Apolipoprotein E2(Arg\textsuperscript{158}→Cys) Frequency in a Hyperlipidemic French-Canadian Population of Apolipoprotein E2/2 Subjects

Determination by Synthetic Oligonucleotide Probes

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An underlying cause of type III hyperlipoproteinemia is the presence of variant forms of apolipoprotein (apo) E that are defective in binding to apo E, low density lipoprotein receptors. This disorder is associated almost exclusively with the apo E2/2 phenotype. However, structural and functional heterogeneity have been demonstrated within this phenotype. The apo E2(Arg\textsuperscript{158}→Cys) variant, displaying 1% of normal apo E3 binding activity, is the most defective known form. In this study, we describe a method in which a pair of 19-mer synthetic oligonucleotide probes were used to distinguish between DNA coding for arginine or cysteine at position 158 in apo E. The specificity of the probes was demonstrated by using DNA from subjects whose apo E protein sequence or phenotype was known. The probes were used to screen a French-Canadian population of 34 apo E2/2 subjects to determine the frequency of the apo E2(Arg\textsuperscript{158}→Cys) variant. All 34 subjects, most of whom displayed clinical or biochemical features of type III hyperlipoproteinemia, were found to be homozygous for apo E2(Arg\textsuperscript{158}→Cys), strongly suggesting that this variant is the most common form of apo E2 within this ethnic and clinical population. In addition, the utility of this approach in detecting new apo E mutations was demonstrated when DNA from one of the apo E33 control subjects, whose family has a history of hyperlipidemia and coronary artery disease, reacted with both probes. This result suggests that this subject is heterozygous for normal apo E3 and a new apo E3 variant that is likely to be functionally equivalent to apo E2(Arg\textsuperscript{158}→Cys).

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Human apolipoprotein (apo) E is a component of several classes of plasma lipoproteins and, because of its ability to interact with lipoprotein receptors, apo E plays an important role in directing the metabolism of triglyceride- and cholesterol-containing lipoproteins.\textsuperscript{1} The structures of the protein and its gene are known.\textsuperscript{2,3,4} A combination of experimental approaches has demonstrated that a region of the protein in the vicinity of residues 140 to 160 is critical for interaction with apo B,E-LDL receptors (for review, see Reference 5). Apo E, which exhibits a genetically determined polymorphism at the level of the structural gene, has several variants,\textsuperscript{5} many of which display defective receptor binding activity. The most common isoforms in the population are designated E2, E3, and E4 and are identified by isoelectric focusing. This heterogeneity results in six phenotypes: E2/2, E3/3, E4/4, E4/2, E4/3, and E3/2.\textsuperscript{6} The apo E3/3 phenotype is the most prevalent one in several different populations.\textsuperscript{7–13} The most common variants differ by arginine-cysteine interchanges at positions 112 and 158. The apo E4, apo E3, and apo E2 variants contain Arg/Arg, Cys/Arg, and Cys/Cys, respectively, at positions 112/158.\textsuperscript{14}

The apo E2/2 phenotype is commonly associated with the lipid disorder type III hyperlipoproteinemia, or dysbetalipoproteinemia.\textsuperscript{15,16} Affected subjects have elevated plasma concentrations of both cholesterol and triglyceride because their lipoprotein remnants are not cleared effectively. These individuals are also predisposed to develop premature atherosclerosis. The lipoprotein remnants are of hepatic and intestinal origin\textsuperscript{17} and are referred to as beta-very low density lipoproteins (β-VLDL). The presence of this lipoprotein class is one of the hallmarks of the disorder. Although there is structural heterogeneity within the apo E2/2 phenotype (apo E2(Arg\textsuperscript{158}→Cys), apo E2(Arg\textsuperscript{158}→Cys), apo E2(Arg\textsuperscript{158}→Ser), and apo E2([Lys\textsuperscript{112}→Gln]),\textsuperscript{18–20} the apo E2 variants all exhibit impaired interaction with apo B,E(LDL) receptors.\textsuperscript{1,5} These defective-binding apo E variants are an underlying cause of the accumulation of lipoprotein remnants in type III hyperlipoproteinemia.\textsuperscript{21} Of all the variants, apo E2(Arg\textsuperscript{158}→Cys) is the most defective, possessing approximately 1% of nor-
mal apo E3 binding. Previous studies have indicated that the cysteine-arginine interchange at position 158 probably affects binding in an indirect manner.

