Normolipemic dysbetalipoproteinemia and hyperlipoproteinemia type III in subjects homozygous for a rare genetic apolipoprotein E variant (apoE1)[1]

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Abstract A family with three heterozygote and two homozygote carriers of the rare apolipoprotein E1 isoform was detected by isoelectric focusing. One of the homozygous patients had type III hyperlipidemia, while the other showed normolipemic dysbetalipoproteinemia. Restriction fragment length analysis as well as allele specific oligonucleotides were used to identify the structural alterations forming the abnormal ε1 genotype. Comparison with the most common ε3 allele showed that two base exchanges A for G in codon 127 and T for G in codon 158 (Asp for Gly and Cys for Arg, respectively) are responsible for the amino acid substitution which causes the charge shift observed in isoelectric focusing. The same defects have been described in the only previously characterized apoE1 (Weisgraber et al. 1984. *J. Clin. Invest.* **73:** 1024–1033). In addition to the study by Weisgraber and coworkers, who reported on a heterozygous patient, we here describe the metabolic and clinical consequences of a homozygosity for this rare allele. Changes in lipoprotein metabolism, as well as in clinical phenotypes, were exactly identical to those seen in patients homozygous for the ε2 allele, which has in common with the ε1 allele the mutation in codon 158, but lacks the substitution in codon 127. In addition, lipoprotein profiles of the ε3/ε1 heterozygotes were indistinguishable from those of ε3/ε2 heterozygotes. Therefore, we conclude that the additional mutation in codon 127 that characterizes the ε1 allele is of no functional importance in vivo. — **Steinmetz, A., N. Assefbarkhi, C. Eltze, K. Ehlenz, H. Funke, A. Pies, G. Assmann, and H. Kaffarnik.** Normolipemic dysbetalipoproteinemia and hyperlipoproteinemia type III in subjects homozygous for a rare genetic apolipoprotein E variant (apoE1). *J. Lipid Res.* 1990. **31:** 1005–1013.

Supplementary key words apolipoprotein mutant • type III hyperlipoproteinemia • RFLP • allele specific oligonucleotides

The genetic polymorphism of apolipoprotein E (apoE) first described by Utermann and collegues (1, 2) was later shown by Zannis an coworkers (3, 4) and Utermann, Steinmetz, and Weber (5) to be determined mainly by three alleles at the apoE structural gene locus. According to a generally accepted nomenclature (6), three common alleles (ε2, ε3, and ε4) code for three isoforms apoE2, E3, and E4, thus leading to six phenotypes, apoE2/2, E3/3, E4/4 and E3/2, E4/2, and E4/3. The charge difference between the three proteins was shown to be due to cysteine for arginine substitutions in positions 112 and 158 of the amino acid sequence (7–9). Furthermore, acidic bands of each major protein arise from variable sialylation of these isoforms (4). Homozygosity for apoE2 (112 Cys, 158 Cys) leads to dysbetalipoproteinemia and, in some cases, to type III hyperlipoproteinemia (1, 10–12). This common apoE2 variant exhibits diminished binding activity to the apoB,E receptor (9, 13–15).

Recently, several additional rare mutants of apoE were reported, many of them being structurally analyzed. Indistinguishable from apoE2 by one-dimensional IEF are apoE2 (Arg_{145}→Cys) (9), apoE2 (Lys_{146}→Gln) (16), and apoE2 (Arg_{136}→Ser) (17). In addition, mutants described by Havel et al. (18), Havekes et al. (19), McLean et al. (20), and Menzel and Utermann (21) cannot be distinguished from normal apoE3. The strutural defect underlying the mutant reported initially by Havel et al. (18) was recently described by Rall et al. (22) as heterozygosity for

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS, sodium dodecylsulfate; CTAB, cetyltrimethylammoniumbromide; IEF, isoelectric focusing.

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normal apolipoprotein E3 (112 Cys, 158 Arg) and mutant apoE3 (112 Arg, Arg_{142}→Cys). Furthermore, variants focusing in the apoE5 (23, 24) and apoE7 positions have been reported (25). The apoE5 variant turned out to be a 3-Glu to Lys mutation (26), whereas the apoE7 protein results from simultaneous Glu to Lys mutations in position 244 and 245 of the amino acid sequence (27, 28). More acidic variants of apoE focusing in the apoE1 position were published (21, 24, 29, 30). The structural defect in one of the apoE1 variants has been analyzed by Weisgraber and coworkers (30) (apoE: Gly_{127}→Asp, Arg_{158}→Cys). This variant was defective in competing with labeled LDL for binding to the apoB,E receptor of human skin fibroblasts (30).

