Genetic and Phenotypic Heterogeneity in Familial Lecithin: Cholesterol Acyltransferase (LCAT) Deficiency

Six Newly Identified Defective Alleles Further Contribute to the Structural Heterogeneity in This Disease

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Abstract

The presence of lecithin:cholesterol acyltransferase (LCAT) deficiency in six probands from five families originating from four different countries was confirmed by the absence or near absence of LCAT activity. Also, other invariate symptoms of LCAT deficiency, a significant increase of unesterified cholesterol in plasma lipoproteins and the reduction of plasma HDL-cholesterol to levels below one-tenth of normal, were present in all probands. In the probands from two families, no mass was detectable, while in others reduced amounts of LCAT mass indicated the presence of a functionally inactive protein. Sequence analysis identified homozygous missense or nonsense mutations in four probands. Two probands from one family both were found to be compound heterozygotes for a missense mutation and for a single base insertion causing a reading frame-shift. Subsequent family analyses were carried out using mutagenic primers for carrier identification. LCAT activity and LCAT mass in 23 genotypic heterozygotes were approximately half normal and clearly distinct from those of 20 unaffected family members. In the homozygous patients no obvious relationship between residual LCAT activity and the clinical phenotype was seen. The observation that the molecular defects in LCAT deficiency are dispersed in different regions of the enzyme suggests the existence of several functionally important structural domains in this enzyme. (*J. Clin. Invest.* 1993. 91:677–683.) Key words: genetic disease • (automated) DNA sequencing • mutagenic polymerase chain reaction primers • corneal opacities • foam cells

Introduction

Familial lecithin: cholesterol acyltransferase (LCAT)[1] deficiency is an autosomal recessive disease caused by the absence of LCAT activity in plasma (1). LCAT is synthesized in the liver and released into the plasma (2) where it functions in the transfer of the sn-2 fatty acid of phosphatidylcholine to the 3-OH group of cholesterol. Cholesterol esters that are formed in this enzymatic reaction are subsequently transferred into the hydrophobic core of plasma lipoproteins, preferentially into HDL (3). The LCAT reaction participates in a process of reverse cholesterol transport whereby excess cellular cholesterol is transferred to the liver from where it can leave the body by bile acid formation or direct secretion into the bile.

In LCAT deficiency, the composition, shape, and concentration of all plasma lipoproteins is abnormal. Mature HDL, whose formation crucially depends on the LCAT reaction, are absent from the plasma of affected patients. In addition, erythrocyte membranes of affected patients contain abnormally high amounts of unesterified cholesterol and phosphatidylcholine (1).

Clinically, familial LCAT deficiency frequently presents with corneal opacities, normochromic anemia, and proteinuria. Foam cells are present in bone marrow and glomeruli and focal and segmental glomerulosclerosis may develop. Progressive renal failure is a common complication in the fourth and fifth decade of life. Furthermore, premature atherosclerosis has been observed in some patients with familial LCAT deficiency (1, 4).

Currently, about 30 cases of homozygous familial LCAT deficiency have been reported from 11 countries in Europe and North America, and from India and Japan. However, there are no reliable figures on the prevalence of this disease. For a secluded area in Norway, however, the heterozygote frequency has been estimated to be as high as 4% (1).

Previously, we have reported that the basic defect in fish eye disease, a disorder in which LCAT selectively does not esterify cholesterol contained in small size lipoprotein particles, is caused by a missense mutation resulting in a substitution of isoleucine for threonine at residue 123 of the LCAT protein (5). Recently, two other mutations in families with fish eye disease (6, 7) and five different mutations in classical familial LCAT deficiency have been reported (8–11).

In this report we characterize the molecular basis of LCAT deficiency in five families from four different countries (France, Denmark, Canada, and Italy [two cases]). We show

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1. *Abbreviations used in this paper:* FED, fish eye disease; LCAT, lecithin:cholesterol acyltransferase.

six new mutations in these patients. Familial LCAT deficiency therefore exhibits both genetic and clinical heterogeneity.

