Two Different Allelic Mutations in the Lecithin-Cholesterol Acyltransferase Gene Associated with the Fish Eye Syndrome

Lecithin-Cholesterol Acyltransferase (Thr_{123} → Ile) and Lecithin-Cholesterol Acyltransferase (Thr_{347} → Met)

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Abstract

We have elucidated the genetic defect in a 66-yr-old patient with fish eye syndrome (FES) presenting with severe corneal opacities and hypoalphalipoproteinemia. The patient's plasma concentration of high density lipoprotein (HDL) cholesterol was reduced at 7.7 mg/dl (35.1–65.3 mg/dl in controls) and the HDL cholesteryl ester content was 31% (60–80% in controls); however, total plasma cholesteryl esters were similar to normal (60% of total cholesterol vs. a mean of 66% in controls). The patient's plasma cholesterol esterification rate was slightly reduced at 51 nmol/ml per h (control subjects: 61–106 nmol/ml per h), whereas lecithin-cholesterol acyltransferase (LCAT) activity, assayed using a HDL-like exogenous proteoliposome substrate, was virtually absent (0.9 nmol/ml per h vs. 25.1–27.9 nmol/ml per h in control subjects). DNA sequence analysis of the proband's LCAT gene revealed two separate C to T transitions resulting in the substitution of Thr_{123} with Ile and Thr_{347} with Met. The mutation at codon 347 created a new restriction site for the enzyme Nla III. Analysis of the patient's polymerase chain reaction–amplified DNA containing the region of the Thr_{347} mutation by digestion with Nla III confirmed that the proband is a compound heterozygote for both defects. The patient's daughter, who is asymptomatic despite a 50% reduction of LCAT activity, is heterozygous for the Thr_{123} → Ile mutation. Our data indicate that the regions adjacent to Thr_{123} and Thr_{347} of LCAT may play an important role in HDL cholesterol esterification, suggesting that these regions may contain a portion of the LCAT binding domain(s) for HDL. (*J. Clin. Invest.* 1992. 89:499–506.) Key words: corneal opacities • DNA sequence analysis • hypoalphalipoproteinemia • lecithin-cholesterol acyltransferase activity • restriction fragment length polymorphism

Introduction

Fish eye disease, which was first described in 1979 as a rare genetic disorder of lipid metabolism inherited as a recessive trait, is characterized by severe corneal opacities and hypoalphalipoproteinemia (1). To date the clinical and biochemical features of three Swedish, one Canadian, and two German patients with fish eye disease have been reported in detail (1–13). The initial studies presented strong evidence for a defect in lecithin-cholesterol acyltransferase (LCAT)[1] activity in these subjects. LCAT, an enzyme synthesized by hepatocytes and secreted into the plasma, is responsible for the esterification of virtually all free cholesterol in plasma. Cholesteryl esters formed by LCAT are subsequently incorporated primarily into HDL particles and are transported in plasma associated with HDL or transferred to VLDL or LDL by the cholesteryl ester transfer protein (4).

The most striking biochemical finding in the Swedish patients was a 90% reduction in plasma HDL cholesteryl ester concentration, but normal cholesterol esterification rates as well as normal levels of total plasma cholesteryl esters (1–3). Cross-incubation studies using HDL- or VLDL- and LDL-depleted plasma demonstrated that plasma from these subjects lacked the ability to esterify HDL cholesterol, whereas the esterification of VLDL and LDL cholesterol was similar to normal (7–9). In vitro incubation experiments with plasma from these patients gave no evidence for either the presence of a plasma inhibitor of the enzyme or for an impaired cholesteryl ester transfer activity (6, 10, 12). Therefore, two separate LCAT activities were postulated and designated as α-LCAT and β-LCAT, assuming separate enzyme activities on apo A– and apo B–containing lipoprotein particles, respectively (8–11). The three Swedish subjects were proposed to have a selective deficiency in α-LCAT activity and their disorder was termed fish eye disease. This condition differs from classic LCAT deficiency in which patients have a total loss of LCAT activity and a virtual absence of cholesterol esterification in plasma. Furthermore, the subjects with fish eye disease had no premature atherosclerosis, anemia or renal disease (4), which are the clinical features of classic LCAT deficiency.