Because of the structural heterogeneity within the apo E22 phenotype, we wanted to determine the frequency of variants within a type III hyperlipoproteinemic or apo E2/2 population. A limited screening of six German apo E2/2 subjects, who ranged from hypolipidemic to hyperlipidemic, showed that all were homozygous for apo E2(Asp-158→Cys). That study involved protein sequencing, which is not an effective method for screening large populations. One possible alternative method is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of VLDL. Under appropriate conditions, apo E2(Asp-158→Cys) migrates more slowly than other known apo E2 mutants, although its molecular weight is essentially identical to the other forms. However, the usefulness of this method for general screening is also questionable, because it has been reported that an apo E3 mutant also migrates anomalously. In addition, to avoid the possibility that other, unknown variants associated with type III hyperlipoproteinemia might also show this effect, we sought an alternative screening method.

Previous studies have demonstrated that a single nucleotide difference in genomic DNA can be detected by hybridization with synthetic oligonucleotide probes by taking advantage of differences in the "melting" temperature of a perfectly matched DNA-DNA hybrid and that of a mismatched hybrid. This approach has been used successfully to detect specific mutations in the β-globin gene and the α-antitrypsin gene. Recently, this approach has been used to distinguish between arginine and cysteine at residue 158 in apo E. In that study, the DNA from 15 subjects, chosen so that the ε2 allele was overrepresented, was examined to determine whether the genotypes determined by oligonucleotide probes agreed with the phenotype determined by isoelectric focusing, assuming that the apo E2(Asp-158→Cys) is the most common form of apo E2. Agreement was found in 14 subjects.

In the fifteen, who had an apo E2/2 phenotype, the DNA reacted with both probes, indicating that one of the ε2 alleles did not specify apo E2(Asp-158→Cys). It was not determined whether the ε2 allele represented one of the previously described apo E2 variants or a new form.

In the present study, oligonucleotide probes were also designed to distinguish between arginine and cysteine at the clinically important position 158. Our focus, however, was to determine the relative frequency of the apo E2(Asp-158→Cys) variant in a French-Canadian population of apo E2/2 subjects, most of whom express type III hyperlipoproteinemia.

**Methods**

**DNA Samples**

Genomic DNA was prepared from the total blood cell pellet obtained by centrifugation of EDTA-treated blood from control and apo E2/2 subjects. All subjects participating in these studies gave their informed consent. A recombinant cosmI fragment containing the 1.9-kilobase (kb) EcoRI fragment of the apo E4 gene was prepared as previously described and served as a control for the Arg-158 probe.

**Oligonucleotide Synthesis and Labeling**

Oligonucleotide probes were synthesized by the methoxyphosphoramidite method on an Applied Biosystems ABI 380B Synthesizer (Foster City, CA) and were purified by polyacrylamide gel electrophoresis. Probes were 5'-labelled with [γ-32P]ATP by polynucleotide kinase. Briefly, [γ-32P]ATP (0.5 mCi, specific activity > 5,000 Ci/mmol; Amersham, Lexington, MA) was dried under vacuum and incubated for 30 minutes at 37°C in 10 μl of 0.05 M Tris-HCl (pH 7.5), 0.01 M MgCl2, 5 mM dithiothreitol, 0.1 mM spermidine, and 0.1 mM EDTA, containing 600 ng of oligonucleotide and 10 U of polynucleotide kinase (Pharmacia P-L Laboratories, Piscataway, NJ). The unincorporated [γ-32P]ATP was separated from labeled oligonucleotide on a Nersorb cartridge (DuPont, Boston, MA) according to the manufacturer's instructions.

