Genetic variation in human apolipoprotein E

M. R. Wardell, P. A. Suckling, and E. D. Janus[1]

Departments of Cardiology and Biochemistry, The Princess Margaret Hospital, Christchurch, New Zealand

Abstract Genetic variation in human apoprotein E was studied using the technique of isoelectric focusing applied to delipidated very low density lipoprotein from 426 Christchurch blood donors and 7 patients with type III hyperlipoproteinemia. Six phenotypes were distinguishable by the relative proportions of the apoprotein E isoforms. In the blood donors, observed frequencies for these were: E3/3 = 51.4%; E4/3 = 25.0%; E4/4 = 1.0%; E3/2 = 20.0%; E4/2 = 1.2%; and E2/2 = 1.4%. All seven patients with type III hyperlipoproteinemia exhibited phenotype E2/2. Family studies of apoprotein E variants support a mode of inheritance controlled by three alleles acting at one gene locus. On this basis, the alleles occurred in the blood donor population with frequencies of 0.72 for the $\epsilon 3$ allele, 0.12 for the $\epsilon 2$ allele, and 0.16 for the $\epsilon 4$ allele. The $\epsilon 2$ allele influenced plasma cholesterol. The mean plasma cholesterol in subjects heterozygous for the $\epsilon 2$ allele was 5.32 mmol/l, very significantly less ($P < 0.01$) than the mean of 5.84 mmol/l in an age- and sex-matched group of subjects without this allele in their genotype. The mean plasma cholesterol value of 4.92 mmol/l for the five individuals homozygous for the $\epsilon 2$ allele was also significantly less ($P < 0.05$) than for age- and sex-matched subjects without the $\epsilon 2$ allele, whose mean was 5.80 mmol/l.—**Wardell, M. R., P. A. Suckling, and E. D. Janus.** Genetic variation in human apolipoprotein E. *J. Lipid Res.* 1982. **23:** 1174–1182.

Supplementary key words blood donor survey • type III hyperlipoproteinemia • cholesterol • genetic variants • isoelectric focusing • very low density lipoproteins

Shore and Shore (1, 2) first recognized an "arginine-rich protein" component in normal very low density lipoprotein (VLDL). Subsequently Utermann (3) designated this component apoprotein E and demonstrated that it showed microheterogeneity when subjected to isoelectric focusing. These workers then reported that the specific deficiency of apoprotein E-3 could be used as a qualitative marker in subjects with type III hyperlipoproteinemia (4). Others have confirmed the apoE-3 abnormality in this disorder (5–7). However, more recently, studies using two-dimensional electrophoresis have shown that what appears to be an apoE-3 deficiency on one-dimensional isoelectric focusing is, in fact, a shift of the apoE focusing pattern due to a charge variation (8).

Detection of the apoE pattern with no apparent protein migrating in the E-3 position has greatly improved the specificity of diagnosis of type III hyperlipoproteinemia when compared to earlier definitions based on electrophoretic criteria (9, 10) and the cholesterol enrichment of VLDL (11–13). This apoprotein E pattern on its own, however, does not result in type III hyperlipoproteinemia. It is only when this abnormal apoE pattern occurs together with genes for monogenic or polygenic forms of familial hyperlipidemia, that type III hyperlipoproteinemia results (14).

The genetic polymorphism of apoE was originally explained with a model that used two genes. One gene, with alleles E^n and E^d, controlled the expression of two of the major E isoproteins, E-2 and E-3. A second but closely linked gene with alleles E_4^+ and E_4^0 governed the presence or absence of the E-4 isoprotein (15, 16). This model, however, did not satisfactorily explain all observed apoE heterogeneity. The currently accepted genetic model postulates that three alleles operate at a single genetic locus to produce inherited variation in apoprotein E and that additionally post-translational glycosylation of the major E isoproteins contributes to the six observed apoE phenotypes (17, 18). Single charge differences found between the major E isoforms (8) have been verified by amino acid sequence data (19), which showed that the three major apoE isoforms E-2, E-3, and E-4 differed by single amino acid interchanges involving cysteine and arginine.

Apoprotein E has been implicated in a number of metabolic functions including cholesterol transport in the plasma (20), interaction with cell receptors (21, 22), and lipoprotein recognition and uptake by the liver (23–25). In addition, plasma lipid values between groups with differing apoE phenotypes have been shown to differ significantly (26). A number of different systems of nomenclature have been used in referring to the genetic variants of apoprotein E and this has resulted in much confusion. A new, unified nomenclature has now been agreed upon to describe the apoE phenotypes and

Abbreviations: VLDL, very low density lipoproteins (d < 1.006 g/ml); LDL, low density lipoproteins (d 1.006–1.063 g/ml); LDL_2, low density lipoproteins (d 1.019–1.063 g/ml); HDL_c, canine high density lipoproteins (d 1.02–1.063 g/ml).

[1] To whom correspondence should be addressed.

in this report we correlate existing nomenclature with the new uniform system of nomenclature (27).

