The Role of Lecithin: Cholesterol Acyltransferase for Lipoprotein (a) Assembly

Structural Integrity of Low Density Lipoproteins Is a Prerequisite for Lp(a) Formation in Human Plasma

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Abstract

The composition of lipoproteins in the plasma of patients with LCAT deficiency (LCAT-D) is grossly altered due to the lack of cholesteryl esters which form the core of normal lipoproteins. When plasma from LCAT-D patients and their relatives was examined we found that nine heterozygotes had plasma Lp(a) levels of 2–13 mg/dl whereas none of 11 affected homozygous individuals from different families contained detectable amounts of Lp(a) in their plasma. Therefore, the binding of apo(a) to LDL density particles was studied in vitro using LDL density fractions prepared from patients, and recombinant apo(a) [r-apo(a)], which was expressed and secreted by transfected COS-7 cells. The LDL from heterozygotes were chemically indistinguishable from normal LDL and homogeneous with regard to morphology, whereas the crude LDL floating fraction from homozygotes consisted of a heterogeneous mixture of large vesicles, and small spheres resembling normal LDL. The LDL density fraction from the LCAT-D patient lacked almost completely cholesteryl esters. Incubation of LCAT-D plasma with active LCAT caused a substantial augmentation of the original subfraction which morphologically resembled normal LDL. Using r-apo(a) and normal LDL or LDL of heterozygous individuals, apoB:r-apo(a) complexes were formed when incubated at 37°C in vitro for 20 h. In contrast, the total LDL floating fraction from a homozygous LCAT-D patient failed to form apoB:r-apo(a) complexes. After treatment with active LCAT, a significant apoB:r-apo(a) association was observed with LCAT-D LDL density particles. Our data emphasize the importance of the integrity of LDL structure and composition for the formation of Lp(a). In addition, we demonstrate that the absence of LCAT activity has a fundamental impact on the regulation of plasma Lp(a) levels. (*J. Clin. Invest.* 1994. 94:2330–2340.) Key words: LCAT deficiency • Lp(a) assembly • recombinant apo(a) • LDL structure • core lipids

Introduction

Lipoprotein (a) [Lp(a)][1] is a highly atherogenic and potentially thrombogenic lipoprotein with unknown physiological function(s) (reviewed in references 1–5). It consists of a lipid core, which is almost indistinguishable from that of LDL, and a protein moiety composed of apoB-100 and apo(a). The protein components are thought to be linked by a disulfide bridge (6–8), however, the nature of apo(a):apoB-100 binding has not yet been characterized in detail. Apo(a) has a unique structure with high homology to plasminogen (9). Most notably, the kringle- (K-) IV- and K-V-like structures, as well as the protease domain of plasminogen are found in apo(a). Although a specific interaction of Lp(a) with the LDL receptor has been demonstrated in vivo (10) and in vitro (11, 12), results from binding experiments with Lp(a) to liver cells in vitro indicated that the main physiological pathway of Lp(a) catabolism is very likely to differ from that of LDL (13).

The characterization of polymorphic forms of apo(a) represents the basis for the understanding of Lp(a) homeostasis (14–17). To date, 34 isoforms of apo(a) with variable molecular mass have been identified in human plasma (18). The size of each apo(a) isoform is determined genetically by the number of K-IV repeats and is highly negatively correlated with both apo(a) mRNA concentrations in the liver and plasma Lp(a) levels (14–17, 19). Sib-pair analyses and twin studies revealed an inheritance of apo(a) levels of up to 95% (20–22).

However, the variable number of K-IV-like repeats of apo(a) is not the only factor to determine Lp(a) levels in plasma. Sequence variations in the apo(a) gene locus have been identified that affect plasma Lp(a) levels (23). In diseases such as familial hypercholesterolemia, patients were found to have approximately threefold higher Lp(a) values than normocholesterolemics (24, 25), implying a possible involvement of the LDL receptor in Lp(a) catabolism. Heterozygous individuals with familial defective apoB-100 (FDB), the LDL of whom fail to bind to the LDL receptor (26), but who exhibit normal LDL receptor activity, also have increased Lp(a) levels (27). However, a smaller portion of defective apo B was detected in Lp(a) from these individuals than in their LDL, indicating that the LDL receptor may not be involved in Lp(a) removal from plasma. There are additional defects of lipoprotein metabolism known to affect the metabolism of Lp(a). Patients with abetalipoproteinemia not only lack LDL but also normal Lp(a) (28). In these patients, small amounts of apo(a), part of which was present in an uncomplexed form, was found only in the *d*

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1. *Abbreviations used in this paper:* CE, cholesteryl esters; FC, free cholesterol; HDL-(C), HDL cholesterol; K, kringle; LCAT, lecithin: cholesterol acyltransferase; LCAT-D, LCAT deficiency; Lp(a), lipoprotein (a).

> 1.21 g/ml bottom fraction. Subjects with familial hypobetalipoproteinemia, caused by a mutation in the apoB gene, exhibited Lp(a) levels of approximately half that of their normal relatives (29).

In this report, we addressed the question of whether or not Lp(a) production and assembly would be impaired in lecithin: cholesterol acyltransferase (LCAT) deficiency. LCAT catalyzes the esterification of free cholesterol (FC) with a lecithin-derived free fatty acid to produce cholesteryl esters (CE) and lysolecithin. The enzyme acts preferentially on HDL. The CE formed in HDL are then transferred, under normal physiological conditions, to lipoproteins of lower densities, in particular to LDL, forming the lipid core of the particle (reviewed in reference 30). LCAT deficiency (LCAT-D) is an inherited disease, where generally all lipoproteins have abnormal lipid compositions and consequently are abnormally shaped (31, 32). These alterations of lipoprotein composition and morphology are caused by a reduction of CE content within the lipoproteins. In the course of this investigation, we observed that none of homozygously affected individuals within two Austrian LCAT-D families that were examined, nor an additional seven other homozygotes, had detectable amounts of Lp(a) in their plasma. Here we show that active LCAT is required to render the structure of particles of LDL density conducive to interaction with apo(a) such that apoB:apo(a) complexes can form.

Methods

Blood samples. Venous EDTA blood was collected from all donors after overnight fasting. Plasma was isolated by low speed centrifugation at 1,500 *g* at 4°C.

Materials. We obtained nitrocellulose from Hoefer Sci. Instrs. (San Francisco, CA). Horseradish peroxidase-labeled protein A and ECL light detection system were from Amersham Intl. (Buckinghamshire, England). All other materials were from previously described sources (12).

Lipid, lipoprotein, and apolipoprotein analyses. TC and CE values were determined by the CHOD-iodide method using a test kit from E. Merck (Darmstadt, Germany). Test kits for TG and PL were from bioMerieux (Marcy l'Étoile, France) and for HDL-C from Immuno AG (Vienna, Austria). Lp(a) and apolipoproteins A-I, B, E, and D were quantitated by electroimmunodiffusion (rocket electrophoresis) as previously described (33). ApoD was expressed as percentage of a standard value of poolplasma. Protein determination was performed by a modified method of Lowry et al. (12).

LCAT assay. Determination of LCAT activity using an artificial proteoliposome assay has been previously described (34). LCAT activity was expressed as nmol/ml per h.

Characterization of apo(a) genotypes. The procedure characterizing apo(a) DNA phenotypes was performed by pulsed-field gel electrophoresis as previously reported (17, 35).

