Studies of familial type III hyperlipoproteinemia using as a genetic marker the apoE phenotype E2/2

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Abstract Clinical symptoms, lipoprotein patterns, and apoE phenotypes were determined in 17 individuals with type III hyperlipoproteinemia (type III HLP) and in their relatives and spouses. The apoE phenotype E2/2 occurred in 15 type III HLP probands (88%) and the apoE phenotype E4/2 was found in 2 probands. In each of the families studied, the apoE phenotype inheritance was compatible with a model we previously proposed in which apoE is determined at a single genetic locus with three common alleles. The apoE phenotypes E4/4, E3/3, and E2/2 represent homozygosity for the apoE alleles $\epsilon 4$, $\epsilon 3$, and $\epsilon 2$, respectively, whereas the apoE phenotypes E4/3, E3/2, and E4/2 represent heterozygosity for the apoE alleles $\epsilon 4/\epsilon 3$, $\epsilon 3/\epsilon 2$, and $\epsilon 4/\epsilon 2$, respectively. Plasma lipids in 69 relatives of type III HLP probands were analyzed by apoE phenotype and revealed no significant differences between phenotypes in the levels of cholesterol, triglyceride, or HDL cholesterol. However, there were differences between the apoE phenotypes in LDL cholesterol levels ($P = 0.01$) and in the ratio of VLDL cholesterol/total triglyceride (ratio) ($P < 0.01$). Relatives with the apoE phenotype E2/2 had the lowest LDL cholesterol levels and the highest ratios. Of these eleven individuals with the apoE phenotype E2/2 who were not type III HLP probands, two males were taking lipid-lowering drugs, one male had mild angina at age 59, five individuals had ratios >0.25 and two had ratios >0.30 with the ratios for males (0.28 ± 0.06) significantly greater than the ratios for females (0.17 ± 0.06) ($P < 0.01$), and seven had evidence of floating βVLDL on lipoprotein electrophoresis. In addition, when compared to a control group in the general population, the whole group of relatives had normal cholesterol and HDL cholesterol levels, slightly low LDL cholesterol levels, and almost twice elevated triglyceride levels. In summary, *a*) a very strong but not invariate association exists between type III HLP and the apoE phenotype E2/2 with some type III HLP individuals having the apoE phenotype E4/2; *b*) apoE phenotype inheritance is determined by three alleles at a single genetic locus; *c*) relatives of type III HLP probands, no matter what their apoE phenotype, have on the average nearly twofold elevated plasma triglyceride levels compared to a control population; and *d*) non-proband type III HLP individuals with the apoE phenotype E2/2 have been identified. As a group these individuals, particularly the males, show a tendency to express type III HLP, but clearly genetic or environmental factors other than the apoE phenotype E2/2 are required for the full phenotypic expression of this disease.—**Breslow, J. L., V. I. Zannis, T. R. SanGiacomo, J. L. H. C. Third, T. Tracy, and C. J. Glueck.** Studies of familial type III hyperlipoproteinemia using as a genetic marker the apoE phenotype E2/2. *J. Lipid Res.* 1982. **23:** 1224–1235.

Supplementary key words apoE phenotype • LDL • plasma triglycerides • plasma cholesterol • βVLDL

Familial type III hyperlipoproteinemia (type III HLP), familial dysbetalipoproteinemia, broad β or floating β disease is a condition characterized by elevated cholesterol and triglyceride levels, and by a cholesterol-enriched very low density lipoprotein designated floating β lipoprotein or βVLDL (1–4). Affected individuals may rarely have yellowish lipid deposits in the creases of the palms of the hands called xanthoma striata palmaris (1, 4–9). In addition, eruptive and tuberous xanthomas are occasionally seen (4). Coronary heart disease and peripheral vascular disease both occur prematurely in type III HLP (4, 9). Men tend to present in the fourth decade of life with clinical symptoms of ischemic vascular disease, whereas women develop symptoms usually after the menopause (4, 9). Obesity or hypothyroidism each facilitate the expression of the disease (4, 10). In type III HLP, the plasma lipid elevation in the form of βVLDL is thought to be due to the accumulation in plasma of remnants of triglyceride-rich lipoproteins that are normally cleared rapidly from the circulation and are not present in large amounts in normal plasma (11–14). In addition to βVLDL, type III HLP patients have increased plasma apoE levels (15–19). The clinical laboratory diagnosis of type III HLP in an individual with

Abbreviations: type III HLP, type III hyperlipoproteinemia; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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(or without) xanthomas and ischemic vascular disease is based on a combination of criteria which includes detection of a broad β band on lipoprotein electrophoresis of whole plasma in different supporting media (20), ultracentrifugation of plasma at its own density with the subsequent demonstration of a floating β migrating lipoprotein (βVLDL) on electrophoresis (2), and the demonstration of an elevated ratio of VLDL cholesterol to total triglyceride (ratio 0.3) and a triglyceride concentration between 150–1000 mg/dl (3, 21, 22). Although there are no good prevalence data, it has been estimated that an unequivocal diagnosis of type III hyperlipoproteinemia can be made in 1/1,000 to 1/10,000 individuals in the population (4, 23).

Unfortunately, in many individuals the clinical diagnosis of type III HLP can be elusive. For example, individuals with hyperlipidemia, with or without xanthomas and symptoms of ischemic vascular disease, may not show a broad β band, βVLDL, or an elevated ratio (20). In other cases, individuals screened for risk factors for coronary artery disease may be asymptomatic but show normal, mildly, or moderately elevated plasma lipids with a broad β band, βVLDL, or an elevated ratio. Finally, an individual with overt clinical symptoms and plasma lipid abnormalities may alter his diet, accomplish weight reduction, undergo drug treatment, or have his hypothyroidism treated, with disappearance of xanthomas and symptoms of ischemic vascular disease and normalization of plasma lipids and lipoproteins (8–10, 24, 25). In fact, the responsiveness of type III HLP to treatment leads one to conjecture that many individuals in the population may have the tendency to develop overt signs of this condition but healthful diet, weight, thyroid status, and endogenous estrogen levels (in women) may prevent expression of this condition. These considerations suggest that the underlying metabolic defect in type III HLP may be considerably more common than 1/1,000 to 1/10,000 (4, 23), but to adequately assess this a molecular marker for the condition is needed that is more accurate than the clinical signs and plasma lipid abnormalities currently used.