In this study the genetic variants of the E apoprotein system and their effects on plasma cholesterol were investigated in 426 Christchurch blood donors and in 7 patients with type III hyperlipoproteinemia.

MATERIALS AND METHODS

Subjects

Twelve ml of blood was taken from each blood donor by antecubital venipuncture into vacutainer tubes containing dry disodium EDTA as anticoagulant (1.5 mg/ml). The plasma was separated from the red cells within 2 hr. The blood donors ranged in age from 16 to 65 years and were non-fasting.

Preparation of very low density apoproteins

Chylomicrons were floated from the plasma into an overlayered solution (d 1.006 g/ml) by ultracentrifugation for 30 min at 17,500 rpm (20,000 *g*) and 4°C in an MSE 40.3 angle-head rotor. The chylomicrons were discarded and a small aliquot of the subnatant plasma was taken for cholesterol and triglyceride measurement and for lipoprotein electrophoresis. The chylomicron-free plasma was again overlayered with d 1.006 g/ml solution and VLDL was separated by ultracentrifugation for a further 16 hr at 40,000 rpm (105,000 *g*) at 4°C. A small aliquot of VLDL was taken for cholesterol and triglyceride estimation.

The very low density lipoproteins were delipidated at −20°C with two washes in acetone–ethanol 1:1 (v/v) and a further rinse in diethyl ether as described by Warnick et al. (6). The delipidated apoproteins were dissolved in 8 M urea containing Tris (hydroxymethyl) aminomethane-HCl (20 mmol/l, pH 8.4).

Isoelectric focusing of apoproteins

Urea (6.6 g) was dissolved in deionized distilled water to a final volume of 12 ml and mixed with 4.5 ml of stock acrylamide solution (30 g acrylamide, 0.8 g N,N′-methylene bisacrylamide dissolved to 100 ml with deionized distilled water). Ampholine (0.8 ml), pH 3.5–7, (LKB) was added and the mixture was degassed under reduced pressure for 2 min. Fifty μl of N,N,N′,N′-tetramethyl ethylenediamine was then added and the mixture was cooled on ice. One ml of freshly prepared potassium persulfate (15 mg/ml) was added and the acrylamide solution was immediately poured to a height of 7.5 cm in matched tubes 0.5 cm i.d. and 12 cm long. The tubes had previously been cleaned thoroughly and siliconized using dichlorodimethyl silane (10% in toluene). Deionized distilled water was carefully overlayered onto the gel solution to exclude oxygen from the polymerization process and to ensure a flat top on the gel. Polymerization took place over 6 hr.

After polymerization, the gels were prefocused at 110 V and 4°C for 1 hr using 20 mmol/l NaOH in the upper (cathode) chamber and 10 mmol/l phosphoric acid in the lower (anode) chamber.

Once the gels were prefocused, the upper (cathode) solution was discarded. The electrolyte was removed completely from the top of the gel tubes by gently shaking the inverted apparatus. The dissolved apoproteins (approximately 400 μg total) were pipetted onto the gels in a volume of 0.35 ml and carefully overlayered with fresh cathode buffer (20 mmol/l NaOH). The gels were then focused at 250 V for 16 hr.

When focusing was complete the gels were fixed in 12% trichloroacetic acid solution for 30 min. They were then washed in three changes of 10% ethanol over 1 hr and stained with Coomassie brilliant blue R250 for 20 min at 60°C. The background was destained in ethanol–acetic acid–water 10:10:80 at 60°C until clear. Each batch of twelve gels was carefully aligned and photographed to facilitate band identification.

Scanning densitometry

The focused gels were scanned in a Beckman model 34 spectrophotometer at 580 nm. The relative quantities of the isoproteins were calculated by integration of peak areas.

Cholesterol and triglyceride determination

Total plasma and VLDL cholesterol and triglyceride were determined on an Abbott Bichromatic Analyzer 100 using Abbott enzymatic reagents.

Lipoprotein electrophoresis

Lipoprotein electrophoresis was carried out according to the Helena Laboratories method on cellulose acetate strips. Each run was done at 166 V constant voltage for 10 min and stained with oil red O.

Nomenclature

For the most part, the nomenclature used in the text is that of the new uniform system of nomenclature. The nomenclature originally used by us is in parentheses. Table 1 shows the phenotype/genotype nomenclature of three groups of investigators and the proposed uniform system of nomenclature.