Density gradient ultracentrifugation. For isolation of LDL density fractions, ultracentrifugation with two different gradients was used. Method 1 (referred to as UC-method 1) was a three step nonlinear gradient (36) using NaBr density solutions of 1.100 and 1.050 g/ml, and deionized water. This gradient was used to obtain "total LDL density" fractions. If not otherwise stated, LDL floating particles were isolated by this method. In method 2 (referred to as UC-method 2), a multistep gradient was used to further fractionate particles ot LDL density from a homozygous LCAT-D patient. The density of the plasma or incubation mixture was adjusted with solid NaBr to density 1.100 g/ml. This solution was then carefully overlayed with 10 different NaBr density solutions (0.8 ml each) starting with d = 1.100 g/ml and decreasing incrementally by 0.010 g/ml per solution to a final d = 1.010 g/ml. Subsequently, the tube (total volume ~ 12 ml) was filled with deionized water to the top (~ 2 ml). Each plasma or incubation mixture was centrifuged in duplicate. One of the duplicate tubes contained Coomassie brilliant blue as protein dye (36) to facilitate documentation and identification of lipoprotein fractions. Lipoproteins in the second tube were not stained and were used for further experiments and analyses. After centrifugation in a Beckman SW-41 rotor at 40,000 rpm for 20 h at 15°C, either total lipoprotein fractions (UC-method 1) or 0.5 ml-fractions (UC-method 2) were carefully aspirated.

Determination of buoyant densities. Following density gradient ultracentrifugation, densities of lipoprotein fractions were calculated upon determination of the weight of 400 μl of lipoprotein solution and were expressed as g/ml.

Treatment of LCAT-D plasma with active LCAT. LCAT was partially purified from a Lp(a) negative proband as previously described (34) with the modification that the purification procedure included only density gradient ultracentrifugation and chromatography on phenyl sepharose. The partially purified LCAT-fraction was contaminated slightly with albumin, apoA-I, and apoD, but was free of apoB as identified by SDS-PAGE. Using ApoA-I proteoliposomes as substrate (34), the enzyme preparation had a specific activity of ~ 0.2 U/mg. 0.7 ml of LCAT-D plasma were incubated in the presence of bovine serum albumin (1% final concentration) and 100 mM Tris/HCl, pH 7.4, with 5 mg of partially purified enzyme in a final volume of 3.0 ml at 37°C for 2 h. After incubation, the LDL floating fraction was isolated (UC-method 1) and further characterized.

SDS-PAGE. SDS-PAGE was performed as previously reported (12). Aliquots of lipoprotein samples containing 5 μg of protein were delipidated by extraction with chloroform:methanol (2:1 vol/vol), solubilized in 20 μl of electrophoresis buffer containing dithiothreitol (0.1 M final concentration) and subjected to SDS electrophoresis in a 4.5–18% polyacrylamide gel. Proteins were stained with 0.1% Coomassie brilliant blue.

Lipoprotein electrophoresis. Lipoprotein electrophoresis was performed on glass plates in 0.5% agarose as previously described (34). Briefly, 6 μl of plasma was applied onto the gel using a narrow slit plastic template, the samples were allowed to diffuse into the gel, and electrophoresis proceeded at 50 mA for 90 min. After fixation with 0.5% sulfosalicylic acid, the gel was dried over night at 20°C. Lipoproteins were visualized with 1% Sudan Black B (Serva, Heidelberg, Germany), dissolved in 60% ethanol (vol/vol). The destaining solution was 60% ethanol.

SDS agarose gel electrophoresis and transfer of proteins onto nitrocellulose. Composition and preparation of the SDS agarose gels have been described elsewhere (37). Briefly, the gel contained 1.5% ultrapure agarose (Gibco BRL, Ltd, Paisley, UK) in 90 mM Tris-borate buffer, pH 7.4, 2 mM EDTA, and 0.1% SDS. Electrophoresis was performed in a Max Submarine agarose gel unit (Hoefer Sci. Instrs.) at 25 W for 2.5 h using 45 mM Tris-borate buffer, pH 7.4, 2 mM EDTA, and 0.1% SDS as running buffer. Immediately after electrophoresis, proteins were transferred electrophoretically overnight at 4°C onto nitrocellulose (Hoefer Sci. Instrs.).

Immunoblotting. After incubation of nitrocellulose strips for 1 h in a buffer containing 20 mM Tris, pH 7.4, 150 mM NaCl, 5% skim milk (referred to as immunoblotting buffer, IBB), detection of apo(a) with an affinity-purified antibody against apo(a) (12) (20 μg/ml, in IBB), or in control experiments with a polyclonal antibody against apoB which did not cross-react with apo(a) was performed for 2 h at 20°C. After washing the nitrocellulose filters twice with IBB for 15 min, they were incubated with horseradish peroxidase-labeled protein A (1:1000, in IBB) for 2 h at 20°C. Bands were visualized by ECL light detection upon autoradiography for 1 min.

Production of recombinant apo(a) [r-apo(a)]. An apo(a) expression plasmid (pSG5-XL) was constructed from several apo(a) cDNA clones (9). DNA sequences coding for the 5′ untranslated region (UTR), signal sequence, 18 K-IV-repeats, as well as the K-V- and the protease domains were assembled by standard recombination techniques (38) in a two step cloning strategy. First step: production of a construct with 15 K-IV repeats (*pSG5-L*): A 1735 bp EcoRI–HhaI fragment from

λ18 encoding the 5′ UTR, signal sequence, 4 K-IV repeats (1–4) and 299 bp of K-IV-5 was fused to a 4669-bp fragment from λ41 encompassing the latter 48 bp of K-IV-27, K-IV-28 through K-IV-37, K-V, protease domain and 67 bp of 3′ UTR. Second step: to achieve a construct with 18 K-IV-repeats (*pSG5-XL*), a 2077 bp EcoRI–HhaI fragment from λ18, encoding the 5′ UTR, signal sequence, K-IV repeats 1–5 and 294 bp of K-IV-6 was fused to a 5353-bp fragment from pSG5-L encompassing the latter 48 bp of K-IV-3, K-IV-4, 294 bp of K-IV-5, the last 48 bp of K-IV-27, K-IV repeats 28–37, K-V, protease domain, and 67 bp of 3′ UTR. The complete apo(a) cDNA fragment was ligated into the EcoRI site of the expression vector pSG 5, which contained the Simian virus (SV 40) early promoter, rabbit β-globin intron II, and the polyadenylation signal (39). Cesium chloride density gradient ultracentrifugation was used to isolate plasmid DNA for subsequent transfection experiments.

Transferrinfection of pSG5-XL and r-apo(a) analysis. High level expression of r-apo(a) was obtained from COS-7 cells, cultured in serum-free (10% LPDS) DME, using the "transferrinfection" methodology as previously reported (40). The r-apo(a)-containing medium was withdrawn after 48 h and the amount of r-apo(a) was determined by a sandwich DELFIA. The recombinant protein migrated slightly faster than the F-apo(a) isoform of the standard serum (Immuno AG) upon SDS-agarose gel electrophoresis. A detailed description of the transfection procedure will be reported elsewhere (40a).

Incubation of LDL density particles with r-apo(a). The ability of different LDL floating particles to form apoB:r-apo(a) complexes was tested in vitro using r-apo(a)-containing medium and different apoB-containing lipoproteins of LDL density. The medium contained approximately 0.2 μg of r-apo(a)/ml. Defined amounts of LDL or LDL floating particles, calculated on the basis of apoB content, specified in the legends for Figs. 5, *A* and *B*, and 6, were added to the medium of transfected COS-7 cells. Incubation of lipoproteins with r-apo(a)-containing medium was performed in an incubator at 37°C for 20 h. After incubation, aliquots of the incubation mixtures were immediately analyzed by SDS agarose gel electrophoresis and immunoblotting.

