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Apolipoprotein E3-Leiden Contains a Seven-Amino Acid Insertion That Is a Tandem Repeat of Residues 121–127*

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Apolipoprotein (apo) E3-Leiden is a variant of apoE that is associated with dominant expression of type III hyperlipoproteinemia and that is defective in binding to the low density lipoprotein receptor. Therefore, the structure of apoE3-Leiden was investigated. Upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis, apoE3-Leiden and its 22-kDa amino-terminal thrombolytic fragment migrated with a higher than normal apparent molecular weight. The structural abnormality of apoE3-Leiden was determined by sequencing its CNBr-, tryptic-, and *Staphylococcus aureus* V8 protease-generated peptides. In contrast to normal apoE3, which has a cysteine at residue 112, apoE3-Leiden does not contain any cysteine and has an arginine at position 112 (as does apoE4, which also completely lacks cysteine). The basis for the molecular weight difference was determined to be a seven-amino acid insertion that is a tandem repeat of residues 121–127 of normal apoE3, *i.e.* Glu-Val-Gln-Ala-Met-Leu-Gly, resulting in apoE3-Leiden having 306 amino acids rather than 299. The negatively charged glutamyl residue within the insertion compensates for the arginine substitution at residue 112; thus apoE3-Leiden focuses in the E3 position. The low density lipoprotein receptor binding activities of both intact apoE3-Leiden and its 22-kDa thrombolytic fragment were determined in an *in vitro* assay. Although apoE3-Leiden had only about 25% of normal binding activity, its 22-kDa thrombolytic fragment had nearly normal binding, suggesting that the carboxyl-terminal domain of apoE3-Leiden modulates the receptor binding function of its amino-terminal domain.

Apolipoprotein (apo)[1] E3-Leiden is a rare variant of apoE that is associated with the lipid disorder type III hyperlipoproteinemia and that has an impaired ability to bind to the low density lipoprotein (LDL) receptor on cultured human fibroblasts and HeLa cells (1, 2). Type III hyperlipoproteinemia is associated with genetic mutants of apoE that bind defectively to lipoprotein receptors (3, 4). This defective binding causes impaired clearance of chylomicron and very low density lipoprotein remnants (β-VLDL) (5, 6), which accumulate in the plasma and cause both hypercholesterolemia and hypertriglyceridemia. The most common apoE variant associated with this disorder is apoE2(158 Arg→Cys) (4), which exhibits only ~1% of the *in vitro* receptor binding activity of normal apoE3 (7). This variant is associated with a recessive mode of inheritance of type III hyperlipoproteinemia; the vast majority of all type III subjects are homozygous for apoE2(158 Arg→Cys), and heterozygotes almost never develop the disorder (4). Furthermore, although ~1% of the population is homozygous for apoE2(158 Arg→Cys), only a small fraction of these individuals develop hyperlipidemia, even though all possess β-VLDL (8, 9). Therefore, not only is the mode of inheritance recessive, but other factors, environmental or genetic, are required for expression of type III hyperlipoproteinemia in these individuals (8, 9).

In contrast to apoE2(158 Arg→Cys), three other apoE variants display an absolute correlation between the presence of only one allele for the variant and the occurrence of type III hyperlipoproteinemia, indicating that these mutants are associated with dominant, rather than recessive, expression of this disorder (2, 10, 11). The first example was established in two generations of the apoE3-Leiden (V.) kindred (2). Six of eight family members examined are heterozygous for this variant and express type III disease (2). The two unaffected family members are homozygous for normal apoE3. In addition, the apoE3-Leiden mutant constituted the vast majority of the total apoE in affected subjects (2), indicating that it is either produced at a greater rate than the normal isoform or is catabolized at a much slower rate. A second variant that results in a dominant mode of expression of type III hyperlipoproteinemia is apoE3(112 Cys→Arg, 142 Arg→Cys) (10). Six family members from four generations are heterozygous for this variant and express type III hyperlipoproteinemia, whereas family members lacking this variant do not express the disorder (10). A third such variant is apoE1-Harrisburg(146 Lys→Glu); heterozygosity for this variant results in dominant expression of type III hyperlipoproteinemia in five family members from three generations (11). These latter two mutants are also defective in their interaction with the LDL receptor (10, 11).

