The Functional Characteristics of a Human Apolipoprotein E Variant (Cysteine at Residue 142) May Explain Its Association with Dominant Expression of Type III Hyperlipoproteinemia

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Type III hyperlipoproteinemia typically is associated with homozygosity for apolipoprotein (apo) E2(Arg$^{158}$ → Cys). Dominant expression of type III hyperlipoproteinemia associated with apoE phenotype E3/3 is caused by heterozygosity for a human apoE variant, apoE3(Cys$^{112}$ → Arg, Arg$^{142}$ → Cys). However, this apoE variant was not separable from the normal apoE3 in these patients' plasma because the two proteins have identical amino acid composition, charge, and molecular weight. Therefore, to determine the functional characteristics of this protein, we used recombinant DNA techniques to produce this apoE variant in bacteria. We also produced a non-naturally occurring variant, apoE(Arg$^{142}$ → Cys), that had only the cysteine substituted at residue 142. These two apoE variants were purified from cell lysates of the transfected Escherichia coli by ultracentrifugal flotation in the presence of phospholipid, by gel filtration chromatography, and by heparin-Sepharose chromatography. Both Cys$^{142}$ apoE variants bound to lipoprotein receptors on human fibroblasts with only about 20% of normal binding activity. Therefore, cysteine at residue 142, not arginine at residue 112, is responsible for the decreased receptor binding activity of the variants. Cysteamine treatment and removal of the carboxyl-terminal domain had little effect on the binding activity, whereas both modulate the receptor binding activity of apoE2(Arg$^{158}$ → Cys). The mutation at residue 142 decreased the binding activity of apoE to both heparin and the monoclonal antibody 1D7 (this antibody inhibits receptor binding of apoE), whereas apoE2(Arg$^{158}$ → Cys), which is associated with recessive expression of type III hyperlipoproteinemia, binds normally to both. The Arg$^{112}$, Cys$^{142}$ variant predominates 3:1 over normal apoE3 in the very low density lipoproteins of plasma from an affected subject, as assessed by differential reactivity with the antibody 1D7. The unique combination of functional properties of the Arg$^{112}$, Cys$^{142}$ variant provides a possible explanation for its association with dominant expression of type III hyperlipoproteinemia.

Human apolipoprotein (apo)$^1$ E, a 299-residue apolipoprotein that is a component of several classes of lipoproteins, plays a key role in the receptor-mediated uptake of these lipoproteins (1). The three major alleles of apoE code for three major isoforms (E2, E3, E4), which can be distinguished by isoelectric focusing (2); apoE3 is the most frequent and is considered the wild type, and apoE4 and -E2 are considered variants (3). Whereas apoE3 and apoE4 bind normally to receptors, apoE2 is defective in interacting with receptors, and this dysfunction has been shown to be an underlying cause of type III hyperlipoproteinemia (3). This protein, apoE2(Arg$^{158}$ → Cys), has only 1% of the normal activity in in vitro assays (4). Type III hyperlipoproteinemia is almost invariably associated with homozygosity for apoE2(Arg$^{158}$ → Cys); however, while about 1% of all individuals have the E2/2 phenotype, only 2-10% of them actually develop type III hyperlipoproteinemia (5). This indicates that other factors are required for overt expression of the hyperlipidemia (3, 5).

In 1983, Havel et al. (6) described members of a family of Salvadoran origin who had type III hyperlipoproteinemia but whose apoE phenotype was E3/3. In 1989, Rall et al. (7) reported that this type III hyperlipoproteinemia is caused by heterozygosity for an apoE3 variant with arginine at residue 112 and cysteine at residue 142 (Cys$^{112}$ → Arg, Arg$^{142}$ → Cys). All the subjects with this variant have β-very low density lipoproteins and other characteristics of type III hyperlipoproteinemia in spite of the presence of normal E3. The transmission of this disorder is apparently a dominant trait, whereas typical type III hyperlipoproteinemia results from the inheritance of two alleles for apoE2(Arg$^{158}$ → Cys) and therefore appears to be a recessive trait.