**Genomic DNA Analysis**

Genomic DNA (20 μg) and a genomic equivalent of plasmid DNA were digested with the restriction endonucleases EcoRI and BamHI (BioLabs, Beverly, MA) according to the manufacturer's instructions. The digests were electrophoresed on 0.8% agarose gels, and the DNA was denatured with 1.5 M NaCl, 0.5 M NaOH, neutralized with 3 M NaCl, 0.5 M Tris-HCl, pH 7.5, and transferred to nitrocellulose acetate membranes (Sartorius, West Coast Scientific, Emeryville, CA) according to the method of Southern. The membranes were then dried for 2 hours at 80°C in a vacuum oven. The dried membranes were prehybridized for 4 hours at 58°C in 0.3 ml/cm² of hybridization buffer: 6× NET (1× = 0.15 M NaCl, 0.001 M EDTA, 0.015 M Tris-HCl, pH 8.0), 5× Denhardt's (1× = 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone), 0.05% Nonidet, 2% dextran sulfate, yeast RNA (100 μg/ml), and bovine serum albumin (1 mg/ml). The membranes were then hybridized overnight at 58°C in 0.012 ml/cm² of the hybridization buffer containing 150 to 150 ng of 32P-labeled oligonucleotide (specific activity of 0.5 to 2.0×10⁸ cpm/μg). Membranes were washed twice for 3 minutes each at 52°C in 2× SSC (1× SSC = 0.15 M NaCl, 0.001 M sodium citrate, pH 7.0) containing 0.1% SDS and once for 1 minute in 0.1× SSC and 0.1% SDS at 60°C for the Arg-158 probe and at 57°C for the Cys-158 probe. Autoradiography was performed with Trimax cassettes with 12B intensifying screens and Xm X-ray film (3M, St. Paul, MN). The autoradiograms were read independently by three individuals. The DNA from each type III patient was examined at least twice. If the examiners could not agree initially on an interpretation, a third digestion was examined. This approach resolved all ambiguities, and a consensus was arrived at in each case.

**Patients**

Blood samples were obtained from 35 unrelated French-Canadian patients attending the Lipid Clinic of the Clinical Research Institute of Montreal. All patients were fasting when the samples were drawn. Thirty-four of the patients were known to have the apo E22 phenotype.
recombinant clone of the EcoRI fragment from an ε4 allele, which also codes for arginine at residue 158, served as one of the controls in these studies (Figure 1, Lane 1).

Based on the approach of Wallace and associates, two 19-mer oligonucleotide probes were designed and synthesized (Table 1). The Arg-158 probe is complementary to the apo E3 anticoding sequence, and the Cys-158 probe is complementary to the apo E2 coding sequence. The mismatch for each probe was positioned at the center of the 19-mer to maximize the difference in stability between an exact and inexact complement. Based on the equation:

\[ T_m = 4 \times (\text{number of cytosine and guanine residues}) + 2 \times (\text{number of adenine and thymine residues}) \]

where \( T_m \) = melting temperature of the hybrid, the exact DNA hybrids for the Arg-158 and Cys-158 probes are predicted to melt at 64°C and 62°C, respectively. The optimal hybridization conditions to detect the mismatch were established experimentally with the cloned apo E4 EcoRI fragment by systematically varying the temperature and duration of hybridization and the stringency of the washing procedure.

