Identification and characterization of a novel apolipoprotein E variant, apolipoprotein E3' (Arg_{136}→His): association with mild dyslipidemia and double pre-β very low density lipoproteins

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Abstract Apolipoprotein (apo) E mediates the removal of chylomicron and VLDL remnants from plasma. In a proband with mild hyperlipidemia and a family history of premature coronary artery disease, we have identified a new mutant of apoE with an isoelectric point close to but distinct from that of apoE3. Sequencing of the apoE gene from this subject (JB) revealed that the subject was heterozygous for a G to A substitution in codon 136, resulting in the substitution of histidine for arginine; therefore, we have designated this isoform apoE3' (Arg_{136}→His). Examination of the proband's kindred revealed that the nine carriers (all heterozygotes) of the variant isoform displayed a twofold elevation in the concentration of very low density lipoprotein (VLDL) cholesterol (40 ± 8 mg/dl) and triglyceride (109 ± 19) compared to the nine noncarriers (19 ± 3 and 55 ± 13, respectively). In all carriers, the VLDL displayed an abnormal double pre-β pattern upon electrophoresis. The low density lipoprotein receptor-binding activity of purified apoE3' (Arg_{136}→His) when complexed with DMPC was slightly defective (80% of the activity of normal apoE). The mutant apoE also displayed a reduced affinity for heparin compared to apoE3. As both of these biochemical parameters are known to be important in VLDL clearance, the defects associated with this variant are likely responsible for the increase in VLDL observed in carriers. None of the carriers displayed clinical features of type III hyperlipoproteinemia, suggesting that the relatively mild dyslipoproteinemic phenotype associated with this variant might be associated with recessive expression of this disorder. However, the abnormal VLDL phenotype appears to be dominantly expressed.—**Minnich, A., K. H. Weisgraber, Y. Newhouse, L-M. Dong, L-J. Fortin, M. Tremblay, and J. Davignon.** Identification and characterization of a novel apolipoprotein E variant, apolipoprotein E3' (Arg_{136}→His): association with mild dyslipidemia and double pre-β very low density lipoproteins. *J. Lipid Res.* 1995. **36:** 57–66.

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Apolipoprotein (apo) E mediates the binding of very low density lipoproteins (VLDL) and chylomicron remnants to the low density lipoprotein (LDL) and putative remnant receptors, thereby mediating the rapid removal of these lipoproteins from plasma (1). ApoE is polymorphic, with three common isoforms (apoE2, apoE3, and apoE4) that differ at two positions, residues 112 and 158, and that are designated according to their relative pI values. The most common isoform is apoE3, which contains cysteine and arginine, respectively, at the polymorphic sites; apoE2 and apoE4 contain cysteine and arginine at both sites (1). It is known that apoE isoforms influence plasma concentrations of cholesterol and LDL, with subjects with apoE4 having elevated levels compared to subjects without this isoform (2). In addition, it has been suggested that apoE isoform type is a determinant of risk for coronary artery disease (2). With respect to receptor-binding activity, apoE3 and apoE4 bind with equal affinity, while apoE2 is defective, displaying approximately 1% the binding activity of apoE3 and apoE4 (3).

Type III hyperlipoproteinemia (HLP) is a disorder characterized by the accumulation of chylomicron and VLDL remnants in plasma and premature atherosclerosis (4). Although homozygosity for apoE2 is necessary for

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; C, cholesterol; TG, triglyceride; DMPC, dimyristoylphosphatidylcholine; HLP, hyperlipoproteinemia; IEF, isoelectric focusing; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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the expression of type III HLP, it is not sufficient by itself. Additional genetic or environmental factors are required for type III HLP expression in apoE2 homozygotes, indicating a low level of penetrance and a recessive inheritance pattern with this isoform (4). However, a few rare apoE variants demonstrate a high degree of penetrance with dominant transmission of type III HLP (5–12). With one exception (13), all of the apoE variants associated with dominant expression involve substitutions of a basic amino acid within the receptor-binding region (residues 134–150).

The biochemical properties of several apoE variants, which confer dominant expression, have been described and include altered receptor binding activity, interaction with heparin, and lipoprotein distribution (14). To date, only apoE2 appears to be associated with recessive expression. This issue is of clinical interest due to the atherogenicity of the type III HLP phenotype. However, Yamamura et al. (15) also observed an association of mutant forms of apoE [apoE5, apoE7 (Glu_{244}→Lys, Glu_{245}→Lys)] with ischemic heart disease in the absence of type III HLP. Apart from these observations, the effects of apoE variants not associated with dominant expression of type III HLP on risk for atherosclerosis are not well studied.

At present, large-scale screening for apoE mutants is limited to detecting charge differences by isoelectric focusing (IEF). The extent to which this approach underestimates the frequency of point mutations in apoE is not known but is likely to be significant. Here we report the detection by isoelectric focusing of a new variant of apoE3, containing histidine substituted for arginine at position 136. This variant, referred to as apoE3' (Arg_{136}→His), displays slightly reduced affinity for both the LDL receptor and heparin. Evaluation of the proband's kindred indicates that this variant is associated with mild dyslipidemia, characterized by an abnormal VLDL electrophoretic pattern, likely associated with recessive expression of type III HLP, and possibly associated with increased risk for cardiovascular disease.