Rall et al. (7) demonstrated that in patients with the Arg$^{112}$, Cys$^{142}$ variant the total apoE from the d < 1.02 g/ml lipoprotein fraction of their plasma has about 20% of normal binding activity. However, the variant apoE could not be separated from normal apoE3 because both have an identical amino acid composition, charge, and molecular weight. Therefore, to determine the characteristics of this variant, it was necessary to produce this protein by recombinant DNA techniques.

In this study we produced this apoE variant by expression in bacteria and investigated its binding activity to the low density lipoprotein (LDL) receptor. We also tested its binding affinity for heparin and monoclonal antibody 1D7 (which inhibits receptor binding activity of apoE) because their binding sites are located in the region of residues 140-150 (8, 9), and arginine at residue 142 could be one of the most important

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$^*$ This work was supported in part by National Institutes of Health Program Project Grant HL41633. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: apo, apolipoprotein; VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); bp, base pair(s); DMPC, dimyristoylphosphatidylcholine; IDL, intermediate density lipoprotein(s); HDL, high density lipoprotein(s).

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residues for these interactions. We report that the substitution of cysteine for arginine at residue 142 in apoE causes a reduction in binding to the LDL receptor and to heparin and antibody 1D7. We also demonstrate that the Arg

apoE variant predominates in the very low density lipoproteins (VLDL) from the plasma of an affected subject.

**MATERIALS AND METHODS**

**Construction of Human Apolipoprotein E Variant Expression Vectors**—Two plasmids were designed: one coded for the naturally occurring variant (Cys

amino acids 112 and 142 when arginine is present at these sites (13). Therefore, the HhaI genotyping method for apoE (13) can be performed as described (16). Charge modification with cysteamine was performed as described (16).

**Receptor Binding Assays**—Receptor binding assays of isolated apoE-dimyristoylphosphatidylcholine (DMPC) complexes were performed using cultured human fibroblasts with 

**Heparin Binding and Monoclonal Antibody 1D7 Binding**—The affinity of apoE for heparin was measured as the concentration of NH4HCO3, at which the protein eluted from a heparin-Sepharose column (9). Briefly, the test protein was bound to the heparin-Sepharose column, washed with 15 mM NH4HCO3 containing 0.1% β-mercaptoethanol, and eluted with a 15–750 mM NH4HCO3 gradient containing 0.1% β-mercaptoethanol. The elution profile was recorded at 280 nm, and 2-ml samples were collected. The concentration of NH4HCO3 was calculated from the conductivity of the fractionated sample.

**Lipoprotein Distribution of the Apolipoprotein E Variant in the Plasma of an Affected Subject**—Plasma was obtained from one of the affected subjects (III-4 in Refs. 6 and 7) by centrifugation at 2000 rpm for 15 min at 4 °C. A 200-μl sample of plasma was subjected to

FIG. 1. Isotyping of apoE by HhaI digestion of polymerase chain reaction-amplified DNA. A, schematic representation of expected fragments representing three isotypes of apoE. HhaI digestion sites are shown by inverted triangles, and the relevant correspondence amino acid sites in the DNA are indicated. Fragments smaller than 20 bp are not included. B, polyacrylamide gel (15%) electrophoresis of HhaI-digested amplified DNA. Lane 1, subcloned patient DNA (Arg

sulfate-polyacrylamide gel electrophoresis, were performed as described (16). Charge modification with cysteamine was performed as described (16).

**Receptor Binding Assays**—Receptor binding assays of isolated apoE-dimyristoylphosphatidylcholine (DMPC) complexes were performed using cultured human fibroblasts with 125I-LDL as competitor (17). Charge modification with cysteamine was performed as described (18). Either bacterially produced apoE3 or apoE3 obtained from human plasma was used as a control because both have nearly identical receptor binding activities (12). Direct binding assays were performed as described (19), using apoE radiolabeled to specific activities similar to those previously reported (19).