DNA sequence analysis and Southern blot hybridization have established the presence of only one gene locus for the LCAT gene on chromosome 16, indicating that α-LCAT and β-LCAT are activities of the same enzyme (14). The LCAT gene, which has been assumed to be genetically defective in fish eye disease, was analyzed in one of the Swedish patients by Southern blot hybridization, but no deletions, insertions or rearrangements were detected (15). Recently, however, in four

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Received for publication 31 May 1991 and in revised form 26 September 1991.

The Journal of Clinical Investigation, Inc.
Volume 89, February 1992, 499–506

1. *Abbreviations used in this paper:* CER, cholesterol esterification rate; LCAT, lecithin-cholesterol acyltransferase; PCR, polymerase chain reaction.

unrelated fish eye disease patients from one German and two Dutch kindreds, DNA sequence analysis identified a single point mutation in the LCAT gene, resulting in the substitution of threonine by isoleucine at position 123 (16, 17). This defect has been proposed to be one of the underlying molecular defects leading to fish eye disease.

In this article we report the elucidation of the molecular defects of a German subject with fish eye disease and his healthy daughter. The proband is a compound heterozygote for the $Thr_{123} \rightarrow$ Ile mutation and a previously undescribed $Thr_{347} \rightarrow$ Met mutation in the LCAT gene.

Methods

Proband. The healthy German patient, born in 1925, was referred for medical evaluation because of increasing corneal opacifications.

Blood samples. Fasting venous blood samples were collected in EDTA, and plasma for lipoprotein analysis was separated by centrifugation (1,500 *g*, 15 min) followed by storage at 4°C. Aliquots for LCAT assays were kept at −70°.

Lipoprotein and apolipoprotein analysis. Lipoproteins were isolated by sequential ultracentrifugation as previously described (18). The following density fractions were obtained: VLDL ($d < 1.006$ g/ml), IDL (1.006–1.019 g/ml), LDL (1.019–1.063 g/ml), HDL_2 (1.063–1.121 g/ml), HDL_3 (1.121–1.210 g/ml), and VHDL (1.210–1.250 g/ml). Plasma triglycerides, total and free cholesterol were quantitated colorimetrically by the glycerolphosphate-oxidase peroxidase and cholesterol-oxidase peroxidase reactions, respectively, using commercial standardized test kits (Boehringer, Mannheim, Federal Republic of Germany). Phospholipids were assayed chromatographically as described (19). Qualitative and quantitative lipoprotein electrophoresis was performed as reported elsewhere (20). Apo A-I isoproteins were analyzed by two-dimensional gel electrophoresis (21). Plasma levels of apo A-I were quantitated by nephelometry, using a standardized test kit (Boehringer). Apo A-II was assayed by a specific ELISA as previously reported (22).

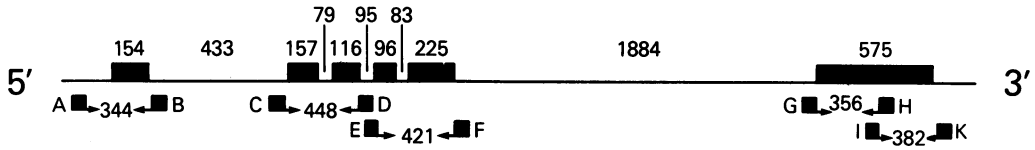
Plasma cholesterol esterification rate (CER) and LCAT activity. CER and LCAT activity were determined as previously reported (23). The CER is defined as the rate of esterification of cholesterol contained within the endogenous lipoproteins of plasma and is comparable to the total plasma LCAT activity. LCAT activity is defined as the ability of plasma to esterify cholesterol in an exogenously presented HDL-like proteoliposome substrate. Plasma samples from the proband, his daughter and from three healthy control subjects were assayed for CER and LCAT activity both in native and in VLDL- and LDL-depleted plasma. LDL and VLDL were precipitated with phosphotungstate-$MgCl_2$. This procedure does not interfere with the enzymic analysis of unesterified HDL cholesterol, the fractional rate of cholesterol esterification, or the gradient gel electrophoretic pattern of HDL subclasses in the supernatant (23).

DNA isolation. High molecular weight chromosomal DNA was isolated from white blood cells using an automated nucleic acid extractor (model 340A, Applied Biosystems, Inc., Foster City, CA).

