Genetic disorders of HDL metabolism: from model to mechanism

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Carriers of Lecithin: Cholesterol Acyltransferase Gene Mutations have Accelerated Atherogenesis as Assessed by Carotid 3T-MRI

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Abstract

Objectives. Aim of this study was to investigate the role of reduced Lecithin:cholesterol acyltransferase (LCAT) function on atherogenesis using 3 Tesla carotid magnetic resonance imaging (MRI) and B-mode ultrasound.

Background. The role of low high-density lipoprotein cholesterol (HDL-c) as a causal factor in atherogenesis has recently been questioned. LCAT plays a key role in HDL-c metabolism.

Methods. Carotid 3.0 Tesla MRI and B-mode ultrasound measurements were performed in 40 carriers of LCAT gene mutations and 40 controls, matched for age. Patients with cardiovascular disease were excluded.

Results. Carriers had 31% lower LCAT activity levels and 38% decreased HDL-c levels (both p<0.001 vs. controls). Carriers presented with a 10% higher normalized wall index (0.34±0.07 vs 0.31±0.04, p=0.002), a 22% higher mean wall area (17.3±8.5mm² vs 14.2±4.1mm², p=0.01), and a 22% higher total wall volume (1039±508mm³ vs 851±247mm³, p=0.01 vs. controls) as measured by MRI. The prevalence (20 vs 5, p=0.002) and the total volume (102mm³ vs 3mm³) of atherosclerotic plaque components on MRI relating to lipid-rich tissue or calcification were also higher in carriers than controls. All differences retained significance after adjustment for age, gender, blood pressure, LDL-c, BMI, smoking and family history of cardiovascular disease. Common carotid intima-media thickness measured with ultrasound was increased in carriers by 12.5% (0.72±0.33mm vs 0.64±0.15mm, p=0.14).

Conclusion. Carriers of LCAT gene mutations exhibit increased carotid atherosclerosis, indicating an increased risk for cardiovascular disease. The present findings imply that raising LCAT activity may be an attractive target in cardiovascular prevention strategies.
**Introduction**

A low plasma high-density lipoprotein cholesterol (HDL) level is among the strongest risk factors for cardiovascular disease (CVD). One of the mechanisms by which HDL is considered to convey atheroprotection is the removal of excess cholesterol from lipid-laden foam cells in the artery wall and transport it to the liver for fecal excretion, a process referred to as “reverse cholesterol transport.” A crucial enzyme in HDL metabolism is lecithin:cholesterol acyl transferase (LCAT). This plasma enzyme, produced in the liver and small intestine, is predominantly associated with HDL and esterifies free cholesterol using apolipoprotein A-I (apoA-I) as a cofactor. Homozygotes for deleterious mutations in the LCAT gene are characterized by near complete HDL-c deficiency (~90% reduction), while heterozygotes have profoundly reduced HDL-c levels (~40% reduction) compared to normal.

To date, the relation between LCAT and atherosclerosis has been a matter of debate. Animal studies have not been able to provide a clear answer, as both LCAT knockout models as well as LCAT overexpression models yielded mixed results with respect to atherogenesis as was recently reviewed. Human studies have also been conflicting. Hovingh et al. reported that carriers of LCAT gene mutations have increased carotid intima-media thickness (IMT as quantified by B-mode ultrasound imaging) compared to family controls. In contrast, Calabresi et al. recently reported that carotid IMT was decreased in carriers while using the same ultrasound methodology. These contradictory outcomes are difficult to interpret and may result from population differences. Furthermore, a limitation of previous studies is that carotid ultrasound lacks statistical power to reliably measure arterial wall thickness in small population studies, since ultrasound provides two dimensional longitudinal images, where atherosclerosis is a three dimensional eccentric developing disease. Magnetic resonance imaging (MRI) might overcome these imaging limitations, as it enables transverse three dimensional imaging of atherosclerosis at high resolution with excellent interscan reproducibility.

In the present study, we set out to assess the relationship between LCAT and carotid atherosclerosis using carotid 3.0 Tesla MRI, in parallel to carotid B-mode ultrasound imaging, comparing carriers of LCAT mutations and controls. We hypothesized that carriers of LCAT gene mutations had increased atherosclerosis compared to controls.

