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Research Article

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[…]

Variable Expression of Familial Dysbetalipoproteinemia in Apolipoprotein E*2(Lys146→Gln) Allele Carriers

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Abstract

Genetic and biochemical studies were carried out in 96 relatives of six independently ascertained probands with familial dysbetalipoproteinemia (FD) carrying the APOE*2 (Lys146→Gln) allele. Compared to noncarriers, the 40 heterozygous APOE*2(Lys146→Gln) allele carriers exhibited markedly increased mean levels of cholesterol and triglyceride in the very low density lipoproteins (VLDL) (1.89±0.37 vs 0.30±0.27 and 1.86±0.37 vs 0.68±0.27 mmol/liter, respectively) and plasma apolipoprotein (apo)E levels (28.1±1.6 vs 4.6±1.1 mg/dl), which is characteristic for FD.

By means of a pedigree-based maximum likelihood method we calculated that carrier-status accounted for 57% and 71%, respectively, of the total variance of the ratio (VLDL + IDL)-cholesterol/plasma triglyceride and plasma apoE levels.

APOE*2(Lys146→Gln) and APOE*3-Leiden allele carriers were found to differ significantly in: (*a*) plasma apoE levels, (*b*) in the amounts of triglycerides in the VLDL and VLDL + IDL fraction, and (*c*) in the amount of cholesterol in the VLDL and VLDL + IDL fraction relative to the amount of triglyceride in these fractions. In the APOE*2 (Lys146→Gln) allele carriers the VLDL and VLDL + IDL fraction is relatively rich in triglycerides as compared with that in APOE*3-Leiden carriers. We hypothesize that these two rare mutations of apoE both lead to dominantly inherited forms of FD along different underlying metabolic defects. (*J. Clin. Invest.* 1994. 94:1252–1262.) Key words: dominant mode of inheritance • family studies • apoE3-Leiden • type III hyperlipoproteinemia

Introduction

Apolipoprotein E (apoE) is one of the major protein constituents of chylomicron and very low density lipoprotein (VLDL) remnants, and serves as a ligand for the receptor-mediated uptake of these lipoprotein particles by hepatic lipoprotein receptors (1, 2). Defects in apoE may lead to an impaired clearance of these particles from the blood stream (3), and, as a consequence, plasma levels of cholesterol, triglycerides, and apoE are elevated (familial dysbetalipoproteinemia, FD)[1] (4). In FD patients the chylomicron and VLDL remnant particles (β-VLDL) are enriched in cholesterol, leading to a strong predisposition for coronary and/or peripheral artery disease in these patients. Clinical abnormalities like palmar xanthomas and tubero-eruptive xanthomas are present in approximately 50% of the patients at the time of initial diagnosis (4).

Three common genetic variants of apoE were originally identified by isoelectric focusing and designated apoE2, apoE3, and apoE4 (5, 6). Molecular characterization showed that the apoE2 and apoE4 isoforms were encoded by APOE*2 (Arg158→Cys) and APOE*4(Cys112→Arg) alleles, each differing from the wild type APOE*3 allele by single point mutations at codons 158 and 112, respectively (7–10). Homozygosity for the APOE*2(Arg158→Cys) allele is present in the vast majority of patients with FD. However, in caucasian populations only 1 to 4% of all APOE*2(Arg158→Cys) homozygotes develop hyperlipoproteinemia. This suggests that additional genetic and/or environmental factors are required for its manifestation (11, 12).

A number of studies demonstrated that the domain of apoE between amino acids 131–150 is responsible for the high affinity binding to the LDL receptor (13–16). This receptor binding is suggested to occur by the direct ionic binding of some of the positively charged amino acid residues in the binding domain of apoE with negatively charged amino acid residues in the ligand binding domain of the LDL receptor. Heterozygosity for apoE variants with mutations removing a positively charged amino acid in the binding domain have been described in patients with FD: apoE3(Cys112→Arg;Arg142→Cys) (17, 18), apoE1-Harrisburg(Lys146→Glu) (19, 20), apoE4-Philadelphia(Glu13→Lys;Arg145→Cys) (21, 22), apoE2-Christchurch(Arg136→Ser) (23, 24), and apoE2(Arg145→Cys) (24, 25). For some of these apoE variants, i.e., apoE3(Cys112→Arg;Arg142→Cys), apoE1-Harrisburg(Lys146→Glu), and apoE4-Philadelphia(Glu13→Lys;Arg145→Cys), association with a dominant inheritance pattern of FD has been reported (18–20, 22).

In patients of Dutch descent two additional apoE variants have been found with dominant association of FD: apoE2 (Lys146→Gln) (26, 27) and apoE3-Leiden (28–32). The

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1. *Abbreviations used in this paper:* BMI, body mass index; FD, familial dysbetalipoproteinemia; LPL, lipoprotein lipase.

apoE2(Lys146→Gln) variant was first described by Rall et al. (33).

In a previous paper, we described the dominant inheritance of FD in APOE*3-Leiden carriers of a large multigeneration family (32). Because a large number of gene carriers could be studied, we were able to perform statistical analyses of the expression of FD in the APOE*3-Leiden allele carriers (32). In this study, the identification of eight probands and their family members allowed us to carry out a similar study with APOE*2 (Lys146→Gln) allele carriers. We performed an extensive family study on six pedigrees of FD probands with the APOE*2 (Lys146→Gln) allele, who probably share common ancestry. Lipid and lipoprotein analyses revealed a strong association of the APOE*2(Lys146→Gln) allele with FD, most likely caused by an impaired conversion of VLDL into IDL. Comparison of the present results with those obtained in our previous apoE3-Leiden family study (32), implies that both of these two rare mutations in the APOE gene cause dominantly inherited forms of FD but along different underlying metabolic defects.

Methods

Subjects. Through the screening of hyperlipidemic patients for biochemical and genetic parameters a total number of eight, independently ascertained, FD probands were identified exhibiting heterozygosity for the rare APOE*2(Lys146→Gln) allele. Probands M.N., F.D., and F.V. have previously been described by Smit et al. (26, 27), and were originally identified in the Lipid Clinic in Leiden. Later, proband F.H. was also identified among the Leiden patients. Probands R.v.D. and A.B. were identified as FD patients in the Lipid Clinic in Nijmegen. Proband R.L. was identified in the Lipid Clinic in Amsterdam. M.d.H. was identified in Cape Town, South Africa, after having an acute myocardial infarction. He is of Belgian desent, and was born close to the Dutch–Belgian border.

EDTA blood samples were collected after overnight fasting. Plasma was separated from cells by centrifugation at 500 *g* for 10 min at room temperature, and was used for lipid and lipoprotein analysis. Secondary causes of hyperlipidemia were excluded by standard laboratory tests. Genomic DNA was isolated from leukocytes using standard procedures (34).

APOE genotyping. APOE genotyping of the common polymorphisms at codons 112 and 158 was performed by polymerase chain reaction (PCR) of the region encompassing both polymorphic sites, digestion of the PCR products with restriction enzyme HhaI and electrophoresis on polyacrylamide gel as described earlier (30, 32, 35).

