revealed an abnormal Southern restriction pattern (with eight different enzymes), suggesting that there was a homozygous deletion encompassing exon 9 of the LPL gene (3). We thus submitted the region between the middle of intron 8 and the beginning of exon 10 to PCR amplification. Two fragments, P1A and P1B (**Fig. 1**), were prepared.

Blot hybridization (**Fig. 2**) of the proband PCR products revealed an abnormally sized product, suggesting a deletion of about 2.1 kb. Sequencing (**Fig. 3** and **Fig. 4**) showed that the deletion spanned exactly 2.136 kb. The deletion observed in the two separate proband clones (P1A and P1B) began at nt 910 of intron 8. As shown in Figs. 1 and 4, this deletion included the end of intron 8 (0.120 kb), the whole of exon 9 (0.105 kb), ended at nt 1911 of intron 9 (1.911 kb).

As we have previously sequenced the first 0.721 kb of intron 9 (Co in Fig. 1) (4), we only had to amplify the domain starting several nucleotides upstream of nt 721 of intron 9 and ending at the beginning of exon 10 to determine the rest of the sequence. Accordingly, two separate clones, C1 and C2, were prepared from two normal control subjects. Their nucleotide sequences were identical.

It can be observed (**Fig. 5**) that in the normal control subject there are inverted repeats that can be base-paired over 14 nt. These repeats were situated in introns 8 (913–927) and 9 (1893–1911). The inverted repeat in intron 9 which base-pairs with intron 8 belongs to an Alu sequence (see below).

Proband is homozygous for the deletion

The proband is a 27-year-old French man who, since the age of 9 years, has had attacks of acute or subacute pancreatitis associated with eruptive xanthomatosis, and hepatosplenomegaly. Episodic free dieting was marked by an elevation of triglyceride levels (5200 mg/dl) in keeping with elevated chylomicron levels in fasting plasma. There was no LPL activity in postheparin plasma

TABLE 1. Synthetic oligonucleotides used

A/For PCR

Y_a = CTG *GGA TCC* ATC TCA ACC TGT CTC CGC $A^{421(I.8)}$
Y_b = GTC *GGA TCC* AGA TGC TAA GAG ATG GCA $GA^{738(I.8)}$
N1 = GTC *GGA TCC* CCT TTA GGG CTA ATC CAT $G^{636(I.9)}$
U = TTT TTG TTT AGG CCT GAA GTT $TC^{(17.E8)}$
W = TCA CAT CCA TTT TCT TCC $AC^{1027(I.8)}$
Z = GGG *CTG CAG* TTC TTT GTT CTG TAG ATT $CG^{9(E.10)}$
N4 = GCG *CTG CAG* TTC TTT GTT CTG TAG ATT $CG^{9(E.10)}$
V = GGG GTC TAA AGT GAA GGA $AGA^{18(I.8)}$
X = CCA GTC AGC TTT AGC $CCA^{11(I.9)}$

B/For sequencing

$OL._P$:
PBR = (see $OL._C$)
S1 = ^{579}GTC ATT TGC TTG TAT CAC $C^{597(I.8)}$
S3 = ^{797}CTG CAT GCC TGT CTA TCT $AA^{816(I.8)}$
S5, S7, S9, S11 (See "$OL._C$")
PBU,A2, A4, A6, A8, A10 (See $OL._C$)
A12 = ^{717}CCA TCT CTT AGC ATC $TGC^{734(I.8)}$

$OL._C$:
PBR = TTA TGC TTC CGG CTC GTA TG
S31 = ^{737}TTC AAC CCT CCA CAC $ATC^{754(I.9)}$
S33 = ^{1039}CGA AAC CAC CTC TCT $ACT^{1056(I.9)}$
S35 = ^{1261}CCC TAT GTG TAA CAT $CTT^{1278(I.9)}$
S37 = ^{1381}ACT CAG AGA CAA CCC $CAA^{1398(I.9)}$
S39 = ^{1582}TGA GCC AGG GAA AAT $AAC^{1599(I.9)}$
S41 = ^{1756}CCA GTT TTG CTC TTG $CTG^{1773(I.9)}$
S5 = ^{2085}TCT GCC AGA GCA TCT ACA $AC^{2104(I.9)}$
S7 = ^{2357}CTA GAA TGT GGT GGC GTG $AT^{2376(I.9)}$
S9 = ^{2632}CCT GAA CTT TAT CTA GGA $GG^{2651(I.9)}$
S11 = ^{2851}CCT CCA AAA ATG ATG CAC $CT^{2870(I.9)}$
PBU = TCC GCT ATT ACG CCA GCT G
A2 = ^{3010}GGA TGG AAA AAG CCT $TCA^{2993(I.9)}$
A4 = ^{2790}GGC CTG TTC CAT TTC ATT $TC^{2771(I.9)}$
A6 = ^{2530}AAT GAC CAG CCT GGC CAA $TA^{2511(I.9)}$
A8 = ^{2269}CTG TTC TGT CAG TAA TCC $TA^{2250(I.9)}$
A10 = ^{2047}TAA AAC TGC ATC TAG $GGC^{2030(I.9)}$
A30 = ^{1815}GTT GCA GTG AGC TGA $GAT^{1798(I.8)}$
A32 = ^{1590}CCT GGC TCA ATA CCA $CTA^{1573(I.8)}$
A34 = ^{1382}GTG ATA TGG TCA CAG $TGA^{1365(I.8)}$
A36 = ^{1115}TGC CGA GTA GCT GGG $ATT^{1098(I.8)}$
A38 = ^{1056}AGT AGA GAG GTG GTT $TCG^{1039(I.8)}$
A40 = ^{790}TTC CTG TAA CCC ATT $AGC^{778(I.8)}$

