methods

Non-denaturing polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinemia

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Abstract **Dysbetalipoproteinemia, an uncommon but highly atherogenic mixed hyperlipidemia due to the accumulation of remnants of triglyceride-rich lipoproteins, is characterized by cholesterol-enriched VLDL that migrates in the β-position on agarose gels. The demonstration of a broad β-band on agarose gel electrophoresis of plasma is an insensitive method and ultracentrifugation is an impractical method of diagnosing this condition. Non-denaturing polyacrylamide gradient gel electrophoresis (PGGE) was investigated as a screening method for the diagnosis of dysbetalipoproteinemia. A minigel procedure separating the Sudan Black prestained apolipoprotein B (apoB)-containing lipoproteins on a 2–8% polyacrylamide gel at 4°C overnight was analyzed for ultracentrifugally and genetically proven dysbetalipoproteinemic subjects as well as matched controls for mixed hyperlipidemia. Visual inspection revealed that the presence of only small VLDL- and IDL-like particles in untreated patients was highly sensitive (72%) and specific (95%) for dysbetalipoproteinemia. Videodensitometric analysis of area under the curve for large and small VLDL, as well as IDL and LDL, permitted even better discrimination in subjects whose profiles included some staining in the LDL-like region. A ratio of area under the curve of more than 0.5 for IDL-LDL allowed for a specificity of 100% and a sensitivity of 89% for the diagnosis of dysbetalipoproteinemia. This modified PGGE system may be useful in screening for dysbetalipoproteinemia.**—Blom, D. J., P. Byrnes, S. Jones, and A. D. Marais. **Non-denaturing polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinemia. *J. Lipid Res.* 2003.** 44: **212–217.**

Supplementary key words Type III hyperlipidemia • remnants • apolipoprotein E • method

Dysbetalipoproteinemia (Type III hyperlipidemia) is an uncommon genetic disorder of lipoprotein metabolism with a prevalence of about one to five per 5,000 (1). It is characterized by the accumulation of remnant particles, derived from triglyceride-rich lipoproteins (TGRL), resulting in the elevation of serum total cholesterol, triglycerides, and apolipoprotein E (apoE) levels in almost all patients. Chylomicron- and VLDL-remnants are cleared from the circulation by hepatic lipoprotein receptors [LDL-receptor (LDLR), LDL-receptor-related protein, and heparin sulfate proteoglycans (HSPG)] (2). ApoE is the ligand that mediates binding of remnant lipoproteins to hepatic lipoprotein receptors (3). Accumulation of chylomicron- and VLDL-remnants (β-VLDL), which are enriched in cholesteryl ester, leads to the development of premature coronary and/or peripheral atherosclerotic vascular disease (4, 5).

In nearly all cases of dysbetalipoproteinemia apoE is dysfunctional, but similar phenotypes may be seen with apoE deficiency (6, 7) or hepatic lipase deficiency (8). Three common alleles occur at the apoE locus, designated ε2, ε3, and ε4 (9, 10). The commonest molecular cause of dysbetalipoproteinemia is the homozygous presence of the ε2 allele (9). The apoE2 molecule binds poorly to the LDLR (11) and the dyslipidemic phenotype is expressed in the presence of additional permissive factors such as diabetes, obesity, hypothyroidism, or renal failure.

The diagnosis of dysbetalipoproteinemia is important as atherosclerotic complications are common, occur early, and the dyslipidemia responds well to therapy (1, 12, 13). Furthermore, an accurate diagnosis allows appropriate genetic counselling. Although cutaneous, tendinous and the highly characteristic palmar crease xanthomata may occur, they are only found in a minority of patients (1, 4, 14), and differentiation from other types of mixed hyperlipidemia is therefore seldom possible on clinical grounds alone. Laboratory diagnosis has been based on the dem-

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Abbreviations: HSPG, heparin sulfate proteoglycans; PGGE, polyacrylamide gradient gel electrophoresis; R_f, retardation factor; TEMED, tetramethylethylenediamine; TGRL, triglyceride-rich lipoproteins.

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onstration of remnant accumulation or analysis of apoE. Multiple diagnostic criteria have been proposed over the years, but there is still no universally accepted single diagnostic test.