*Detection of the APOE*2(Lys146→Gln) mutation.* Identification of APOE*2(Lys146→Gln) allele carriers was performed by PCR using a mutagenic amplification primer assay (Fig. 1). Primer 3012 5′ GGC-ATCGCGGAGGAGAGCAGCT-3′ (nucleotides 3848–3867, non-coding strand) was designed with a nucleotide mismatch (underlined) as compared to the wild type APOE sequence (36). In the case of the APOE*2(Lys146→Gln) allele a PvuII restriction site is introduced due to a base pair substitution specific for this allele and the nucleotide mismatch in the primer. PCR was performed using this primer and primer 398 5′-GCGGGCACGGCTGTCCAAGG-3′ (nucleotides 3678–3697, coding strand). The reaction mixture included 50 pmol of each primer, 0.5 μg genomic DNA, 0.2 mM deoxyribonucleotide triphosphates (dNTPs), 0.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 200 μg/ml BSA, 0.1 U of Taq polymerase (superTaq) (Boehringer Mannheim GmbH, Mannheim, Germany) and 10% dimethylsulphoxide (vol/vol) in a total volume of 50 μl. Amplification was performed for 32 cycli of 1 min at 95°C, 30 s at 55°C, and 1 min 30 s at 72°C, with an initial denaturation period of 4 min. Some 15 μl of PCR products were digested with restriction enzyme PvuII according to recommendations of the supplier (Pharmacia Fine Chemicals, Piscataway, NJ). Thereafter, fragments were separated on a neutral 7.5% polyacrylamide gel, stained with ethidium bromide, and photographed on a UV source. Digestion of the PCR product of a heterozygous APOE*2 (Lys146→Gln) carrier with PvuII, will result in patterns consisting of three fragments: an undigested fragment of 189 bp, originating from the normal allele, and 171-bp and 18-bp fragments, originating from the mutant allele. The 18-bp fragment is not visible in Fig. 1.

*Haplotyping of APOE*2(Lys146→Gln) alleles.* Genomic DNA obtained from informative family members in branches of all six pedigrees was used for Southern blot analysis. Seven different restriction fragment length polymorphisms (RFLPs) in the APOE-C1-C2 gene cluster were tested (37–39). For analysis of the polymorphic HpaI-site, located in the promoter region of the APOC1 gene (40), PCR was performed according to Nillesen et al. (41). Subsequently, PCR products were digested using restriction enzyme HincII, which reveals the same polymorphism. In addition, the $(TG)_n(AG)_m$ microsatellite marker in the first intron of APOC2 was analyzed by PCR, using primer set Mfd5CA/MfdGT (42, 43).

Genealogical studies. Genealogical studies were carried out using information obtained from different sources: interviews with family members, parish archives from the 17th and 18th centuries and local civil registration, population, and census records from the 19th and 20th centuries.

Lipid and lipoprotein analysis. For the isolation of VLDL + IDL ($d < 1.019$ g/ml), 2 ml of plasma was brought to a density of 1.019 g/ml with potassium bromide and overlayered with a 3.5-ml solution of sodium chloride ($d = 1.019$ g/ml) in a 10.4-ml centrifuge tube fitting the 50 Ti fixed-angle rotor (Beckman Instruments, Geneva, Switzerland). VLDL + IDL was aspirated from the top (1 ml fraction) after centrifugation at 106,000 *g* for 16 h at 4°C. For the isolation of VLDL ($d < 1.006$ g/ml), 2 ml of plasma was overlayered with a 2.5-ml solution of NaCl ($d = 1.006$ g/ml) in a 5-ml tube fitting the 40 Ti swing-out rotor (Beckman Instruments). VLDL was aspirated from the top (1 ml fraction) after centrifugation at 90,000 *g* for 16 h at 4°C. High density lipoprotein (HDL) was determined in the infranatant after precipitation of IDL and LDL (44).

Plasma and lipoprotein cholesterol concentrations in VLDL ($d < 1.006$), VLDL + IDL ($d < 1.019$), and HDL fractions were measured using the CHOD-PAP kit (No. 236691; Boehringer Mannheim GmbH). Plasma and lipoprotein triglycerides were measured using the GPO-PAP kit (No. 701904; Boehringer Mannheim GmbH). IDL-cholesterol was calculated as the difference between VLDL + IDL-cholesterol ($d < 1.019$ g/ml) minus VLDL-cholesterol ($d < 1.006$ g/ml). LDL-cholesterol ($1.019 < d < 1.063$ g/ml) was calculated using the formula: LDL-cholesterol = plasma-cholesterol − (VLDL-cholesterol + IDL-cholesterol + HDL-cholesterol).

Agarose electrophoresis for the detection of β-VLDL was performed as described by Demacker et al. (45).

ApoE quantification. ApoE concentrations were measured by sandwich ELISA. Affinity purified polyclonal goat anti-human apoE antibodies were used for coating. Affinity purified polyclonal rabbit anti-human apoE antibodies were used as second antibodies. As second antibody swine anti-rabbit IgG antibodies conjugated to horseradish peroxidase were used. Color development was performed using tetramethylbenzidine. Pool plasma with known apoE level and obtained from healthy individuals was used as a standard.

Statistics. For the statistical analyses we considered allele carriers and noncarriers of six different families, implying that, in a strict sense, there were only six independent observations. Therefore, to test for the statistical significance of the effect of the mutant APOE*2 (Lys146→Gln) allele on the lipoprotein traits in these six families, we used a pedigree-based maximum likelihood method developed by Lange et al. (46), in which for a given pedigree of n individuals a vector of observations ($\mathbf{x}$) is defined and a vector of expected values [$\mathbf{E}(\mathbf{x})$], that can depend on measured variables such as sex or measured genotype. The covariances between the residual part of the observations, i.e., the part that is not accounted for by the measured genotype, depend on the relationships between the pedigree members and on the genetic model assumed for the observations. Throughout we have modeled the variances not accounted for by the measured genotype as consisting of additive genetic and random environmental variance, recognizing that

*Table I. Clinical Characteristics of the APOE*2(Lys146→Gln) Probands at the Time Point of Diagnosis*

	Probands with the APOE*2(Lys146→Gln) allele							
	M.N.	F.D.	F.V.*	R.L.	F.H.	R.v.D.‡	A.B.§	M.d.H.
Age	41	31	36	40	66	48	41	58
Gender	F	M	M	M	M	F	M	M
Plasma cholesterol (mmol/liter)	18.4	13.9	39.3	12.9	12.4	8.0	7.1	9.3
Plasma triglycerides (mmol/liter)	17.9	5.3	33.3	8.3	3.5	2.7	3.7	3.6
VLDL-cholesterol/plasma triglycerides (mmol/mmol)	2.11‖	2.09‖	1.50‖	0.78	1.15	1.06	0.71	N.D.
β-VLDL¶	+	+	+	+	+	+	+	+
Second APOE allele	E*3	E*3	E*3	E*2	E*3	E*4	E*3	E*4
Clinical signs**	+	+	+	+	+	+	+	+

Plasma samples were collected after an overnight fasting. N.D.: not determined. * Proband F.V. was very overweight at timepoint of sampling. ‡ Proband R.v.D. was following dietary guidelines. § Proband A.B. was under medication (gemfibrozil). ‖ Ratio cholesterol/triglycerides (mmol/mmol) was measured in VLDL after centrifugation of for 30′ at 30,000 *g* at $d < 1.006$ to remove chylomicrons. ¶ Presence (+) of β-VLDL, as revealed by agarose electrophoresis of VLDL $d < 1.006$ g/mL fraction. ** Presence of xanthomas and/or coronary artery disease/peripheral vascular disease.

the genetic part may also reflect environmental influences shared by family members. However, our main interest is to test for the influence of the measured genotype, and not to explain the remaining variance. For a given $\mathbf{E}(\mathbf{x})$ and expected covariance matrix Σ, the log likelihood of obtaining the observation vector $\mathbf{x}$ is: $L = -1/2\ln|\Sigma| - 1/2[\mathbf{x} - \mathbf{E}(\mathbf{x})]'\Sigma^{-1}[\mathbf{x} - \mathbf{E}(\mathbf{x})]$ + constant; where ′ denotes matrix transpose.