So far, many of the rare mutants have been found in patients with type III hyperlipoproteinemia (16–20, 22, 29), a lipid disorder usually associated with homozygosity for apoE2 (10), characteristic accumulation in plasma of remnants of triglyceride-rich lipoproteins, with elevation of plasma triglycerides and cholesterol and a high risk to develop premature atherosclerosis (for reviews see 31–34).

In the present study we describe two subjects homozygous for a rare mutant apoE1 in a family, one affected with normolipidemic dysbetalipoproteinemia and the other with hyperlipemic dysbetalipoproteinemia (type III hyperlipoproteinemia).

METHODS

Subjects

The index patient MB, of Turkish origin, 31 years old, body mass index 23.9, was referred to the outpatient clinic of the University Hospital at Marburg. He suffered from tuberous xanthoma of the elbows, knees, and hips and expressed xanthochromia striata palmaris. No further abnormalities were found upon physical examination.

Serum triglycerides were 403 mg/dl; total serum cholesterol was 752 mg/dl. Liver, kidney, and thyroid function were found to be normal. Oral glucose tolerance was impaired (fasting blood glucose was 98 mg/dl; 30 min, 1 h, and 2 h after 100 g oral glucose load plasma concentrations were 180, 230, and 160 mg/dl, respectively).

The patient's sister, SB, 16 years old, body mass index 24.4, revealed no abnormalities upon physical examination and routine blood chemistry. Serum triglyceride and cholesterol levels were 155 and 164 mg/dl, respectively.

Lipid and apoprotein quantitation

Cholesterol and triglyceride levels were determined with standard enzymatic tests (Boehringer Mannheim, FRG). HDL cholesterol was analyzed using the phosphotungstate precipitation procedure (Boehringer Mannheim, FRG). Apolipoproteins A-I and B were quantified by nephelometry (Array System, Beckman Instruments Inc., Fullerton CA). Apolipoprotein E levels were measured by electroimmunodiffusion (Immuno Chemicals, Heidelberg, FRG).

Plasma lipoprotein separation

Plasma lipoproteins were separated from EDTA plasma by density gradient ultracentrifugation as described by Redgrave, Robert, and West (35) in a SW-41 type rotor (Beckman Instruments, Inc., Fullerton CA). After centrifugation, tubes were punctured from the bottom and 0.5-ml fractions were collected. Lipoprotein-containing fractions were identified by measuring cholesterol.

Lipoprotein electrophoresis was carried out with the Polygram System (Beckman Instruments, Inc.).

Isoelectric focusing (IEF) and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Fractions containing VLDL after density gradient ultracentrifugation were pooled and subjected to a second step ultracentrifugation at density d 1.006 g/ml, 50000 rpm, 18 h, in a 50.3 Ti-type rotor (Beckman Instruments). Apolipoprotein E isoforms were analyzed from delipidated VLDL by isofocusing on Immobiline gels, pH 4–7, as described by Baumstark and colleagues (36). In addition, another aliquot of delipidated VLDL was subjected to SDS-PAGE as described by Utermann and coworkers (37).

DNA analysis

Oligonucleotide melting with allele-specific oligonucleotides was done as described (38). Some minor modifications of this procedure include the reduction of sample DNA to 5 μg, the omission of the electrophoretic purification step of the radiolabeled oligonucleotides in some experiments, and, in the case of low specific activities of the radiolabeled oligonucleotides ($< 10^8$ dpm/μg), the incubation of nitrocellulose membranes with mineral oil. The latter procedure largely enhances signal intensity during autoradiography (39). Oligonucleotides specific for the alleles 127 Gly and 127 Asp were chemically synthesized using cyanoethyl chemistry on a Gene Assembler (Pharmacia, Freiburg, FRG) (38). Temperatures for optimal discrimination of the two alleles were determined empirically. They were 63°C for 127 Gly and 60°C for 127 Asp.

Twenty-one base-long oligonucleotides were used as primers for PCR amplification. The amplification reaction was carried out in 50 μl buffer recommended by the supplier of the Taq polymerase (Cetus), 1 μg patient DNA, and final concentrations of 200 μM dNTPs and 0.1 μg primers. Initial denaturation at 100°C for 10 min was followed by the addition of 3 U Taq polymerase and 30 cycles of 96°C (2 min) and 70°C (1 min) incubations. Fifty percent of the crude product was transferred to nylon membranes using a slot-blot apparatus (Schleicher und

Schuell, Dassel, FRG). Hybridization was done using the same conditions as described for genomic DNA. Hybridization temperatures were 65°C in all cases.