Lipoprotein phenotypes in dysbetalipoproteinemia have been described by ultracentrifugation and electrophoresis. The presence of a broad-β band on electrophoresis of plasma in agarose gels and the demonstration of β-migrating lipoproteins in the ultracentrifugally isolated fraction less dense than 1.006 g/ml (β-VLDL) have been held to be diagnostic (15). Plasma electrophoresis has insufficient sensitivity because only 42% of samples positive for β-VLDL display a broad-β band (16). β-VLDL by paper electrophoresis was less discriminatory than VLDL analysis when the two techniques were compared (17). Concordance between β-VLDL positivity and VLDL composition ratios considered diagnostic has ranged between 47% and 83% (18, 17). Other electrophoretic techniques have included refinements in agarose gel electrophoresis (19), immunoelectrophoresis (20), combined polyacrylamide gel, and paper electrophoresis (16) and agarose electrophoresis with precipitation of VLDL by magnesium chloride/heparin (21, 22).

Abnormal cholesterol enrichment of the VLDL fraction isolated by ultracentrifugation (17, 23, 24), reflects the accumulation of remnant particles when compared to normal VLDL. Ratios of cholesterol to triglycerides by mass within VLDL of more than 0.42 (23) or 0.35 (24) have been proposed as diagnostic. A ratio of cholesterol in VLDL to plasma triglyceride of more than 0.30 (17) or 0.25 (24) may also be regarded as diagnostic or suggestive. A molar ratio of cholesterol in remnant like particles (isolated by immuno-affinity gels containing antibodies to apoB-100 and apoA-I) to plasma triglycerides of more than 0.23 has also been proposed as a diagnostic criterion (25).

The apoE phenotype can be determined by isoelectric focusing of apolipoproteins, while the three common genotypes are now most commonly determined by PCR and analysis of restriction fragment length polymorphism (26).

Chemical analysis of VLDL composition or electrophoresis of isolated lipoprotein fractions is impractical as ultracentrifugation is required. Electrophoretic techniques are more widely available, although interpretation of results may often be subjective. ApoE genotyping is diagnostic for apoE2 homozygotes, but may fail to detect the less common mutations of apoE that are often dominantly expressed (3). As all the described diagnostic techniques are labour and resource intensive, it is desirable to have a screening test, to best select patients with mixed hyperlipidemia that require further analysis.

PGGE separates particles according to size and has mainly been used in studies analysing LDL and HDL subspecies as an alternative to ultracentrifugation (27, 28, 29). In 1990, we developed a PGGE method to analyze the size distribution of all apoB-containing lipoproteins to explore the previously reported relationship between LDL particle size and cardiovascular disease (30). We noted specific patterns in patients with dysbetalipoproteinemia and analyzed these further.

METHODS

The patients who consented to full diagnostic workup all attended the University of Cape Town/Groote Schuur Hospital Lipid Clinic. The clinical and laboratory records including electrophoreses were traced and reviewed retrospectively. All electrophoreses have been performed by a single person (Pamela Byrnes) and previously reported on by a single observer (A. David Marais).

We evaluated our experience of PGGE in patients with dysbetalipoproteinemia proven on *a*) elevated levels of serum cholesterol and triglycerides at presentation, and *b*) VLDL cholesterol (VLDL-C) to VLDL-triglyceride ratio of ≥0.42 (23) or VLDL-C to plasma triglyceride ratio of ≥0.30 (17), and *c*) homozygosity for the ε2 allele or carriers of the apoE2 (Arg145Cys) mutation that has previously been shown to be associated with dysbetalipoproteinemia (31).

PGGE in patients with dysbetalipoproteinemia was compared with the results obtained in a control group of patients with mixed hyperlipidemia, in whom dysbetalipoproteinemia was initially clinically suspected but excluded by ultracentrifugation. Patients were selected as controls if they met all the following criteria: *a*) total cholesterol more than 7 mmol/l; *b*) plasma triglycerides between 3 and 10 mmol/l; *c*) VLDL compositional analysis consistently showing a ratio by mass of cholesterol to triglyceride within VLDL of less than 0.35 and a ratio of VLDL-C to plasma triglycerides of less than 0.25; and *d*) apoE genotype (if known) is not predictive of dysbetalipoproteinemia. We included patients in the control group based on their lipid values at the time of ultracentrifugation, which in the majority of patients was before lipid-modifying treatment was given.