Oligonucleotides. Based on the published human LCAT sequence (14), oligonucleotide primers were synthesized by the phosphoramidite method on a DNA synthesizer (model 380B, Applied Biosystems, Inc.) and desalted on NAP-5 columns (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). For subcloning procedures, cleavage sites for the restriction enzymes EcoRI and HindIII were incorporated into each oligonucleotide. Primer sequences A–K and their locations with respect to the human LCAT gene are depicted in Fig. 1.

DNA amplification by the polymerase chain reaction (PCR). 1 μg each of genomic DNA from the proband, his 34-yr-old daughter, and a control subject were amplified as previously described (24–26) using an automated DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). Each reaction mixture contained 1 μg each of two oligonucleotide primers in a solution of 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, and 200 μM each dATP, dCTP, dGTP, and dTTP as well as 5 U of Taq DNA polymerase (Bethesda Research Laboratories, Gaithersburg, MD). 30 cycles were performed, each consisting of denaturation at 95°C for 45 s, annealing at 55°C for 1 min, and polymerization at 72°C for 1 min.

DNA sequence analysis. Amplified DNA was digested with EcoRI and HindIII under the conditions recommended by the manufacturer (Bethesda Research Laboratories). Restriction fragments were subjected to 1.5% low melting point agarose (Bethesda Research Laboratories) gel electrophoresis, extracted with 0.5 M NaCl saturated phenol and ligated into M13 vectors mp18 and mp19 (Bethesda Research Laboratories) (27). Single-stranded DNA sequencing was performed by the dideoxynucleotide chain termination method (28) using T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH).



PCR Primers

5′ 3′	5′ 3′
A: GGCTCCCTGAAGCTTTGCCCCTTT	B: TGGCGTGGTGAATTCGGGGCCTGG
C: CACGGGGGGAAGCTTGAGTCCAGA	D: CTTCAACCTGAATTCTAGCCATCA
E: GCATGGCCCAAGCTTCCGGTGCTG	F: TGGACCTAAGAATTCGAGGCCTTC
G: TCCCTGTCCAAGCTTGCTCCATAT	H: CGTGTAGGGGAATTCGTGGTCGTA
I: CTGGTGTGGAAGCTTACTGTCTTT	K: CTTCAACCTGAATTCTAGCCATCA

Figure 1. Location and sequence of the oligonucleotide primers used for the PCR. The genomic structure of the human LCAT gene is illustrated in the upper panel. Six exons, represented by black bars, are interrupted by five introns, designated by lines. The filled squares and arrows indicate the location of the primers and the direction of the PCR, respectively. The numbers indicate the length of the PCR-generated products in base pairs and the lengths of introns and exons of the LCAT gene. The sequences of the primers are shown in the lower panel. Incorporated restriction sites for the restriction enzymes EcoRI and HindIII are underlined.

Table I. Plasma Lipid Composition of Healthy Controls, the Proband, and His Daughter, Compared with the Previously Reported Swedish Patients

	Germany			Sweden		
	Controls (90th perc.)	Proband *1925	Daughter *1957	*1899; †	*1908; †	*1914; †
Cholesterol (*md/dl*)	142–219	215	203	248	227	215
TG (*mg/dl*)	60–165	149	76	510	288	325
HDL chol. (*mg/dl*)	35–65	8	40	7	7	5
HDL chol. ester (%)	60–80	31	71	—	26	20
HDL TG (*mg/dl*)	12–45	4	12	7	4	3
HDL PL (*mg/dl*)	81–152	38	108	—	—	—
LDL chol. (*mg/dl*)	63–148	125	141	175	162	149
LDL chol. ester (%)	60–76	62	70	62	—	—
LDL TG (*mg/dl*)	21–53	70	33	135	127	169
LDL PL (*mg/dl*)	61–103	128	114	—	—	—
VLDL chol. (*mg/dl*)	5–20	13	6	64	39	42
VLDL chol. ester (%)	46–65	60	35	—	—	—
VLDL TG (*mg/dl*)	29–100	45	33	368	153	123
VLDL PL (*mg/dl*)	10–62	24	14	—	—	—
Reference	—	—	—	(3)	(5)	(5)

Symbols: *, born; †, deceased. Abbreviations: perc., percentile; TG, triglycerides; chol., cholesterol; PL, phospholipids. The reference values represent the data of the 90th percentile determined in 120 healthy control subjects.