The search for a molecular marker for type III HLP has led to studies of apoproteins that exist on the surface of the lipoproteins and which, to a large extent, determine the fate of these particles (26–31). In this regard, apoE has been studied extensively, since it appears to be important in mediating the hepatic clearance of chylomicron remnants, the particle that accumulates in the plasma of type III HLP individuals (29–31). Utermann and his colleagues (32–36) were the first to demonstrate an apoE abnormality in type III HLP patients and suggest polymorphism of apoE in humans. They separated apoE isoproteins on the basis of one-dimensional isoelectric focusing and showed an apparent deficiency of one of these isoproteins, designated apoE-III, in patients with type III HLP (32–36). It was proposed that the apparent apoE-III deficiency was the result of defective post-translational modification of another apoE isoprotein apoE-II to apoE-III. The early genetic models proposed by these investigators could not satisfactorily explain the genetic polymorphism of apoE (36). Recent studies in our laboratory, utilizing high resolution two-dimensional polyacrylamide gel electrophoresis, have shown a complex array of apoE isoproteins in plasma which upon further study we showed were due to both post-translational modification of apoE with carbohydrate chains containing sialic acid and a common genetic polymorphism of apoE in the human population (37–39). According to the currently accepted genetic model, human apoE is specified at a single structural gene locus with three alleles, $\epsilon 4$, $\epsilon 3$, and $\epsilon 2$. Individuals homozygous for these alleles have the apoE phenotypes E4/4, E3/3, and E2/2, respectively. Heterozygotes for these alleles have the apoE phenotypes E4/3, E3/2, and E4/2, which correspond to genotypes $\epsilon 4/\epsilon 3$, $\epsilon 3/\epsilon 2$, and $\epsilon 4/\epsilon 2$, respectively. In addition, in our previous studies, six type III HLP individuals were found to have the apoE phenotype E2/2 (37–39). For the description of human apoE, a new uniform nomenclature system has been agreed upon and is used in this manuscript (40).

In the current study, we have determined clinical signs, plasma lipids and lipoproteins, and apoE phenotypes in 17 individuals, suspected of having familial type III HLP, and their families. This study confirms our proposed model for the inheritance of the apoE phenotypes and indicates that homozygosity for the apoE allele $\epsilon 2$, apoE phenotype E2/2, occurs in most but not all type III HLP individuals. Two type III HLP patients were identified who were only carriers of the apoE allele $\epsilon 2$ and had the apoE phenotype E4/2. Relatives of type III HLP patients were studied with regard to those factors that contribute to the expression of the disease.

METHODS

Patient selection

Lipids, lipoproteins, and the apoE phenotypes were determined in 17 individuals being followed by the Cincinnati Lipid Research Clinic who were suspected of having type III HLP (by existing clinical laboratory criteria). For the purpose of this study, dysbetalipoproteinemia was defined by the presence of both βVLDL and a ratio of VLDL cholesterol to plasma triglyceride (ratio) of $\geq$0.30. Suspected dysbetalipoproteinemia was characterized by the presence of βVLDL and a borderline ratio of 0.25 to 0.29 (3, 21, 22). Six probands

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TABLE 1. Type III hyperlipoproteinemia probands[a]

Fam.	Kin. No.[b]	Age	Sex	ApoE	CHOL	CHOL[c]	TG	TG[c]	HDL	HDL[c]	LDL	LDL[c]	Ratio VLDL Chol/Plasma TG	βVLDL[d]	Medicine
		yrs						*mg/dl*							
01	I-1	37	M	E2/2	186	176	295	282	29	30	78	71	0.27		Nicobid
02	I-1	48	F	E2/2	281	254	244	247	35	28	171	156	0.31	Y	Atromid
03	II-3	19	F	E2/2	165	189	205	284	41	35	82	101	0.20	Y	
04	II-1	46	M	E4/2	290	267	297	267	35	37	177	162	0.26	Y	Atromid
05	I-1	26	M	E2/2	458	464	424	432	38	38	174	178	0.58	Y	Atromid
06	I-3	59	M	E2/2	152	110	89	34	53	56	81	53	0.20	Y	Atromid
07	I-1	38	M	E4/2	199	187	205	190	29	30	99	91	0.35	Y	
08	I-1	52	M	E2/2	342	310	414	373	34	36	128	107	0.43	Y	Atromid
09	I-4	57	M	E2/2	225	186	194	143	34	37	131	105	0.31	Y	Atromid
10	I-1	59	M	E2/2	293	251	308	253	39	42	120	92	0.44	Y	Atromid
11	I-1	62	M	E2/2	298	251	432	372	35	39	154	123	0.22	Y	Atromid
12	I-1	40	M	E2/2	348	333	702	683	21	22	124	114	0.29	Y	Atromid
13	I-1	45	M	E2/2	349	327	330	302	35	37	117	103	0.59	Y	
14	II-3	30	M	E2/2	259	259	235	235						Y	
15	I-1	44	M	E2/2	239	219	182	156	49	51	137	124	0.30	Y	Atromid
16	I-2	49	F	E2/2	215	186	149	149	40	33	101	85	0.50	Y	Atromid
17	II-2	54	F	E2/2	366	328	424	411	38	31	140	118	0.44	Y	

[a] All probands were chemically euthyroid and without other overt endocrine abnormalities.
[b] The Kin. No. (kinship numbers) have been assigned to all first degree relatives and are indicated in Fig. 2.
[c] These lipid values were adjusted for age (30 years) and sex (male).
[d] Y indicates that βVLDL was present; a blank space indicates that βVLDL was absent.

were referred to the clinic because they had palmar and tuberous xanthomas, ischemic vascular disease, and elevated cholesterol and triglyceride levels (see **Table 1,** Fam 2, 5, 8, 9, 14, 16). Five probands had only the lipid abnormality that was detected because of either a family history of early heart disease or routine screening by their physicians. These individuals did not have xanthomas or ischemic vascular disease (see Table I, Fam 1, 11, 12, 13, 17). Six probands were selected from a population-based screening for lipid abnormalities during the Cincinnati Lipid Research Clinic's Princeton school prevalence study when lipoprotein quantification revealed an abnormal ratio (41) (see Table I, Fam 3, 4, 6, 7, 10, 15). In each case, as many family members as were available were sampled.