METHODS

Subjects

The proband (JB), a 42-year-old male, was referred to the lipid clinic at the Clinical Research Institute of Montreal for hypercholesterolemia and a family history of coronary artery disease on his paternal side. Four years before, he had consulted for palpitations, and a mitral valve prolapse was diagnosed. Except for palpitations, he was completely asymptomatic. He consumed a typical North American diet of 2800 kcal, 24% protein, 35% fat, 37% carbohydrates, and 4% alcohol, rich in refined sugar, saturated fat, and cholesterol (512 mg/day). On physical examination he measured 1.76 m and weighed 73.4 kg (BMI 23.6). His supine blood pressure was 112/70 with a heart rate of 60/min. His lipoprotein profile showed a moderate hyperlipidemia (cholesterol, 269; LDL-cholesterol, 176; triglyceride, 222 mg/dl). Fasting blood sugar was 79 mg/dl, and he had a mild elevation of γGT at 212 U/L (normal range = 10–75), tentatively ascribed to alcohol ingestion with mild steatosis, but no evidence of hypothyroidism, renal dysfunction, or obstructive liver disease. His uric acid was 4.6 mg/dl. There was no clinical manifestation of hyperlipidemia or atherosclerosis, and the only positive clinical finding was the presence of a mid-systolic click on cardiac examination. The ECG showed a brachycardia with nonspecific signs of delayed intraventricular conduction. There was no aortic calcification on X-rays of the chest and abdomen, and the abdominal echogram revealed no aortic dilation. An American Heart Association step II diet was prescribed, which resulted in a lowering of total plasma cholesterol from 269 to 219 mg/dl and of triglycerides from 222 to 70 mg/dl over a period of approximately 4 months.

Lipoprotein lipids and protein

Plasma was prepared from venous blood of fasting subjects by low-speed centrifugation at 4°C in the presence of 1.5 mg/ml EDTA. Lipoproteins were isolated by ultracentrifugation at d 1.006 g/ml to obtain VLDL, and by precipitation of apoB in the d > 1.006 g/ml fraction to separate LDL from high density lipoproteins (HDL) (16). Plasma and lipoprotein cholesterol and triglyceride (TG) concentrations were determined enzymatically on an automated analyzer (Abbott Biochromatic Analyzer model 100, Abbott Laboratories, Pasadena, CA). Plasma total and d > 1.006 g/ml apoB concentrations were determined by electroimmunoassay (17). Isoelectric focusing of VLDL apolipoproteins was performed according to the method of Bouthillier, Sing, and Davignon (18). Cysteamine and neuraminidase treatments were performed as described (18, 19). Bands were visualized by staining with Coomassie blue G-250, or by immunoblotting with a goat anti-human apoE antisera (supplied by International Immunology Corp., Marietta, CA) (20) or with monoclonal antibodies 6C5 and 1D7 (kindly provided by Drs. R. W. Milne and Y. L. Marcel) (21). Densitometric scanning of Coomassie blue-stained gels was performed with a model E-C densitometer (Mandel, St. Petersburg, FL) and integration of the resulting peaks with Chromatochart software (v. 2.0). Scanning of photographs was performed with an imaging densitometer (Model GS-670, Bio-Rad Laboratories, Richmond, CA) and integration of peaks with Image Analysis software (Bio-Rad). Plasma lipoproteins were subjected to agarose gel electrophoresis (22) with the Paragon Electrophoresis system (Beckman Instruments Inc., Fullerton, CA) and visualized by Sudan Black staining. The gels were scanned as above.

DNA analysis

DNA was extracted from white blood cells with an Applied Biosystems 340A automated extractor (Foster City, CA). The fourth exon of the apoE gene was sequenced by the method of Higuchi and Ochman (23). Briefly, a phosphorylated and a nonphosphorylated primer [nt 3513–3546 and 4342–4311, respectively (24)], encompassing the fourth exon of the apoE gene, were used to generate an 830-bp DNA fragment with the polymerase chain reaction (PCR). The phosphorylated strand was degraded with lambda exonuclease to produce single-stranded DNA as the sequencing template. Dideoxy chain-termination sequencing (25) was performed with 10% of the PCR product with internal primers. The mutation (see Results) was also detected with a modification of the *Hha*I cleavage method of Hixson and Vernier (26). An amplified segment (nt 3915–3795) of the fourth exon of the apoE gene was digested with *Hha*I restriction endonuclease. The resulting fragments were resolved on a 15% nondenaturing polyacrylamide gel in a Tris-borate-EDTA buffer system (27).

Isolation and characterization of apoE isoforms

Apolipoprotein E was purified from the d < 1.02 g/ml lipoproteins by Sephacryl S-300 HR gel chromatography, as described previously (28). The apoE3' (Arg_{136}→His) variant was isolated from the total apoE of a subject heterozygous for this isoform and apoE4 by covalent thiopropyl-Sepharose chromatography (28). Recombinant apoE•dimyristoylphosphatidylcholine (DMPC) complexes were prepared, and LDL receptor-binding assays were performed with the use of cultured fibroblasts, as described (29). Affinity to heparin was performed by applying purified apoE (50 μg) to a heparin-Sepharose column (30), equilibrated with 20 mM Tris-Cl (pH 7.5). The column was eluted at a rate of 0.5 ml/min with a linear gradient of 0.0–1.5 M NaCl in Tris-Cl (pH 7.5), and the eluate was monitored by UV absorbance at 280 nm.