Technique of gradient gel electrophoresis

Polyacrylamide mini-gels (6 cm long) were freshly cast. A 30% stock solution of acrylamide with 2.7% bisacrylamide was used to prepare an 8% solution of acrylamide in a buffer containing 13.6 g% Tris and 30% (v/v) glycerol at pH 8.8. A 2% acrylamide solution was prepared in Tris 13.6 g%. Polymerisation was activated in 4 ml of each solution by adding 30 μl of ammonium persulphate (100 mg/ml) and 1.5 μl of tetramethylethylenediamine (TEMED). The activated solutions were placed in the two chambers of a gradient maker with a small magnet in each chamber to permit mixing as the gel was poured from the chamber containing the 8% acrylamide solution. The stacking gel contains 3% acrylamide stock in 1.68 g% Tris at a pH of 6.8. Once the separation gel has set, the stacking gel solution is polymerized by adding 100 μl of ammonium persulphate and 4.0 μl of TEMED. Using this solution and a comb, 15 lanes are prepared for electrophoresis.

Fasted EDTA-plasma samples were prestained for lipids with Sudan Black using a similar method to Gambert et al. (29). Briefly, 100 μl of plasma was incubated with 50 μl of lipid stain (1% w/v Sudan Black in ethylene glycol) for an hour at 4°C and then spun for 20 min at 10,000 *g*. Equal volumes of supernatant and saturated sucrose were mixed and 12 μl of the resultant solution was loaded per well.

Gels were run for 12–18 h at 130 V at 4°C in a Tris Glycine (pH 8.3) buffer utilising the BioRad Minigel apparatus. The gels were subsequently analyzed without any further processing.

The gels were visually inspected and reports used a simple terminology that avoids inferring lipoprotein separation by density. The bands corresponding to LDL species usually appeared sharp and well defined and migrated to the distal fifth of the gel (anodal end). Migration of lipoproteins on successive gels was assessed relative to two control lanes, in which previously selected plasma with a known large (A) and small (B) LDL species were run.

Gels were calibrated with lipoprotein fractions (VLDL1, VLDL2, IDL, and LDL) isolated by sequential density-gradient ultracentrifugation, according to a previously described method (32). These fractions were prestained for lipids as described above and then electrophoresed on the same gel. Optical density profiles were obtained by video densitometry and the migration range of each lipoprotein fraction was expressed in terms of the retardation factor (R_f), for which the beginning of the separation gel was taken as zero and the peak of small dense LDL (B species) as 1.0, as this is the apoB-containing lipoprotein that migrates furthest in this system. VLDL1 (S_f 60–400) has a R_f of 0.2–0.45, VLDL2 (S_f 20–60) has a R_f of 0.45–0.7, IDL (S_f 12–20) has a R_f of 0.7–0.85, and LDL (S_f 0–12) has a R_f of 0.85 to1.0. Chylomicrons are found at $R_f < 0.2$.

Gels run after June 1998 were electronically captured with the Bio-Rad GelDoc 1000 system (Bio-Rad). Gels are placed in a photographic chamber and images are captured by a mounted video camera. The image is digitalized and transferred to a personal computer for further analysis with the BioRad MultiAnalyst software (Bio-Rad) or alternatively raw optical density and migration distance data can be exported for analysis with other software. Baseline optical density was measured for each gel at the beginning of the separation gel but between lanes. The value obtained was subtracted from the optical density readings for the lanes.

We categorized the lipoprotein staining in each lane into one of four patterns that are readily recognized on visual inspection (**Figs. 1**, **2**). Briefly, pattern A is VLDL-IDL only, pattern B is VLDL-IDL with faint LDL, and pattern C is VLDL-IDL with dominant LDL, while pattern D is LDL only. We quantitatively analyzed all gels from dysbetalipoproteinemic patients that could be closely matched for lipid values at the time of positive ultracentrifugation to gels from control patients. We only analyzed gels where ultracentrifugation and electrophoresis were done on the same sample. We calculated the relative proportions of the area under the curve for each of the following R_f segments 0.0–0.2, 0.2–0.45, 0.45–0.7, 0.7–0.85, 0.85–1.0, and 1.0–1.1.