To understand further the apoE mutations that are associated with dominant expression of type III hyperlipoproteinemia, we examined the structural abnormality in apoE3-Leiden. In this report we describe a new type of apoE mutation, in which an insertion of seven amino acids appears to be responsible for an unusual characteristic of apoE3-Leiden receptor binding.

MATERIALS AND METHODS

Family History—The clinical features of members of the V. kindred have been described previously (1, 2). All six individuals possessing

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[1] The abbreviations used are: apo, apolipoprotein; β-VLDL, very low density lipoproteins with β electrophoretic mobility; DMPC, dimyristoylphosphatidylcholine; HPLC, high-performance liquid chromatography; LDL, low density lipoproteins; SDS, sodium dodecyl sulfate.

the mutant apoE3-Leiden isoform had β-VLDL and elevated plasma concentrations of cholesterol, triglycerides, and apoE (2). In addition, four individuals had xanthomas, including palmar xanthomas (2), which have been described only in type III hyperlipoproteinemia (4). Apolipoprotein E3-Leiden, after being purified and recombined with egg phosphatidylcholine, also was defective in binding to LDL receptors on both cultured human fibroblasts and HeLa cells (1, 2).

Isolation and Characterization of Apolipoprotein E3-Leiden—Lipoproteins of $d < 1.02$ g/ml were prepared from EDTA-treated plasma from the propositus (subject II-6 in the V. kindred (2)), and apoE was isolated from this fraction as described previously (7, 12). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed on 10–20% gradient slab gels using the buffer system of Laemmli (13) but substituting 2-amino-2-methyl-1,3-propanediol for Tris; the protein was stained with Coomassie Brilliant Blue R-250. Analytical isoelectric focusing was performed on 5% polyacrylamide gels containing 8 M urea and 2% Ampholine, pH 4–6 (Pharmacia LKB Biotechnology Inc.), according to the method of Pagnan *et al.* (14) with the modification previously described (15). Purified apoE was modified with cysteamine as described (7). Preparative Immobiline isoelectric focusing was performed on 5% polyacrylamide slab gels containing 6 M urea and having a pH gradient of 5.5–6.5 (16).

Structural Analysis—Apolipoprotein E3-Leiden was digested with CNBr (Pierce), and the released peptides were separated by Sephadex G-50 chromatography as previously described (17). To generate thrombolytic fragments, apoE3-Leiden (1 mg/ml in 0.1 M NH_4HCO_3) was incubated at 23 °C with 1% thrombin (a gift from Dr. J. W. Fenton II of the New York Department of Health, Albany) (w/w) for 2 h and then with 2% thrombin for a further 2 h. The digest then was lyophilized, dissolved, and separated as previously described (12). Digestion with L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin (Cooper Biomedical, Freehold, NJ) and separation of the tryptic peptides by two-dimensional paper mapping were performed as previously described (18), except that apoE3-Leiden was not carboxymethylated and 0.5 mg of digest was applied to each map. Apolipoprotein E3-Leiden (1 mg/ml in 0.1 M NH_4HCO_3) was incubated with *Staphylococcus aureus* V8 protease (ICN) (40:1, w/w) at 37 °C for 18 h. The digest then was lyophilized and redissolved at a concentration of 10 mg/ml in 1 M NH_4OH. Two large V8 peptides that were insoluble in this basic solution were dissolved in 1 M formic acid. The base-soluble V8 peptides (0.5 mg) were separated by two-dimensional paper mapping in a fashion identical to that for the tryptic peptides.

The tryptic and V8 digests or impure peptides from two-dimensional paper mapping were also separated by reverse-phase high-performance liquid chromatography (HPLC) (Beckman Model 334, Beckman Instruments, Fullerton, CA) on a Waters Delta Pak C_{18}-100Å stainless steel column (15 cm × 3.9 mm, 5 μm, Waters Chromatography Division, Millipore Corp., Milford, MA) maintained at 36 °C. Digests were separated using a stepwise gradient of two buffers: buffer A, 0.1% trifluoroacetic acid; and buffer B, 80% acetonitrile with trifluoroacetic acid added so that the absorbance of buffer B at 214 nm was identical to that of buffer A. Injections of 50 μg of digest were made to the column equilibrated with 90% buffer A and 10% buffer B. Buffer B then was increased as follows: to 15% in 10 min, 25% in another 25 min, 50% in another 30 min, 60% in another 10 min, and finally to 100% in another 10 min. The eluate was monitored at 214 nm, and peptides were collected manually.

