

Increased Prevalence of Apolipoprotein E₄ in Type V Hyperlipoproteinemia

G. GHISELLI, E. J. SCHAEFER, L. A. ZECH, R. E. GREGG, and H. B. BREWER, JR.,
*Molecular Disease Branch, National Heart, Lung, and Blood Institute,
National Institutes of Health, Bethesda, Maryland 20205*

ABSTRACT Type V hyperlipoproteinemia (HLP) is characterized clinically by hepatosplenomegaly, occasional eruptive xanthomas, and an increased incidence of pancreatitis. These patients have striking hypertriglyceridemia due to increased plasma chylomicron and very low density lipoprotein concentrations in the fasting state, without a deficiency of lipoprotein lipase or its activator protein, apolipoprotein (apo) C-II. ApoE, a protein constituent of triglyceride-rich lipoproteins, has been implicated in the receptor-mediated hepatic uptake of these particles. ApoE has three major alleles: E², E³, and E⁴, and the products of these alleles are apoE₂, apoE₃, and apoE₄, respectively. ApoE phenotypes were determined in 30 type V HLP patients as well as in 37 normal volunteers. Among the type V patients, 33.3% were noted to be homozygous, and 40.0% heterozygous for E⁴ (normal, 2.7 and 21.6%, respectively). These data suggest that apoE₄ may play a role in the etiology of the hyperlipidemia in a significant number of type V HLP patients.

INTRODUCTION

Apolipoprotein (apo) E is a major protein constituent of plasma very low density lipoproteins (VLDL)¹ (1, 2). Studies in animals and man indicate that apoE may play a crucial role in the regulation of lipoprotein metabolism and is possibly involved in the atherogenic process (3). ApoE has been reported to be important in the receptor-mediated uptake by the liver of tri-

glyceride-rich lipoproteins, especially chylomicron remnants (4-6). Isoelectrofocusing (IEF) of delipidated VLDL protein by polyacrylamide gel electrophoresis (PAGE) demonstrates the presence of three major apoE bands in man: E₂, E₃, and E₄ (7, 8). These apoE bands differ in their isoelectric point due to arginine for cysteine substitutions at two positions in the polypeptide chain (9).

ApoE has been proposed to be inherited at a single genetic locus with three common alleles (8). The three gene products are designated as apoE₂, apoE₃, and apoE₄. In the plasma of any given individual either one major apoE form is observed (homozygote) or two major forms are noted (heterozygote). A population study in central Germany revealed the following frequency of apoE phenotypes: E₃ (58.9%), E_{3/4} (24.3%), E_{2/3} (13.1%), E₄ (2.7%), and E₂ (1.0%) (7, 10).² A study of normal individuals in the United States yielded similar distributions except that E_{2/4} was seen in 3.3% of the subjects (8). Most patients with type III hyperlipoproteinemia (HLP) have been shown to have the E₂ phenotype (7, 10), and apoE from type III HLP on plasma lipoproteins is degraded at a lower fractional catabolic rate than is normal apoE in normal and type III HLP subjects (11). Recently we have shown that type III HLP may also be due to apoE deficiency (12).

Plasma apoE concentrations have been shown to be increased in most patients with type III HLP and type V HLP (13-16). Type V patients may have hepatosplenomegaly, lipemia retinalis, eruptive xanthomas, and pancreatitis (17). These patients have striking hy-

This paper was presented in part at the Annual Meeting of the American Federation of Clinical Research, Wash., DC, 8 May 1982.

Received for publication 22 March 1982 and in revised form 19 May 1982.

¹ Abbreviations used in this paper: apo, apolipoprotein; HLP, hyperlipoproteinemia; HDL, high density lipoprotein; IEF, isoelectrofocusing; LDL, low density lipoprotein; PAGE, polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein.