Tests of thyroid, hepatic, renal, endocrine function, history of medication, and alcohol intake were carried out in probands and their hyperlipidemic relatives, which documented the primary nature of their hyperlipidemias.

Lipid, lipoprotein, and apoE phenotype determination

Blood was obtained after a 12-hr fast and plasma was prepared as previously described (38). Total cholesterol and triglycerides were determined by the Technicon Autoanalyzer II method as specified by the laboratory manual of the Lipid Research Clinics (42, 43). Agarose electrophoresis of whole plasma and the $d < 1.006$ and $d > 1.006$ gm/ml fractions was performed to ascertain whether βVLDL was present (44). The levels of HDL, LDL, and VLDL cholesterol were determined from the ultracentrifugally separated plasma by Lipid Research Clinic (LRC) methodology. Another aliquot of plasma was used for lipoprotein fractionation by ultracentrifugation, also following the guidelines of the LRC laboratory manual. During this procedure, VLDL was isolated from 5 ml of plasma after the specimen was overlayed with 1 ml of normal saline and centrifuged in a Beckman L5-65 ultracentrifuge for 18 hr at 120,000 *g* in a 40.3 rotor. Each VLDL specimen was dialyzed overnight against 4 liters of water at 4°C, lyophilized, and used directly as a source of apoE for two-dimensional polyacrylamide gel electrophoresis. In this analysis, lyophilized VLDL was dissolved in O'Farrell's lysis buffer (45). Two-dimensional polyacrylamide gel electrophoresis was carried out using O'Farrell's method (45) with previously described modifications (38). For the second dimension, the focused cylindrical gels were placed on SDS slab gels 17 × 20.5 cm with a thickness of 0.75 mm. The separating gel (11.7% acrylamide and 0.32% bisacrylamide) and the stacking gel (4.4% acrylamide and 0.12% bisacrylamide) were prepared according to Davis (46). Each sample was run alone or after it was mixed with VLDL of known apoE phenotype. The former analysis determined whether the unknown phenotype was homozygous or heterozygous for the apoE alleles, and the latter analysis determined the isoelectric point relationship of the unknown to the known apoE phenotypes described previously (37–39). The plasma lipid and lipoprotein measurements were done in the laboratory of the Cincinnati Lipid Research

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Clinic. The dialyzed, lyophilized VLDL was then shipped to Boston for apoE phenotyping.

RESULTS

Recent studies in our laboratory have shown that the complex apoE patterns seen by two-dimensional gel electrophoresis result from genetic variation and post-translational modification of apoE (38). According to the currently accepted genetic model proposed by us (38, 39), there exist three alleles ($\epsilon 4$, $\epsilon 3$, and $\epsilon 2$) at the structural apoE gene locus which specify three homozygous (E4/4, E3/3, E2/2) and three heterozygous (E4/3, E3/2, E4/2) apoE phenotypes (**Fig. 1**). Analysis of apoE phenotypes in 117 normal volunteers yielded apoE allele frequencies of $\epsilon 4 = 0.12$, $\epsilon 3 = 0.74$, $\epsilon 2 = 0.14$, and calculated apoE phenotype frequencies of E4/4 = 0.014, E3/3 = 0.55, E2/2 = 0.02, E4/3 = 0.18, E3/2 = 0.21, E4/2 = 0.03.

Clinical data, lipid and lipoprotein levels, and apoE phenotypes for the 17 probands studied are indicated in Table 1. The six individuals who had palmar and tuberous xanthomas, ischemic vascular disease, and hyperlipidemia, as well as the five individuals who had only the lipid abnormality which was detected because of either a family history of early heart disease or routine screening by their physicians, all had the apoE phenotype E2/2. Four of the six individuals selected by population-based screening for lipid abnormalities, when lipoprotein quantification revealed an abnormal ratio, had the apoE phenotype E2/2, while two had the apoE phenotype E4/2. Therefore, in the probands with type III HLP, the apoE phenotype E2/2 occurred with a frequency of 88% and the apoE phenotype E4/2 occurred with a frequency of 12%. It is of interest that 13 of the 17 probands were male.

In **Fig. 2,** the apoE phenotypes and genotypes of 15 of the probands and their first-degree relatives are indicated. In this study, we describe several matings, not previously observed, that are critical to the single genetic locus, three allele model of apoE inheritance we have proposed (38, 39). In family 6, we describe a mating between two individuals with two different homozygous apoE phenotypes, E3/3 and E2/2, giving rise to offspring with only the heterozygous apoE phenotype E3/2. In families 3 and 14, two normal parents with the apoE phenotype E3/2 (carriers of the allele $\epsilon 2$) produced offspring homozygous for the apoE allele $\epsilon 2$ with the apoE phenotype E2/2 and type III HLP. Finally, in family 4, parents with the apoE phenotypes E4/3 and E4/2, heterozygotes for the apoE allele $\epsilon 4$, produced offspring with the apoE phenotype E4/4. This observation provides direct genetic evidence that the apoE

Fig. 1. Schematic presentation of the one structural locus, three-allele model of apoE inheritance and nomenclature of apoE alleles and phenotypes. The closed circles represent the major asialo apoE isoproteins.

allele $\epsilon 4$ is at the same locus as the apoE alleles $\epsilon 3$ and $\epsilon 2$. The current study also shows that the frequency observed for the apoE phenotypes of offspring in families where both parents were sampled were highly compatible by χ^2 analysis with the expected frequencies of the apoE phenotypes based on the proposed model of apoE inheritance (**Table 2**) ($\chi^2 = 5.98$, $0.3 < P < 0.4$, degrees of freedom 5). In no case did matings produce children with apoE phenotypes incompatible with the parental phenotypes.

