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Research Article

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Apolipoprotein E2-Christchurch (136 Arg → Ser)

New Variant of Human Apolipoprotein E in a Patient with Type III Hyperlipoproteinemia

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Abstract

The primary structure of apolipoprotein E (apo E) was investigated in seven type III hyperlipoproteinemic patients with the apo E-2/2 phenotype. Six of the patients had identical two-dimensional tryptic peptide maps. These differed from the normal apo E3 map by the altered mobility of a single peptide. Amino acid analysis and sequencing showed that apo E2 in these patients had a substitution of 158 Arg → Cys. The presence of this mutation in six of the seven type III patients confirms that this is the most common form of apo E2.

The seventh type III patient had a unique map with a new peptide resulting from a substitution of 136 Arg → Ser. He was heterozygous for this and for the more common apo E2 (158 Arg → Cys) variant. His very low-density lipoprotein contained approximately five times more apo E2 (136 Arg → Ser) than apo E2 (158 Arg → Cys), as determined by cysteamine treatment and peptide mapping. This new apo E2 mutant thus appears to contribute significantly to the patient's hyperlipidemia.

Introduction

Recent understanding of the pathophysiology and genetics of dyslipoproteinemias has resulted in a classification based on the structure and function of apolipoproteins, enzymes, and cellular receptors involved in lipoprotein metabolism (1). Mutations in the apolipoprotein genes of humans have been identified at both the amino acid and DNA levels. Some of these have profound effects on lipoprotein metabolism and are associated with atherosclerosis.

Familial type III hyperlipoproteinemia (type III)[1] is a genetic disorder of lipid metabolism, characterized by elevated levels of plasma cholesterol and triglycerides and by the presence of abnormal lipoproteins. These, the cholesterol-enriched, β-migrating very low-density lipoproteins (β-VLDL) (2), give rise to the characteristic broad-β electrophoretic pattern. They comprise chylomicron remnants, containing apolipoprotein B-48, apolipoprotein E (apo E), and VLDL remnants containing apolipoprotein B-100 and apo E (3). Affected patients have an increased risk of premature atherosclerosis.

Type III is associated with a variant form of apo E, apo E2. Affected subjects are often homozygous for apo E2, but heterozygosity for this variant has also been reported in association with this disease (4–8). Individual cases of type III have also been observed in association with the apo E-3/3 phenotype (9) and in an individual with a deficiency of apo E (10).

The variant apo E2 alone is not sufficient to cause type III, however, as only ~ 2% of all apo E2 homozygotes have this disease. Type III is currently believed to result from two gene defects, one in the apo E structural gene and the other in a gene causing hyperlipidemia, probably familial combined hyperlipidemia (2, 11).

Three variants of apo E2 have been described. Each of these differs from apo E3 by a single amino acid substitution (6, 12–14). The most common has a mutation of 158 Arg → Cys (13), but mutations of 145 Arg → Cys (14) and 146 Lys → Gln (6) have also been reported in individuals with type III hyperlipoproteinemia.

Apo E binds to two distinct high-affinity receptors mediating internalization and catabolism of lipoprotein particles. The first of these, apo B,E or low-density lipoprotein (LDL) receptor, is present in most tissues. Apo E has a much higher affinity than apolipoprotein B for this receptor because of the potential of apo E–containing particles to form multiple interactions with several sites on the receptor (15–17). The second is the apo E or remnant receptor, which is found in hepatic tissues only and is able to recognize particles containing apo E but not apo B (18).

The determination of receptor-binding affinity of known variants (6, 13, 14, 19–21), and the blocking of receptor binding by monoclonal antibodies to apo E (22) have established that its receptor-binding domain lies between residues 140 and 150. The positively charged amino acids in this region form ionic bonds with negatively charged residues in the consensus sequence, which is repeated seven times in the ligand-binding region of the apo B,E (LDL) receptor (23). The positively charged arginyl residue at position 158 is thought to maintain the correct conformation of the receptor-binding domain (24).

In this investigation, six of seven type III patients were found to be homozygous for apo E2 (158 Arg → Cys), confirming this as the most common apo E2 isoform. The seventh was heterozygous for this and for a new apo E2, apo E2 (136 Arg → Ser), which was the predominant VLDL isoform in this subject. His daughter, who had the apo E-3/2 phenotype, also possessed the new protein.

