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Complete and Partial LCAT Deficiency are Differentially Associated with Atherosclerosis

Running Title: *Oldoni et al.; LCAT Deficiency and Atherosclerosis*

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Abstract

Background—Lecithin:cholesterol acyltransferase (LCAT) is the sole enzyme that esterifies cholesterol in plasma. Its role in the supposed protection from atherogenesis remains unclear since mutations in LCAT causing Fish-Eye Disease (FED) or Familial LCAT Deficiency (FLD) have been reported to be associated with more or instead less carotid atherosclerosis, respectively. This discrepancy may be associated with the loss of cholesterol esterification on only apolipoprotein (apo) A-I (FED) or on both apoA-I and apoB-containing lipoproteins (FLD), an aspect that has thus far not been investigated.

Methods—Seventy-four heterozygotes for LCAT mutations recruited from Italy and the Netherlands were assigned to FLD (n=33) or FED (n=41) groups and compared to 280 controls. Subclinical atherosclerosis was assessed using carotid intima-media thickness (IMT).

Results—Compared to controls, total cholesterol was lower by 16% (-32.9 mg/dL) and 7% (-14.9 mg/dL), and HDL cholesterol was lower by 29% (-16.7 mg/dL) and 36% (-20.7 mg/dL) in the FLD and FED groups, respectively. FLD subjects displayed a significant 18% lower LDL cholesterol compared with FED (101.9±35.0 vs 123.6±47.4 mg/dL, P=0.047) and controls (122.6±35.0 mg/dL, P=0.003). Remarkably, all three IMT parameters were lower in FLD compared to FED and controls (accounting for age, sex, BMI, smoking, hypertension, family history of cardiovascular disease and plasma lipids). After additional correction for nationality and ultrasonographic methods, average and maximum IMT remained significantly lower when comparing FLD to FED (0.59mm vs 0.73mm, P=0.003, and 0.87mm vs 1.24mm, P<0.001, respectively). By contrast, the common carotid IMT (corrected for age, sex, BMI, smoking, hypertension, family history of cardiovascular disease, and plasma lipids) was higher in FED compared to controls (0.69mm versus 0.65mm, P=0.05), but this significance was lost after adjustment for nationality and ultrasonographic machine.

Conclusions—In this head-to-head comparison, FLD and FED mutations were shown to be associated with decreased and increased atherosclerosis, respectively. We propose that this discrepancy is related to the capacity of LCAT to generate cholesterol esters on apoB-containing lipoproteins. While this capacity is lost in FLD, it is unaffected in FED. These results are important when considering LCAT as a target to decrease atherosclerosis.

Key Words: LCAT deficiency; carotid IMT; atherosclerosis

Clinical Perspective

What is new?

- Half-century of LCAT research has established its important function in HDL metabolism; however, the role of this enzyme in human atherogenesis still remains controversial.
- In this study, we tested the hypothesis that genetic mutations causing complete LCAT deficiency (FLD) or partial LCAT deficiency (FED) are differentially associated with carotid atherosclerosis in carriers of LCAT mutations.
- Using carotid intima-media thickness (IMT) as a measure of atherosclerosis, we demonstrate that carriers of LCAT mutations leading to FLD exhibit less carotid atherosclerosis, indicating a reduced risk of cardiovascular disease.
- By contrast, carriers of LCAT mutations leading to FED show marginally more atherosclerosis.
- The association of mutations in LCAT with subclinical atherosclerosis appears to be related to the capacity of LCAT to esterify cholesterol on apoB-containing lipoproteins since the abnormal LCAT present in FED is only active on this class of lipoproteins.

What are the clinical implications?

- These findings bear relevance for pharmaceutical strategies that target LCAT such as the recombinant enzyme for replacement therapy and, more importantly, small molecules aimed at increasing LCAT activity which previously has been thought to decrease risk of atherosclerosis.

Introduction

Genetic LCAT deficiency is a rare autosomal recessive disorder due to mutations in the *LCAT* gene.[1] Importantly, these mutations translate into two different clinical presentations known as Familial LCAT Deficiency (FLD; OMIM# 245900), and Fish-Eye Disease (FED; OMIM# 136120). Compound heterozygosity or homozygosity for mutations causing either FLD or FED is characterized by near absent HDL cholesterol.[2] Furthermore, heterozygosity for mutations in LCAT causes up to a 40% decrease in HDL cholesterol.[3, 4] In FLD cases, the lack of LCAT catalytic activity is complete thereby affecting the ability of the enzyme to esterify free cholesterol on both HDL and LDL. As a consequence, cholesteryl esters (CE) are virtually absent in plasma. By contrast, mutations causing FED result in a loss of the capacity of LCAT to esterify cholesterol only in HDL and yet retains the ability to esterify cholesterol on apolipoprotein (apo)B-containing lipoproteins (VLDL and LDL). As a result, plasma CE are mainly associated with LDL particles.[2] Clinical manifestations of a loss of LCAT activity are only observed in subjects who are homozygotes or compound heterozygotes for LCAT mutations.[2] Corneal opacity is the most common feature in FLD and FED cases whereas anaemia, proteinuria and renal disease are clinical features in only FLD cases.[2] Of note, glomerulosclerosis is the major cause of morbidity and mortality in FLD cases as it ultimately leads to renal failure.[2] The pathogenesis of renal disease in FLD is not completely understood, but it is likely related to the accumulation of abnormal lipoproteins including large molecular weight LDL particles[5] and lipoprotein X (LpX).[6]