Restriction fragment length polymorphism analysis. Sequences surrounding the mutation identified in exon 6 of the human LCAT gene were amplified from genomic DNA of the proband, his daughter, and a control subject using the primer pair L (5'-GAAGGCTGG-TACCTGTGGCTGCAG-3') and M (5'-GCTGAAGACCAGGTT-GAGATGCTG-3'). PCR products were gel-purified and digested with 8 U of the polymorphic restriction enzyme Nla III for 4 h at 37°C as outlined by the supplier (New England Biolabs, Beverly, MA). Restriction digests were separated on a 2% agarose (FMC BioProducts, Rockland, ME) minigel supplemented with 1% low melting point agarose at 30 mA for 3.5 h. DNA was stained with ethidium bromide.

Results

Lipoprotein and apolipoprotein analysis. Lipoprotein analyses of the proband and his daughter are summarized in Table I and compared with the data available from the Swedish patients and healthy control subjects (n = 120). The patient's plasma triglyceride and cholesterol levels were in the normal range, while HDL cholesterol was reduced to ~ 14% of the mean control value. Both HDL subfractions, HDL_2 and HDL_3, were reduced in cholesterol to the same extent. 31% of the HDL cholesterol was esterified compared to a mean of 74% in normal subjects in this age group. LDL from the patient showed no abnormalities in content and proportional distribution of free and esterified cholesterol; however, triglycerides were increased by ~ 50%. VLDL particles had normal levels of total cholesterol and triglycerides, but cholesterol esters in this subfraction tended to be slightly higher than in control individuals. Quantitative lipoprotein electrophoresis revealed a virtually absent α-band (5 vs. > 35 mg/dl in controls), an elevated β-band (206 vs. < 170 mg/dl) and a low pre-β-band (4 vs. < 36 mg/dl). Two-dimensional gel electrophoresis demonstrated a normal apo A-I isoprotein pattern. Plasma concentrations of apo A-I (38 vs. 87–155 mg/dl in controls) and apo A-II (26 vs. 30–45 mg/dl in controls) were decreased.

Plasma CER and LCAT activity. Results of the LCAT assays are summarized in Table II. The CER in the proband was only slightly reduced, whereas LCAT activity measured by an HDL-like proteoliposome assay was < 5% of that present in healthy subjects. Analysis of CER in LDL- and VLDL-depleted plasma revealed that 34–41% of the activity was present on HDL particles in control subjects, whereas in the proband's plasma only 12% of CER was associated with HDL particles.

Table II. Plasma CER and LCAT Activity of Healthy Controls, the Proband and His Daughter, in Comparison to the Previously Reported Cases from Sweden

	Canada: controls (n = 3)	Germany		Sweden	
		Proband *1925	Daughter *1957	*1908; †	*1914; †
CER (*nmol/mol per h*)	61–106 [23–43]	51 [6]	114 [31]	72	69
LCAT activity (*nmol/ml per h*)	25.1–27.5 [20.8–23.9]	0.9 [0.9]	13.8 [13.9]	1.4	0.8
Reference	—	—	—	(7)	(7)

Symbols: *, born; †, deceased. The numbers in brackets represent the CER and LCAT activity in VLDL- and LDL-depleted plasma.

