Familial type III hyperlipoproteinemia (HLP) is characterized by the accumulation of cholesterol-rich remnants (β-VLDL). The differential diagnosis of type III HLP is clinically important because patients with type III HLP develop premature coronary artery disease (CAD) and peripheral atherosclerosis and because type III HLP responds well to dietary treatment and fibrin acid derivatives (1, 2). Pathogenetically, type III HLP is related to dysfunctional isoforms of apolipoprotein (apo) E. At the APOE gene locus, three common alleles exist, designated e2, e3, and e4 (3–5). apoE2 is defective in its binding to lipoprotein receptors (6, 7). Because of the impaired catabolism of chylomicron and VLDL remnants, individuals homozygous for apoE2 reveal detectable amounts of β-VLDL in their plasma. β-VLDLs are atypical lipoproteins with a density <1.006 kg/L and β-mobility on agarose gel electrophoresis. Compared with normal VLDL, β-VLDLs are cholesterol-enriched; compared with normal LDL, they are relatively enriched in triglycerides. More than 90% of patients with type III HLP are homozygous for apoE2, but only ~1 in 20 individuals carrying the E2/2 phenotype finally develops type III HLP (8). Those homozygous carriers of apoE2 having small amounts of β-VLDL in their plasma not sufficient to produce overt hyperlipidemia have been classified as suffering from normalolipidemic dysbetalipoproteinemia. The term type III HLP, in contrast, is applied to hyperlipemic individuals only.

Clinical characteristics such as palmar, tendon, and/or tubero-eruptive xanthomas do not occur in all individuals with type III HLP. To establish biochemically the diagnosis of type III HLP, the following criteria have been applied in this study: (a) presence of increased cholesterol and triglycerides at 2500 mg/L or more, (b) an increased ratio of VLDL-cholesterol (VLDL-C) to VLDL-triglycerides (VLDL-TG; >0.4), and (c) an increased ratio of VLDL-C to total triglycerides (>0.3) (9–11).

We studied in total 1317 sera from women and men, ages 20–65 years. Among the participants with apoE phenotypes other than E2/2 (n = 1288) were 468 CAD patients recruited at the University Hospital, Freiburg or at the Benedikt-Kreutz-Klinik, Bad Krozingen, and 820 clinically healthy individuals, recruited at the Rheintalklinik, Bad Krozingen or employees of the BASF, Ludwigshafen. Twenty-nine apoE2 homozygotes were studied, including 21 samples obtained at the University Hospital of Heidelberg. In 8 of the 12 individuals with type III HLP, signs of atherosclerosis were present (66%). Two of the 17 apoE2 homozygotes without manifest type III HLP showed CAD (12%). Informed consent was obtained from each individual participating in this study; all procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Blood was drawn after an overnight fast and allowed to clot at room temperature; serum was obtained by centrifugation at 1500g for 15 min. All analyses were performed within 2 days after blood collection at the University Hospital of Freiburg. Cholesterol and triglycerides, without blanking for glycerol, were determined enzymatically (Boehringer Mannheim) using a Hitachi type 747 (total cholesterol and triglycerides) or a Wako 30R analyzer (supernates of precipitation reactions), respectively. The total CVs for the methods were <2%.

LDL-like lipoproteins were isolated using 100 μL of serum and 1000 μL of dextran sulfate (DS)/MgCl₂ precipitation reagent (Quantolip™, Immuno GmbH) and incubated for at least 10 min. The resulting precipitate was separated by a 5-min centrifugation, and the cholesterol and triglyceride content of the soluble lipoproteins (mainly VLDL and HDL) was measured in the supernate. Cholesterol and triglycerides associated with the precipitated lipoproteins (LDL-C₁₂₅ and LDL-TG₁₂₅, respectively) were calculated as total cholesterol or triglycerides minus supernatant cholesterol or triglycerides, respectively.

A combined ultracentrifugation (UC) and precipitation assay was used as the comparison method (12, 13). In this method, the recoveries of the bottom fraction (LDL plus HDL) was between 97% and 102%, whereas the recoveries of the top fraction (VLDL) was between 75% and 103%. All lipid measurements of one sample—lipids and lipoprotein fractions—were performed in the same analytical runs. The between-day CVs for LDL-C₁₂₅, LDL-TG₁₂₅, LDL-C_UC, and HDL-C_UC were below 3% and 4%, respectively.

apoE Phenotyping was performed by isoelectric focusing on agarose and immunofixation (14, 15). Regression analyses were performed using the method of Passing and Bablok (16).