Epidemiologic studies have clearly established the inverse association between HDL cholesterol and risk of cardiovascular disease (CVD).[7] Thus, individuals suffering from near complete loss of HDL or even half normal HDL are hypothesized to be at very high CVD risk. In

a first study by Ayyobi et al., however, 2 homozygotes and 7 heterozygotes from a large Canadian kindred with FLD have been followed for 25 years, during which time no vascular events were reported in any of the carriers aged up to 70 years.[8] Interestingly, the homozygotes had only minimally greater carotid intima media thickness (IMT) and no plaques, while some IMT abnormalities were observed in four out of seven heterozygotes.[8] In a much larger carotid IMT study in the Netherlands, measurements were performed in 9 carriers of two mutant *LCAT* alleles, 47 carriers of one mutant *LCAT* allele and 58 age and gender-matched family controls. All individuals belonged to 5 families of whom the index cases all presented with FED.[3] Heterozygous carriers were reported to have significantly greater carotid IMT compared to family controls and two had premature cardiovascular events.[3] This population was later on re-assessed by measuring carotid artery wall thickness by 3.0-T carotid magnetic resonance imaging showing that these carriers of LCAT mutations had thicker carotid artery walls and 32% more plaque component compared to family controls.[9] In sharp contrast, a study in Italian families in which the majority of the index cases suffered from FLD showed significantly smaller carotid IMT in carriers of LCAT mutations compared to age-gender matched controls, with a convincing distinct gene-dose effect.[4]

These contrasting prior findings suggest the question: Does the development of preclinical atherosclerosis follow a different course in partial LCAT deficiency (FED) compared to complete LCAT deficiency (FLD)? It is critical that this question is addressed since the answer will influence the effectiveness as well as safety of pharmaceutical strategies that target LCAT. These include both enzyme replacement therapy to treat FLD[10] but, more importantly, small molecules aimed at increasing LCAT activity to decrease risk of atherosclerosis.[11] In this light, we have tested the hypothesis that FLD and FED are two disease entities with different

associations with atherosclerosis. Our results highlight remarkable discrepancies between the two LCAT disease entities in a first head to head comparison.

Methods

The raw data that support the findings of this study are available from the corresponding authors upon reasonable request.

Subjects

A total of 375 subjects were included in this study, 168 recruited from Italy and 207 from the Netherlands. Among the 168 Italian subjects, 55 were carriers of LCAT mutations in previously identified families,[12, 13] while 113 were controls recruited among blood donors. From 207 Dutch subjects, 40 were carriers of LCAT mutations and 167 were controls (comprising 137 family members[9] and 30 age-gender matched healthy controls).

A total of 16 individuals were carriers of two mutant *LCAT* alleles (homozygotes or compound heterozygotes), 11 of whom were biochemically[12] diagnosed with FLD, all Italians, while 5 subjects (4 Italians and 1 Dutch) were classified as FED. Seventy-nine individuals carried one mutant *LCAT* allele and were assigned to either the FLD or FED group on the basis of all available data. Specifically, heterozygotes for mutations known to cause FLD or FED in homozygotes were assigned FLD or FED, respectively. In case such information was not present (in families in which the index cases were compound heterozygotes), we used biochemical information that was obtained through *in vitro* characterization of the respective mutants.[4] In case such information was not available, the subjects were excluded from our study which was the case in 5 individuals. Following, of the 74 remaining heterozygotes, 33 (26 Italians and 7 Dutch) and 41 (9 Italians and 32 Dutch) subjects were classified as FLD and FED, respectively.

A detailed and complete overview of the LCAT mutations and associated biochemical phenotype can be found in the supplemental material, including mutation carriers that were not included because no appropriate data was present to assign them to either the FLD or FED group (Supplemental Table I).

Personal and family history of CVD and cardiovascular risk factors were surveyed by questionnaires. Personal and family history of CVD was not specifically used to include or exclude subjects for this study. Biometrics including blood pressure were recorded and fasting blood samples were collected. Hypertension was defined as a blood pressure over 140/90 mm Hg or use of antihypertensive medication. None of the participants was taking medication known to affect plasma lipid/lipoprotein levels. All subjects were fully informed about the modalities of the study and signed informed consent. All procedures were approved by the institutional Ethic Committees.

Biochemical Analysis

Plasma total and HDL cholesterol and triglyceride levels were determined with certified enzymatic methods. In addition, LDL and non-HDL cholesterol were calculated by the Friedewald's equation and as the difference between total and HDL cholesterol, respectively. In the case of triglycerides levels>400 mg/dL (2 carriers and 2 controls), LDL cholesterol was assessed using a direct measurement method.

Carotid Ultrasonography

Carotid IMT was performed using standardized techniques for all carriers of *LCAT* gene mutations and controls. All ultrasonographic scans were performed with the subjects laying in the supine position. Right and left carotid artery segments (common carotid artery, bifurcation, and internal carotid artery) were identified according to specific landmarks (i.e. flow divider and

crest of the bifurcation) and imaged over a length of 10 mm according to a fixed lateral transducer angle (lateral). In both the Netherlands and Italy, the affected families are living across the two Nations. All carriers and controls who agreed to participate in the Netherlands were asked to attend the Department of Vascular Medicine in Amsterdam for IMT assessment, whereas all carriers and controls who agreed to participate in Italy were asked to attend the Lipid Clinic of the Center E. Grossi Paoletti in Milan. In Italy, when carriers could not travel to Milan, the sonographer moved to the family's site.