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1. *Abbreviations used in this paper:* apo E, apolipoprotein E; LDL, low-density lipoprotein; PTH, phenylthiohydantoin; type III, type III hyperlipoproteinemia; VLDL, very low-density lipoprotein.

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Methods

Patients. The subjects in this study were selected from a group of type III hyperlipoproteinemic patients with the homozygous apo E-2/2 phenotype, in whom lipoprotein kinetic studies had been performed (25). Five subjects with the most severely impaired VLDL fractional catabolic rates and longest VLDL-to-LDL mean conversion times were selected to determine whether this impairment was related to structural heterogeneity in apo E2.

Two further patients with the apo E-2/2 phenotype (D.W.B. and D.B.Y.) were selected to represent cases with lipoprotein metabolic parameters more typical of type III hyperlipoproteinemia. All seven patients were undergoing clofibrate treatment. Subject F.J.T. (apo E-3/3) had secondary hyperlipidemia due to diabetes mellitus. Plasma cholesterol and triglyceride levels were determined enzymatically (Abbott Laboratories, South Pasadena, CA).

Plasma lipoprotein isolation. The $d < 1.006$ g/ml lipoproteins (VLDL) were prepared from EDTA plasma by centrifugation for 16 h at 50,000 rpm in a type 60Ti rotor (Beckman Instruments, Inc., Fullerton, CA) (26). After centrifugation, the supernatant lipoproteins were delipidated with two washes of acetone/ethanol (1:1 vol/vol) and one wash with diethyl ether (27). The first extraction with acetone/ethanol used a lipoprotein-to-organic solvent volume ratio of 1:6.

Isoelectric focusing. VLDL apoproteins were analyzed by isoelectric focusing, either on polyacrylamide gel rods as previously described (28) or on a 7.5% vertical slab gel of 1 mm thickness, using identical conditions. Cysteamine modification of either VLDL, apo VLDL, or purified apo E, before isoelectric focusing, was performed as described previously (19, 29).

Apo E isolation. Apo E was separated from apo VLDL by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a modification of the method of Laemmli (30). A 1-mm thick vertical slab gel was prepared consisting of a 5–20% linear acrylamide gradient. 15 mg of apo VLDL dissolved in 2 ml of 0.125 M Tris-HCl, pH 6.5, 2% (wt/vol) SDS, 10% (vol/vol) glycerol, and 3% (vol/vol) β-mercaptoethanol was applied to each gel. A constant current of 50 mA was used until the voltage reached 300 V. This voltage was then maintained until the end of the run. The protein bands were located using 0.25 M KCl (31). The apo E band was cut out and eluted from the gel with distilled water. Six preparative gels were run on each patient. The apo E was pooled, dialyzed against 10 mM Tris-HCl, pH 8.0, 0.05% SDS to remove KCl, and lyophilized. The dried residue was dissolved in 2 ml of distilled water and the apo E was precipitated, free of SDS, with 15 vol of acetone containing 1% (vol/vol) HCl. The apo E pellet was washed twice more with acid/acetone, and its purity was assessed by isoelectric focusing.

Carboxymethylation and carboxamidomethylation. Apo E was reduced and carboxymethylated or carboxamidomethylated using a modification of the method of Nelson et al. (32). Apo E was dissolved in 0.1 M Tris-HCl, pH 8.0, 6 M urea at 5 mg/ml. Dithiothreitol was added to give a final concentration of 2 mM, and reduction was allowed to proceed for 18 h under nitrogen. A 2.5-fold molar excess of iodoacetic acid or iodoacetamide over total thiol groups was added, and the reaction was allowed to proceed in the dark for 45 min. The solutions were then dialyzed extensively in the dark against 0.1 M NH_4HCO_3.

Two-dimensional peptide mapping. *S*-Carboxymethyl apo E (5 mg/ml in 0.1 M NH_4HCO_3) was incubated with TPCK-trypsin (2% wt/wt) at 37°C (33). After 2 h, the digest was lyophilized and redissolved at a concentration of 10 mg/ml in distilled water, and was stored frozen until required.

