Role of apolipoproteins E and C in type V hyperlipoproteinemia

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Abstract Type V hyperlipoproteinemia is characterized by elevations of chylomicron (CM) and very low density lipoprotein (VLDL) triglycerides. The development of this lipid disorder involves a multitude of metabolic derangements including deficient clearance of triglycerides and/or their increased output aggravated by obesity, diabetes, alcohol intake, or use of some hormones. Some studies have suggested that the apolipoprotein E4 phenotype is involved in this dyslipoproteinemia but this concept is still a matter of controversy. Therefore, we determined the apoE phenotype in 21 patients with severe hypertriglyceridemia classified as type V. Their apoE4 gene frequency was 0.595 which is 2.6-fold higher ($P < 0.001$) than that in the Finnish population. Correspondingly, their apoE3 gene frequency was lower than that in the normal population. No differences were noted in plasma lipoproteins of the apoE4 phenotypes and the other type V subjects. The apolipoprotein C-II and C-III distribution was similar to that in normolipidemic subjects. The results suggest that apoE4 may be involved in the development of type V hyperlipoproteinemia. —**Kuusi, T., M-R. Taskinen, T. Solakivi, and R. Kauppinen-Mäkelin.** Role of apolipoproteins E and C in type V hyperlipoproteinemia. *J. Lipid Res.* 1988. **29:** 293–298.

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Apolipoprotein E is a normal constituent of plasma chylomicrons (CM) and very low density lipoproteins (VLDL) (1). This apolipoprotein is supposed to mediate the hepatic uptake of CM and VLDL remnants through its ability to bind to the high affinity B/E receptors on the liver cell surface (2). ApoE also has a function in the transport of cholesterol by the high density lipoproteins (HDL) (2, 3). This apolipoprotein has a genetic polymorphism with three common alleles E2, E3, and E4 which determine six different phenotypes (E2/2, E3/3, E4/4, E4/2, E4/3, and E3/2) (4, 5). ApoE2 is a nonfunctional mutant having a single amino acid interchange (arg 158→cys 158) which makes it unable to bind to the B/E receptor (6). The apoE2/2 phenotype is a prerequisite for the development of type III hyperlipoproteinemia (7). The other mutant, apoE4, has a single cys→arg interchange at the locus 112 (8). It has been suggested that this apoE plays a role in the development of type V hyperlipoproteinemia, but this concept is still a matter of controversy (9–12).

Type V hyperlipoproteinemia is a complex lipid disorder which is characterized by the presence of both fasting chylomicronemia and an elevation of the VLDL level (13). Most patients with type V hyperlipoproteinemia have a dual defect in triglyceride metabolism, showing both overproduction of triglycerides and a clearance defect of VLDL. This form of hyperlipoproteinemia is relieved by a fat-free diet and/or is converted to type IV hyperlipoproteinemia. On the other hand, type V hyperlipoproteinemia is aggravated by factors causing enhanced output of triglycerides, such as poor control of diabetes, obesity, or alcohol intake, which lead to the excessive accumulation of CM and VLDL (13). Since the phenotype of the type V patients can vary considerably, a group of patients with a long follow-up in the Lipid Outpatient Clinic was selected to evaluate the significance of apoE phenotype in type V hyperlipoproteinemia. In addition we examined their apoC-II/C-III distribution because decreased ratio of these apolipoproteins has been suggested to be causally related to severe hypertriglyceridemia (14).

SUBJECTS AND METHODS

Patients

Twenty-one unrelated probands with type V hyperlipoproteinemia, eighteen men and three women, were recruited for the study in 1985–1986 from the Lipid Out-

Abbreviations: CM, chylomicrons; VLDL, very low density lipoproteins; HDL, high density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HL, hepatic lipase; LPL, lipoprotein lipase; IEF, isoelectric focusing.