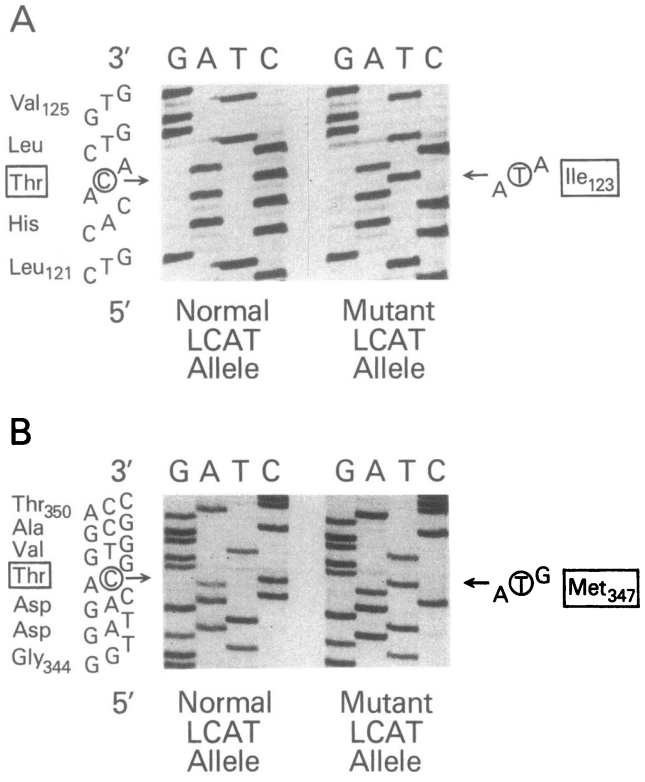


Figure 2. DNA sequence analysis of the two alleles coding for the enzyme LCAT in a patient with fish eye syndrome. The upper panel shows an autoradiogram of a sequencing gel illustrating the mutation in exon 4, the lower panel demonstrates the base substitution in exon 6, as indicated by the arrows.

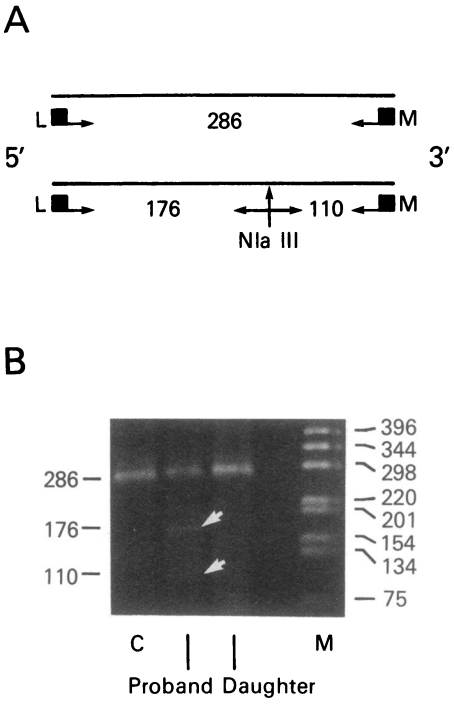


Figure 3. Schematic illustration of the region spanning the mutation in exon 6 of the LCAT gene and agarose gel electrophoresis of PCR-amplified DNA after digestion with the restriction enzyme Nla III. A portion of the sixth exon of the human LCAT gene was amplified using primers M and L. The polymorphic cleavage site for the enzyme Nla III is indicated and the sizes of the resulting restriction fragments are illustrated. The lower panel contains the electrophoretogram of PCR-amplified DNA from a control subject (C), the proband, and his daughter after restriction enzyme digestion with Nla III. A nucleic acid molecular size standard is shown in lane M and the size of the fragments in base pairs is given on the right side. The lengths of the cleaved and uncleaved PCR products are illustrated on the left side.

The daughter had a 50% reduction in LCAT activity, but a normal CER in native as well as in VLDL- and LDL-depleted plasma.

DNA sequence analysis. The protein coding regions of the LCAT gene were sequenced in the patient and in a normal control using PCR-amplified DNA as a template (Fig. 2). Sequence analysis of the LCAT gene from the proband revealed two point mutations: (*a*) a C to T substitution at nucleotide 13 of exon 4, resulting in the conversion of amino acid 123 of the mature protein, threonine (ACA), to isoleucine (ATA), and (*b*) a C to T substitution at nucleotide 364 of exon 6, replacing threonine (ACG) at position 347 with methionine (ATG). Analysis of these regions in the LCAT gene of the daughter demonstrated that she is heterozygous for the base substitution in exon 4, while the DNA sequence of exon 6 was identical to the normal allele.

Polymorphic restriction enzyme sites. Computer analysis of the mutant LCAT sequence (MacVector, International Biotechnologies Inc., New Haven, CT) revealed a new restriction site for the enzyme Nla III (5′-CATG/-3′) due to the C to T mutation in exon 6. The base substitution in exon 4 neither created nor eliminated an enzyme cleavage site. Digestion of PCR products spanning the region of the $Thr_{347} \rightarrow$ Met mutation with Nla III confirmed the results of the DNA sequence analysis. The 286-bp fragment obtained by PCR amplification of the patient's DNA was partially cleaved into an abnormal 176- and a 110-bp fragment, establishing that the proband is heterozygous for this mutation. Digestion of PCR-amplified DNA from the daughter and from a control subject demonstrated the presence of only one uncleaved fragment (Fig. 3), indicating that both had normal DNA sequences at this locus.