In the Netherlands, an Acuson 128XP/10v equipped with a 7.0-MHz linear-array transducer was used to obtain B-mode ultrasound images of carotid arteries by two blinded sonographers. In Italy, ultrasound scanning was also performed by two blinded sonographers by using an ESAOTE TECHNOS machine, equipped with a multi-frequency probe of 8.5 MHz. When one of the two sonographers moved to the family's site around the country, ultrasound scanning was performed by using a portable device (Logiq E compact ultrasound portable system; GE) equipped with a 5.0-13.0 MHz linear array transducer. In the Netherlands images were saved as JPEG image files, whereas in Italy the whole ultrasonographic scan was recorded on sVHS videotapes from three transducer angles (anterior, lateral and posterior). In order to be congruent with Dutch ultrasonographic protocol, only images from the lateral angle were considered. All IMT measurements were centralized in Milan and performed by a single expert reader blinded for the genotype of the subjects, using dedicated software (M'Ath, Metris SRL France) that allows the semi-automatic edge detection of the echogenic lines of the intima-media complex. Whenever possible, carotid images were measured in at least 3 different frames. Measurements of left and right common carotids were averaged to calculate the mean common carotid IMT.

Measurements of the three segments (common carotid artery, bifurcation, and the internal carotid artery) of left and right carotids were averaged to calculate the mean IMT (Average IMT); the highest IMT value among the three segments of the two carotids was defined as the Maximum IMT.

Statistical Analysis

Data were summarized as mean±SD or as mean and 95% confidence interval (CI) for normally distributed variables, with median and inter-quartile range (IQR) for non-normally distributed variables and with n (%) for categorical variables. Carotid IMT variables were log-transformed before multivariable analysis and were presented as geometric means and 95% CI. Unadjusted pairwise comparisons between groups were performed by Wilcoxon rank-sum test, chi-square test, or Fisher exact test, as indicated. Multivariable analysis was performed by ANCOVA using three different models: model 1, adjusted for age and sex; model 2, as model 1 with additional adjustment for BMI, smoking, hypertension, family history of cardiovascular disease, total cholesterol, HDL cholesterol and triglycerides; model 3, as model 2 with further adjustments for nation and ultrasonographic machine. P-values below 0.05 were considered as significant, except when appropriate Bonferroni correction was applied. All tests were two-sided and were performed by SAS v. 9.4 (Sas Institute Inc., Cary, NC, USA).

Results

Demographic Characteristics and Lipid Levels

Individuals from the previously reported Italian and Dutch families with mutations in LCAT were combined and analysed following identical methods. For the current analysis, we excluded homozygotes and compound heterozygotes to account for possible referral bias as these patients

may have been initially studied because of cardiovascular complications. The final cohort includes 74 proven heterozygous carriers of *LCAT* gene mutations (33 FLD and 41 FED subjects) and 280 controls. Table 1 summarizes the demographic, lifestyle and clinical characteristics of the subjects included. Statistical comparisons were made as FED vs FLD subjects, FED subjects vs controls and FLD subjects vs controls, respectively.

Age and gender distribution were very similar amongst all 3 study groups, while also BMI and hypertension were not significantly different between all groups. In addition, the FED and FLD groups were also similar for smoking status. Personal history of CVD was significantly increased in FED compared to controls, while family history of CVD was comparable in the 3 groups.

With respect to lipids, total cholesterol was significantly lower in FLD and FED subjects compared to controls (167.5 ± 39.0 vs 200.4 ± 38.5 mg/dL in FLD vs controls, P<0.0001; 185.5 ± 52.5 vs 200.4 ± 38.5 mg/dL in FED vs controls, P=0.03) but there were no statistically significant differences in total cholesterol between FLD and FED subjects. FLD subjects exhibited a statistically significant 18% (101.9 ± 35.0 vs 123.6 ± 47.4 mg/dL) lower LDL cholesterol compared to FED subjects (P=0.047) and controls (P=0.003). However, LDL cholesterol was close to identical when comparing FED subjects with controls (123.6 ± 47.4 vs 122.6 ± 35.0 mg/dL). Mutations related to FLD or FED were associated with very similar HDL cholesterol levels, i.e. 29-36% lower levels compared to controls (41.5 ± 13.2 vs 37.5 ± 14.3 mg/dL; P<0.0001 for both compared to controls). Non-HDL cholesterol tended to be lower in FLD (126.0 ± 40.5 mg/dL) compared to controls (142.2 ± 39.7 mg/dL) and FED subjects (148.0 ± 50.9 mg/dL; P=0.03 and P=0.08, respectively) but, as for LDL cholesterol, it was similar between FED subjects and controls (148.0 ± 50.9 vs 142.2 ± 39.7 mg/dL). Finally, fasting

plasma triglycerides were significantly higher in FLD (97.0 mg/dL (77.0; 144.0)) and FED subjects (100.2 mg/dL (80.8; 145.9)) compared to controls (86.1 mg/dL (59.7; 123.0); P=0.03 and P=0.02, respectively)), but without differences between the FLD and FED groups.

Carotid IMT in FLD and FED Mutation Carriers

Carotid IMT values are presented using three different statistical adjustments: model 1, adjusted for age and sex; model 2, same as model 1 with additional adjustment for BMI, smoking, hypertension, family history of CVD, total cholesterol, HDL cholesterol and triglycerides; model 3, same as model 2 with further adjustments for nationality and ultrasonographic machine (Table 2 and Supplemental Figure I). Overall, FLD subjects exhibited significantly lower IMT compared to FED subjects and controls among all the three carotid variables. Specifically, common carotid IMT was lower in FLD subjects compared to FED subjects (0.62mm (0.55-0.69) vs 0.69mm (0.60-0.76), P=0.003). This remained highly statistically significant after correction for additional confounders (P<0.001) but was lost after correction also for nation and ultrasonographic machine (P=0.091). Common carotid IMT was also significantly lower in FLD subjects compared to controls but only after correction for confounders (0.62mm (0.55-0.69) vs 0.65 (0.55-0.74), P=0.028). In contrast, common carotid IMT was significantly greater in FED compared to controls when adjusted for age and gender (0.69mm (0.60-0.76) vs 0.65 (0.55; 0.74), P=0.005). Upon adjustment for confounders, only a trend to significance was observed (P=0.05). When nationality and ultrasonographic machine were taken into account, the difference was no longer statistically significant.