² In this report apoE alleles are designated as E², E³, and E⁴, and the gene products of these alleles are apoE₂, apoE₃, and apoE₄, respectively. The six apoE genotypes are E²/E², E³/E³, E⁴/E⁴, E²/E³, E²/E⁴, and E³/E⁴. The corresponding phenotypes, as determined by IEF PAGE, for homozygotes are E₂, E₃, and E₄, and for heterozygotes E_{2/3}, E_{2/4}, and E_{3/4}, respectively. The major apoE isoforms on IEF PAGE are designated as the E₂, E₃, and E₄ bands.

pertriglyceridemia in the fasting state due to elevations of both chylomicrons and VLDL (17). The purpose of this study was to investigate apoE phenotypes in type V HLP patients. We observed a higher than expected prevalence of E⁴ homozygosity and heterozygosity, consistent with the concept that apoE₄ may play a role in the pathogenesis of type V HLP.

METHODS

30 type V hyperlipoproteinemic patients were selected from the dyslipoproteinemic patients attending the clinic of the Molecular Disease Branch at the National Institutes of Health. The mean age of these patients was 51±8 yr (mean±SD). Subjects were diagnosed as having type V hyperlipoproteinemia by the following criteria: (a) plasma triglyceride above 1,000 mg/dl, (b) chylomicrons and VLDL present in fasting plasma as determined by lipoprotein electrophoresis (18), (c) no evidence of lipoprotein lipase deficiency (19), and (d) no plasma apoC II deficiency (20). No patients had secondary causes of hyperlipidemia other than diabetes mellitus (40%) and obesity. Almost all patients were on low fat (20% of calories) diets when sampled for this study, and none were receiving medication known to affect plasma lipids except for diabetic subjects who required insulin. 37 normal subjects were also analyzed for the apoE phenotype. Their mean age was 27±4 yr.

Blood was obtained in 0.1% EDTA after an overnight fast, and the plasma was separated at 4°C in a refrigerated centrifuge. Plasma cholesterol and triglyceride levels were measured with a Technicon Auto Analyzer II (Technicon Instruments Corp., Tarrytown, NY) (18). VLDL were separated by ultracentrifugation at 1.006 g/ml at 39,000 rpm for 18 h at 4°C in Beckman 40.3 rotors (Beckman Instruments, Inc., Fullerton, CA), and the lipoprotein fractions were recovered by tube slicing (21). HDL cholesterol was measured after heparin-manganese precipitation of the 1.006 g/ml plasma infranate. Low density lipoprotein (LDL) cholesterol was calculated by subtraction of the HDL cholesterol from the 1.006 g/ml plasma infranate cholesterol value (18). VLDL, 1.006 g/ml infranate, and HDL cholesterol levels were determined by automated techniques as described for plasma. Lipoprotein electrophoresis for lipoprotein phenotyping was performed on the plasma and the 1.006-g/ml supernate and infranate (18).

VLDL were isolated by ultracentrifugation as previously described and washed a second time by the same technique.

The isolated lipoproteins were desalted by passage through a Sephadex G-25 prepacked column (Pharmacia AB, Uppsala, Sweden) (6 × 1.5 cm), equilibrated, and eluted with 0.08 M ammonium bicarbonate. VLDL were then lyophilized and delipidated with chloroform/methanol (3:1, vol/vol). VLDL apoproteins were solubilized in 8.6 M urea and 0.1 M Tris-HCl (pH 8.2) just before electrophoresis. Aliquots were assayed for the protein content by the Bradford method (22). Isoelectrofocusing of the VLDL apoE was performed on 7.5% polyacrylamide slab gels. Serva (Serva AB, Heidelberg, Federal Republic of Germany) ampholines were added to the acrylamide solution to produce a pH range between 4 and 6. 30 µg of VLDL apolipoproteins were loaded on the gels and electrofocused for 16 h at 250 V, with cooling of the electrophoretic cell at 4°C. Lower and upper buffers were 0.01 M phosphoric acid and 0.02 M sodium hydroxide, respectively. Gels were stained in 300 ml of 12.5% trichloroacetic acid containing 15 ml of Coomassie Blue G 250 (1% as stock solution) and preserved in 5% acetic acid. The major apoE isoforms electrofocused between pH 5.4 and 5.8 as determined by internal standards. The results from the slab gel isoelectrofocusing were confirmed by two-dimensional electrophoresis (8) in 25 of the 30 type V subjects and in 10 of the normal individuals.