Amino acid analyses were performed using two systems. Phenylthiocarbamyl amino acid analysis (19) was performed on all peptides from both two-dimensional paper (18) and HPLC maps. For the larger thrombolytic and CNBr fragments and the intact protein, amino acid analysis was performed on a Beckman 121MB analyzer as described previously for apoE (17). Cysteine was determined as cysteic acid according to the method of Moore (20).

Automated peptide sequencing was performed on either a Beckman Model 890M spinning cup sequencer as previously described (17) or on an Applied Biosystems 477A pulsed liquid sequencer (Applied Biosystems, Foster City, CA) equipped with an on-line 120A analyzer. Lyophilized CNBr peptides were dissolved in water for sequencing. Tryptic and V8 peptides that had been located on the paper map with fluorescamine (Aldrich) were eluted with acetic acid:ethanol:water (1:1:8, v/v/v) and sequenced. Peptides eluted from the HPLC were applied directly to the sequencer.

Receptor binding assays were carried out with cultured human fibroblasts using ^{125}I-labeled LDL as the competitor (21).

RESULTS

Electrophoretic Characterization of Apolipoprotein E3-Leiden—When apoE3-Leiden, isolated from the $d < 1.02$ g/ml lipoproteins of the propositus of the V. kindred, was subjected to SDS-polyacrylamide gel electrophoresis, a single band with an apparent molecular mass approximately 1–2 kDa greater than normal apoE3 was seen (Fig. 1). In the gel system described under "Materials and Methods," this is the only apoE variant to have a different electrophoretic mobility. In another gel system in which apoE2(158 Arg→Cys) has a retarded mobility (22), Havekes *et al.* (2) have reported that apoE3-Leiden also has a retarded mobility, migrating between normal apoE3 and apoE2(158 Arg→Cys). When apoE3-Leiden was digested with thrombin, the amino-terminal 22-kDa fragment also displayed a slower electrophoretic mobility than the normal apoE3 22-kDa fragment, while the carboxyl-terminal 12-kDa fragment of apoE3-Leiden migrated in a position identical to the 12-kDa fragment from normal apoE3 (Fig. 1). This indicated that the property responsible for the aberrant electrophoretic mobility of this variant resided within the 22-kDa thrombolytic fragment.

Upon isoelectric focusing, apoE3-Leiden focused in the E3 position (Fig. 2), in agreement with previous reports (1, 2). After cysteine residues were modified with cysteamine (7), only a small portion shifted to the E4 position (Fig. 2). This confirmed the earlier report that while most of the apoE did not contain cysteine, a small fraction of it did (2). The minor band that had shifted to the E4 position after cysteamine treatment was purified by preparative Immobiline isoelectric focusing. Amino acid analysis of this material showed that it contained 1.3 mol of cysteine (determined as cysteic acid)/mol of protein, confirming that the second allelic product expressed in the propositus is normal apoE3 (2).

Structural Characterization of Apolipoprotein E3-Leiden—The amino acid compositions of apoE3-Leiden and normal apoE3 were compared. It was found that apoE3-Leiden did not contain cysteine, but no other compositional differences were readily apparent (data not shown). This was also the case when the amino acid compositions of the respective 22-kDa thrombolytic fragments were compared (data not shown).

The gel filtration separation profile of the CNBr peptides of apoE3-Leiden on Sephadex G-50 is shown in Fig. 3. This profile is similar to that for normal apoE3 (17, 23) except for

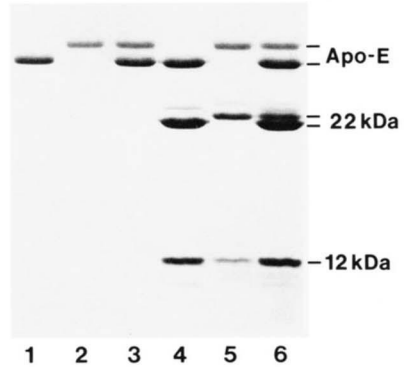

FIG. 1. **Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of apoE3-Leiden and its thrombolytic fragments.** *Lane 1*, 1 μg of Immobiline-purified normal apoE3; *lane 2*, 1 μg of Immobiline-purified apoE3-Leiden; *lane 3*, a 1:1 mixture of the samples used in *lanes 1* and *2*; *lane 4*, 5 μg of normal apoE3 thrombolytic digest; *lane 5*, 5 μg of apoE3-Leiden thrombolytic digest; *lane 6*, a 1:1 mixture of the samples used in *lanes 4* and *5*. Digestion with thrombin (2% w/w) was limited to 30 min at 23 °C. The positions of intact apoE and the 22- and 12-kDa thrombolytic fragments are indicated.