Plasma lipids were analyzed in 69 relatives of type III HLP probands and their spouses by apoE phenotype (**Tables 3, 4, 5, and 6**). In this group of individuals, the apoE phenotypes containing the $\epsilon 2$ allele predominated (E3/2, E4/2, E2/2) and, for purposes of analysis, it was necessary to pool the data for the apoE phenotypes that do not contain the $\epsilon 2$ allele (E4/3, E4/4, and E3/3). In addition, since there were significant differences between the groups of individuals with different apoE phenotypes with regard to age, the lipid values were all adjusted by age and sex to 30 years old and male. The adjustment was made on the basis of the total group studied, excluding persons on lipid-lowering medicines. This group included 77 individuals, 55 females and 22 males, whose mean age was 30 years. The adjustment parameters were derived by multiple regression analysis and are given in Table 5. This analysis showed no significant differences between relatives with different apoE phenotypes in cholesterol, triglycerides, or HDL cholesterol levels either before or after age and sex ad-

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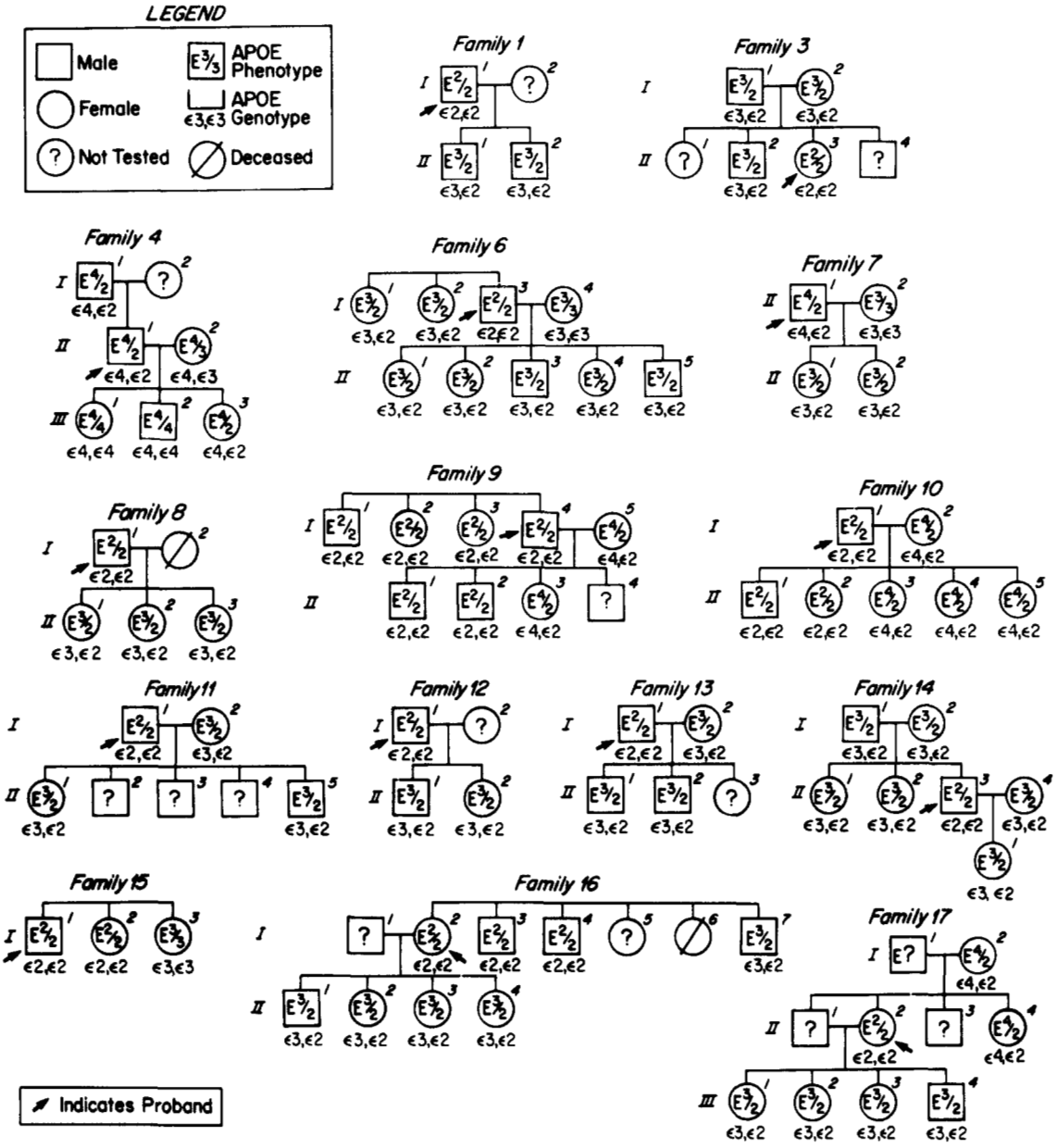

Fig. 2. Pedigrees of 15 families showing the transmission of type III HLP, as well as the transmission of the apoE phenotypes.