The hybridization conditions for the oligonucleotide probes were verified by examining a series of controls. The DNA from a type III subject, DR, who is homozygous for apo E2(Arg158→Cys) served as a control for the Cys-158 probe (Figures 2A and 2B, Lanes 2). The DNA from a subject with the apo E3/3 phenotype whose apo E was confirmed by protein sequencing to have an arginine at position 158 (Rall SC, Jr., unpublished observations) served as the control for the Arg-158 probe (Figures 2A and 2B, Lanes 4). Additional controls were from subjects with an apo E3/2 phenotype (Figures 2A and 2B, Lanes 3) and an apo E3/3 phenotype (Figures 2A and 2B, Lanes 5). As shown in Figure 2A, the Arg-158 probe hybridized specifically to the 1.9-kb fragment from the subject whose apo E has been demonstrated to have arginine at position 158 (Lane 4) or from subjects presumed to have an arginine at this position based on their phenotype (Lanes 3 and 5). In a similar manner, the Cys-158 probe hybridized specifically to the 1.9-kb fragment from subjects whose apo E has been demonstrated to contain a cysteine at position 158 (Figure 2, Lane 2) or from a subject with the apo E2 isoform (Figure 2, Lane 3). Other fragments detected by the two probes (in addition to the 1.9-kb fragment) migrated with approximate lengths of 5.5, 4.0, and 1.3 kb; these most likely represent other closely related, complementary sequences that would be expected to occur in the genome as a result of chance or, in the case of the larger fragments, as a result of incomplete digestions. The appearance of additional fragments appears to be more common with the Arg-158 probe (compare panels A and B in Figures 2 and 3). Although digestion of genomic DNA with EcoRI alone should be sufficient to yield the 1.9-kb fragment, inclusion of BanHI in the digestion mixture improved the yield of the 1.9-kb EcoRI fragment and led to more consistent results.

A clinical summary of the 34 apo E2/2 subjects (and one control apo E3/3 subject) is presented in Table 2. In all but one apo E2/2 patient (PB, #28, who has type IV

Figure 1. Hybridization of apo E cDNA to restriction endonuclease-digested human genomic DNA. Genomic DNA (20 μg) was digested with EcoRI/BamHI, was electrophoresed on 0.8% agarose gels, was transferred to nitrocellulose, and was then hybridized to a 32P-labeled apo E cDNA. Lane 1, cloned EcoRI genomic fragment from ε4 allele; Lanes 2 to 4, genomic DNA from three different individuals. The nucleotide lengths indicated at the left were determined using the HindIII-digested fragments of bacteriophage λ DNA as markers.

Most had been clinically monitored for several years, and the baseline values for lipids and lipoproteins were known for each patient.

Results

As previously reported, when human genomic DNA is digested with EcoRI/BamHI, fragments of 2.3 and 1.9 kb can be detected with 32P-labeled apo E cDNA probes (Figure 1, Lanes 2 to 4). The 1.9-kb fragment represents an EcoRI fragment and contains the entire fourth exon of the apo E gene; this exon specifies the carboxyl-terminal three-fourths of the protein (residues 62 to 299). The EcoRI fragment contains the substitution site of interest (residue 158) and is of a useful size for this study.
Table 1. Gene Sequence and Synthetic Oligonucleotide Probes in Region of Residue 158 of Apolipoprotein E

| Apolipoprotein E | Sequence |
|------------------|----------|
| Normal apo E3    | ...GAC  |
|                  | CTG CAG AAG | CGC CTG GCA GTG TAC... |
| Apo E2(Arg<sub>158</sub>-Cys) | ...GAC  |
|                  | CTG CAG AAG | CGC CTG GCA GTG TAC... |
| Apo E3 antcoding sequence | 3' ... GAC GTC TTC GCG GAC CGT C ... 5' |
| Arg-158 probe    | 5'      |
| Apo E2 antcoding sequence | 3' ... GAC GTC TTC ACG GAC CGT C ... 5' |
| Cys-158 probe    | 5'      |
| Apo E3 coding sequence | 5' ... CTG CAG AAG |
|                  | TGC CTG GCA G ... 3' |

The gene sequence is taken from Reference 4. Apo=apoprotein.

A.  

B.  

