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The genetic defect of the original Norwegian lecithin:cholesterol acyltransferase deficiency families

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Three of the original Norwegian lecithin:cholesterol acyltransferase (LCAT) deficiency families have been investigated for mutations in the gene for lecithin:cholesterol acyltransferase by DNA sequencing of the exons amplified by the polymerase chain reaction. A single T→A transversion in codon 252 in exon 6 converting Met(ATG) to Lys(AAG) was observed in all homozygotes. In spite of the identical mutation, the disease phenotypes differed in severity. This was not reflected in the expression of LCAT in the heterozygotes.

Lecithin:cholesterol acetyltransferase (LCAT) deficiency; Mutation; Reverse cholesterol transport

1. INTRODUCTION

Lecithin:cholesterol acyltransferase (LCAT) is synthesized in the liver and transported in plasma complexed with high density lipoprotein (HDL). It participates in the reverse transport of cholesterol from peripheral tissues to the liver by catalyzing the esterification of cholesterol. The LCAT gene consists of 6 exons and is located in the q22.1 region of chromosome 16 spanning approx. 4,200 bp of genomic DNA [1]. The mature protein consists of 416 amino acids.

The LCAT gene is known to be responsible for two heritable metabolic diseases, fish eye disease (FED) and familial LCAT deficiency. FED was first discovered in Sweden by Carlson and Philipson [2]. The disease is characterized by dyslipoproteinemia and corneal opacities. The most striking abnormality is the reduced HDL cholesterol content (90% reduction compared to normal values). In the Swedish FED patients the LCAT activity was about 15% of that of normal plasma. However, the percentage of esterified cholesterol in plasma was found to be normal. Carlson et al. reported that there were two apparently different LCAT activities involved in cholesterol esterification, one using HDL cholesterol as a substrate (α-LCAT activity) and another using LDL and VLDL (low and very low density lipoprotein, respectively) cholesterol as substrates (β-LCAT activity) [3–5]. From this FED is characterized as an α-LCAT deficiency. We have recently sequenced the LCAT gene from two of the original Swedish FED patients and found that they have a mutation in codon 10 causing the conversion of Pro^{10} (CCG) to Leu (CTG) [6].

Familial LCAT deficiency was discovered by Norum and Gjone in 1967 [7]. To date more than 50 patients have been identified from approx. 30 families spread all over the world [8]. The mode of inheritance is autosomal recessive. The phenotypic expression of the disease varies widely and the clinical symptoms include renal failure, proteinuria, anemia, corneal opacity, lipoprotein abnormalities, premature atherosclerosis, reduced levels of plasma cholesterol esters and high levels of plasma cholesterol and lecithin. The LCAT activity in plasma is absent or extremely low in these patients and immunological studies have revealed that the LCAT protein mass is 0–60% of the normal values [9].

The LCAT genes from several patients have been studied and altogether 13 mutations have been reported (J. Albers, personal communication). We report here the mutation in patients from three of the four Norwegian families. Among these is the first family described by Norum and Gjone [7]. All three families originate from the same rather isolated area. They have been traced back five to seven generations and no link between them has been found [10]. As previously reported [7,11,12] these patients have the same laboratory phenotype (no detectable LCAT activity and the LCAT mass is 10–25% of control values), but rather variable clinical pictures with regard to severity. It was therefore of interest to determine if they carried one or several different mutations.

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Abbreviations: FED, fish eye disease; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; PBS, phosphate-buffered saline; RT, room temperature, VLDL, very low density lipoprotein.

2. MATERIALS AND METHODS

2.1. *Subjects*

Family 1. A female patient (M.R.) and her half-sister (K.R.) from the father's first marriage.

Family 3. A female patient (D.J.) and her daughter (G.E.)

Family 4. A female patient (E.R.) and her mother (M.J.), father (A.J.), sister (M.F.) and son (T.R.).

Clinical and laboratory findings of all the patients and their relatives have been described elsewhere [7,11,12]. Albers et al. have previously reported the LCAT activity and the LCAT mass for all the subjects except G.E. and T.R. [13]. Blood samples were drawn into EDTA-containing Vacutainers and processed within 20 h.