Immunoquantitation of r-apo(a) in apoB:r-apo(a) complexes. The amount of r-apo(a) specifically bound to apoB was determined in some experiments by a double antibody DELFIA. 96 well plates (Costar Corp., Cambridge, MA) were coated with polyclonal affinity purified antibody against human apo(a). The antibody exhibited no cross-reaction with human apoB-100. Nonspecific binding sites were blocked with 250 μl of 0.5% bovine serum albumin for 30 min. 200-μl aliquots of samples from incubation mixtures were added to the wells and incubated for 2 h at 20°C. After three washing steps with 50 mM Tris/HCl, pH 7.7 (DELFIA buffer), a polyclonal antibody against affinity purified human apoB-100, labeled with Eu according to the manufacturer's protocol (Pharmacia, Uppsala, Sweden), was added to the wells. The incubation proceeded for 2 h at 20°C. Excess of antibody was removed by two washing steps with DELFIA buffer. After addition of 200 μl of enhancement solution (Pharmacia), fluorescence was determined after 15 min in a DELFIA reader (Pharmacia-Wallac). For determination of the total amount of apo(a), the polyclonal anti-apo(a) antibody was used for the coating and detection step. An Lp(a) reference serum (Immuno AG) was used as an internal control in each set of experiments. The assay system was found to be linear between 1 and 100 ng of Lp(a) per well.

Electron microscopy. Solutions containing lipoproteins (~ 0.1 mg/ml) were transferred to carbon-coated copper grids. Lipoproteins were allowed to adhere, and excess of the samples were removed by touching the edge of the grids with filter paper. After washing the grids twice with one drop of deionized water, each grid received one drop of 1% phosphotungstic acid. After removing excess of phosphotungstic acid, grids were air-dried and investigated in a Philips EM 300 electron microscope. Instrumental magnification was 35,500.

Results

Clinical chemical data of families with LCAT-D. Table I summarizes the clinical chemical data of family members of both the W as well as of the S family from Austria. The LCAT-D patients presented with clinical symptoms typical for the disease such as bilateral corneal opacities, hemolytic anemia, and glomerulosclerosis (41), the latter condition leading to kidney transplantation in one individual within each family. Expectedly, LCAT activities in affected individuals were undetectable. In addition, other biochemical features typical of LCAT-D such as extremely low HDL-C, low apoA-I, apoD, and elevated apoE levels (not shown) were observed. A very high TG value was observed in one patient (9) of the W family. In all four affected persons, the presence of LpX (42) could be demonstrated upon electrophoresis in agarose gels (Fig. 2 *B*). A detailed clinical report of the two families (E. Steyrer et al., manuscript submitted for publication) will be published separately.

Determination of apo(a) phenotypes and Lp(a) values in two LCAT-D families. Fig. 1 illustrates the pedigrees of the two affected families, the W family, and the S family, and summarizes data from Lp(a) quantitation, apo(a) phenotyping, and apo(a) genotyping (S family only). The results of Lp(a) quantitation and apo(a) phenotyping demonstrated that none of the four homozygotes for LCAT-D had detectable Lp(a) in their plasma. Lp(a) concentrations of nonaffected members of both LCAT-D families ranged between 2 and 13 mg/dl. Apo(a) phenotypes S3 and S4 were determined for obligatory heterozygotes of the W family. The apo(a) isoforms S2/S4 and S4 were observed for S family members I.S. and S.S., respectively. Lp(a) was undetectable in the plasma of the father (J.M.) of the LCAT-D patient of the S family. The minimal amount of apo(a) that can be detected in our assay system is 20 μg/dl. Pulsed-field gel electrophoresis of DNA samples of the S family revealed the following apo(a) alleles: J.M., 22/25; G.S., 22/25; I.S., 14/22; and S.S., 22/24. For reasons of sample availability all further studies were performed with plasma of the S family. In an earlier study with four Norwegian families affected with LCAT-D (described in references 43 and 44), Lp(a) was measured by electroimmunodiffusion which has a detection limit of 0.5 mg Lp(a)/dl. Lp(a) was not detectable (G. M. Kostner and E. Gjone, unpublished observations) in any of the following seven homozygous individuals (family 1, A.R., I.S., R.M; family 2, D.J., L.G.; family 3, L.G.; family 4, E.R.). Unfortunately, these patients are not at our disposal and therefore data of Lp(a) phenotyping and genotyping can not be provided.

Lipopoproteins in LCAT-D. Upon density gradient ultracentrifugation, a substantial amount of the apoB-containing lipoproteins of G.S. floated in the VLDL density range (Fig. 2 *A*). In the normal LDL density region only a faintly stained band was visible, whereas the remaining lipoproteins distributed as a "smear" between the LDL and HDL density range. In both heterozygotes J.M. and I.S., intense staining at the very top of the tubes, typical of VLDL, indicated elevated VLDL-TG concentrations as well. Their LDL floated in the same density range as LDL from a normolipemic control person (N.). S.S. had a normal lipoprotein pattern with low LDL levels. Lipoprotein electrophoresis of G.S.'s plasma (Fig. 2 *B*) revealed a strongly reduced α-lipoprotein band and an additional lipoprotein fraction that migrated cathodically, and most likely represented LpX. The main lipoprotein fraction of G.S. migrated in the β/pre-β position. The heterozygous proband J.M. displayed strong β and pre-β bands that were clearly separated.

LCAT-treatment of lipoproteins in LCAT-D. The lipid core formed by CE is essential for LDL structure, therefore, in vitro

Table I. Clinical Chemical Data of Two Families with LCAT-D

	W family										
Proband	1	2*	3	4	5	6	7	8	9*	10*	11
LCAT act (nmol/ml per h)	61	0	54	119	53	40	62	62	0	0	84
TC (mg/dl)	202	101	220	215	216	213	226	191	267	157	202
FC/TC	0.24	0.88	0.26	0.25	0.27	0.28	0.28	0.26	0.89	0.99	0.32
HDL-C (mg/dl)	50	16	32	38	27	40	29	30	10	6	32
TG (mg/dl)	58	65	88	142	116	95	123	142	726	182	276

	S family				
Proband	J.M.	G.S.*	I.S.	S.S.	Normal values
LCAT act (nmol/ml per h)	40	0	59	69	60–120
TC (mg/dl)	156	123	211	198	<220
FC/TC	0.28	0.96	0.28	0.19	0.25–0.35
HDL-C (mg/dl)	33	6	33	71	>35
TG (mg/dl)	169	310	154	52	>150

LCAT act, LCAT activity. * Homozygous LCAT-D individuals. See Fig. 1 for pedigrees of the two families.

reconstitution of a CE core in LCAT-D LDL density particles would be expected to normalize their morphology. To test this conjecture, partially purified LCAT from a Lp(a) negative donor was added to the plasma of G.S. Upon incubation, the CE content in total plasma increased considerably, depending whether or not partly purified cholesteryl ester exchange/transfer protein (CETP) was added to the incubation mixture. Typically, up to 14% of total cholesterol was found in esterified form after incubation with LCAT alone. In the presence of CETP, the plasma CE content raised from 1 to 31% (not shown). Density gradient ultracentrifugation of LCAT-treated plasma revealed two distinct fractions within the LDL density range, referred to as GS/LDL density particles/LCAT/F1 (mean density 1.05 g/ml) and GS/LDL density particles/LCAT/F2 (mean density 1.07 g/ml).

Electron microscopy of various lipoprotein fractions. The morphology of all fractions with LDL density used in our studies was analyzed by electron microscopy (Fig. 3). The LDL density fraction of LCAT-D plasma (Fig. 3 *A*) consisted to a major extent of large particles of vesicular structure, and to a minor subfraction of small particles (mean diameter 21.3±1.6 nm) that were slightly smaller in diameter than normal LDL (mean diameter 22.8±2.8 nm) (Fig. 3 *D*). Upon ultracentrifugation by UC-method 2, LCAT-D plasma lipoprotein fractions were further separated. After centrifugation, twelve 0.5-ml fractions from densities 1.015 to 1.100 g/ml were collected and further analyzed (Figs. 3 and 4) as described below.