Methods

Subjects. Six patients with LCAT deficiency from five families and 43 of their relatives were analyzed. Initially, the presence of LCAT deficiency in the homozygous patients was verified by the absence or near absence of LCAT activity and by an increased cholesterol:cholesterol ester ratio in plasma. Plasma lipids and lipoproteins as well as LCAT-specific parameters are shown in tables in the Results section. The proband in the French family (family A) is a 39-yr old male, who was hospitalized in 1985 for proteinuria and microscopic hematuria. He had an elevated blood pressure (140/100 mmHg), diffuse corneal opacities, and proteinuria of 3–4 g/d. He did not show anemia or signs of cardiac failure or coronary heart disease. Although plasma creatinine level and creatinine clearance were normal at that time, a renal biopsy showed the presence of severe mesangial proliferation and deposition of membranoid material, associated with diffuse irregular thickening of glomerular basal membranes. Interstitial lesions, particularly foam cell deposition, were not found. For the following five years he was treated with nonsteroid antiinflammatory drugs. During that time plasma creatinine concentrations gradually increased from 90 μmol/liter to 133 μmol/liter. The Danish proband in family B is now 48 yr old and was originally seen at a hospital in 1988 for complaints of leg edema. He presented with homogenous bilateral corneal opacifications, which were apparently present since childhood, elevated blood pressure (180/105 mmHg), normochromic anemia (7.0 mmol/liter hemoglobin), and a proteinuria of 6.5 g/d. Plasma creatinine was 106 μmol/liter and urea nitrogen was 9.3 mmol/liter. 2 yr later, hemoglobin was reduced to 6.0 mmol/liter, plasma creatinine was 171 μmol/liter, and urea nitrogen was 10.2 mmol/liter. A kidney biopsy taken in 1990 showed ischemic glomerular damage and mesangial proliferation. The two probands in the Canadian family (family C) are of Italian and Dutch descent. A clinical and biochemical description of this family has been reported (12, 13). We have analyzed the LCAT gene of the Sardinian patient previously described by Utermann (14, 15) (family D). A kidney biopsy taken in 1977 showed membranoproliferative glomerulonephritis and the presence of foam cells. The patient has been on hemodialysis since February 1981. A coronary angiography in 1981 did not show signs of coronary heart disease. A bone marrow biopsy taken in 1988 showed clusters of macrophage-derived foam cells. In 1990 he presented to Münster University Clinics because of an infected cardiac pacemaker. Biochemical data in Table I are from this visit. The 38-yr old male Italian patient in family E presented with corneal opacifications, proteinuria (3 g/d), and normochromic anemia (red blood cells 3.79×10^{12}/liter) with increased numbers of target cells. Urea nitrogen, plasma creatinine, and endogenous creatinine clearance were normal. A renal biopsy showed interstitial and glomerular accumulation of lipids. The family of this patient was not available for analysis.

Determination of LCAT mass and activity. Blood and EDTA-blood were collected from all probands after an overnight fast. Plasma lipid and lipoprotein concentrations were determined by standard procedures (16). LCAT activity was determined using exogenous (17) and endogenous (18) substrates. In the exogenous assay an artificial substrate particle composed of egg yolk lecithin, unesterified cholesterol, and apolipoprotein A-I is used, while in the endogenous assay radiolabeled cholesterol is added to plasma at 4°C and the resulting esterification is measured after incubation at 37°C. Results from this latter assay are termed cholesterol esterification rate (CER). LCAT mass was determined by radioimmunoassay using polyclonal antibodies as previously described (19). Phenotype assignment was based on LCAT activity measured with the use of exogenous substrate. 20 nmol/ml per h, which is > 2 SD below the mean for normals (17), was used as the cutoff between normals and heterozygotes; all probands had LCAT activities below 2 nmol/ml per h.

Sequence analysis. The LCAT genes of the homozygous patients were sequenced directly from single stranded DNA templates prepared by two rounds of PCR amplification (20) following a previously described protocol (5).