Figure 2. Hybridization of synthetic apo E oligonucleotide probes to EcoRI/BamHI-digested human genomic DNA. Genomic DNA was treated as described in Figure 1. A. Hybridization to the Arg-158-specific probe. B. Hybridization to the Cys-158-specific probe. Lanes 1, cloned EcoRI genomic fragment; Lanes 2, DNA from an individual homozygous for apo E2(Arg<sub>158</sub>-Cys); Lanes 3, DNA from subject with E3/2 phenotype; Lanes 4, DNA from an individual homozygous for apo E3; and Lanes 5, DNA from a subject with the E3/3 phenotype.

hyperlipoproteinemia), a diagnosis of type III hyperlipoproteinemia was made, based on the presence of typical clinical or biochemical manifestations, or both. Typical clinical features included palmar crease pigmentation (orange or brownish), xanthoma striata palmaris, tubero-eruptive xanthomas of elbows or knees, and/or eruptive xanthomas with an orange or brownish-orange discoloration. Biochemical manifestations included the presence of \( \beta \)-VLDL in the \( d<1.006 \) g/ml ultracentrifugation fraction, a broad-beta band in the plasma, and a VLDL cholesterol/plasma triglyceride ratio >0.30 in the untreated state. Among the 34 subjects, 17 (50%) had one or more of the
typical clinical features and 11 (32%) had coronary artery disease (angina pectoris and/or myocardial infarction or previous coronary artery bypass surgery) (Table 2). One subject (SO, #23) had concomitant familial hypercholesterolemia with xanthoma tendinosum and a high concentration of LDL cholesterol (222 mg/dl); two others with elevated LDL cholesterol concentrations (DD, #27, and FL, #33) probably had superimposed familial combined hyperlipidemia.

Examples of the hybridization results from four subjects are shown in Lanes 4 through 7, respectively, of Figures 3A and 3B, and the results from all the subjects are summarized in Table 2. All 34 apo E2/2 subjects were homozygous for cysteine at position 158. The DNA in Lanes 4, 5, and 7 reacted only with the Cys-158 probe, indicating that these three subjects were homozygous for cysteine at this position. During the course of this study, some apo E3/3 subjects were included as additional controls. One of these controls (subject M-PD, #35 in Table 2) turned out to be of particular interest. The DNA of this subject (Lanes 6 in Figures 3A and 3B) reacted with both probes, demonstrating that this subject is heterozygous for two forms of apo E. Although this subject's phenotype is apo E3/3, one apo E allele specifies cysteine (rather than arginine) at residue 158, indicating that this subject possesses a previously undescribed ε3 allele.

Cysteamine modification of VLDL from this subject indicates that both forms of apo E contain one cysteine residue.

Discussion

In this study, a method was developed in which 19-mer synthetic oligonucleotide probes were used to detect cysteine-arginine interchanges at position 158 in human apo E. The specificity of the method was demonstrated using DNA from subjects whose apo E has been shown by protein sequencing to have either cysteine or arginine at residue 158. The utility of the method as a screening tool was established by determining the frequency of cysteine at position 158 in a French-Canadian population of 34 apo E2/2 homozygotes, most of whom express type III hyperlipoproteinemia. This approach could be extended to other sites in apo E at which mutations have been found to result in clinical abnormalities. One advantage of this molecular approach is that a specific pair of probes can be designed for each particular amino acid substitution site. Knowledge of the precise nature of an apo E mutation could aid in tracing the genetic history of an associated type III hyperlipoproteinemia.