Maps were run on No. 1 paper (Whatman Inc., Clifton, NJ) using 0.8 mg of digest. Electrophoresis (3 kV for 55 min) was performed at pH 6.4 in pyridine/acetic acid/water (100:4:900 vol/vol), followed by chromatography for 20 h in the upper phase of pyridine/isoamyl alcohol/water (6:6:7 vol/vol) (34). Internal markers of dansyl-arginine and dansyl-sulphonic acid were incorporated to determine electrophoretic mobilities and calculate peptide molecular weights (35).

Analytical maps were stained with fluorescamine (0.004% wt/vol in acetone containing 2% vol/vol pyridine), ninhydrin (0.2% wt/vol in acetone containing 2% vol/vol pyridine) or cadmium-ninhydrin (36) for the detection of peptides. Specific staining procedures were used to locate peptides containing divalent sulphur, tyrosine/tryptophan, histidine, and arginine (36–38). Preparative maps were run on 1.5 mg of digest and stained with 0.002% fluorescamine in acetone.

Amino acid analysis. Peptides were eluted in ethanol/acetic acid/water (1:1:8 vol/vol), and hydrolyzed under vacuum (110°C for 18 h), using 6 M HCl/1% phenol (39). Hydrolyzates were redried from ethanol/water/triethylamine (2:2:1 vol/vol) and then reacted with 15 μl of coupling reagent (ethanol/triethylamine/water/phenylisothiocyanate 7:1:1:1 vol/vol) for 20 min at room temperature. The phenylthiocarbamyl amino acid derivatives were quantified following reverse-phase high-pressure liquid chromatography (HPLC) on a Nova Pak column (Waters Associates, Milford, MA) at 36°C. The HPLC system consisted of two Waters model 570 solvent delivery pumps, a model 680 automated gradient controller, and a model 441 fixed wavelength detector with a 254-nm filter.

Sequence analysis. *N*-Terminal analysis was performed by the dansyl procedure (40), and dansyl–amino acids were identified by chromatography on 2.5 × 2.5-cm polyamide plates (41). Manual microsequence analysis was carried out as described by Tarr (42), except that the coupling reagent was the same as that described above for amino acid analysis, and conversion to phenylthiohydantoin (PTH) derivatives was effected with 50% trifluoroacetic acid (43). PTH–amino acids were identified using the same column and HPLC system as for amino acid analysis. The initial solvent was 35 mM sodium acetate (pH 5.0)/acetonitrile (5:1), and the second solvent was 60% isopropanol in distilled water.

Results

Case histories. The seven type III patients in this study were part of a group of 32 type III subjects with the apo E-2/2 phenotype, whose plasma and lipoprotein lipid values have already been reported (44). All were undergoing dietary and clofibrate treatment that had successfully lowered their plasma cholesterol and triglyceride levels to normal values (Table I).

One of these patients, J.W.T., a 43-yr-old male, was referred to the Christchurch Lipid Clinic in 1981 after showing repeated high plasma cholesterol and triglyceride levels in excess of 10 mmol/liter and 5 mmol/liter, respectively. His hyperlipidemia was not due to diabetes, excessive alcohol intake, or obesity. Physical examination showed him to be 20% underweight. The patient was shown to have familial type III hyperlipoproteinemia with β-VLDL (Fig. 1 *A*), a homozygous apo E-2/2 phenotype (Fig. 1 *B*), and a VLDL cholesterol-to-triglyceride ratio of 1.88. There was a family history of high lipid levels; his father had elevated cholesterol and triglyceride levels, whereas his sister and two daughters had elevated plasma cholesterol (Table II). He was placed on a diet low in animal fat and was prescribed clofibrate, 1 g twice a day. This treatment led to a reduction in plasma cholesterol and triglyceride levels to 4.65 mmol/liter and 1.63 mmol/liter, and a reduction in the VLDL cholesterol-to-triglyceride ratio to 0.75. J.W.T. had no symptoms of cardiovascular disease at the time of this study.

Isoelectric focusing. The complex isoelectric focusing pattern exhibited by apo E results from posttranslational desialylation superimposed on genetic polymorphism (45, 46). As apo E3 is the most common form in the population (28, 45, 47), it is regarded as the parent (24) or normal isoform (48).