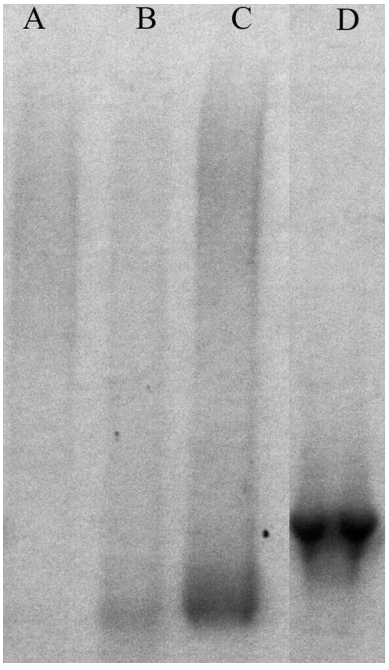

Fig. 1. Polyacrylamide gradient gel electrophoresis (PGGE) for dysbetalipoproteinemia. Patterns A to D are visible from left to right. The D pattern has a large LDL species while the C pattern has a small dense LDL species for which the retardation is set as 1.0. The B pattern demonstrates a small amount of small dense LDL while the A pattern contains only lipoproteins in the VLDL-IDL range.

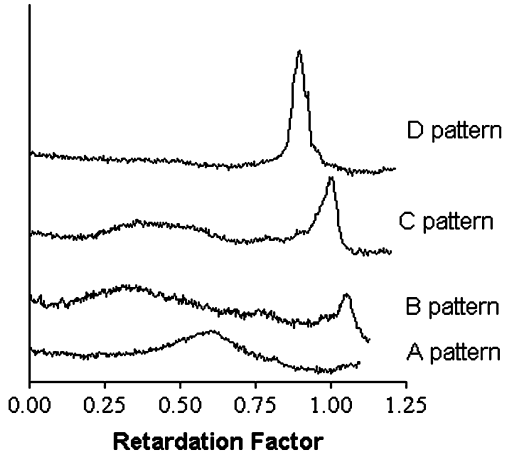

Fig. 2. Optical density tracings of representative PGGE patterns. Migration distance is expressed as retardation factor (R_f), with small dense LDL set as $R_f = 0$. Pattern A shows only VLDL-IDL. Pattern B contains VLDL and a small amount of small dense LDL species. Pattern C contains VLDL and a large amount of small dense LDL. Pattern D contains only large LDL species.

Agarose gel electrophoresis was performed according to the manufacturer's instructions using the Beckman Paragon system (Beckman Instruments). Samples were interpreted as being positive for a broad β-band if there was uniform staining in the β- and pre-β area without a discernable interruption between these two bands.

Triglycerides and cholesterol in plasma and lipoprotein fractions were determined by standard enzymatic techniques using automated analyzers in a routine chemical pathology laboratory that implemented standard quality control procedures. HDL-C was measured in the supernatant after precipitation of apoB-containing lipoproteins.

For isolation of VLDL (density < 1.006 g/ml), plasma was adjusted to density 1.30 g/ml with potassium bromide and layered beneath a saline/EDTA solution of density 1.006 g/ml. The specimen was centrifuged for 16 h at 100,000 *g* in a Beckman SW40 rotor. VLDL was recovered and lipids were determined by standard enzymatic methods. All ratios were expressed in terms of mass.

ApoE genotyping was performed according to the method described by Hixson and Vernier (26). ApoE2 (Arg145Cys) genotype was detected after *Bbv*I digestion and restriction fragment analysis (33) of the same 244 bp fragment of DNA amplified for detection of the common allelic variants.

Statistical analysis was performed using GraphPad InStat. Results are expressed as mean ± SD. Analysis of variance between groups of continuous variables was performed using the unpaired Student's *t*-test (Welch corrected). Discontinuous variables were analyzed by chi-square test. $P < 0.05$ was taken as statistically significant.

