

The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes

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Abstract Lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes represent a group of rare genetic disorders of HDL metabolism that have been the subject of a large number of clinical, biochemical, and genetic studies. Of special interest are patients with LCAT-related disorders with severe HDL deficiency and the apparent absence of premature atherosclerosis. This finding is inconsistent with the general concept that low HDL cholesterol levels are an obligate risk factor for atherosclerosis. In this review, we describe 36 natural mutations in the LCAT gene that result in either familial LCAT deficiency (FLD) or the milder phenotype known as fish-eye disease (FED). We propose a new classification of the natural mutations of the LCAT gene that are described to date. The defects are divided into four classes based on both the clinical and biochemical characterization of the patient and data that were obtained from the functional assessment of the mutant proteins. We define FLD-associated mutations that underlie a complete or nearly complete loss of LCAT activity due to null mutations (Class 1), and missense mutations (Class 2), respectively. In addition, we distinguish two classes of FED-associated mutations (Classes 3,4) that underlie a partial impairment of LCAT activity but differ in their lipoprotein substrate specificity. In addition, we review the evidence of atherosclerosis in subjects with LCAT deficiency syndromes. The observation that 6 (all males) of a total of 19 FED subjects suffered from premature CAD (as defined by <55 years of age and <60 years of age for women and men, respectively) challenges the earlier assumption that the FED phenotype is not associated with increased risk of CAD. However, premature CAD remains an unusual clinical complication in FLD subjects.—**Kuivenhoven, J.A., H. Pritchard, J. Hill, J. Frohlich, G. Assmann, and J. Kastelein.** The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes. *J. Lipid Res.* 1997. **38**: 191–205.

Supplementary key words fish-eye disease • familial LCAT deficiency • mutation • genetics • human • lipoproteins • HDL deficiency

LCAT GENE AND PROTEIN

The human lecithin:cholesterol acyltransferase (LCAT) gene is localized in the q21–22 region of chromosome 16 (1) and is primarily expressed in the liver (2). It consists of 6 exons separated by 5 introns and encompasses a total of 4.2 kilobases (2). The human LCAT mRNA of approximately 1550 bases encodes a protein of 416 amino acids with a hydrophobic leader sequence of 24 residues (2–4). The mature protein has a calculated M_r of 47,090 that contains four N-linked glycosylation sites that have been shown to be functional (5–7). Plasma LCAT has an apparent M_r of 63 kD (2; 5) on SDS gels and therefore the carbohydrate content is estimated to represent approximately 25% of total LCAT mass. The enzyme is secreted into the plasma compartment where it primarily resides on circulating HDL particles (8) which contain its principal activator protein apolipoprotein A-I (apoA-I) (9, 10). The active site of the protein has been identified by comparison with other serine-type esterases (3) and comprises the sequence Gly-X-Ser*-X-Gly (3, 4) where the Ser* represents the putative active site serine. Site-

Abbreviations: apo, apolipoprotein; BHK, baby hamster kidney; CAD, coronary artery disease; CE, cholesteryl ester; CER, cholesterol esterification rate; FC, free cholesterol; FED, fish-eye disease; FLD, familial LCAT deficiency; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LpA-I, HDL particles that contain only apoA-I; LpA-I:A-II, HDL particles that contain both apoA-I and apoA-II; TG, triglyceride; VLDL, very low density lipoprotein; RCT, reverse cholesterol transport; CETP, cholesterol ester transfer protein.

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directed mutagenesis of the serine residue confirmed the functional importance of this site (11). The enzyme has both a phospholipase A₂ activity and an acyltransferase activity. At the surface of lipoproteins, it preferably catalyzes the transacylation of the *sn*-2 fatty acid of lecithin to the free 3 β -OH group of cholesterol whereby lysolecithin and cholesteryl ester are formed (12, 13). The preferred natural substrate for LCAT is plasma high density lipoprotein (HDL) although the majority of the substrate cholesterol originates from other lipoproteins or peripheral tissues (14). Whereas LCAT is primarily associated with HDL in plasma (15), the enzyme is also shown to be active in the low density lipoprotein (LDL) fraction (15–19). Tracing the cholesteryl ester formation in plasma devoid of cholesteryl ester transfer protein (CETP) activity, 73% of the esterified cholesterol was found in the HDL fraction, 25% in LDL, and 1% in very low density lipoprotein (VLDL) (19). Both particle size (20, 21) and its surface charge (22) mediate LCAT activity. Although LCAT is well conserved among mammalian species, the substrate and positional specificity of LCAT proteins from different species varies considerably (23).

GENETIC DISORDERS OF LCAT METABOLISM

Mutations in the human LCAT gene underlie either familial LCAT deficiency (FLD) or fish-eye disease (FED) which are both inherited in an apparently autosomal recessive manner. As these LCAT deficiency syndromes have been extensively reviewed (24–26), we will only briefly illustrate the clinical and biochemical features of these disorders.

Familial LCAT deficiency

FLD was first reported in 1967 by Norum and Gjone (27). The probands of a Norwegian family were characterized by the virtual absence of plasma LCAT activity, severely reduced content of both cholesteryl esters (CE) and plasma LCAT concentration (28). Furthermore, this disorder was characterized by HDL deficiency and lipid changes in both VLDL and LDL fractions. The major clinical findings included corneal opacification, anemia, proteinuria, and renal disease (25).

In 1992, Skretting et al. (29) reported that the probands of this FLD family were homozygous for a single nucleotide substitution in exon 6 of the LCAT gene, which resulted in the exchange of a methionine for a lysine residue at position 252 of the mature protein. In vitro expression of this missense mutation was recently shown to result in the production of a fully inactive LCAT enzyme (30).

Fish-eye disease

A second genetic disorder of LCAT metabolism, known as FED or partial LCAT deficiency, was extensively studied by Carlsson and Philipson (31–33) in two families of Swedish origin. In contrast to FLD, the FED patients presented with no clinical signs except for the characteristic dense age-dependent corneal opacification. The probands were characterized by HDL deficiency, elevated triglyceride (TG) levels (31, 32), and demonstrated several additional lipoprotein abnormalities (33–35). An apparently normal ability of the LCAT enzyme to esterify cholesterol in plasma, a partial reduction in LCAT concentration, and a normal of percentage plasma CE clearly distinguished this disorder from FLD (31, 33). Further biochemical analysis of the LCAT protein revealed paradoxical results, in so far that plasma LCAT activity was severely reduced despite near normal cholesterol esterification rates (CER) (36). In addition, it was shown that the CE content of HDL was extremely low (37) whereas the relative CE content of VLDL and LDL was normal (33). It was suggested that LCAT exhibits two activities in normal plasma: alpha-LCAT activity; specific for lipoproteins that migrate with alpha-mobility upon gel electrophoresis (i.e., HDL), and beta-LCAT activity that is specific for pre-beta, and beta-migrating lipoproteins (i.e., VLDL and LDL, respectively) (36, 38, 39). Thus, FED was classified as alpha-LCAT deficiency whereas FLD was proposed to result from a lack of both alpha- and beta-LCAT activities. However, it was clear, after cloning of the LCAT gene, that alpha- and beta-LCAT activity represented two functional aspects of the same protein, as only one LCAT gene is present in humans (2).

In 1991, Funke et al. (40) described the first LCAT gene defect that was associated with FED in a kindred of German origin and in an unrelated Dutch family (41). Homozygosity for a missense mutation in exon 4 resulting in T123I was shown to be associated with the FED phenotype. In support of the proposed alpha- and beta-LCAT activity (38), O et al. (42) showed that recombinant mutant protein had lost the ability to esterify cholesterol in the HDL pool while it retained the capability to esterify cholesterol on other lipoproteins. The latter LCAT activity in FED appears to prevent the development of severe clinical symptoms as described for subjects with FLD. This illustrates the physiological importance of the LCAT reaction on apoB-containing lipoproteins.

Mutations of the LCAT gene

The above description of FLD and FED clearly indicates that LCAT gene defects can result in different biochemical and clinical phenotypes. With the advent of

TABLE 1. Mutations that underlie LCAT deficiency syndromes

Defect	Exon	Clinical Phenotype		References ^a
		FLD	FED	
Homozygous mutations				
1. C-insertion (codons 9, 10) ^b	1	X		(62, 63, 88, 89)
2. P10L	1		X	(31–33, 67)
3. G30S	2	X		(47, 90)
4. Y83-stop	3	X		(61)
5. A93T ^c	3	X		(24, 46, 61)
6. T123I	4		X	(40–42)
7. N131D	4		X	(48)
8. R140H	4	X		(55)
9. G141-insertion	4	X		(64)
10. L209P	5	X		(24, 61)
11. N228K	6	X		(64)
12. R244G	6	X		(63, 91)
13. M252K	6	X		(27, 29, 92, 93)
14. M293I	6	X		(63, 64, 69, 70, 94, 95)
15. L300-deletion	6	X		(60, 96)
16. T321M	6	X		(61, 97, 98)
17. G344S	6	X		(56)
18. G-deletion (codon 264) ^b	6	X		(56)
Heterozygous mutations				
1. P10Q R135Q	1, 4		X	(49)
2. L32P T321M	2, 6	X		(63)
3. G33R 30 bp Insertion (codon 4)	1, 2	X		(65)
4. Y83-stop Y156N	3, 5	X		(53)
5. T123I Y144C	4, 4		X	(68)
6. T123I Intron 4 defect (IVS4:T-22C)	4, intron 4		X	(50)
7. T123I T347M	4, 6		X	(58)
8. T123I Unknown	4, ?		X	(82)
9. R135W A-insertion (codon 376) ^b	4, 6	X		(24, 61, 99, 100)
10. R147W Unknown	4, ?	X		(24, 81, 101)
11. G183S A-T substitution/C-deletion (codon 120) ^b	4, 5	X		(63, 102)
12. M252K N391S	6, 6		X	(30, 59, 66)
13. T321M C-deletion (codon 168) ^b	5, 6	X		(57)
14. R399C C-insertion (codons 9, 10) ^b	6, 1	X		(54)

^aReferences include clinical, biochemical, and genetic reports on the LCAT gene defects presented.