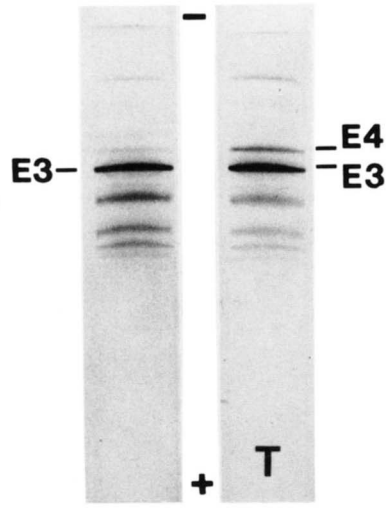

FIG. 2. **Analytical isoelectric focusing of apoE3-Leiden.** The *left gel* is untreated apoE3-Leiden; the *right gel* is cysteamine-treated (*T*) apoE3-Leiden. The cathode (−) is at the top, and the anode (+) is at the bottom. The focusing positions of apoE3 and apoE4 are indicated.

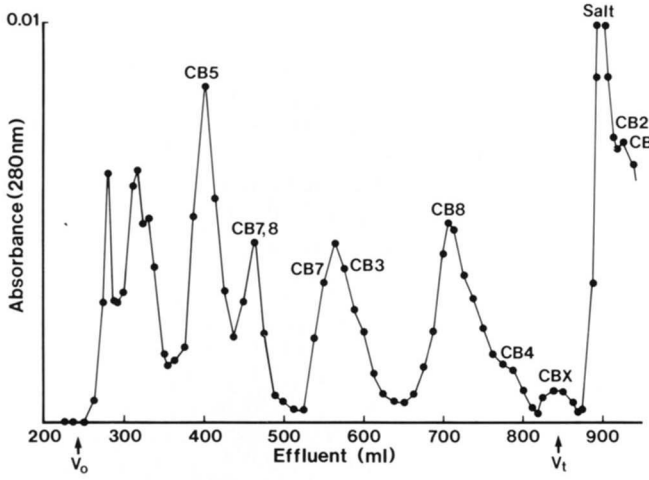

FIG. 3. **Sephadex G-50 chromatography of a CNBr digest of apoE3-Leiden.** Seven milligrams of apoE3-Leiden (5 mg/ml in 70% formic acid) was digested with a 30-fold excess (w/w) of CNBr at 23 °C for 24 h. After lyophilization, the digest was dissolved in 2 ml of 20% formic acid and chromatographed on a 2.5 × 190-cm Sephadex G-50 column in 0.02 N HCl at a flow rate of 15 ml/h. The peptide designations are those of Rall *et al.* (23). Because peptide CB1 chromatographs poorly and is recovered in very low yield (23), it is not indicated in the figure. The new peptide that is not present in normal apoE3 is labeled *CBX*.

the presence of a new peak (*CBX*) eluting at the column volume. Amino acid analysis of the apoE3-Leiden peptide corresponding to CB4 (residues 109–125 in normal apoE) indicated an absence of cysteine but the presence of an extra arginine (Table I). This difference was confirmed by sequencing: apoE3-Leiden CB4 had arginine at the position corresponding to residue 112, just as occurs in apoE4, which also completely lacks cysteine. The peptide corresponding to CB5 (residues 126–218 in normal apoE) was sequenced for 61 cycles and was found to be identical to that of normal apoE3 (data not shown). The composition of the new CNBr fragment, CBX, is given in Table I, and its sequence was Leu-Gly-Glu-Val-Gln-Ala-Met. Because this CNBr peptide does not occur in normal apoE, apoE3-Leiden was digested with trypsin to locate its position in the sequence.