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patient Clinic of the Meilahti Hospital. The type V diagnosis was based on the level of plasma triglycerides above 1000 mg/dl, the presence of chylomicrons upon ultracentrifugation, and on the exclusion of lipoprotein lipase (LPL) and apoC-II deficiencies. The patients were aged between 30 and 58 yr with mean ± SEM of 45.2 ± 0.84 yr; the mean ± SEM relative body weight was 127.3 ± 3.0% (range 108–159%). Secondary causes of hypertriglyceridemia other than diabetes (52%) and obesity were excluded. The purpose of the study was explained to the patients and informed consent was obtained. The study protocol was approved by the Ethical Committee of Meilahti Hospital. Blood samples for lipoprotein and apolipoprotein analysis were taken in the morning at 8 AM after an overnight fast. This was followed by intravenous injection of 100 IU/kg of heparin for the collection of postheparin plasma into precooled tubes 5 and 15 min later.

Controls

The reference values of serum lipoproteins and postheparin plasma lipase activities were from 24 women and 50 men from the staff of Meilahti Hospital and from the office staff of two companies. They volunteered in response to a written invitation. The mean ± SEM age was 47.0 ± 1.2 yr (range 30–62 yr) for men and 46.4 ± 1.7 yr (range 30–62 yr) for women. The mean relative body weight was 112.6 ± 2.1% (range 83.6–157.6%) in men and 110.6 ± 3.3% (range 85.7–146.9%) in women. The apolipoprotein C distribution was studied in a subgroup of eleven normolipidemic subjects.

Lipid and lipoprotein analyses

Serum and lipoprotein cholesterol and triglyceride concentrations were determined enzymatically with commercially available kits from Boehringer GmbH, FRG (No 236691 and No 297771, respectively) in a Kone Olli-C analyzer (Kone Ltd., Espoo, Finland). CM were separated by ultracentrifugation for 30 min at 2×10^4 rpm using a Kontron TFT 45.6 rotor in a Beckman L7-70 ultracentrifuge. VLDL, LDL, and HDL were then isolated by sequential ultracentrifugation at 10^5 g_{av} and at +4°C (15). We used spinning times of 18 hr, 20 hr, and 72 hr at densities of 1.006 g/ml, 1.063 g/ml, and 1.21 g/ml for the separation of VLDL, LDL, and HDL, respectively. The postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) were determined by an immunochemical assay using specific anti-HL serum for the inactivation of HL in the assay of LPL (16).

ApoE phenotyping

Lipoproteins of density <1.019 g/ml were separated in the Beckman L7-70 ultracentrifuge operated at 10^5 g_{av} for 18 hr. Duplicate aliquots (50–100 μg of protein) were incubated with either 10 μl of 10% (w/v) cystamine (Sigma, St. Louis, MO) in 0.1 M ammonium bicarbonate or 10 μl of mercaptoethanol for 3 hr at 37°C followed by delipidation with ethanol–ethyl ether 3:1 and 3:2 (v/v), and finally by two extractions with ethyl ether. The proteins were dried under nitrogen, followed by an overnight incubation at room temperature in 100 μl of a solution containing 8 M urea and 20% (w/v) sucrose in 10 mM Tris-HCl buffer, pH 8.6. The solution for the 2-mercaptoethanol-treated sample also contained 10 mM dithiothreitol. ApoE phenotyping was carried out by the isoelectric focusing (IEF) method of Pagnan et al. (17) with modifications described in detail earlier (18, 19). Briefly, 1.5-mm thick polyacrylamide gels were used in an LKB 2001 vertical electrophoresis unit with 2% Ampholine, pH 4–6 (LKB, Bromma, Sweden) in 8 M urea. Aliquots (60 μl) of the sample and the cystamine-treated sample were pipetted after the polymerization of the gels into adjacent slots filled with 25 μl/ml Ampholine in 5% (w/v) sucrose. The gel was then focused for 40 min at 300 volts followed by 4 hr at 500 volts, fixed and stained using Coomassie BB R-250. They were then destained until the background was clear.