2.2. *DNA*

Genomic DNA was extracted from blood samples by using an ABI 340A nucleic acid extractor. The cells were lysed in 9 volumes of lysis buffer (0.32 M sucrose, 10 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, 1% Triton X-100) on ice for 5 min. Nuclei and cell debris were pelleted at 1,500 × *g* for 15 min. The pellet was washed in 5 ml PBS, centrifuged at 1,500 × *g* for 10 min and resuspended in PBS before loading on the extractor. As controls DNA samples from 10 healthy individuals were sequenced.

2.3. *Amplification of genomic DNA*

Primers (19–25 bases) for PCR amplification were synthesized (Fig. 1) using a Beckman System 200A DNA Synthesizer. The amplification was carried out in 100 μl using 0.8–1 μg genomic DNA, 2.5 U Taq polymerase (Promega), 0.2 mM dNTP and 1 μM of each primer in a buffer provided with the enzyme (50 mM KCl, 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100). Initial denaturation at 96°C was followed by 26 cycles of 96°C (2 min), 56°C (2 min) and 72°C (3 min). The products were purified by electrophoresis in 1.5–2% agarose. The DNA was electrophoresed on to DEAE membranes (NA45, Schleicher and Schuell). The membranes were washed in 50 mM LiCl for 5 min at RT and DNA was eluted from the membrane into 1.5 M LiCl at 65°C in 45 min. The DNA was extracted once with phenol/chloroform and ethanol precipitated. Alternatively, the DNA was excised from the gel and centrifuged through Whatman 3MM [14].

2.4. *Sequencing of the PCR products*

The sequencing primers were identical to the primers used for PCR. Manual sequencing by the dideoxy method was done using the Sequenase kit (USB) with the following modification: DNA (0.25 pmol) was denatured in a total volume of 20 μl by adding NaOH to a 0.2 M final concentration. After 5 min at RT, DNA was precipitated by adding 5 μl 7.5 M ammonium acetate and 75 μl ethanol. The DNA was dissolved in Sequenase buffer and primer (20 pmol) was added. T7 polymerase (Pharmacia) was used. Throughout all reactions NP-40 was present at a final concentration of 0.5%.

2.5. *LCAT activity*

LCAT activity in plasma was measured by a method using proteoliposomes containing apolipoprotein A-I (Sigma) (apo-A-I:lecithin:cholesterol, 0.8:250:12.5 (molar ratio)) as substrate. The substrate was preincubated for 20 min with human serum albumin (Sigma) (final concentration 0.5%). Plasma and mercaptoethanol (final concentration 5 mM) were added, the mixture was incubated for 60 min at 37°C and the enzyme activity was assayed by measuring the conversion of cholesterol to cholesterol ester after separation by thin-layer chromatography [15].

3. RESULTS

3.1. *Amplification and sequencing*

We have previously shown by Southern blotting analysis of genomic DNA from the Norwegian patients that no major insertions or deletions in their LCAT gene existed [16]. We were therefore looking for minor changes, single base mutation or small deletions/insertions. The PCR primers (Fig. 1), gave different combinations of fragments. Sequencing of all six exons and parts of introns revealed only a single A→T transversion in codon 252 in exon 6 causing a missense mutation converting Met(ATG) to Lys(AAG) (Fig. 2). The sequencing ladder showed only a single band in this position (A) indicating that the patients are homozygous for the mutation. The half-sister (K.R.) of the patient from family 1 (M.R.), did not have the mutation and the daughter (G.E.) of the patient from family 3 (D.J.) was a heterozygote, having two bands in this position (A+T). From family 4 we sequenced DNA from the parents of the patient (M.J. and A.J.), her son (T.R.) and her sister (M.F.). They were all heterozygous for the mutation, having two bands in the sequencing ladder (A+T) (data not shown).