The joint log-likelihood of obtaining all pedigrees is the sum of the log-likelihood of the separate pedigrees. Estimation involves selection of parameter values under a specific model that maximizes the joint likelihood of all pedigrees. The likelihoods obtained for different models can be compared with chi-squared difference tests where $\chi^2 = 2(L_1 - L_0)$ and L_1 and L_0 denote the log likelihood for the general (H_1) and the constrained (H_0) hypothesis. The degrees of freedom (df) for this test are equal to the number of independent parameters between H_1 and H_0. (47). The Fisher package (46) was used for genetic modeling. Ascertainment correction was carried out by conditioning on the probands.

To test for the difference between the APOE*2(Lys146→Gln) and APOE*3-Leiden allele with respect to their effect on plasma lipoprotein levels, a nested multivariate analysis of variance (MANOVA) was performed, with individuals nested within families. Data from the probands of each pedigree were discarded. Also, one pedigree with only one carrier for the APOE*2(Lys146→Gln) mutation in addition to the proband was omitted from the analysis. To test which individual lipid traits are responsible for the difference between both groups, a univariate analysis of variance (ANOVA) was used.

Results

Probands. Eight independently ascertained probands, heterozygous for the APOE*2(Lys146→Gln) allele, were identified among FD patients attending different Lipid Clinics as indicated in Methods. In Table I clinical and lipoprotein parameters are shown for the probands at their first visit, unless indicated otherwise. Three of these probands (M.N., F.D., and F.V.) have been described before (26, 27). All probands showed clinical signs of FD, including tuberoeruptive xanthomas, palmar streaks, or coronary and/or peripheral vascular disease. ApoE phenotyping and genotyping revealed that five probands carry the common APOE*3 allele as second allele. Two probands carry the APOE*4(Cys112→Arg) allele, whereas one proband carries the APOE*2(Arg158→Cys) allele. In all probands the apoE phenotype is changed into the E4/E3 instead of the E4/E4 phenotype after cysteamine treatment of plasma (results not shown). Similar incomplete modification patterns have been found earlier for probands M.N., F.D., and F.V. (26). Definite identification of the APOE*2(Lys146→Gln) mutation in these probands was confirmed by PCR followed by PvuII digestion (Fig. 1).

Genealogical investigations and haplotype analyses. Family

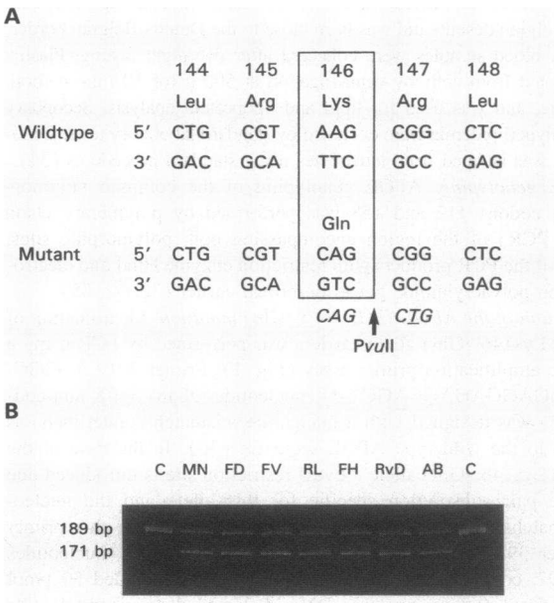

Figure 1. Analysis of APOE*2(Lys146→Gln) carriers by PCR using a mutagenic amplification primer. (*A*) A relevant part of the wildtype and mutant APOE sequence is shown. The mutated codon 146 is indicated by a box. By PCR using a mutagenic primer an additional mutation is introduced (underlined T), thereby resulting in a PvuII restriction enzyme cutting site in the mutant PCR product. (*B*) 2% agarose gel electrophoresis of PCR products using amplification primers 398 and 3012 after PvuII restriction enzyme digestion. The lanes represent probands MN, FD, FV, RL, FH, RvD, and AB. Two healthy APOE*3/APOE*2(Arg158→Cys) heterozygotes were loaded as controls (C). Proband M.d. H. gave identical bands but is not shown in the figure.