Oligonucleotides are shown 5' to 3'. The left-hand column corresponds to the sense sequence, and the right-hand column to the antisense sequence. Numbering is as in Fig. 4. $OL._P$: oligonucleotides used for proband sequencing. $OL._C$: oligonucleotides used for control sequencing. Restriction sites introduced to facilitate the cloning of PCR products are shown in italics (N4 differs from Z by the restriction site. As the HindIII site is more convenient than the PstI site for hydrolysis buffer conditions, N4 was subsequently used instead of Z). I.7 = Intron 7, I.8 = Intron 8, I.9 = Intron 9, E.8 = Exon 8, E.10 = Exon 10.

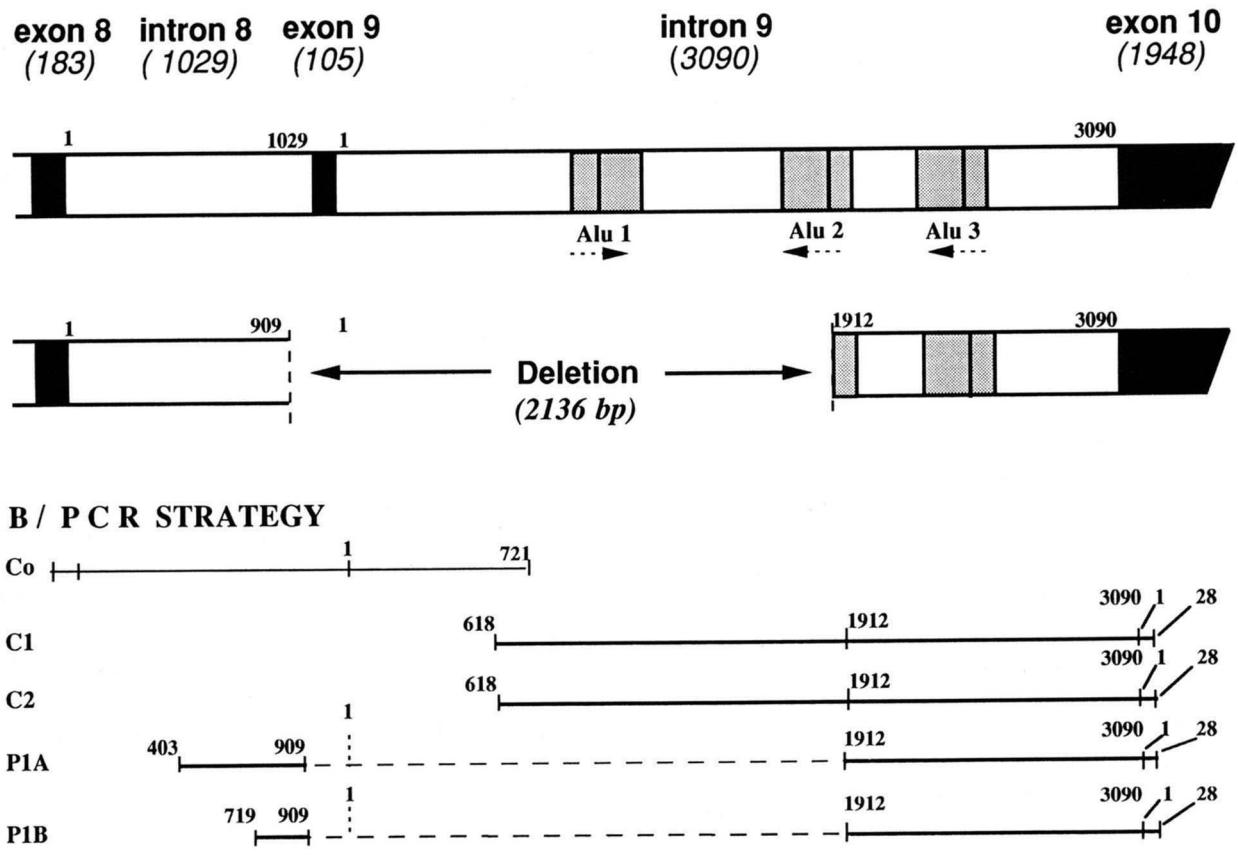

Fig. 1. Schematic representation of the proband LPL gene deletion. (A) 3' normal LPL gene is shown at the top of the figure. The length (in nucleotides) of the exons (solid segments) and introns (open segments) is given in parentheses. The orientations of the three Alu repeats in intron 9 are indicated by short arrows (for numbering see Fig. 4). The propositus 3' gene with the observed deletion of 2136 bp is shown beneath the normal 3' gene. The deletion includes the end of intron 8 (120 nt, from nt 910 to 1029), the whole of exon 9 (105 nt), and two-thirds of intron 9 (1911 nt). (B) C0, C1, C2 are three normal control subjects. C0, clone obtained from a genomic library that we had previously sequenced up to nt 721 of intron 9 (4); C1 and C2, PCR clones obtained in this work (from nt 618 of intron 9 to nt 28 of exon 10); $P1_A$ and $P1_B$, the two proband PCR clones, from nt 403 ($P1_A$), or 719 ($P1_B$), of intron 8, to nt 28 of exon 10. The exons and introns are numbered individually as in Fig. 4.