Restriction fragment lengths were determined as previously described (40). In brief, 5 μg of genomic DNA was incubated overnight at 60°C with 20 U of Taq I (Boehringer Mannheim, FRG). Resulting DNA fragments were separated by electrophoresis in 1.2% agarose gels run at 20 V/cm in Tris-borate-EDTA (TNE) buffer (41). Hybridization was done with radiolabeled apoE cDNA (38) after transfer to nylon membranes (Biodyne A, Pall, Dreieich, FRG).

For haplotype analysis we used Nco I, Taq I, and Bgl I restriction fragment length polymorphism at the apoC-II locus (42), which is located approximately 40 kb 3' of apoE (43).

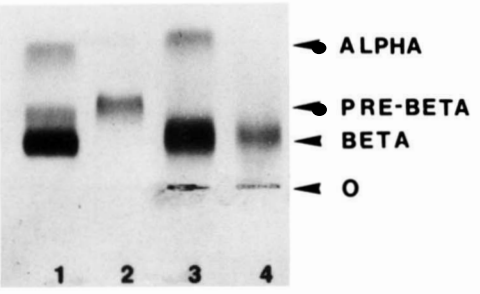

Fig. 1. Lipoprotein electrophoresis of normal control plasma (1) and normal VLDL (2) (isolated by ultracentrifugation) and of plasma (3) and VLDL (4) from the proband (MB). Note the broad beta band in the proband's plasma (3) and the proband's beta-migrating VLDL (4); O, origin.

RESULTS

Characterization of the B-family

The index patient (MB) exhibited typical clinical signs of type III hyperlipoproteinemia. He had developed yellowish tuberous xanthoma on the elbows and knees at the age of 25. At that time hyperlipidemia was disclosed. He was never grossly overweight and never before followed a lipid-lowering diet. Various lipid lowering medications had been tried including derivatives of clofibrate but normolipidemia had not been achieved. At the time he was seen in our outpatient clinic, he was not taking the full dose of the prescribed fibrate derivative and showed a pronounced hyperlipidemia (triglyceride 403 mg/dl; cholesterol 752 mg/dl). Impaired glucose tolerance was the only other abnormality seen in laboratory tests. Physical examination was normal except for the tuberous and palmar xanthoma.

From the members of his family we were able to examine both parents, a younger brother, and a sister. Three other sisters live in Turkey and could not be studied. There was no family history of premature atherosclerosis.

Characterization of lipoprotein and apolipoprotein E patterns

Lipoprotein electrophoresis of the proband's (MB) plasma revealed a broad-beta pattern. The electrophoretic patterns of the parents and the brother and sister were classified normal. VLDL isolated from MB's plasma showed beta-mobility in addition to normal pre-beta-migration. The sister's VLDL also showed a beta-migrating fraction; VLDL of the other family members had only normal pre-beta mobility. Lipoprotein analyses by electrophoresis of the proband's plasma and VLDL are shown in **Fig. 1**.

The proband's apoE focused as a major band characteristic of a homozygous pattern one charge unit more acidic than apoE2. This position is usually occupied by sialylated derivatives of apoE2, E3, and E4, or by asialo E1. In addition, several isoforms could be detected at even more acidic positions. The intensity of the minor isoform bands decreased upon neuraminidase digestion, simultaneously increasing the intensity of the protein band in the E1 position (data not shown). Thus, the peptide in the apoE1 position is unlikely to be a sialylated derivative of one of the common E-isoproteins but itself exhibits several degrees of sialylation. In accordance with the conventional nomenclature (6) and previously reported apoE proteins in this position (21, 24, 29, 30), we determined this variant protein to be an apoE1 isoform.

Isofocusing analysis of the available family members revealed another homozygous apoE1 pattern in the sister (SB) and heterozygous apoE3/1 patterns in both parents (OB, FB) and the brother (IB). This established the vertical transmission of the apoE1 protein. The isofocusing pattern of the proband's VLDL and, for comparison, an apoE3/2 and apoE3/3, and apoE4/3 pattern are shown in **Fig 2**. The pedigree of the B-family is given in **Fig. 3**.