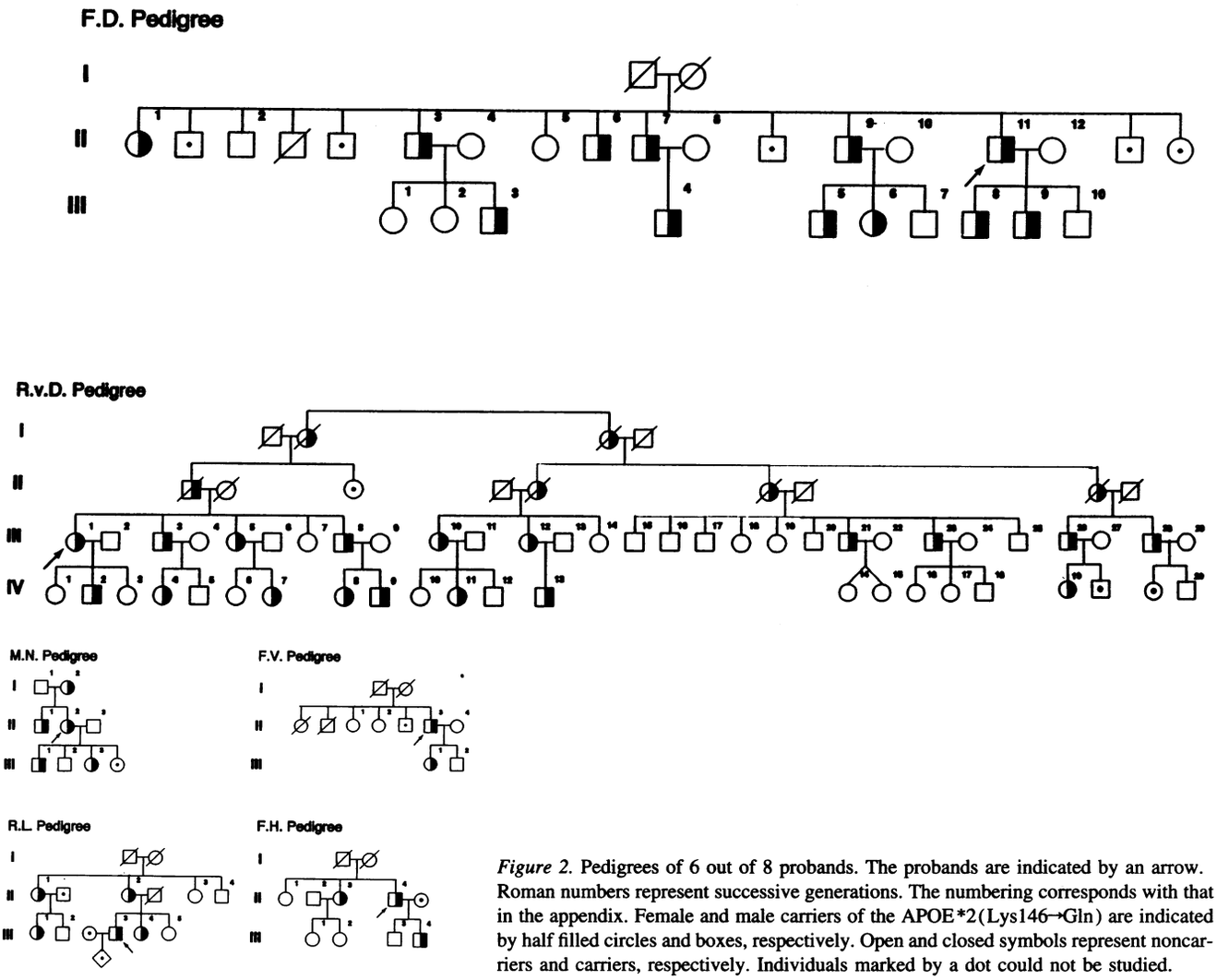

Figure 2. Pedigrees of 6 out of 8 probands. The probands are indicated by an arrow. Roman numbers represent successive generations. The numbering corresponds with that in the appendix. Female and male carriers of the APOE*2(Lys146→Gln) are indicated by half filled circles and boxes, respectively. Open and closed symbols represent noncarriers and carriers, respectively. Individuals marked by a dot could not be studied.

studies could be performed for six out of eight probands, yielding 96 individuals. Heterozygosity for the APOE*2 (Lys146→Gln) allele was observed in 40 family members. Family pedigrees are presented in Fig. 2.

Since most of the probands originate from the southwestern part of the Netherlands, we investigated whether common ancestry could be demonstrated. So far, genealogical studies revealed a common ancestry only for probands M.N. and R.L. This ancestor (in the 10th generation) was born in the early 17th century in the southwestern part of the Netherlands.

Extended haplotypes were constructed of the APOE-C1-C2 gene cluster for all pedigrees, using restriction enzymes HpaI, DraI, BglI, NcoI, BamHI, AvaII, BanI, and TaqI. We found that in all six pedigrees shown in Fig. 2, the APOE*2(Lys146→Gln) mutation cosegregated with one unique haplotype that occurred only twice among eighteen unrelated non-carrier haplotypes. This unique haplotype is designated HpaI1, DraI2, BglI2, NcoI1, BamHI2, AvaII1, BanI2 and TaqI2, whereby 1 and 2 stand for absence and presence of cutting site, respectively. Additional evidence for the presence of a unique APOE*2 (Lys146→Gln) allele in all pedigrees was obtained by including the $(TG)_n(AG)_m$ microsatellite marker in intron 1 of APOC2 (43). In all 40 APOE*2(Lys146→Gln) allele carriers, a fragment of 149 bp (designated allele G7) was observed, whereas this allele was not detected in any of the 56 noncarriers. In a caucasian population this allele was found to have a very low frequency of about 3% (43).

Lipid and lipoprotein levels in carriers versus non-carriers. Plasma samples of six probands and the 96 additional family members were studied for lipid and lipoprotein parameters (see Appendix for individual data). To avoid possible ascertainment bias, in all statistical calculations the data of the probands were excluded. The descriptive statistics of allele carrying (n = 40) and noncarrying (n = 56) family members are presented in Table II. As expected for subjects with FD characteristics, the mean levels are elevated for all lipid traits except for LDL and HDL cholesterol. Compared to noncarriers, the APOE*2 (Lys146→Gln) allele carriers exhibited markedly increased mean levels of cholesterol and triglyceride in the very low density lipoproteins (VLDL) (1.89±0.37 vs 0.30±0.27 and 1.86±0.37 vs 0.68±0.27 mmol/liter, respectively) and plasma apolipoprotein (apo)E levels (28.1±1.6 vs 4.6±1.1 mg/dl), characteristic for FD. Strikingly, the mean intermediate density lipoprotein (IDL)-cholesterol level was only slightly elevated in carriers (0.54±0.03 vs 0.29±0.03 mmol/liter). Also the mean ratio (VLDL + IDL)-cholesterol/plasma triglyceride is

*Table II. Descriptive Statistics (means±standard errors) of the APOE*2(Lys146→Gln) Allele Carriers and Their Non-carrier Relatives*

	APOE*2(Lys146→Gln) allele carriers (n = 40)	Non-carrier relatives (n = 56)
BMI*	23.3±0.8	24.9±0.6
Plasma TG	2.69±0.42	1.31±0.30
VLDL-TG	1.86±0.37	0.68±0.27
Plasma Chol	6.68±0.36	5.94±0.29
VLDL-Chol	1.89±0.37	0.30±0.27
IDL-Chol	0.54±0.03	0.29±0.03
LDL-Chol	2.74±0.65	3.03±0.18
HDL-Chol	1.37±0.07	1.53±0.05
(VLDL + IDL)-Chol/Plasma TG	0.85±0.03	0.50±0.02
ApoE	28.1±1.6	4.6±1.1

Means and asymptotic standard errors were estimated from "best-fit" models using the maximum likelihood estimate procedures implemented in the Fisher program. Probands were excluded for these quantitative analyses. * All levels are expressed as mmol/liter, except BMI (kg/m^2) and apoE (mg/100 ml).

strongly elevated. The statistical analyses of these results are presented in Table III.

We considered allele carriers and noncarriers of six different families, implying that, in a strict sense, there were only six independent observations. Therefore, to test for the effect of the mutant APOE*2(Lys146→Gln) allele in these six families, we used a pedigree-based maximum likelihood method developed by Lange et al. (46). The principles of this method of statistical analyses are briefly described in Methods. The statistical analyses of the lipoprotein levels (Table II) are shown in Table III as log-likelihood estimates for six models (as indicated in the legends of Table III). In this table we also present, when significant, the percentage of the total variance that can be explained by gender and carrier status, respectively. It is obvious that gender significantly influences levels of plasma and VLDL triglyceride, as well as VLDL, IDL, and HDL cholesterol concentrations. Gender explains 12.1% of the total variance of HDL cholesterol levels.

From Table III we also conclude that carrying the APOE*2 (Lys146→Gln) allele does significantly affect plasma levels of plasma triglyceride and all lipoprotein levels considered, but not the level of total plasma cholesterol. It is also apparent from this table that the carrier status explains the total variance of these traits for about 25 to 35%. As expected, carrier status is responsible for 57% of the variance in the ratio (VLDL + IDL)-cholesterol/plasma triglyceride, whereas 71% of the total variance of the apoE level is explained by the carrier status. In addition, Table III shows that all traits, except plasma-, IDL-, and LDL-cholesterol, are also influenced by additional genetic factors.