Sequencing of fluorescence-labeled DNA was done using the A.L.F. sequencer (Pharmacia, Freiburg, Federal Republic of Germany). In this case 24 nucleotides, composed of the M13 universal primer sequence, were added to the 5′ end of one of the two primers of a PCR primer pair. Specifically, for subsequent use with fluorescent sequencing the 3′ portion of the LCAT gene was PCR amplified with 5′ CGACGTTGTAAAACGACGGCCAGTGCTCTATGAGGATGG-TGATGACAC 3′ as the upper strand primer and 5′ CTCGACCCGTG-GGATCTACATGTC 3′ as the lower strand primer. In the upper strand primer the first 24 nucleotides represent the M13 universal primer sequence and the next 24 nucleotides are nucleotides 4798–4821 in the LCAT sequence reported by McLean et al. (2); the lower strand primer is composed of nucleotides 5182–5205. Double strand PCR amplification was done by 30 cycles of denaturation (1 min, 95°C), annealing (1 min, 62°C), and extension (30 s, 72°C) of 100 μl reaction mixture containing 500 ng genomic DNA, dNTPs (200 μM), the two primers (0.2 μM), PCR buffer (Perkin-Elmer Cetus Instruments, Düsseldorf, Federal Republic of Germany), and 2.5 U Taq polymerase (Beckman Instruments, Palo Alto, CA). No prolonged initial denaturation step was used; the extension time of the last cycle was 7 min. The resulting 431-bp PCR product was purified by agarose gel electrophoresis (3% NuSieve, 0.5% SeaPlaque, FMC Corp., Rockland, ME) at 10 V/cm and subsequent electroelution. Desalting and concentration was done as described (21). Single strand production was done using 0.001 parts of the purified product from the double strand PCR reaction as template. Concentrations of the upper and lower strand primers were 10 nM and 300 nM, respectively. 45 amplification cycles and no final extension were used in an otherwise unchanged protocol. The purified product was dried in a vacuum centrifuge. Sequence analysis was done with the fluorescence-labeled M13 primer of the AutoRead sequencing kit (Pharmacia) and all of the reaction product from the asymmetric PCR as template following the protocol of the kit supplier.

Genotype determinations. Genotypes were determined with the use of mutagenic PCR primers, a method described in principle by Kumar and Barbacid (22) and Kumar and Dunn (23). We have previously used this method in the determination of carriers of the fish eye disease (FED) mutation (5). Primer synthesis and purification were done as described (21).

Results

The presence of LCAT deficiency in the five investigated families was verified by the absence or near absence of LCAT activity from the plasma of the probands. Other hallmarks of this disease, an increased cholesterol to cholesterol ester ratio in plasma and the almost complete absence of HDL cholesterol, were also seen in all six patients. In addition, LCAT mass was absent or severely reduced in all probands. Also, several first degree relatives of the probands showed marked changes in these parameters. The clinical symptoms of the probands are summarized in Table I; biochemical parameters are listed in Table II.

The genetic nature of the disease was ascertained by the identification of several individuals in each family with LCAT activities below 20 nmol/ml per h, an activity that is 2 SD below normal (17). Additional data on the heterozygote as well as the normal relatives from the affected families are shown in Table II. Clinical abnormalities in heterozygotes were not seen. Of note are the marked reductions in HDL cholesterol and apo A-I concentrations in the heterozygotes of

Table I. Clinical Characteristics of Six Homozygous LCAT-deficient Patients from Five Families

Family	Sex	Age at examination	Corneal opacities	Anemia	Target cells	Proteinuria	Plasma creatinine	Renal disease	Congenital heart disease
						g/d	*μmol/liter*		
A	m	38	+	−	−	3–4	133	+*	−
B	m	47	+	+	(+)‡	6.5	171	+*	−
C1§	m	18	+	+	+	5.9	63	+*	−
C2§	f	24	+	+	+	−	54	−	−
D	m	46	+	+	+	ND	884	+*	−
E	m	39	+	+	+	3	111	(+)*	−

* For a more detailed description refer to subjects in the Methods section. ‡ No target cells but otherwise irregularly shaped erythrocytes present. § Data are from reference 12.

kindreds A, B, and C compared to the unaffected family members.