Lipoproteins were further analyzed in all family members by density gradient ultracentrifugation. The proband (MB) showed a profile typical for hyperlipidemic dysbetalipoproteinemia. The sister (SB) was shown to have normolipidemic dysbetalipoproteinemia usually seen in normolipidemic apoE2/2 subjects. Lipoproteins in the three family members with apoE3/1 heterozygosity showed a normal density profile. **Fig. 4** shows the results from density gradient ultracentrifugation. Plasma lipid and apoprotein determinations in the B family members are given in **Table 1**.

Structural analysis of the variant apoE1 protein

The results from cysteamine modification as well as the presence of dysbetalipoproteinemia in the two homozygous family members suggested that the apoE1 in the family was likely caused by an additional mutation in the ε2 gene. It has been shown earlier that the common apoE2

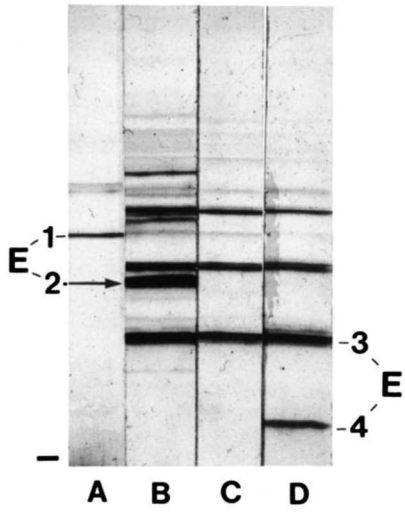

Fig. 2. Determination of the proband's apolipoprotein E phenotypic pattern as E1/1 (A) from VLDL. VLDL was isolated by density gradient ultracentrifugation and then subjected to isofocusing on immunobilized pH gradient gels (pH 4–7) (36). For comparison, apoE3/2 (B), apoE3/3 (C), and apoE4/3 (D) patterns are shown (Coomassie Blue staining).

(112 Cys, 158 Cys) but not other apoE2 variants exhibit slightly higher apparent molecular weight upon SDS-PAGE as compared to apoE3 and E4 (37). As demonstrated in **Fig. 5**, the apoE1 of this family also showed a slightly shorter migration in SDS-PAGE than apoE3. All apoE1 isoproteins in the family showed the same slow migration. This suggests that the two cysteine residues present in the apoE1 isoforms reside at positions 112 and 158. The localization of the two cysteine residues in these positions (112 and 158) was further confirmed by a recently developed oligonucleotide melting procedure (38). Allele-specific oligonucleotides for the 112 Cys and 158 Cys allele, respectively, gave strong hybridization signals with genomic DNA from all family members, while the oligonucleotide specific for the alternative alleles yielded no detectable signals in the homozygous patients. In carriers of the apoE3 isoform, the presence of a 158 Arg allele was demonstrated (data not shown).

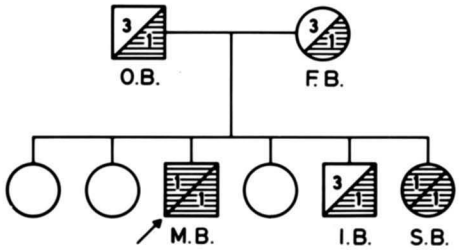

Fig. 3. Pedigree of the B-family. The proband MB and his sister SB have homozygous apoE1/1 patterns. Both parents, OB and FB, are heterozygous, apoE3/1, as is the brother IB. Open symbols represent sisters of the proband who were not available for the study.