An increased ratio of (VLDL + IDL)-cholesterol/plasma triglyceride indicates the presence of β-migrating VLDL particles. Like in the APOE*2(Lys146→Gln) carrying FD probands, the presence of β-migrating VLDL particles was also clearly

Table III. Results of Maximum Likelihood Analyses on the Quantitative Traits and BMI in the ApoE2(Lys146→Gln) Pedigrees

	A	B	C	D	E	F	Best model	Total variance	Percent variance explained by: Gender	Carrier status
Df for difference test		1	2	3	1	1				
BMI	−167.97	−168.01	−168.69	−170.85	−172.09	−181.15*	E	16.0		
Plasma TG	−93.55	−96.48*	−94.09	−94.26	−101.32*	−103.72*	D	3.88	3.8	25.5
VLDL-TG	−81.09	−84.92*	−81.56	−81.77	−88.35*	−92.37*	D	3.06	4.9	26.1
Plasma Chol	−113.40	−113.80	−114.12	−116.33	−118.03	−118.53	F	4.33		
VLDL-Chol	−91.55	−93.66*	−91.69	−91.80	−101.49*	−95.13*	D	3.46	2.6	24.4
IDL-Chol	127.44	125.35*	127.09	126.33	107.26*	126.33	D/F	0.04	2.8	36.4
LDL-Chol	−37.33	−37.35	−37.94	−39.39	−52.16*	−41.24	F	1.30		33.2
HDL-Chol	81.78	72.72*	81.72	79.51	75.38*	71.22*	D	0.11	12.1	25.6
(VLDL + IDL)-Chol/Plasma TG	136.30	136.07	135.13	133.77	92.67*	133.46	F	0.05		56.9
ApoE	−236.60	−237.82	−238.68	−238.98	−294.29*	−241.36*	D	196		71.3

Log-likelihood estimates for six models and, when significant, the percent of total variance explained by gender and carrier-status are shown. * indicates a significant deterioration of a model when compared with the preceding best fitting one. Model definition: *A,* Most general model allowing for: (*i*) differences in mean values for the 7 different genotypes, (*ii*) gender-difference, (*iii*) an additive genetic influence, and (*iv*) random environmental variability; *B,* No gender difference; *C,* All carriers have the same means; *D,* All noncarriers have the same means; *E,* No difference between carriers and noncarriers; *F,* No additive genetic variance allowed. Testing procedure: (1), Model B is tested against model A. When twice the difference in log-likelihoods of these models is higher than the χ^2 corresponding to df = 1 and P = 0.05 (3.84) than this indicates a significant gender difference. (2) Model C (with df = 2) is tested against model B as described above. When model B was significantly different from model A, then model C was tested against model A. (3) Model D (with df = 3) is tested against model C. When model C differs significantly from model B than D is tested against model B. (4) Model E (with df = 1) is tested against model D or, in case of a significant deterioration of model D, against model C. (5) Model F (with df = 1) is tested against model E or, in case of a significant deterioration of model E, against model D. The best model is the most parsimonous model: e.g., for plasma triglycerides this is model D, with significant deteriorations in models B, E, and F. This shows that there are (*i*) significant differences between the carriers and noncarriers; (*ii*) significant gender differences; (*iii*) significant additive genetic variability (by genes other than APOE); and (*iv*) there is no variability between the carriers and between the noncarriers.

*Table IV. Differences between APOE*2(Lys146→Gln) and APOE*3-Leiden Allele Carriers: Results of Nested Univariate ANOVA*

	apoE2(Lys146→Gln) (n = 39)	apoE3-Leiden (n = 37)	Combined (n = 76)	Univariate ANOVA
Plasma TG	3.23±2.64	2.19±0.83	2.75±2.03	0.009
VLDL-TG	2.37±2.36	1.17±0.65	1.82±1.84	0.001
Plasma Chol	6.70±2.90	7.10±2.12	6.91±2.55	0.981
VLDL-Chol	2.29±2.58	1.56±1.08	1.98±2.03	0.039
HDL-Chol	1.24±0.21	1.34±0.33	1.28±0.28	0.054
(VLDL + IDL)-Chol/ Plasma-TG	0.86±0.21	1.28±0.33	1.06±0.34	0.000
ApoE	28.9±11.6	22.8±7.8	26.0±10.4	0.004
(VLDL + IDL)-Chol	2.80±2.64	2.96±1.75	2.88±2.25	0.692
(VLDL + IDL)-TG	2.53±2.46	1.57±0.81	2.05±1.90	0.003
VLDL-Chol/Plasma-TG	0.66±0.18	0.66±0.23	0.66±0.21	0.789
(VLDL + IDL)-Chol/ (VLDL + IDL)-TG	1.22±0.36	1.89±0.45	1.56±0.53	0.000
VLDL-Chol/VLDL-TG	1.00±0.27	1.29±0.32	1.14±0.33	0.000

Probands were excluded from these analyses. Nested univariate ANOVA was used to estimate the significance of the difference between the two carrier groups.

detectable in the 36 other carriers, whereas only in four APOE*-2(Lys146→Gln) allele carriers the presence of these particles was less evident (see Appendix). From the results presented in Tables II and III, we conclude that most plasma lipid and lipoprotein parameters characteristic for FD were significantly elevated in the group of carriers when compared with the group of noncarriers.

*Comparison between APOE*2(Lys146→Gln) and APOE*3-Leiden allele carriers.* The unique availability of two large groups of carriers either of the APOE*2(Lys146→Gln) allele described in this study and the APOE*3-Leiden allele (32), enabled us to compare the specific effects of these two rare apoE mutations on the expression patterns of FD. As in both groups the carriers are not unrelated (family members of six and five different families, respectively), statistically significant differences in lipoprotein traits between these two groups of carriers were analyzed using a nested multivariate analysis of variance (MANOVA), with individuals nested within families and excluding the respective probands. With this analysis, we found that the APOE*2(Lys146→Gln) allele carriers differ significantly from the APOE*3-Leiden allele carriers considering the lipoprotein traits presented in Table IV ($P < 0.001$). Nested univariate ANOVA of these lipid traits revealed that the statistically significant differences between APOE*2(Lys146→Gln) and APOE*3-Leiden allele carriers are due to differences in: (a) plasma apoE levels, (b) in the amounts of triglycerides in the VLDL and VLDL + IDL fraction, and (c) in the amount of cholesterol in the VLDL and VLDL + IDL fraction relative to the amount of triglyceride in these fractions (cholesterol/triglyceride ratios). From Table IV we conclude that in the APOE*2(Lys146→Gln) allele carriers the VLDL and VLDL + IDL fraction is relatively rich in triglycerides as compared with that in APOE*3-Leiden allele carriers.

Discussion

In general, FD is associated with homozygosity for the APOE*2(Arg158→Cys) allele and is manifested as a recessively inherited multifactorial disease, i.e., additional factors, genetic and/or environmental, are needed for its expression. For these "classical" FD patients age, nutritional status, obesity, and gender are important factors in the expression of the disease (4).

Next to the recessive form of FD, rare apoE variants exist for which heterozygosity is associated with the expression of FD. These variants include apoE3(Cys112→Arg;Arg142→Cys), apoE1-Harrisburg(Lys146→Glu), apoE4-Philadelphia (Glu13→Lys;Arg145→Cys), and apoE3-Leiden (18, 19, 22, 32). Thus far, all family members showing heterozygosity for one of these rare mutant forms of apoE had more or less severe hyperlipidemia. Thus for these variants, FD is inherited in a dominant fashion with a high penetrance, although with variable expression.

In 1983 Rall et al. (33) reported heterozygosity for the APOE*2(Lys146→Gln) allele in two unrelated males with FD. Later, we reported three independently ascertained Dutch families in which heterozygosity for the APOE*2(Lys146→Gln) allele appeared to be associated with the expression of FD (26, 27). Although in these studies evidence was obtained for a dominant mode of inheritance of FD, the number of APOE*2 (Lys146→Gln) allele carriers was too low to study the expression of FD in these carriers by statistical analyses, like we have been able to carry out for the extended multigeneration apoE3-Leiden family (32). However, recent screening of FD patients for the presence of the APOE*2(Lys146→Gln) allele resulted in the identification of five additional index patients (Table I).