RESULTS

We found 64 patients with dysbetalipoproteinemia that fulfilled the inclusion criteria. A total of 251 PGGE were performed on these patients, of which 247 were technically satisfactory. Of the 64 patients, 43 (67%) were homozygous for the ε2 allele, while 20 (31%) had the apoE2 (Arg145Cys) mutation. One patient carried the apoE2

TABLE 1. Presenting data in patients and controls with mixed hyperlipidemia

	Dysbetalipoproteinemia	Control	*P*
Total cholesterol at presentation (mmol/l)	11.86 ± 4.97	10.05 ± 3.63	$P = 0.01$
Plasma triglycerides at presentation (mmol/l)	7.5 ± 5.45	6.7 ± 8.8	$P = 0.50$
HDL-C at presentation (mmol/l)	1.02 ± 0.37	1.06 ± 0.35	$P = 0.53$
Cholesterol/triglyceride in VLDL (mass ratio)	0.54 ± 0.19	0.25 ± 0.04	$P < 0.0001$
Cholesterol in VLDL/plasma triglycerides (mass ratio)	0.36 ± 0.09	0.18 ± 0.03	$P < 0.0001$

The dysbetalipoproteinemia group was made up of 65 subjects (32 male and 32 female). The Control group had 79 subjects (39 male and 40 female).

(Lys146Gln) mutation. Fifty-seven of the patients had agarose gel electrophoretograms available for analysis but only 24 (42%) had a broad β-band.

There were 79 control patients from whom 230 PGGE were available for analysis. Fifty-eight of the control patients had agarose gels available for analysis, of which nine (15%) were interpreted as suggestive of a broad β-band. Patients and controls are further described in **Table 1**. Since dysbetalipoproteinemic patients and controls were not specifically matched, the initial total cholesterol was found to be significantly higher in dysbetalipoproteinemic patients compared with controls (11.86 vs. 10.05 mmol/l), while the triglycerides (7.5 vs. 6.7 mmol/l) and HDL-C (1.02 vs. 1.06 mmol/l) did not differ significantly.

Electrophoretic patterns assigned by the inspection of the PGGE of 64 patients and 79 controls are compared in **Table 2**. Dysbetalipoproteinemic patterns and mixed hyperlipidemia patterns differed significantly ($P < 0.0001$). Patients with dysbetalipoproteinemia have less LDL-like particles visible on their gels; in 43% of patients no LDL-sized particles are visible. Lipid staining is mainly found in the areas corresponding to VLDL2 and IDL as a broad smear across the R_f range from about 0.3 to 0.85. The most intense staining was seen in the range of R_f from 0.5–0.85.

We analyzed the PGGE of 39 patients (Table 2) with dysbetalipoproteinemia that had PGGE performed before any lipid-modifying treatment was prescribed at the discretion of the treating physician. About 50% of patients received fibrates, while statins were used in 11%. Statin and fibrate combination therapy was given to 31% of patients and some patients took niacin either alone or in combination with a fibrate or statin. PGGE from untreated patients differed significantly both from the gels of control patients ($P < 0.0001$) and from dysbetalipoproteinemic patients who were analyzed in the treated state ($P = 0.0002$). Seventy-two percent of untreated patients had no detectable particles of LDL-size range, while in only 8% there was a distinct band visible in this range. Amongst the untreated patients none had staining of LDL-sized particles only (pattern D).

Thirteen patients had electrophoresis performed before treatment and when their hyperlipidemia was well controlled. Four of the eight patients that initially had no particles staining in the LDL-size range subsequently developed faint bands in this area. For the remaining patients, the patterns seen on electrophoresis originally did not change with therapy. The pattern on presentation in these 13 patients was not significantly different ($P = 0.31$) from the pattern in the other untreated dysbetalipoproteinemic patients.