^bPremature truncation as the result of a frameshift.

^cOccurring in a patient who also carries an R158C mutation (double compound heterozygote).

PCR-based strategies for genomic DNA sequencing, a worldwide search for gene mutations causing these disorders ensued. To date, 36 mutations of the LCAT gene have been described for which there is strong evidence that they are causative for the clinical phenotype of either FLD or FED (Table 1). Of these mutations, 18 occur in the homozygous form whereas the remaining mutations occur in compound heterozygous subjects. In the latter case, the association of any specific mutation with a distinct clinical phenotype must not be over-interpreted as any one mutation might have a biochemical phenotype of either FLD or FED. This issue will be further discussed in the next section.

From the localization of all mutations that have now been reported, it is evident that they are highly dispersed over the entire LCAT gene and that the exact

site of the mutation does not allow us to predict the associated biochemical or clinical phenotype (see Table 1). In addition, it is noteworthy that the LCAT gene is highly conserved among mammalian species (43). In fact, no genetic polymorphisms have been described for the human LCAT gene although there exists some confusion over the functionality of a non-conserved arginine residue at codon 158 (44–46).

To date, all natural mutations of LCAT appear to directly affect LCAT function but this might be the result of a strong selection bias as only DNA in patients with LCAT deficiency syndromes has been sequenced. However, even conservative amino acid substitutions like glycine to serine at position 30 were shown to completely abolish normal LCAT function (47). In line with this concept, only a small number of mutations

are associated with a partial impairment of LCAT function, i.e., FED. Taken together, this indicates that the overall LCAT protein structure is vulnerable to small changes.

PITFALLS IN THE BIOCHEMICAL ANALYSIS OF LCAT GENE DEFECTS

Multiple expression systems

In order to further investigate the molecular basis of LCAT deficiency syndromes, a number of investigators have recreated and expressed natural mutants of LCAT in mammalian cell culture (30, 42, 46, 48–57). These studies have helped to explain the various clinical and biochemical phenotypes of patients who carry LCAT gene mutations.

In this review, we have categorized the results of these studies and we propose a classification system of the different biochemical phenotypes observed. Whilst the functional assessment of mutations has generally provided similar results, there are occasional discrepancies. This might be due to differences in the expression systems used, i.e., baby hamster kidney cells (BHK) (30, 48–50), COS-1 (42, 46, 56, 57), COS-6 (51), COS-7 (54, 55), and human embryonic kidney-293 cells (52, 53). Furthermore, differences in the intracellular processing of recombinant proteins and the various rates of expression might be the cause of discrepancies in phenotype assignment. Also, different laboratories have used variable substrates in the evaluation of the enzyme activity of the recombinant proteins and different antibodies for the assessment of LCAT concentrations (30, 46, 51, 52). These methodological dissimilarities may have resulted in differences in apparent specific enzyme activity and lipoprotein substrate selectivity.

Compound heterozygosity

Defects of LCAT have been frequently classified according to their associated clinical phenotype in the patient in which they were identified (51, 52, 58). For defects that occurred in the homozygous form, this posed no problem as the phenotype of the patient is determined by only one species of mutant LCAT. However, this type of classification has caused confusion in the interpretation of the effects of allelic variants that have been identified in compound heterozygous subjects. This problem can be illustrated by our study of a FED patient who was a compound heterozygote for P10Q and R135Q (49). Functional assessment of these defects revealed that P10Q mutation resulted in an enzyme with partial activity while the R135Q allele encoded for

a catalytically inactive protein. It was proposed that the P10Q defect determined the biochemical and clinical phenotype of the patient in the presence of non-functional LCAT_{R135Q}. Using the classification method described above, the R135Q defect would be misclassified as an FED mutation but it is clear that homozygosity for this defect would in reality result in the more severe FLD phenotype.

Intermediate biochemical phenotype

An additional issue that should be further highlighted is the description of LCAT gene mutations that are associated with the FED phenotype, yet have an intermediate biochemical phenotype when analyzed in vitro. These are mutations that cause a biochemical phenotype that is between those causing a total loss of catalytic activity (FLD) and those that result in a partial loss of activity against HDL only (classical FED) (30, 48, 59, 60). The studies of these mutants clearly show that LCAT gene defects result in a range of biochemical phenotypes with both mild and severe disturbances in the ability of the mutant LCAT to esterify cholesterol in HDL and/or LDL.

CLASSIFICATION OF LCAT GENE MUTATIONS

In an attempt to resolve some of the above issues, we have reviewed both the clinical and biochemical characterization of the patients, and the data that were obtained from the functional assessment of the mutant proteins (where available). In doing this, we focused on the following in vivo criteria: 1) LCAT activity, i.e., the activity of plasma LCAT towards exogenous apoA-I-containing proteoliposomes (HDL analogues); 2) the activity of plasma LCAT towards endogenous lipoproteins which is generally referred to as cholesterol esterification rate; 3) the ratio of plasma FC to EC; 4) plasma LCAT concentration; and 5) the clinical manifestations of the disease.

While interpreting the in vitro data of the mutant proteins, we addressed (when possible) 1) the specific activity of the mutant protein while using HDL-analogues and native LDL as substrates and, 2) the activity of the mutant towards heat-inactivated plasma. This information was used in conjunction with the zygosity of the defect(s) in the patients studied. As the secretion of recombinant proteins appeared to vary with the expression system as discussed above, we also used data on the patient's plasma LCAT concentration (where available). Using the above criteria, we have categorized

TABLE 2. Classes of LCAT gene defects

Class	Biochemical Phenotype	Defect	References ^a
1. Null mutations causing FLD	Total loss of catalytic activity. LCAT mass: (virtually) absent.		
1.		30 bp Insertion (codon 4)	(65)
2.		C-insertion (codons 9, 10)	(54, 62, 63)
3.		Y83-stop	(53, 61)
4.		G141-insertion	(64)
5.		Intron 4 defect (IVS4:T-22C)	(50)
6.		A-T substitution/C-deletion (codon 120)	(63)
7.		C-deletion (codon 168)	(57)
8.		G-deletion (codon 264)	(56)
9.		A-insertion (codon 376)	(24, 61)
2. Missense mutations causing FLD	Total loss of catalytic activity. LCAT mass: normal/reduced/ absent.		
1.		G30S	(47)
2.		L32P	(51, 63)
3.		G33R	(65)
4.		A93T	(24, 46, 51, 61)
5.		R135W	(24, 30, 51, 61)
6.		R135Q	(49)
7.		R140H	(55)
8.		R147W	(24, 51, 52, 101)
9.		Y156N	(52, 53)
10.		G183S	(63)
11.		L209P	(24, 30, 51, 61)
12.		N228K	(52, 64)
13.		R244G	(63)
14.		M252K	(29, 30, 59)
15.		T321M	(51, 57, 61, 63)
16.		G344S	(56)
17.		T347M	(30, 51, 52, 58)
18.		R399C	(54)
3. Missense mutations and minor deletions causing FED	Partial loss of activity against a) LDL, or b) both HDL and LDL. LCAT mass: reduced.		
1.		N131D	(48)
2.		L300 deletion	(30, 52, 60)
3.		N391S	(30, 59)
4. Missense mutations causing FED	Partial loss of activity against HDL only. LCAT mass: reduced.		
1.		P10L	(30, 51, 52, 67)
2.		P10Q	(49)
3.		T123I	(30, 40, 42, 50–52, 58, 68)
5. Unclassified mutations			
1.		Y144C	(68)
2.		M293I	(52, 64, 69, 70)
3.		R158C	(24, 44–46, 51, 52)

In the absence of data on the functional assessment of LCAT mutations, we have categorized both homozygous (e.g., ref. 47) and heterozygous mutations (e.g., ref. 65) that were identified in subjects with the FLD phenotype as either class 1, 2 defects because only the (virtual) absence of LCAT activity appears to result in the clinical phenotype of FLD.

^aThe references quoted are reports that describe the identification of the respective LCAT gene defects and (when available) literature on the functional assessment of these mutations.

all reported mutations of the LCAT gene into four distinct classes which are shown in **Table 2**.

Class 1

This class contains null mutations of the LCAT gene that are, as a consequence, associated with a total loss of catalytic activity and the clinical phenotype of FLD in homozygous subjects. These defects can directly in-

roduce stop codons, (53, 61) cause defective splicing of LCAT mRNA (50), or underlie frameshifts that eventually result in the synthesis of truncated/non-sense LCAT (24, 54, 56, 57, 61–63). In addition, two reports describe insertions of more than one nucleotide (64, 65). A specific 3 bp deletion at codon 300 (60) has been difficult to classify and is discussed in the next section on Analysis of confounding mutations. In order to illus-

trate the characteristic features of this class of mutations, we recently described a single nucleotide substitution in intron 4 of the *LCAT* gene (50). This defect was shown to cause lack of normal expression of the affected allele and was shown to be associated with half normal *LCAT* concentrations, *LCAT* activity, and HDL cholesterol levels in heterozygous subjects.