Comparison of mammalian LCAT sequences. Analysis of the deduced amino acid sequences of human (14), mouse (29), and rat (30) LCAT revealed ~ 85% identity. Comparison of these sequences demonstrated that the threonine residues at positions 123 and 347 are conserved in evolution in all three species.

Primary and secondary structure prediction. Both LCAT mutations cause the replacement of a relatively hydrophilic threonine by hydrophobic amino acids, resulting in a decreased average hydrophilicity (31) and protein chain flexibility (32), and an increased hydropathic index (33) for the adjacent regions (PC/GENE, IntelliGenetics, Mountain View, CA). Residue 347 is part of the sequence with the highest degree of hydrophilicity and antigenicity in the LCAT gene spanning amino acids 342–347. The methionine substitution decreases the average hydrophilicity of this segment from 1.93 to 1.78, but it remains the region with the highest antigenicity.

The computer-predicted secondary structures of the mutant LCAT enzyme were also affected by these two amino acid

substitutions. Threonine at position 123 is proposed to be part of an extended conformation of residues 119–129 (34) or 123–129 (35) or of a β-sheet conformation of amino acids 117–127 (36) depending on the mathematical model applied for these predictions. Replacement by isoleucine increases the propensity for β-sheet (36) and changes the extended conformation to include amino acids 120–129 (34) or 122–129 (35). The substitution with methionine results in a higher α-helix propensity (36) and in the formation of a new region of helical structure, spanning either amino acids 346–349 (35) or 345–349 (36), or the α-helical structure present in the normal LCAT becomes an extended conformation to include residues 343–353 (34).

Discussion

Several different lipoprotein disorders are associated with HDL deficiency and corneal opacification. These include Tangier disease (37), LCAT deficiency (38), fish eye disease (1), apo A-I, C-III deficiency (39), apo C-III, A-I, A-IV deficiency (40), fish eye-like dyslipoproteinemia (13), and specific apo A-I variants (41, 42). The degree of corneal opacity, however, ranges from mild cloudiness in Tangier disease to severe opacification in fish eye disease and does not correlate with the degree of HDL reduction or the risk of premature coronary artery disease. The pathophysiological mechanism responsible for the clinical manifestation of central corneal lipid deposits is as yet unknown and it has not been definitely established if the different degrees of corneal opacification are features of a continuous process.

Of particular interest are the patients characterized clinically by severe corneal opacifications and very low plasma HDL levels. The prominent corneal opacification led to the designation of fish eye disease in the original three Swedish patients with α-LCAT activity deficiency. The genetic defect in these subjects has not been reported. A young patient (born in 1969) from Canada has been described with a defect in α-LCAT activity similar to the Swedish patients; however, the patient clinically has only arcus. The molecular defect has not been ascertained, and this patient's condition has been designated as resembling fish eye disease (13). Recently, four patients with severe corneal opacifications and absent α-LCAT activity comparable to the Swedish patients have been reported, and the molecular defect in these subjects was found to be a point mutation in the LCAT gene (16, 17). In addition, another subject with severe corneal opacification, reduced α-LCAT activity and HDL deficiency has been identified. In this case the genetic defect was a frame shift mutation in the apo A-I gene (42). Thus, defects in both the LCAT and the apo A-I genes as well as the yet unknown defects in the Swedish and the Canadian patients result in the clinical features of severe corneal opacifications with HDL deficiency. Based on these results we propose to designate the condition of patients with the clinical features of severe corneal opacifications and HDL deficiency as fish eye syndrome to reflect the heterogeneity of the molecular defects now identified in patients previously designated as having fish eye disease.

DNA sequence analysis of the LCAT gene in our patient revealed the presence of a C to T point mutation in one allele, converting Thr_{123} to Ile as previously reported (16, 17). This mutation was also detected in one allele of the proband's daughter. The patient's other allele was found to be affected by a second C to T point mutation, which replaced threonine at position 347 with methionine. DNA sequence analysis and digestion with the polymorphic restriction enzyme Nla III established that the patient is heterozygous for both nucleotide substitutions, whereas the daughter inherited only the $Thr_{123} \rightarrow$ Ile mutation. The proband reported here therefore represents the first compound heterozygote for a defect in the LCAT gene resulting in fish eye syndrome, suggesting that the regions around amino acid 123 and residue 347 are both essential for normal LCAT activity.