Since VLDL composition is variable even in patients with established dysbetalipoproteinemia, we performed detailed quantitative analysis of PGGE patterns when positive ultracentrifugation in dysbetalipoproteinemic patients coincided with lipid estimation and PGGE. These dysbetalipoproteinemic patients were closely matched for lipid concentrations to controls who underwent the same investigations simultaneously. In the nine dysbetalipoproteinemic patients analyzed, significant differences in the relative proportion of area under the curve occupied by lipoproteins of differing size were found. In the dysbetalipoproteinemic patients the R_f segment in which LDL-sized particles are seen (0.85–1.0) occupied only 15.6% (±12.3) of the total area under the curve, while in the controls this area was 44.6% (±12.3) of the total area ($P < 0.0001$). In the dysbetalipoproteinemic patients, significantly more area under the curve was occupied by particles of the VLDL2-IDL size range. Despite the significant differences in percentage area under the curve for the various R_f segments, there is still considerable overlap of absolute values and a ratio of area under the curve of R_f segment 0.7–0.85/R_f segment 0.85–1.0 was found to be the best discriminator between dysbetalipoproteinemic patients and mixed hyperlipidemia patients. A ratio of more than 0.5 was found to have a sensitivity of 89% and a specificity of 100% in discriminating dysbetalipoproteinemic subjects in this sample.

TABLE 2. Reports of PGGE patterns in mixed hyperlipidemia

	Pattern			
	A	B	C	D
Dysbetalipoproteinemic patients (all reports)	105 (43%)	72 (29%)	50 (20%)	20 (8%)
Untreated dysbetalipoproteinemic patient reports	28 (72%)	8 (20%)	3 (8%)	0 (0%)
Mixed hyperlipidemia reports	12 (5%)	7 (3%)	171 (74%)	40 (18%)

A, VLDL2 and IDL only (R_f 0.45–0.85); B, VLDL and IDL, faint staining in LDL region (R_f 0.85–1.0); C, VLDL and IDL, prominent staining in LDL region (R_f 0.85–1.0); D, LDL only (R_f 0.85–1.0).

DISCUSSION

There are few reports in the literature describing PGGE as a diagnostic tool in the evaluation of patients with suspected dysbetalipoproteinemia. Masket (16) in 1978 described 27 dysbetalipoproteinemic patients (diagnosed by the presence of β-VLDL) that had undergone non-gradient polyacrylamide gel electrophoresis. Of 118 samples, 113 (96%) had no discernible LDL band on polyacrylamide gel electrophoresis, while a definite β-band was seen on paper electrophoresis. This combination was only found in three of 228 (1%) samples from patients with other forms of hyperlipidemia. These findings, however, were not reproducible in a second study (18), although agarose gel electrophoresis was used instead of paper electrophoresis. In 1978, Chew (34) reported the results of PGGE in a single patient with dysbetalipoproteinemia. In the untreated state several dense bands were seen below the VLDL zone, while there was no well-defined LDL band. After treatment a LDL band was visible and the dense bands below the VLDL zone disappeared.

In this paper, we investigated the lipoprotein patterns in a specifically modified PGGE procedure that yields good separation not only of LDL particles but also of TGRL and their remnants. Our experience (data not shown) indicated that a 2–8% acrylamide gradient in the separation gel permitted the best resolution of ultracentrifugally prepared lipoprotein fractions (32). The creation of wells in the stacking gel requires an acrylamide concentration of at least 3%, as lower acrylamide content does not impart adequate rigidity to the gel. In our experience, the relatively small migration distance in the stacking gel does not significantly influence lipoprotein separation.

Current commercially available calibration beads were impractical to use as internal standards because they are not easily detected by our scanning system. Our standardization therefore relies on using large and small LDL particles selected from a wide variety of samples. We have established that samples can be frozen at −20°C for up to 3 months without deterioration. Repeated small dense LDL samples can therefore be used to provide a consistent marker for determining the retardation factors derived in this study. We estimate that the error in the LDL particle size is not likely to exceed 1.5 nm and suggest that this would not materially affect the calculations presented here.

In this study, we based the diagnosis of dysbetalipoproteinemia on a combination of phenotypic criteria (hyperlipidemia and positive VLDL compositional analysis) as well as requiring the presence of a compatible apoE genotype. We excluded dysbetalipoproteinemia if the lowest proposed ratios of cholesterol-enrichment in VLDL were not reached. The control group was chosen from a group of patients with a mixed hyperlipidemia in whom dysbetalipoproteinemia had been initially suspected, leading to ultracentrifugal analysis. We believe this is the most appropriate control group with which to compare dysbetalipoproteinemia. We excluded patients with marked hypertriglyceridemia at the time of ultracentrifugation, as severe hypertriglyceridemia often results in non-diagnostic ratios (18, 17, 23, 24), even in dysbetalipoproteinemic subjects.