RESULTS

Fig. 1 shows the six apoE phenotypes as they appeared on stained gels after isoelectric focusing. Slightly

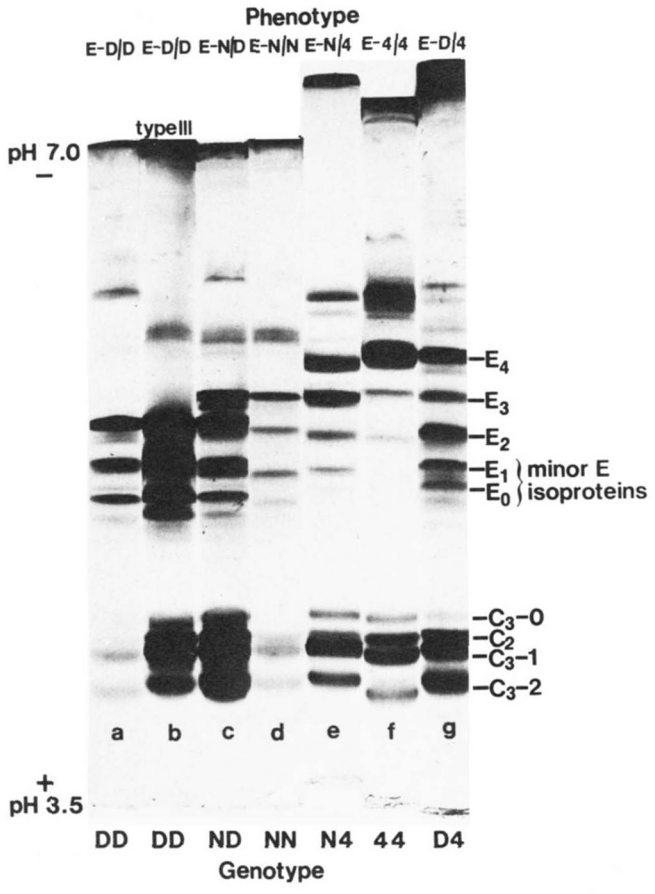

Fig. 1. Analytical isoelectric focusing in polyacrylamide gels of urea-soluble apo-VLDL isolated by ultracentrifugation. All six observed phenotypes are shown.

more than 1.0% of all subjects had no band migrating in the E-3 region and these individuals were designated phenotype E2/2 (apoE-D/D). All seven patients with type III hyperlipoproteinemia were found to have this phenotype and one is shown in Fig. 1. A marked increase in the concentration of apoE (as seen in Fig. 1b) was characteristic of type III patients compared with blood donors of phenotype E2/2 (apoE-D/D) (Fig. 1a).

Seventy-two percent of all subjects had two major bands in the apoprotein E region of the gels corresponding to the E-2 and E-3 isoproteins. Within this group two apoprotein E phenotypes exist and were distinguished according to the relative proportions of the E-2 and E-3 isoproteins. In most of these subjects E-3 appeared as a more intense band than E-2 and these individuals were designated phenotype E3/3 (apoE-N/N) (Fig. 1d). When the E-2 isoprotein was present in greater concentration than the E-3 isoprotein, the subjects were phenotype E3/2 (apoE-N/D) (Fig. 1c) (15).

In 27% of the blood donors, a third major E isoprotein, E-4, was present. This band has also been observed in 27–30% of the subjects in other studies (6, 7, 28). Within this group the three apoE phenotypes, E4/3 (apoE-N/4), E4/4 (apoE-4/4), and E4/2 (apoE-D/4) exist. These three phenotypes are shown in Fig. 1, e, f, and g, respectively.

Fig. 2 shows stained gels of the three homozygous states of the apoE system. It clearly shows the shift of the total apoE pattern. In the case of the phenotype E4/4 (apoE-4/4) the entire pattern is shifted cathodally relative to the pattern in phenotype E3/3 (apoE-N/N). In the subject with phenotype E2/2 (apoE-D/D), the entire pattern is shifted anodally relative to the pattern of phenotype E3/3 (apoE-N/N).

Visual appraisal of the gels is often not sufficient to assign a definite phenotype to an individual and for this reason all gels were scanned as shown in **Fig. 3.** After integration of the E-2 and E-3 peak areas, subjects were phenotyped according to their E-2/E-3 ratios.