(3) and the LPL protein mass was absent from both pre- and postheparin plasma, a phenomenon that corresponds to class I in Brunzell's classification (6). ApoC-II and hepatic lipase activity were normal (3).

The proband recently had a female child; her leukocyte DNA had all the features of heterozygosity, with a normal exon 9 (like the mother) and a deletion of exon 9 plus the end of intron 8 and two-thirds of intron 9 (like the proband). The proband exhibits only the deletion (**Fig. 6**).

Identification of Alu sequences in intron 9

Nucleotide sequencing of DNA from a normal control subject had to be done to define the limits of the deletion. This enabled us to identify three Alu sequences in intron 9 (Figs. 1 and 4). The first (nt 952 to 1231) is inserted in the direct orientation, while the second (nt 1711 to 2046) and the third (nt 2291 to 2616) are oriented in the opposite direction. The first Alu sequence was not found in the proband, as it was located in the deleted region. Half of the second Alu sequence was deleted; one nucleotide of the linker and the whole left arm remained. Only the third Alu sequence was complete. The direct orientation of the three Alu sequences in intron 9 of the normal control subject can be aligned with the consensus sequence established from 125 known Alu sequences by Jurka and Smith (7) (**Fig. 7**).

Being transposed into the genome at different stages of primate evolution, Alu sequences can be used as paleogenetic markers. The sequence to be dated must first be placed in one of the two Alu subfamilies (7–10), Alu-J (Jurka) and Alu-S (Smith). This classification is based on different base preferences for a number of diagnostic sequence positions. By following the procedure proposed by Jurka and Smith (7) and described in **Fig. 8** (top panel),

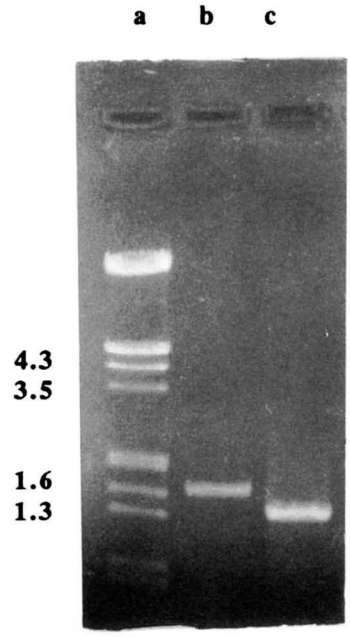

Fig. 2. Agarose gel electrophoresis (1%) of proband PCR products; a = ladder (4.3, 3.5, 1.6, 1.3 kb); b = $P1_A$ = PCR product obtained with primers Ya and Z (expected size: 3.832 kb, observed size: about 1.7 kb); c = $P1_B$ = PCR product obtained with primers Yb and Z (expected size: 3.516 kb, observed size about 1.4 kb). The presumed ~2.1 kb deletion was confirmed by sequencing (see Fig. 4). The primers used for PCR are listed in Table 1.

we found that the Alu repeats belonged to the Alu-S subfamily, which itself is divided into three subclasses, Alu-Sa, Alu-Sb, and Alu-Sc (7). By using another set of diagnostic positions, also shown in Fig. 8 (low panel), the Alu sequences were found to belong to the Alu-Sa subclass.

DISCUSSION

Various missense point mutations underlying human LPL deficiencies have been reported in recent years (11). They seem to be preferentially situated in exons 4, 5, and 6, which code for essential domains of LPL. A deletion has been described in a compound heterozygote who inherited a 6-kb deletion from his mother and a 2-kb insertion from his father (12). Recently, Ma et al. (13) reported a 4-bp deletion in exon 4 resulting in a frameshift mutation expected to produce a truncated protein. The proband and his affected brother were heterozygous for this deletion and also for a partial duplication of exon 6. Two other members of this family, who were heterozygous for the 4-bp deletion, were healthy.

The homozygous LPL molecular defect reported here consists of a deletion of the end of intron 8, the whole of exon 9, and about two-thirds of intron 9. This is the first example of such a deletion. Sequence analysis of the deletion breakpoints revealed that a stretch of 14 oppositely oriented nucleotides flanked the gene rearrangement, one