3.2. *LCAT activity*

Albers et al. [13] have previously reported the LCAT activity for all our subjects except for G.E. and T.R. We have repeated these measurements and confirm their results, i.e. LCAT activity was very low in the patients (family 1, M.R.; family 3, D.J.; family 4, E.R.), and the

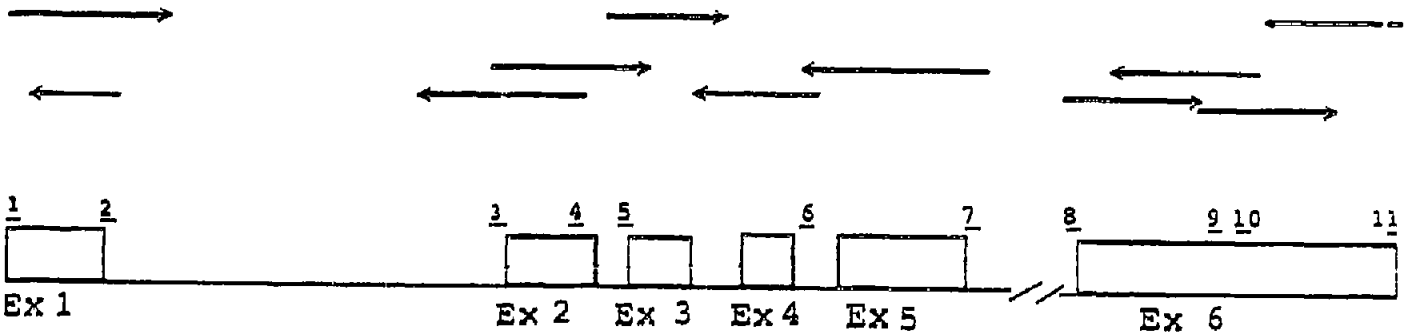

Fig. 1. Schematic representation of the LCAT gene. The exons are boxed and arrows above the exons show the orientation and length of the sequence readings. Oligonucleotides numbered 1–11 were used for PCR amplification and/or sequencing. The exons are 182, 157, 116, 96, 225 and 597 nucleotides, respectively. The amplified fragments were: 204 (exon 1, primers 1 and 2), 2000 (exon 1–exon 2, primers 1 and 4), 552 (exon 2–exon 4, primers 3 and 6), 620 (exon 3–exon 5, primers 5 and 7), and 608 (exon 6, primers 8 and 11).

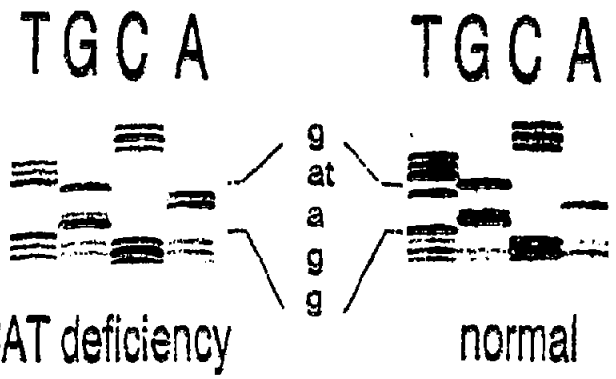

Fig. 2. Direct sequencing of PCR amplified DNA. A single-nucleotide exchange (T→A) in codon 252 in exon 6 of the LCAT gene is the defect in the Norwegian LCAT deficiency patients.

heterozygotes had 44–60% of normal values in repeated assays (Table I).

In one of the three families the disease phenotype is less severe than in the remaining two. In families 1 and 4 differences in symptoms can be explained by the patients being of different ages. In family 3, however, the propositus has reached the age of 77 without kidney failure. This does not correlate with the expression of LCAT in the heterozygotes of the various families (Table I) and is most likely due to the influence of modifying genes acting through other mechanisms. This patient with the less severe phenotype lacks the large molecular weight LDL which is typical of the other LCAT patients [11,17] and may thus be partially protected.