The tryptic peptides were mapped on a two-dimensional paper system (18). There were three new peptides present on the apoE3-Leiden map (Fig. 4) as compared with the tryptic map of normal apoE3. Two of these new peptides had net negative charge, and one had net positive charge (Table I). One of the new acidic peptides and the new basic peptide, labeled T13* and T13**, respectively (Fig. 4), arose as a result of the arginine-for-cysteine substitution at position 112, yielding a new tryptic cleavage site in apoE3-Leiden. The sequence of the second new acidic peptide, labeled T15* (Fig. 4), identified the position of the 7-residue CBX sequence in apoE3-Leiden. In normal apoE3, peptide T15 is a 15-residue peptide encompassing residues 120–134 (23). However, the apoE3-Leiden peptide T15* was a 22-residue peptide (Table I) with the sequence Gly-Glu-Val-Gln-Ala-Met-Leu-Gly-Glu-Val-Gln-Ala-Met-Leu-Gly-Gln-Ser-Thr-Glu-Glu-Leu-Arg, which contained the CBX sequence in its entirety. Inspection of this sequence also indicated that this peptide contains the normal T15 sequence, with the 7-residue sequence Glu-Val-Gln-Ala-Met-Leu-Gly inserted after Gly-127. This 7-residue insertion is a direct repeat of the preceding 7 residues, which are residues 121–127 in normal apoE.

Two-dimensional paper mapping of the *S. aureus* V8 proteolytic peptides confirmed these results. Two new peptides were found on the apoE3-Leiden map compared with the normal apoE3 map (data not shown). A new basic peptide was found that encompassed residues 110–121 and resulted from the substitution of arginine-for-cysteine at position 112 in apoE3-Leiden (V8 16, Table I). A new acidic peptide with the sequence Val-Gln-Ala-Met-Leu-Gly-Glu resulted from the introduction of a new glutamyl cleavage site within the insertion (V8 17*, Table I). The expected normal peptide encompassing residues 122–132 was also found on the apoE3-Leiden map (V8 17, Table I). A summary of the peptide results demonstrating the occurrence of arginine at residue 112 and the presence of the 7-residue insertion in apoE3-Leiden is presented in Fig. 5. A variety of other thrombolytic, CNBr, tryptic, and V8 peptides encompassing almost the entire apoE sequence showed no other differences from normal apoE (data not shown). Although apoE3-Leiden has arginine at position 112, making it "E4-like," it focuses in the same position as normal apoE3 because of the presence of the negatively charged glutamic acid residue in the 7-residue insertion.

Receptor Binding Activities of Apolipoprotein E3-Leiden and Its 22-kDa Thrombolytic Fragment—Because the total isolated apoE consisted of at least 90% of the variant apoE3-Leiden isoform (2), it was used directly, without further purification, for receptor binding analysis in an *in vitro* assay (21). As shown in Fig. 6, the apoE3-Leiden·dimyristoylphosphatidylcholine (DMPC) complexes were defective in binding to the LDL receptors on human fibroblasts, in agreement with the findings of Havekes and co-workers (1, 2), who used apoE3-Leiden·egg phosphatidylcholine complexes. A logit-log plot of the binding data in Fig. 6 indicated that apoE3-Leiden possesses about 25% of normal apoE3 receptor binding activity (calculated from the 50% competition point). In contrast, when the 22-kDa thrombolytic fragment of apoE3-Leiden was complexed with DMPC, its receptor binding activity was nearly identical to normal apoE3·DMPC (Fig. 6) and the normal apoE3 22-kDa thrombolytic fragment (24). In this respect, apoE3-Leiden behaves like apoE2(158 Arg→Cys), in that the receptor binding activity of this 22-kDa thrombolytic fragment is also greater than that of the intact molecule (25).

TABLE I

Amino acid compositions of selected peptides from apoE3-Leiden

Tryptophan and cysteine were not determined. Numbers in parentheses were determined by sequencing. ND = not determined.