Fractions 2–4 (GS/LDL density particles/P2-4) contained mostly LDL-like particles (Fig. 3 *B*) with a mean diameter of 24.6±3.5 nm but also particles with vesicular structure. Large particles containing apoB, albumin, and low amounts of apoC proteins, were mainly found in fractions 6–8 (Fig. 3 *C*). Fractions 9–12 floated in a mean density range of 1.085 g/ml and were composed mainly of small particles similar to discoidal HDL (electron micrographs not shown).

The first of the two LDL floating fractions from LCAT-D plasma after LCAT treatment (GS/LDL density particles/LCAT/F1) was shown to contain two populations of lipopro-

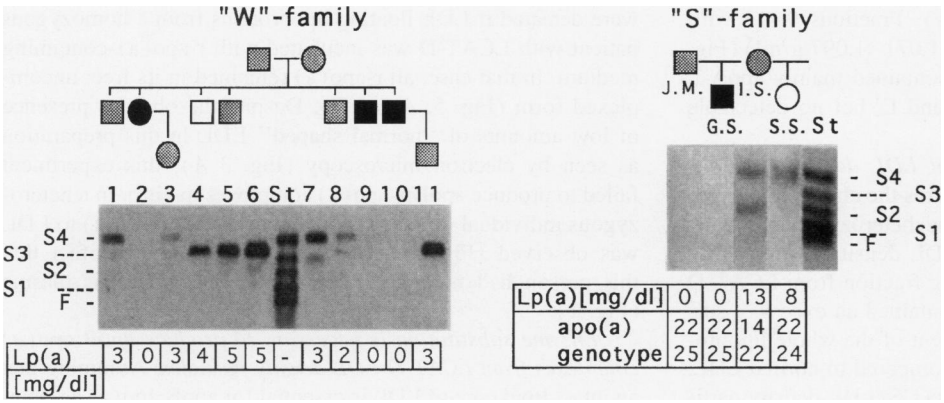

Figure 1. Pedigrees of LCAT-D families, Lp(a) concentrations, apo(a) protein isoforms and genotypes. (*Open symbol*) normal individual; (*black symbols*) homozygous for LCAT-D, (*punctated symbols*) heterozygotes. 2 μl of plasma (10 μl were used in the case of J.M. and G.S.) were diluted with electrophoresis buffer containing mercaptoethanol (5% final concentration) to 35 μl, boiled for 5 min and subjected to SDS agarose gel electrophoresis. After transfer of proteins onto nitrocellulose, immunobloting was performed with affinity-purified polyclonal rabbit anti-apo(a) IgG (20 μg/ml final concentration), followed by incubation with HRP-labeled protein A (dilution 1:1000). Bands were visualized by ECL light detection. Autoradiography was for 1 min. St, apo(a) standard obtained from Immuno AG. The positions of apo(a) isoforms are indicated. Lp(a) concentrations in plasma were determined by rocket electrophoresis, and apo(a) genotypes were determined by pulsed-field gel electrophoresis. Initials of probands of W family are not specified.

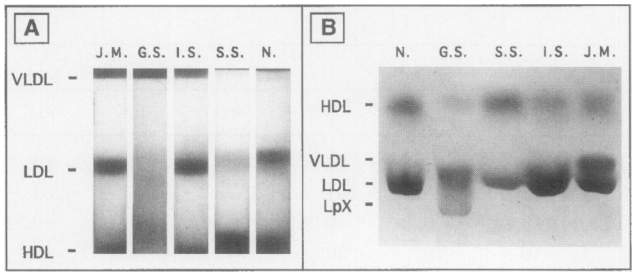

Figure 2. Lipoprotein status of LCAT-D family members. (*A*) Density gradient ultracentrifugation of plasma from the S LCAT-D family. 2 ml of plasma from S family members, and from a normolipemic individual (N.) were stained with 10 μl of a 5% Coomassie brilliant blue solution, adjusted to d = 1.100 g/ml, and carefully overlayed with solutions of d = 1.100, 1.050 g/ml, and deionized water. Density gradient ultracentrifugation (UC-method 1) was performed at 15°C for 20 h at 40,000 rpm. The position of lipoproteins according to reference plasma (N.) is indicated. (*B*) Lipoprotein electrophoresis of plasma from the S LCAT-D family. 6 μl of plasma from each proband was subjected to lipoprotein electrophoresis in 0.5% agarose gels as described in Methods. After fixation with 0.5% sulfosalicylic acid, the gel was air-dried overnight at 20°C. Lipoproteins were visualized with 1% Sudan black b. The position of lipoproteins from normal plasma (N.) is indicated.

teins by electron microscopy. One part of this fraction with a mean density of 1.050 g/ml was indistinguishable in terms of morphology from normal LDL, the other part consisted of larger vesicle-like particles (Fig. 3 *E*). The second LDL floating fraction from G.S. plasma after LCAT treatment (GS/LDL density particles/LCAT/F2) with a mean density of 1.070 g/ml contained primarily large particles (not shown) and resembled the preparation shown in Fig. 3 *C*. Fig. 3 *F* represents LDL from the plasma of the father (J.M.) of the LCAT-D patient. The preparation is very uniform (mean diameter 22.4±2.1 nm) and is indistinguishable in terms of morphology from normal LDL.

Apolipoprotein content of LCAT-D LDL density subfractions. LCAT-D LDL floating subfractions, after density gradient ultracentrifugation (UC-method 2), were characterized by SDS polyacrylamide gel electrophoresis and analyzed for apoB content and buoyant density. Fig. 4 demonstrates that all fractions contained both albumin, typical of contamination by LpX, and apoE (42), the values of which increased from lower to higher densities. Up to 72% of the total protein content within fractions 1–4 was apoB (Fig. 4, *bottom table*). Fractions 9–12 with "hallmarks" of discoidal HDL_E (d = 1.071–1.097 g/ml) (Fig. 4, electron micrographs not shown) contained mainly apoA-I, small amounts of apolipoproteins C and E, but no detectable amounts of apoB.

Chemical composition of different LDL density fractions from LCAT-D families. Table II compares the chemical composition of normal LDL, LDL from the heterozygous proband J.M., and abnormal lipoproteins of LDL density from LCAT-D patient G.S. The crude LDL floating fraction from LCAT-D plasma, prepared by UC-method 1, contained an excess of surface lipids. Most notably, the PL content of the whole lipoprotein fraction was increased by 100% compared to normal LDL. The subfraction from LCAT-D plasma (GS/LDL density particles/P2–4), isolated by UC-method 2, had a low CE content which was partially compensated for by an increased amount of TG.

After LCAT treatment, the amount of "LDL-like" particles increased considerably in the GS/LDL density particles/LCAT/F1-fraction and comprised ~ 60% of the total of lipoproteins as judged by evaluation of electron micrographs. This lipoprotein fraction was still heterogeneous in size (Fig. 3 *E*) and exhibited as a whole an increased content of CE and decreased concentrations of PL and FC compared to untreated GS/LDL density particles. The minor subfraction (GS/LDL density particles/LCAT/F2) was still devoid of CE. J.M.'s LDL had a normal chemical composition and surface lipid/core lipid ratio, consistent with the normal particle shape observed with electron microscopy.