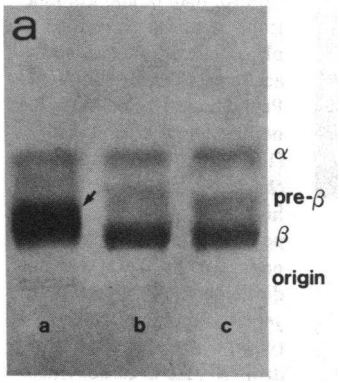
All seven type III patients were shown to have the apo E-2/2 phenotype by analytical isoelectric focusing. The control subject (F.J.T.) had the apo E-3/3 phenotype.

Cysteamine treatment was used to determine the number of

Table I. Description of Subjects on Whom Apo E Primary Structural Studies Were Performed

Subject	Age	Sex	Height	Weight	Apo E phenotype	Plasma lipid levels			
						Before clofibrate treatment		After clofibrate treatment	
						Cholesterol	Triglyceride	Cholesterol	Triglyceride
			cm	*kg*		*mmol/liter*	*mmol/liter*	*mmol/liter*	*mmol/liter*
F.J.T.	51	M	—	—	E-3/3	7.1	5.1	—	—
D.B.Y.	54	M	168	84.7	E-2/2	7.9	8.4	5.8	3.0
D.W.B.	48	M	159	73.2	E-2/2	10.1	7.6	5.4	2.6
L.L.	49	M	172	80.6	E-2/2	13.5	6.9	4.5	2.4
J.S.F.	30	M	194	84.5	E-2/2	9.5	15.8	6.9	6.6
D.D.	51	F	161	68.2	E-2/2	23.3	8.9	5.0	2.7
A.D.F.	38	M	170	70.0	E-2/2	10.0	3.4	5.6	1.5
J.W.T.	43	M	175	60.0	E-2/2	11.6	6.7	4.7	1.6

cysteine residues in apo E. This reagent adds a positive charge to cysteine residues and the resultant modified apo E has an altered pattern on isoelectric focusing (12). As expected, cysteamine treatment caused the apo E–3/3 isoform pattern of F.J.T. to shift toward the cathode, and thus to resemble the apo E–4/4 phenotype. This indicated the presence of one cysteine residue (data not shown). With cysteamine, the apo E isoform pattern from six of the seven type III patients migrated two charge units more cathodally, indicating the presence of two cysteine residues (Fig. 1 *B*).



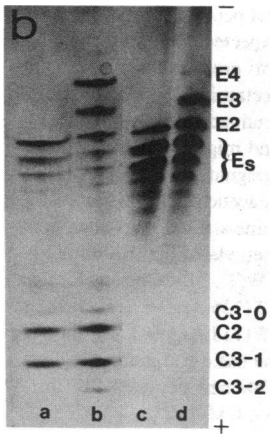


Figure 1. (*A*) Lipoprotein electrophoresis on cellulose acetate of fasting plasma. (Lane *a*) J.W.T., showing the broad-β band characteristic of type III hyperlipoproteinemia (*arrow*). Chylomicrons can also be seen at the origin. (Lanes *b* and *c*) Normal subjects. (*B*) Analytical isoelectric focusing of apo VLDL and purified apo E, before and after modification of cysteine residues. (Lane *a*) apo VLDL from a type III patient (subject L.L.) homozygous for apo E2 (158 Arg → Cys); (lane *b*) cysteamine-treated apo VLDL from L.L.; (lane *c*) purified apo E2 from J.W.T.; (lane *d*) cysteamine-treated apo E2 from J.W.T.

The pattern was consistently different in J.W.T. After cysteamine modification, a major apo E isoprotein changed its migration by only one charge towards the cathode, whereas a minor band focused two charge units more cathodally (Fig. 1 *B*). This indicated the presence of two apo E2 proteins: one with the expected two cysteines, and one with only a single cysteine residue.

Peptide mapping and structural analysis. A set of at least three analytical maps was run on each tryptic digest to permit full staining characterization. In this way peptides containing tryptophan/tyrosine, histidine, arginine, and divalent sulphur were specifically located. Peptides were designated according to the nomenclature of Rall et al. (13), and although the mobility and staining characteristics of individual peptides provided strong evidence of their identity, the final designation was based on amino acid analysis.