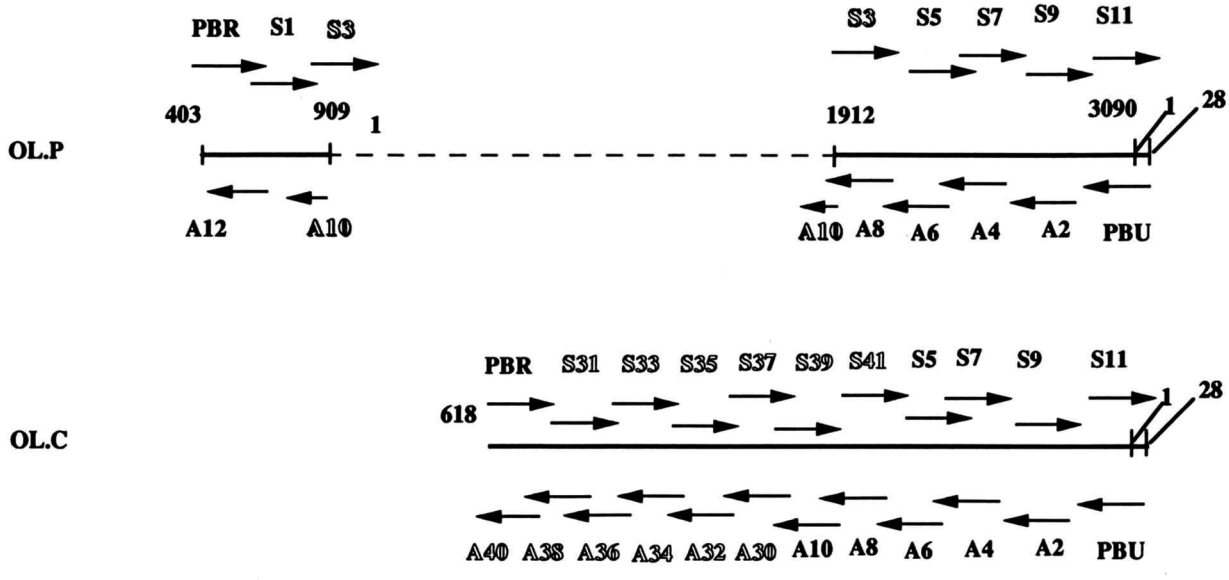

Fig. 3. Sequencing strategy. The sequences of the oligonucleotides used for sequencing are given in Table 1; OL.P = oligonucleotides used for proband sequencing; OL.C = oligonucleotides used for control sequencing.