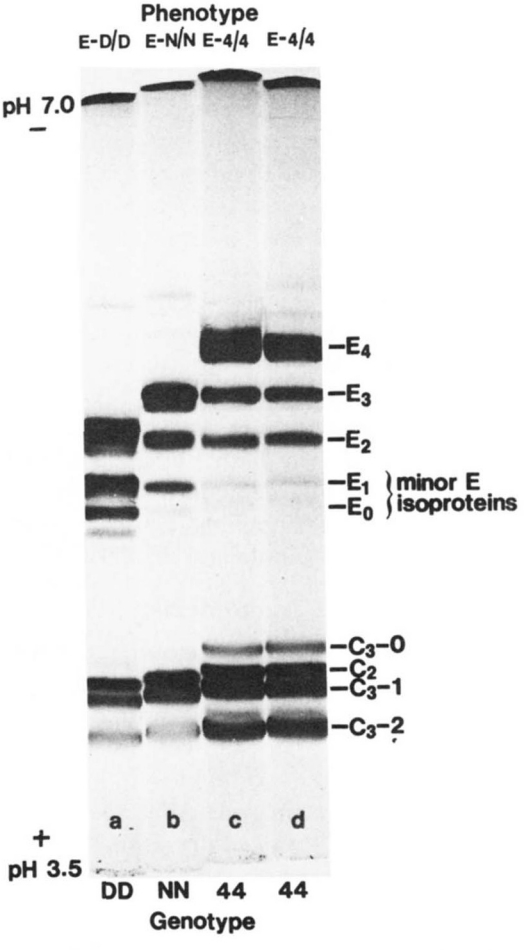

Fig. 2. Analytical isoelectric focusing in polyacrylamide gels of urea-soluble apo-VLDL isolated by ultracentrifugation. The three homozygous states are shown to demonstrate the entire apoE pattern shift. a, A subject with type III hyperlipoproteinemia homozygous for the ϵ2(D) allele. b, A subject homozygous for the ϵ3(N) allele. c and d, Two brothers homozygous for the ϵ4(4) allele.

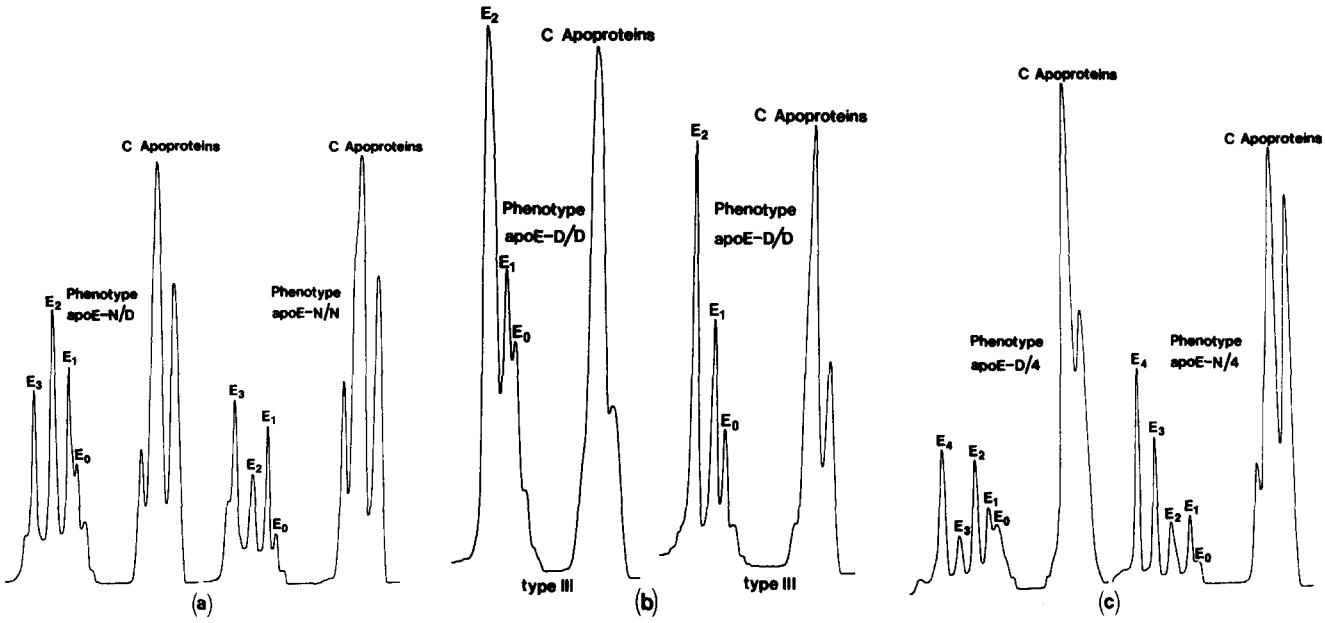

Fig. 3. Scanning densitometric patterns (580 nm) of focused gels showing: (a), phenotypes E3/3 (apoE-N/N) and E3/2 (apoE-N/D); (b), phenotype E2/2 (apoE-D/D) from two patients with type III hyperlipoproteinemia; (c), phenotypes E4/3 (apoE-N/4) and E4/2 (apoE-D/4).

A histogram plot of the E-2/E-3 ratios of all the blood donors is shown in **Fig. 4** and on the basis of this the cutoff point of 1.00 was derived to distinguish between phenotypes. The phenotypes E3/3 (apoE-N/N), E4/3 (apoE-N/4), and E4/4 (apoE-4/4) have E-2/E-3 ratios less than 1.00. The two phenotypes E3/2 (apoE-N/D) and E4/2 (apoE-D/4) both have E-2/E-3 ratios greater than 1.00 and the phenotype E2/2 (apoE-D/D) stands out as a separate group with much higher E-2/E-3 ratios. This trimodal distribution obtained by plotting E-2/E-3 ratios is very similar to that reported by Utermann et al. (29) which is shown for comparison. The two phenotypes E4/3 (apoE-N/4) and E4/4 (apoE-4/4), which appear together in this histogram, may be distinguished from each other by visual appraisal of the focused apoE patterns. Subjects with phenotype E4/4 show a distinct apoE pattern shift towards the cathode (Fig. 2) with minor E isoproteins notably diminished. In addition the E-4 band is the most prominent isoform. In contrast, the subject with phenotype E4/3 (apoE-N/