Fig. 2 *A* shows the parent apo E3 map from the normal control. The basic peptide T22 is of particular interest. It is one of only four tyrosine-containing peptides and had the expected amino acid composition Leu, Ala, Val, Tyr, Gln, Ala, Gly, Ala, Arg.

Peptide T22 was missing in six of the seven homozygous apo E2 type III patients and was replaced by a new peptide containing tyrosine and sulphur. When iodoacetamide had been used to derivatize the apo E, the new peptide retained its net positive charge, but when iodoacetic acid was used, the new

Table II. Plasma Lipid Levels and Apo E Phenotypes in the J.W.T. Kindred

Relative	Age	Plasma cholesterol	Plasma triglyceride	Apo E phenotype
		mmol/liter	*mmol/liter*	
J.W.T.	43	11.6	6.7	E-2/2
Father (G.W.T.)	78	7.9	2.6	E-3/2
Mother (J.T.)	74	6.7	2.1	E-3/2
Sister (G.C.)	44	6.2	1.5	E-3/2
Daughter (K.T.)	10	5.8	1.4	E-3/2
Daughter (V.T.)	7	6.5	1.5	E-3/2
Wife (L.T.)	39	7.2	1.3	E-3/3

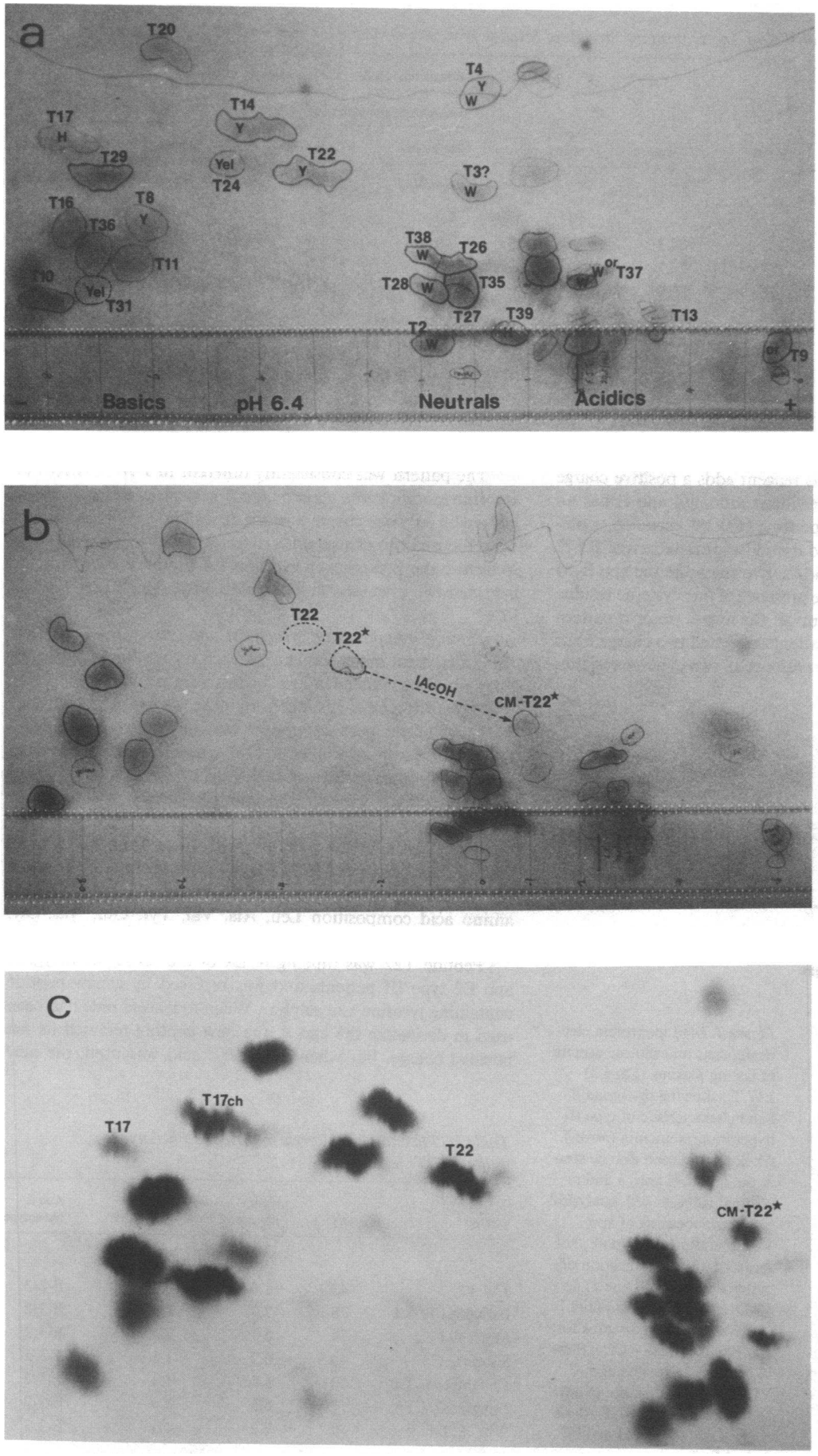


Figure 2. Tryptic peptide maps of apolipoprotein E. (*A*) Control map of apo E3. Electrophoresis was performed at pH 6.4. Basic, neutral, and acidic peptides are indicated. Peptides are labeled using the nomenclature of Rall et al. (13). Some specific staining characteristics are shown. H, histidine-positive; Y, tyrosine-positive; and W, tryptophan-positive spots. *Yel* and *or* indicate peptides that stained yellow and orange, respectively, with cadmium-ninhydrin. (*B*) Map of carboxymethylated apo E2 (158 Arg → Cys). Peptide T22 is missing and replaced by a new neutral peptide, CM-T22*. As expected, when the apo E2 was prederivatized with iodoacetamide, the new peptide retained its positive charge and migrated in the position designated T22*. IAcOH, iodoacetic acid. (*C*) Fluorescamine-stained map of carboxymethylated apo E2 from J.W.T., heterozygous for apo E2 (136 Arg → Ser) and apo E2 (158 Arg → Cys), showing the basic and neutral regions. Note new peptides T17Ch and CM-T22*.