The finding that all 34 apo E2/2 subjects were homozygous for cysteine at position 158 is consistent with the results from two protein sequencing screens. In the first, six German apo E2/2 subjects were found to be homozy-
Table 2. Clinical Summary of Subjects

| Subject | Sex/age | TC (mg/dl) | TG (mg/dl) | VLDL-C/TG | CAD† | Xanthoma‡ | Phenotype§ | Cys-158¶ | Arg-158∥ |
|---------|---------|------------|------------|-----------|------|-----------|------------|----------|---------|
| 1 MT    | M/52   | 382        | 600        | 0.41      | +    | E2/2      | +          | -        | -       |
| 2 JG    | M/32   | 466        | 705        | 0.49      | +    | E2/2      | +          | -        | -       |
| 3 BD    | M/35   | 515        | 659        | 0.50      | +    | E2/2      | -          | -        | -       |
| 4 J-LM  | M/45   | 550        | 1452       | 0.33      | +    | E2/2      | +          | -        | -       |
| 5 AV    | M/58   | 455        | 808        | 0.41      | +    | E2/2      | +          | -        | -       |
| 6 RT    | M/49   | 329        | 390        | 0.41      | +    | E2/2      | +          | -        | -       |
| 7 HD    | F/56   | 400        | 995        | 0.30      | -    | E2/2      | +          | -        | -       |
| 8 J-GG  | M/56   | 330        | 928        | 0.27      | +    | E2/2      | +          | -        | -       |
| 9 MF    | F/55   | 437        | 421        | 0.59      | -    | E2/2      | +          | -        | -       |
| 10 BD   | M/54   | 268        | 411        | 0.41      | +    | E2/2      | -          | -        | -       |
| 11 GR   | M/54   | 345        | 1086       | 0.22      | -    | E2/2      | +          | -        | -       |
| 12 AM   | M/50   | 405        | 695        | 0.36      | -    | E2/2      | +          | -        | -       |
| 13 MB   | F/64   | 222        | 249        | 0.38      | -    | E2/2      | +          | -        | -       |
| 14 RC   | F/62   | 326        | 492        | 0.32      | -    | E2/2      | +          | -        | -       |
| 15 JC   | F/74   | 760        | 1290       | 0.52      | +    | E2/2      | +          | -        | -       |
| 16 EB   | M/60   | 418        | 1820       | 0.20      | +    | E2/2      | +          | -        | -       |
| 17 LM   | M/47   | 206        | 308        | 0.38      | -    | E2/2      | +          | -        | -       |
| 18 RD   | M/55   | 565        | 846        | 0.55      | -    | E2/2      | +          | -        | -       |
| 19 CB   | M/41   | 274        | 253        | 0.39      | -    | E2/2      | +          | -        | -       |
| 20 AH   | M/44   | 304        | 705        | 0.29      | -    | E2/2      | +          | -        | -       |
| 21 JL   | M/56   | 379        | 598        | 0.40      | +    | E2/2      | +          | -        | -       |
| 22 LH   | M/28   | 189        | 159        | 0.52      | -    | E2/2      | +          | -        | -       |
| 23 SO   | M/46   | 444        | 404        | 0.41      | -    | E2/2      | +          | -        | -       |
| 24 JA   | M/55   | 180        | 189        | 0.36      | +    | E2/2      | +          | -        | -       |
| 25 RT   | M/40   | 460        | 1231       | 0.30      | -    | E2/2      | +          | -        | -       |
| 26 JB   | M/42   | 182        | 238        | 0.45      | -    | E2/2      | +          | -        | -       |
| 27 DD   | M/33   | 593        | 606        | 0.60      | +    | E2/2      | +          | -        | -       |
| 28 PB   | M/45   | 246        | 606        | 0.23      | -    | E2/2      | +          | -        | -       |
| 29 RG   | M/31   | 264        | 292        | 0.30      | +    | E2/2      | +          | -        | -       |
| 30 RL   | M/44   | 261        | 268        | 0.48      | +    | E2/2      | +          | -        | -       |
| 31 J-FM | M/55   | 198        | 205        | 0.33      | -    | E2/2      | +          | -        | -       |
| 32 JD   | M/32   | 656        | 2607       | 0.21      | -    | E2/2      | +          | -        | -       |
| 33 FL   | F/50   | 349        | 194        | 0.47      | -    | E2/2      | +          | -        | -       |
| 34 GD   | M/48   | 258        | 190        | 0.54      | -    | E2/2      | +          | -        | -       |
| 35 M-PD | F/71   | 236        | 402        | 0.21      | -    | E3/3      | +          | -        | -       |