Residues	CB4	CBX	T13*	T13**	T15*	V8 16	V8 17*	V8 17
Asx	1.0 (1)		1.8 (2)		0.4	0.8[a] (1)		0.2
Glx	4.2 (4)	2.0 (2)	1.2 (1)		6.9 (7)	2.8 (2)	1.9 (2)	4.3 (4)
Ser			0.1	0.2	1.0 (1)			0.8 (1)
Gly	2.1 (2)	1.1 (1)	1.1 (1)	0.6[b] (1)	2.9[a] (3)	2.1 (2)	1.1 (1)	1.0 (1)
His								
Arg	2.6 (3)		1.0 (1)	1.2 (1)	1.0 (1)	2.9 (3)		
Thr					0.9 (1)	0.2		0.7 (1)
Ala	1.1 (1)	1.0 (1)	1.1 (1)	0.2	2.1 (2)	0.4	1.0 (1)	0.8 (1)
Pro						0.3		
Tyr	0.7 (1)					0.9 (1)		
Val	3.0 (3)	1.0 (1)	1.0 (1)		2.1 (2)	2.1 (2)	0.8[a] (1)	1.0[a] (1)
Met	0.5[b] (1)	0.5[b] (1)	1.0 (1)	0.2	1.8 (2)		0.8 (1)	0.9 (1)
Ile			0.1	0.1	0.3		0.1	0.1
Leu	1.1 (1)	1.0 (1)	0.7[a] (1)	0.2	3.0 (3)	1.3 (1)	1.1 (1)	0.9 (1)
Phe								
Lys	0.3				0.1			
Total residues	17	7	9	2	22	12	7	11
Method of isolation	Sephadex G-50	Sephadex G-50	2-D[c] paper peptide map	2-D paper peptide map	2-D paper peptide map	HPLC peptide map	2-D paper peptide map	2-D paper peptide map
Electrophoretic mobility, pH 6.4	ND	ND	Acidic	Basic	Acidic	Basic	Acidic	Acidic
Yield of peptide (%)	19	25	45	40	16	24	21	22
Amount sequenced (pmol)	15,200	875	2,130	630	600	350	400	900
Initial yield (pmol)	8,509	857	690	205	129	214	282	528
Repetitive yield (%)	91.0 Val 8-3	ND	92.8 Asp 7-4	ND	94.1 Avg 7aa[d]	93.3 Avg 3aa	ND	90.3 Gln 7-2

[a] The amino-terminal residues of the peptides, which were reacted with fluorescamine prior to amino acid analysis.

[b] Determined as homoserine and homoserine lactone, based on leucine equivalents.

[c] 2-D, two-dimensional.

[d] Avg, average; aa, amino acid residues.

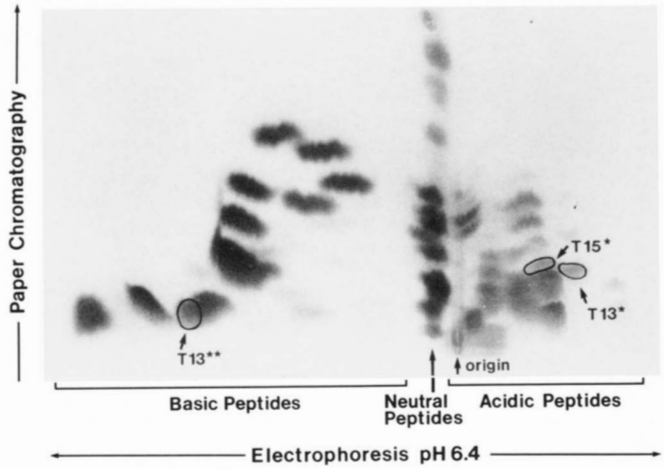

FIG. 4. **Two-dimensional tryptic peptide map of apoE3-Leiden.** The tryptic digest (0.5 mg) was applied at the origin and electrophoresed at 3 kV for 55 min in a pH 6.4 pyridine-acetate buffer (pyridine:acetic acid:water, 100:4:900, v/v/v) in the first dimension and chromatographed in the ascending direction in the second dimension for 20 h in the upper phase of pyridine:isoamyl alcohol:water (6:6:7, v/v/v) (13). The peptides are designated as in Rall *et al.* (23). The new peptides in apoE3-Leiden are labeled with *asterisks*.

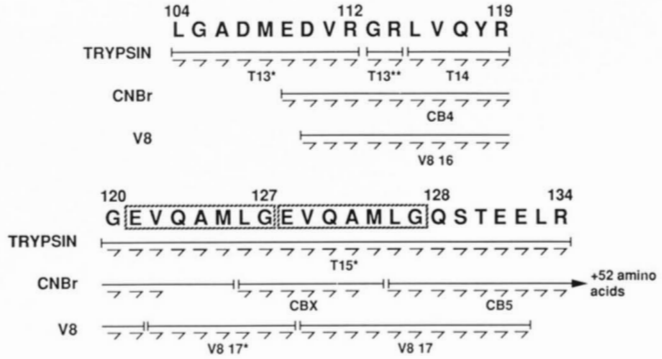

FIG. 5. **Partial primary structure of apoE3-Leiden in the region where differences from normal apoE3 are found.** The sequences obtained for the tryptic, CNBr, and V8 peptides (see also Table I) are indicated by the *half-arrows* (—›). The tryptic peptide T14 is identical to that of normal apoE3 and is not included in Table I but is given here for completeness. Likewise, the CNBr peptide CB5 was sequenced for 61 cycles and was found to be identical to that of normal apoE3. The *boxed residues* indicate the sequence that is tandemly repeated. The *numbers* refer to residue numbers in normal apoE (23).