RESULTS

Identification of the mutant apoE isoform

Isoelectric focusing of VLDL apolipoproteins from the proband (JB) revealed two prominent bands in the approximate position of apoE3 that, though separable, were much more closely spaced than the common apoE isoforms, i.e., apoE2, apoE3, and apoE4. Both bands were identified as apoE with specific anti-human apoE polyclonal and monoclonal antibodies (data not shown) and persisted after neuraminidase treatment (**Fig. 1**). Treatment with cysteamine resulted in both bands shifting one charge unit in the basic direction (Fig. 1), indicating that both apoE bands contain one cysteine residue and that

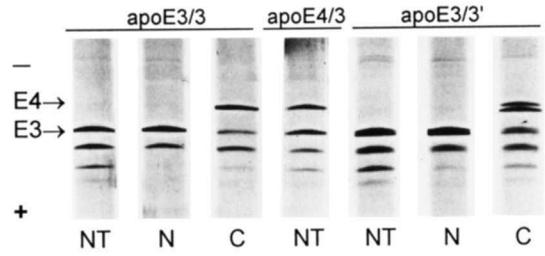

Fig. 1. Isoelectric focusing of VLDL apolipoprotein E. VLDL (150 μg) from subjects with apoE3/3, apoE4/3, and apoE3/3' phenotypes were electrophoresed in a pH gradient of 4–6 (bottom to top in figure). Lanes marked NT, N, and C contain apoVLDL not treated, treated with neuraminidase, and treated with cysteamine, respectively. Apolipoprotein E isoforms are indicated on the left-hand side of the figure.

one band was likely apoE3. Improved resolution of the bands, obtained by use of a pH gradient of 5–7, revealed that one band coelectrophoresed with apoE3 while the second was slightly more acidic (data not shown). Two-dimensional sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed the apparent M_r of the second apoE band to be similar to that of apoE3 (data not shown).

DNA sequencing of exon 4 of the apoE gene from the proband revealed that the subject was heterozygous for a G to A substitution at nucleotide 3818 within codon 136 [numbering as in (24)] (**Fig. 2**). This substitution would result in a missense mutation giving rise to a histidine for the normally present arginine at position 136 in the protein. The remainder of the sequence of exon 4 was identical to that of apoE3, indicating that the mutant differed from apoE3 at this single position and confirming that the other allele was a normal apoE3. The lower pKa of histidine relative to arginine also is consistent with the slightly more acidic pI of the variant observed on IEF gels. Thus, this new apoE isoform was designated apoE3' (Arg_{136}→His). The sequence of third exon of the apoE gene was identical to the published sequence (24).

DNA analysis and characterization of the JB kindred

Isoelectric focusing of apo VLDL revealed the apoE3/3' phenotype in six additional members of the JB family (**Fig. 3**). For family members with apoE4, the presence or absence of apoE3' was determined by co-focusing VLDL from an apoE3/3 homozygote.

As the G to A substitution in the mutant apoE gene from the proband eliminates a *Hha*I restriction site, enzymatically amplified DNA also could be screened for this mutation. With the primers used for amplification, the presence of a 44-bp DNA fragment after *Hha*I digestion indicates the presence of the mutation (**Fig. 4**). When family members were screened, the 44-bp fragment segregated with the double-band IEF pattern without exception (data not shown).

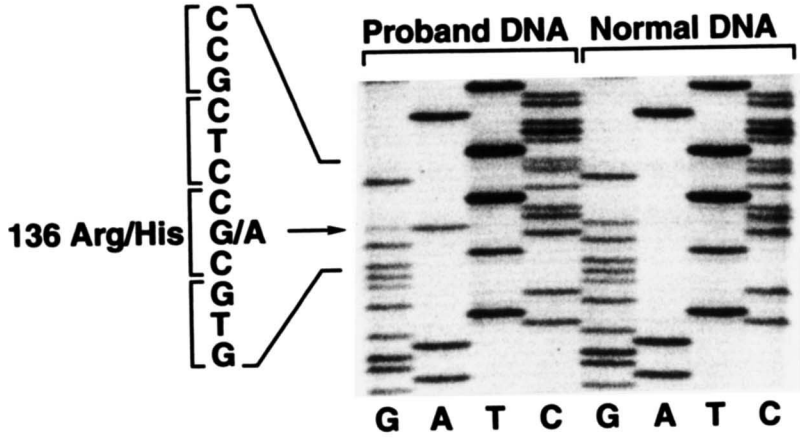

Fig. 2. DNA sequencing of the fourth exon of the apoE gene from proband and an apoE3/3 subject. Autoradiogram of a sequencing gel of genomic DNA from the proband and DNA from an apoE3/3 subject (normal) showing the mutation. The substitution at nucleotide 3818 in the proband DNA results in a histidine for arginine substitution at position 136 in apoE.