Apolipoprotein C-II and C-III determination

This was carried out according to the method of Catapano et al. (20) modified for slab gels. VLDL was first isolated by ultracentrifugation for 18 hr at 10^5 g_{av} in the Beckman L7-70 ultracentrifuge. This was followed by delipidation of an aliquot containing 200 μg of protein with acetone–diethyl ether 1:1 (v/v). The dried proteins were then dissolved in 6 M urea in 10 mM Tris-HCl buffer, pH 8.2, followed by IEF (in the LKB slab gel apparatus described above) with Ampholine, pH 3.5–6.5, for 2 hr at a constant power of 25 W. The gels were then fixed, stained with Coomassie blue BB R-250, and destained as described above. The areas of apoC-II and C-III-0, -1, and -2 were then measured with an ultraviolet scanner.

Statistical methods

The statistical analysis of the data was carried out using the BMDP software (BMDP Statistical Software Inc., Los Angeles, CA) in a Digital Vax computer. The observed and expected phenotype frequencies were tested by the chi-square test, and the differences between the mean values by unpaired *t*-test. The standard deviations of the gene frequencies were calculated as described earlier (19).

RESULTS

Plasma lipid and lipoprotein cholesterol concentrations for the type V patients are compared to the lipid values

TABLE 1. Lipoproteins and postheparin plasma lipoprotein lipase and hepatic lipase activities for type V patients and normal healthy subjects

	Men		Women	
	Type V (n = 18)	Normal (n = 50)	Type V (n = 3)	Normal (n = 24)
Triglycerides, mg/dl	1332 ± 307	127 ± 10[a]	841 ± 175	92 ± 11[a]
Cholesterol, mg/dl	345 ± 37	243 ± 8[b]	249 ± 43	210 ± 9[c]
LDL-C, mg/dl	88 ± 13	153 ± 5[a]	78 ± 9	129 ± 6[a]
HDL-C, mg/dl	33 ± 3	52 ± 2[a]	30 ± 1	60 ± 2[a]
Lipoprotein lipase (μmol/hr per ml)	19.5 ± 1.5	24.9 ± 1.0[b]	15.9 ± 3.5	27.2 ± 1.5[b]
Hepatic lipase (μmol/hr per ml)	35.2 ± 3.6	30.6 ± 1.9[c]	31.0 ± 3.6	18.7 ± 1.1[b]

Values are given as means ± SEM.
[a] $P < 0.001$; [b] $P < 0.01$; [c] not significant; denotes statistical significance of difference between type V patients and control subjects by unpaired *t*-test.

of 74 normal subjects in **Table 1**. The mean plasma triglyceride concentration of all type V patients was 1262 ± 266 mg/dl and the corresponding cholesterol concentration was 330 ± 33 mg/dl. Thus, the patients had higher serum cholesterol levels than the healthy subjects, although both LDL and HDL cholesterol were lower than in normal subjects. The VLDL cholesterol/plasma triglyceride ratio of type V subjects was 0.22 which is comparable with reported values in the literature (9). Table 1 also includes the postheparin plasma LPL and HL activities. Thus, the LPL activities were lower in both male and female type V patients in comparison with the normolipidemic subjects ($P < 0.01$ in both sexes). The range of LPL values in type V patients was from 3.95 to 29.9 μmol/hr per ml. The hepatic lipase activities of type V patients were similar in men but higher in women ($P < 0.01$) in comparison with the corresponding values in normal subjects (Table 1).

The distributions of apoC-II and C-III were investigated to evaluate their possible contribution to the type V hyperlipoproteinemia (**Table 2**). The distribution of the C apolipoproteins appeared to be comparable with that observed in 11 normal subjects thereby excluding an abnormal apoC-II/C-III ratio as a cause for hypertriglyceridemia.