Average IMT and maximum IMT were profoundly lower in FLD compared to FED subjects (0.59mm (0.56-0.79) versus 0.73mm (0.66-0.88), P<0.001, and 0.87mm (0.72-1.30) versus 1.24mm (0.95-1.41), P<0.001, respectively). The differences remained statistically

significant in all the tested models (Table 2). The same held true when comparing FLD subjects and controls (0.59mm (0.56-0.79) versus 0.72mm (0.62-0.83), P<0.001, and 0.87mm (0.72-1.30) versus 1.12mm (0.95-1.45), P<0.001, respectively). Regarding average and maximum IMT, FED subjects were not different from controls regardless of the statistical model used (Table 2).

Upon inclusion of carriers of two mutant *LCAT* alleles (homozygotes/compound heterozygotes, Supplemental Tables II and III) the analysis yielded very similar results (Supplemental Table IV).

Discussion

Despite 50 years of research the role of LCAT in the pathogenesis of atherogenesis remains unclear. For example, opposite findings were reported by Hovingh et al.[3] and Calabresi et al.[4] who described more and less carotid atherosclerosis in carriers of *LCAT* gene mutations, respectively. It is important to emphasize, however, that Hovingh et al [3] only studied families in which the probands were diagnosed with FED while Calabresi et al.[4] almost exclusively investigated families in which the probands suffered from FLD. In the current study, we have carefully discriminated between these two LCAT disease entities on the basis of clinical and biochemical features. This was anticipated to be important because LCAT in FED can still esterify cholesterol on apoB-containing lipoproteins, while such activity is missing in FLD.

After assignment of carriers to FLD or FED groups, we first tested the hypothesis that mutations that are associated with FLD or FED have different impacts on the development of atherosclerosis. To this end, the previously described Dutch and Italian cohorts were combined and used to re-read all carotid IMT images. The study groups and controls had a very similar cardiovascular risk profile. The final result shows that mutations causing FLD and FED are

indeed differentially associated with subclinical atherosclerosis as determined by IMT. Specifically, the FLD group presented with significantly lower IMT compared to controls among all three carotid measurements thereby validating previous findings.[4] The FED group, however, showed a slight increase of only the common carotid IMT compared to controls as reported earlier.[3] The latter was also in agreement with an additional report on carotid 3.0-T magnetic resonance imaging data in the same individuals.[9]

In addressing our hypothesis, the FLD group showed significantly lower IMT values across all carotid segments compared to the FED group. To account for possible imbalances between Italian and Dutch study sites, all IMT measurements were additionally adjusted for nationality and ultrasonographic machine: average and maximum IMT in FLD remained highly significantly lower compared to FED which supports the validity of our findings.

Since the major cardiovascular risk factors were similar in the FLD and FED groups, the identified changes in IMT may be related to differences in plasma lipid fractions resulting from different functions in the mutant LCAT proteins. Notably, LDL cholesterol levels were 18% lower in FLD compared to FED subjects. In view of the established causal role of LDL cholesterol in atherogenesis, this may explain the observed differences in subclinical atherosclerosis between the FLD and FED groups when considering the life-long impact of this detrimental lipid feature. This was recently highlighted through studies of mutations in another gene where a 28% reduction of LDL cholesterol observed in carriers of loss of function mutations in *PCSK9* were shown to be associated with a remarkable 88% reduction of the risk of coronary heart disease.[14] Non-HDL cholesterol was also non-significantly reduced in FLD compared to FED and controls. Since the reduction of HDL cholesterol levels was similar in both the FLD and FED groups compared to controls, this parameter is unlikely to account for

differences in IMT between the groups. We cannot exclude, however, that HDL subclasses or sizes of LDL and VLDL particles are associated with our observations.[15]

Placing our results in a broader perspective, others have shown that low LCAT activity and high pre-β1 HDL concentration are strong positive risk markers for ischemic heart disease but independent of HDL cholesterol. [16] We previously showed that LCAT concentration is not associated with HDL cholesterol levels or future coronary artery disease[17] and also reported that a low plasma concentration and activity of LCAT is not associated with carotid atherosclerosis.[18] When considering the relations between LCAT and HDL cholesterol, the above findings are not in line with those obtained through families with FLD and FED. This may be explained by relatively large reductions of LCAT activity in carriers of LCAT mutations, while the variation of LCAT concentrations in other studies was marginal by comparison.[17, 18]

When considering the relation between HDL cholesterol and CVD, the first report on a strong inverse association between HDL cholesterol and atherosclerosis[7, 19] has been confirmed by many other investigators. There is, however, little evidence from studies in humans that this relation has a causal basis.[20] In considering the role of LCAT in HDL metabolism, a recent Mendelian Randomization study showed that low HDL cholesterol associated with common LCAT gene variation is not associated with an altered risk of myocardial infarction.[21] In line with the notion that this relation may not be causal, the current study shows that a marked assumedly lifelong 29-36% reduction of HDL cholesterol levels in carriers of LCAT mutations is not associated with a markedly greater carotid IMT. In fact, we recapitulate that carriers of FLD with very marked hypoalphalipoproteinemia, surprisingly appear significantly protected against carotid atherosclerosis.