**Heparin Binding and Monoclonal Antibody 1D7 Binding**—The affinity of apoE for heparin was measured as the concentration of NH4HCO3, at which the protein eluted from a heparin-Sepharose column (9). Briefly, the test protein was bound to the heparin-Sepharose column, washed with 15 mM NH4HCO3 containing 0.1% β-mercaptoethanol, and eluted with a 15–750 mM NH4HCO3 gradient containing 0.1% β-mercaptoethanol. The elution profile was recorded at 280 nm, and 2-ml samples were collected. The concentration of NH4HCO3 was calculated from the conductivity of the fractionated sample.

**Binding Affinity**—The binding affinity of the 22-kDa fragment of the variant apoE for monoclonal antibody 1D7 was measured in a competitive binding assay against the 22-kDa fragment of normal apoE3, which was coated in the wells as described (8). Immunoblot analysis with 1D7 was conducted using 125I-sheep anti-mouse immunoglobulin G as a second antibody.

For both heparin binding and IDT competition experiments, bacterially produced 22-kDa fragments were used. This was done to avoid any possible difficulties due to the intact protein being tetrameric in free solution (the 22-kDa fragment is monomeric) (20) or to the existence of a second heparin-binding site in the carboxyl-terminal domain (9).
Superoxide 6 chromatography (1 × 30 cm, Pharmacia LKB Biotechnology Inc.). Lipoproteins were separated at a flow rate of 0.5 ml/min at ambient temperature in 150 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA, pH 7.4. Fifty-five 0.5-ml fractions were collected. The VLDL, intermediate density lipoprotein (IDL)/LDL, and high density lipoprotein (HDL) fractions were determined by measurement of cholesterol (Spectrum cholesterol reagent, Abbott Laboratories) and triglycerides (Triglycerides GPO Reagent Set, Boehringer Mannheim). The pooled VLDL, IDL/LDL, and HDL fractions were concentrated using Centricon filters (Amicon). Lipid-binding proteins were precipitated with Cab-O-Sil (Sigma) and eluted with gel loading buffer (21). Approximately equal amounts of total apoE from each fraction were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel (12%). Proteins were transferred to nitrocellulose and immunoblotted with antibody 1D7 and 125I-sheep anti-mouse immunoglobulin G and autoradiographed for 2 h. The autoradiographs were then subjected to densitometric analysis.

RESULTS

Purification of Apolipoprotein E Variants and Protein Analysis—The intact apoE variants were purified as summarized in Fig. 2A. In the bacterial pellet, an apoE band could not be distinguished clearly (lane 1). After phospholipid flotation, apoE became a major component (lane 2). Pure apoE was obtained after further steps of gel filtration (lane 3) and heparin affinity chromatography (lane 4). The 22-kDa fragment was purified by the same methods (Fig. 2B); however, it did not need the final heparin affinity chromatography step because the 22-kDa fragment of the apoE variant was pure after gel filtration (Fig. 2B, lane 3). To ascertain the isoelectric focusing position and the number of cysteines of these variants, isoelectric focusing was performed without and with cysteamine treatment. These variants showed the expected charge and cysteine number (data not shown).

Receptor Binding Assays—Receptor binding activities of the variants were determined in an in vitro competition assay, using the 50% competition point from logit-log plots of the binding data as a measure of activity. Both the naturally occurring Arg112, Cys142 variant and the non-naturally occurring Cys112, Cys142 variant were defective in binding to lipoprotein receptors on cultured human fibroblasts. A representative competition experiment is shown in Fig. 3, and a summary of all experiments is presented in Table I. The Arg112, Cys142 variant averaged 21% of normal binding, and the Cys112, Cys142 variant slightly less; the difference was mostly due to one experiment (no. 2 in Table I).

Direct binding assays were performed with 125I-apoE/DMPC complexes to confirm the competition data. From Scatchard analysis of the binding data, normal apoE had a \( K_d = 0.10 \text{nM} \), the same as previously reported by Pitas et al. (19). In contrast, the Arg112, Cys142 variant had a \( K_d = 0.27 \text{nM} \) and the Cys112, Cys142 variant had a \( K_d = 0.24 \text{nM} \). The variants had slightly higher \( B_{max} \) compared with normal apoE (1.4 ng/35-mm dish versus 1.1 ng/35-mm dish). Therefore, the variants display a reduced affinity for binding to LDL receptors.