Plasma VLDL cholesterol (VLDL-C) and triglyceride concentrations were approximately doubled in subjects with either apoE3/3' or apoE4/3' phenotypes compared to those without the variant (**Table 1**). This difference was not due to differences in age (40 ± 6.4 vs. 42 ± 7.2 years for carriers and noncarriers, respectively) or sex (7 M, 2 F vs. 6 M, 3 F in carrier and noncarrier groups, respectively). Plasma VLDL apoB concentrations did not differ significantly between the two groups. In generation I, subject I-2 had a myocardial infarction at age 52, subjects I-3 and I-4 had coronary bypass surgery at ages 60 and 58, respectively, and subjects I-5, I-8, and I-9 died suddenly of cardiac arrest at ages 60, 57, and 64, respectively.

Agarose gel electrophoresis of fasting plasma from an apoE3' ($Arg_{136} \rightarrow His$) carrier revealed a broad β-pattern (**Fig. 5A**), while an abnormally migrating band was observed in isolated VLDL, compared to the normal position of LDL and VLDL (Fig. 5A). This band displays a mobility between the β-VLDL typically observed in type III HLP patients and the pre-β-VLDL in normal subjects

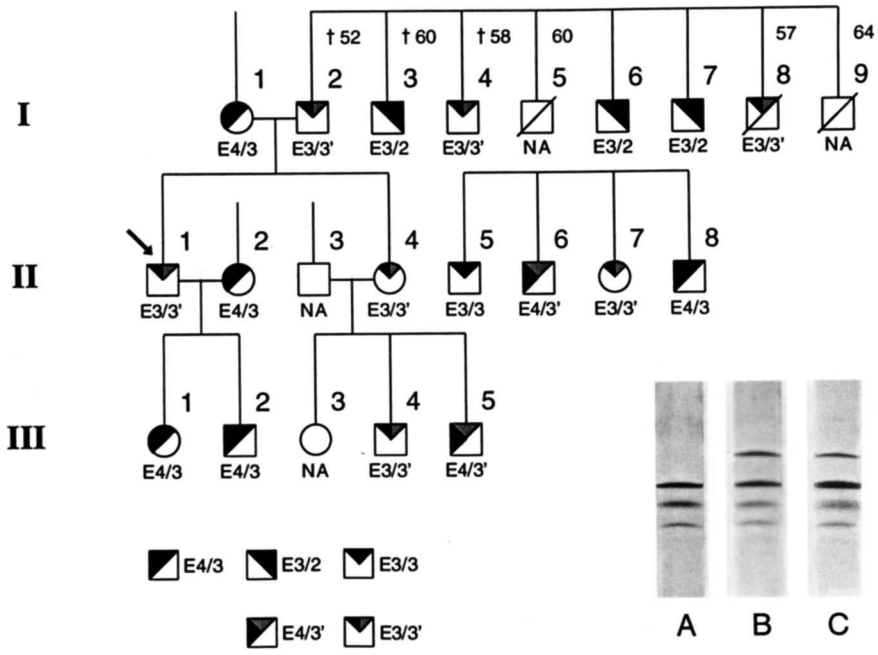

Fig. 3. Apolipoprotein E phenotype of JB kindred. Left panel, pedigree. The arrow indicates the proband, JB. Slashes through the symbols indicate that the subject is deceased. Crosses above symbols indicate evidence for cardiovascular disease, a myocardial infarction in I-2 and coronary bypass surgery in I-3 and I-4. Numbers above symbols indicate ages of death or cardiovascular events. Right panel, isoelectric focusing of VLDL apoE: A, from a subject with the apoE3/3 phenotype; B, from subject II-6; C, from a mixture of equal quantities of protein from A and B. The two closely spaced bands near the position of apoE3 in addition to the apoE4 indicate an apoE E4/3' phenotype.

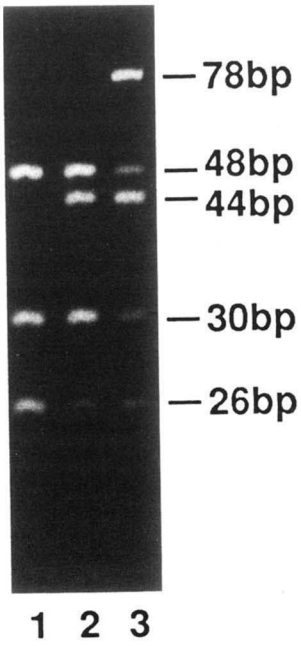

Fig. 4. *Hha*I digest of amplified DNA from an apoE3' (Arg_{136}→His) carrier and from a noncarrier. Exon 4 of the apoE gene was subjected to PCR between nucleotides 3601 and 3914. The amplified DNA was digested with *Hha*I and electrophoresed on a nondenaturing polyacrylamide gel: lane 1, a member of the JB kindred with an apoE3/3 phenotype; lane 2, proband JB; and lane 3, a subject with E2(Arg_{136}→Cys)/E2 (Arg_{158}→Cys).

(Fig. 5A). Within the JB kindred, this abnormal VLDL band was observed in all carriers of the apoE3' variant but not in noncarriers (Fig. 5B). As the VLDL-C/TG ratio of carriers was greater than 0.3 (characteristic of type III HLP) in fewer than half of the carriers, abnormal VLDL does not appear to represent typical β-VLDL, but rather double pre-β-VLDL. Thus, double pre-β-VLDL appears to mark the lipoprotein phenotype associated with apoE3' (Arg_{136}→His) in the JB kindred.