Class 2

This class contains missense mutations that cause a complete or nearly complete loss of catalytic activity of *LCAT*. As a consequence, cholesterol esterification as measured against heat-inactivated or artificial substrates is virtually absent. Homozygous subjects present with normal (47) or reduced plasma *LCAT* concentrations (most cases) in the virtual absence of *LCAT* activity which in turn causes the clinical and biochemical characteristics of FLD. To date, numerous mutations have been shown to be associated with this phenotype (Table 2). The previously described M252K defect is a good representative for this class of defects (29). This single amino acid substitution was shown to result in a complete loss of *LCAT* activity and to underlie FLD in homozygous subjects (30, 59).

Class 3

A third class of mutations encode *LCAT* proteins that cause an intermediate phenotype in between those described for classes 1,2 and class 4, respectively. These are either missense mutations (30, 48) or minor deletions (60). Specifically, these mutant proteins are characterized by either a selective partial loss of activity using LDL as substrate (60), or a combined partial loss of activity against both HDL and LDL (30, 48). In vivo, homozygous subjects have half-normal *LCAT* concentration and moderately reduced CER and present with the clinical phenotype of FED. To date, only three mutations with this phenotype have been reported. First, functional assessment of the N131D mutation revealed that this mutant exhibited a near complete loss of activity against HDL (5% of wild type recombinant *LCAT*) in conjunction with a 75% reduction in activity towards LDL as substrate (as compared to wild type) (48). Second, the L300Del, which also caused the clinical phenotype of FED in homozygotes, was reported to have abnormal function towards HDL and LDL (60). This specific defect will be further discussed in the section on Analysis of confounding *LCAT* mutations. The third mutation that meets the criteria of this class of mutations is the N391S defect which was identified in a compound heterozygous patient who suffered from FED (30, 66). In vitro characterization showed that the *LCAT*_{N391S} exhibited had reduced activity against both HDL (18% of wild type) and LDL (53%

of wild type) and therefore was similar to the N131D defect.

Class 4

In line with the original hypothesis on FED by Carlson and Holmquist (38), we defined a fourth class of defects that cause the typical FED phenotype: i.e., missense mutations that result in the specific loss of activity against HDL analogues while the activity against apoB-containing lipoproteins is largely unaffected. The latter activity results in near normal esterification of cholesterol in heat-inactivated plasma. In vivo, homozygous subjects present with a specific loss of *LCAT* activity towards HDL analogues whereas CER will be either normal or moderately reduced. This is classic FED. Only three defects identified so far clearly meet the criteria of this class. First, the P10L defect, a mutation that was identified in the original FED patients (33, 67) from Sweden, was shown to exhibit this phenotype (30). Second, a nucleotide exchange in the same codon that resulted in the exchange of proline for a glutamine (P10Q) at this position was shown to exhibit similar properties (49). Finally, the most frequent FED mutation, i.e., T123I (40, 41, 58), has also been shown to have specifically lost its activity towards HDL (30, 42). Furthermore, these FED r*LCAT* proteins were shown to be secreted at near normal levels which is in agreement with the near normal levels of *LCAT* in plasma of these patients. A novel case of FED that results from compound heterozygosity for T123I and Y144C will be discussed below (68).

Class 5

We have not been able to classify three defects, and therefore introduced the category of unclassified mutations. *a) Y144C*: to date, all mutations in both homozygous and compound heterozygous FED subjects have been characterized in vitro except for the Y144C defect. This mutation was recently identified in a subject who also carried the FED-associated T123I mutation (68). In this case, we cannot deduce the effect of the Y144C mutation on *LCAT* function as we cannot distinguish between carriers of two FED alleles and carriers of one FLD and one FED allele. In the absence of in vitro expression data, we cannot classify this mutant and therefore included this defect in the category of unclassified mutations (Table 2). The difficulties that we encountered in classifying *b) M293I*, *c) R158C*, are described in the section on Analysis of confounding *LCAT* mutations.

Interesting aspects

From Table 2, it appears that the region specified by amino acids 123–156 is of interest for further study of

LCAT. First, arginine residues in this region are essential for LCAT activity as the loss of this positively charged residue at positions 135, 140, and 147 all underlie FLD (24, 49, 55). Second, mutations in this small region are not only associated with the severe FLD phenotype (class 2) but also with the milder FED phenotype (class 3,4: T123I, N131D). These data might indicate that this region of the protein is of importance to its interaction with lipoproteins, and therefore regulates lipoprotein substrate specificity. Indirect evidence for the hypothesis that differences between the biochemical characteristics of LCAT mutants originate from variations in binding properties was provided by Klein et al. (52). These investigators have shown that a large number of LCAT defects (including R147W) do not seem to affect the catalytic activity of the enzyme.

Another aspect which is of interest comes from our studies of recombinant LCATs that were produced by BHK cells (30, 48, 49). It appears that the activity of the LCAT enzyme towards HDL analogues is more easily disrupted than its activity towards native LDL. While this is, by definition, true for class 4 defects, it also holds true for class 2 (R135W; R135Q; M252K; T347M) and class 3 defects (N131D; N391S; L300Del), although to a less pronounced degree. To illustrate this observation, we have presented the catalytic activities of four representative LCAT mutants of class 1, 2, 3, and 4 defects against both HDL analogues (proteoliposomes) and native LDL in Fig. 1.

Summary

Both class 1 and class 2 gene defects underlie the clinical phenotype of FLD in homozygous subjects. The mutant proteins share the biochemical feature of a complete or nearly complete loss of LCAT activity; however, the molecular background that underlies this phenotype differs: i.e., the mutant proteins (if present) are the result of non-sense mutations and missense mutations in classes 1 and 2, respectively. Furthermore, classes 3 and 4 share the feature of causing the clinical phenotype of FED. However, these classes differ in their biochemical features, in that class 3 mutations are associated with varying substrate specificities towards different lipoprotein classes, whereas class 4 defects are characterized by only a specific loss of activity against HDL (classical FED). In order to provide a better insight in the background of the above classification, we have attempted to show the biochemical features of all four classes in Fig. 2. It is important, however, to note that one should not overestimate the arbitrary borders of the proposed categories as the effects of mutations on LCAT function appear to range from a complete loss of catalytic activity to a specific partial loss of activity towards different lipoprotein substrates.

ANALYSIS OF CONFOUNDING LCAT MUTATIONS

In attempting to classify all LCAT gene mutations in Table 2, we had to address several confusing issues related to the diagnosis of FLD and FED and to discordant phenotype assignment. These problems have arisen for both homozygous and compound heterozygous gene defects as is described below.

Homozygous mutations

R158C. There exists considerable confusion over the effect of the R158C mutation on LCAT function. This mutation was originally described by Assmann, von Eckardstein, and Funke (24) in a proband with FLD who was double homozygous for both R158C and A93T. In addition, Luc et al. (44) and Duverger et al. (45) reported the marked decrease of LCAT activity in the HDL fraction in a proband with clinical FED which appears to result from this defect. However, the in vitro analysis from our laboratory (46) revealed that the double mutant (rLCAT_{R158C/A93C}) had lost catalytic activity while rLCAT_{R158C} protein exhibited near normal activity against both HDL and heat-inactivated plasma. Thus, we proposed that the A93T defect was responsible for the biochemical defect in the FLD patient whereas the R158C mutation was merely a co-inherited natural polymorphism of LCAT. This assumption was strengthened by the fact that the residue at position 158 differed in four mammalian LCAT proteins; a finding which was true for only three other residues in the primary structure of the LCAT protein. By contrast, the in vitro analysis by Klein et al. (52) differed considerably: their LCAT_{R158C} was shown to exhibit markedly reduced activity against both HDL and heat-inactivated plasma. By contrast, Qu et al. (51) provided data that supported the hypothesis that the A93T mutation determined the biochemical phenotype in the patient.

The differences in the effects of this mutation on LCAT function cannot, in our opinion, be explained by the use of different expression systems. Whether this defect causes FED or not could be clarified by a more elaborate report on the biochemical phenotype and genetic analysis of the patient who was reported to be homozygous for this mutation and of the family members (44). This case clearly shows the need of both in vivo and in vitro data in order to be able to classify defects of the LCAT gene. Until this problem is resolved, we included the R158C defect in the category Unclassified in Table 2.