Taken together, our data show that carriers of FLD mutations exhibit less carotid atherosclerosis while FED present with slightly more atherosclerosis. We propose that this discrepancy is related to the capacity of LCAT to esterify cholesterol on apoB-containing lipoproteins (Figure 1). This is supported by significantly lower LDL cholesterol in carriers of FLD mutations compared to carriers of FED mutations. Further studies are, however, warranted to improve insight into possible mechanisms underlying the observed differences in the two LCAT syndromes. Such studies which we were not able to perform here, may encompass measures of lipoprotein composition and size, and cholesterol esterification. In closing, we would like to emphasize that our findings may bear consequences for therapeutic approaches to increase LCAT activity to fight atherosclerosis.

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Disclosures

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Table 1. Demographic, Clinical, and Lipid/Lipoprotein Data in Heterozygous Carriers of *LCAT* Gene Mutations and Controls

	FLD Carriers	FED Carriers	Controls	*P value* (FED vs FLD)*	*P value* (FED vs controls)*	*P value* (FLD vs controls)*
n.	33	41	280			
Italian/Dutch	26/7	9/32	113/167			
Gender (M, %)	18 (54.5%)	27 (65.9%)	152 (54.3%)	0.32 †	0.16 †	0.98 †
Age (y)	44.8 ± 19.3	44.5 ± 13.8	43.9 ± 13.3	0.76	0.69	0.47
BMI (kg/m^2)	25.0 ± 4.2	25.2 ± 4.6	24.9 ± 3.9	0.74	0.34	0.78
Smoking status				0.29 ‡	0.006 ‡	0.08 ‡
Never (n, %)	23 (69.7%)	25 (62.5%)	138 (49.3%)			
Former (n, %)	5 (15.2%)	3 (7.5%)	82 (29.3%)			
Current (n, %)	5 (15.2%)	12 (30.0%)	60 (21.4%)			
Personal history of CVD (n, %)	2 (6.1%)	5 (12.2%)	8 (2.9%)	0.45 ‡	0.016 †	0.28 ‡
Family history of CVD (n, %)	18 (54.5%)	21 (51.2%)	180 (64.3%)	0.77 †	0.11 †	0.27 †
Hypertension (n, %)	9 (27.3%)	14 (34.1%)	70 (25%)	0.53 †	0.21 †	0.78 †
Total Cholesterol (mg/dL)	168 ± 39	186 ± 53	200 ± 39	0.12	0.03	<0.0001§
LDL Cholesterol (mg/dL)	102 ± 35	124 ± 47	123 ± 35	0.047	0.83	0.003
HDL Cholesterol (mg/dL)	42 ± 13	38 ± 14	58 ± 16	0.14	<0.0001§	<0.0001§
Triglycerides (mg/dL)	97 (77; 144)	100 (81; 146)	86 (60; 123)	0.96	0.02	0.03
Non-HDL Cholesterol (mg/dL)	126 ± 41	148 ± 51	142 ± 40	0.08	0.60	0.03
Ratio LDL-C/HDL-C	2.2 (1.9; 3.4)	3.4 (2.2; 4.2)	2.1 (1.6; 2.8)	0.02	<0.0001§	0.09

Data are expressed as mean±SD or as geometric mean and 95% CI or as percentage where indicated.
* by Wilcoxon rank-sum test, except: † chi-square test, ‡ Fisher exact test
§ P<0.002 (Bonferroni correction)

Table 2. IMT of Carotid Arteries in Heterozygous Carriers of *LCAT* Gene Mutations and Controls