The plasma lipid profile in our proband is consistent with those reported for the three Swedish patients and demonstrates the typical laboratory findings of hypoalphalipoproteinemia and decreased HDL cholesteryl ester concentrations. Normal levels of VLDL and LDL cholesteryl esters suggest a normal β-LCAT activity on apo B–containing particles, which results in plasma concentrations of total cholesteryl esters in these patients similar to those of control subjects. A markedly increased triglyceride content was observed in the proband's LDL, but not in VLDL, although the increase in triglyceride content was not as high as that reported for the three Swedish patients. The loss of α-LCAT activity, demonstrated by the virtual absence of esterification of an exogenous HDL-like substrate or in VLDL- and LDL-depleted plasma, and the nearly normal cholesterol esterification rate in plasma are consistent with previous reports from the patients from Sweden. The proband's heterozygous daughter had a 50% reduction in α-LCAT activity, but no abnormalities in lipoprotein profile. This suggests that one normal LCAT allele is sufficient for HDL cholesterol esterification. This may also indicate that under normal conditions LCAT activity is not the rate-limiting step in the net rate of cholesterol esterification. Similarly, the decreased plasma enzyme levels reported in heterozygotes for classic LCAT deficiency were adequate to maintain a normal plasma esterification rate (43, 44).

It may be speculated that both regions of the mature LCAT protein affected by the mutations reported here participate in binding of LCAT to HDL particles and are part of a complex interaction between LCAT, the lipid substrates and activator protein(s) on the surface of the lipoprotein particle. Under normal conditions, LCAT is presumably continuously transferred from mature, spherical HDL to newly secreted nascent HDL particles with only a small portion of the LCAT enzyme associated with apo B containing lipoprotein particles (45). However, the equilibrium state and distribution of the LCAT enzyme in plasma may be shifted towards LDL and VLDL in the Swedish patients and in our proband. Biochemical data strongly suggest that the only enzymically active domain of LCAT consists of a serine residue at position 181 (46–48), whereas the two free cysteines at positions 31 and 184 appear not to be an integral part of the catalytic domain, as recently demonstrated by site-directed mutagenesis of the LCAT gene (49). The normal CER in the Swedish subjects and in our patient indicates that the catalytic site of the enzyme is intact, pointing to a defective interaction of LCAT with HDL particles in these patients.

Several molecular defects in the LCAT gene have now been identified in classic LCAT deficiency (Fig. 4), but molecular defects affecting the putative enzymically active site of LCAT have not yet been reported. A striking observation is that a single amino acid substitution ($Arg_{135} \rightarrow$ Trp), which is located

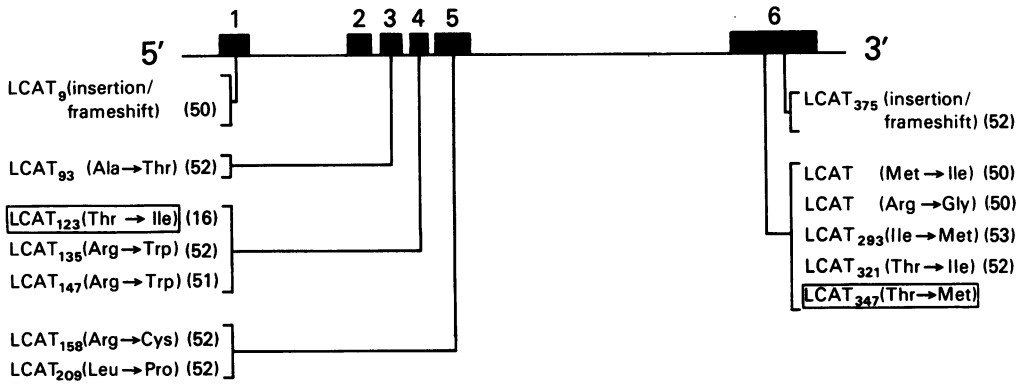


Figure 4. Summary of genetic defects identified in the human LCAT gene to date. The black boxes with the numbers represent the 6 exons of the human LCAT gene. Mutations associated with class II LCAT defects causing fish eye syndrome are boxed, all other genetic defects are class I LCAT defects resulting in classical LCAT deficiency. References are given in parentheses.

only 12 residues carboxyl terminal from the Thr_{123} → Ile mutation results in the severe clinical manifestation of classic LCAT deficiency with glomerulosclerosis, hemolytic anemia, and atherosclerosis. The predicted secondary structure of the LCAT protein spanning exons 1–5 has been reported (46); however, a conclusive interpretation of the mutant gene loci is currently not available, since the sites for lipid binding and cofactor interactions in LCAT are unknown. The conformation of exon 6 encoding nearly half of the mature protein has not been elucidated to date, but would be of great interest, since several molecular defects involving this region have been identified.