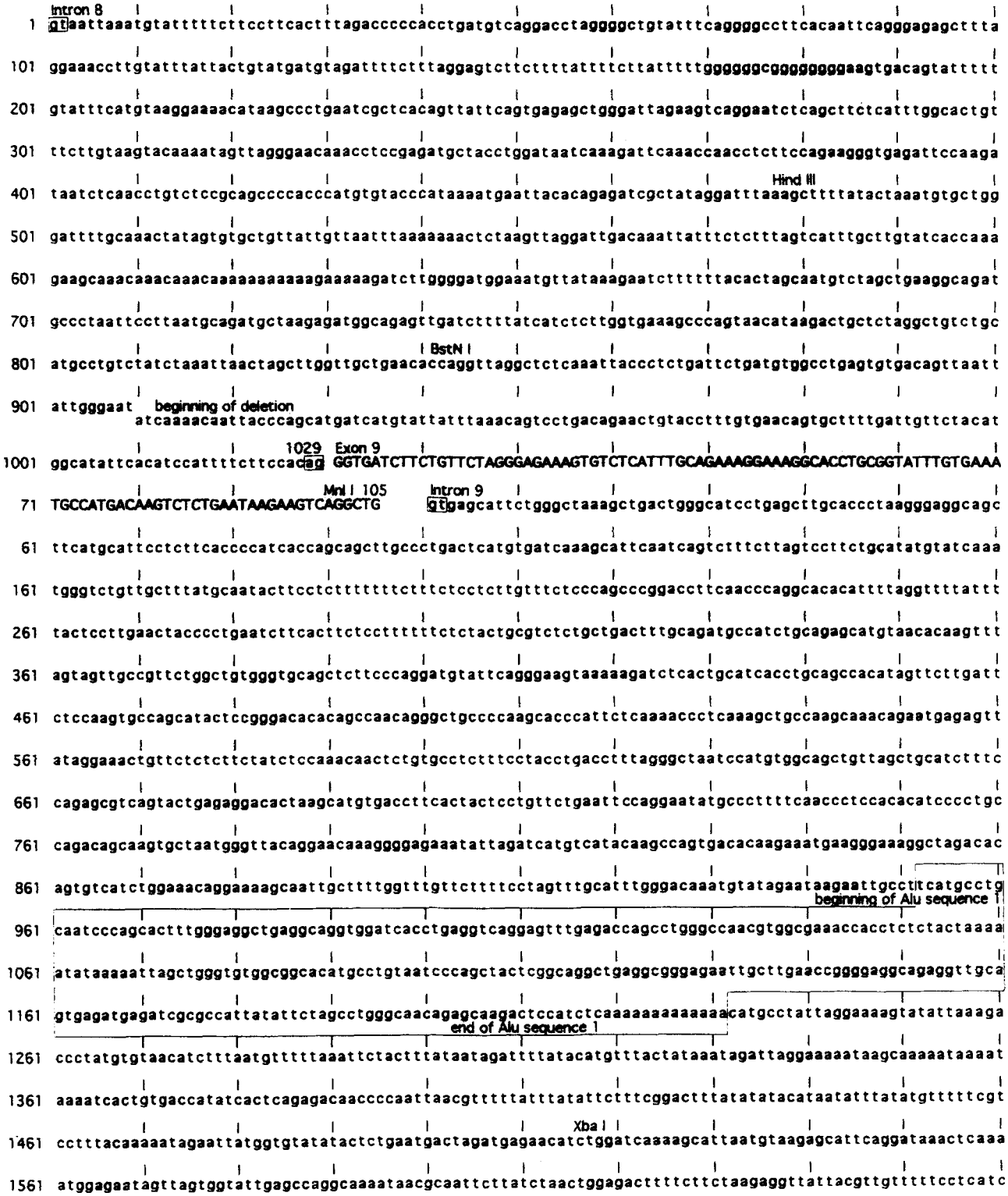

Fig. 4. The 4435 nt sequence from the 5' region of LPL intron 8 to the 5' region of exon 10, and the proband deletion (GenBank/EMBL accession No. Z46943). The nucleotide sequence is a composite of overlapping clones from three normal control subjects: C_0 (our previous results (4), and C1, C2 (present results)). The borders of the deletion (nt 910 of intron 8 and nt 1911 of intron 9) were determined after sequencing two separate proband PCR clones. The deletion (2136 nt) thus includes the end of intron 8 (120 nt out of 1029), the whole of exon 9 (105 nt) and two-thirds of intron 9 (1911 nt out of 3090 nt). Three Alu sequences were identified in intron 9. The first is in the direct orientation, while the second and third are in the opposite orientation (the second and third are complete, while the first lacks the first 16 nt, see Fig. 7). For the meaning of C_0, C_1, C_2, $P1_A$, $P1_B$, refer to Fig. 1 legend. For clarity, a gap has been artificially introduced between the end of exon 9 and the beginning of intron 9, and between the end of intron 9 and the beginning of exon 10. The restriction sites indicated above the sequence are polymorphic.

inside intron 8, the other inside the second Alu repeat of intron 9. Alu repeats have sometimes been involved in homologous recombination, resulting in deletion of part of an exon or intron. The best-known examples are those where the deletion is bracketed by two Alu sequences, as in the LDL receptor gene defect reported by Lehrman et al. (14). In other cases, the recombination involves only one Alu sequence with an exon (15) or an intergenic sequence (16). In another example, the gene rearrangement does not involve an Alu sequence itself but the repeat flanking the Alu sequence (12, 17). In this case, which affects the LPL gene, recombination (between the repeat flanking the intron 6 Alu, and exon 6) resulted in a partial duplication (~2kb) of exon 6 and intron 6.

At a given point of DNA replication, after the DNA strands separated, a stem-loop structure, with base-pairing of 14 nt, may have formed between the sequence in intron 8 and the oppositely oriented second Alu sequence in intron 9. A break may then have occurred, such that intron 8 was cleaved at position 909–910 and intron 9 at position 1911–1912. This would have been followed by ligation of the two DNA fragments. The 2.136-kb DNA sequence between these cleavage points may have dissociated or been destroyed by nucleases. The question that arises is why this recombination would lead to a deletion in very few subjects.

Interestingly, of the 2136 nt deleted from the proband LPL gene, only 105 nt would have been coding. These

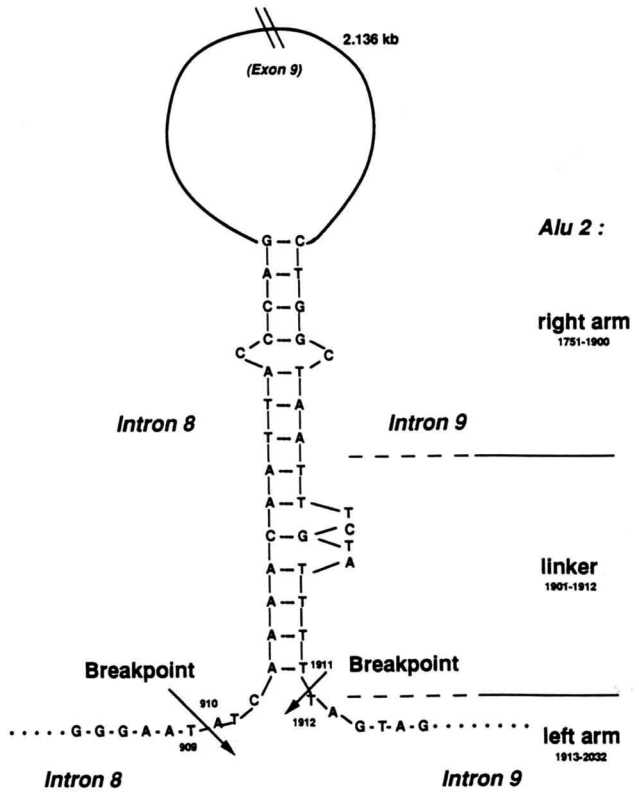

Fig. 5. Possible secondary structure in the region of the breakpoints (intron 8 and intron 9). The figure represents the hypothetical stem-loop with its potential hairpin structure just 5' and 3' of the deletion. (To simplify, only one DNA strand is represented.)

correspond to the nucleotides of the entirety of exon 9, that encodes the C-terminus from aa 415 to 448 (and Lys 414 which is encoded by the last two nt of exon 8 + the first nt of exon 9). Previous structure–function studies of the LPL gene have shown that the major domains involved in catalytic activity are encoded by exons 4, 5, and 6, while exon 9 did not seem to play a role in catalytic activity. Recently, however, Davis et al. (18) deduced from experiments with chimeric lipases (LPL and HL) that heparin binding was associated with the C-terminal domain, i.e., downstream of the 229–370 region previously assumed to be exclusively responsible for this property. The interaction of LPL with heparin, or with heparan sulfate proteoglycan of endothelial cell membranes, is thought to be ionic, with binding between the negative charges of heparin and positively charged LPL aa. As in the other LPL candidate regions for heparin binding, there is a concentration of positively charged residues in the C-terminal domain. Moreover, exon 9 codes for Cys 418 and 438, which form the fifth disulfide bridge of LPL and are thus likely to play an important role in secondary structure. Until now, only one missense mutation has been described in exon 9; it involved Ser447 (→stop) (19, 20) causing synthesis of an LPL missing two aa. In fact, the latter are not necessary for LPL activity, and this mutation has been likened more to a polymorphism rather than to a pathogenic defect.