justment (Table 4). However, there were differences between the apoE phenotypes in adjusted LDL cholesterol levels ($P = 0.01$) and in the ratio ($P < 0.01$) (Table 4). Individuals with the apoE phenotypes E3/2, E4/2, and E2/2, which contain the ε2 allele, had lower adjusted LDL cholesterol levels than individuals with the apoE phenotypes E4/3, E4/4, and E3/3 ($P < 0.01$), which do not contain the apoE allele ε2. Individuals with the apoE phenotype E2/2, homozygosity for the apoE allele ε2, had the lowest adjusted LDL cholesterol levels. Individuals with the apoE phenotypes E3/2 and E4/2, heterozygotes for the apoE allele ε2, had intermediate adjusted LDL cholesterol levels that were significantly lower than the adjusted LDL cholesterol levels of individuals with the apoE phenotypes E4/3, E4/4, and E3/3 ($P < 0.01$), but barely missed being significantly higher than the adjusted LDL cholesterol levels of individuals with the apoE phenotype E2/2 ($0.05 < P < 0.10$). With regard to the ratios, individuals with the apoE phenotype E2/2 had significantly higher ratios than individuals with the apoE phenotypes E4/3, E4/4, E3/3 ($P < 0.05$), E3/2 ($P < 0.01$), and E4/2 ($P < 0.05$). The ratios were not significantly different between the apoE phenotypes E4/3, E4/4, E3/3, noncarriers of the ε2 allele, and the apoE phenotypes E3/2 and E4/2, carriers of the ε2 allele. The eleven rela-

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tives with the apoE phenotype E2/2 had a mean ratio of 0.23 ± 0.08 (Table 4). The six males in this group had a mean ratio of 0.28 ± 0.06, which was significantly higher than the mean ratio for the five females in this group of 0.17 ± 0.06 ($P < 0.01$). In fact, the mean ratio for the females with the apoE phenotype E2/2 was exactly the same as the mean ratio for the entire group of relatives. The group of relatives with the apoE phenotype E2/2 included two males taking lipid-lowering drugs, one male with mild angina at age 59, and five out of six males and two out of five females with βVLDL observed after ultracentrifugation and lipoprotein electrophoresis.

Plasma lipid levels in the entire group of 69 relatives of type III HLP probands were also compared to measurements made by the Lipid Research Clinic (LRC) in control populations (47). In the relatives, the adjusted mean plasma lipid levels were: cholesterol 188 mg/dl, triglycerides 185 mg/dl, HDL cholesterol 44 mg/dl, and LDL cholesterol 109 mg/dl (Table 4). The LRC data indicate that 30-year-old white males would have the following plasma lipid levels: cholesterol 186 mg/dl, triglycerides 113 mg/dl, HDL cholesterol 45 mg/dl, and LDL cholesterol 122 mg/dl. (These values are the average of the means given for white males—visit 2, random sample for the 25–29 and 30–34-year-old age groups.) These data suggest that relatives of type III HLP probands have normal cholesterol and HDL cholesterol levels, but slightly low LDL cholesterol levels, and almost twice elevated triglyceride levels. Analysis of the frequency distribution of the adjusted triglyceride levels in all first-degree relatives of type III HLP probands revealed that most but not all of the values occurred in a single Gaussian distribution (**Fig. 3**).

DISCUSSION

In recent studies, we have suggested that apoE in humans is specified at a single structural gene locus with three common alleles, ε4, ε3, ε2 (38, 39). These three apoE alleles give rise to three homozygous (E4/4, E3/3, and E2/2) and three heterozygous (E4/3, E3/2, and E4/2) apoE phenotypes. The current family studies provide unequivocal support for this model of apoE inheritance. Our genetic model has also recently been confirmed by both amino acid sequence and other chemical data by Weisgraber, Rall, and Mahley (48, 49). According to their data, the apoE phenotypes E4/4, E3/3, and E2/2 result from amino acid substitutions at two sites in the apoE polypeptide. The ε4 gene product E4 contains arginine at both sites, the ε3 gene product E3 contains one cysteine and one arginine, and the ε2 gene product E2 contains two cysteines. It is reasonable to speculate that the structural apoE gene mutation is most likely the cause of the delayed hepatic clearance of the remnant apoE-rich lipoproteins seen in type III HLP (50–52).

The current study also explores the use of the apoE phenotype as a molecular marker to aid in the diagnosis of familial type III HLP. In 17 individuals with this condition, the apoE phenotype E2/2 occurred with a frequency of 88% and the apoE phenotype E4/2 occurred with a frequency of 12%. In the general population, these two phenotypes occur with frequencies of 2% and 3%, respectively. The six cases of familial type III HLP previously studied (37–39) all had the apoE phenotype E2/2 and together with the current study indicate a strong association of type III HLP and the apoE phenotype E2/2. These data provide justification for the use of this genetically determined trait as a mo-

TABLE 2. Expected versus observed apoE phenotypes of offspring in families where both parents were sampled

Family	Parents	Number of Children	Children (Observed)	Children (Expected)
3	E3/2, E3/2	2	1 E3/2, 1 E2/2	0.5 E3/3, 1 E3/2, 0.5 E2/2
4	E4/2, E4/3	3	2 E4/4, 1 E4/2	0.75 E4/4, 0.75 E4/3, 0.75 E3/2, 0.75 E4/2
6	E2/2, E3/3	5	5 E3/2	5 E3/2
7	E4/2, E3/3	2	2 E3/2	1 E4/3, 1 E3/2
9	E2/2, E4/2	3	1 E4/2, 2 E2/2	1.5 E4/2, 1.5 E2/2
10	E2/2, E4/2	5	3 E4/2, 2 E2/2	2.5 E4/2, 2.5 E2/2
11	E2/2, E3/2	2	2 E3/2	1 E3/2, 1 E2/2
13	E2/2, E3/2	2	2 E3/2	1 E3/2, 1 E2/2
14	E3/2, E3/2	3	2 E3/2, 1 E2/2	0.75 E3/3, 1.5 E3/2, 0.75 E2/2

	E4/4	E3/3	E2/2	E4/3	E3/2	E4/2	Total
Expected[a]	0.75	1.25	7.25	1.75	11.25	4.75	27
Observed	2	0	6	0	14	5	27

[a] A χ^2 analysis with five degrees of freedom indicated that the expected and observed apoE phenotypes were compatible with a χ^2 of 5.98 ($0.3 < P < 0.4$).