We also measured the receptor binding activities of a 1:1 mixture of each variant apoE and normal E3 because Rall et al. (7) had shown that the total apoE purified from the d < 1.02 g/ml lipoprotein fraction from the plasma of patients had 19–26% of normal activity. This artificial 1:1 mixture exhibited only ~40% of control activity (Table I), although this activity was higher than that of the previously reported plasma mixture. To determine whether the manner by which the mixture was prepared had an effect on receptor binding activity, we made two further 1:1 mixtures: a 1:1 protein mixture that had been denatured by guanidine before recombination with DMPC, and a 1:1 mixture of DMPC complexes previously formed with either apoE3 or the apoE variant. All three mixtures bound almost equally, indicating that the method of mixing had no impact on binding activity (data not shown).

To assess the effect of cysteamine treatment on receptor binding, the apoE variants were incubated with cysteamine before being complexed with DMPC. Modifications were verified by isoelectric focusing (data not shown). Cysteamine treatment increased the binding activity of the Arg112, Cys142.

![Fig. 2](image-url)  
**Fig. 2.** Coomassie Blue-stained sodium dodecyl sulfate-polyacrylamide gel of purification steps of apoE variants. A, apoE variant with Cys112, Cys142. Lane 1, fermented material; lane 2, after dipalmitoylphosphatidylcholine flotation; lane 3, after Sephacryl S-300 gel filtration chromatography; lane 4, after heparin-Sepharose chromatography. The molecular weight markers are in the far left lane. B, 22-kDa fragment of apoE variant with Arg112, Cys142. Lane 1, fermented material; lane 2, after dipalmitoylphosphatidylcholine flotation; lane 3, after gel filtration; lane 4, authentic 22-kDa fragment of apoE3 (indicated by 22K).

![Fig. 3](image-url)  
**Fig. 3.** Receptor binding activities of apoE variants. The receptor binding activities of apoE variants were determined by competition for 125I-human LDL binding to cultured human fibroblast receptors at 4°C. A, apoE variant with Cys112, Cys142; C, apoE variant with Arg112, Cys142; O, apoE3 (control).
variant by 68% (n = 2) and of the Cys112, Cys142 variant by 45% (n = 2), values similar to the 30% activation for control apoE3 (4). Thus, cysteamine has little effect on these variants, in marked contrast to its 14-fold effect (n
apoE3 (4). Thus, cysteamine has little effect on these variants, in marked contrast to its 14-fold effect (n = 1) on apoE2, a finding in agreement with previous data (4, 18).

To assess the effect of removing the carboxyl-terminal domain, purified 22-kDa fragments were tested for receptor binding activity. The binding activity of the 22-kDa fragment of the Arg15**, Cys142 variant was only 47% (n = 3) of that of the normal apoE3 22-kDa fragment. Cysteamine treatment of the variant 22-kDa fragment did not increase the activity significantly (21%) (n = 3). This also contrasts markedly with cysteamine treatment of the 22-kDa fragment of apoE2 (Arg156
Cys) whose binding activity increased over 6-fold (i.e. to normal) after cysteamine treatment (18).

Heparin Binding Affinity—To assess the effect of the mutation at residue 142 on heparin binding, heparin-Sepharose gradient chromatography of the 22-kDa fragments of normal apoE3 and the Arg1**, Cys142 variant was conducted. The 22-kDa fragment of normal apoE3 eluted at a salt concentration of 105 mM NH4HCO3 (Fig. 4, upper panel), whereas the variant 22-kDa fragment eluted at 63 mM NH4HCO3 (Fig. 4, lower panel). This indicated that the presence of Cys142 leads to a weaker affinity for heparin and that Arg142 is a major contributor to apoE-heparin binding.