peptide, CM-T22*, was electrically neutral (Fig. 2 *B*). The implied new cysteine in CM-T22* was confirmed by amino acid, *N*-terminal, and sequence analysis. A sequence/composition of CM-Cys-Leu-Ala, Val, Tyr, Gln, Ala, Gly, Ala, Arg was established for this aberrant peptide. The complete absence of the normal T22 peptide indicated that these six individuals were in fact homozygous for apo E2 (158 Arg → Cys).

J.W.T. had a unique peptide map that differed from the other type III maps in two respects. First, T22 was present in addition to CM-T22*, the former being present in substantially higher yield. Second, a completely new basic peptide (T17Ch) was observed with a mobility of +0.65 relative to aspartic acid (Fig. 2 *C*). This new peptide stained slowly with cadmium-ninhydrin suggesting a valine *N*-terminal (36) and it also stained positively for histidine and arginine.

As there are only two histidine-containing peptides in apo E (T17 and T39), one of these peptides must be implicated, or the mutation has introduced a new histidine. This latter possibility can be excluded, because the presence of a third histidine, with its positive charge, would not result in the expression of the apo E-2/2 phenotype observed in J.W.T.

The loss of positive charge from peptide T39 could only produce a new acidic peptide, and not a basic one. This leaves T17 as the only possible source of the new peptide. Considering its electrophoretic mobility (+0.65) and its specific staining characteristics, the aberrant peptide (T17Ch) can result only from the mutation of arginine 136 to a neutral residue.

To confirm these deductions and to establish the precise mutation, peptides T17, T17Ch, T22, and CM-T22* were eluted from preparative fluorescamine-stained maps of J.W.T. and subjected to structural analysis.

Amino acid analysis of T22 and CM-T22* (Table III) indicated that they were identical, except that CM-T22* contained an additional residue of carboxymethyl cysteine. Initial sequences of Leu-Ala-Val and CM-Cys-Leu-Ala respectively were obtained for these peptides, confirming the substitution as 158 Arg → Cys.