Five different apoE phenotypes were observed by analytical IEF (**Table 3**). ApoE isoform phenotypes were compared with the distribution of apoE phenotypes in 21 subjects calculated by the Hardy-Weinberg formula using the apoE gene frequencies reported earlier for the Finnish population (19). More than a half of the normal population has the apoE3/3 phenotype, whereas only 10% of the type V subjects had this phenotype. In contrast, about 81% of the type V subjects had an apoE4-containing phenotype which is detectable in only 39% of normal Finns. The apoE-phenotype distribution was significantly different from that in the normal Finnish population ($\chi^2 = 15.75$, $P < 0.001$). Accordingly, the apoE4 gene frequency (± SD) of the type V subjects was 0.595 ± 0.093 which is 2.6-fold higher ($P < 0.001$) than the gene frequency of 0.227 ± 0.013 in the Finnish population (19). Instead, the apoE3 gene frequency (± SD) of 0.310 ± 0.077 in type V hyperlipidemic patients was markedly lower than the respective gene frequency (± SD) of 0.733 ± 0.017 in the normal population ($P < 0.001$). Although the apoE gene locus is known to contribute to the regulation of plasma lipoproteins, no differences were observed in lipoprotein lipids of type V subjects with or without the apoE4 isoform (**Table 4**).

DISCUSSION

ApoE phenotypes were first investigated in the association with the type III hyperlipoproteinemia (7, 21–23). In fact, studies on this lipid disorder led to the discovery of the apoE allele system (7). The role of apoE phenotypes has also been investigated in other dyslipoproteinemias. Interestingly, only in type V hyperlipoproteinemia has apoE phenotype appeared to play some role (9, 10). In other hyperlipoproteinemias, type I, II, and type IV, the apoE phenotype distribution is comparable with that in normolipidemic subjects (10, 21–23).

TABLE 2. Apolipoprotein C distribution by analytical isoelectric focusing

	Type V Subjects	Normal Subjects
	%	
ApoC-III-0	8.25 ± 0.84	8.21 ± 2.48
ApoC-II	23.35 ± 0.67	21.60 ± 0.71
ApoC-III-1	39.02 ± 0.85	41.91 ± 0.98
ApoC-III-2	29.20 ± 0.79	28.44 ± 1.55

The gels were scanned and the mean ± SEM percentages of the total area are given for apoC-III-0, apoC-II, apoC-III-1, and apoC-III-2. Normal distribution is for 11 normolipidemic subjects.

TABLE 3. Observed phenotype frequency of apolipoprotein E in type V patients in comparison with those obtained according to the Hardy-Weinberg formula for 21 subjects using the gene frequencies of the Finnish population (19)

ApoE Phenotype	Type V Patients Observed (%)		Normal Population Expected
E4/4	7	(33)	1
E4/3	8	(38)	8
E4/2	3	(14)	0
E3/3	2	(10)	11
E3/2	1	(5)	1
E2/2	0	(0)	0

Recent studies have documented the association between high LDL cholesterol and the apoE4 phenotype on one hand and low LDL cholesterol and the apoE2 phenotype on the other (19, 24–26). Indeed, about 16% of the genetic variance of LDL cholesterol is due to variation of the apoE gene locus (24). Accordingly, the apoE3/2 subjects have about 20% lower and the apoE4/3 subjects 10% higher levels of LDL cholesterol than subjects with the apoE3/3 phenotype (25). The present type V subjects had markedly lower LDL cholesterol concentrations than normal subjects although they had increased frequency of apoE4 which should elevate the LDL levels. The low LDL cholesterol is probably a reason why there were no further differences in LDL cholesterol between the type V subjects with and without the apoE4 phenotype. The observed dissociation between apoE phenotype and LDL cholesterol is of interest. Low LDL implies that conversion of VLDL to IDL and LDL is impaired or the removal of LDL is enhanced. Type V subjects frequently display reduced removal capacity of triglyceride-rich particles (13, 27–30). When VLDL production is excessive, as in type V hyperlipidemia, the compensatory capacity of the removal system is exceeded. The impaired hydrolysis of triglyceride-rich particles interrupts the VLDL-IDL-LDL cascade and explains the lowering of both LDL and HDL in type V patients (13). In accord with this the postheparin plasma LPL activity was subnormal or within low normal range in our type V patients. The LPL activity may be selectively deficient in muscle and/or adipose tissue in type V hyperlipoproteinemia (13). Unfortunately, the tissue LPL deficiency is not always properly reflected in the postheparin plasma LPL activity (13). However, some patients had totally normal LPL activity as well as normal apoC-II and C-III. In these subjects other unknown pathophysiological mechanisms must prevail.