Sequence analysis of the LCAT genes of the probands in all cases showed deviations from the wild type sequence reported by McLean and co-workers (2). In four of the five families the patients were homozygous for their genetic defect. The two probands in the Canadian family (family C) were compound heterozygotes with one missense mutation and a frameshift mutation due to a single base insertion. Four of the six defective alleles identified in the five families carried missense mutations (codon 209: CTT- > CCT, Leu- > Pro [family A]; codon 93: GCC- > ACC, Ala- > Thr and on the same allele codon 158: CGC- > TGC, Arg- > Cys [family B]; codon 135: CGG- > TGG, Arg- > Trp [one of the defective alleles in family C]; and codon 321: ACG- > ATG, Thr- > Met [family D]). A single base insertion (introduction of an adenine between codons 375 and 376) that gives rise to a frameshift (the other defective allele in family C) and a nonsense mutation in codon 83 (TAC- > TAA) (family E) were the two other mutations observed. These defects are shown in Fig. 1 and summarized in Table III. No other deviations from the wild type sequence were detected in the rest of the gene.

In all cases the presence of the mutations was confirmed by restriction fragment length analysis of PCR-amplified DNA. In the absence of naturally occurring restriction sites that could distinguish between the wild type and mutant alleles we introduced allele-specific restriction sites into the PCR fragments with the use of mutagenic primers. This method was also used

Table II. Biochemical Characteristics of the Homozygous, Heterozygous, and Unaffected Individuals from Families with Familial LCAT Deficiency

Family	*n*	Total cholesterol	Free cholesterol:total cholesterol	Triglycerides	HDL cholesterol	apo A-I	Cholesterol esterification rate	LCAT activity	LCAT mass
		mg/dl		*mg/dl*	*mg/dl*	*mg/dl*	*nmol/h per ml*	*nmol/h per ml*	*mg/liter*
Homozygous patients									
A		185	0.51	380	0	48	0	0	0.13
B		565	0.88	1,480	7	40	0	1.3	ND
C1*		183	0.87	180	5	48	0	0	0
C2*		106	0.87	110	5	48	0	0	0
D		89	0.92	186	ND	ND	ND	1.4‡	0.30§
E		123	0.86	341	12	36	ND	1.8	0
Heterozygous patients									
A	11	119±16	0.30±0.05	97±32	26±3	94±10	101±21	16.1±2.2	2.42±0.38
B	5	206±39	0.34±0.03	234±223	36±12	ND	112±18	16.6±3.6	ND
Cm‖	3	214±18	0.27±0.01	153±77	37±8	118±16	73±9	14.4±2.6	ND
Ci‖	5	225±25	0.25±0.01	122±18	33±9	108±20	67±12	15.1±1.6	ND
Unaffected patients									
A	5	161±49	0.24±0.07	109±59	42±13	126±25	97±18	24.9±1.9	4.53±0.43
B	5	214±69	0.29±0.01	82±38	62±16	ND	102±22	26.8±4.2	ND
C‖	10	208±33	0.23±0.02	85±24	57±12	144±24	63±18	27.9±1.3	ND

In two families (D and E) only the probands were analyzed. * Data were taken from reference 12. ‡ Data were taken from reference 14; the reported value was multiplied with 50 to correct for the 50-fold higher normal range in our current report. § Data were taken from reference 28. ‖ Data were from reference 17. Cholesterol esterification rate is LCAT activity assay using endogenous substrates; see Methods. C1 and C2 refer to the two affected probands in family C. Cm and Ci denote heterozygotes in family C with a missense mutation and a base insertion, respectively.