M293I. Sakuma et al. (69) originally reported markedly reduced LCAT activity (15% of normal), and a marked reduction in CER in the plasma of two Japanese probands. These subjects presented with corneal opacities in absence of proteinuria or kidney dysfunction. However, the authors (69) did not refer to the charac-

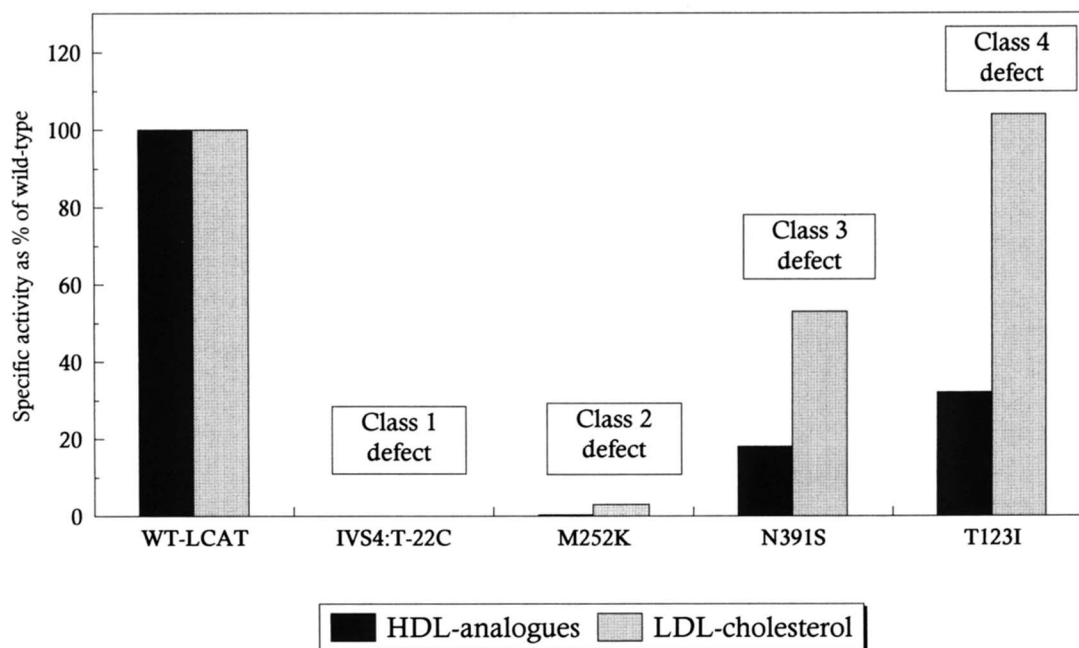


Fig. 1. This histogram gives the catalytic activity of four representative LCAT mutants of class 1, 2, 3, and 4 defects against both HDL analogues (proteoliposomes) and native LDL. Functional assessment of these mutations of the LCAT gene was realized by stable transfection of BHK cells with pNUT constructs that contained mutagenized LCAT cDNA (30,49,50).

teristics of FED but only of FLD. In a further description of the genetic background of this phenotype, the underlying M293I defect was also reported as a FLD defect (64, 70). By contrast, Klein and co-workers (52) classified M293I as a FED-associated mutation. However, their *in vitro* analysis revealed that the mutant protein exhibited a near total loss of catalytic activity as measured against HDL-analogues in conjunction with the absence of the ability to esterify cholesterol in heat-inactivated plasma (52). Taken together, the data on the patients suggest FED while the *in vitro* data indicate that this mutant exhibits the characteristics of FLD. This discrepancy indicates the need for additional data on the lipoprotein specificity of LCAT_{M293I}. The presence of target cells (a clear hallmark for FLD) in both probands further complicates the classification of this defect. Therefore, we have decided to include this mutation as Unclassified in Table 2.

L300DEL. Homozygous probands for this defect were originally reported to suffer from FED. They presented with reduced activity towards HDL-analogues, severely reduced LCAT concentrations, and markedly reduced CER (60). However, *in vitro* analysis of this defect by two independent laboratories has provided controversial results. Klein et al. (60) showed that the rLCAT_{L300DEL} was poorly secreted by embryonic kidney cells, and that it exhibited normal or even elevated activ-

ity towards HDL analogues (52, 60). By contrast, our laboratory provided data that showed that rLCAT_{L300DEL} was catalytically inactive while it was secreted at near normal levels when using BHK cells to express this mutant LCAT (30). Although these data indicate the need for additional analysis, they clearly show that this mutation differs from the other mutations in class 4 that cause FED.

Compound heterozygous mutations

Two defects may previously have been misclassified as a result of an overinterpretation of mutations that occur as compound heterozygotes.

L32P. This defect was originally identified in a compound heterozygous FLD patient who carried the T321M mutation on the second allele (63). However, Qu et al. (51) reported that this mutation was associated with FED. As these investigators showed that the LCAT_{L32P} protein was catalytically inactive (51), we classified this mutation as a class 2 defect.

T347M. This defect was originally identified in a compound heterozygous FED subject who also carried the T123I defect (58). According to the associated clinical phenotype, this mutation was reported to be associated with FED. Subsequent functional assessment, however, showed that the T347M defect exhibited a total loss of activity against HDL analogues (30, 51, 52) while its ac-

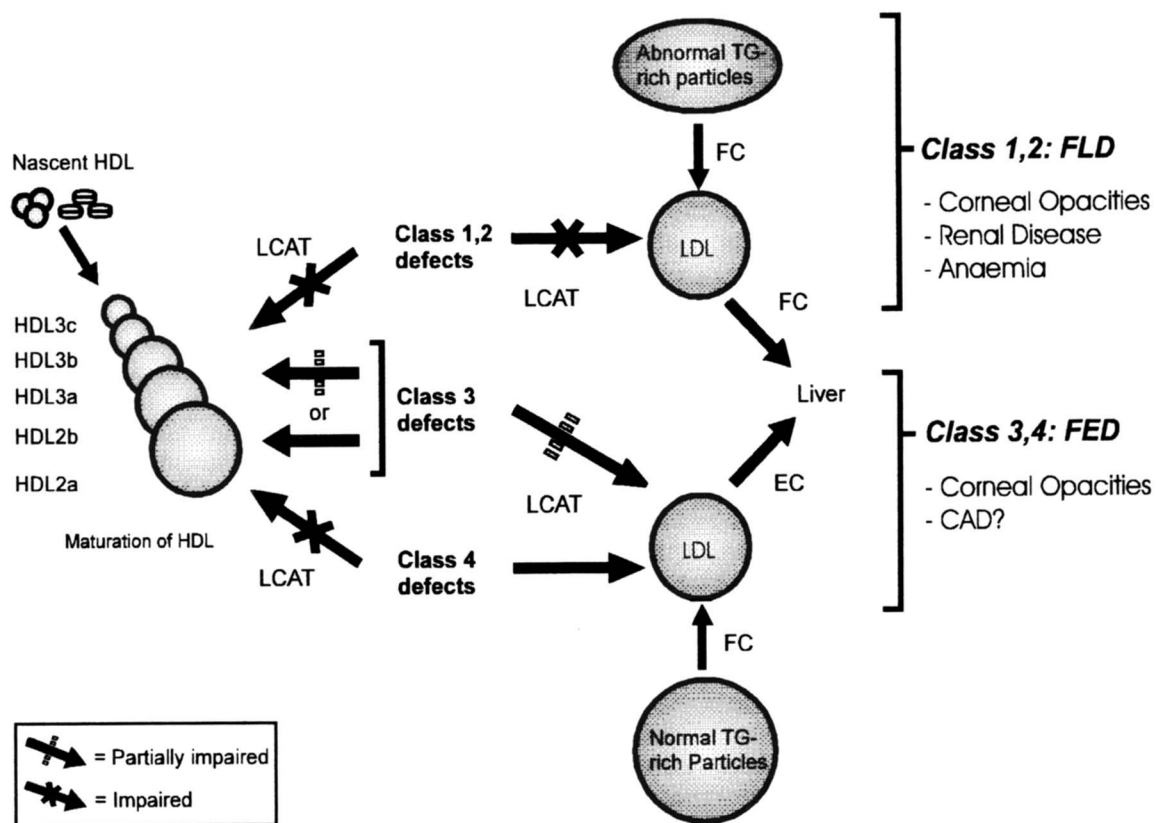


Fig. 2. This figure illustrates the biochemical characteristics of the four different classes of LCAT mutations. Class 1,2: null mutations and missense mutations that result in a loss of activity against both HDL and LDL as substrates, and underlie the clinical phenotype of FLD. Class 3: missense mutations or minor deletions that either cause a selective partial loss of activity using LDL as substrate (60) or a combined partial loss of activity against both HDL and LDL (30, 48). These defects underlie the clinical phenotype of FED. Class 4: missense mutations that result in the specific loss of activity against HDL and underlie the FED phenotype (30, 48, 60).

tivity towards LDL was severely reduced. In addition, it as shown that this mutant was unable to esterify cholesterol in heat-inactivated plasma (52). Taken together, LCAT_{1347M} represents a catalytically inactive enzyme and was therefore defined as a class 2 defect.

ATHEROSCLEROSIS IN LCAT DEFICIENCY SYNDROMES

A central but somewhat controversial issue in LCAT research is the role of this enzyme in the protection against atherosclerotic vascular disease. LCAT clearly plays a central role in reverse cholesterol transport (RCT), and it has generally been accepted that high esterification rates promote the efflux of cholesterol from peripheral tissues. In doing so, LCAT appears to be responsible for the maturation of the HDL pool. Evidence for this comes from studies of LCAT deficiency syndromes in which only nascent type HDL accumu-

lated in plasma (25). However, despite the virtual absence of mature HDL in subjects who are homozygous for LCAT gene mutations, these patients have generally been reported not to manifest premature atherosclerosis (24, 25, 61). This interesting finding has even led to the frequent description of FED as an example of a hereditary HDL deficiency that is not associated with premature CAD. It has been proposed that the absence of CAD might be related to the fact that the RCT is only partially impaired in FED patients as cholesterol esterification in apoB-containing lipoproteins is not impaired. However, this hypothesis cannot account for the absence of CAD in FLD patients. In addressing this issue, Glomset, Norum, and King (71) originally postulated that the residual HDL in FLD would be a good acceptor for cellular cholesterol. However, recent evidence by Ohta et al. (72) suggested that these lipoproteins are not as good acceptors of cholesterol as originally proposed.