Several patients of the present study had an apoE4-containing phenotype. In fact, the distribution of apoE phenotypes was closely similar to that observed by Ghiselli et al. (9, 10) and Gregg, Zech, and Brewer (12). Thus the current data support their proposal for the role of apoE4 in the development of type V hyperlipoproteinemia. The majority of the patients in the present study had been followed over years because of severe hypertriglyceridemia. This is probably the reason for the similarity of the apoE phenotype distribution to that observed by Ghiselli et al. (9); this research group also studied well-characterized type V patients (12). The molecular basis for the accumulation of both CM and VLDL in the apoE4 subjects with type V is not established. Recent findings on the role of apoE phenotypes in the lipoprotein metabolism in liver and intestine offer possibilities for speculation on underlying mechanisms for hyperlipidemia in apoE4 subjects.

ApoE4 is considered to enhance the hepatic uptake of remnant lipoproteins, although the receptor binding of this isoform in vitro does not differ from that of the most common apoE3 isoform (8, 19, 26, 31). In vivo this isoform is catabolized at a rate twice that of the normal apoE3 isoform, suggesting that apoE4 is more effective than apoE3 in modulating direct CM and VLDL remnant uptake in the liver (31). The consequent down-regulation of the B/E receptors by increased cholesterol influx from the remnants could explain the elevation of LDL cholesterol levels in normolipidemic apoE4 subjects (19, 24–26, 32).

The elevation of hepatic cholesterol as a result of remnant uptake can increase the secretion of biliary

TABLE 4. Lipoproteins and postheparin plasma lipoprotein lipase and hepatic lipase activities for type V men with and without the apoE4 phenotype

	Type V Patients	
	With ApoE4 (n = 15)	Without ApoE4 (n = 3)
Triglycerides, mg/dl	1340 ± 355	1294 ± 625[a]
Cholesterol, mg/dl	359 ± 43	266 ± 28[a]
LDL-C, mg/dl	92 ± 14	63 ± 27[a]
HDL-C, mg/dl	34 ± 3	24 ± 7[a]
Lipoprotein lipase, μmol/hr per ml	20.7 ± 1.3	13.7 ± 5.7[a]
Hepatic lipase, μmol/hr per ml	36.0 ± 4.2	31.3 ± 5.3[a]

Values are given as mean ± SEM.
[a]Not significant by unpaired *t*-test.

cholesterol and bile acids (33). Interestingly, the apoE phenotype is also involved in the absorption of cholesterol from the intestine, apoE4 subjects absorbing cholesterol more efficiently than the apoE2 phenotypes (34). Variation in cholesterol absorption, on the other hand, explains about 15% of the variation of serum LDL cholesterol (35). If the bile acid secretion varies according to the apoE phenotype, the capacity for micelle formation would be different and, consequently, also cholesterol absorption. Since absorption of dietary triglycerides also requires formation of micelles, the availability of excess bile acids would promote the absorption of dietary fats. In fact, the apoE4 phenotypes have enhanced clearance of dietary fat labeled with retinyl palmitate (36).