In vitro functional analysis of apoE3' (Arg_{136}→His)

In order to gain insight into whether the elevated VLDL levels and the abnormal VLDL migration pattern in carriers might result from a defective catabolism of the variant apoE, the relative quantities of the variant versus apoE3 or apoE4 were determined. The resolution of apoE3' (Arg_{136}→His) and apoE3 by densitometric scanning of IEF gels was not sufficient to allow accurate measurements; however, scanning of the photograph in Fig. 1 was successful and revealed an apoE3':apoE3 ratio of approximately 1.17, indicating more apoE3' than apoE3 on VLDL (**Fig. 6**). Densitometric scanning of IEF gels from VLDL of the two nonaffected subjects, II-8 and III-2, from the kindred with an apoE4/3 phenotype revealed apoE4:apoE3 ratios of 1.33 and 1.24, respectively, reflecting the higher affinity of apoE4 for VLDL compared to apoE3 (31–33). In contrast, the VLDL apoE4:apoE3' ratios for the two carriers with this phenotype, II-6 and III-5, were 0.70 and 0.71, respectively, indicating slightly more apoE3' than apoE4 (Fig. 6). In 245 unrelated French-Canadian patients from our lipid clinic, the VLDL apoE4:apoE3 ratio was 0.91 ± 0.16 (mean ± SEM).

The apparent higher levels of the mutant apoE3' (Arg_{136}→His) relative to apoE3 or apoE4 might be due to a greater affinity for VLDL. Alternatively, this apparent overabundance may be due to the slower clearance rate of the apoE3' (Arg_{136}→His) from plasma relative to apoE3 or apoE4. The latter possibility was tested by comparing the LDL receptor-binding activity of apoE3' (Arg_{136}→His) and apoE3 in fibroblasts (**Fig. 7**). The availability of a subject (II-6, Fig. 3) with an apoE4/3' (Arg_{136}→His) phenotype enabled the separation of the mutant from apoE4 by thiopropyl covalent chromatography. As judged by isoelectric focusing, the apoE3' variant was completely free of apoE4 (data not shown). The LDL-receptor binding of the apoE3' variant was approximately 80% of normal ($P < 0.002$), measured as the concentration of protein required to displace 50% of bound ^{125}I-labeled LDL from fibroblast receptors (Fig. 7). Thus, the apparent overabundance of apoE3' (Arg_{136}→His) in VLDL, and the abnormal lipoprotein phenotype's segregating with this variant, are associated with a slightly defective LDL receptor-binding activity.

As interactions between lipoproteins and heparin-like proteoglycans have been implicated in lipolysis of VLDL and in the clearance of chylomicron and VLDL remnants (34), the heparin affinities of the variant and normal apoE isoforms were compared, and purified apoE was subjected to heparin-Sepharose chromatography (**Fig. 8**). Normal apoE3 and apoE4 purified from plasma of subject II-6 (with an apoE4/E3' (Arg_{136}→His) phenotype) eluted at

TABLE 1. Plasma lipid, lipoprotein, and apoB concentration in JB kindred

	Affected Subjects	Nonaffected Subjects
Plasma TG	137 ± 21	77 ± 15
Plasma C	229 ± 16	198 ± 18
VLDL-C	40 ± 8[a]	19 ± 3
VLDL-TG	109 ± 19[a]	55 ± 13
VLDL-C/TG	0.29 ± 0.02	0.27 ± 0.02
LDL-C	139 ± 12	130 ± 16
HDL-C	49 ± 3	48 ± 2
Plasma apoB	119 ± 12	108 ± 14
LDL-apoB	101 ± 9	96 ± 10
VLDL apoB[b]	18 ± 4	12 ± 4

Affected subjects are subjects in kindred with apoE3'; nonaffected subjects are members of kindred who lack apoE3'. Results are expressed in mg/dl, and values are means ± SEM; n = 9 affected and 9 control subjects. C, cholesterol; TG, triglyceride.

[a] $P < 0.05$ vs. noncarriers by Student's *t*-test.

[b] VLDL apoB concentration was calculated by subtracting LDL apoB from total.