Dysbetalipoproteinemia is a highly dynamic metabolic disorder, lipid levels and lipoprotein composition may fluctuate widely. Patients may be normolipidemic and have detectable β-VLDL as the only lipid abnormality or severe hypercholesterolemia and hypertriglyceridemia may be present. In this study, we analyzed PGGE from dysbetalipoproteinemic patients with a wide spectrum of lipid levels. The cholesterol levels at time of electrophoresis ranged from 4.8 mmol/l to 34.3 mmol/l, while triglyceride levels fluctuated between 1.6 mmol/l and 24 mmol/l.

In our experience, severe hypertriglyceridemia causes a shift towards larger VLDL and chylomicrons, but the small number of subjects precludes a formal evaluation for diagnostic criteria. Patients with dysbetalipoproteinemia often have low levels of LDL-C secondary to impaired lipolytic processing of VLDL to IDL, as apoE2 in high concentrations displaces apoC-II from VLDL particles (3). In addition, apoE2 is not an effective enhancer of hepatic lipase mediated lipolysis of IDL to LDL (3) and competes poorly with apoB at the LDLR. Our experience supports these observations, but it would be of interest to study dysbetalipoproteinemia in the premorbid state to better understand the pathogenesis of the dyslipoproteinemia. For the same reason, it would be of interest to study the changes in PGGE of dysbetalipoproteinemic subjects undergoing treatment with different drugs.

In untreated dysbetalipoproteinemic subjects, staining only in the VLDL2-IDL region is the most commonly found pattern. This pattern is readily distinguished visually from all the other patterns. Diagnostic sensitivity is 72% for this pattern with a specificity of 95%. This "classical" pattern reflects the accumulation of excess remnants with impaired production of LDL and in our experience is more useful as an initial diagnostic pointer than the presence of a broad β-band on agarose gel electrophoresis. In patients with a visible LDL band as well as a suggestive TGRL pattern, quantitative analysis of area under the curve is useful to discriminate between dysbetalipoproteinemia and other dyslipidemias. This is preferred to the subjective interpretation of whether the LDL band is faint or prominent. An assessment of the proportion of LDL-sized particles relative to larger lipoproteins (IDL sized) should be made. Lipoprotein [a] may be a potential confounding factor, as it is of similar size to IDL and consequently stains in the same area. Lipoprotein [a], however, usually stains as a distinct band if present in high concentrations (usually more than 50 mg/dl) and not as diffusely as IDL, and is therefore readily recognized. Quantitative gel analysis should not be undertaken in the presence of this band, or the sample should be treated with a reducing agent and electrophoresed again.

If staining in the VLDL2-IDL region together with faint staining in LDL region is taken as diagnostic, sensitivity improves to 92% with specificity of 92%. No patient had staining only of LDL-size particles in the untreated state. This pattern was only observed in some patients who had achieved excellent control of lipid values on therapy.

Although this relatively simple and inexpensive technique holds promise as a diagnostic test, there may be some potential problems. The in-house gels may be somewhat ir-

reproducible: we compensate for this by relating migration of samples to that of the standards carried from gel to gel. The subjective interpretation of whether there is faint or prominent staining in the LDL area is overcome by quantitation of staining within the various gel segments, but an experienced single observer may also minimize variability. As with nearly all phenotypic tests for dysbetalipoproteinemia, diagnostic accuracy is limited at the extremes of lipid values.

The advantages of this technique include better diagnostic accuracy than agarose gel electrophoresis, at relatively low cost, in the evaluation of patients with mixed hyperlipidemia as well as better targeting of investigations such as ultracentrifugation and genotyping. We routinely perform PGGE on all patients referred to our clinic and admitted to the coronary care unit of our hospital and have diagnosed dysbetalipoproteinemia in several patients in whom this disorder was initially not suspected.

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