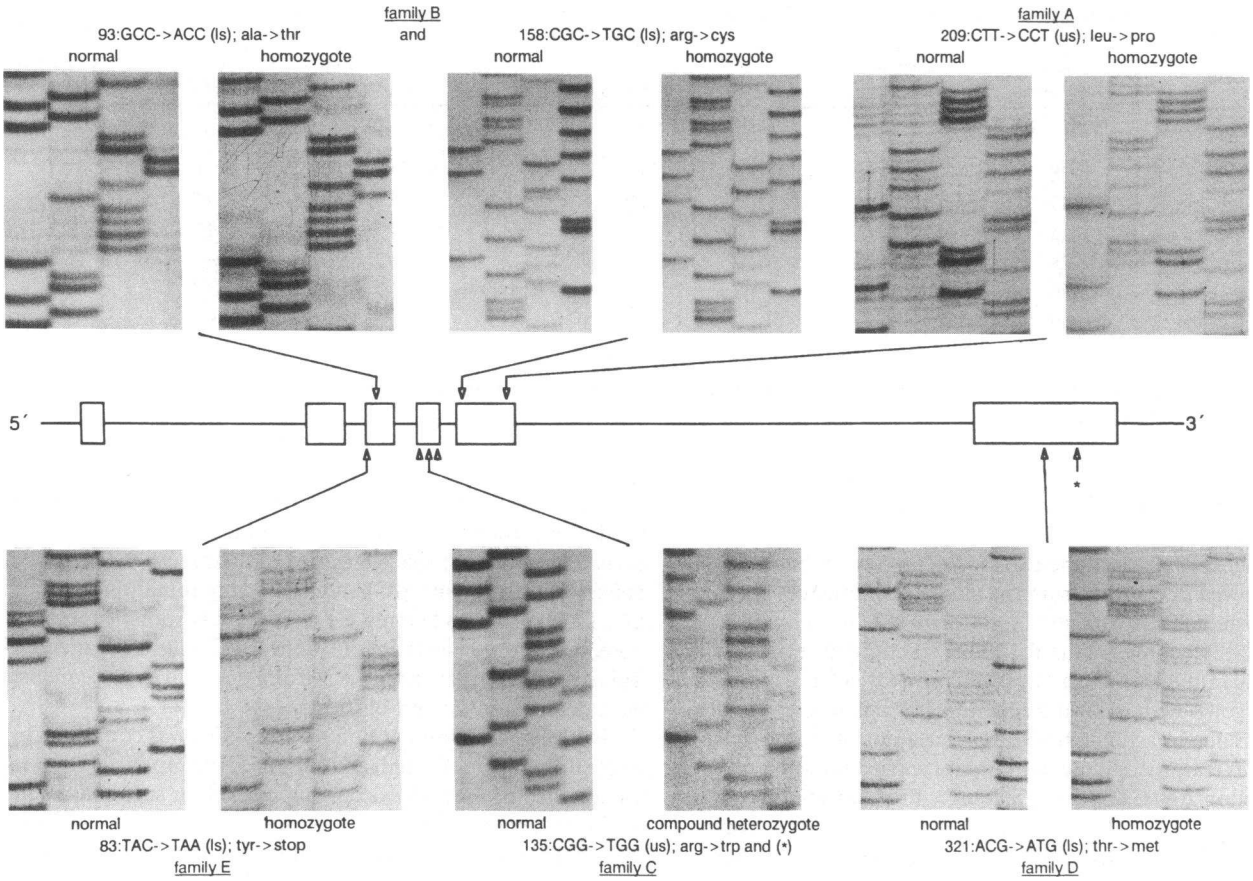

Figure 1. Identification and localization of defects causative for familial forms of LCAT deficiency. The upper part of the figure shows a schematic drawing of the LCAT gene in which the locations of the newly identified defects are indicated by arrows. Arrowheads point to previously identified defects (5, 8). The photo inserts show partial sequences of the areas in which the defects occur; for comparison the respective areas of the wild type alleles are also shown. The mutations are marked by arrowheads. All sequencing gels have been loaded with A, C, G, and T reactions, from left to right. The codon changes are given as they occur in the upper strand in all cases. For better orientation the actually sequenced strand is indicated. Codon numeration is done as in reference 2. The arrow in the sixth exon marked with a star indicates the location of a single base (adenosine) insertion that gives rise to a frameshift. Sequence analysis of this part of the gene was done by semiautomated fluorencence-labeled sequencing (data not shown).