Reconstitution of apoB:apo(a) complexes. In order to study the assembly of apoB-containing lipoproteins with r-apo(a), normal LDL and LDL from individuals heterozygous for LCAT-D, as well as lipoproteins of LDL density from the homozygous LCAT-D patient were incubated with r-apo(a)-containing serum-free medium from transfected COS-7 cells for 20 h at a molar apoB:r-apo(a) ratio of approximately 1:1. The reconstitution of apoB:r-apo(a) complexes was demonstrated by agarose electrophoresis followed by immunoblotting. Incubation of normal LDL r-apo(a) led to the formation of two bands (Fig. 5 *A*, lane *3*) which were identified by incubation with anti-apo(a). Only the slow migrating band in this figure, labeled as apoB:r-apo(a), reacted with both anti-apoB and anti-apo(a) (data not shown). This indicated that part of the r-apo(a) had formed a complex with apoB-100. We ascertained (not shown in this figure but described elsewhere) (40a) that the apoB:r-apo(a) complex dissociated completely in the presence of 1% mercaptoethanol. The simultaneous addition of 50 μmol/liter of ϵ-amino caproic acid and normal LDL to the r-apo(a)-containing medium completely abolished the formation of the apoB:r-apo(a) complex.

However, once this complex was formed, addition of ϵ-amino caproic acid could not disrupt the putative disulfide bridge, and the apoB:r-apo(a) complex remained detectable upon immunoblotting. (40a) Lanes *1* and *2* in Fig. 5 *A* are control incubations of medium from nontransfected cells (lane *1*) with LDL or apo(a)-containing medium in the absence of LDL (lane *2*). The addition of 0.5–5 μg of normal LDL to r-apo(a)-containing medium led to the binding of a substantial amount of free apoB to r-apo(a) as demonstrated by the additional slow migrating band which reacted with both antibodies against apoB and apo(a).

In contrast, not even traces of apoB:r-apo(a) complexes were detected if LDL floating lipoproteins from a homozygous patient with LCAT-D was incubated with r-apo(a)-containing medium. In that case, all r-apo(a) remained in its free, uncomplexed form (Fig. 5, *A* and *B*). Despite the obvious presence of low amounts of "normal shaped" LDL in this preparation as seen by electron microscopy (Fig. 3 *A*), this experiment failed to produce apoB:r-apo(a) complexes in vitro. In a heterozygous individual (J.M.), normal association of apo(a) to LDL was observed (Fig. 5 *A*, lanes *9–11*), despite the fact that this person had no measurable Lp(a) present in his plasma (Fig. 1).

Enzyme substitution is necessary to produce apoB:apo(a) complexes from LCAT-D LDL density particles. Assuming that an intact lipid core of LDL is essential for apoB:apo(a) association, in vitro reconstitution of a CE core in LCAT-D LDL density particles should improve the efficiency of complex formation between these particles and r-apo(a). To test this hypothesis, plasma of the LCAT-D patient was first incubated

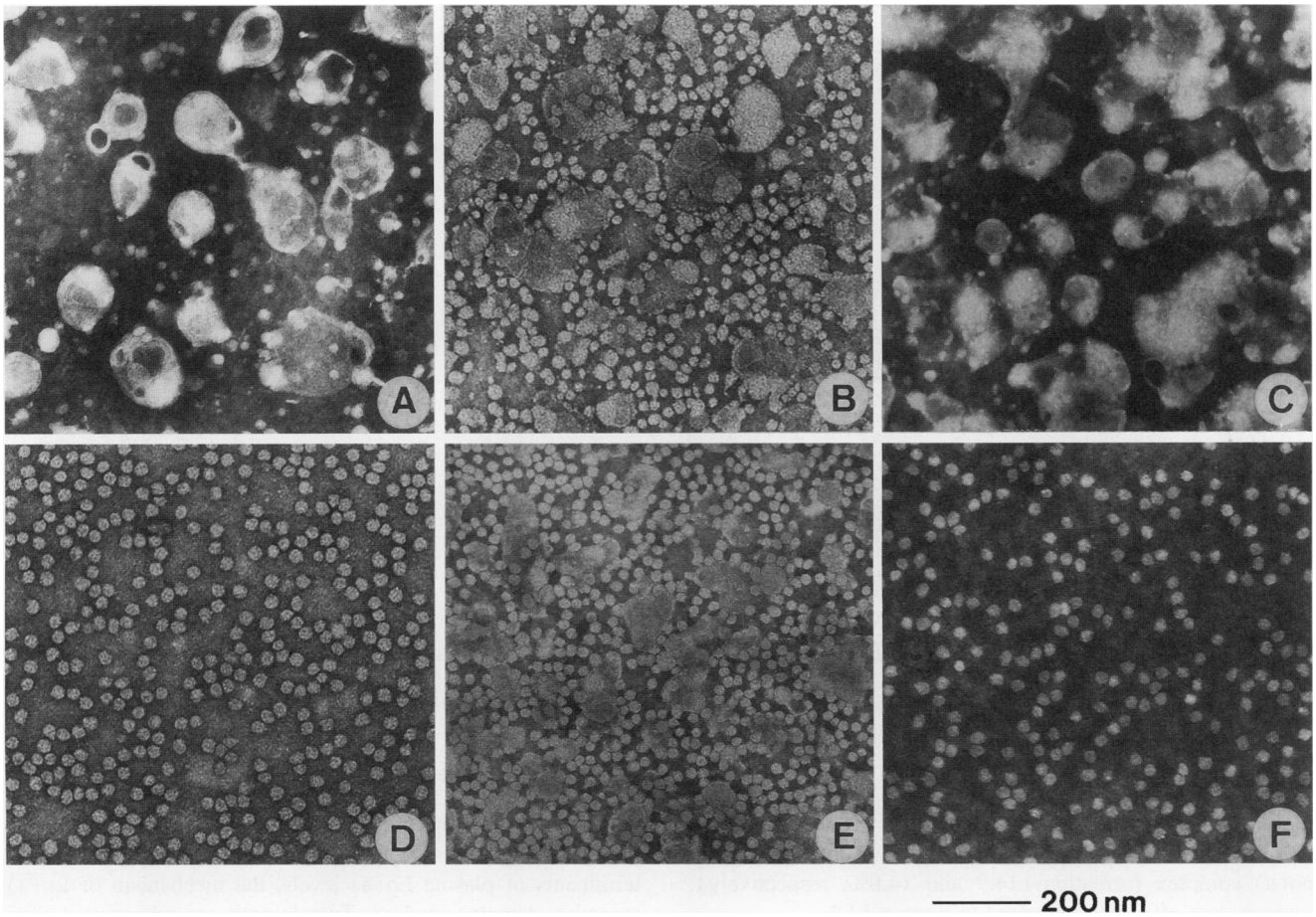

Figure 3. Electron micrographs of lipoproteins from S family members following density gradient ultracentrifugation. *A, B, C,* and *E,* lipoprotein fractions from homozygous LCAT-D individual G.S.; *D,* LDL from a normolipemic proband; *F,* LDL from heterozygous proband J.M.. In *A, D, E,* and *F,* lipoproteins were separated by UC-method 1; in *B* and *C* by UC-method 2, respectively. (*A*) lipoproteins of $d = 1.02-1.07$ g/ml, (*B*) lipoproteins of $d = 1.016-1.043$ g/ml, (*C*) lipoproteins of $d = 1.046-1.071$ g/ml, (*D*) normal LDL, (*E*) LDL floating fraction after LCAT-treatment (GS/LDL density particles/LCAT/F1), (*F*) LDL from J.M. Lipoproteins were transferred to carbon-coated copper grids (for details see Methods) and electron micrographs were taken at an instrumental magnification of 35,500. The bar indicates 200 nm.

with LCAT. Afterwards the reaction mixture was subjected to density gradient ultracentrifugation and the two fractions that floated in the LDL density range were isolated.