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TABLE 3. Relatives of type III hyperlipoproteinemia probands

Fam.	Kin. No.[a]	Age	Sex	ApoE	CHOL	CHOL[b]	TG	TG[b]	HDL	HDL[b]	LDL	LDL[b]	Ratio VLDL Chol/Plasma TG	βVLDL[c]	Medicine
		yrs						*mg/dl*							
11		13	F		154	189	83	178	56	50	80	107	0.22		
16		15	F		125	157	45	135	45	39	75	99	0.11		
16		10	F	E4/3	154	194	78	181	47	41	98	128	0.12		
01	II-2	5	M	E3/2	133	170	57	104	43	40	83	107	0.05		
03	I-1	46	M	E3/2	201	178	284	254	35	37	112	97	0.19		
12	II-1	15	M	E3/2	140	162	50	78	46	44	94	108	0.01		
12	II-2	17	F	E3/2	160	188	110	194	44	38	103	125	0.12		
03	I-2	46	F	E3/2	196	173	112	120	58	51	119	106	0.17		
01	II-1	8	M	E3/2	124	156	133	174	42	40	75	96	0.05		
06	I-1	55	F	E3/2	284	245	175	160	59	52	195	172	0.17		
06	I-2	62	F	E3/2	217	165	126	92	60	53	134	103	0.18		
06	II-1	31	F	E3/2	216	219	64	112	44	38	164	169	0.13		
13	II-2	21	M	E3/2	137	150	94	111	41	40	79	88	0.18		
06	II-3	27	M	E3/2	232	236	366	372	49	49	129	132	0.15		
06	II-4	26	F	E3/2	155	167	137	198	57	51	78	89	0.15		
06	II-5	18	M	E3/2	132	150	153	176	45	44	64	75	0.15		
17	III-2	31	F	E3/2	149	152	53	101	58	52	80	85	0.04		
17	III-3	28	F	E3/2	144	153	67	122	64	58	67	76	0.19		
16	I-7	51	M	E3/2	480	449	1872	1832	13	15	85	65	0.20		Atromid
06		1	M	E3/2	165	207	76	131	50	47	105	133	0.13		
07	II-1	12	F	E3/2	191	228	88	185	51	45	127	155	0.15		
07	II-2	8	F	E3/2	154	198	109	217	47	41	81	113	0.24		
08	II-1	26	F	E3/2	194	206	118	179	33	27	133	144	0.22		
08	II-2	23	F	E3/2	163	180	88	157	68	62	86	101	0.10		
13	II-1	24	M	E3/2	124	133	57	68	43	42	71	77	0.18		
08	II-3	20	F	E3/2	166	189	59	135	58	52	88	106	0.34		
14	II-2	26	F	E3/2	220	232	82	143							
16		13	F	E3/2	143	178	94	189	32	26	90	117	0.22		
14	I-2	65	F	E3/2	294	237	147	105							
06	II-2	29	F	E3/2	142	149	56	109	76	70	58	66	0.14		
11		9	M	E3/2	167	198	108	148	53	51	89	109	0.23		
14	III-1	3	F	E3/2	134	187	83	204							
17	III-1	31	F	E3/2	177	180	80	128	89	83	72	77	0.14		
16		13	F	E3/2	145	180	77	172	52	46	78	105	0.19		
14	II-1	28	F	E3/2	184	193	77	132							
17	III-4	26	M	E3/2	155	161	68	76	52	52	94	98	0.13		
11	II-1	37	F	E3/2	185	178	129	161	41	35	111	109	0.23		
11	II-5	13	M	E3/2	175	200	185	217	40	38	101	117	0.18		
16	II-1	24	M	E3/2	202	211	168	179	49	48	119	125	0.20		
03	II-2	20	M	E3/2	166	181	80	99	46	45	110	120	0.13		
16	II-4	31	F	E3/2	244	247	172	220	71	65	166	171	0.04		
16	II-2	30	F	E3/2	141	146	127	177	33	27	88	94	0.16		
14	I-1	65	M	E3/2	204	153	76	10							
16	II-3	28	F	E3/2	121	130	63	118	44	38	65	74	0.19		
04	I-1	79	M	E4/2	275	203	241	149	39	45	172	125	0.26		
16		11	F	E4/2	125	164	57	157	43	37	75	104	0.12		
04	III-3	20	F	E4/2	163	186	72	148	59	53	92	110	0.17		
09	II-3	21	F	E4/2	193	214	162	236	44	38	122	139	0.14		
10	II-3	27	F	E4/2	168	178	91	149	56	50	96	106	0.18		
17	I-2	77	F	E4/2	143	65	98	25	30	23	93	44	0.09		
10	II-4	20	F	E4/2	179	202	110	186	77	71	81	99	0.19		
10	II-5	15	F	E4/2	151	183	130	220	63	57	76	100	0.15		
17	II-4	46	F	E4/2	191	168	159	167	36	29	128	115	0.17		
04	III-2	23	M	E4/4	254	264	158	171	45	44	182	189	0.17		
04	III-1	24	F	E4/4	308	324	160	226	16	10	208	222	0.19		
16		19	F	E3/3	143	167	64	143	38	32	97	116	0.13		
06		5	M	E3/3	111	148	77	124	27	24	75	99	0.12		
06		8	F	E3/3	179	223	85	193	50	44	116	148	0.15		
15	I-3	46	F	E3/3	177	154	74	82	57	50	109	96	0.15		
16		20	F	E3/3	158	181	117	193	34	28	103	121	0.18		
09	I-1	52	M	E2/2	180	148	250	209	33	35	87	66	0.27	Y	

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TABLE 3. (*Continued*)

Fam.	Kin. No.[a]	Age	Sex	ApoE	CHOL	CHOL[b]	TG	TG[b]	HDL	HDL[b]	LDL	LDL[b]	Ratio VLDL Chol/Plasma TG	βVLDL[c]	Medicine
09	I-2	55	F	E2/2	252	213	322	307	45	38	153	130	0.17	Y	
09	I-3	55	F	E2/2	225	186	214	199	49	42	145	122	0.14	Y	
09	II-1	30	M	E2/2	224	224	106	106	38	38	150	150	0.34	Y	
09	II-2	27	F	E2/2	148	158	158	216	40	34	81	91	0.17		
15	I-2	51	F	E2/2	209	177	133	128	71	64	104	86	0.26		
10	II-1	28	M	E2/2	144	147	136	140	37	37	69	71	0.28	Y	
10	II-2	24	F	E2/2	141	157	83	149	92	86	40	54	0.11		
16	I-3	59	M	E2/2	247	205	494	439	31	34	96	68	0.24	Y	Atromid
16	I-4	60	M	E2/2	217	173	210	153	32	35	107	78	0.37	Y	Norlutat
06		1	M	E2/2	128	170	89	144	56	53	54	82	0.20		