As the whole end of intron 8 (120 nt) is deleted in the proband, the A nucleotide branchpoint involved in pre-

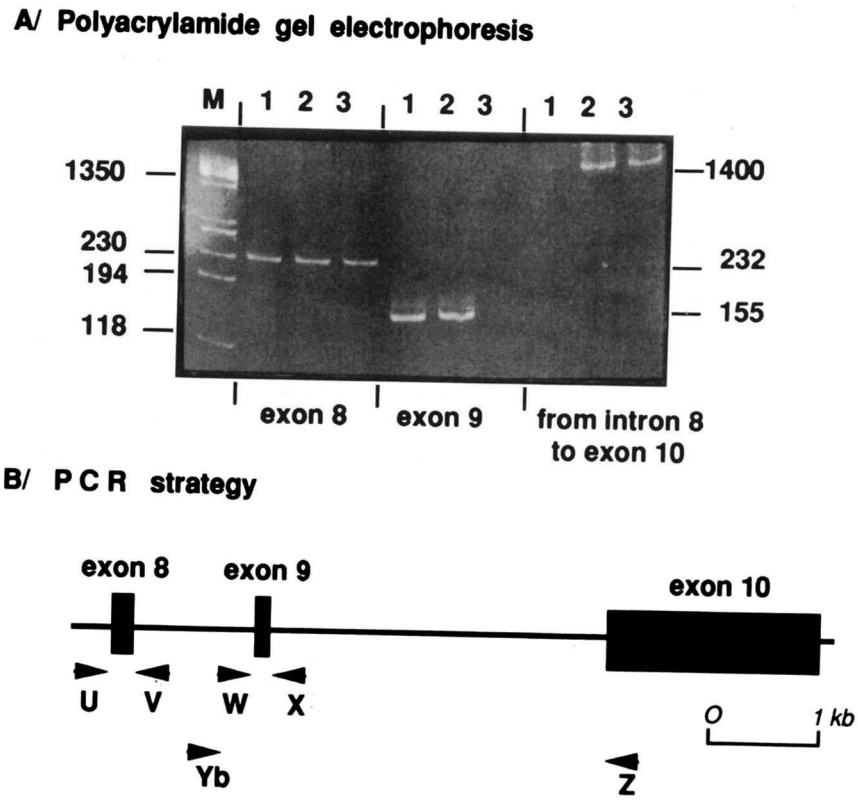

Fig. 6. Acrylamide gel electrophoresis of spouse, daughter, and proband PCR products. (A) Polyacrylamide gel electrophoresis of PCR products obtained with different primers (see legend B below) on genomic DNA from 1: spouse (unaffected), 2: daughter (obligate heterozygote) 3: proband (homozygous). M = markers (1350, 230, 194 and 118 nt); exon 8: a band is present in all three subjects; exon 9: a band is present only in the spouse and daughter; from intron 8 to exon 10: a band is present only in the daughter and proband, with an estimated size of only 1.4 kb, because of the deletion (2136 nt) which includes the end of intron 8 (120 nt), the whole of exon 9 (105 nt), and two-thirds of intron 9 (1911 nt). (B) Schematic representation of the normal LPL gene from exon 8 to exon 10 (solid segment: exon; line: intron), with location of the primers used to amplify exon 8 (primers U, V), exon 9 (primers W, X), and from the end of intron 8 to the beginning of exon 10 (primers Yb and Z). The primers used for PCR are listed in Table 1.