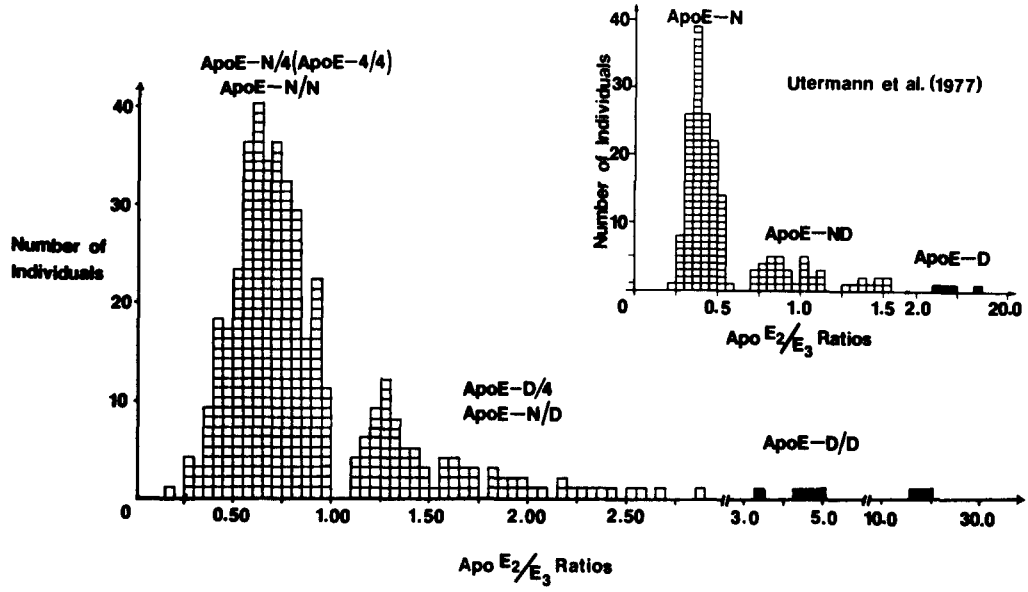

Fig. 4. Histogram plot of apoE-2/E-3 ratios of all blood donors in this study alongside a similar trimodal distribution reported by Utermann et al. (29).

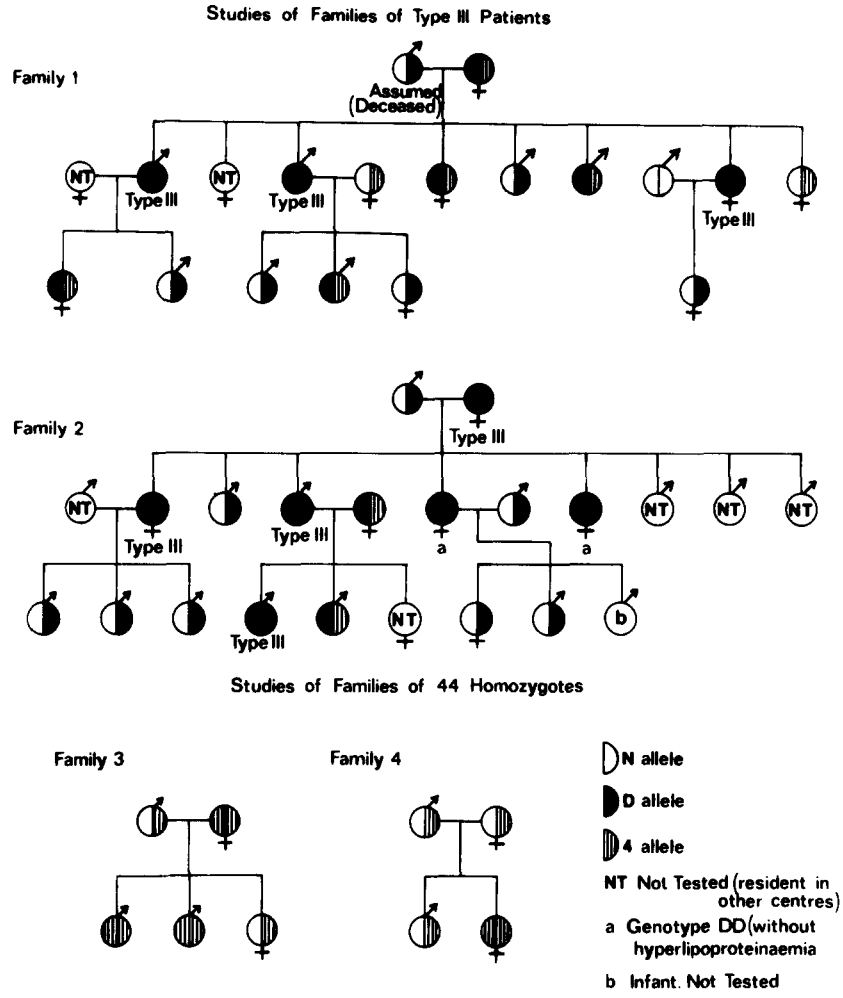

Fig. 5. Family studies showing the inheritance of the three apoE alleles.