[a] The Kin. No. (kinship numbers) have been assigned to all first degree relatives and are indicated in Fig. 2.
[b] These lipid values were adjusted for age (30 years) and sex (male).
[c] Y indicates that βVLDL was present; a blank space indicates that βVLDL was absent.

lecular marker for type III HLP. In addition, the results of the current study suggest genetic heterogeneity in the population of individuals with type III HLP. The two individuals with the E4/2 phenotype at this time show no symptoms, but do manifest the lipid abnormality consistent with type III HLP. These individuals carry the apoE allele ε2 and it is possible that carriers of this allele are at risk for type III HLP under circumstances yet to be identified. It is also possible that in some individuals type III HLP may not be associated with a particular apoE phenotype but may be the result of a defect in the hepatic apoE receptor or in other still unidentified steps involved in apoE catabolism (53).

Type III HLP has been believed to be a single gene disorder on the basis of the occurrence of this disease in siblings in certain families and in a parent and one or more children in other families (1, 4, 7, 9, 54, 55). Since the former example suggests recessive inheritance and the latter dominant, confusion has existed as to the true mechanism of inheritance of this disease. Our work

TABLE 4. Plasma lipids of relatives of type III hyperlipoproteinemia probands according to apoE phenotype

Phenotype	Age	Chol	Chol[a] Adj.	TG	TG[a] Adj.	HDL	HDL[a] Adj.	LDL	LDL[a] Adj.	Ratio VLDL Chol/Plasma TG
	yr				*mg/dl*					
E4/3, E4/4, E3/3[b]	19 ± 13	186 ± 64 N = 8	207 ± 61 N = 8	102 ± 39 N = 8	164 ± 46 N = 8	39 ± 13 N = 8	34 ± 13 N = 8	124 ± 46 N = 8	140 ± 45 N = 8	0.15 ± 0.03 N = 8
E3/2	27 ± 16	182 ± 63 N = 41	190 ± 52 N = 41	154 ± 282 N = 41	192 ± 269 N = 41	50 ± 14 N = 36	46 ± 13 N = 36	100 ± 31 N = 36	108 ± 28 N = 36	0.16 ± 0.07 N = 36
E4/2	35± 26	176 ± 43 N = 9	174 ± 44 N = 9	124 ± 57 N = 9	160 ± 60 N = 9	50 ± 15 N = 9	45 ± 15 N = 9	104 ± 32 N = 9	105 ± 26 N = 9	0.16 ± 0.05 N = 9
E2/2	40 ± 19	192 ± 45 N = 11	178 ± 26 N = 11	200 ± 122 N = 11	199 ± 97 N = 11	48 ± 19 N = 11	45 ± 16 N = 11	99 ± 38 N = 11	91 ± 30 N = 11	0.23 ± 0.08 N = 11
All relatives	29 ± 19	183 ± 57 N = 69	188 ± 49 N = 69	151 ± 224 N = 69	185 ± 211 N = 69	48 ± 15 N = 64	44 ± 14 N = 64	103 ± 35 N = 64	109 ± 33 N = 64	0.17 ± 0.07 N = 64
Differences between subclasses[c]	$P = 0.05$	NS	NS	NS	NS	NS	NS	NS	$P = 0.01$	$P < 0.01$

[a] Since there were significant differences between the groups of individuals with different apoE phenotypes with regard to age, the lipid values were adjusted for age and sex to 30 years and male and are shown in the columns marked Adj. The adjustment was made on the basis of the total group studied, excluding persons on lipid-lowering medicines, and the parameters used are given in Table 5.
[b] In the population studied, there were relatively few individuals with the apoE phenotypes E4/3, E4/4, and E3/3 and for data analysis it was necessary to pool the data for these apoE phenotypes.
[c] Analysis of variance was performed to see if there were significant differences between the apoE phenotypes with respect to lipid parameters.

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TABLE 5. Parameters used to adjust plasma lipid levels for age and sex[a]

Plasma Lipid	Coefficient of Linear Regression	Y Intercept
	ml/dl per year	*mg/dl*
Cholesterol		
Men (n = 22)	1.462	136
Women (n = 55)	1.771	131
Triglyceride		
Men (n = 22)	1.886	91
Women (n = 55)	2.624	40
HDL cholesterol		
Men (n = 22)	−0.112	46
Women (n = 55)	−0.021	52
LDL cholesterol		
Men (n = 22)	0.957	79
Women (n = 55)	1.183	73

[a] The adjustment parameters for the lipids were determined on the basis of the total group studied, excluding persons on lipid-lowering medicines. This group included 77 individuals, 55 females and 22 males, whose mean age was 30 years. The adjustment parameters were derived by multiple regression analysis.

showing that the apoE phenotype E2/2 is a marker for most patients with type III HLP, along with an understanding of the inheritance of the apoE phenotypes, now allows a better understanding of the genetic transmission of type III HLP. Individuals with the apoE phenotype E2/2 are homozygotes for the apoE allele $\epsilon 2$ and the genetic tendency to type III HLP would therefore be recessive in most cases. This is indicated by non-type III HLP parents who carry the apoE allele $\epsilon 2$ and produce offspring with type III HLP and the apoE phenotype E2/2 (Families 3, 14). In addition, type III HLP parents with the apoE phenotype E2/2 who marry individuals who do not carry the apoE allele $\epsilon 2$, produce no children with type III HLP and the apoE phenotype E2/2 (Family 6 and suggested by Families 1, 8, 12, 16). In some families, an individual with type III HLP and the apoE phenotype E2/2 marries a phenotypically normal individual who carries the apoE allele $\epsilon 2$. These individuals can have the apoE phenotypes E3/2 or E4/2 and comprise 21% and 3% of the population, respectively. Half of the offspring of these marriages would be homozygous for the apoE allele $\epsilon 2$ and possess the apoE phenotype E2/2 and the genetic tendency to type III HLP (Families 9 and 10). The vertical transmission of type III HLP in such families suggests dominant inheritance, but this is a pseudodominant pattern due to the common frequency of the $\epsilon 2$ gene in the population (15%). Finally, some individuals with type III HLP (probands 4 and 7) have the apoE phenotype E4/2 and only have one copy of the $\epsilon 2$ gene and may inherit the tendency to type III HLP in a truly dominant fashion.