**Methods**

*Study Design*

In this study the extent of carotid atherosclerosis in subjects with LCAT gene mutations and age-matched controls was compared. The study was conducted at the Academic Medical Center in Amsterdam, The Netherlands from October 2008 to October 2009.
The study protocol was reviewed and approved by the institutional review board and all subjects gave written informed consent. The 3 probands of the families in which we identified LCAT mutations, had presented to the ophthalmologists with corneal clouding, after which they were referred to our lipid clinic. Subsequently, we performed genetic testing in their family members to identify subjects with LCAT mutations. Carriers of molecularly diagnosed LCAT mutations (DNA and LCAT activity) were enrolled in this study, irrespective of their age and gender. Probands with cardiovascular disease and their family members were excluded. For the control group, family members of the included carriers were asked to participate in the study, comprising first, second or third degree family members or spouses. They were included if they could be matched for age with a carrier. As insufficient numbers of unaffected family controls (N=19) volunteered, we complemented the control group with unrelated controls (N=21) recruited by advertisement. Exclusion criteria for both carriers and controls were history of CVD, prior carotid surgery or any contraindication for MRI.

**Questionnaire, Biometric and Biochemical Measurements**

Presence of cardiovascular risk factors, use of medication and family history of CVD, were assessed by a questionnaire. Brachial artery blood pressures were measured using an oscillometric blood pressure device (Omron 705IT). Presence of hypertension was defined as a systolic blood pressure (SBP) >140 mmHg, a diastolic blood pressure (DBP) >90 mmHg or use of antihypertensive medication. Weight and length were measured to calculate body mass index (BMI).

EDTA plasma obtained through venous blood samples were obtained after overnight fasting and stored using standardized protocols. Plasma total cholesterol, HDL-c and triglyceride levels were analyzed using a commercially available enzymatic methods (Westburg, USA) on a Cobas Mira autoanalyzer (Roche, Switzerland). Low-density lipoprotein cholesterol (LDL-c) levels were calculated using the Friedewald equation. LCAT activity was measured using a proteoliposome substrate as previously described.

**Carotid Magnetic Resonance Imaging**

Scans were obtained in a 3.0 Tesla Philips whole-body scanner, using a single-element microcoil with a diameter of 5cm. Cardiac gated axial T1-weighted Turbo Spin Echo image stacks were acquired at end-diastole using double inversion recovery preparation and active fat suppression. Sequence parameters were: slice thickness 3 mm, imaging matrix size 240, FOV of 60 x 60 mm, non-interpolated pixel size 0.25 x 0.25 mm, TE 9 ms, TR according to the subjects’ heart rate (approximately 900 ms), echo train length 7, echo train duration 63 ms. To localize the left and right common carotid artery and carotid bifurcation, axial Magnetic Resonance Angiography (MRA) images were acquired using a Time of Flight (TOF) sequence. These images together with projection images were used for positioning the scan planes perpendicular to the vessel at a predefined distance.
distal to the flow divider. Ten slices were scanned of the distal 3.0 cm of the left and right common carotid artery. The slices were located from 9 mm to 39 mm proximal to the carotid flow divider. Each carotid was scanned individually. A total of 20 images were obtained per scan. All images were saved in DICOM format. Standardized equipment and protocols were used for image storage and data management. The imaging protocol and image analysis have been described previously\textsuperscript{12,13}.

Quantitative image analysis was performed using semi-automated measurement software (VesselMass, Leiden University Medical Center, The Netherlands)\textsuperscript{14}. One reader analyzed all the images, blinded for group and any other data of the participants. Mean wall area (MWA), lumen area (LA), outer wall area (OWA), and total wall volume (TWV) were measured. Normalized wall index (NWI) was calculated as:

\[ \text{NWI} = \frac{\text{MWA}}{\text{OWA}}. \]

Also prevalence of plaque components (PC) and total PC volume (mm\textsuperscript{3}) was assessed. PC was defined as a T1-weighted image on which an area of decreased signal intensity within the artery wall was identified. Previous studies have shown that areas in the artery wall with decreased signal intensity on T1-weighted images represent either lipid-rich tissue or calcification\textsuperscript{15}. Prevalence of PC was reported as the total number of images per group that showed PC. Also the volume of PC’s was quantified, and reported as the sum of all PC volumes of all subjects per group.

**Carotid Ultrasound Imaging**

Carotid B-mode ultrasound scans of the left and right common, bulb and internal carotid arterial far walls were assessed in a single-angle imaging protocol, with the transducer axis parallel to a virtual ear-to-ear line, according to our standardized protocol as previously described\textsuperscript{16}. One experienced and certified sonographer performed all scans, and one reader analyzed all the images, blinded for group and any other data of the participants. Images were analyzed quantitatively off-line by one certified image analyzer using validated software (eTrack, Academic Medical Center, The Netherlands). The primary ultrasound parameter was defined as mean common carotid intima-media thickness (CCIMT, defined as the average far wall IMT of the left and right distal 1cm of the common carotid artery). A secondary ultrasound endpoint entailed the mean carotid IMT (IMT), defined as the average far wall IMT of the left and right common, bulb and internal carotid arterial wall segments.