J.W.T. was heterozygous for this mutation and for one involving peptide T17. Amino acid analysis (Table III) indicated that T17Ch contained two additional amino acids, valine and serine. The expected initial sequence of Leu-Ala was confirmed for T17; that of T17Ch was Val-Ser-Leu, indicating a mutation of 136 Arg → Ser. The resultant loss of peptide T16 (Val-Arg) was not noted on peptide mapping because of the heterozygosity and because the sequence Arg-Val-Arg recurs further along the molecule.

Table III. Amino Acid and N-Terminal Analyses of Apo E Tryptic Peptides T22, CM-T22, T17, and T17Ch from J.W.T.*

Amino acid	T22	CM-T22*	T17	T17Ch
Glu	1.4	1.0	—	—
CM-Cys	0	0.9	—	—
Ser	—	—	1.0	2.1
Gly	1.2	1.3	—	—
His	—	—	1.0	1.0
Arg	0.9	0.9	1.3	1.0
Ala	3.2	3.2	1.0	1.0
Tyr	0.8	0.9	—	—
Val	1.0	1.1	0	1.0
Leu	0.9	1.1	1.9	2.3
Yield (*nmol*)	14.7	3.0	3.3	15.7
N-Terminal	Leu	CM-Cys	Leu	Val

Amino acid mean data from four separate hydrolysates. *N*-Terminals determined as dansyl derivatives.

Both cysteamine treatment and peptide mapping indicated a considerable excess of apo E2 (136 Arg → Ser) over the usual apo E2 gene product. This was confirmed by quantitative elution and amino acid analysis of the appropriate peptides. The ratio of T17Ch to T17 was 4.8:1, and the ratio of T22 to CM-T22* was 4.9:1.

In an attempt to trace the inheritance of apo E2 (136 Arg → Ser), we obtained plasma from the sister and the eldest daughter of the proband. (The father died at age 81 and the youngest daughter did not consent to a blood donation.) The amount of isolated VLDL from the sister was too low to yield a sufficient quantity of apo E for peptide mapping.

The eldest daughter was found to have inherited the apo E2 (136 Arg → Ser) mutant from her father, as evidenced by the presence of peptide T17Ch. When quantitative amino acid analysis was performed on her two peptides, T17Ch and T17, the ratio was found to be 1.5:1. At the age of ten, K.T. had an elevated cholesterol level, but now, five years later, her cholesterol is normal (4.4 mmol/liter). As yet she does not exhibit any symptoms of type III hyperlipoproteinemia, although her plasma triglyceride has increased from 1.4 to 3.8 mmol/liter.

Discussion

In each of six unrelated patients with type III hyperlipoproteinemia and the apo E-2/2 phenotype, we showed that their apo E2 was identical to that which has been postulated as the most common apo E2 variant (24). We thus confirm that a substitution of cysteine for arginine at position 158 is the most common mutation giving rise to the apo E2 isoprotein.

The seventh type III patient was heterozygous for this and for a new variant, apo E2 (136 Arg → Ser). The new 136 Arg → Ser substitution is consistent with the apo E-2/2 phenotype, with the cysteamine modification experiments and alterations in peptide mobility observed on mapping. This substitution can arise from a point mutation of CGC → AGC occurring in the apo E3 gene. Apo E2 (136 Arg → Ser) is the fourth apo E2 structural variant to be characterized and the second containing only one cysteine. The primary structures of nine apo E isoproteins have now been determined (Table IV).

Secondary structure predictions (54, 55) suggest that position 136 is part of an α-helix that extends between residues 130 and 150 (13, 24), encompassing the receptor-binding domain from 140 to 150 (22). Although the replacement of arginine 136 by serine would not be expected to affect the internal stability of the helix, the loss of a putative ionic bond involving this arginine could be expected to result in altered alignment of the helix. In this way, a substitution at position 136 could alter receptor binding, as happens in the case of apo E2 (158 Arg → Cys) (24), which also lies outside the recognized receptor-binding domain.