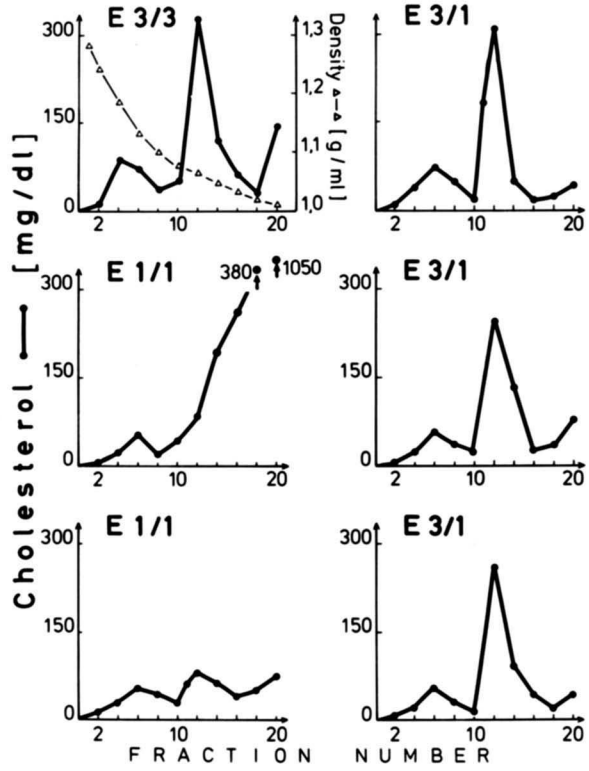

Fig. 4. Density gradient ultracentrifugation of plasma from the B-family members indicating typical dysbetalipoproteinemia in the E1 homozygous subjects. After the run in a SW 41-type rotor, tubes were punctured from the bottom and 0.5-ml fractions were collected. Lipoprotein-containing fractions were identified by cholesterol determination. Upper left graph represents a control gradient from a normlipemic apoE3/3 subject. The density of the fractions is represented by open triangles. Fractions 3–8 contain HDL, 10–16 LDL, 17–19 IDL, and 19–21 VLDL. Counterclockwise, adjacent to the control gradient, are shown the gradients of the type III hyperlipoproteinemic proband and his normolipidemic sister, also exhibiting the typical dysbetalipoproteinemia. The gradients from the plasma of the apoE3/1 members follow in the right panel of the figure (brother IB, mother FB, and father OB).

Using protein sequencing techniques, Weisgraber and colleagues (30) located the structural basis for their apolipoprotein E1 variant at position 127 of the amino acid sequence where they found an aspartic acid residue instead of the normal glycine. Assuming a point mutation responsible for this change, the DNA sequence for codons 126 and 127 in this allele should read CTCGAC instead of CTCGGC (20) and thus create an additional cleavage site for the restriction endonuclease Taq I.

A Taq I restriction fragment length analysis was carried out to test whether the underlying structural defect in the apoE1 of the B family was identical with the one reported by Weisgraber et al. (30). The result of this analysis is shown in **Fig. 6**. A 2.0-kb fragment, which is expected from a computer-derived restriction map based on the apoE gene sequence reported by Paik et al. (44), is only seen in the E3 control subjects and the heterozygous

TABLE 1. Lipids and apoproteins in the B-family

Identification	Total Cholesterol	Serum Triglycerides	HDL-Cholesterol	Apolipoprotein E	Apolipoprotein B	Apolipoprotein A-I	Apolipoprotein E phenotype
				mg/100 ml			
MB, proband	752	403	32	30.4	83.2	135	Apo E 1/1
SB, sister	164	155	56	17.6	54.8	144	Apo E 1/1
FB, mother	260	145	52	8.0	92.6	159	Apo E 3/1
OB, father	215	85	52	6.6	95.8	154	Apo E 3/1
IB, brother	240	110	44	6.7	99.0	149	Apo E 3/1

Lipid and apolipoprotein parameters of the B-family members. The overall increase of apoE in the two homozygous apoE1/1 subjects is obvious. Note the low apoB level in the sister (SB) with normolipidemic dysbetalipoproteinemia.

members of the B-family, but not in the homozygous subjects. Instead, in the homozygous carriers of the apoE1 isoform, an abnormal 1.6-kb-long Taq I fragment is observed, which is also present in all heterozygote carriers of apoE1. As the previously described apoE (Gly_{127}→Asp, Arg_{158}→Cys) (30) may be caused by a mutation which at the same time gives rise to a 1.6-kb-long Taq I restriction fragment, it is possible that the mutation in the B-family is the same. In Fig. 6 it is demonstrated, however, that in the vicinity of codon 127 there are two more sites where a point mutation can create a 1.6-kb-long Taq I fragment and at the same time cause an amino acid change that adds a negative charge to apoE.

To positively identify the mutation underlying the apoE1 isoform in the B-family we synthesized two oligonucleotides (for sequences see **Fig. 7**) that can specifically discriminate between the wild type and the aspartic acid mutation at position 127. **Fig. 8** shows the oligonucleotide melting result of PCR-amplified DNA for all members of the B-family. There clearly is no hybridization of the wild type detecting oligonucleotide with the DNA from the apoE1 homozygote individuals, whereas hybridization with the asp-coding oligonucleotide is positive. In two examples, OB and MB, these results were confirmed by oligonucleotide melting using unamplified DNA (data not shown). It is thus confirmed that the apoE1 in the B-family is apoE (Gly_{127}→Asp, Arg_{158}→Cys).

Haplotype analysis gave identical results (apoE:112C, 127A, 158C; apoC-II: B2, N2, T1) for the apoE1 coding allele of the two parents. The two homozygote apoE1 carriers were homozygote for this haplotype.