4. DISCUSSION

We have sequenced the LCAT gene from 3 of the 4 Norwegian families suffering from familial LCAT deficiency. Among these is the first family ever to be described [7]. The patients have the same mutation but their clinical pictures vary in severity and in laboratory findings. We were therefore interested in determining if they carried more than one mutation. We found that all the patients had the same mutation, namely a single base exchange in codon 252 causing a conversion of Met(ATG)→Lys(AAG). No other replacements were detected. The half-sister (K.R.) of the patient from family 1 (M.R.) did not have the mutation. Assuming that their common father was heterozygous for the mutation and the half-sister's mother was not, the chance for her to inherit the mutation is only 25%. She also has a normal LCAT activity. The daughter (G.E.) of the patient from family 3 (D.J.) was found to be heterozygous for the mutation having two bands in the same position, A and T. This subject has a reduced (59%) LCAT activity in plasma. This was also the case for the subjects tested from family 4. They were all heterozygous for the mutation and their LCAT activity was reduced to 44–60% of normal value.

Table I

LCAT activity in plasma of hetero- and homozygotes

Family	Subject	Enzyme activity (nmol·ml^{-1})	Esterification (% of normal)
1	1-2 (normal)	82	100
	1-1 (homozygote)	0.4	0.5
3	3-2 (heterozygote)	48	60
4	4-1 (homozygote)	1.3	1.5
	4-2 (heterozygote)	44	54
	4-4 (heterozygote)	36	44
	4-5 (heterozygote)	49	60

Plasma was incubated with proteoliposomes at 37°C and cholesterol ester was separated from cholesterol by thin layer chromatography. The results are presented as % activity relative to normal plasma.

Albers et al. [13] suggested that the apparently healthy members of these families were obligate heterozygotes. The finding of reduced LCAT activity in plasma, indicated that they had only one normal allele for the LCAT gene. Our results confirm this hypothesis and show that they really are heterozygotes by the observation of two bands at the same position in the sequencing ladder.

The mutation we report here is different from those previously reported. The two disease phenotypes associated with deficiencies in the LCAT gene, the classical LCAT deficiency and the somewhat less serious FED, both seem to be highly allelic disorders. For classical LCAT deficiency altogether 14 different mutations have been reported, ten of these are point mutations, three frameshifts and one is a three-base insertion which maintains the reading-frame. For FED three mutations are known [6,18,19]. The LCAT amino acid sequence is highly conserved between mouse, rat and human and all the point mutations except one are in fully conserved positions. The affected positions for the homozygous LCAT patients are L209P [20] N228K [21]. R244G (J.J. Albers, personal communication) M293I [21,22], T321I [20] and the mutation reported here, M252K. For the compound heterozygotes the affected positions are A93T and R158K [20], R135W and a frameshift at codon 375 [20], R147W and another unknown mutation [23]. In one case the dipeptide R140–A141 is altered to R140–G141–A142 [21]. In addition two other frameshift mutations have been found associated with the LCAT phenotype, codons 10→16 and codons 120→228 (J.J. Albers, personal communication). For FED so far, mutations have been observed at P10L [6], T123I [18,19] and T347M, the latter only in a compound heterozygote with T123I [19]. The active site of LCAT involves Ser^{181}, Cys^{31}, Cys^{184} and an unknown histidine residue [24–27]. None of the mutations so far known to cause familial LCAT deficiency are located directly in the sequence coding for the active site residues. The nature of the defects in enzyme action caused by these mutations is

unclear. They may be due to an abnormality in apolipoprotein binding or an(other) alteration in secondary structure. The first possibility is supported by the fact that a frameshift mutation in the gene for apolipoprotein A-I causes LCAT deficiency [28]. There are probably separate binding sites on the enzyme for HDL and LDL/VLDL particles. One might suggest that the N-terminal region is involved in binding HDL particles since FED is associated with mutations in codon 10 and codon 123. Different or more complex structures are involved in interactions with LDL/VLDL particles. The existence of two different disease phenotypes as well as the variation within familial LCAT deficiency can eventually be explained by the biochemical changes caused by the various mutations. Among the Norwegian families there is phenotypic variation in spite of identical mutations. This clearly points to the importance of interaction with other genes in the determination of the phenotype.

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