RESULTS

Plasma lipid and lipoprotein cholesterol concentrations for the type V HLP subjects studied are shown in Table I, and the data are compared with those obtained for the normal population. Type V HLP patients had mean plasma triglyceride levels (1,640 mg/dl) and VLDL cholesterol concentrations (309 mg/dl) that were significantly (*P* < 0.01) higher than normal, and decreased HDL cholesterol values consistent with previous observations (23). The mean VLDL cholesterol/plasma triglyceride ratio in type V HLP was 0.19, similar to normal. The mean triglyceride level in our patients before institution of the low-fat (20% of total calories) diet was 3,024 mg/dl.

Six different isoform patterns for apoE were observed. Their typical appearance on slab gel IEF are shown in Fig. 1. ApoE isoform phenotype frequencies in type V HLP as compared with three normal populations are shown in Table II. 10 of the 30 type V

TABLE I
Plasma Lipid and Lipoprotein Cholesterol Concentrations in Normal and Type V Hyperlipoproteinemic Subjects*

Subjects	Plasma		Cholesterol		
	Cholesterol	Triglycerides	VLDL	LDL	HDL
	mg/dl				
Normal, <i>n</i> = 37	178±34	82±29	12±7	133±33	54±15
Type V, <i>n</i> = 30	426±131‡	1640±537‡	309±201‡	96±50‡	21±8‡

* Mean±SD.

‡ Significantly different (*P* < 0.01) from normal as determined by *t* test analysis.

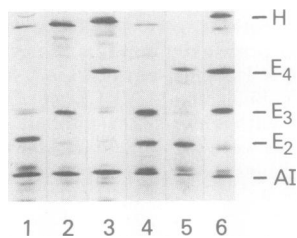


FIGURE 1 Isoelectric focusing of VLDL apoproteins from plasma of patients with type V hyperlipoproteinemia. Lipoprotein isolation and delipidation was performed as described in the text. 30 μ g of apo-VLDL were loaded in each well. Six different phenotypes are shown: (1) E₂; (2) E₃; (3) E₄; (4) E_{2/3}; (5) E_{2/4}; and (6) E_{3/4}. Only the zone of the gels showing apoH, the E₄, E₃, and E₂ bands, and apoA-I are presented. Apoproteins have been identified by comparison with purified standards.

subjects screened (33.3%) had the E₄ phenotype, in striking contrast to that observed for normal subjects (2.7%). Over 50% of the normal population were found to have the E₃ phenotype. In contrast only two of the 30 (6.7%) type V patients had this phenotype. In a normal population the following apoE allele frequencies were noted: E², 9.5%; E³, 77.0%; and E⁴, 13.5%. Utermann et al. (7, 10) have reported for a population of 490 normal individuals the following apoE allele frequencies: E², 7.6%; E³, 77.6%; and E⁴, 14.8%. Zannis and Breslow (8) have obtained similar results: 17.2%, 72.2%, and 10.6%, respectively. In this study of type V HLP subjects the allele frequency for E² was 15.0%; for E³, 33.3%; and for E⁴, 51.7%. A significantly ($P < 0.01$) higher frequency of the E⁴ allele was therefore observed in type V patients. There was no significant

difference in the lipid and lipoprotein levels of the type V patients having the E₄ phenotype when compared with the other type V subjects. No sex differences in the allele frequencies in our patients or in normals (7, 10) have been observed. In addition, no significant differences in these frequencies were observed when diabetic and nondiabetic patients were compared.