Antibody 1D7 Binding Affinity—To assess the effect of the mutation at residue 142 on the binding to monoclonal antibody 1D7, a competition study was performed using antibody 1D7 and the 22-kDa fragments of apoE. The mutation at residue 142 substantially decreased the binding activity of apoE to 1D7 (Fig. 5A). The concentration at which the Arg15**, Cys142 variant 22-kDa fragment displaced 50% of the 1D7 from the immobilized apoE3 22-kDa fragment was 23.2 μg/ml (n = 4), compared with 0.81 μg/ml (n = 4) for the 22-kDa fragment of normal apoE3. Therefore, this apoE variant has only about 3.5% of normal affinity for antibody 1D7. Furthermore, immunoblot analysis indicated that antibody 1D7 failed to detect the variant apoE2 22-kDa fragment under the incubation and washing conditions used (Fig. 5B).

Lipoprotein Distribution of the Arg1**, Cys142 Variant—To measure the relative amounts of the Arg1**, Cys142 variant and normal apoE3 directly in the lipoprotein fractions from a type III hyperlipoproteinemic subject heterozygous for the variant (7), we took advantage of the immunoreactivity difference of the variant for antibody 1D7 (see Fig. 5). As shown in Fig. 6, antibody 1D7, which detects essentially only normal apoE, detected much less apoE in the VLDL fraction compared with either the IDL/LDL or HDL fractions. The polyclonal antibody, which detects equally the variant and normal apoE, was used to normalize any differences in the total amount of apoE among fractions (Fig. 6). Densitometric comparisons indicated that normal apoE3 accounted for 27% of the total apoE in VLDL, 50% in IDL/LDL, and 55% in HDL. Therefore, the Arg1**, Cys142 variant is overrepresented in the VLDL fraction from the type III hyperlipoproteinemic subject, there being almost three times more variant than normal apoE3 in this fraction.

DISCUSSION

A human apoE variant with cysteine substituted for arginine at residue 142 is known to be associated with dominant transmission of the genetic lipid disorder type III hyperlipoproteinemia (7). We have produced this variant in bacteria and investigated the impact of this particular substitution on several important interactions: apoE binding to LDL receptors; its affinity for the monoclonal antibody 1D7, which blocks apoE binding to those receptors; and its affinity for heparin. Arginine 142 is one in a cluster of six basic residues in the 140–150 region of apoE that has previously been implicated in these interactions (1, 8, 9). Crystallographic analysis has shown that these basic residues occur in an
stition at residue 142 has a profound effect on all three interactions. This substitution reduces receptor binding to about 20% that of normal apoE3. Because the naturally occurring human variant also has a substitution of arginine for cysteine at residue 112, we also produced a Cys112, Cys142 variant so that we also could assess the role of residue 112 in receptor binding. This latter variant was only slightly less active than the Arg112, Cys142 variant, indicating that most, if not all, of the receptor binding defect is due to the presence of Cys142 and not to the substitution at residue 112. This result is in agreement with previous findings that showed that there was no difference in receptor binding between apoE3 (cysteine at residue 112) and apoE4 (arginine at residue 112) (4). With the exception of apoE2(Arg58→Cys), which has about 1% of normal activity, the ~20% receptor binding activity of the Arg112, Cys142 apoE variant is the lowest of the naturally occurring variants. Using site-specific mutagenesis, Lalazar et al. (15) reported two apoE variants that have lower binding activity than these variants with Cys142: one with a substitution of alanine for lysine at residue 143 (9%), and the other with proline for leucine at residue 144 (13%). This suggests that residues 142–144 are especially crucial for binding to the LDL receptor.