To gain further insight into the metabolic basis of the absence of CAD in LCAT deficiency syndromes, Rader

et al. (73) investigated the *in vivo* kinetics of HDL subfractions in both FLD and FED. This analysis provided evidence of accelerated clearance of HDL particles that contained both apoA-I and apoA-II (LpA-I:A-II) as compared to the catabolism of the HDL that contained only apoA-I (LpA-I). It was concluded that low plasma HDL in either disorder resulted from a preferential hypercatabolism of apoA-II and LpA-I:A-II. As LpA-I may represent the antiatherogenic potential of HDL (74–76), these investigators proposed that the LpA-I fraction in LCAT-deficient patients would still be able to maintain an effective RCT. The presence of intact LpA-I metabolism and an increased LpA-I/LpA-I:A-II ratio was proposed to underlie the apparent absence of a high risk of premature atherosclerosis in both disorders. However, these conclusions were in disagreement with the results of subsequent investigations that showed that both LpA-I and LpA-I:A-II of FLD patients were poor acceptors of intracellular cholesterol from macrophage foam cells (72) and it appeared that the removal of cellular cholesterol from foam cells by LpA-I required LCAT activity (72, 77). Therefore, it was concluded that factors other than the apoA-I-mediated RCT had to be considered in explaining why FLD patients are apparently not at high risk for premature atherosclerosis. In addition, they postulated that a reduced generation of atherogenic CE-rich LDL could possibly compensate for the loss of apoA-I-mediated RCT. In this respect, the authors referred to the studies of Fielding et al. (78) who showed that apoA-I-free, apoE-rich HDL was a potent mediator of cholesterol efflux from cholesterol-loaded fibroblasts. More recently, von Eckardstein and co-workers (79) have shown that several quantitatively minor HDL subfractions as well as LDL might contribute to the RCT. These particles include not only apoE-containing HDLs (79) but also HDL that contain only apoA-IV (80), which might compensate for the loss of apoA-I-containing particles in the RCT in hereditary HDL deficiencies such as FLD, FED, Tangier disease, and apoA-I deficiency.

All of the aforementioned studies make the assumption that the incidence of premature CAD in patients with FLD and FED is not increased. However, we have already reported a case of premature CAD in a 38-year-old male proband with FED in the absence of additional risk factors (49). In an earlier report on FED (40) two homozygous probands were also reported to suffer from CAD at ages 50 and 60; however, at that time, no connection with impaired LCAT function was suggested. The recent characterization of two additional FED patients from an unrelated family who also suffer from premature atherosclerosis (48) indicates that we must not overinterpret the apparent absence of risk of disease in this disorder. In addition, we have suggested

that heterozygous carriers of the N131D mutation might be at increased risk for atherosclerosis as a result of atherogenic changes in lipoproteins, e.g., the presence of small dense LDL, low HDL, and high fractional esterification rates in HDL (48). In this respect, we note that the high apoB levels in our Dutch patients might be related to an enhanced risk for atherosclerosis (41, 48, 49). Despite the general absence of detailed data on coronary atherosclerosis in subjects with LCAT deficiency syndromes, we tried to obtain some insight in the number of CAD cases described to date. In FLD, CAD is generally absent although there is evidence of early coronary atherosclerosis in a few cases (65) and in first degree family members of FLD patients (57, 81). By contrast, of all 19 patients with FED known to date, 6 subjects from 5 families, all males, have now been reported to suffer from premature CAD (40, 48, 49, 82) (as defined by the presence of myocardial events before the age of 55 for men and 60 for women). We have summarized the data on CAD in FED in **Table 3**. The apparent absence of CAD in FLD when compared with FED is a subject that needs further clarification. This observation could be related to the presence of LDL-associated LCAT activity in FLD as shown in Fig. 2. Overall, we are of the opinion that the small number of patients described and the potential referral bias prevent meaningful conclusion. However, we wish to emphasize that despite the general absence of premature atherosclerosis in LCAT-related genetic disorders, there is accumulating evidence for the occurrence of CAD in several cases, especially in male subjects with class 3 and 4 mutations, i.e., FED.

CONCLUSIONS AND FUTURE RESEARCH

The elucidation and *in vitro* characterization of natural mutations of LCAT have provided better insight into the biochemical characteristics of this enzyme. However, the molecular mechanisms that underlie the biochemical phenotype of either FLD or FED remain unclear. To further elucidate the molecular basis of the heterogenic expression of LCAT mutations, studies on the catalytic activities and binding properties of the recombinant mutant proteins towards different lipoprotein substrates should be completed. Although we are convinced that additional natural mutations of the LCAT gene will provide a better understanding of LCAT function, we estimate that the impact of the natural mutation on the structure and function of LCAT can be fully interpreted only when a 3-dimensional model of this protein is available.

We already indicated that the relatively small number

TABLE 3. Premature CAD in FED cases

Kindred	Country of Origin	Molecular Defect(s)	Gender	Age at Investigation	Presence of CAD	Age of Onset	References
1.	Sweden	P10L	F	61	—		(31, 33)
			F	76	—		
2.	Sweden	P10L	F	70	—		(32, 33)
3.	Canada	M252K/N391S	M	16	—		(59, 66)
4.	Germany	T123I	M	57	+	50	(40)
			M	68	+	60	
5.	The Netherlands	T123I	M	32	—		(40, 41)
			M ^a	37	—		
6.	Germany	T123I/T347M	M	66	—		(58)
7.	Algeria	L300-deletion	M	53	—		(60, 96)
			F	49	—		
8.	The Netherlands	N131D	M	58	+	43	(48)
			M	64	+	54	
9.	The Netherlands	P10L/R135Q	M	38	+	38	(49)
			F	63	—		
10.	Australia	T123I/Y144C	F	63	—		(68)
11.	The Netherlands	T123I/IVS4:T-22C	F	44	—		(50)
			F	46	—		
12.	United Kingdom	T123I/unknown	F	52	—		(82)
			M	76	+	50	

We here present an extended table that was originally shown in one of our previous reports (49).

^aThis man was previously reported to suffer from premature CAD (49). However, recent angiographic data prove him free of coronary atherosclerosis to date.

of patients with LCAT deficiency syndromes only allows us to speculate on the relationship between LCAT function and atherosclerosis (49). However, the study of families with LCAT deficiency syndromes might still provide new insights. The suggested role of non-apoA-I-mediated RCT in the protection against premature CAD (79) could be further delineated in FED probands with premature CAD as this has been carried out only in probands without CAD. In addition, it remains to be solved whether heterozygotes for LCAT gene defects are at increased risk for atherosclerosis as the result of the unfavorable lipoprotein changes (48, 49). An alternative and likely better way to assess the role of LCAT in atherogenesis is to determine LCAT activity and CER (in both whole plasma and VLDL/LDL-depleted plasma) in well-defined large populations of both CAD cases and controls.

To date, the use of transgenic animals enables the investigation of the in vivo characteristics of LCAT and its mutants. More importantly, these studies allow us to obtain better insight into the relationship between LCAT and atherogenesis although the reports on this subject are controversial to date (83–87). In addition, these animals might be useful to define whether LCAT gene therapy protects against atherosclerosis, or alternatively, will be useful to treat subjects who suffer from LCAT deficiency syndromes. Transgenics that overexpress specific FLD and FED mutants might, finally, help to elucidate the pathogenesis of the renal changes in

FLD and corneal changes seen in both FLD and FED. Several of these studies are underway in our combined laboratories. ■■

We wish to thank Heiko Wiebusch and Harald Funke whose expertise has been invaluable to the identification of LCAT gene defects over the past few years. The project was supported by grants from the Dutch Heart Foundation (nr. 89201), the Medical Research Council of Canada, the BC Heart Foundation, the Deutsche Forschungsgemeinschaft (SU179/1-1), and National Institutes of Health grant HL 30086. Dr. J. J. P. Kastelein is a clinical investigator of the Dutch Heart Foundation.

Manuscript received 16 July 1996 and in revised form 6 November 1996.