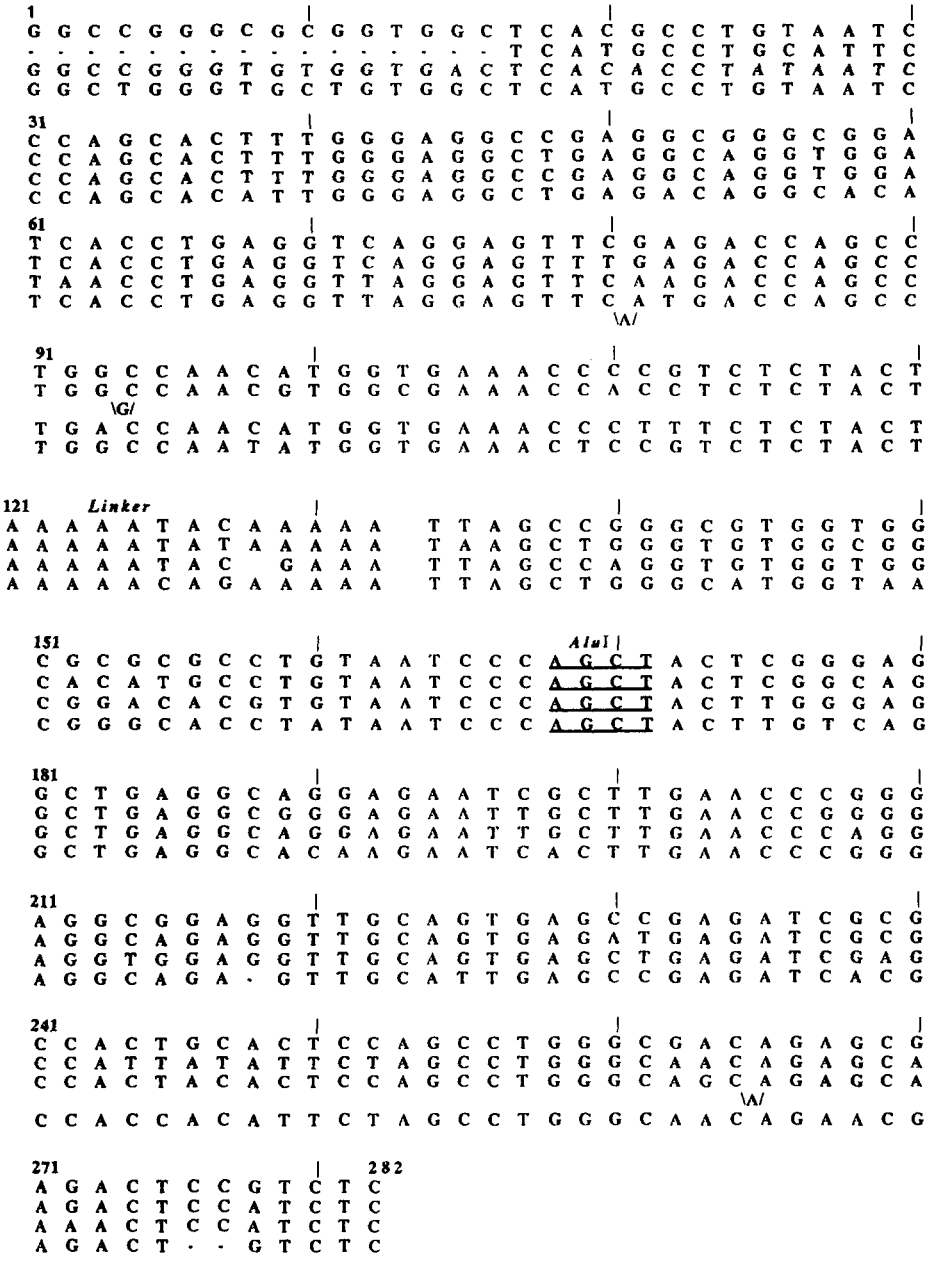

Fig. 7. The three Alu sequences in intron 9. Top line: consensus sequence proposed by Jurka and Smith (7). Bottom three lines: intron 9, present results (from Fig. 4). Sequence 1: direct nt 952–1218 (the first 16 nt are lacking). Sequence 2: complementary inverted nt 1751–2032. Sequence 3: complementary inverted nt 2331–2610. The hallmarks of Alu sequences, a 120-bp left arm and a 149-bp nearly homologous right arm, connected by the A-rich linker, and the Alu I restriction site, are indicated. Note the insertion between nt 93 and 94 in Alu sequence 1, the insertion between nt 264 and 265, and the gap (nt 129) in sequence 2, and the insertion between nt 80 and 81 and the three gaps (nt 218, 276, 277) in sequence 3. Below is indicated the identity of the sequences adjacent to the Alu sequence, in order to facilitate recognition of the three Alu sequences; in normal figures: numbering according to Fig. 4; in italics: numbering according to Fig. 7.

Alu sequence 1: (Fig. 4) : ...ttgcct **tcat** .... **tctc** aaaaaa...
952 1218

(*Fig. 7*) : **tcat** .... **tctc** *direct orientation*
17 *284*

Alu sequence 2: (Fig. 4) : ...ttcctt **gaga** .... **ggcc** ctagat...
1751 2032

(*Fig. 7*) : **ctct** .... **ccgg** *opposite orientation*
282 *1*

Alu sequence 3: (Fig. 4) : ...attttt **gaga** .... **agcc** agatgc...
2331 2610

(*Fig. 7*) : **ctct** .... **tcgg** *opposite orientation*
282 *1*

FAMILIES *ALU*-J AND *ALU*-S :

	57	63	65	70	71	94	101	106	163	194	204	208	220	233	275
Alu-J	A	G	T	C	C	G	A	G	G	G	G	A	C	T	C
Alu-S	C	A	C	G	T	C	G	A	A	A	A	G	T	A	T
Alu 1 intron 9	T	A	C	G	T	C	G	A	A	A	A	G	T	A	T
Alu 2 intron 9	T	A	C	G	T	C	G	A	A	A	A	A	T	A	T
Alu 3 intron 9	C	A	C	G	T	C	G	A	A	A	A	G	T	A	T

Alu 1→ 14/15 = Alu S
Alu 2→ 13/15 = Alu S
Alu 3→ 15/15 = Alu S

SUBFAMILIES OF *ALU*-S (-Sa, -Sb and -Sc) :