A fascinating feature of this case is that the product of the apo E2–Christchurch gene comprises 80% of the apo E in J.W.T.'s VLDL, the remaining 20% being apo E2 (158 Arg → Cys). Although an increased expression of the apo E2–Christ-

Table IV. Human Apolipoprotein E Polymorphism

Name	Charge relative to parent E3	Allele	Molecular defect	Receptor binding activity relative to E3	Disease association	Reference
				%		
E-Suita	+4	—	—	—	Types IIa, IIb, IV; cerebral and myocardial infarction	49
E5	+2	ϵ5	—	—	Types IIa, IIb, IV	50
E4	+1	ϵ4	$Cys_{112} \rightarrow Arg$	100 (19)	Type V; increased plasma cholesterol	14
E3	0	ϵ3	Parent	—	None	13
E3*	0	ϵ3*	$Ala_{99} \rightarrow Thr$, $Ala_{152} \rightarrow Pro$	—	Unknown	51
E3**	0	ϵ3**	$Cys_{112} \rightarrow Arg$, $Arg_{142} \rightarrow Cys$	<20 (21)	Unknown	24
E3-Leiden	0	ϵ3-Leiden	—	Defective	Type III	52
E2	−1	ϵ2	$Arg_{158} \rightarrow Cys$	<2 (19)	Type III	13
E2*	−1	ϵ2*	$Arg_{145} \rightarrow Cys$	45	Type III	14
E2**	−1	ϵ2**	$Lys_{146} \rightarrow Gln$	40	Type III	6
E2-Christchurch	−1	ϵ2Ch	$Arg_{136} \rightarrow Ser$	41 (58)	Type III	—
E1	−2	ϵ1	$Gly_{127} \rightarrow Asp$, $Arg_{158} \rightarrow Cys$	4	Hypertriglyceridemia, variable hyperlipidemia	20
E-Bethesda	−2	^{c}B	—	—	Type III	53
E-Deficiency	—	— (ϵ_{null})	—	—	Type III	10

church gene cannot be discounted as an explanation, it is improbable. Of some 300 characterized point mutations in hemoglobin (56), none result in increased expression, though missplicing of a pseudo-intron in hemoglobin E (β26 Glu → Lys) results in decreased expression (57). The preponderance of apo E2 (136 Arg → Ser) is most likely due to a perturbation of protein structure rather than altered gene expression. In the daughter of J.W.T., who has the apo E-3/2 phenotype, apo E2 (136 Arg → Ser) only represented 60% of the apo E circulating in her VLDL.

A possible explanation for the different apo E2 isoform ratios found in the father and daughter is that one normal allele gives enough functional apo E to clear particles, whereas when both alleles are abnormal, another slower mechanism exists for the removal of these particles, thus allowing for the build-up of their remnants. The longer circulatory time of the remnants may be associated with different exchange rates of the two apo E2 isoforms with other lipoprotein classes, or apo E2 (136 Arg → Ser) may have a slower catabolic rate than apo E2 (158 Arg → Cys).

Whatever the basis for the relative increase in apo E2 (136 Arg → Ser), it is clear that this variant contributes significantly to the hyperlipidemia in J.W.T. The lipoprotein turnover studies showed J.W.T. had a markedly reduced VLDL fractional catabolic rate (0.045 h^{-1}), compared with other type III patients with the apo E-2/2 phenotype (mean = 0.078±0.018 h^{-1}) (25). Similarly, his mean conversion time of VLDL to LDL (32.7 h) was markedly longer than the mean for other homozygous apo E-2/2 type III patients of 27.4±3.02 h (25).

To date it has not been possible to isolate pure apo E2 (136 Arg → Ser) for receptor-binding assays. However, a genetically engineered site-directed mutant of 136 Arg → Ser showed only 41% of normal apo E3 receptor binding to apo B,E (LDL) receptors on cultured human fibroblasts (58).

Preliminary experiments in our laboratory have shown that apo E2 (158 Arg → Cys) and apo E2 (136 Arg → Ser) have the same heparin affinity when chromatographed on heparin sepharose under denaturing conditions. This is significant because the heparin-binding and receptor-binding domains coincide (59). The structural perturbations caused by this substitution will ultimately need to be interpreted in terms of three-dimensional structure when this becomes available.

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