We also determined the receptor binding activities of the cysteamine-treated Arg112, Cys142 variant and its 22-kDa fragment, because cysteamine treatment and removal of the carboxyl-terminal domain have a profound effect on the receptor binding activity of apoE2(Arg58→Cys) (18). Both of these modifications enhanced the receptor binding of apoE2(Arg58→Cys) by an order of magnitude, and the cysteamine-treated 22-kDa fragment of apoE2(Arg58→Cys) actually had normal binding (18). In contrast, the binding activity of the Arg112, Cys142 variant was enhanced relatively little by either modification, and cysteamine treatment of its 22-kDa fragment failed to normalize binding activity. This relative lack of susceptibility to modulation of receptor binding activity supports the notion that the cluster of basic residues in the 140–150 region binds through direct ionic interaction with the negatively charged residues of the ligand-binding domain of the LDL receptor (1), whereas the substitution of cysteine at residue 158 in apoE2(Arg58→Cys) indirectly affects receptor binding by local conformational perturbations (18).

It has been reported that the total apoE from the subjects heterozygous for the Arg112, Cys142 variant had 19–26% of normal receptor binding activity (7). Based only on peptide yields from sequence data, Rall et al. (7) inferred that the total apoE isolated from the d < 1.02 g/ml lipoproteins from the plasma of subjects with this variant was approximately a 1:1 mixture of the variant and normal apoE3. Because our results indicated that the isolated Arg112, Cys142 variant had 20% of normal binding by itself, we also determined the binding activity of 1:1 mixtures of the variant and normal apoE3. These artificial mixtures had approximately double the binding activity of the variant alone, suggesting that it is unlikely that the total apoE from the subjects is in a 1:1 ratio; instead, it is probable that the defective variant apoE predominates, at least in the d < 1.02 g/ml lipoproteins, in spite of the previous estimation (7). This conclusion was confirmed directly by the data in Fig. 6, in which the defective variant apoE predominates almost 3:1 over normal apoE3 only in the VLDL fraction from the plasma of an affected subject.

We have demonstrated that the substitution at residue 142 also reduces apoE affinity for heparin (Fig. 4). Previously, Weisgraber et al. (9) showed that the high affinity heparin-binding site on apoE coincided with the receptor-binding site, with the cluster of basic residues again being implicated as

### FIG. 5. Effect of the mutation at residue 142 on the immunoreactivity with monoclonal antibody 1D7. A, competition analysis. The binding of the 22-kDa fragments of apoE to antibody 1D7 was determined by competition for the 22-kDa fragment of apoE3 coated in the well. The data are presented in the logit-log transformation. B, 22-kDa fragment of Arg112, Cys142 variant; C, 22-kDa fragment of normal apoE3. B, immunoblot analysis. Five micrograms of the 22-kDa fragment of apoE was applied to each lane. After staining with Coomassie Blue, the protein in the gel was transferred to nitrocellulose paper. Transferred proteins were incubated with antibody 1D7 followed by 125I-sheep antibody against mouse immunoglobulin G. Autoradiography was performed for 16 h.

### FIG. 6. Distribution of the Arg112, Cys142 variant among an affected subject’s plasma lipoproteins. Plasma was fractionated on a Superose 6 column. The resultant VLDL, LDL/LDL, and HDL fractions were pooled, concentrated, and the apoproteins precipitated with Cab-O-Sil. After dissolution in gel running buffer, the proteins were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel (12%) and blotted to a nitrocellulose filter. After incubation with antibody 1D7 and autoradiography for 16 h (upper panel), the filter was incubated with the polyclonal antibody and autoradiographed for 2 h (lower panel). Because of the much shorter exposure time required for the polyclonal antibody, the contribution of radioactivity from the 1D7 incubation (upper panel) contributes only minimally to the radioactivity in the lower panel. The band below apoE in the upper panel is recognized nonspecifically by the 125I-sheep anti-mouse secondary antibody since that band is present when incubation with 1D7 is omitted.
crucial for the interaction. Our results also support this contention. The physiological significance of the heparin interaction with apoE is uncertain, but heparin is known to interact with lipoproteins, and heparin has been shown to play a role in lipoprotein processing (23) and even in the development of atherosclerosis (24). If this is the case, then the reduced affinity of the Arg^{112}, Cys^{142} variant for heparin-like molecules may interfere with normal processing of triglyceride-rich lipoproteins and contribute to the accumulation of these lipoproteins in the plasma of affected subjects.