These fractions as well as untreated LCAT-D LDL density particles were then tested for their capacity to associate with r-apo(a) (Fig. 5 *B*). The lipoprotein fraction that resembled normal LDL (GS/LDL density particles/LCAT/F1) (see electron micrograph in Fig. 3 *E*) did form complexes although less efficiently than normal LDL of an equivalent apoB concentration most likely because only part of it represented LDL with normal shape. The LDL floating particles of G.S. that were not treated with LCAT did not produce any apoB:r-apo(a) complexes.

Concentration-dependent apoB:r-apo(a) complex formation with different lipoproteins of LDL density. We additionally examined the effect of varying the concentrations of all of the fractions, isolated from the plasma of the LCAT-D patient which were expected to contain "LDL-like" particles, on the formation of apoB:r-apo(a) complexes. The affinity of these fractions for r-apo(a) was compared to that of normal LDL. As demonstrated in Fig. 6, incubation of r-apo(a) with LDL from a normal proband yielded apoB:r-apo(a) bands at concentrations as low as 0.25 μg of apoB/ml. At this concentration, no other LCAT-D LDL density fraction resulted in the formation of apoB:r-apo(a)-complexes. Notably, after LCAT treatment, one lipoprotein fraction of LDL density (GS/LDL density particles/LCAT/F1) produced detectable amounts of the apoB:r-apo(a) complex at 1.0 μg of apoB/ml, whereas GS/LDL density particles/LCAT/F2 did not associate with r-apo(a) at all (Fig. 6). The low abundance LDL-like fraction of untreated G.S. plasma (GS/LDL density particles/P2–4) did form apoB:r-apo(a) complexes, but only at the highest concentration tested (5.0 μg of apoB/ml) (Figs. 5, *A* and *B*).

Immunoquantitation of r-apo(a) in apoB:r-apo(a) complexes. In order to determine the amounts of apoB:r-apo(a) complexes formed by incubation of r-apo(a) with normal LDL or LDL-like fractions of the homozygous patient, a double antibody DELFIA was performed (Table III). When 5 μg apoB/ml of normal LDL or of J.M. LDL were incubated for 20 h at 37°C, ~ 25% of r-apo(a) was found to be in a stable complex with apoB. This complex could not be dissociated by addition of 0.1% Tween-20, 0.1% Triton X-100, or 5% NaCl. Using the crude LDL floating fraction of the homozygous patient (GS/LDL density particles), only trace amounts of apoB:r-apo(a) complex were produced. In contrast, the incubation of GS/LDL

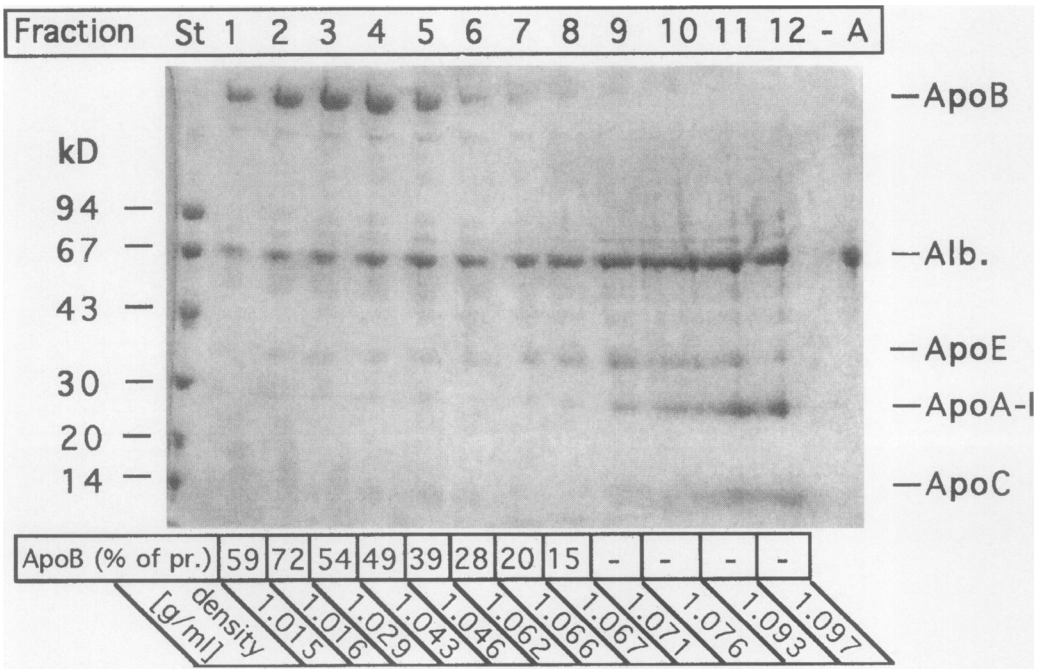

Figure 4. SDS gel electrophoresis, apoB content, and bouyant densities of LCAT-D lipoprotein fractions. After density gradient ultracentrifugation (UC-method 2), aliquots of lipoprotein samples containing 5 μg of protein were delipidated by extraction with chloroform: methanol (2:1 vol/vol), solubilized in 20 μl of electrophoresis buffer containing dithiothreitol (0.1 M final concentration) and subjected to SDS electrophoresis in a 4.5–18% polyacrylamide gel. Protein bands were stained with 0.1% Coomassie brilliant blue. The apoB content of lipoprotein fractions, expressed as percent of total protein value (% of pr.), was determined by rocket electrophoresis. Determination of densities of lipoprotein fractions was performed gravimetrically. Corresponding apolipoproteins are indicated at right. St, molecular mass protein standard, the molecular masses are labeled at left; A, human serum albumin.

density particles/P2-4 and of GS/LDL density particles/LCAT/F1 with r-apo(a) resulted in a considerable apoB:r-apo(a) complex formation (14.9 and 14.0%, respectively), however, less effective compared to normal LDL.

Discussion

The factors controlling Lp(a) biosynthesis and plasma Lp(a) levels are poorly understood. In earlier studies we and others demonstrated that Lp(a) plasma levels are mainly determined by the rate of synthesis (10, 45). While processes leading to the formation of mature Lp(a) are undoubtedly significant determinants of plasma Lp(a) levels, the mechanism of Lp(a) assembly remains unclear. Two hypotheses regarding Lp(a) assembly are currently discussed. First, Lp(a) is produced and secreted as a mature particle (intracellular assembly), or second, apo(a) is secreted by liver cells as such, and associates with LDL in the plasma compartment to form Lp(a) (extracellular assembly). Experimental data obtained so far clearly indicate that extracellular assembly seems more likely. (*a*) Apo(a) can be secreted in vitro from transfected cells (46, 47) and in vivo in transgenic mice (48) in its uncomplexed form. (*b*) LDL:apo(a) complexes were not detected intracellularly in apo(a)-transfected HepG2 cells or primary baboon hepatocytes (46). (*c*) Apo(a) binds specifically and strongly to human LDL extracellularly in transfected cell media, or when r-apo(a) was centrifuged through sucrose gradients containing LDL (49), or in the plasma of transgenic animals (48, 50, 51). (*d*) It is also unlikely that the liver secretes a mature particle since one major constituent of the core lipid of Lp(a) is cholesteryl linoleate produced in plasma by the action of LCAT and exchange/transfer processes. (*e*) Patients with abetalipoproteinemia have uncomplexed apo(a) and only minimal amounts of apoB-100:apo(a) heterodimers in their plasma (28).