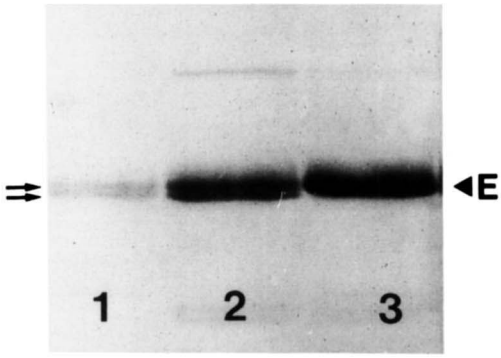

Fig. 5. Sodium dodecylsulfate polyacrylamide gel electrophoresis of VLDL apolipoproteins as described by Utermann and colleagues (37). After density gradient ultracentrifugation, the VLDL-containing fractions were pooled and recentrifuged for 18 h at 50,000 rpm at density d 1.006 g/ml. After delipidation in acetone–ethanol 1:1 (v/v) at −20°C, apoproteins were applied to the gel. Only the apoE region of the gel is shown. Lane 1 shows apoVLDL of an apoE3/2 control subject. Lanes 2 and 3 contain apoVLDL of an apoE3/1 heterozygous and an apoE1/1 homozygous family member, respectively. Note the slightly higher apparent molecular weight of the apoE2 peptide and of the apoE1 variant as compared to apoE3. The faster moving band is missing in the apoE1/1 homozygous sample (3).

DISCUSSION

The three-allele mode (3–5) of the structural apolipoprotein E gene locus has recently been expanded by a variety of mutations adding to the three most commonly occurring alleles ε2, ε3, and ε4 (reviewed in the introduction section). These mutations either give rise to proteins located within the most common isofocusing positions (E2–E4) (9, 16–20, 22) or apoE isoforms in positions more basic than E4 (23–28) or more acidic than E2 (21, 24, 29, 30). The mutant proteins focusing outside the apoE2–E4 region lead to new IEF phenotypes. So far, only patients heterozygous for rare apoE isoforms have been described. Several subjects were reported with apoE focusing with one of their major isoform in the E1 position (21, 24, 29, 30), presenting either type III hyperlipidemia, hypertriglyceridemia, or normolipidemia.

Two subjects described in this report are homozygous for a rare apoE1 protein. Both exhibit dysbetalipoproteinemia. The index patient is hyperlipemic, expressing type III hyperlipidemia; his sister, also homozygous for apoE1, is normolipidemic. Dysbetalipoproteinemia is usually associated with homozygosity for the ε2 (112 Cys, 158 Cys) allele. After cysteamine modification, the apoE isoform