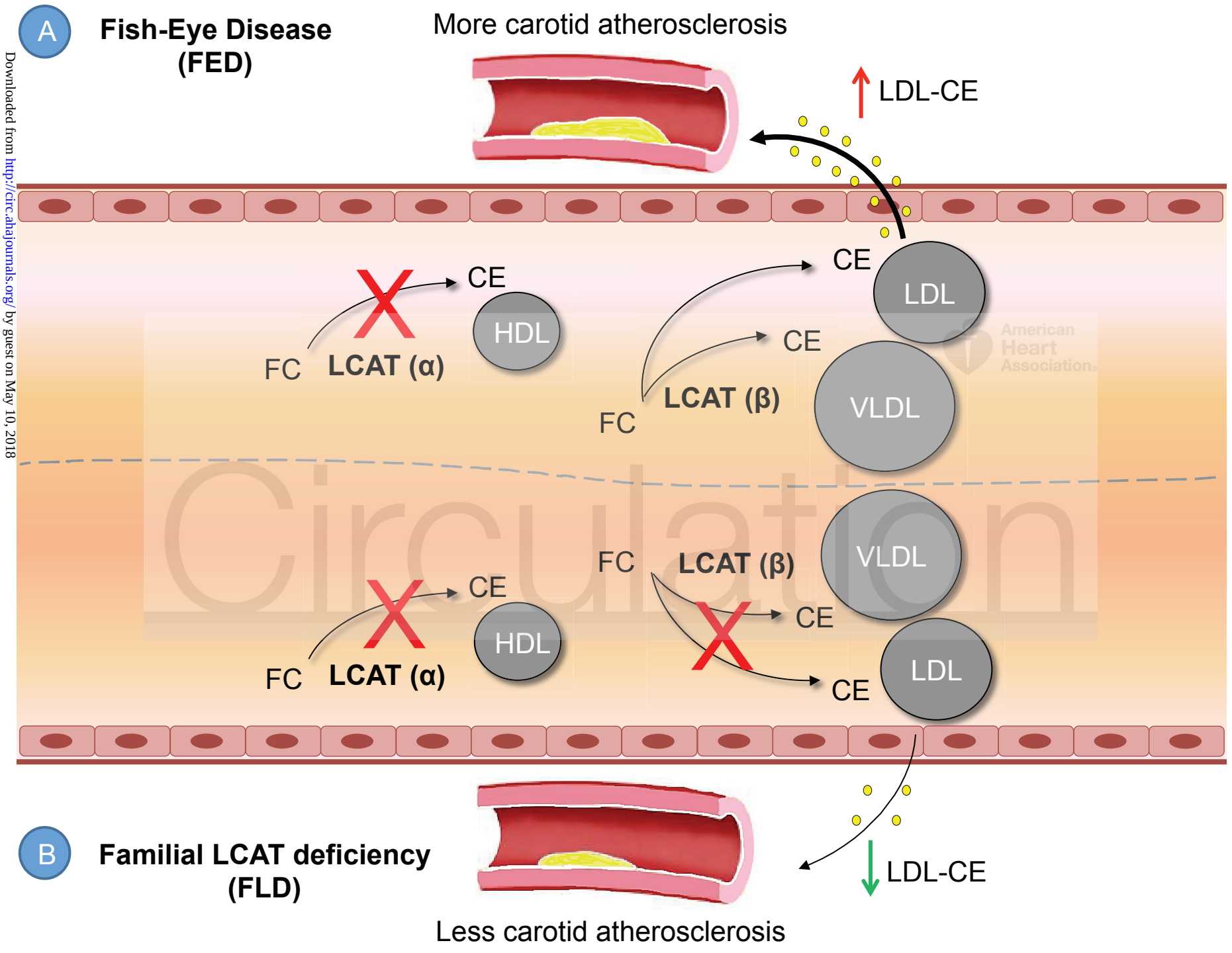
	FLD Carriers n=33	FED Carriers n=41	Controls n=280	FED vs FLD Beta (SE), *P values*			FLD vs controls Beta (SE), *P values*			FED vs controls Beta (SE), *P values*		
				Models			Models			Models		
				1	2	3	1	2	3	1	2	3
Common carotid IMT, mm	0.62 (0.55; 0.69)	0.69 (0.60; 0.76)	0.65 (0.55; 0.74)	0.053 (0.018), *0.003**	0.060 (0.018), *<0.001**	0.030 (0.018), *0.091*	-0.018 (0.014), *0.201*	-0.033 (0.015), *0.028*	-0.014 (0.015), *0.356*	0.036 (0.013), *0.005**	0.026 (0.014), *0.050*	0.016 (0.013), *0.206*
Average IMT, mm	0.59 (0.56; 0.79)	0.73 (0.66; 0.88)	0.72 (0.62; 0.83)	0.063 (0.017), *<0.001**	0.072 (0.017), *<0.001**	0.051 (0.017), *0.003**	-0.047 (0.013), *<0.001**	-0.063 (0.014), *<0.001**	-0.052 (0.015), *<0.001**	0.017 (0.012), *0.172*	0.009 (0.013), *0.504*	0.000 (0.012), *0.975*
Maximum IMT, mm	0.87 (0.72; 1.30)	1.24 (0.95; 1.41)	1.12 (0.95; 1.45)	0.116 (0.025), *<0.001**	0.122 (0.026), *<0.001**	0.097 (0.028), *<0.001**	-0.111 (0.020), *<0.001**	-0.115 (0.022), *<0.001**	-0.098 (0.024), *<0.001**	0.004 (0.018), *0.809*	0.007 (0.020), *0.726*	-0.001 (0.020), *0.951*

Data are geometric means and (95% CI). Beta coefficient are computed on log-transformed variables.
Model 1: adjusted for age and gender
Model 2: as model 1 plus BMI, smoking, hypertension, family history of CVD, total cholesterol, HDL-C and triglycerides
Model 3: as model 2 plus nationality and ultrasonographic machine
* $P<0.006$ (Bonferroni correction for 9 independent comparisons)

Figure Legend

Figure 1. Illustration of the likely underlying mechanism(s) leading to discrepancies in atherosclerosis in FLD and FED subjects

In an attempt to explain the differences in atherosclerosis observed between FLD and FED subjects we depicted the relevant underlying biological mechanism(s). LCAT esterifies cholesterol on either HDL (α activity) or LDL (β activity). In carriers of LCAT mutations, the α activity (encoded by the FED allele) is compromised, but cholesteryl ester (CE) can still be generated on pro-atherogenic (V)LDL through normal β activity (encoded by the mutant and wild-type LCAT allele) (Figure 1, A). In contrast, the reduction of both α and β activity in carriers of FLD mutations has a larger effect on overall plasma CE and on atherogenic apoB-containing lipoproteins compared to FED (Figure 1, B). FC free cholesterol; CE, cholesteryl esters.



Complete and Partial LCAT Deficiency are Differentially Associated with Atherosclerosis

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SUPPLEMENTAL MATERIAL

Supplemental Table I. LCAT Mutations and Associated Biochemical Phenotype

Sample Origin	Amino acid position	Type of Mutation	Reference AA	Altered AA	Biochemical phenotype	Genotype	Number of carriers
ITA	83	Missense	Tyr	stop	FLD	Heterozygote	1
ITA	91	Missense	Ser	Pro	FLD	Heterozygote	1
ITA	141	Missense	Ala	Thr	FED	Heterozygote	1
ITA	147	Missense	Arg	Trp	FLD	Heterozygote	6
ITA	181	Missense	Ser	Asn	FLD	Heterozygote	1
ITA	218	Missense	Lys	Asn	FLD	Heterozygote	3
ITA	244	Missense	Arg	His	FED	Heterozygote	4
ITA	274	Missense	Thr	Ile	FLD	Heterozygote	5
ITA	309	Missense	Val	Met	FLD	Heterozygote	2
ITA	372	Missense	Leu	Arg	FLD	Heterozygote	3
ITA	stop(nt977-981)	Stop	/	/	FLD	Heterozygote	2
ITA	delG, stop 16	Del, stop	/	/	FLD	Heterozygote	2
ITA	delG,Thr13--Met	Del, missense	/	/	FED	Heterozygote	4
ITA	317	Missense	Val	Met	Unknown	Heterozygote	2
ITA	277	Missense	Asp	Asn	Unknown	Heterozygote	2
ITA	122	Missense	His	Tyr	Unknown	Heterozygote	1
ITA	91/141	Missense	Ser/Ala	Pro/Thr	FED	Compound Heterozygote	2
ITA	147	Missense	Arg	Trp	FLD	Homozygote	3