From six pedigrees together, we were able to identify 40 additional APOE*2(Lys146→Gln) allele carriers among a total of 96 first degree relatives. Haplotype analysis of the APOE-C1-C2 gene cluster in these six families revealed a unique haplotype cosegregating with the APOE*2(Lys146→Gln) allele and thus pointing to common ancestry. Preliminary analysis suggested that one of the remaining two probands did not carry the same haplotype, indicating that the APOE*2(Lys146→Gln) mutation may be a recurrent mutation. Of the apoE2(Lys146→Gln) FD patients described by Rall et al. (33) haplotyping was not possible as additional family material was not available. At present, we have preliminary data that at least three additional unrelated hyperlipidemic patients carrying the APOE*2(Lys146→Gln) allele do exist among Dutch FD patients. Taken the FD populations of all the three lipid clinics together, about 8% of all index cases were heterozygous carriers of the APOE*2(Lys-146→Gln) allele.

A relatively large number of 40 allele carriers (excluding the probands) allowed us now to perform statistical analyses regarding the expression of FD. Moreover, it enabled us to compare the effect of the APOE*2(Lys146→Gln) allele on the expression of FD with that of our previously reported APOE*3-Leiden allele (32) and to evaluate as to whether different underlying metabolic defects are involved.

Previously, to analyze the effect of the dominant APOE*3-Leiden allele on the expression of FD by investigating family members of the different probands, we used the conventional statistical analyses of Mann–Whitney rank sum test and multiple regression analyses after logaritmic transformation of the dependent variables (32). However, since the subjects included are not unrelated and come from only a small number of different families, this type of statistical analysis is in fact not fully appropriate, as the number of independent observations is limited. The use of nonparametric tests does not overcome this problem of nonindependent samples. However, since then we

learned that with a new generation of statistical programs, e.g., Mendel and Fisher, it is possible, indeed, to study the influence of measured alleles on quantitative traits among relatives under different models. In this study we were not hampered by the limited number of independent observations (families) anymore, as we used the pedigree-based maximum likelihood method developed by Lange et al. (46) for analyzing the effect of the apoE2(Lys146→Gln) on quantitative lipoprotein traits relevant for FD. In addition, by using the nested multi- and univariate analysis of variance, we were also able to compare the effect of the APOE*2(Lys146→Gln) allele on the expression of FD with that of the previously described APOE*3-Leiden allele (32), in an appropriate way.

The results clearly show that the apoE2(Lys146→Gln) variant is, like the rare apoE variants mentioned above, invariably associated with the expression of FD, but the phenotypic expression varies to a large extent. Although gender does contribute to the total variance in quantitative FD-related lipoprotein traits to a certain extent, it is obvious that the APOE*2(Lys146→Gln) allele carrier status is more important in the expression of FD characteristics in these families.

Using nested multivariate analysis of variance, we found that APOE*2(Lys146→Gln) and APOE*3-Leiden allele carriers significantly differ with respect to the expression of FD (Table IV): (*a*) in the APOE*2(Lys146→Gln) allele carriers, the increased lipid levels occurred mainly in the VLDL fraction rather than in the IDL fraction, whereas in the apoE3-Leiden subjects the increased plasma cholesterol is due more specifically to an elevated IDL level, like that observed in "classical" FD patients with homozygosity for the APOE*2(Arg158→Cys) allele (48). In addition, in the APOE*2(Lys146→Gln) allele carriers the VLDL + IDL lipoprotein fraction is relatively enriched in triglycerides as illustrated by the much lower ratio of cholesterol to triglycerides in this fraction (Table IV); (*b*) separate quantitative isoelectric focusing experiments of plasma and the different lipoprotein fractions, showed that in the APOE*3-Leiden allele carriers the apoE3-Leiden variant is much more abundant than the common apoE protein. This predominance is most prominent in the VLDL and IDL density range (32) and is due to an enhanced-binding affinity of the apoE3-Leiden protein to the VLDL lipoprotein fraction (49). Such a selective accumulation of the mutant apoE protein on the VLDL and IDL lipoprotein particles has also been reported for the apoE3(Cys112→Arg;Arg142→Cys) variant (50). In contrast, in the APOE*2(Lys146→Gln) allele carriers, a predominance of the mutant apoE protein relative to its normal apoE counterpart was not observed (26, 27).

These differences strongly suggest different metabolic defects underlying the dominant mode of inheritance of FD for the rare apoE2(Lys146→Gln) and apoE3-Leiden variants. In the case of apoE3-Leiden subjects, the predominance of apoE3-Leiden converts the $d < 1.019$ g/ml lipoprotein fraction into "apparent" homozygosity for the binding-defective apoE3-Leiden protein. This apparent homozygosity will, possibly in combination with a modulating effect of the abundant apoE3-Leiden protein on the conformation of the remaining normal apoE, lead to an impaired receptor-mediated clearance of these lipoprotein particles from the circulation. Such a mechanism is also supported by the observation that in the apoE3-Leiden family, the APOE*2(Arg158→Cys) allele as counter allele results in a more severe FD phenotype, whereas the APOE*4(Cys112→Arg) allele acts in the opposite way (32).

For the apoE2(Lys146→Gln) variant, this mechanism does not hold true. It has been suggested by Mahley and co-workers (51) that the loss of any positively charged amino acid residue within the receptor-binding domain of apoE, between amino acid residue 131 and 150, affects the binding of apoE to the receptor by reducing the ionic interaction. This interaction is probably not easily modified by environmental factors and lipid composition of the lipoprotein particle. As a consequence, these authors hypothesize that mutations in the 131–150 segment of apoE result in "permanent" receptor-binding defects. This hypothesis is supported by experiments using apoE variants made by site-directed mutagenesis (13). In line with this reasoning, we found that VLDL lipoproteins, isolated from APOE*2(Lys146→Gln) allele carriers, are also defective in binding to the LDL receptor in vitro, but much less defective than VLDL from subjects with homozygosity for the common APOE*2(Arg158→Cys) allele (M. Mulder and L. Havekes, unpublished observations). It is unlikely that this relatively little impaired binding of the apoE2(Lys146→Gln) protein to the receptor is the major cause of the dominant behavior of this protein in the expression of FD. From the results presented, we hypothesize that in the apoE2(Lys146→Gln) subjects, the conversion of VLDL into IDL is impaired. Very recently, we obtained some evidence that this reduced VLDL into IDL conversion is due to an impaired lipolysis of these particles by the enzyme lipoprotein lipase (M. Mulder and L. Havekes, unpublished results). An impaired conversion into IDL would eventually lead to a less efficient binding of these lipoproteins to the LDL receptor in the liver, despite the presence of normal apoE protein, and in that way, might explain the dominant behavior of the apoE2 (Lys146→Gln) protein in the expression of FD.