REFERENCES

1. Teisberg, P., E. Gjone, and B. Olaisen, 1975. Genetics of LCAT (lecithin:cholesterol acyltransferase) deficiency. *Ann. Hum. Genet.* **38**: 327–331.
2. McLean, J., K. Wion, D. Drayna, C. Fielding, and R. Lawn. 1986. Human lecithin-cholesterol acyltransferase gene: complete gene sequence and sites of expression. *Nucleic Acids Res.* **14**: 9397–9406.
3. Yang, C. Y., D. Manoogian, Q. Pao, F. S. Lee, R. D. Knapp, A. M. Gotto, Jr., and H. J. Pownall. 1987. Lecithin:cholesterol acyltransferase. Functional regions and a structural model of the enzyme. *J. Biol. Chem.* **262**: 3086–3091.
4. McLean, J., C. Fielding, D. Drayna, H. Dieplinger, B. Baer, W. Kohr, W. Henzel, and R. Lawn. 1986. Cloning

- and expression of human lecithin:cholesterol acyltransferase cDNA. *Proc. Natl. Acad. Sci. USA* **83**: 2335-2339.
5. O, K., J. S. Hill, X. Wang, R. McLeod, and P. H. Pritchard. 1993. Lecithin:cholesterol acyltransferase: role of N-linked glycosylation in enzyme function. *Biochem. J.* **294**: 879-884.
 6. Qu, S. J., H. Z. Fan, F. Blanco-Vaca, and H. J. Pownall. 1993. Effects of site-directed mutagenesis on the N-glycosylation sites of human lecithin:cholesterol acyltransferase. *Biochemistry*. **32**: 8732-8736.
 7. Francone, O. L., L. Evangelista, and C. J. Fielding. 1993. Lecithin-cholesterol acyltransferase: effect of mutagenesis at N-linked oligosaccharides attachment sites on acyl acceptor specificity. *Biochim. Biophys. Acta*. **1166**: 301-304.
 8. Francone, O. L., A. Gurakar, and C. Fielding. 1989. Distribution and functions of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in plasma lipoproteins. Evidence for a functional unit containing these activities together with apolipoproteins A-I and D that catalyzes the esterification and transfer of cell-derived cholesterol. *J. Biol. Chem.* **264**: 7066-7072.
 9. Fielding, C. J., V. G. Shore, and P. E. Fielding. 1972. A protein cofactor of lecithin:cholesterol acyltransferase. *Biochem. Biophys. Res. Commun.* **46**: 1493-1498.
 10. Akanuma, Y., S. Yokoyama, M. Imawari, and H. Itakura. 1978. A role of apoA-I in LCAT reaction. *Scand. J. Clin. Lab. Invest. Suppl.* **38**[Suppl. 150]: 40-47.
 11. Francone, O. L., and C. J. Fielding. 1991. Structure-function relationships in human lecithin:cholesterol acyltransferase. Site-directed mutagenesis at serine residues 181 and 216. *Biochemistry*. **30**: 10074-10077.
 12. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9**: 155-167.
 13. Glomset, J. A. 1962. The mechanism of the plasma cholesterol esterification reaction: plasma fatty acid transferase. *Biochim. Biophys. Acta*. **65**: 128-135.
 14. Marcel, Y. L. 1982. Lecithin:cholesterol acyltransferase and intravascular cholesterol transport. *Adv. Lipid Res.* **19**: 85-136.
 15. Chen, C. H., and J. J. Albers. 1982. Distribution of lecithin-cholesterol acyltransferase (LCAT) in human plasma lipoprotein fractions. Evidence for the association of active LCAT with low density lipoproteins. *Biochem. Biophys. Res. Commun.* **107**: 1091-1096.
 16. Barter, P. J. 1983. Evidence that lecithin:cholesterol acyltransferase acts on both high-density and low-density lipoproteins. *Biochim. Biophys. Acta*. **751**: 261-270.
 17. Barter, P. J., G. J. Hopkins, and L. Gorjatschko. 1984. Comparison of human plasma low- and high-density lipoproteins as substrates for lecithin:cholesterol acyltransferase. *Biochim. Biophys. Acta*. **792**: 1-5.
 18. Knipping, G., A. Birchbauer, E. Steyrer, J. Groener, R. Zechner, and G. M. Kostner. 1986. Studies on the substrate specificity of human and pig lecithin:cholesterol acyltransferase: role of low-density lipoproteins. *Biochemistry*. **25**: 5242-5249.
 19. Rajaram, O. V., and P. J. Barter. 1985. Reactivity of human lipoproteins with purified lecithin:cholesterol acyltransferase during incubations in vitro. *Biochim. Biophys. Acta*. **835**: 41-49.
 20. Barter, P. J., G. J. Hopkins, and L. Gorjatschko. 1985. Lipoprotein substrates for plasma cholesterol esterification. Influence of particle size and composition of the high density lipoprotein subfraction 3. *Atherosclerosis*. **58**: 97-107.
 21. Sparks, D. L., and P. H. Pritchard. 1989. The neutral lipid composition and size of recombinant high density lipoproteins regulates lecithin:cholesterol acyltransferase activity. *Biochem. Cell Biol.* **67**: 358-364.
 22. Sparks, D. L., G. M. Anantharamaiah, J. P. Segrest, and M. C. Phillips. 1995. Effect of the cholesterol content of reconstituted LpA-I on lecithin:cholesterol acyltransferase activity. *J. Biol. Chem.* **270**: 5151-5157.
 23. Liu, M., J. D. Bagdade, and P. V. Subbaiah. 1995. Specificity of lecithin:cholesterol acyltransferase and atherogenic risk: comparative studies on the plasma composition and in vitro synthesis of cholesteryl esters in 14 vertebrate species. *J. Lipid Res.* **36**: 1813-1824.
 24. Assmann, G., A. von Eckardstein, and H. Funke. 1991. Lecithin:cholesterol acyltransferase deficiency and fish-eye disease. *Curr. Opin. Lipidol.* **2**: 110-117.
 25. Glomset, J. A., G. Assmann, E. Gjone, and K. R. Norum. 1989. Lecithin:cholesterol acyltransferase deficiency and fish eye disease. In *The Metabolic and Molecular Bases of Inherited Disease*. C. R. Scriver, A. L. Beaudet, W. S. Sly, J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, Inc. New York. 1933-1951.
 26. McIntyre, N. 1988. Familial LCAT deficiency and fish-eye disease. *J. Inher. Metab. Dis.* **11**: 45-56.
 27. Norum, K. R., and E. Gjone. 1967. Familial plasma lecithin:cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab. Invest.* **20**: 231-243.
 28. Albers, J. J., and G. Utermann. 1981. Genetic control of lecithin-cholesterol acyltransferase (LCAT): measurement of LCAT mass in a large kindred with LCAT deficiency. *Am. J. Hum. Genet.* **33**: 702-708.
 29. Skretting, G., J. P. Blomhoff, J. Solheim, and H. Prydz. 1992. The genetic defect of the original Norwegian lecithin:cholesterol acyltransferase deficiency families. *FEBS Lett.* **309**: 307-310.
 30. Hill, J. S. 1994. The molecular pathology of lecithin:cholesterol acyltransferase deficiency. Dissertation.
 31. Carlson, L. A., and B. Philipson. 1979. Fish-eye disease: a new familial condition with massive corneal opacities and dyslipoproteinemia. *Lancet*. **ii**: 921-923.
 32. Carlson, L. A. 1979. A further case fo fish-eye disease (Letter). *Lancet*. **ii**: 1376-1377.
 33. Carlson, L. A. 1982. Fish eye disease: a new familial condition with massive corneal opacities and dyslipoproteinemia. *Eur. J. Clin. Invest.* **12**: 41-53.
 34. Forte, T. M., and L. A. Carlson. 1984. Electron microscopic structure of serum lipoproteins from patients with fish eye disease. *Arteriosclerosis*. **4**: 130-137.
 35. Turner, P. R., L. A. Carlson, C. Cortese, S. Rao, B. Marehah, N. E. Miller, and B. Lewis. 1984. Studies of lipoprotein metabolism in a patient with fish-eye disease. *Eur. J. Clin. Invest.* **14**: 273-277.
 36. Carlson, L. A., and L. Holmquist. 1985. Paradoxical esterification of plasma cholesterol in fish eye disease. *Acta Med. Scand.* **217**: 491-499.
 37. Carlson, L. A., and L. Holmquist. 1983. Studies on high density lipoproteins in fish eye disease. *Acta Med. Scand.* **213**: 177-182.
 38. Carlson, L. A., and L. Holmquist. 1985. Evidence for deficiency of high density lipoprotein lecithin:cholesterol acyltransferase activity (alpha-LCAT) in fish eye disease. *Acta Med. Scand.* **218**: 189-196.
 39. Holmquist, L., and L. A. Carlson. 1987. Alpha-lecithin:cholesterol acyltransferase deficiency. Lack of both phospholipase A2 and acyltransferase activities charac-