**Outcome Parameters**

Normalized wall index (NWI) was the primary outcome parameter of the study. A priori, based on previous study data and assuming a two-sided $\alpha$ of 0.05 and a $\beta$ of 0.2 (power of 80%), we calculated a sample of at least 38 subjects per group was required to detect a 0.02 difference in NWI between groups. Secondary MRI outcome parameters were MWA (mm\textsuperscript{2}), and TWV (mm\textsuperscript{3}). Secondary ultrasound outcome parameters were CCIMT
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(mm), and IMT (mm). Exploratory endpoints were PC prevalence (n) and total PC volume (mm³) assessed by MRI.

Statistical Analysis

Continuous variables are expressed as means ± standard deviations (SD), unless otherwise specified. Differences in demographic, biometrical and biochemical parameters between carriers of LCAT gene mutations and controls were assessed using unpaired Student’s t-tests or Chi square tests, where appropriate. Differences in carotid imaging parameters between carriers of LCAT gene mutations and controls were assessed using unpaired Student’s t-tests, unless otherwise specified. In addition, a multivariate model was used with generalized estimating equations in the SAS procedure GENMOD to account for age, gender, hypertension (SBP >140 mmHg, DBP >90 mmHg or use of antihypertensive medication), LDL-c, BMI, smoking, family history of CVD and correlations within families due to clustering of genetic and/or environmental factors. To compare the agreement between MRI and US scans within patients we assessed the intraclass correlation coefficients (r) and mean paired difference between MWT (MRI) and CCIMT (ultrasound). Statistical analyses were done using SPSS (Statistical Package for the Social Sciences) version 16.0 and SAS package (SAS Institute Inc.). The authors had full access to the raw data and take responsibility for its integrity.

Results

Population Characteristics

We studied 40 carriers of LCAT gene mutations (from 14 families of Dutch descent) and 40 age-matched controls of which 19 were family members and 21 were unrelated individuals. Mutations in the LCAT gene can either cause loss of enzymatic activity on only HDL (α-activity), or loss of activity on both HDL and LDL (α- and β-activity respectively). Clinically, this translates into two different autosomal recessive disorders: fish eye disease (FED, only loss of α-activity) and familial LCAT deficiency (FLD, loss of both α- and β-activity). Whereas both FED and FLD patients present with low HDL-c, only FLD patients also exhibit lower LDL-c levels. Of the carriers, 38 had one mutant LCAT allele, while 2 were homozygotes for a defect which underlied clinically manifest FED (corneal opacification). Thirty-three out of the 40 carriers had a mutation of which is known that it causes FED when present on both alleles. Four individuals were heterozygotes for a mutation which is known to cause FLD when present on both alleles. Finally, 3 subjects carried LCAT gene point mutations of which it is unknown whether they cause FED or FLD when present on both alleles (no homozygous patients described). Table 1 summarizes the demographic, lifestyle, and clinical characteristics of carriers and controls. Age, gender, smoking, alcohol use, blood pressure, diabetes, fasting glucose level, fasting insulin level, Homeostatic Model Assessment index (HOMA) index, hypertension and the Framingham risk score were similar. BMI tended to be higher in the carriers, but this
LCAT mutation carriers have increased atherosclerosis assessed by MRI.

Table 1. Characteristics in Carriers of LCAT Gene Mutations and Controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carriers of LCAT gene mutations (n=40)</th>
<th>Controls (n=40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.4 (13.0)</td>
<td>42.3 (14.1)</td>
<td>0.97</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>27 (68)</td>
<td>23 (58)</td>
<td>0.36</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>25.7 (4.0)</td>
<td>24.5 (3.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>6 (15)</td>
<td>6 (15)</td>
<td>1.0</td>
</tr>
<tr>
<td>Alcohol use (units per week)</td>
<td>5.8 (5.5)</td>
<td>7.8 (7.9)</td>
<td>0.22</td>
</tr>
<tr>
<td>Medication use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>11 (28)</td>
<td>1 (3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>3 (8)</td>
<td>0 (0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Niacin</td>
<td>3 (8)</td>
<td>0 (0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Fibrate</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>0.31</td>
</tr>
<tr>
<td>Asprin</td>
<td>3 (0)</td>
<td>0 (0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>131 (13)</td>
<td>128 (13)</td>
<td>0.29</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>78 (9)</td>
<td>76 (9)</td>
<td>0.26</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td></td>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.2 (0.8)</td>
<td>5.3 (0.9)</td>
<td>0.49</td>
</tr>
<tr>
<td>Fasting insulin (mU/L)</td>
<td>5.1 (8.9)</td>
<td>5.2 (7.3)</td>
<td>0.94</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.2 (2.1)</td>
<td>1.5 (2.4)</td>
<td>0.67</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0.31</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>175.4 (44.1)</td>
<td>190.8 (41.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>126.5 (33.4)</td>
<td>123.6 (31.4)</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>34.1 (13.9)</td>
<td>54.4 (16.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>104.4 (80.8 – 146.2)</td>
<td>82.3 (55.3 – 126.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>103.1 (25.6)</td>
<td>100.7 (23.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dL)</td>
<td>119.9 (31.9)</td>
<td>150.6 (25.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LCAT activity (nmol·mL⁻¹·h⁻¹)</td>
<td>9.24 (2.95)</td>
<td>12.85 (2.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Framingham Risk Score</td>
<td>3.9 (4.6)</td>
<td>3.1 (4.6)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Values are indicated as mean ± SD unless otherwise indicated. Male sex, smokers, medication use, hypertension, diabetes: p for X² test; for other parameters: p for Students’ t-test. LCAT is lecithin:cholesterol acyltransferase, HOMA index is Homeostatic Model Assessment index, hypertension was defined as systolic blood pressure > 140mmHg, diastolic blood pressure > 90 mmHg or use of antihypertensive medication. For triglycerides we report median and interquartile range; P for T-test after log-transformation. Framingham Risk Score shows the 10-year risk for coronary heart disease.