ITA	274/83	Missense/stop	Thr/Tyr	Ala/stop	FED	Compound Heterozygote	1
ITA	218	Missense	Lys	Asn	FLD	Homozygote	3
ITA	(nt977-981)/181	Stop/missense	Ser	Stop/Asn	FLD	Compound Heterozygote	2
ITA	372	Missense	Leu	Arg	FLD	Homozygote	2
ITA	274	Missense	Thr	Ile	FLD	Homozygote	1
ITA	277/317	Missense	Asp/Val	Asn/Met	FED	Compound Heterozygote	1
NL	10	Missense	Pro	Gln	FED	Heterozygote	6
NL	83	Stop	Tyr - Term	/	FLD	Heterozygote	4
NL	123	Missense	Thr	Ile	FED	Heterozygote	8
NL	131	Missense	Asn	Asp	FED	Heterozygote	17
NL	244	Missense	Arg	His	FED	Heterozygote	1
NL	309	Missense	Val	Met	FLD	Heterozygote	3
NL	123	Missense	Thr	Ile	FED	Homozygote	1

Supplemental Table II. Demographic, Clinical, and Lipid/Lipoprotein Data in Carriers of *LCAT* Gene Mutations (including homozygotes/compound heterozygotes) and Controls

	FLD Carriers	FED Carriers	Controls	*P value* (FED vs FLD)*	*P value* (FED vs controls)*	*P value* (FLD vs controls)*
n.	44	46	280			
Italian/Dutch	37/7	13/33	113/167			
Gender (M, %)	26 (59.1%)	31 (67.4%)	152 (54.3%)	0.41 †	0.10 †	0.55 †
Age (y)	43.1 ±17.2	44.5 ± 15.2	43.9 ± 13.3	0.56	0.80	0.75
BMI (kg/m^2)	24.5 ± 4.1	25.0 ± 4.4	24.9 ± 3.9	0.48	0.48	0.63
Smoking status				0.026 ‡	0.007 ‡	0.237 ‡
Never (n, %)	27 (61.4%)	28 (62.2%)	138 (49.3%)			
Former (n, %)	12 (27.3%)	4 (8.9%)	82 (29.3%)			
Current (n, %)	5 (11.4%)	13 (28.9%)	60 (21.4%)			
Personal history of CVD (n,	2 (4.6%)	6 (13%)	8 (2.9%)	0.26 ‡	0.007 ‡	0.63 ‡
Family history of CVD (n, %)	27 (61.4%)	24 (52.2%)	180 (64.3%)	0.38 †	0.12 †	0.71 †
Hypertension (n, %)	13 (29.5%)	16 (34.8%)	70 (25.0%)	0.59 †	0.16 †	0.52 †
Total Cholesterol (mg/dl)	160 ± 47	178 ± 56	200 ± 39	0.13	0.003	<0.0001§
LDL Cholesterol (mg/dl)	100 ± 40	119 ± 48	123 ± 35	0.08	0.38	0.001§
HDL Cholesterol (mg/dl)	35 ± 17	35 ± 16	58 ± 16	0.83	<0.0001§	<0.0001§
Triglycerides (mg/dl)	107 (85; 150)	103 (81; 149)	86 (60; 123)	0.70	0.006	0.001§
Non-HDL Cholesterol (mg/dl)	125 ± 45	144 ± 52	142 ± 40	0.13	0.98	0.02
Ratio LDL-C/HDL-C	2.9 (2.1; 3.8)	3.5 (2.4; 5.0)	2.1 (1.6; 2.8)	0.08	<0.0001§	0.002§

Data are expressed as mean ± SD or as geometric mean and 95% CI or as percentage where indicated.

* by Wilcoxon rank-sum test, except: † chi-square test, ‡ Fisher exact test. § $P<0.002$ (Bonferroni correction).

Supplemental Table III. Demographic, Clinical, and Lipid/Lipoprotein Data in Carriers of Two Mutant *LCAT* Alleles and Controls

	FLD Carriers	FED Carriers	Controls	*P value* (FED vs FLD)*	*P value* (FED vs controls)*	*P value* (FLD vs controls)*
n.	11	5	280			
Italian/Dutch	11/0	4/1	113/167			
Gender (M, %)	8 (72.7%)	4 (80.0%)	152 (54.3%)	1.00†	0.38†	0.36†
Age (y)	44.2 ±26.2	37.9 ±6.3	43.9 ±13.3	0.57	0.70	0.05
BMI (kg/m^2)	23.3 ±3.8	24 ±3.1	24.9 ±3.9	0.43	0.57	0.14
Smoking status				0.17†	1.00†	0.04†
Never (n, %)	4 (36.4%)	3 (60.0%)	138 (49.3%)			
Former (n, %)	7 (63.6%)	1 (20.0%)	82 (29.3%)			
Current (n, %)	0 (0%)	1 (20.0%)	60 (21.4%)			
Personal history of CVD (n, %)	0 (0%)	1 (20%)	8 (2.9%)	0.31†	0.15†	1†
Family history of CVD (n, %)	9 (81.8%)	3 (60%)	180 (64.3%)	0.55†	1†	0.34†
Hypertension (n, %)	4 (36.4%)	2 (40%)	70 (25%)	1.00†	0.60†	0.48†
Total Cholesterol (mg/dl)	138 ±61	120 ±52	200 ±39	0.73	0.002	0.001
LDL Cholesterol (mg/dl)	93 ±57	81 ±41	123 ±35	0.59	0.03	0.11
HDL Cholesterol (mg/dl)	15 ±9	11 ±9	58 ±16	0.28	0.0001§	<0.0001§
Triglycerides (mg/dl)	114 (85; 328)	121 (119; 190)	86 (60; 123)	1.00	0.07	0.006
Non-HDL Cholesterol (mg/dl)	123 ±59	110 ±52	142 ±40	0.82	0.13	0.35
Ratio LDL-C/HDL-C	4.7 (3.4; 22.5)	6.3 (4.1; 22.2)	2.1 (1.6; 2.8)	0.89	0.0007§	0.0001§