Because of the new data available from DNA sequence analyses in fish eye syndrome and LCAT-deficient subjects, we propose a classification of primary LCAT deficiency syndromes, correlating clinical and biochemical features with the underlying defect in LCAT activity and the associated LCAT gene defect (Table III). The class I defect is characterized by absent or extremely low levels of CER and LCAT activity, indicating a total functional loss of LCAT activity. This could be the consequence of molecular defects in the LCAT gene resulting in (*a*) a null allele, (*b*) a synthesis defect, (*c*) an enzyme with impaired binding to both HDL and VLDL-LDL, or (*d*) a functionally inactive enzyme. The presence of two class I alleles results therefore in the clinical features of classic LCAT deficiency. Class II defects are characterized by a partial loss in LCAT function, namely in α-LCAT activity, and a nearly normal CER, but a virtual absence of LCAT activity using the proteoliposome assay. In this case, the catalytic domain is presumably intact as indicated by the capability of the enzyme to generate normal levels of plasma cholesteryl esters, but the interaction with HDL particles is defective. Patients with two class II alleles, who are either homozygous for one defect or compound heterozygous for two class II defects, as described here, develop corneal opacities, but no atherosclerosis or renal disease. LCAT deficient patients who are compound heterozygotes with one class I allele and one class II allele would be anticipated to have intermediate biochemical abnormalities as well as variable clinical features. A potential third variant of LCAT defects, which could exclusively affect the interaction of the LCAT enzyme with apo B–containing lipoprotein particles is conceiv-

Table III. Primary LCAT Deficiency Syndromes

Category	Historical designation	Cholesterol esterification assays	Clinical features	Cholesteryl ester concentrations	Associated LCAT gene defects
Class I LCAT defect (combined α/β-LCAT activity deficiency)	LCAT deficiency	CER low–zero LCAT activity very low–zero	Corneal opacities Hypoalphalipoproteinemia Glomerulosclerosis Normochromic anemia Atherosclerosis	Plasma CE ↓ HDL CE ↓↓ VLDL and LDL CE ↓	$LCAT_9$ (Frameshift) $LCAT_{93}$ (Ala → Thr) $LCAT_{135}$ (Arg → Trp) $LCAT_{147}$ (Arg → Trp) $LCAT_{158}$ (Arg → Cys) $LCAT_{209}$ (Leu → Pro) $LCAT_{293}$ (Ile → Met) $LCAT_{321}$ (Thr → Ile) $LCAT_{375}$ (Frameshift) $LCAT_{exon6}$ (Arg → Gly) $LCAT_{exon6}$ (Met → Ile)
Class II LCAT defect (α-LCAT activity deficiency)	Fish eye disease	CER 50–100% of normal LCAT activity very low—zero	Corneal opacities Hypoalphalipoproteinemia	Plasma CE normal or slightly reduced HDL CE ↓↓ VLDL and LDL CE normal	$LCAT_{123}$ (Thr → Ile) $LCAT_{347}$ (Thr → Met)

Symbols: ↓, decreased; ↓↓, markedly decreased. Abbreviation: CE, cholesteryl ester.

able; however, this disorder may be of minimal clinical importance owing to the high cholesteryl ester transfer rate from HDL to VLDL and LDL.

In conclusion, we have described the first compound heterozygote for a class II LCAT defect resulting in fish eye syndrome. The defect involves two separate point mutations in the LCAT gene resulting in partial LCAT deficiency, presumably due to defective binding of the enzyme to HDL. Additional studies will be required to further elucidate the functions of the different domains of LCAT and to determine the reason why class I and class II molecular defects result in such different clinical sequelae.

Acknowledgment

Dr. Klein is a recipient of a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft.

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