	65	66	78	88	95	100	153	163	197	200	219
Alu-Sa	C	T	T	G	C	T	C	A	C	T	G
Alu-Sb	-	-	A	T	T	C	G	G	G	G	C
Alu-Sc	-	-	A	T	C	T	T/G	G	C	T	G
Alu 1 intron 9	C	T	T	G	C	T	C	A	T	T	G
Alu 2 intron 9	C	T	T	G	C	T	G	A	T	T	G
Alu 3 intron 9	C	T	T	G	C	T	G	A	C	T	G

Alu 1 → 10/11 = Alu Sa
Alu 2 → 9/11 = Alu Sa
Alu 3 → 10/11 = Alu Sa

Fig. 8. Classification of the Alu sequences into families and subfamilies according to Jurka and Smith (7). Top panel: comparison was made between the diagnostic positions (top line: 57, 63 etc.) of the Alu-J and Alu-S consensus sequences (7, 10), with those of the three intron 9 Alu sequences (from Fig. 7). With more than nine identities, the three Alu repeats belong to the Alu-S family. Lower panel: Subclasses of the Alu-S family (Alu-Sa, -Sb, and -Sc) are based on the diagnostic positions (top line: 65, 66 etc...) (7, 10). With more than five identities, the three Alu repeats belong to the Alu-Sa subfamily.

mRNA splicing must be absent, as it is usually located approximately 20 to 50 nt upstream of the 3' end of the intron. Conversely, the A branchpoint of intron 9 must be present, as 1179 nt upstream of the 3' end of intron 9 are conserved. Splicing between exon 8 and the normally untranslated exon 10, skipping exon 9, is conceivable. In this case, the truncated protein would be missing the last 35 aa after Lys 413, which are normally encoded by exon 9. Moreover, the last two nt of exon 8 (AA) associated with the first nt of exon 10 (A) would not give rise to a stop codon, as is the case when the last two nt of exon 9 (TG) associated with the first nt of exon 10 (A). Thus, the beginning of exon 10 could encode an extra 39 aa, up to the next stop codon. This putative protein would therefore have 35 aa less (exon 9) and 39 aa more (beginning of exon 10). It was not possible to characterize the LPL protein of this patient. LPL mass was measured using the monoclonal antibody 5D2 (21). The epitope recognized by this antibody is residue 400 of mature LPL, in the C-terminal domain (22). As this epitope is encoded by exon 8, it should be present in the putative truncated product. A protein LPL with a different C-terminal moiety could be synthesized but degraded before transfer to endothelial cells. Indeed, loss of the last disulfide linkage could affect the stability of the LPL protein.

The presence of three Alu sequences in LPL intron 9 is surprising. Indeed, there is usually about one Alu sequence every 4 kb in the human genome, but these three Alu sequences are contained in only 1.66 kb; furthermore, together with the Alu sequence we have previously characterized in intron 7 (4), the four Alu sequences span only 2.93 kb.

The second and third Alu sequences in intron 9 are complete, while the first has a truncated 5′ end (16 nt missing); direct flanking repeat at the 5′ extremity of the transposition is missing. An Alu sequence situated in the 5′ end of the ϵ-globin gene (23) has the same type of truncation (i.e., the 16 nt 5′ and the flanking repeat). It seems reasonable to suppose that, in these cases, deletion occurred after retroposition. A 5′ truncation (39 nt) has been described in the Alu sequence of the factor IX gene (24), but the Alu element is flanked by the appropriate direct repeats, suggesting that it was inserted before completion of reverse transcription.

The two Alu sequences we have previously identified in introns 6 and 7 (4) are both members of the Alu-Sa family, like the three Alu sequences found in intron 9. Approximate dating of the different Alu subfamilies indicates that Alu-Sa may have been transposed into the primate genome only 40 million years ago (9).

Funke et al. (25) have described a BstNI (CC(A/T)GG) polymorphism in the LPL gene, by using a probe recognizing the end of intron 8, exon 9, and the beginning of intron 9. Similarly, Heinzmann et al. (26) have described Hind III (AAGCTT) and XbaI (TCTAGA) polymorphisms located in intron 8 and intron 9, respectively. However, the sequence of these introns was unknown at the time. Now that intron 8 and intron 9 have been sequenced, we can deduce that the Hind III polymorphism is situated at nt 477 in intron 8, BstN1 at nt 841 in intron 8, and XbaI at nt 1515 in intron 9. The allelic frequencies were found to be as follows: Hind III (27): 75% for the +allele (containing the site, as is the case for P1A and Co, Fig. 1), and 25% for the −allele (without the site); Bst NI(26): 70% for the +allele (as for P1A, P1B and Co), and 30% for the −allele; XbaI(27): 83% for the −allele (as for C1 and C2), and 17% for the +allele.

We thank I. J. Forsythe (Prof. Hayden's laboratory) for determining LPL mass, and J. P. Lagarde and D. Dabit for excellent technical assistance. We thank Béatrice Pelletier and Maryse Artz for typing the manuscript. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (grant CRE 900 203 to J.E. and 91CN45 to PB) and from Université Paris VI (grant DRED no. EA273 to JE and no. 92166 to PB).

Manuscript received 23 May 1994 and in revised form 22 August 1994.

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