To date, a possible regulatory role for hepatic B/E receptors in the metabolism of other (apoE-containing) lipoproteins than LDL is totally unknown. It can be speculated that the down-regulation of the B/E receptors could also contribute to a defective clearance of CM, VLDL, and their remnants in the apoE4 phenotype. This would be of functional importance if their flux to liver is increased by disturbances in normal lipolytic cascade. We suggest that the combination of apoE4 phenotype and/or enhanced VLDL production together with subnormal or low removal capacity aggravates hypertriglyceridemia to such an extent that it manifests as type V hyperlipoproteinemia. This outlined hypothesis remains to be established by further studies.

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REFERENCES

1. 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.
2. Mahley, R. W. 1982. Atherogenic hyperlipoproteinemia. The cellular and molecular biology of plasma lipoproteins altered by dietary fat and cholesterol. *Med. Clin. N. Am.* **66:** 375–400.
3. Basu, S. K., Y. K. Ho, M. S. Brown, D. W. Bilheimer, R. G. W. Anderson, and J. L. Goldstein. 1982. Biochemical and genetic studies on the apolipoprotein E secreted by mouse macrophages and human monocytes. *J. Biol. Chem.* **25:** 9788–9795.
4. Zannis, V., P. W. Just, and J. L. Breslow. 1981. Human apolipoprotein E isoprotein subclasses are genetically determined. *Am. J. Hum. Genet.* **33:** 11–24.
5. Rall, S. C., K. H. Weisgraber, and R. W. Mahley. 1982. Human apolipoprotein E. The complete amino acid sequence. *J. Biol. Chem.* **257:** 4171–4178.
6. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1982. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine–arginine interchange at a single site. *J. Biol. Chem.* **257:** 2518–2521.
7. Utermann, G., M. Hees, and A. Steinmetz. 1977. Polymorphism of apolipoprotein E and occurrence of dysliproteinaemia in man. *Nature.* **269:** 604–607.
8. Weisgraber, K. H., S. C. Rall, and R. W. Mahley. 1981. Human E apoprotein heterogeneity. Cysteine–arginine interchange in the amino acid sequence of the apo-E isoforms. *J. Biol. Chem.* **256:** 9077–9083.
9. Ghiselli, G., E. J. Schaefer, L. A. Zech, R. E. Gregg, and H. B. Brewer, Jr. 1982. Increased prevalence of apolipoprotein E4 in type V hyperlipoproteinemia. *J. Clin. Invest.* **70:** 474–477.
10. Ghiselli, G., R. E. Gregg, L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1982. Phenotype study of apolipoprotein E isoforms in hyperlipoproteinemic patients. *Lancet.* **ii:** 405–407.
11. Stuyt, P. M., A. F. M. Stalenhoef, P. N. M. Demacker, and A. van 't Laar. 1982. Hyperlipoproteinemia type V and apolipoprotein E4. *Lancet.* **ii:** 934.
12. Gregg, R. E., L. A. Zech, and H. B. Brewer, Jr. 1983. Apolipoprotein E alleles in severe hypertriglyceridemia. *Lancet.* **1:** 353.
13. Nikkilä, E. A. 1983. Familial lipoprotein lipase deficiency and related disorders of chylomicron metabolism. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown, editors. 5th ed. McGraw-Hill Book Co. Inc., New York. 622–642.
14. Carlson, L. A., and G. Wahlberg. 1978. Relative increase in apolipoprotein CII content of VLDL and chylomicrons in a case with massive type V hyperlipoproteinemia by nicotinic acid treatment. *Atherosclerosis.* **31:** 77–84.
15. Havel, R. J., H. S. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
16. Huttunen, J. K., C. Ehnholm, P. K. J. Kinnunen, and E. A. Nikkilä. 1975. An immunochemical method for selective measurement of two triglyceride lipases in human postheparin plasma. *Clin. Chim. Acta.* **63:** 335–347.
17. Pagnan, A., R. J. Havel, J. P. Kane, and L. Kotite. 1977. Characterization of human very low density lipoproteins containing two electrophoretic populations: double prebeta lipoproteinemia and primary dysbetalipoproteinemia. *J. Lipid Res.* **18:** 613–622.
18. Weisgraber, K. H., and R. W. Mahley. 1978. Apoprotein (E-AII) complex of human plasma lipoproteins. I. Characterization of this mixed disulfide and its identification in a high density lipoprotein subfraction. *J. Biol. Chem.* **253:** 6281–6288.
19. Ehnholm, C., M. Lukka, T. Kuusi, E. A. Nikkilä and G. Utermann. 1986. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. *J. Lipid Res.* **27:** 227–235.
20. Catapano, A. L., R. L. Jackson, E. B. Gilliam, A. M. Gotto, Jr., and L. C. Smith. 1978. Quantification of apoC-II and apoC-III of human very low density lipoproteins by analytical isoelectric focusing. *J. Lipid Res.* **19:** 1047–1052.
21. Breslow, J. L., V. I. Zannis, T. R. SanGiacomo, J. L. H. C. Third, T. Tracy, and C. J. Glueck. 1982. Studies of familial type III hyperlipoproteinemia using as a genetic marker the apoE phenotype E2/2. *J. Lipid Res.* **23:** 1224–1235.
22. Utermann, G., I. Kindermann, H. Kaffarnik, and A. Steinmetz. 1984. Apolipoprotein E phenotypes and hyperlipidemia. *Hum. Genet.* **65:** 232–236.
23. Davignon, J., C. F. Sing, S. Lussier-Cacan, and D. Buthillier. 1984. Xanthelasma, latent dyslipoproteinemia and atherosclerosis: contribution of apoE polymorphism. *In* Latent