If it is assumed that Lp(a) assembly in fact occurs in the extracellular compartment by complexing apo(a) to mature LDL, the morphology of LDL should be crucial for this process. To approach this hypothesis, we investigated the assembly of Lp(a) using r-apo(a) and apoB-containing particles from LCAT-D patients as these lipoproteins are known to have a grossly altered structure (32, 43, 44, 52, 53). Analysis of Lp(a) levels in seven Norwegian plasma samples from patients homozygous for LCAT-D revealed that none of the samples contained detectable amounts of Lp(a) (G.M. Kostner and E. Gjone, un-

Table II. Chemical Composition of Various Lipoprotein Preparations from Probands G.S. and J.M. Compared to Normal LDL

	Protein	FC	CE	PL	TG
Normal LDL	23	11	40	22	4
GS/LDL density particles	17	22	1	43	17
GS/LDL density particles/P2–4	13	23	2	41	21
GS/LDL density particles/LCAT/F1	15	23	8	36	17
GS/LDL density particles/LCAT/F2	16	27	2	45	10
JM LDL	25	11	36	22	7

Values are given as percent of total LDL (wt/wt). GS/LDL density particles, LDL floating particles from the homozygous LCAT-D individual; GS/LDL density particles/P2-4, low abundance spherical LDL floating particles from the homozygous LCAT-D individual, fractions 2–4 upon density gradient ultracentrifugation (method 2); GS/LDL density particles/LCAT/F1 (F2), LDL floating particles from the homozygous LCAT-D individual after LCAT-treatment of LCAT-D plasma and density gradient ultracentrifugation, fraction 1 (F1) or fraction 2 (F2); JM LDL, LDL from the heterozygous father of the affected LCAT-D individual.

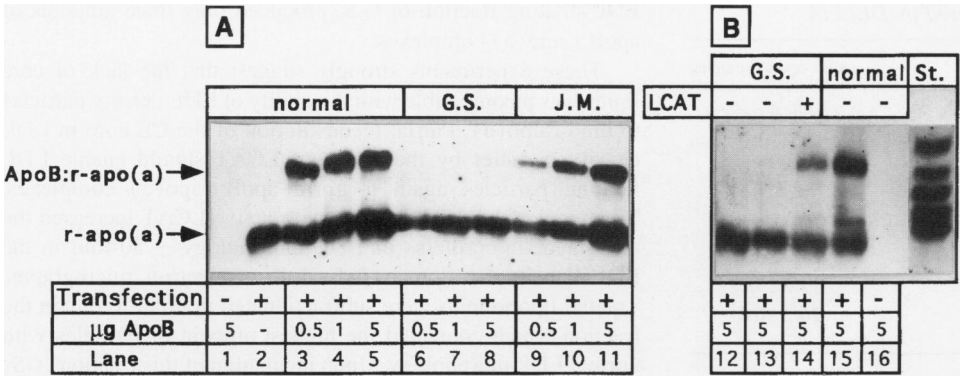

Figure 5. Reconstitution of apoB:r-apo(a) complexes. (*A*) Medium containing 0.2 µg/ml of r-apo(a) (lanes *2–16*) was incubated at 37°C for 20 h with the indicated amounts of LDL from a normolipemic individual (normal) (lanes *1–5*), the homozygote's (G.S.) LDL density particles (lanes *6–8*), and LDL from a heterozygous proband (J.M.) of the S family (lanes *9–11*). After SDS agarose gel electrophoresis and transfer of proteins onto nitrocellulose, immunobloting was performed with affinity-purified polyclonal rabbit anti apo(a) IgG (20 µg/ml final concentration), followed by incubation with HRP-labeled protein-A (dilution 1:1000). Bands containing r-apo(a) were visualized by ECL light detection. Exposure to x-ray film was for 1 min. (*B*) Two independently isolated LDL floating fractions from the homozygous LCAT-D individual G.S. (lanes *12* and *13*, respectively), LDL density particles from the same proband after LCAT treatment of LCAT-D plasma (LCAT +) (lane *14*), and normal LDL (lanes *15* and *16*) (5 µg of protein each) were incubated with r-apo(a) at 37°C for 20 h. SDS agarose gel electrophoresis and subsequent steps for detection of apo(a)-containing bands were the same as in A. St., apo(a) protein standard containing the following apo(a)-isoforms from top to bottom: S4, S3, S2, S1, F. The positions of uncomplexed r-apo(a), and of apoB:r-apo(a) complex are indicated by arrows.

published observations). In subsequent experiments, the same observation was made in four affected members of two LCAT-D families from Styria, Austria. In this investigation, we demonstrated that all four homozygous individuals lacked Lp(a) and apo(a), despite the fact that all other family members had Lp(a) levels between 2 and 13 mg/dl. The only exception was the father of G.S. (J.M.), an obligatory heterozygote, who also lacked Lp(a). The apo(a) genotype of the homozygote of the S family was investigated by pulsed-field gel electrophoresis. Apo(a) alleles with 22 and 25 K-IV repeats were identified. To investigate if Lp(a) deficiency was caused by structural alterations of LDL density particles in LCAT-D, lipoproteins of affected and nonaffected members of the LCAT-D S family were isolated by density gradient ultracentrifugation and further characterized. Both obligatory heterozygous parents had LDL with normal morphology and chemical composition. The LDL-floating fraction of the homozygous individual (G.S.), however, was not only reduced in concentration, but also exhibited a heterogeneous particle size distribution and a grossly altered morphology. Two main particle populations were identified within the LDL density range. First, LDL-like spheres (*d* 1.016–1.043 g/ml) which in contrast to normal LDL lacked CE yet had apoB as the main apolipoprotein constituent. Second, LpX-like particles (*d* 1.046–1.071 g/ml) that mainly contained albumin, apoB, apoE, and small amounts of apoC. This finding is in agreement with data on the lipoprotein particle heterogeneity in LCAT-D previously published by several laboratories (reviewed in reference 53). Treatment of LCAT-D plasma with active LCAT in vitro resulted in a considerable increase in the plasma CE content as well as in the amount of the patient's LDL density particles which were morphologically comparable to normal LDL. All lipoprotein fractions of normal individuals as well as heterozygous and homozygous LCAT-D patients were tested for their ability to form complexes with r-apo(a). Such in vitro complex formation has been reported by several authors (47, 54). Lipoproteins were incubated with serum-free cell medium containing r-apo(a) for 20 h at 37°C. Formation of the apoB:apo(a) complex was observed with normal LDL and LDL from heterozygous LCAT-D individuals. In this case, a slow migrating band that reacted with both anti-apo(a) and anti-apoB was identified upon agarose gel electrophoresis and immunoblotting. The addition of mercaptoethanol but not of SDS to the sample led to the disappearance of the apoB:apo(a) band which led us to assume that this complex is stabilized by a disulfide bridge. In additional experiments described in detail elsewhere (40a), we also demonstrated that

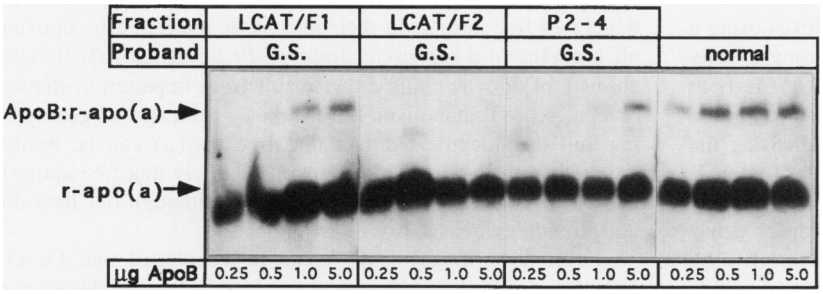

Figure 6. Concentration-dependent formation of apoB:r-apo(a) complexes with different LDL density particles. Four different concentrations of lipoprotein fractions from G.S., and normal LDL were incubated with r-apo(a) at 37°C for 20 h and subjected to SDS agarose gel electrophoresis. After transfer of proteins onto nitrocellulose, immunoblotting was performed with affinity-purified polyclonal rabbit anti-apo(a) IgG (20 µg/ml final concentration), followed by incubation with HRP-labeled protein a (dilution 1:1000). Bands were visualized by ECL light detection. Exposure to x-ray film was for 1 min. G.S./LCAT/F1, top floating fraction 1 of LDL density from LCAT-D plasma after LCAT treatment (mean density 1.05 g/ml); G.S./LCAT/F2, fraction 2 within LDL density from LCAT-D plasma after LCAT treatment (mean density 1.07 g/ml); GS/P2-4, LDL-like particles of LCAT-D plasma after UC-method 2, pool of fractions 2–4; normal, LDL from a normolipemic proband. The positions of uncomplexed r-apo(a), and of apoB:r-apo(a) complex are marked by arrows.