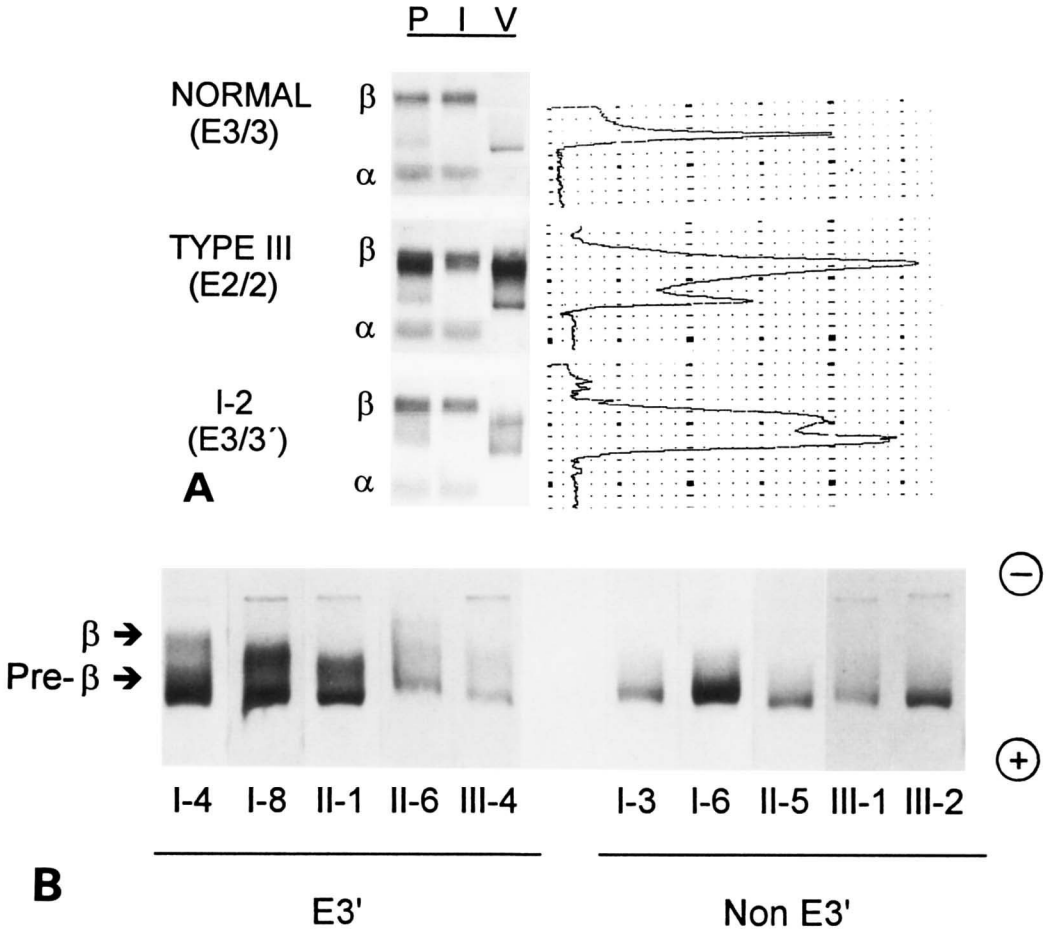

Fig. 5. Agarose gel electrophoresis of plasma lipoproteins. Panel A: left, Sudan black-stained lipoproteins after agarose gel electrophoresis; right, densitometric scan of electrophoresed VLDL. P, plasma; I, infranatant (d > 1.006 g/ml density fraction); V, VLDL (d < 1.006 g/ml fraction). This panel shows the mobility of the abnormal VLDL band in subject I-2 in juxtaposition with those of lipoproteins with known mobility: β, (lanes P and I in all subjects and lane V in a type III subject), α (lanes P and I for all subjects), and pre-β (lane V for a normal subject). Plasma VLDL cholesterol concentrations (mg/dl) and VLDL-C/TG (w/w) were 64 and 0.33 for an apoE3' (Arg_{136}→His) (apoE3') carrier (LB), 169 and 0.61 for a subject with type III HLP, and 18 and 0.22 for a normolipidemic subject (normal). Panel B shows the electrophoretic mobility of VLDL from 10 members of the JB kindred (indicated as in Fig. 3): 5 carriers of apoE3' and 5 noncarriers. Approximate β- and pre-β mobility were determined by comparison to whole plasma, as in panel A.

approximately 0.50 M NaCl, while the apoE3' variant eluted at 0.42 M. ApoE2 eluted at approximately 0.48 M. The results indicate that apoE2 binds heparin with slightly lower affinity than does apoE3 or apoE4, and that the apoE3' variant binds with lower affinity than do all three common apoE isoforms.

DISCUSSION

The present paper reports the detection and characterization of a naturally occurring point mutation in apoE. Isoelectric focusing of native and neuraminidase-treated VLDL apolipoproteins from the proband revealed two very closely spaced bands, one migrating in the position of apoE3 and the other one slightly more acidic. Both bands reacted with anti-apoE antibodies, and cysteamine treatment revealed that the abnormal band contained one cysteine residue, as does apoE3. The relationship of the more acidic isoform with apoE3 was confirmed by sequencing PCR-amplified genomic DNA from the proband. Sequencing revealed that the subject was heterozygous for a G to A substitution at nucleotide 3818. This substitution results in substitution of histidine for the normally present arginine at position 136. This new apoE variant was therefore designated apoE3' (Arg_{136}→His). Isoelectric focusing of VLDL from 17 members of the proband's family revealed 8 additional carriers of apoE3' (Arg_{136}→His). The mutation also could be identified by loss of a *Hha*I restriction site and in every case, the DNA mutation segregated with the presence of the apoE3' (Arg_{136}→His) protein isoform.