We first wished to compare the results for LDL-C obtained with the DS precipitation method with those obtained with UC. In this comparison, we excluded 45 samples in which a recently developed algorithm indicated that the DS precipitation was incomplete because of

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Simple Precipitation-based Method for the Screening of Type III Hyperlipoproteinemia, Matthias Nauck, Lioba Glatt, Winfried März, Giso Feussner, and Heinrich Wiendl (1 University Hospital of Freiburg, Department of Clinical Chemistry, Hugstetter Strasse 55, 79106 Freiburg i. Br., Germany, and 2 University Hospital of Heidelberg, Department of Endocrinology, D-69115 Heidelberg, Germany; author for correspondence: fax 49-761-270 3444, e-mail manauck@mlz200.ukl.uni-freiburg.de)
fatty acid concentrations exceeding 2 mmol/L (17–19). Alternatively, samples can be diluted with bovine serum albumin before precipitation, which will overcome the effect of free fatty acids, as described for lipoprotein electrophoresis [Nauck et al., unpublished results, and Ref. (20)].

In the remaining 1243 samples with apoE phenotypes other than apoE2/2, LDL-C_DS showed a good agreement to LDL-C_UC (y = 1.07x – 154 mg/L; r = 0.969) (19). Recent work demonstrated that DS/MgCl₂ precipitation co-precipitates VLDL (19).

In apoE2 homozygotes with normolipidemic dysbetalipoproteinemia, the two methods corresponded with each other (r = 0.953; y = 0.79x + 304 mg/L), indicating that co-precipitation of β-VLDL does not markedly affect apparent LDL-C_DS values. In individuals with type III HLP, however, virtually no correlation between LDL-C_DS and LDL-C_UC was observed (r = −0.126). The LDL-C_DS was on average twice as high as LDL-C_UC, suggesting the amounts of β-VLDL.

The accumulation of triglyceride-enriched β-VLDL can be detected by analyzing the triglyceride content of the DS precipitate. We compared the ratio of LDL-TG_DS to LDL-C_DS between individuals with type III HLP and other types of HLP. Eight samples with type III HLP were matched with 16 samples with other types of HLP according to total cholesterol and total triglycerides. The ratio of LDL-TG_DS to LDL-C_DS was significantly higher in type III HLP, but this did not allow unequivocal diagnosis of type III HLP because this ratio may be increased in other types of HLP as well (Table 1).

In Fig. 1A, absolute values of LDL-TG_DS are plotted vs LDL-C_DS, including values for 18 samples from apoE2 homozygotes. All samples from individuals with type III HLP (n = 8) had increased LDL-TG_DS >950 mg/L and LDL-C_DS >1750 mg/L, whereas all samples from subjects with normolipidemic dysbetalipoproteinemia did not meet these criteria. Twenty-one samples of 1243 samples with phenotypes other than apoE2/2 (1.69%) exceeded the two threshold concentrations. The proportion of actual type III HLP among the suspicious samples thus was 28% (8 of 29). When calculated on the basis of all samples, the sensitivity to detect type III HLP by applying the combined thresholds of LDL-TG_DS >950 and LDL-C_DS >1750 mg/L was thus 100%, and the specificity was 98.3%. The specificity dropped to 92% if only samples with LDL-C_DS >1750 mg/L were considered. To verify these results, we analyzed another 11 completely independent serum samples from other individuals homozygous for apoE2 without type III HLP, whereas eight homozygotes for apoE2 without type III HLP were correctly classified as unsuspicious.

### Table 1. Lipid values for patients with type III HLP (n = 8) and other hyperlipoproteinemias (n = 16).

<table>
<thead>
<tr>
<th>Type III</th>
<th>No type III</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Total triglycerides, mg/L</td>
<td>4333 ± 903</td>
<td>4300 ± 855</td>
</tr>
<tr>
<td>Total cholesterol, mg/L</td>
<td>3440 ± 626</td>
<td>3070 ± 361</td>
</tr>
<tr>
<td>HDL-C, mg/L</td>
<td>410 ± 48</td>
<td>430 ± 103</td>
</tr>
<tr>
<td>LDL-TG_DS/LDL-C_DS</td>
<td>0.82 ± 0.19</td>
<td>0.55 ± 0.14</td>
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*For total cholesterol and total triglyceride concentrations, the data were matched for each patient with deviations of <500 mg/L.

* NS, not significant.
of HLP existed. Because our diagnostic criteria have been chosen in favor of sensitivity rather than specificity, we recommend confirming the diagnosis of type III HLP in suspicious samples using apoE phenotyping and/or apoE genotyping along with preparative UC and/or lipoprotein electrophoresis.

In conclusion, the advantage of the proposed procedure is that the screening for increased LDL-C concentrations includes the option to detect high concentrations of atherogenic LDL-like particles with an abnormal composition by simultaneous determination of triglycerides and cholesterol after precipitation with DS and Mg$^{2+}$ ions. Without this information, type III HLP will frequently be overlooked. Our procedure is inexpensive and can be performed in every laboratory because it needs no specialized equipment.

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