4) (Fig. 1e) has prominent anodal minor E isoproteins and the E-3 and E-4 bands are present in similar concentrations. Furthermore, the phenotype E4/2 has even more prominent minor E isoproteins and the E-2 and E-4 bands are of comparable intensity. The three heterozygous patterns (Fig. 1c, e, and g) are as expected from the three possible pair combinations of the homozygous patterns (Fig. 2).

ApoE phenotyping of the families of type III patients was carried out. Similarly, apoE phenotyping was undertaken in the families of subjects thought to be homozygous for the E-4 state. Examples of these family studies are seen in **Fig. 5** and give strong support to the three-allele genetic model of apoE heterogeneity.

The frequencies of the six phenotypes are given in **Table 1**. These results are consistent with a genetic

TABLE 1. Phenotype frequencies (%)

Christchurch (n = 426)			Utermann (Ref. 16) (n = 717)		Zannis and Breslow (Ref. 17) (n = 61)			Proposed Uniform System of Nomenclature for ApoE (Ref. 27)	
Phenotype	Genotype	Freq.	Phenotype	Freq.	Phenotype	Genotype	Freq.	Phenotype	Genotype
apoE-N/N	NN	51.41	apoE-N/4$^-$	55.92	βIII	ϵIII/ϵIII	49	E3/3	$\epsilon3\epsilon3$
apoE-N/4	N4	25.12	apoE-N/4$^+$	24.12	αII	ϵIII/ϵII	15	E4/3	$\epsilon4\epsilon3$
apoE-4/4	44	0.94			βII	ϵII/ϵII	2	E4/4	$\epsilon4\epsilon4$
apoE-N/D	ND	19.95	apoE-ND/4$^-$	15.62	αIII	ϵIII/ϵIV	31	E3/2	$\epsilon3\epsilon2$
apoE-D/4	D4	1.17	apoE-ND/4$^+$	3.09	αIV	ϵII/ϵIV	3	E4/2	$\epsilon4\epsilon2$
apoE-D/D	DD	1.41	apoE-D/4$^-$	1.25	βIV	ϵIV/ϵIV	0	E2/2	$\epsilon2\epsilon2$

TABLE 2. Genetic models of apoprotein E polymorphism

Christchurch (n = 426) (1 gene, 3 alleles) Allele Frequencies	Utermann (Ref. 16) (n = 717) (2 linked genes) Haplotype Frequencies	Zannis and Breslow (Ref. 17) (n = 61) (1 gene, 3 alleles) Allele Frequencies	Proposed Uniform System of Nomenclature for ApoE (Ref. 27) (1 gene, 3 alleles) Alleles
N = 0.72	$apoE^n/E4^- = 0.77$	$\epsilon III = 0.72$	$\epsilon 3$
D = 0.12	$apoE^d/E4^- = 0.09$	$\epsilon IV = 0.17$	$\epsilon 2$
4 = 0.16	$apoE^n/E4^+ = 0.14$	$\epsilon II = 0.11$	$\epsilon 4$
	$apoE^d/E4^+$ = 4th possible haplotype		

model where three common alleles act at a single genetic locus to produce the observed apoE polymorphism. The genotypes that correspond to the phenotypes of this study are also given in Table 1, while the allele frequencies obtained from this population survey are given in **Table 2.** The results for the genetic models proposed by other workers and the proposed uniform system of nomenclature are shown in these tables for comparison (16, 17).

Utermann, Pruin, and Steinmetz (26) have reported significantly different lipid parameters between phenotypes. In our study the $\epsilon 4$ allele was found to have no influence on plasma lipid parameters. In particular, mean lipid parameters from seven subjects homozygous for the $\epsilon 4$ allele (four from the blood donor study and three from subsequent family studies) were tested against corresponding mean lipid values from a group of age- and sex-matched blood donors of phenotype E3/3. No significant differences were found using Student's *t* test in plasma cholesterol, plasma triglyceride, VLDL cholesterol, and VLDL triglyceride. The group of blood donors with a single dose of the $\epsilon 2$ allele in their genotype had a mean plasma cholesterol of 5.32 mmol/l. This was very significantly lower ($P < 0.01$ using Student's *t* test) than the mean plasma cholesterol of 5.84 mmol/l found in a group of age- and sex-matched subjects without the $\epsilon 2$ allele in their genotype but who may have had the $\epsilon 3$ or $\epsilon 4$ alleles (**Table 3**). The mean plasma cholesterol of 4.92 mmol/l for the five blood donors without type III hyperlipoproteinemia but with two $\epsilon 2$ alleles making up their genotype was also significantly lower ($P < 0.05$) than the mean of 5.80 mmol/l found in an age- and sex-matched group of subjects without the $\epsilon 2$ allele. Plasma triglyceride and VLDL cholesterol and triglyceride levels were also considered to see if the $\epsilon 2$ allele influenced their values. No significant difference between phenotypes was demonstrated for any of these parameters.