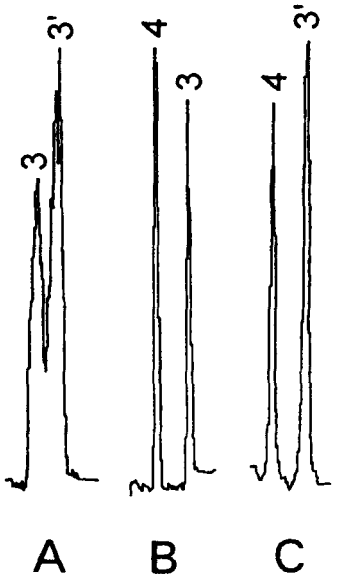

Fig. 6. Densitometric scanning of apoE isoforms in VLDL. VLDL was subjected to isoelectric focusing and the photographs of the gels were subjected to densitometric scanning as described in Methods: lane A, subject with apoE3/3' (Arg_{136}→His) phenotype; lane B, apoE4/3; lane C, apoE4/3' (Arg_{136}→His).

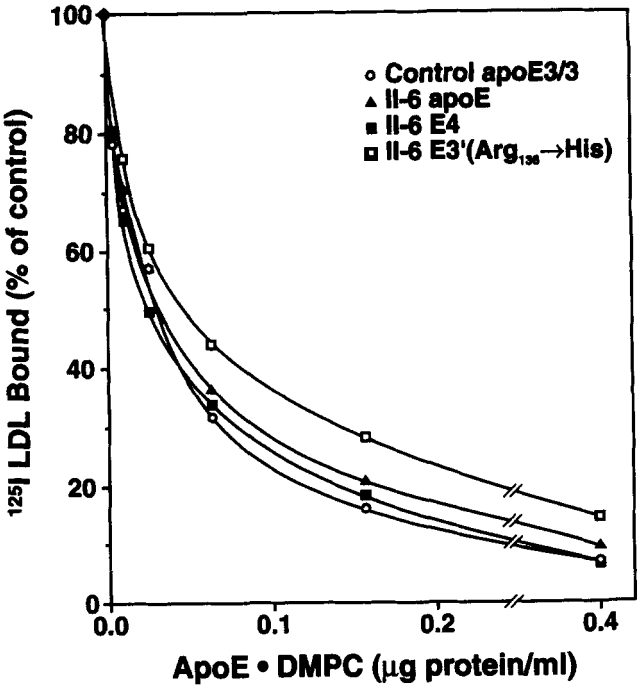

Fig. 7. Binding of apoE • DMPC complexes to LDL receptors. Apolipoprotein E was isolated from plasma of a control subject with an apoE3/3 phenotype and from subject II-6 (Fig. 3) with the apoE4/3' (Arg_{136}→His) phenotype. Complexes were prepared from DMPC and total apoE (II-6 apoE, Control apoE3/3) or mutant (II-6 apoE3' (Arg_{136}→His) and normal (II-6 apoE4) purified from VLDL of subject II-6. Binding to cultured human fibroblasts at 4°C was determined by competition for ^{125}I-labeled human LDL for each preparation of complexes. Each point represents the average of three different determinations performed in duplicate. Values from the three experiments were normalized to control apoE3 set at 100% and are expressed as percent of control. Mean values of the amount of apoE4 vs. apoE3' (Arg_{136}→His) from subject II-6 to displace 50% of bound ^{125}I-labeled LDL were compared with a paired *t*-test.

The mutant apoE allele displays vertical transmission through three generations within the JB kindred. Heterozygous carriers displayed approximately twice the VLDL cholesterol and triglyceride plasma concentrations compared to unaffected individuals. An abnormal VLDL with electrophoretic mobility between that of β-VLDL and pre-β-VLDL was observed in every carrier of the apoE variant, but in none of the unaffected subjects. These two aspects, therefore, mark the lipoprotein phenotype segregating with the mutant apoE. The presence of β-VLDL is also frequently noted in apoE2 homozygotes, even in the absence of type III HLP (J. Davignon, unpublished data). Although no apoE3' (Arg_{136}→His) homozygotes were detected, the absence of clinical features of type III HLP, e.g., palmar xanthomas, suggests that the mutation may confer recessive expression of type III HLP similar to the expression conferred by apoE2. However, it is interesting that the abnormal VLDL phenotype appears to be dominantly expressed in carriers of this variant.

Several lines of evidence indicate the importance of arginine-136 for physiological function of apoE. This residue is conserved in eight of nine species (bovine: histidine) (35), and the $apoE2_{Christchurch}$(Arg_{136}→Ser) appears to segregate with dyslipidemia (35). Finally, site-directed mutagenesis of arginine-136 to serine results in defective LDL receptor-binding activity (40% of normal) (29). Binding of apoE to the LDL and putative remnant receptors is thought to be mediated by basic arginines and ly-

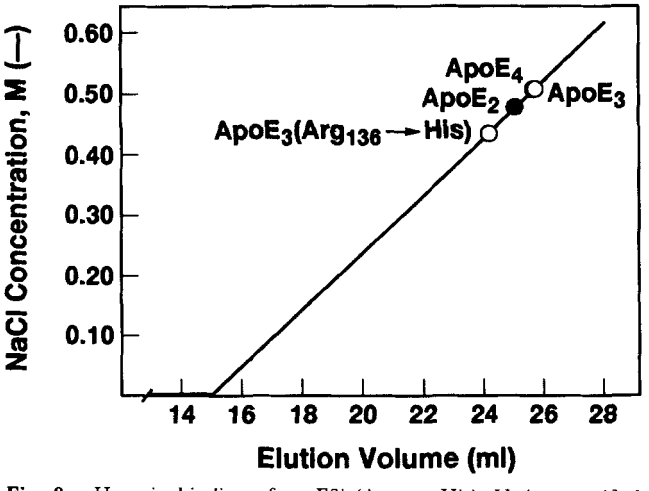

Fig. 8. Heparin binding of apoE3' (Arg_{136}→His). Various purified apoEs (50 μg) were applied to a heparin-Sepharose affinity column equilibrated with 20 mM Tris-Cl, pH 7.5. The column was eluted with a linear salt gradient from 0.0 M to 1.0 M NaCl in 20 mM Tris-Cl buffer, pH 7.5. Elution was monitored at 280 nM.