Data are expressed as mean±SD or as geometric mean and 95% CI or as percentage where indicated.

* by Wilcoxon rank-sum test, except: † Fisher exact test. § $P<0.002$ (Bonferroni correction).

Supplemental Table IV. IMT of Carotid Arteries in Carriers of *LCAT* Gene Mutations (including homozygotes/compound heterozygotes) and Controls

	FLD Carriers n=44	**FED Carriers n=46**	**Controls n=280**	**FED vs FLD Beta (SE), *P values***			**FLD vs controls Beta (SE), *P values***			**FED vs controls Beta (SE), *P values***		
				Models			**Models**			**Models**		
				1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Common carotid IMT, mm	0.60 (0.54; 0.69)	0.69 (0.59; 0.77)	0.65 (0.55; 0.74)	0.049 (0.016), *0.002*§	0.049 (0.016), *0.002*§	0.023 (0.016), *0.160*	-0.015 (0.012), *0.220*	-0.027 (0.014), *0.053*	-0.010 (0.014), *0.480*	0.034 (0.012), *0.005*§	0.022 (0,013), *0.110*	0.013 (0.012), *0.310*
Average IMT, mm	0.58 (0.55; 0.77)	0.73 (0.64; 0.90)	0.72 (0.62; 0.83)	0.062 (0.015), *<0.001*§	0.065 (0.015), *<0.001*§	0.047 (0.015), *0.002*§	-0.049 (0.012), *<0.001*§	-0.061 (0.013), *<0.001*§	-0.051 (0.013), *<0.001*§	0.014 (0.012), *0.240*	0.004 (0.013), *0.770*	-0.004 (0.012), *0.730*
Maximum IMT, mm	0.83 (0.70; 0.94)	1.20 (0.89; 1.44)	1.12 (0.95; 1.45)	0.123 (0.023), *<0.001*§	0.123 (0.023), *<0.001*§	0.100 (0.025), *<0.001*§	-0.127 (0.017), *<0.001*§	-0.124 (0.020), *<0.001*§	-0.108 (0.022), *<0.001*§	-0.004 (0.017), *0.830*	-0.001 (0.019), *0.970*	-0.008 (0.019), *0.690*

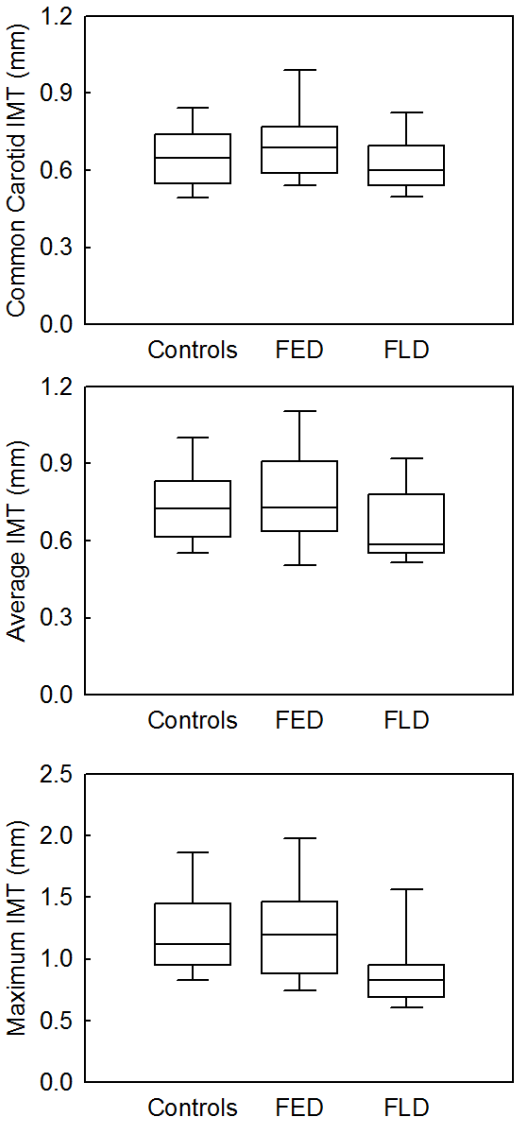
Data are geometric means and (95% CI). Beta coefficient are computed on log-transformed variables.

Model 1: adjusted for age and gender

Model 2: as model 1 plus BMI, smoking, hypertension, family history of CVD, total cholesterol, HDL-C and triglycerides

Model 3: as model 2 plus country and ultrasonographic machine

§ $P<0.006$ (Bonferroni correction for 9 independent comparisons)



Supplemental figure I. IMT of Carotid Arteries in Carriers of *LCAT* Gene Mutations and Controls

Common Carotid, Average IMT and Maximum IMT values in control subjects (n=280), FED (n=46) and FLD (n=44) carriers. Boxes indicate the median value with the 25th and 75th percentiles; capped bars indicate the 10th and 90th percentiles.