- teristic of high density lipoprotein lecithin:cholesterol acyltransferase in fish eye disease. *Acta Med. Scand.* **222**: 23–26.
40. Funke, H., A. von Eckardstein, P. H. Pritchard, J. J. Albers, J. J. P. Kastelein, C. Droste, and G. Assmann. 1991. A molecular defect causing fish eye disease: an amino acid exchange in lecithin-cholesterol acyltransferase (LCAT) leads to the selective loss of alpha-LCAT activity. *Proc. Natl. Acad. Sci. USA.* **88**: 4855–4859.
 41. Kastelein, J. J. P., P. H. Pritchard, D. W. Erkelens, J. A. Kuivenhoven, J. J. Albers, and J. J. Frohlich. 1992. Familial high-density-lipoprotein deficiency causing corneal opacities (fish eye disease) in a family of Dutch descent. *J. Int. Med.* **231**: 413–419.
 42. O, K., J. S. Hill, X. Wang, and P. H. Pritchard. 1993. Recombinant lecithin:cholesterol acyltransferase containing a Thr123 to Ile mutation esterifies cholesterol in low density lipoprotein but not in high density lipoprotein. *J. Lipid Res.* **34**: 81–88.
 43. Shaw, S. 1996. The characterization and in vivo metabolism of recombinant LCAT. Dissertation.
 44. Luc, G., L. Elkhailil, Z. Majd, R. Bakir, P. Poulain, B. Lacroix, N. Duhal, and J. C. Fruchart. 1994. Fish eye disease: structural and metabolic abnormalities of high density lipoproteins. *Atherosclerosis.* **109**: 121 (Abstr.)
 45. Duverger, N., H. G. Klein, G. Luc, J. C. Fruchart, J. J. Albers, and H. B. Brewer, Jr. 1993. Identification of a novel mutation in the LCAT gene resulting in fish eye disease with alpha-LCAT activity. *Circulation.* **88**: I-423 #2274 (Abstr.)
 46. Hill, J. S., K. O. X. Wang, and P. H. Pritchard. 1993. Lecithin:cholesterol acyltransferase deficiency: identification of a causative gene mutation and a co-inherited protein polymorphism. *Biochim. Biophys. Acta.* **1181**: 321–323.
 47. Owen, J. S., H. Wiebusch, P. Cullen, G. F. Watts, V. L. M. Lima, H. Funke, and G. Assmann. 1996. Complete deficiency of plasma lecithin-cholesterol acyltransferase (LCAT) activity due to a novel homozygous mutation (Gly-30-Ser) in the LCAT gene. *Hum. Mutat.* **6**. In press.
 48. Kuivenhoven, J. A., E. J. G. M. van Voorst tot Voorst, H. Wiebusch, S. M. Marcovina, H. Funke, G. Assmann, P. H. Pritchard, and J. J. P. Kastelein. 1995. A unique genetic and biochemical presentation of fish-eye disease. *J. Clin. Invest.* **96**: 2783–2791.
 49. Kuivenhoven, J. A., A. F. H. Stalenhoef, J. S. Hill, N. M. Demacker, A. Errami, J. J. P. Kastelein, and P. H. Pritchard. 1996. Two novel molecular defects in the LCAT gene are associated with fish eye disease. *Arterioscler. Thromb. Vasc. Biol.* **16**: 294–303.
 50. Kuivenhoven, J. A., H. Wiebusch, P. H. Pritchard, H. Funke, R. Benne, G. Assmann, and J. J. P. Kastelein. 1996. A mutation in aariat branchpoint sequence causes intron retention: a novel molecular basis for hereditary disease. *J. Clin. Invest.* **98**: 358–364.
 51. Qu, S. J., H. Z. Fan, F. Blanco-Vaca, and H. J. Pownall. 1995. In vitro expression of natural mutants of human lecithin:cholesterol acyltransferase. *J. Lipid Res.* **36**: 967–974.
 52. Klein, H. G., N. Duverger, J. J. Albers, S. Marcovina, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1995. In vitro expression of structural defects in the lecithin-cholesterol acyltransferase gene. *J. Biol. Chem.* **270**: 9443–9447.
 53. Klein, H. G., P. Lohse, N. Duverger, J. J. Albers, D. J. Rader, L. A. Zech, S. Santamarina-Fojo, and H. B. Brewer, Jr. 1992. Two different allelic mutations in the lecithin:cholesterol acyltransferase (LCAT) gene resulting in classic LCAT deficiency: LCAT (tyr83 to stop) and LCAT (tyr156 to asn). *J. Lipid Res.* **33**: 49–58.
 54. Miettinen, H., H. Gylling, I. Ulmanen, T. A. Miettinen, and K. Kontula. 1995. Two different allelic mutations in a Finnish family with lecithin:cholesterol acyltransferase deficiency. *Arterioscler. Thromb. Vasc. Biol.* **15**: 460–467.
 55. Steyrer, E., S. Haubenwallner, G. Hörl, W. Giessauf, G. M. Kostner, and R. Zechner. 1995. A single G to A nucleotide transition in exon IV of the lecithin:cholesterol acyltransferase (LCAT) gene results in an Arg140 to His substitution and causes LCAT-deficiency. *Hum. Genet.* **96**: 105–109.
 56. Moriyama, K., J. Sasaki, F. Arakawa, N. Takami, E. Maeda, A. Matsunaga, Y. Takada, K. Midorikawa, T. Yanase, G. Yoshino, S. M. Marcovina, J. J. Albers, and K. Arakawa. 1995. Two novel point mutations in the lecithin:cholesterol acyltransferase (LCAT) gene resulting in LCAT deficiency: LCAT (G873 deletion) and LCAT (Gly344Ser). *J. Lipid Res.* **36**: 2329–2343.
 57. Miller, M., K. Zeller, P. C. Kwiterovich, J. J. Albers, and G. Feulner. 1995. Lecithin:cholesterol acyltransferase deficiency: identification of two defective alleles in fibroblast cDNA. *J. Lipid Res.* **36**: 931–938.
 58. Klein, H. G., P. Lohse, P. H. Pritchard, D. Bojanovski, H. Schmidt, and H. B. Brewer, Jr. 1992. Two different allelic mutations in the lecithin-cholesterol acyltransferase gene associated with the fish eye syndrome. Lecithin-cholesterol acyltransferase (Thr123 → Ile) and lecithin-cholesterol acyltransferase (Thr347 → Met). *J. Clin. Invest.* **89**: 499–506.
 59. Hill, J. S., K. O. X. Wang, and P. H. Pritchard. 1993. Genetic and biochemical heterogeneity in fish eye disease (FED). *Circulation* **88**: I-423 #2272 (Abstr.)
 60. Klein, H. G., S. Santamarina-Fojo, N. Duverger, M. Clerc, M. F. Dumon, J. J. Albers, S. Marcovina, and H. B. Brewer, Jr. 1993. Fish eye syndrome: a molecular defect in the lecithin-cholesterol acyltransferase (LCAT) gene associated with normal alpha-LCAT-specific activity. Implications for classification and prognosis. *J. Clin. Invest.* **92**: 479–485.
 61. Funke, H., A. von Eckardstein, P. H. Pritchard, A. E. Hornby, H. Wiebusch, C. Motti, M. R. Hayden, C. Datchet, B. Jacotot, U. Gerdes, O. Faergeman, J. J. Albers, N. Colleoni, A. Catapano, J. Frohlich, and G. Assmann. 1993. 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. *J. Clin. Invest.* **91**: 677–683.
 62. Bujo, H., J. Kusunoki, M. Ogasawara, T. Yamamoto, Y. Ohta, T. Shimada, Y. Saito, and S. Yoshida. 1991. Molecular defect in familial lecithin:cholesterol acyltransferase (LCAT) deficiency: a single nucleotide insertion in LCAT gene causes a complete deficient type of the disease. *Biochem. Biophys. Res. Commun.* **181**: 933–940.
 63. McLean, J. W. 1992. Molecular defects in the lecithin:cholesterol acyltransferase gene. In *High Density Lipoproteins and Atherosclerosis III*. N. E. Miller and A. R. Tall, editors. Elsevier Science Publishers B. V. Amsterdam. 59–65.
 64. Gotoda, T., N. Yamada, T. Murase, M. Sakuma, N. Murayama, H. Shimano, K. Kozaki, J. J. Albers, Y. Yazaki, and Y. Akanuma. 1991. Differential phenotypic expression by three mutant alleles in familial lecithin:cholesterol acyltransferase deficiency. *Lancet.* **338**: 778–781.
 65. Wiebusch, H., P. Cullen, J. S. Owen, D. Collins, P. S.