APPENDIX

Data of all family members including probands

				Plasma	VLDL	Plasma	VLDL	IDL	LDL	HDL					
no.*	BMI‡	age§	sex	triglycerides (mmol/l)		cholesterol (mmol/l)					ratio¶	APOE genotype¶	β-VLDL	plasma apoE	clinical symptoms
Family proband R.L.															
II-1	26.6	76	F	2.11	1.45	7.15	1.84	0.77	3.07	1.47	1.24	3/*	+	26.4	
II-2	25.9	74	F	2.30	1.51	5.82	1.34	0.67	2.39	1.42	0.87	3/*	+	34.8	M
II-3	26.6	71	F	1.41	0.82	5.22	0.50	0.28	2.99	1.45	0.55	3/3	-	5.8	
II-4	31.0	67	M	0.86	0.50	4.94	0.30	0.19	3.05	1.40	0.57	3/3	-	4.7	
III-1	20.0	45	F	1.16	0.58	4.44	0.49	0.31	2.23	1.41	0.69	3/*	+	10.5	
III-2	27.2	46	M	3.03	2.64	5.89	0.88	0.22	3.26	1.53	0.36	3/3	-	4.7	
III-3	21.3	40	M	2.54	1.80	5.50	1.99	0.68	1.72	1.11	1.05	2/*	+	31.3	P;X
III-4	20.6	38	F	1.64	0.98	4.05	0.98	0.36	1.52	1.19	0.82	4/*	+	12.9	
III-5	20.6	36	F	1.22	0.58	4.52	0.33	0.22	2.10	1.87	0.45	3/2	-	4.6	

Family proband F.D.															
II-1	21.0	61	F	1.87	1.13	5.68	1.05	0.49	2.56	1.58	0.82	3/*	+	26.4	M
II-2	21.0	59	M	0.92	0.37	5.40	0.19	0.19	3.68	1.34	0.41	3/3	-	6.1	
II-3	19.0	55	M	2.32	1.30	6.85	1.52	0.64	3.47	1.22	0.93	3/*	+	27.1	
II-4	23.7	52	F	1.61	0.93	6.40	0.42	0.38	4.06	1.54	0.50	3/3	-	6.4	
II-5	21.2	54	F	0.96	0.43	5.50	0.34	0.30	3.28	1.58	0.67	3/3	-	6.0	
II-6	21.5	53	M	3.52	2.97	4.98	1.65	0.32	1.77	1.24	0.56	3/*	+	26.9	M
II-7	25.3	51	M	4.64	3.49	6.22	2.75	0.57	1.82	1.08	0.72	3/*	+	24.9	M;X
II-8	25.7	41	F	1.50	0.75	4.37	0.50	0.22	2.43	1.22	0.48	3/3	-	4.3	
II-9	22.0	48	M	3.04	2.05	8.55	2.75	0.79	3.84	1.17	1.16	3/*	+	29.8	
II-10	25.6	48	F	5.22	4.28	5.30	1.50	0.31	2.68	0.81	0.35	3/3	+	9.0	
II-11	22.8	47	M	2.90	1.83	7.47	1.66	0.59	3.49	1.73	0.78	3/*	+	26.3	P;X
II-12	21.6	46	F	0.66	0.15	4.44	0.16	0.15	2.53	1.60	0.47	3/3	+/-	2.9	
III-1	20.0	25	F	1.38	0.64	6.02	0.29	0.24	3.07	2.42	0.38	3/3	-	5.4	
III-2	19.4	23	F	1.29	0.55	6.69	0.22	0.19	3.60	2.68	0.32	3/3	-	5.6	
III-3	17.6	20	M	1.88	1.22	3.93	0.76	0.25	1.69	1.23	0.54	3/*	+	16.7	
III-4	17.3	11	M	0.57	0.20	3.29	0.27	0.18	1.42	1.42	0.79	3/*	+/-	18.3	
III-5	24.3	23	M	7.73	6.02	9.78	5.05	0.43	3.47	0.83	0.71	3/*	+	39.1	
III-6	19.8	21	F	3.46	2.36	5.80	1.94	0.69	2.06	1.11	0.76	3/*	+	23.4	
III-7	18.1	16	M	2.23	1.64	5.60	0.75	0.21	3.35	1.29	0.43	3/3	-	6.7	
III-8	20.9	19	M	1.68	1.00	3.94	0.83	0.33	1.64	1.14	0.69	3/*	+	18.3	
III-9	19.5	16	M	1.15	0.58	4.51	0.57	0.19	2.21	1.54	0.66	3/*	+	22.3	
III-10	16.5	11	M	0.80	0.30	4.15	0.28	0.09	2.24	1.54	0.46	3/3	+/-	2.1	
Family proband M.N.															
I-1	26.2	82	M	4.62	3.84	4.91	1.32	0.28	2.50	0.81	0.35	3/3	+	5.6	
I-2	32.0	78	F	5.57	4.25	8.96	3.45	0.94	3.35	1.22	0.79	3/*	+	52.6	X
II-1	26.1	59	M	15.59	13.78	20.83	16.24	0.59	2.99	1.01	1.08	3/*	+	75.2	X
II-2	32.0	56	F	7.11	6.07	6.56	4.20	0.50	0.80	1.06	0.66	3/*	+	33.1	P;X;M
II-3	35.2	56	M	1.60	0.97	4.87	0.50	0.28	3.11	0.98	0.49	3/3	-	4.8	M
III-1	26.7	33	M	4.26	3.50	7.20	3.63	0.35	2.16	1.06	0.93	3/*	+	34.6	
III-2	27.8	31	M	1.93	1.45	5.85	0.64	0.27	3.98	0.96	0.47	3/3	_	4.5	
III-3	29.0	27	F	6.48	5.15	6.67	3.77	0.55	1.20	1.15	0.67	3/*	+	36.2	
Family proband F.V.															
II-1	25.8	64	F	3.34	2.19	6.92	1.34	0.92	3.20	1.46	0.68	3/3	+	6.2	
II-2	29.1	61	F	1.97	1.23	7.05	0.66	0.54	4.48	1.37	0.61	3/3	_	6.6	
II-3	28.3	52	M	11.37	8.71	9.77	5.89	0.51	2.12	1.25	0.56	3/*	+	50.2	P;X;M
II-4	23.4	47	F	0.72	0.23	5.77	0.15	0.16	3.34	2.12	0.43	4/3	_	3.4	
III-1	13.4	20	F	1.47	1.00	4.49	0.69	0.23	1.81	1.76	0.63	3/*	+	19.7	
III-2	20.6	18	M	2.12	1.57	4.22	0.57	0.19	2.04	1.42	0.36	4/3	_	3.9	
Family proband R.v.D.															
III-1	23.6	60	F	3.68	2.24	9.74	3.44	1.27	3.88	1.15	1.28	4/*	+	43.3	P;M
III-2	24.5	55	M	3.52	2.45	6.75	1.50	0.27	3.71	1.27	0.50	4/3	_	7.4	
III-3	25.7	59	M	3.87	2.96	7.24	2.56	0.38	3.48	0.82	0.76	3/*	+	34.4	M
III-4	21.5	55	F	1.31	0.72	6.93	0.34	0.19	5.12	1.28	0.40	3/3	_	4.0	
III-5	20.1	53	F	3.27	2.33	7.02	2.57	0.68	2.63	1.14	0.99	3/*	+	34.5	
III-6	23.4	57	M	1.82	1.35	5.76	0.68	0.24	3.70	1.14	0.51	4/3	_	5.9	
III-7	23.8	52	F	1.83	1.19	7.90	0.70	0.38	5.59	1.23	0.59	4/4	_	3.5	
III-8	23.1	43	M	5.35	4.34	8.31	4.05	0.69	2.57	1.00	0.89	4/*	+	30.9	
III-9	20.6	44	F	1.05	0.34	4.30	0.32	0.25	2.13	1.60	0.54	3/2	_	5.1	
III-10	23.1	70	F	1.44	0.78	6.11	0.82	0.57	3.35	1.37	0.97	3/*	+/_	31.6	
III-11	21.2	73	M	1.62	1.22	4.78	0.58	0.11	2.77	1.32	0.43	3/3	-	7.1	
III-12	30.5	67	F	1.67	0.98	5.38	0.83	0.56	2.75	1.24	0.83	3/*	+	37.0	