for genotype assignment for the other family members. To avoid false genotype assignments to heterozygotes due to incomplete restriction enzyme digestion, all mutagenic primers were constructed to introduce the newly created restriction site only in the presence of the mutant allele. Therefore, the presence of a new, but 25 bases shorter, restriction fragment was indicative of the presence of the specific mutation. The sequences of the primers we used in this analysis and the lengths of the resulting diagnostic restriction fragments are listed in Table IV.

Table III. Basic Defects in Six Patients with Familial LCAT Deficiency

Family	Cases	Country	Zygosity	Mutation amino acids	Nucleotides
A	1	France	hom	209: Leu → Pro	CTT → CCT
B	1	Denmark	hom	93: Ala → Thr + 158: Arg → Cys	GCC → ACC + CGC → TGC
C	2	Canada	c-het	135: Arg → Trp	CGG → TGG
				376: Gln → Thr → . . . → 416: End	ATA CAG → ATA ACA G
D	1	Italy	hom	321: Thr → Met	ACG → ATG
E	1	Italy	hom	83: Tyr → End	TAC → TAA

The two mutations in family B are listed in one line to show their location in *cis* on chromosome 16. The two mutations in family C that are located in *trans* are listed in two separate lines. Both probands from this family are compound heterozygotes for these two mutations. The frame-shift mutation and the missense mutation originate from the Italian and the Dutch ancestry of the family, respectively.

Table IV. Mutagenic Primers Used in the Genotype Determination of LCAT-deficient Families

Mutated codon	Mutagenic primer	Primed strand	Site created	Fragment lengths: Wild type	Fragment lengths: Mutant
93: GCC → ACC	5′ GGGACGCGGATCTGGACA*CA*CGGGG 3′	ls	DraIII	360	335
135: CGG → TGG	5′ GAACCTGGTCAACAAT*CCA*TACGTG 3′	us	BstXI	439	419
158: CGC → TGC	5′ TGCAGGCCAGCAGGAGGAGT*AG*TAC 3′	us	ScaI	287	262
209: CTT → CCT	5′ GAGCCACCCCAGGGAG*CCT*CA 3′	ls	SauI	434	413
321: ACG → ATG	5′ CTTTACGGCGTGGGCCTGC*GC*A 3′	us	SphI	311	287

The table shows the nucleotide sequences of the mutagenic PCR primers used in the detection of the indicated mutations. The corresponding primers were placed 287 bp to 439 bp downstream or upstream of these primers. The sequence modifications of the mutagenic primers are printed in italic. The parts of the restriction enzyme recognition sites that are located within the primers are underlined. PCR product fragment lengths resulting after digestion with the appropriate endonuclease of the wild type or mutant allele are indicated. Information on the mutated codons is given as upper strand sequence irrespective of the orientation of the mutagenic primer. The base insertion between codons 375 and 376 was diagnosed by sequencing.

The likelihood for a causal relationship between the observed mutations and the LCAT deficient phenotype was assessed by segregation analysis. Fig. 2 gives an example of this type of analysis, where phenotype assignments were based on plasma LCAT activity and genotypes were assessed by the presence of selectively introduced restriction enzyme cutting sites. No deviations between genotype and phenotype were observed in families A, B, and C. In families D and E only probands were analyzed.

To exclude further the possibility that the identified sequence variations merely represent frequent polymorphisms rather than functionally important mutants we checked the DNA of 150 students from Münster University. None of the reported mutations were seen in any of these DNA samples. Moreover, complete sequence analysis of the LCAT genes from five apparently healthy individuals and of one heterozygote carrier for each of the defective alleles did not yield any difference from the wild type sequence.