Dyslipoproteinemia and Atherosclerosis. J. L. DeGennes, editor. Raven Press, New York. 213–223.
24. Sing, C. F., and J. Davignon. 1985. Role of apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am. J. Hum. Genet.* **37:** 268–285.
25. Robertson, F. W., and A. M. Cumming. 1985. Effects of apoprotein E polymorphism on serum lipoprotein concentration. *Arteriosclerosis.* **5:** 283–292.
26. Lenzen, H. J., G. Assmann, R. Buchwalsky, and H. Schulte. 1986. Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. *Clin. Chem.* **325:** 778–781.
27. Fredrickson, D. S., and R. S. Lees. 1965. A system for phenotyping hyperlipoproteinemias. *Circulation.* **31:** 321–327.
28. Kesäniemi, Y. A., and S. M. Grundy. 1984. Dual defect in metabolism of very low density lipoprotein triglycerides. Patients with type V hyperlipoproteinemia. *J. Am. Med. Assoc.* **18:** 2542–2547.
29. Packard, C. J., J. Shepherd, S. Joerns, A. M. Gotto, Jr., and O. D. Taunton. 1980. Apolipoprotein B metabolism in normal, type IV, and type V hyperlipoproteinemic subjects. *Metabolism.* **29:** 213–222.
30. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St. Hilaire, and B. Lewis. 1982. Relationships between the metabolism of high density and low density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* **12:** 113–120.
31. Gregg, R. E., L. A. Zech, E. J. Schaefer, D. Stark, D. Wilson, and H. B. Brewer, Jr. 1986. Abnormal metabolism of apolipoprotein E4 in humans. *J. Clin. Invest.* **78:** 815–821.
32. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
33. Dietschy, J. M., and J. D. Wilson. 1974. Regulation of cholesterol metabolism. *N. Engl. J. Med.* **282:** 1126–1142.
34. Kesäniemi, Y. A., C. Ehnholm, and T. A. Miettinen. 1987. Intestinal cholesterol absorption efficiency in man is related to apolipoprotein E phenotype. *J. Clin. Invest.* **80:** 578–581.
35. Kesäniemi, Y. A., and T. A. Miettinen. 1987. Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur. J. Clin. Invest.* **17:** 391–395.
36. Weintraub, M. S., S. Eisenberg, and J. L. Breslow. 1987. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J. Clin. Invest.* **80:** 1571–1577.