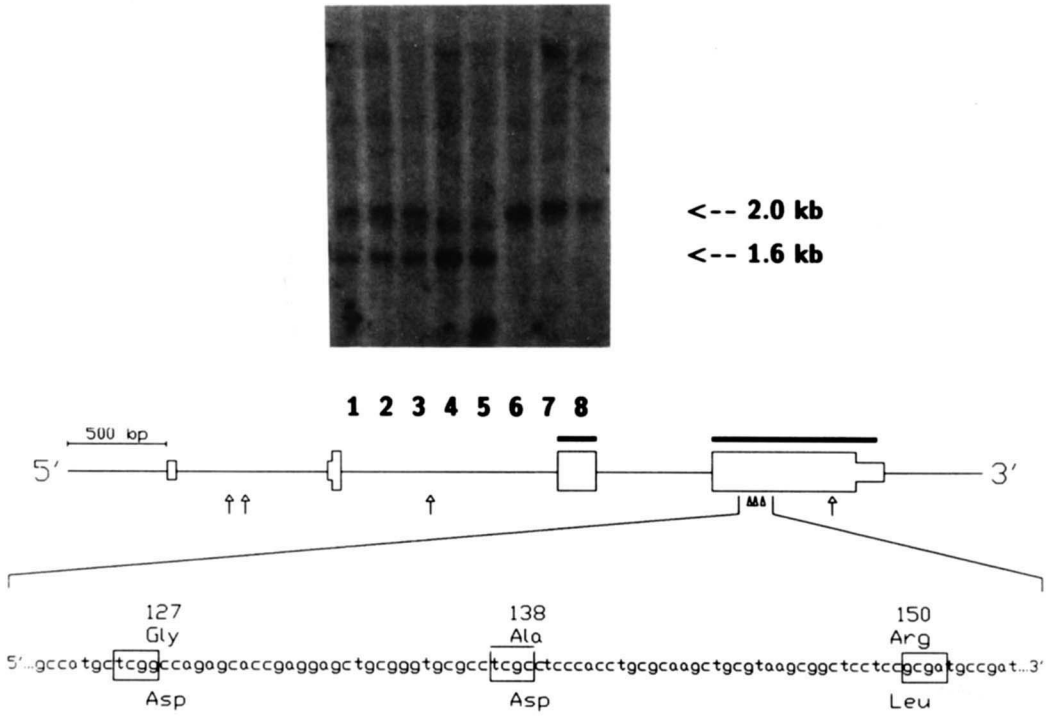

Fig. 6. Taq 1 restriction fragment length analysis. Hybridization was done with apoE cDNA (the cDNA length is indicated as a thick bar above the schematic drawing of the apoE gene). Lanes 1–3 are from heterozygotes of the B-family (IB, OB, FB); lanes 4 and 5 are from homozygote apoE1 carriers (MB in lane 3, SB in lane 5). Lanes 6–8 are from random controls with E3/3 phenotype in IEF. The faint band with about 1.9 kb length likely represents hybridization of the apoE cDNA with a genomic fragment that contains the 3' end of the apoE gene. In the schematic drawing of the apoE gene, arrows point to regular Taq I cutting sites. Arrow heads point to the region where an additional cutting site is expected that leads to the formation of the 1.6 kb fragment. In a detailed view of this region, boxes indicate areas where a single base mutation can cause both the formation of a new Taq I cutting site and a more acidic mutant protein.

patterns of the proband and his sister shifted to resemble a homozygous apoE3 pattern, and the patterns of the three other family members shifted from apoE3/1 to apoE4/3 (data not shown). This documented the presence of two cysteine residues in the E1 isoforms and of one cysteine in the E3 isoforms. The apolipoprotein E1 in all the family was then shown to have the cysteine residues in positions 112 and 158 of the amino acid sequence both by sodium dodecylsulfate-polyacrylamide gel electrophoresis and hybridization experiments using specific oligonucleotide probes. Thus, the apoE1 isoform in this family most probably arose as a result of a mutation in the common $\epsilon 2$ allele (112 Cys, 158 Cys).

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3'.........cggtacgagCcggtctcgtgg.........5'   127 Gly detecting probe
5'...gtgcaggccatgctcGgccagagcaccgaggag...3'
                                              127 Gly allele
3'...cacgtccggtacgagCcggtctcgtggctcctc...5'
5'.........gccatgctc(A/C)gccagagcacc.........3'   127 Asp detecting probe

3'.........cggtacga9(C/T)cggtctcgtgg.........5'   127 Gly detecting probe
5'...gtgcaggccatgctcAgccagagcaccgaggag...3'
                                              127 Asp allele
3'...cacgtccggtacgagTcggtctcgtggctcctc...5'
5'.........gccatgctcAgccagagcacc.........3'   127 Asp detecting probe
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Fig. 7. Sequences of the allele-specific oligonucleotides used in the detection of the apoE1 (137 Gly-Asp) allele.

Second dimension electrophoresis in SDS-polyacrylamide gels after isofocusing in the first dimension showed a slightly higher apparent molecular weight of the variant peptide in E1 position as compared to the apoE3 peptide in the family and apoE3 from control subjects. The minor, more acidic bands of apoE1 had increasingly higher apparent molecular weights indicative of different degrees of sialylation (data not shown). As neuraminidase treatment did not alter the isofocusing position of the major apoE1 plasma isoform and as apoE1 was vertically transmitted in the B-family, we further investigated a possible amino acid exchange in the primary sequence of apoE responsible for the charge shift to the E1 position. Weisgraber and colleagues (30) reported an aspartic acid for glycine exchange in position 127 responsible for the shift to apoE1 in their apoE1 mutant found in a Finnish patient.

Using allele-specific oligonucleotides, we have also been able to identify the mutation causing the apoE1 IEF-phenotype as a glycine to aspartic acid change at position 127

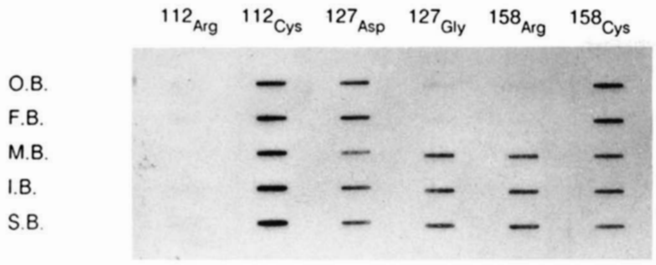

Fig. 8. Hybridization of PCR-amplified patient DNA with allele-specific oligonucleotides under stringent conditions. Top, the codon specificities of the oligonucleotides used for hybridization in the respective lanes are given. The genotypes are thus (112 Cys/112 Cys; 127 Asp/127 Gly; 158 Cys/158 Arg) for the heterozygotes MB, IB, and SB.