- Sharp, H. Funke, and G. Assmann. 1995. Deficiency of lecithin:cholesterol acyltransferase due to compound heterozygosity of two novel mutations (Gly33Arg and 30 bp ins) in the LCAT gene. *Hum. Molec. Genet.* **4**: 143-145.
66. Frohlich, J., G. Hoag, R. McLeod, M. Hayden, D. V. Godin, L. D. Wadsworth, J. D. Critchley, and P. H. Pritchard. 1987. Hypoalphalipoproteinemia resembling fish eye disease. *Acta Med. Scand.* **221**: 291-298.
67. Skretting, G., and H. Prydz. 1992. An amino acid exchange in exon I of the human lecithin:cholesterol acyltransferase (LCAT) gene is associated with fish eye disease. *Biochem. Biophys. Res. Commun.* **182**: 583-587.
68. Contacos, C., D. R. Sullivan, K. A. Rye, H. Funke, and G. Assmann. 1996. A new molecular defect in the lecithin:cholesterol acyltransferase (LCAT) gene associated with fish eye disease. *J. Lipid Res.* **37**: 35-44.
69. Sakuma, M., Y. Akanuma, T. Kodama, N. Yamada, S. Murata, T. Murase, H. Itakura, and K. Kosaka. 1982. Familial plasma lecithin:cholesterol acyltransferase deficiency. A new family with partial LCAT activity. *Acta Med. Scand.* **212**: 225-232.
70. Maeda, E., Y. Naka, T. Matozaki, M. Sakuma, Y. Akanuma, G. Yoshino, and M. Kasuga. 1991. Lecithin-cholesterol acyltransferase (LCAT) deficiency with a missense mutation in exon 6 of the LCAT gene. *Biochem. Biophys. Res. Commun.* **178**: 460-466.
71. Glomset, J. A., K. R. Norum, and W. King. 1970. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: lipid composition and reactivity in vitro. *J. Clin. Invest.* **49**: 1827-1837.
72. Ohta, T., R. Nakamura, Y. Ikeda, J. Frohlich, K. Takata, Y. Saito, S. Horiuchi, and I. Matsuda. 1994. Evidence for impaired cellular cholesterol removal mediated by apoA-I-containing lipoproteins in patients with familial lecithin:cholesterol acyltransferase deficiency. *Biochim. Biophys. Acta.* **1213**: 295-301.
73. Rader, D. J., K. Ikewaki, N. Duverger, H. Schmidt, P. H. Pritchard, J. J. Frohlich, M. Clerc, M. F. Dumon, T. Fairwell, L. Zech, S. Santamarina-Fojo, and H. B. Brewer, Jr. 1994. Markedly accelerated catabolism of apolipoprotein A-II (apoA-II) and high density lipoproteins containing apoA-II in classic lecithin:cholesterol acyltransferase deficiency and fish-eye disease. *J. Clin. Invest.* **93**: 321-330.
74. Stampfer, M. J., F. M. Sacks, S. Salvini, W. C. Willett, and C. H. Hennekens. 1991. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N. Engl. J. Med.* **325**: 373-381.
75. Parra, H. J., D. Arveiler, A. E. Evans, J. P. Cambou, P. Amouyel, A. Bingham, D. McMaster, P. Schaffer, P. Douste-Blazy, and G. Luc. 1992. A case-control study of lipoprotein particles in two populations at contrasting risk for coronary heart disease: the ECTIM study. *Arterioscler. Thromb.* **12**: 701-707.
76. Puchois, P., A. Kandoussi, P. Fievet, J. L. Fourrier, M. Bertrand, E. Koren, and J. C. Fruchart. 1987. Apolipoprotein A-I-containing lipoproteins in coronary artery disease. *Atherosclerosis.* **68**: 35-40.
77. Ohta, T., R. Nakamura, Y. Ikeda, M. Shinohara, A. Miyazaki, S. Horiuchi, and I. Matsuda. 1992. Differential effect of subspecies of lipoprotein containing apolipoprotein A-I on cholesterol efflux from cholesterol-loaded macrophages: functional correlation with lecithin:cholesterol acyltransferase. *Biochim. Biophys. Acta.* **1165**: 119-128.
78. Fielding, C. J., J. Frohlich, K. Moser, and P. E. Fielding. 1982. Promotion of sterol efflux and net transport by apolipoprotein E in lecithin:cholesterol acyltransferase deficiency. *Metabolism.* **31**: 1023-1028.
79. von Eckardstein, A., Y. Huang, S. Wu, H. Funke, G. Nosedá, and G. Assmann. 1995. Reverse cholesterol transport in plasma of patients with different forms of familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol.* **15**: 691-703.
80. von Eckardstein, A., Y. Huang, S. Wu, A. S. Sarmadi, S. Schwarz, A. Steinmetz, and G. Assmann. 1995. Lipoproteins containing apolipoprotein A-IV but not apolipoprotein A-I take up and esterify cell-derived cholesterol in plasma. *Arterioscler. Thromb. Vasc. Biol.* **15**: 1755-1763.
81. Vergani, C., A. L. Catapano, P. Roma, and G. Giudici. 1983. A new case of familial LCAT deficiency. *Acta Med. Scand.* **214**: 173-176.
82. Winder, A. F., J. S. Owen, D. T. Vallance, R. Wray, D. Lloyd-Jones, and P. White. 1993. Fish-eye disease at 76 years. 62nd European Atherosclerosis Society Congress **45** (Abstr.)
83. Mehlum, A., B. Staels, N. Duverger, A. Tailleux, G. Castro, C. Fievet, G. Luc, J. C. Fruchart, G. Olivecrona, G. Skretting, J. Auwerx, and H. Prydz. 1995. Tissue-specific expression of the human gene for lecithin:cholesterol acyltransferase in transgenic mice alters blood lipids, lipoproteins and lipases towards a less atherogenic profile. *Eur. J. Biochem.* **230**: 567-575.
84. Vaisman, B. L., H. G. Klein, M. Rouis, A. M. Bérard, M. R. Kindt, G. D. Talley, S. M. Meyn, R. F. Hoyt, Jr., S. M. Marcovina, J. J. Albers, J. M. Hoeg, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1995. Overexpression of human lecithin:cholesterol acyltransferase leads to hyperalphalipoproteinemia in transgenic mice. *J. Biol. Chem.* **270**: 12269-12275.
85. Francone, O. L., E. L. Gong, D. S. Ng, C. J. Fielding, and E. M. Rubin. 1995. Expression of human lecithin:cholesterol acyltransferase in transgenic mice—effect of human apolipoprotein A-I and human apolipoprotein A-II on plasma lipoprotein cholesterol metabolism. *J. Clin. Invest.* **96**: 1440-1448.
86. Hoeg, J. M., B. L. Vaisman, S. J. Demosky, Jr., S. M. Meyn, G. D. Talley, R. F. Hoyt, Jr., S. Feldman, A. M. Berard, N. Sakai, D. Wood, M. E. Brousseau, S. Marcovina, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. Lecithin:cholesterol acyltransferase overexpression generates hyperalphalipoproteinemia and a nonatherogenic lipoprotein pattern in transgenic rabbits. *J. Biol. Chem.* **271**: 4396-4402.
87. Hoeg, J. M., S. Santamarina-Fojo, B. L. Vaisman, R. F. Hoyt, Jr., S. Feldman, F. Cornhill, E. Herderick, G. Talley, D. Wood, S. Marcovina, and H. B. Brewer, Jr. 1995. Lecithin:cholesterol acyltransferase transgenic rabbits: hyperalphalipoproteinemia and diet-induced atherosclerosis. *Circulation.* **92**(Suppl.): I-359 #1717(Abstr.)
88. Ohta, Y., S. Yamamoto, H. Tsuchida, S. Murano, Y. Saitoh, S. Tohjo, and M. Okada. 1986. Nephropathy of familial lecithin-cholesterol acyltransferase deficiency: report of a case. *Am. J. Kidney Dis.* **7**: 41-46.
89. Murano, S., K. Shirai, Y. Saito, S. Yoshida, Y. Ohta, H. Tsuchida, S. Yamamoto, G. Asano, C. H. Chen, and J. J. Albers. 1987. Impaired intermediate-density lipoprotein triglyceride hydrolysis in familial lecithin:cholesterol acyltransferase (LCAT) deficiency. *Scand. J. Clin. Lab. Invest.* **47**: 775-783.
90. Bron, A. J., J. K. Lloyd, A. S. Fosbrooke, A. F. Winder, and

- R. C. Tripathi. 1975. Primary LCAT-deficiency. *Lancet*. **i**: 928-929.
91. Vrabec, M. P., M. B. Shapiro, E. Koller, D. A. Wiebe, J. Henricks, and J. J. Albers. 1988. Ophthalmic observations in lecithin:cholesterol acyltransferase deficiency. *Arch. Ophthalmol.* **106**: 225-229.
92. Gjone, E., J. P. Blomhoff, R. Holme, T. Hovig, B. Olaisen, A. J. Skarbovik, and P. Teisberg. 1981. Familial lecithin:cholesterol acyltransferase deficiency. *Acta Med. Scand.* **210**: 3-6.
93. Gjone, E., A. J. Skarbovik, J. P. Blomhoff, and P. Teisberg. 1974. Familial lecithin:cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab. Invest. Suppl.* **137**: 101-105.
94. Albers, J. J., C. H. Chen, J. Adolphson, M. Sakuma, T. Kodama, and Y. Akanuma. 1982. Familial lecithin-cholesterol acyltransferase deficiency in a Japanese family: evidence for functionally defective enzyme in homozygotes and obligate heterozygotes. *Hum. Genet.* **62**: 82-85.
95. Albers, J. J., J. Adolphson, C. H. Chen, N. Murayama, S. Honma, and Y. Akanuma. 1985. Defective enzyme causes lecithin-cholesterol acyltransferase deficiency in a Japanese kindred. *Biochim. Biophys. Acta.* **835**: 253-257.
96. Clerc, M., M. F. Dumon, D. Sess, M. Freneix Clerc, M. Mackness, and C. Conri. 1991. A 'fish-eye disease' familial condition with massive corneal opacities and hypolipoproteinemia: clinical, biochemical and genetic features. *Eur. J. Clin. Invest.* **21**: 616-624.
97. Utermann, G., W. Schoenborn, K. H. Langer, and P. Dieker. 1972. Lipoproteins in LCAT-deficiency. *Hum. Genet.* **16**: 295-306.
98. Utermann, G., and H. J. Menzel. 1981. Lecithin-cholesterol-acyltransferase deficiency: autosomal recessive transmission in a large kindred. *Clin. Genet.* **19**: 448-455.
99. Frohlich, J., W. J. Godolphin, C. E. Reeve, and K. A. Evelyn. 1978. Familial LCAT deficiency. Report of two patients from a Canadian family of Italian and Swedish descent. *Scand. J. Clin. Lab. Invest.* **38**: 156-161.
100. Frohlich, J., K. Hon, and R. McLeod. 1972. Detection of heterozygotes for familial lecithin:cholesterol acyltransferase (LCAT) deficiency. *Hum. Genet.* **16**: 295-306.
101. Taramelli, R., M. Pontoglio, G. Candiani, S. Ottolenghi, H. Dieplinger, A. Catapano, J. Albers, C. Vergani, and J. McLean. 1990. Lecithin:cholesterol acyltransferase deficiency: molecular analysis of a mutated allele. *Hum. Genet.* **85**: 195-199.
102. Bethell, W., C. McCulloch, and M. Ghosh. 1975. Lecithin-cholesterol acyltransferase deficiency: light and electron microscopic findings from two corneas. *Can. J. Ophthalmol.* **10**: 494-501.