DISCUSSION

The molecular defect in type V HLP remains to be elucidated. These patients do not have a deficiency of lipoprotein lipase or apoC-II to account for their marked hypertriglyceridemia (19, 20). Similar to type III HLP, type V HLP is often not manifest until middle age (17), suggesting a role for environmental and hormonal factors as well as genetic abnormalities. Type V HLP has been noted to be familial in certain kindreds, and in addition, may be found in families with other forms of HLP such as type II and type IV (24, 25). Precise genetic patterns have not been determined, and it has been suggested that the type V HLP phenotype may be due to a number of different genetic defects (17). VLDL appears to be the most affected lipoprotein fraction in type V HLP. Metabolic studies indicate that these patients have both a threefold increase in synthesis rate as well as a decreased fractional catabolism of VLDL apoB (26). The functional importance of the apoE isoforms in the metabolism of triglyceride-rich lipoproteins is suggested by the fact that type III HLP has been associated either with apoE deficiency (12) or the E₂ phenotype (10). In patients with undetectable plasma apoE there is

TABLE II
Apolipoprotein E Phenotype Frequency in Three Normal Populations and in Patients with Type V HLP

ApoE phenotype nomenclature*			ApoE phenotype frequency†			
1	2	3	NP‡ 1	NP 2	NP 3	Type V HLP
apoE-ND/E4+	β -II	E ₄	2.7% (13)	1.6% (1)	2.7% (1)	33.3% (10)
apoE-N/E4+	α -II	E _{3/4}	24.3% (119)	14.8% (9)	21.6% (8)	36.7% (11)
apoE-D/E4+	α -IV	E _{2/4}	0.0% (0)	3.3% (2)	0.0% (0)	3.3% (1)
apoE-N/E4-	β -III	E ₃	58.9% (289)	49.1% (30)	56.8% (21)	6.7% (2)
apoE-ND/E4-	α -III	E _{2/3}	13.1% (64)	31.2% (19)	18.9% (7)	16.7% (5)
apoE-D/E4-	β -IV	E ₂	1.0% (5)	0.0% (0)	0.0% (0)	3.3% (1)
			100% (490)	100% (61)	100% (37)	100% (30)

* Nomenclature 1 refers to that used by Utermann et al. (7); 2 to that used by Zannis and Breslow (8), and 3 refers to the nomenclature used in this report.

† Phenotype frequencies for the population 1 have been calculated from the data presented by Utermann et al. (7) for a normal population studied in Germany. Phenotype frequencies for normal population 2 have been reported by Zannis and Breslow (8). Normal population 3 is that analyzed in this study.

‡ Normal population.

^{||} The number of the subjects studied is given in parentheses.

accumulation of chylomicron remnants containing apoB-48 and apoA-IV within VLDL, IDL, and LDL density fractions (12). Lipoproteins containing apoE₂ have a reduced uptake by the perfused rat liver (6) and have a delayed clearance in man when compared with lipoproteins containing apoE₃ (11). These data indicate that the apoE and its isoforms may be of important clinical significance and may affect the catabolism of the triglyceride-rich lipoproteins in man. Our results are consistent with the concept that the presence of the E⁴ allele may be a genetic factor that predisposes or contributes to the development of type V HLP. A much higher frequency of this apoE phenotype was observed in the type V HLP patients studied than that noted in normal populations (7, 8, 10). However, only a portion of the patients studied had this phenotype. This suggests that type V HLP may be due to several different molecular defects. The data presented in this report indicate that one form of apoE (apoE₄) may be important in the pathogenesis of type V HLP.