no.*	BMI‡	age§	sex	Plasma triglycerides (mmol/l)	VLDL triglycerides (mmol/l)	Plasma cholesterol (mmol/l)	VLDL cholesterol (mmol/l)	IDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	ratio¶	APOE genotype**	β-VLDL	plasma apoE	clinical symptoms
III-12	27.1	71	M	1.44	0.89	5.06	0.36	0.19	3.10	1.41	0.38	3/3	-	4.8	
III-14	27.7	62	F	2.12	1.45	8.40	0.81	0.47	5.79	1.33	0.60	4/3	+/-	6.7	M
III-15	26.5	61	M	1.45	0.76	7.92	0.47	0.28	5.84	1.33	0.52	4/4	-	1.9	
III-16	22.5	59	M	1.09	0.52	6.78	0.39	0.30	4.79	1.30	0.63	4/4	-	1.7	
III-17	25.0	56	M	1.86	1.19	6.54	0.81	0.26	4.41	1.06	0.58	4/4	-	2.9	
III-18	34.2	54	F	1.58	0.89	7.08	0.66	0.23	4.82	1.37	0.56	4/4	-	3.2	
III-19	33.4	51	F	2.87	1.87	7.35	1.20	0.47	4.56	1.12	0.58	4/4	-	4.1	
III-20	24.6	50	M	1.81	1.07	6.63	0.70	0.26	4.34	1.33	0.53	4/4	+/-	5.2	
III-21	24.7	47	M	1.91	1.00	9.36	1.96	0.84	5.26	1.30	1.47	4/*	+	31.9	
III-22	31.2	46	F	1.02	0.49	5.65	0.40	0.10	3.64	1.51	0.49	3/3	-	4.6	
III-23	26.6	44	M	3.05	1.99	9.25	2.66	0.51	4.60	1.48	1.04	4/*	+	29.7	
III-24	28.3	47	F	0.65	0.10	7.27	0.20	0.21	5.13	1.73	0.63	3/3	-	2.4	
III-25	28.6	42	M	2.33	1.52	9.52	1.10	0.33	6.93	1.16	0.61	4/4	-	5.5	
III-26	24.8	62	M	2.54	1.73	6.21	1.57	0.65	2.89	1.10	0.87	3/*	+	18.5	
III-27	26.3	61	F	3.21	2.19	6.63	1.42	0.60	3.12	1.49	0.63	4/3	+	7.7	M
III-28	25.3	43	M	2.37	1.63	5.81	1.60	0.49	2.44	1.28	0.88	3/*	+	26.4	
III-29	21.7	41	F	1.06	0.41	6.29	0.33	0.21	3.46	2.29	0.51	3/3	-	4.6	
IV-01	20.6	31	F	0.94	0.33	4.91	0.25	0.25	3.17	1.24	0.53	4/4	_	2.3	
IV-02	21.6	29	M	6.48	5.11	7.19	3.91	0.55	1.84	0.89	0.69	4/*	+	34.1	
IV-03	20.4	24	F	1.35	0.57	5.10	0.31	0.14	3.13	1.42	0.33	4/3	_	3.0	
IV-04	24.8	33	F	3.56	2.15	6.14	1.83	0.57	2.11	1.63	0.67	3/*	+	24.1	
IV-05	25.7	31	M	1.14	0.73	5.29	0.33	0.19	3.75	1.02	0.46	3/3	_	4.5	
IV-06	23.1	31	F	1.13	0.59	4.57	0.35	0.18	2.42	1.62	0.47	4/3	_	4.4	
IV-07	20.2	29	F	1.57	1.07	5.26	0.87	0.35	2.60	1.44	0.78	4/*	+	16.8	
IV-08	21.0	18	F	1.90	1.25	4.85	1.04	0.35	2.17	1.29	0.73	4/*	+	22.9	
IV-09	20.1	16	M	1.75	1.26	4.99	1.63	0.46	1.79	1.11	1.19	2/*	+	29.5	
IV-10	24.8	44	F	1.16	0.66	4.86	0.46	0.24	2.92	1.24	0.60	3/3	-	5.9	
IV-11	22.5	39	F	1.33	0.98	3.61	0.70	0.22	1.36	1.33	0.69	3/*	+	10.6	
IV-12	21.2	31	M	2.13	1.39	4.88	1.11	0.42	2.34	1.01	0.72	3/3	-	16.8	
IV-13	29.2	37	M	4.62	3.72	7.78	3.46	0.25	2.88	1.19	0.80	3/*	+	44.4	
IV-14	22.5	20	F	0.47	0.09	4.95	0.18	0.13	2.83	1.81	0.66	4/3	-	2.5	
IV-15	30.8	20	F	1.33	0.66	7.92	0.46	0.21	5.41	1.84	0.50	4/3	-	6.2	
IV-16	24.3	26	F	0.94	0.21	6.32	0.26	0.25	3.90	1.91	0.54	4/3	-	2.2	
IV-17	23.9	19	F	0.94	0.58	5.52	0.49	0.15	3.48	1.40	0.68	4/3	-	2.0	
IV-18	22.1	19	M	1.26	0.76	4.81	0.49	0.21	2.64	1.47	0.56	4/3	-	2.2	
IV-19	19.8	36	F	3.13	2.25	6.69	2.12	0.72	2.65	1.20	0.91	4/*	+	29.3	
IV-20	17.8	14	M	0.63	0.24	4.21	0.31	0.17	1.98	1.75	0.76	3/*	+/-	25.4	
Family proband F.H.															
II-1	25.7	80	F	1.05	0.30	7.07	0.24	0.21	4.88	1.74	0.43	3/3	-	5.9	
II-2	24.7	73	M	1.29	0.69	6.22	0.25	0.15	3.95	1.87	0.31	3/3	-	5.0	M
II-3	20.3	72	F	2.77	1.98	9.49	3.09	0.79	4.54	1.07	1.40	3/*	+	33.7	M
II-4	24.1	70	M	2.29	1.76	5.53	1.26	0.40	2.63	1.24	0.72	3/*	+	25.8	P;M
III-1	21.2	42	F	0.97	0.39	4.47	0.23	0.14	2.93	1.17	0.38	3/3	-	1.7	
III-2	20.6	38	F	1.91	1.13	6.33	0.56	0.37	3.91	1.49	0.49	3/3	+/-	4.1	
III-3	21.0	39	M	1.38	0.94	6.03	0.42	0.15	3.85	1.61	0.41	3/3	-	4.3	
III-4	25.1	36	M	1.78	1.13	7.77	1.08	0.56	5.02	1.11	0.92	3/*	+/-	24.8	

*Family number corresponding with that in pedigrees of Figure 2; ‡BMI: body mass index in weight/height^2 (kg/m^2); §Persons age at the timepoint of bloodsampling in years. ¶Ratio: (VLDL+IDL)-cholesterol/plasma triglycerides; plasma apoE concentrations are given in mg/dl. **APOE genotyping; 2, 3, 4 represent common alleles: APOE*2(Arg158→Cys), APOE*3, APOE*4(Cys112→Arg), respectively; *, represents APOE*2(Lys146→Gln) allele. β-VLDL: presence (+) or absence (-) of β-VLDL as characterized by agarose electrophoresis. ‡Remarks: X: xanthomas present at first visit (palmar or tubero-eruptive type); M: medication, individual is taken lipid lowering drug; P: proband of respective family.

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