DISCUSSION

The primary structure of apoE3-Leiden differs from normal apoE3 in two respects. First, as is the case in apoE4, arginine replaces cysteine at residue 112, making it likely that apoE3-Leiden is derived from the $\epsilon 4$ allele. Second, the 7-residue sequence Glu-Val-Gln-Ala-Met-Leu-Gly encompassing residues 121–127 in normal apoE3 is directly repeated in apoE3-Leiden, resulting in a variant protein that is seven amino acids longer than normal (306 *versus* 299). The additional mass that this repeated segment adds to the variant is calculated to be 729 Da, and both apoE3-Leiden and its 22-kDa

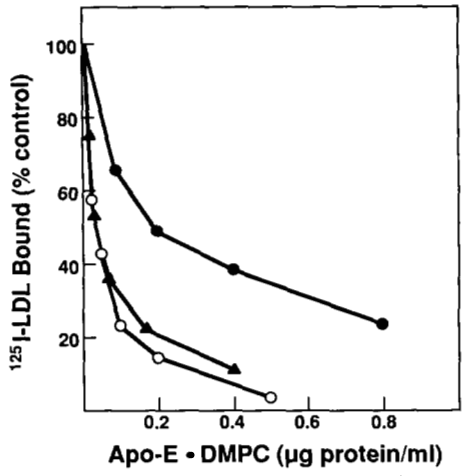

FIG. 6. **Receptor binding activity of apoE3-Leiden and its 22-kDa thrombolytic fragment as assessed in a competition assay.** The apoE·DMPC complexes were mixed at various concentrations with 2 μg of ^{125}I-labeled LDL and incubated with cultured human fibroblasts on 35-mm culture dishes for 4 h at 4 °C. Each point represents the average of two independent experiments. ●, apoE3-Leiden; ▲, the 22-kDa thrombolytic fragment of apoE3-Leiden; ○, normal apoE3 control.

thrombolytic fragment display increased apparent molecular masses on SDS-polyacrylamide gels (Fig. 1). Because the positively charged arginine at residue 112 is compensated for by the negatively charged glutamic acid residue contained within the insertion, this variant focuses in the apoE3 position.

Although we have chosen to indicate the inserted sequence as a repetition of amino acids 121–127, apoE3-Leiden could also have arisen by a repetition of amino acids 120–126. The situation is even more complex at the nucleotide level: there are five possible positions where 21 nucleotides could be repeated tandemly to give the new protein sequence. DNA sequence analysis has confirmed the presence of a 21-base pair duplication that corresponds to amino acids 121–127 (or 120–126).[2]

The mechanism accounting for the 7-residue repeated sequence in apoE3-Leiden is not known. One possibility is that the repeated sequence originated from a recombination event caused by nonhomologous pairing and crossing over during meiosis. Such a recombination event usually occurs when sequences immediately flanking the repeated element are identical or closely homologous for a length of about 10 nucleotides or more (26). However, the nucleotide sequences immediately flanking the repeated sequence in the normal apoE gene show no such similarity (27). If this were the mechanism giving rise to apoE3-Leiden, it also implies that another allele was produced that would contain a deletion of the 21 nucleotides. A second possible mechanism that could give rise to a tandemly repeated sequence is replication slippage (28), which probably occurs by "looping out" of the template strand (deletion) or the growing strand (insertion). This mechanism usually involves one or a few nucleotides in a region of identical nucleotides. However, in the case of apoE3-Leiden, the DNA polymerase transcribing the new strand would have to slip back 21 nucleotides along the template strand.