Discussion

We have identified six different mutations in the LCAT gene in five patients with familial LCAT deficiency. The familial nature of the disease in these patients was determined by extensive pedigree analyses (families A–C) and the previously published findings on family D (15). Low HDL cholesterol levels seen in several first degree relatives of the proband in family E is indicative for the familial nature of the disease also in this family.

In three families we have performed segregation analysis to assess the relationship of genotype with phenotype. In the plasma of genotypically homozygous (or compound heterozygous) patients, none or only minute residual LCAT activity was found, while all genotypic heterozygotes showed a reduction in their LCAT activity to 50–60% of normal. Sequence analysis of all exons, the splice donor and acceptor sites, and of the 5′ consensus regulatory elements yielded only the reported mutations. It thus appears likely that the identified mutations are causative for the observed phenotypes. The possibility that these mutations represent functionally unimportant changes in DNA sequence is unlikely. Such a hypothesis requires the existence of a neighboring gene that interferes with LCAT activity that would have to be in linkage disequilibrium with several allelic mutations in the LCAT gene which are otherwise not found in the population. Moreover, to date no structurally normal LCAT gene has been reported in a patient with confirmed

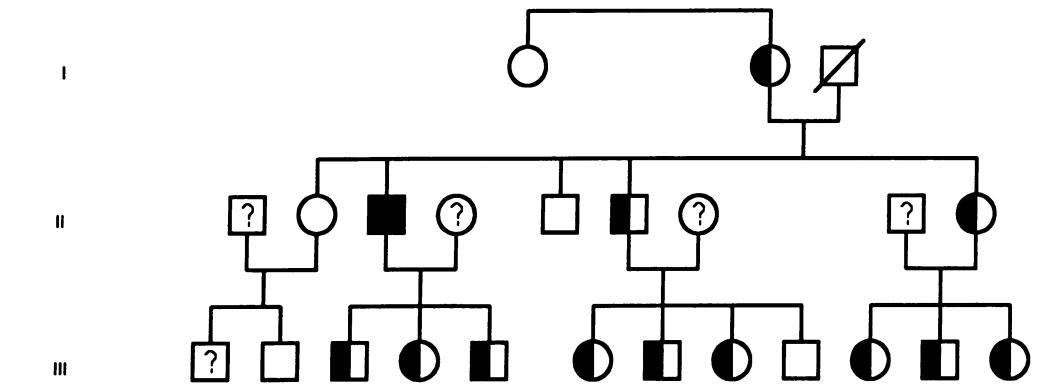

Figure 2. Pedigree, LCAT genotypes, and LCAT mass and activity of family A. Genotype assignment was based on the presence of the Leu-> Pro mutation in codon 209 of the LCAT gene. Question marks or n.d. indicate that the corresponding parameters of these family members have not been analyzed. All heterozygotes had LCAT activities below 20 nmol/ml per h and an LCAT mass below 3.5; all normals had values above these cutoffs.

familial LCAT deficiency. Taken together, there is strong evidence that the mutations we have identified in the LCAT gene are causative for familial LCAT deficiency.

As it is expected for a rare recessive trait, most of the patients carry two identical defective alleles. An exception in this regard is the Canadian family where the two clinically affected patients are compound heterozygotes for two different mutations. This probably reflects the different paternal and maternal ancestral origins for these patients. Since neither LCAT mass nor LCAT activity was detectable in the two affected persons in this family the missense mutation in codon 135 and the frameshift mutation appear to be functionally equivalent. This is further supported by the absence of differences in the biochemical phenotype between heterozygote carriers of the two mutations.