of the mature apoE protein sequence. Although the oligonucleotide melting was done only in one homozygote patient and the 127 Asp signal is weak, the result from this analysis is conclusive, as there was no hybridization signal with the 127 Gly-specific oligonucleotide. The presence of a null allele as the underlying cause of the low signal intensity was furthermore excluded by restriction fragment length analyses, which always showed double dose signal intensities after hybridization with apoE cDNA. The possible existence of a null allele as an explanation of the expression of apoE1 protein only is also excluded by the pedigree apoE phenotype analysis, because both parents are apoE3/1 heterozygous. Thus, the patient was established to be a real homozygote. Familial segregation of apoC-II haplotypes further supports this interpretation.

A mutation at one of the two other candidate loci for a possible creation of an additional Taq I cleavage site (see Fig. 6) instead of a mutation at codon 127 can be excluded from the negative hybridization results with 127 Gly, unless there is a mutation within the sequence overlapped by the 127 Gly oligonucleotide. This, however, is not very likely, as haplotype analysis revealed that the father and the mother have the mutation on alleles with identical haplotypes. Thus we could prove that apoE1 in this family shows the same mutation reported by Weisgraber and colleagues (30). Although we cannot exclude that there are additional amino acid changes in apoE1, the aspartic acid for glycine accounts for the charge difference between the E2 and the E1 positions.

As no specific pattern of hyperlipidemia was associated with the apoE3/1 heterozygous members of this family, the $\epsilon 1$ allele in the single dose apparently does not cause a lipid abnormality per se. Similar to subjects heterozygous for the common $\epsilon 2$ allele, the apoE1 protein in the E3/1 subjects of the family comprises more than 50% of the total apoE as judged from densitometry of isofocusing patterns. No first degree relatives not exhibiting the apoE1 isoform were available as internal controls. Hypertriglyceridemia, as seen in the apoE3/1 patient of Weisgraber and colleagues (30), could not be observed in our family.

Interestingly, both apoE1 homozygous individuals showed a typical dysbetalipoproteinemia. This condition is commonly associated with mutations in the receptor binding region of apoE (45, 46), most frequently occurring in the apoE2 ($Arg_{158} \rightarrow Cys$) protein. As the mutation leading to apoE1 is located in a position far outside the receptor-binding domain and as the apoE1 peptide possesses the 158 cysteine for arginine exchange, it is very likely that the dysbetalipoproteinemia seen in the two homozygous subjects is due to the presence of cysteine at position 158. This assumption is further supported by apoB,E receptor binding studies with the apoE1 ($Gly_{127} \rightarrow Asp$, $Arg_{158} \rightarrow Cys$) carried out by Weisgraber and colleagues (30) that revealed a defective binding of this mutant similar to that of apoE2 ($Arg_{158} \rightarrow Cys$).

Type III hyperlipoproteinemia is currently believed to be a multifactorial disorder. Homozygosity for the common apoE2 (112 Cys, 158 Cys) protein leads to dysbetalipoproteinemia, representing the basic defect on which hyperlipoproteinemia type III develops, when additional factors causing hyperlipoproteinemia are present (31, 32, 34, 47). The impaired glucose tolerance on the basis of dysbetalipoproteinemia in the patient from our family may be one additional factor to cause type III hyperlipoproteinemia. The patient responded well to dietary and drug treatment with a fibrate derivative, an additional argument for the presence of a "typical" type III hyperlipoproteinemia. However all five patients heterozygous for the apoE3 (112 Arg, 142 Cys) mutant described by Rall et al. (22) expressed type III hyperlipoproteinemia, indicating the presence of dominant allele.

None of the persons in the B-family carrying either one or two copies of this $\epsilon 1$ allele exhibited any detectable difference in metabolism or clinical phenotypes when compared to carriers of the more frequent $\epsilon 2$ allele. We therefore conclude that the aspartic acid for glycine exchange in codon 127, which is the only structural difference between the apoE1 and the apoE2 isoforms, is of no functional importance in vivo. It can be expected that further investment in screening programs for apolipoprotein mutants will lead to the identification of additional mutants which will help us in better understanding lipoprotein metabolism.

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