Even though the amino- and carboxyl-terminal structural domains of apoE appear to be independently folded (12, 29), evidence suggests that the carboxyl-terminal domain can modulate the receptor binding function of the amino-terminal domain (25). The receptor binding activity of apoE2(158 Arg→Cys)·DMPC is only 1% of normal apoE3·DMPC (7). However, the apoE2 22-kDa amino-terminal thrombolytic fragment displays a 12-fold increase in binding to the LDL receptor (25). Similarly, when the cysteine at position 158 of apoE2 is converted to a positively charged lysine analogue by reaction with cysteamine, this apoE2 possesses ~13% of the receptor binding activity of normal apoE3 (25). More importantly, the cysteamine-treated apoE2 22-kDa thrombolytic fragment possesses full receptor binding activity (25). This indicates that removal of the carboxyl-terminal one-third of the molecule releases some constraint on the receptor binding function of the amino-terminal domain. Likewise, the carboxyl-terminal domain of apoE3-Leiden also appears to modulate the receptor binding function of its amino-terminal domain, although the mechanism is likely to be different than that for apoE2(158 Arg→Cys). Removal of the carboxyl-terminal domain in this variant increases the receptor binding activity from 25% of normal to nearly normal. Residues 121–127 in apoE3 constitute nearly two full turns of a predicted α-helix (12). The repetition of these residues in apoE3-Leiden would therefore be predicted to increase the length of this helix by two turns. It is conceivable that increasing the dimensions of this segment of the amino-terminal domain leads to an aberrant interaction with the carboxyl-terminal domain that somehow reduces the ability of the molecule to interact with the LDL receptor. However, in the absence of the carboxyl-terminal domain, the amino-terminal domain of apoE3-Leiden can assume a conformation that allows nearly normal binding to occur.

Although the affected subjects are heterozygous for apoE3-Leiden, they all express type III hyperlipoproteinemia (2), indicating that this lipid disorder is transmitted in a dominant fashion in this family. There are two other apoE mutants for which this also appears to be the case: apoE3(112 Cys→Arg, 142 Arg→Cys) (10) and apoE1-Harrisburg(146 Lys→Glu) (11). One factor that may influence dominant *versus* recessive expression of type III hyperlipoproteinemia is the location of the mutation in the apoE polypeptide chain. The site of the mutation may determine whether the receptor binding activity can be modulated by other factors. For example, the binding activity of β-VLDL isolated from a single type III hyperlipoproteinemic patient homozygous for apoE2(158 Arg→Cys) before and after dietary intervention has been shown to vary (30). The β-VLDL isolated from this patient after weight loss possessed a 30-fold higher receptor binding activity than the β-VLDL isolated before weight loss (30). As a result of the diet, the subject's plasma cholesterol concentration decreased drastically (from 725 to 92 mg/dl) and the ratio of cholesterol to triglyceride in his VLDL ($d < 1.006$ g/ml) decreased 3-fold (30). This result suggests that different lipid environments within lipoprotein particles could alter the conformation of the receptor binding domain of apoE2(158 Arg→Cys) and affect its binding activity. The susceptibility of this apoE variant to environmental influences on its receptor binding activity may be the explanation for its association with a recessive mode of inheritance of type III hyperlipoproteinemia.

In contrast, the mutations of both apoE3(112 Cys→Arg, 142 Arg→Cys) (10) and apoE1-Harrisburg(146 Lys→Glu) (11) occur within a putative α-helix (residues 131–150) (25), which is rich in basic amino acids that are thought to provide direct ionic interaction with the acidic residues within the ligand binding domain of the LDL receptor (31). Because it

[2] A. M. J. M. van den Maagdenberg, P. de Knijff, J. A. Gevers Leuven, A. F. H. Stalenhoef, L. M. Havekes, and R. R. Frants, manuscript in preparation.

is unlikely that these mutations disrupt either the α-helix or the conformation of apoE, the receptor binding activity in these cases would not be expected to be influenced by other factors, and dominant expression of type III hyperlipoproteinemia results. In the case of apoE3-Leiden, the nature of the mutation, a 7-amino acid insertion, probably causes a permanent disruption in the receptor binding domain and therefore leads to dominant expression of type III hyperlipoproteinemia. While heterozygosity for three further mutants (apoE2(145 Arg→Cys) (32, 33), apoE2(146 Lys→Gln) (34), or apoE2-Christchurch(136 Arg→Ser) (18, 33)) is associated with type III hyperlipoproteinemia, there are no family studies to ascertain whether these variants also demonstrate a dominant mode of inheritance. Because the nature and location of the amino acid substitution(s) within the polypeptide chain of functionally abnormal mutants of apoE may determine whether type III hyperlipoproteinemia is transmitted as a dominant or recessive characteristic, family studies will be important in determining whether heterozygosity for a receptor binding-defective apoE mutant is in fact sufficient to cause this disorder.

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