The seven patients with type III hyperlipoproteinemia all showed the characteristic broad beta band on cellulose acetate lipoprotein electrophoresis. One blood donor also exhibited this pattern and was subsequently confirmed to have type III hyperlipoproteinemia by the apoE pattern shift on isoelectric focusing. The five blood donors with phenotype E2/2 had normal lipoprotein electrophoretic patterns. The technique of li-

TABLE 3. Lipid parameters (mean ± SD) in age- and sex-matched groups, comparing subjects without the $\epsilon 2$ allele against subjects with a single $\epsilon 2$ allele and subjects homozygous for the $\epsilon 2$ allele

Parameter	No $\epsilon 2$ Allele Phenotypes: E3/3, E4/3, E4/4	Single $\epsilon 2$ Allele Phenotypes: E3/2, E4/2	Two $\epsilon 2$ Alleles Phenotype: E2/2
		mmol/l	
Plasma cholesterol	5.84 ± 1.15**[a] 5.80 ± 0.57*[b]	5.32 ± 1.01**	4.92 ± 0.40*
Plasma triglyceride	1.30 ± 0.64[a] 1.15 ± 0.54[b]	1.36 ± 0.69	1.10 ± 0.29
VLDL cholesterol	0.55 ± 0.49[a] 0.42 ± 0.26[b]	0.54 ± 0.45	0.33 ± 0.18
VLDL triglyceride	1.19 ± 0.82[a] 0.95 ± 0.73[b]	1.20 ± 0.92	0.75 ± 0.27

[a] Subjects with no $\epsilon 2$ allele (mean age ± SD = 33.8 ± 13.3 years; n = 90) matched with subjects with a single $\epsilon 2$ allele (mean age ± SD = 33.8 ± 13.5 years; n = 90).
[b] Subjects with no $\epsilon 2$ allele (mean age ± SD = 32.6 ± 14.2 years; n = 5) matched with subjects with two $\epsilon 2$ alleles (mean age ± SD = 32.6 ± 14.2 years; n = 5).
* $P < 0.05$: ** $P < 0.01$.

poprotein electrophoresis on cellulose acetate has proved useful in preliminary screening for type III hyperlipoproteinemia in patients with both hypercholesterolemia and hypertriglyceridemia.

The two ratios VLDL cholesterol:plasma triglyceride and VLDL cholesterol:VLDL triglyceride were calculated for all blood donors in our study. These ratios have been cited as being indicative of type III hyperlipoproteinemia when greater than 0.3 and 0.42, respectively (when the units used are mg/100 ml) (11–13). We found subjects in this study who did not have type III hyperlipoproteinemia but whose ratios exceeded these limits. This again confirms the unreliability of these earlier criteria in the diagnosis of type III hyperlipoproteinemia.

DISCUSSION

Six apoprotein E phenotypic patterns were resolved by one-dimensional isoelectric focusing according to the E isoprotein migration patterns and the relative intensities of the bands. Six genotypes (corresponding to the observed six phenotypes) would be expected if apoE polymorphism resulted from three alleles acting at one gene locus. Our family studies support this three-allele model by showing that genotypes are inherited in a manner consistent with such a system.

Fig. 2 shows the three homozygous combinations of the three alleles. It has been shown that when apoE is desialated with neuraminidase, acidic and higher molecular weight isoproteins are eliminated (18). Thus the homozygous patterns show heterogeneity as a result of post-translational glycosylation (18). Each homozygote is producing one major apoE polypeptide backbone and the most cathodal band has no sialic acid residues whereas more anodal bands have progressively more sialic acid residues.

Our DD ($\epsilon 2\epsilon 2$), NN ($\epsilon 3\epsilon 3$), and 44 ($\epsilon 4\epsilon 4$) allele combinations have been referred to as the E-2, E-3, and E-4 homozygous states, respectively (19). When the amino acid compositions of protein from the E-2 and E-3 homozygotes were compared, it was observed that the E-2 homozygote protein had one more cysteine (a total of two cysteine residues) and one less residue of arginine than the E-3 homozygote protein (19). Furthermore, protein from an E-4 homozygote was shown to have no cysteine residues but one more arginyl residue than the E-3 protein (19). The amino acid sequences of the cyanogen bromide fragments that contained cysteine have been reported from protein of both E-2 and E-3 homozygous subjects (19). This work showed that substituted for one of the cysteine residues in the E-2 protein was an arginine residue in the E-3 protein (19). The sequence data for the E-4 protein has not been reported, although it would seem from amino acid compositional data that in the E-4 protein an arginyl residue may substitute for the cysteine in the E-3 protein (19).

Heterozygotes can be regarded as combinations of these three basic patterns such that some isoprotein bands observed in heterozygous subjects are mixtures of two or three of the polypeptide backbones with varying numbers of sialic acids.