Table III. Assembly of Lp(a) as Measured by DELFIA

Fraction	apoB:r-apo(a) complex
	% of total r-apo(a)
Normal LDL	24.0±1.0
Normal LDL + 0.1% Tween-20*	23.2±2.0
Normal LDL + 5% NaCl*	22.9±1.2
GS/LDL density particles	0.8±0.03
GS/LDL density particles/P2-4	14.9±0.3
GS/LDL density particles/LCAT/F1	14.0±0.6
JM-LDL	26.2±0.8

1 ml of cell medium containing 0.2 μg of r-apo(a) was incubated at 37°C with various lipoprotein fractions (5 μg of protein/ml) for 20 h, and the amounts of apoB:r-apo(a) complexes were quantitated by DELFIA (see Methods). Values are mean±SD of triplicate analyses and represent the amount of complex formed between r-apo(a) and apoB–100, expressed as percent of total r-apo(a). Normal LDL, LDL from a nomolipemic donor, in the absence or the presence of detergent (Tween-20) or NaCl; GS/LDL density particles, LDL floating particles from the homozygous LCAT-D individual; GS/LDL density particles/P2-4, low abundance spherical LDL floating particles from the homozygous LCAT-D individual, fractions 2–4 upon density gradient ultracentrifugation (method 2); GS/LDL density particles/LCAT/F1, LDL floating particles from the homozygous LCAT-D individual after LCAT treatment of LCAT-D plasma and density gradient ultracentrifugation, fraction 1 (F1); JM-LDL, LDL from the heterozygous father of the affected LCAT-D individual. * Tween-20 or NaCl were added after apoB:r-apo(a) complex formation.

ε-amino caproic acid is unable to disrupt the apoB:apo(a) complex once it is formed, but prevents completely the complex formation if added simultaneously with LDL. These findings are not completely in line with observations of Phillips et al. (49) who concluded from their experiments that a disulfide bridge is nor necessarily formed in apoB:apo(a) complexes. The reason for this discrepancy is not obvious but might relate to fact that a recombinant apo(a) protein of different structure and/or different incubation conditions have been used.

However, the crude LDL floating fraction from the homozygous LCAT-D patient failed to produce complexes. LDL from the heterozygous father (J.M.) of the affected individual were indistinguishable from normal LDL in their ability to form apoB:r-apo(a) complexes, despite the fact that no Lp(a) was detected in the plasma of J.M., indicating that the lack of Lp(a) in this case was not caused by an aberrant LDL.

These results were confirmed in independent experiments upon immunoquantitation of apoB:r-apo(a) complexes using a double antibody DELFIA. The data revealed a strong affinity of normal LDL as well as LDL of J.M. for r-apo(a). In both cases, the amount of r-apo(a) complexed with apoB was around 25% of total r-apo(a) using 5 μg of apoB/ml. Increasing the amount of normal LDL to 20 μg/ml yielded up to 50% of r-apo(a) in complexed form (data not shown). The apoB:r-apo(a) complex was not dissociated by the addition of detergents (Tween-20, Triton X-100) or by 5% NaCl in our DELFIA assay which led us to conclude that covalent binding (disulfide bridge) did stabilize this complex. The low abundant LDL-like fraction (GS/LDL-density particles/P2-4) from the LCAT-D patient did also assemble in vitro with r-apo(a), yet the amount of complex was only ~ 60% that of normal LDL. The crude LDL floating fraction of G.S. produced only trace amounts of apoB:r-apo(a) complexes.

These experiments strongly suggest that the lack of core lipids was incompatible with the ability of LDL density particles to bind r-apo(a). Partial reconstitution of the CE core in LDL density particles by the action of LCAT should enable LDL floating particles again to form apoB:r-apo(a) complexes. Treatment of LCAT-D plasma with active LCAT increased the amount of lipoproteins of LDL morphology ~ 50-fold in the LDL density fraction as judged from electron micrographs. Treated lipoproteins were subfractionated in order to obtain the fraction which contained the highest amount of particles with normal LDL morphology. Upon incubation of this fraction (GS/LDL density fraction/LCAT/F1) with r-apo(a), a heterodimer band containing apo(a) was clearly visible after immunoblotting. Using this fraction, the amount of apoB:r-apo(a) complex determined upon immunoquantitation was similar to that obtained with the low abundant LDL-like fraction (GS/LDL density particles/P2-4) (14.0% of complex compared to 14.9%).

Although at a variable degree, all LDL floating fractions containing substantial amounts of core lipids (e.g., GS/LDL density particles/LCAT/F1, or GS/LDL density particles/P2-4) exhibited a partial association with r-apo(a) as demonstrated by the formation of apoB:r-apo(a) complexes, whereas lipoprotein preparations with low amounts of core lipids (e.g., GS/LDL density particles/LCAT/F2) did not. As expected, normal LDL with a surface lipid/core lipid ratio of 0.75 had the strongest affinity to r-apo(a). This underlined the importance of the presence of core lipids in LDL to facilitate the association of apoB with r-apo(a). It was of interest to note that the low abundance LDL density fraction from the affected patient without LCAT treatment (GS/LDL density particles/P2-4) formed apoB:apo(a) complexes in vitro. Therefore, it can be concluded that CE may be substituted by TG as the core component of LDL to produce LDL with a residual affinity for apo(a). However, the low abundance of this fraction within the total LDL floating particles of LCAT-D plasma as well as the reduced affinity of this fraction for r-apo(a) is very likely responsible for the inability of the crude LDL-floating fraction of G.S. to produce Lp(a)-like particles in a detectable amount in vivo or upon incubation with r-apo(a).

These results suggest that the putative first step in Lp(a) assembly, the docking of apo(a) to the LDL surface, requires a distinct shape and chemical composition of LDL. If apo(a) was produced but incapable of associating with LDL, one would expect the presence of uncomplexed apo(a) in the plasma of LCAT-D patients similarly as described for individuals with abetalipoproteinemia (28). However, none of the examined patients had free apo(a) in their plasma as measured by apo(a) phenotyping at a sensitivity limit of 20 μg of apo(a)/dl. The absence of apo(a) could either result from impaired synthesis or an increased catabolism. Based on data from apo(a) genotyping and the documented fact that free apo(a) can be easily secreted from liver cells, it seems more likely that free apo(a) leaves the circulation faster than Lp(a), although our data do not provide evidence for this hypothesis.

From these experiments it can be concluded that LCAT activity in plasma is required to produce LDL particles of the right morphology and chemical composition to allow Lp(a) formation. In addition, we hypothesize that other disorders of lipoprotein metabolism which affect LDL structure also, may have an impact on plasma Lp(a) levels.

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