It has been shown that the epitope for monoclonal antibody 1D7 lies in the 130–150 region of apoE, and it was postulated that the important residues of the epitope are the cluster of basic residues at positions 142, 143, 145, 146, and 147 (8). In support of this, Weisgraber et al. (8) determined that natural apoE variants with single substitutions at residue 145 or 146 had 49 or 32%, respectively, of normal apoE affinity for antibody 1D7. The results of the present study indicate that the substitution of cysteine at residue 142 leads to a dramatic reduction in apoE affinity for antibody 1D7, to less than 5% of normal (Fig. 5). Thus, Arg^{142} is a critical determinant of the antibody 1D7 epitope.

The functional characteristics of the Arg^{112}, Cys^{142} variant provide a possible explanation for its association with dominant expression of type III hyperlipoproteinemia. The receptor binding defect alone cannot be the explanation for dominant transmission. Although this variant is defective in receptor binding, it is less defective than apoE2(Arg^{142} → Cys) in in vitro assays, and Chappell (25) has demonstrated that β-VLDL from subjects with the Arg^{112}, Cys^{142} variant has higher binding affinity than does β-VLDL from E2/2 subjects.

Part of the explanation for dominant expression may lie in the distribution of the variant apoE. Because any individual triglyceride-rich lipoprotein particle will have multiple copies of apoE and because in heterozygous subjects the normal and defective apoE probably distribute randomly, some lipoprotein particles will have more of the variant than of the normal apoE and vice versa. Over time, those particles with more of the normal apoE will be cleared from the circulation more efficiently, whereas the clearance of particles with more of the receptor binding-defective apoE variant will be retarded. Although this differential distribution of normal and variant apoE also occurs in E3/2 subjects, apoE2(Arg^{145} → Cys) is only slightly increased relative to apoE3 in their triglyceride-rich lipoproteins (5). However, this differential distribution is not nearly as pronounced as the 3:1 ratio demonstrated here for the Arg^{112}, Cys^{142} variant. It is known that the presence of arginine at residue 112, as occurs in this variant, confers upon apoE a preference for association with triglyceride-rich lipoproteins (26). This could account for the observed selective accumulation of the Arg^{112}, Cys^{142} variant in the triglyceride-rich lipoproteins of affected subjects. Thus, it is likely that the natural preference of apoE with Arg^{112} for triglyceride-rich lipoproteins, in conjunction with the receptor binding defect, is a major part of the explanation for this increased selective accumulation. This may also be the case for apoE-Leiden, a variant with Arg^{112} that demonstrates this same selective accumulation and is associated with dominant expression of type III hyperlipoproteinemia (27).

Finally, if the interaction of apoE with heparin-like molecules is important for lipoprotein processing and/or catabolism in vivo, then the lower affinity of the Arg^{112}, Cys^{142} variant for heparin may also be a contributing factor to the accumulation of β-VLDL. This is probably not the case for apoE2(Arg^{145} → Cys), which binds normally to heparin (9), but might be the case for any other apoE variant that has a substitution for a basic residue in the region of the heparin-receptor-binding site (residues 135–150). It is already known that some of these latter variants are associated with dominant expression of type III hyperlipoproteinemia (25).

In summary, we believe that the unique combination of functional properties of the Arg^{112}, Cys^{142} apoE variant, i.e. defective receptor binding, reduced affinity for heparin, and especially its preference for association with triglyceride-rich lipoproteins, leads to excessive accumulation of β-VLDL, which in turn is manifested in dominant expression of type III hyperlipoproteinemia.

**Acknowledgments**—We thank Norberto Torres for excellent technical assistance, Maureen Balestra and Kay Arnold for receptor binding assays, Kurt Haubold for DNA sequencing, Charles Benedict and Tom Rolain for graphic art, Al Averbach for editorial assistance, and Perry Humphrey for manuscript preparation. We also thank Drs. Robert W. Mahley and Thomas L. Innerarity for a critical reading of the manuscript and Dr. Thomas P. Bersot for providing the plasma from patients.

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