*Values for TC (total cholesterol) and TG (triglyceride) expressed as mg/dl; VLDL-C/TG=ratio of very low density lipoprotein cholesterol to plasma triglyceride.
†Coronary artery disease as evidenced by angina, myocardial infarction, or coronary artery bypass surgery, either singly or in combination.
‡Palmar crease pigmentation, xanthoma striata palmari, tuberoueruptive xanthomas, and/or xanthoma tuberosum.
§Apo E phenotypes determined as previously described.39
∥I indicates a probe specific for this residue hybridized with subject's DNA; -- indicates that no hybridization occurred.

results indicate that apo E2(Arg₁₅₈→Cys) is the most common apo E2 variant in populations sharing this ancestry. This conclusion is supported by the results of Utermann et al.,24 who have shown that in German populations the vast majority of subjects with apo E2 display a slow-migrating form of apo E on SDS-PAGE. Presumably, this form is apo E2(Arg₁₅₈→Cys).24 It is possible that defective-binding apo E mutants other than apo E2(Arg₁₅₈→Cys) are more frequent in non-European populations. For example, an apo E2 (Arg₁₅₈→Cys) variant associated with type III hyperli-
poproteinemia has been demonstrated in three unrelated subjects, two of whom were homozygous for this variant.18,35 All three subjects were North American blacks who expressed type III hyperlipoproteinemia, suggesting that in this ethnic population the apo E2(Arg15→Cys) may be frequently associated with type III hyperlipoproteinemia. This question will have to be answered by additional screening studies of patients from different ethnic backgrounds.

The expression of type III hyperlipoproteinemia is not restricted to the apo E2/2 phenotype; it has also been identified in several apo E3/2 and apo E4/2 subjects.24 Furthermore, as has been demonstrated in a Central American family27 and in one Dutch subject,25 the expression of type III hyperlipoproteinemia is not restricted to the presence of apo E2. Clearly, a key feature underlying expression of the disorder is the presence of a form of apo E that possesses defective receptor binding activity.21 It is apparent, even from the limited number of apo E variants whose structure and receptor binding activity are known, that binding activity cannot be inferred from isolectric focusing position.

The potential for detecting new apo E mutants in screening studies using a molecular biology approach was previously indicated by Funke et al.20 and is illustrated again in the present study by the apo E3/3 subject. M-PD (#35 in Table 2). This subject had a mild type IV hyperlipoproteinemia (no detectable β-VLDL) and a family history of hyperlipidemia and coronary artery disease. The fact that both of the hybridization probes reacted with this subject's DNA demonstrated that this individual has two different apo E alleles, although the protein product from each allele focuses in the apo E3 position. In addition, the reaction with the Arg-158 probe suggests that one of the apo E3 forms represents normal apo E3. The reaction with the Cys-158 probe indicates that the other allele in this patient is a previously undescribed variant containing a cysteine-for-arginine substitution at position 158. Isoelectric focusing of cysteamin-modified VLDL from this subject demonstrates that both forms of apo E3 contain one cysteine residue. This indicates that the variant allele probably arises from a point mutation in the e4 allele and most likely has an arginine at position 112. The cysteine-arginine substitution at position 112 in apo E4 does not affect receptor interaction, since apo E3 and apo E4 possess identical binding activities.28 Therefore, this new apo E3 variant would be expected to function like apo E2(Arg15→Cys) and to possess the same defective receptor binding activity, approximately 1% of normal. Thus, the presence of this defective binding form of apo E3 might account for the history of hyperlipidemia and coronary heart disease in this family. This possibility is currently being investigated.

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Index Terms: apo E variants • apo E2(Arg156→Cys) • type III hyperlipoproteinemia • apo E mutant detection • allele-specific oligonucleotide probes