REFERENCES

- Shore, V. G., and B. Shore. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry*. **12**: 502-507.
- Shelburne, F. A., and S. H. Quarfordt. 1974. A new apolipoprotein of human plasma very low density lipoproteins. *J. Biol. Chem.* **249**: 1428-1433.
- Mahley, R. W., and T. L. Innerarity. 1978. Properties of lipoproteins responsible for high affinity binding to cell surface receptors of fibroblasts and smooth muscle cells. *Adv. Exp. Med. Biol.* **109**: 99-127.
- Shelburne, F. A., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65**: 652-658.
- Sherrill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of the canine lipoproteins containing only the E apoproteins by a high affinity receptor. *J. Biol. Chem.* **255**: 1804-1807.
- Havel, R. J., Yu-S. Chao, E. E. Windler, L. Kotite, and L. S. S. Guo. 1980. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **77**: 4349-4353.
- Utermann, G., V. Langenbeck, U. Beisiegel, and W. Weber. 1980. Genetics of the apolipoprotein E system in man. *Am. J. Hum. Genet.* **32**: 339-347.
- Zannis, V. I., and J. L. Breslow. 1981. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and post-translational modification. *Biochemistry*. **20**: 1033-1041.
- Weisgraber, K. H., S. C. Rall, Jr., and R. W. Mahley. 1981. Human E apolipoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J. Biol. Chem.* **256**: 9077-9083.
- Utermann, G., J. Hees, and A. Steinmetz. 1977. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. *Nature (Lond.)*. **269**: 604-607.
- Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia: defective metabolism of an abnormal apolipoprotein E. *Science (Wash., DC)*. **211**: 584-586.
- Ghiselli, G., E. J. Schaefer, P. Gascon, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science (Wash., DC)*. **214**: 1239-1241.
- Havel, R. J., and J. P. Kane. 1973. Primary dysbetalipoproteinemia: predominance of a specific apoprotein species in triglyceride-rich lipoproteins. *Proc. Natl. Acad. Sci. USA.* **70**: 2015-2019.
- Curry, M. D., W. J. McConathy, P. Alaupovic, T. H. Ledford, and M. Popovic. 1976. Determination of human apolipoprotein E by electroimmunoassay. *Biochim. Biophys. Acta.* **439**: 413-425.
- Blum, C. B., L. Aron, and R. Sciacca. 1980. Radioimmunoassay studies of human apolipoprotein E. *J. Clin. Invest.* **66**: 1240-1250.
- Havel, R. J., L. Kotite, J.-L. Vigne, J. P. Kane, P. Tun, N. Phillips, and G. C. Chen. 1980. Radioimmunoassay of human arginine-rich apolipoprotein, apoprotein E. Concentration in blood plasma and lipoproteins as affected by apoprotein E-3 deficiency. *J. Clin. Invest.* **66**: 1351-1362.
- Fredrickson, D. S., and R. I. Levy. 1978. Familial Type 5 hyperlipoproteinemia. In *The Metabolic Basis of Inherited Disease*. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. 4th edition. McGraw Hill Book Co., New York. 643-646.
- Manual of Laboratory Operations, Lipid Research Clinic Program. 1974. Department of Health, Education, and Welfare Publication 75-628. National Heart, Lung, and Blood Institute, Bethesda, MD.
- Krauss, R. M., R. I. Levy, and D. S. Fredrickson. 1974. Selective measurement of two lipase activities in post-heparin plasma from normal subjects and patients with hyperlipoproteinemia. *J. Clin. Invest.* **54**: 1107-1123.
- Breckenridge, W. C., J. A. Little, G. Steiner, A. Chow, and M. Poapst. 1978. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N. Engl. J. Med.* **298**: 1265-1273.
- Havel, R. J., H. A. Eder, and J. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**: 1345-1356.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.
- Schaefer, E. J., R. I. Levy, D. W. Anderson, R. N. Danner, H. B. Brewer, Jr., and W. C. Blackwelder. 1978. Plasma triglycerides in regulation of HDL cholesterol levels. *Lancet*. **II**: 391-393.
- Greenberg, B. H., W. C. Blackwelder, and R. I. Levy. 1977. Primary Type V hyperlipoproteinemia. A descriptive study in 32 families. *Ann. Intern. Med.* **87**: 526-534.
- Fallat, R. W., and C. Glueck. 1976. Familial and acquired Type V hyperlipoproteinemia. *Atherosclerosis*. **23**: 41-62.
- Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. The metabolism of very low density lipoproteins in hyperlipidemia: studies of apolipoprotein B kinetics in man. *Eur. J. Clin. Invest.* **6**: 167-177.