As stated in the introduction, the diagnosis of type III HLP is often elusive and individuals with the classical picture of xanthomas, ischemic vascular disease, and dysbetalipoproteinemia are relatively rare in the population (4–9). Previous evidence suggests that this is the extreme of the clinical spectrum of type III HLP and that the expression of this condition is influenced by many factors including sex, age, diet, obesity, and thyroid status (4, 9, 10). The full clinical spectrum of this condition may now be better appreciated by examining relatives of type III HLP probands who also have the apoE phenotype E2/2. In the current study, eleven such relatives were identified and none had xanthomas, one male had mild angina at age 59, and seven had βVLDL. When compared to relatives with the other apoE phenotypes, there were no differences in cholesterol, triglycerides, or HDL cholesterol, but LDL cholesterol levels were low and the ratio was high. The elevated ratio occurred in the males in this group, whereas the average ratio for the females was the same as for all relatives. The proposed defect in type III HLP of delayed clearance of the remnants of triglyceride-rich particles is thought to result in the accumulation in plasma of par-

TABLE 6. Family study, spouses[a]

Fam.	Age	ApoE	Chol	Chol[b]	TG	TG[b]	HDL	HDL[b]	LDL	LDL[b]	Ratio VLDL Chol/Plasma TG
	yr					*mg/dl*					
4	54	E4/3	257	219	60	47	96	89	158	136	0.05
6	59	E3/3	307	261	219	193	48	41	218	190	0.19
7	39	E3/3	204	193	104	131	54	48	135	131	0.14
9	59	E4/2	270	224	488	462	37	30	112	84	0.25
10	56	E4/2	292	251	740	722	31	24	96	72	0.22
11	58	E3/2	238	193	122	99	51	44	166	139	0.17
13	45	E3/2	193	171	53	64	51	44	171	129	0.04
14	30	E3/2	166	171	43	93					
16	47	E3/2	191	166	51	57	77	70	111	97	0.06

[a] All of the spouses studied were female and none had βVLDL or were taking lipid-lowering medicines.
[b] Indicates adjusted lipid parameters as in Table 4.

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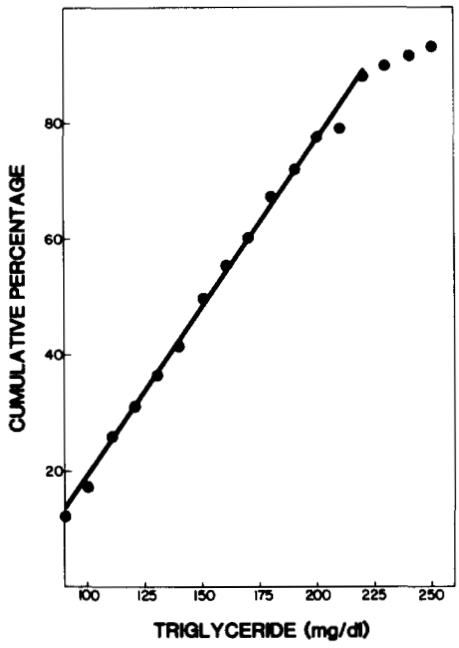

Fig. 3. Frequency distribution of the adjusted triglyceride levels in all first-degree relatives of type III HLP probands.

ticles primarily of d ≤ 1.019 g/ml with approximately equal cholesterol and triglyceride content that are indicated by the measurement of an increased ratio (3, 11–14, 21, 22, 56). Incomplete remnant catabolism (particularly VLDL remnants) could also result in diminished LDL formation and could be indicated by the observed decreased plasma LDL levels (9). Therefore, the lipid data on the relatives with the apoE phenotype E2/2 suggests that these individuals suffer from the underlying metabolic defect in type III HLP. However, the full clinical picture of type III HLP is expressed in a minority of these individuals and whatever expression occurs is favored in males. Further studies of apoE phenotypes in families of other type III HLP patients will be necessary to provide additional data on the factors necessary to cause expression of type III HLP in those with the apoE phenotype E2/2. These studies may also reveal factors that permit some non-E2/2 individuals to express type III HLP.

It has recently been suggested that expression of type III HLP is the result of the simultaneous inheritance of an apoE isoprotein abnormality and another gene for an autosomal dominant hyperlipidemia such as familial hypercholesterolemia or familial combined hyperlipidemia (9, 54, 55, 57–59). The current study revealed that relatives of type III HLP probands had normal cholesterol and HDL cholesterol levels but triglyceride levels that were almost twice normal. The cause of this could be either environmental or genetic and the latter could be either monogenic or polygenic. The frequency distribution of triglyceride levels in all first-degree relatives or in those 25 years or older did not yield a bimodal distribution. This suggests that the cause of the hypertriglyceridemia in these families was in most cases not due to a single dominant gene. The current studies do not rule out the contribution of a single dominant hyperlipidemia gene in the expression of type III HLP in a few of these cases, but proof that this has actually occurred will probably await the recognition of a suitable molecular marker for these familial hyperlipidemias. Our studies suggest that in most cases, type III HLP is expressed in families with a tendency to hypertriglyceridemia based on environmental or polygenic causes or some combination of the two.

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