Utermann (15) originally explained the polymorphism of apoprotein E with his five observed phenotypes in terms of a two-gene model (see Tables 1 and 2). Here the apoE-N/D locus with the two codominant alleles $apoE^n$ and $apoE^d$ gave rise to the phenotypes apoE-N, apoE-ND, and apoE-D. At the second ($apoE_4$) locus, the dominant allele $apoE_4^+$ and the recessive allele $apoE_4^0$ gave rise to the $apoE_4(+)$ and $apoE_4(-)$ phenotypes (15). Further investigation indicated a close linkage of the two apoE loci and the data were explained using a genetic model with three or possibly four haplotypes (Table 2). If the fourth haplotype $apoE^d/4^+$ exists, it is assumed that the dominant allele $apoE_4^+$ is not expressed when coupled with the $apoE^d$ allele (16). Because the $apoE_4^+$ allele exhibits this major exception to its proposed dominant state when coupled to the E^d allele and because amino acid sequence data and family studies support the three-allele model, this two-gene model is not widely accepted.

The three-allele model was first postulated by Zannis, Just, and Breslow (17) who used two-dimensional electrophoretic techniques to resolve six apoE patterns. They observed three phenotypes representing homozygosity for two identical apoE alleles, and three phenotypes representing heterozygosity for two different apoE alleles. Their family studies supported this model. They estimated the frequencies of the three apoE alleles (Table 2) from the observed phenotypes in 61 non-related volunteers (Table 1). In their study subjects with type III hyperlipoproteinemia were homozygous for the ϵIV allele. From their two-dimensional electrophoretic patterns they propose that what Utermann, Hees, and Steinmetz (30) have called apoprotein E-3 deficiency is, in fact, a charge shift, probably resulting from a structural mutation in an apoE gene. These workers concluded that any attempt on their part to correlate their phenotypes with the system of Utermann (16) would be inaccurate.

We see no difficulty in reconciling the two systems. The major apoE subclasses in the work of Zannis et al. (17), in our opinion, correlate exactly to the isoproteins E-2, E-3, and E-4 found in the one-dimensional isoelectric focusing system, and in both systems the bands are one charge shift apart. Corresponding minor isoproteins (E-0, E-1, and others) are also apparent. Weisgra-

ber, Rall, and Mahley (19) have recently reconciled the two systems diagrammatically. Tables 1 and 2 show the phenotypes, genotypes, and allele frequencies we obtained alongside those of Zannis et al. (17) and Utermann (16) which we consider equivalent. Also shown is the proposed uniform system of nomenclature for apoE genotypes and phenotypes (27).

Allowing for the small population studied by Zannis et al. (17) (n = 61), Table 1 shows that the three sets of results for phenotype frequencies are in close agreement. This further supports our view that the two systems for phenotyping apoE variants are readily reconciled.

Utermann et al. (26) reported that the apoE^d allele in his two-gene model of apoprotein E influenced plasma cholesterol levels. We have also found that the $\epsilon 2$ allele within the three-allele genetic system significantly influences plasma cholesterol levels. A very substantial proportion (22%) of our blood donors had the $\epsilon 2$ allele.

Utermann et al. (26) have also reported that their apoE^d allele exerts significant influences on other lipid parameters (serum triglyceride, VLDL cholesterol, and VLDL triglyceride) but these findings were not confirmed in our study. Of particular interest was their finding of a very significantly increased VLDL triglyceride in subjects of phenotype apoE-D/4$^-$ compared with subjects of phenotypes apoE-N/4$^\pm$ (26). Their triglyceride levels in the VLDL fraction only represented 30–35% of the corresponding total serum triglycerides. In the usual situation VLDL triglyceride accounts for an average of 65% of the total plasma triglyceride (31).

Current evidence indicates that apoprotein E has an important role in the binding of lipoproteins to cell receptor sites (32, 33). The apoE-rich HDL$_c$ from cholesterol-fed dogs binds avidly to LDL receptors (22) and there is also evidence that removal of lipoprotein remnants by the liver is modulated by apoE, presumably through the interaction with an apoE receptor on the hepatocyte membrane (34). Thus subjects in whom the apoprotein E is abnormal in structure might be expected to show abnormalities of remnant metabolism. A recent study comparing the metabolism of radioiodinated apoE from normal subjects and patients with type III hyperlipoproteinemia showed that catabolism of apoE from the type III patients was indeed markedly impaired (35). Our current understanding of type III hyperlipoproteinemia is that it represents a combination of a genetically determined cause of increased VLDL levels together with an impaired conversion of chylomicron and VLDL remnants to LDL$_2$ resulting from the abnormal apoE present on the remnant surface.

Further studies are needed to determine whether normolipidemic subjects, homozygous for the $\epsilon 2$ allele, do indeed have a defect in the conversion of chylomicron and VLDL remnants to LDL$_2$. A reduction in the plasma pool of LDL would then be expected, which might explain the observed lower total plasma cholesterol levels in these subjects.

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