A recent report on phenylalanine hydroxylase deficiency showed that different defects in this enzyme were predictive of different levels of enzyme activity and of the severity of the disease (24). Similarly, two different mutations in the arylsulfatase A gene have been found to provide a structural basis for early and late onset of metachromatic leukodystrophy (25). It is thus interesting to know if also in LCAT deficiency different mutations in the enzyme gene are responsible for the variation of clinical symptoms. In the cases reported here, some variability was observed in the age of onset of symptoms and in the severity of clinical complications. As none of the families shared identical mutations, it remains unknown at present if this heterogeneity is genetically determined. The residual level of plasma LCAT activity, however, does not appear to be predictive for associated clinical symptoms, as the clinical symptoms in the patients reported in this paper show a considerable variance despite the absence or near absence of LCAT activity in all cases. Also, the two affected patients in family C, who have identical LCAT defects, show a considerable variability in their clinical phenotypes (12). It may therefore be speculated that the expressivity of the clinical disease is modulated by the absence or presence of additional effects of the environment and of minor genes.

There have been previously reported defects in the LCAT gene. These include missense mutations in codons 147, 228, and 293 (references 8–10), a single nucleotide insertion in exon 1 (11), and an in frame GGC (Arg) insertion between codons 140 and 141 (9), all found in patients with classical familial LCAT deficiency, and missense mutations in codons 10, 123, and 347 (5–7) causing familial partial LCAT deficiency in a disorder termed FED. Including the mutations reported in this paper, 13 different mutant alleles of the LCAT gene are known currently which all are associated with marked impairments of LCAT function. In most cases the loss of LCAT activity is associated with the absence of LCAT mass from the plasma compartment. Exceptions are the mutations in codons 147, 228, and 293, where no or very little LCAT activity but 40–60% of normal mass was found, and the FED defects, in which the ability of LCAT to act on small substrate particles is lost despite the presence of half normal LCAT mass. Other examples of LCAT deficiency, for which the basic defects are as yet unknown, show a reduction in LCAT mass to 20–40% and in LCAT activity to 0.1–13% (1). Based on these observations the existence of different mutations causing LCAT deficiency had been proposed. Recent reports and our current data confirm this hypothesis and, moreover, suggest that LCAT activity may be reduced by one or more of several different mechanisms. From the observations made in FED it has been hypothesized that LCAT may in fact have at least two functionally important domains one of which is the catalytic center and the other a recognition site for small size substrate particles. The identification of mutations in different regions of the LCAT gene that cause familial LCAT deficiency is in support of the presence of several different domains important for LCAT function.

Based on clinical as well as on biochemical differences we can currently define two classes of familial LCAT deficiency: an α-type and an α,β-type. The terminology reflects the inability of different mutant LCAT enzymes to interact with α- and/or β-migrating lipoproteins in vitro. Surprisingly, the currently known mutations in the α-type of LCAT deficiency are not confined to a specific region of the protein. If this relates to complex secondary or tertiary structure requirements for substrate specificity of the enzyme or if it simply reflects the unproven genotype with phenotype relation in two of the defects (6, 7) is currently not known. The α-entity of LCAT deficiency is clearly distinct from LCAT deficiencies of the α,β-type by a normal cholesterol esterification rate and the absence of hematological and nephrological symptoms even at high age. All other LCAT deficiencies with known basic defects are of the α,β-type. As mentioned above variance in the clinical expression of α,β-type LCAT deficiencies is more likely caused by a different genetic and environmental background than by their different basic mutations. Also, it is of interest to note that, different from a previous report (1), the LCAT-deficient patients reported here have no apparent signs of congenital heart disease.

Although a number of easily definable biochemical parameters, like HDL cholesterol or free cholesterol:total cholesterol ratio, are significantly altered in familial LCAT deficiency these findings do not show the consistency required of a diagnostic marker. Therefore, the diagnosis of LCAT deficiency should be based on the absence or near absence of LCAT activity measured with exogenous substrates. A distinction between severe forms (α,β-type LCAT deficiency) and mild forms of the α-type can be made by the measurement of LCAT activity using endogenous substrates. Secondary forms of LCAT deficiency which occur in conjunction with primary HDL deficiency usually show half normal LCAT activity (21, 26, 27). In most cases this condition can be distinguished from heterozygous primary LCAT deficiency by the pattern of inheritance, as most heterozygotes for primary HDL deficiency have near normal LCAT activity.

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