sines in the vicinity of residues 134–150 (36). The effect of the Arg_{136}→Ser mutation has been ascribed to disturbances of ionic interactions between a basic residue on apoE and acidic residues in the ligand-binding domain of the LDL receptor (29).

The slightly more acidic focusing position of the variant relative to apoE3 is consistent with the slightly lower pKa of histidine, which would not be expected to display a full charge difference. Densitometric scanning of isoelectric focusing gels of VLDL from two carriers and two noncarriers revealed a relatively greater amount of the apoE3' variant relative to apoE3 and apoE4. The higher levels of the apoE3' variant relative to apoE4 in the VLDL of carriers suggests either that this isoform is less efficiently catabolized in vivo than is apoE4, or that it has higher affinity for VLDL. The apoE3' variant displayed slightly defective binding ability to the LDL receptor (80% of normal), consistent with the former hypothesis.

Observations of defective lipolysis of VLDL from type III HLP subjects (37, 38) suggest other factors in addition to impaired LDL receptor-binding activity in the etiology of the dysbetalipoproteinemia. Recently it has become apparent that other factors do exist. Such functions include the binding of lipoproteins to cell-surface heparin-like proteoglycans (39–41), which is likely related to a role in mediating triglyceride lipolysis by lipoprotein or hepatic lipase or in the initial trapping and presentation of lipoproteins to be catabolized by hepatic receptors. Thus, a possible explanation for the observed dyslipidemia associated with the apoE3' (Arg_{136}→His), which displays only slightly defective LDL-receptor binding, is that this mutation also interferes with other apoE functions, such as interaction with heparin-like structures associated with lipolysis (42). The consistent observation of abnormal lipoproteins with electrophoretic mobility more similar to pre-β-VLDL than to the β-VLDL typically associated with binding-defective apoE also suggests that a mild lipolytic defect may also contribute to the unusual lipoprotein phenotype in carriers of apoE3' (Arg_{136}→His). Consistent with this hypothesis, the apoE3' variant displayed reduced affinity for heparin compared to apoE3 or apoE4. This observation, along with the observed binding defect, suggests that the presence of this apoE variant may impair VLDL catabolism.

Other apoE variants associated with dominant inheritance of dyslipidemia display the following biochemical characteristics, all of which may contribute to their association with dominant expression of type III HLP. With one exception, all contain a substitution of a nonbasic residue for arginine or lysine between residues 143 and 150 of apoE (5, 7–9, 13) and display impaired LDL-receptor binding. Unlike apoE2, these defects cannot be ameliorated by biochemical modification such as charge alteration with cysteamine (9), removal of the carboxyl terminus (7, 43), or lipid modification (44), implicating the loss of specific interactions by the specific substituted residues rather than a conformational effect as in apoE2. Some (6, 7), derived from apoE4, display increased affinity for VLDL, as does apoE4, and others display reduced heparin affinity (7, 11).

Most recent data have shown that two apoE variants known to confer dominant expression of type III HLP fail to enhance β-VLDL binding to cell-surface heparan sulfate proteoglycans (45), consistent with their low affinity for heparin. As these aspects of known dominant apoE variants differ from apoE2, which displays only slightly defective heparin binding and is associated with recessive inheritance of type III HLP, they possibly constitute a biochemical basis for the dominant inheritance of dyslipidemia (14). Specifically, these observations suggest that heparin affinity may serve as one of the biochemical markers for dominant or recessive inheritance of type III HLP associated with apoE variants. The apoE3' (Arg_{136}→His) displays only slightly reduced LDL-receptor binding and heparin affinity. The phenotype of apoE3' carriers differs from the known dominant variants with respect to the VLDL electrophoretic pattern and degree of hyperlipidemia. The relatively mild effects of the apoE3' variant indicate that the substituted histidine, although less basic, may partially perform the functions of arginine-136.

Schmitz et al. (46) have observed that the proportion of β-VLDL in apoE2/2 homozygotes did not correlate with severity of hypertriglyceridemia. Thus, it is possible that the composition of VLDL, as well as hyperlipidemia per se, may be an important risk factor in type III HLP or in carriers of abnormal apoE isoforms, and that the abnormal VLDL in apoE3' (Arg_{136}→His) carriers, like β-VLDL (9), may bind avidly to macrophages. The apoE3' (Arg_{136}→His) (previously referred to as $apoE3'_{Montreal}$ (47)) appears to be associated with premature cardiovascular disease in the JB kindred; however, confirmation of this possibility awaits the identification of additional carriers in other kindreds.

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