was not statistically significant. Also more lipid lowering medication, especially statins, and ascal were prescribed in the carriers.

Table 1 also gives the results of lipid, (apolipoproteins and LCAT activity measurements. Carriers had 8% lower total cholesterol (p<0.04) which could be attributed to a 38% reduction of HDL-c levels (p<0.001) with similar LDL-c levels in both groups. Plasma
triglyceride levels were 27% higher in carriers compared to controls (p<0.06). Apo B levels were identical while carriers had 20% lower apo A-I levels (p<0.001). LCAT activity levels were 31% lower in carriers compared to controls (p<0.001).

**Carotid MRI and Ultrasound**

Mean values (±SD) of MRI and ultrasound parameters are shown in Table 2. NWI, the primary endpoint of this study, was significantly increased in carriers compared to controls (p=0.02), shown in Figure 1. Statistical corrections for differences in age, gender, hypertension, LDL-c, BMI, smoking, family history of cardiovascular disease (CVD) and clustering of genetic and/or environmental factors in families rendered stronger

<table>
<thead>
<tr>
<th>Table 2. Carotid 3.0 Tesla MRI and B-mode Ultrasound Parameters for Carriers of LCAT Gene Mutations and Controls.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3.0 Tesla MRI</strong></td>
</tr>
<tr>
<td>NWI (0.07)</td>
</tr>
<tr>
<td>MWA (mm²)</td>
</tr>
<tr>
<td>TWV (mm³)</td>
</tr>
<tr>
<td>LA (mm²)</td>
</tr>
<tr>
<td><strong>Plaque Composition Analysis</strong></td>
</tr>
<tr>
<td>Total PC volume (mm³)</td>
</tr>
<tr>
<td><strong>B-Mode ultrasound</strong></td>
</tr>
<tr>
<td>CCIMT (mm)</td>
</tr>
<tr>
<td>IMT (mm)</td>
</tr>
</tbody>
</table>

P1 for the unadjusted model, P2 for multivariate model adjusting for age, gender, BMI, hypertension, LDL-cholesterol, smoking status, family history of cardiovascular disease (CVD) and accounting for clustering of genetic and/or environmental factors in families, *P for X2 test. LCAT is lecithin:cholesterol acyltransferase. NWI is normalized wall index, MWA is mean wall area, TWV is total wall volume, LA is lumen area, PC is plaque component. CCIMT is the mean common carotid intima media thickness. IMT is the average mean intima media thickness of the common, bulb and internal carotid arteries.

**Figure 1.** Comparison of Carotid Atherosclerosis between Carriers of LCAT Gene Mutations and Controls. The mean and 95% confidence intervals for normalized wall index (NWI) measured by 3.0 Tesla MRI are shown for each group. P value is adjusted for age, gender, BMI, hypertension, LDL-cholesterol, smoking status, family history of cardiovascular disease (CVD) and clustering of genetic and/or environmental factors in families.
LCAT mutation carriers have increased atherosclerosis assessed by MRI

statistical significance (p=0.002). NWI in unaffected family controls was similar to that of unrelated controls (0.30 ± 0.04 versus 0.31 ± 0.05, p=0.57). We also assessed differences in plaque composition. The prevalence of plaque component related to lipid-rich tissue or calcification (PC prevalence) was 25% higher (20 versus 6) and total PC volume was 34 times larger in carriers than in controls. Ultrasound CCIMT, and IMT were increased in carriers, but these differences did not reach statistical significance. There was excellent agreement between MWT (MRI) and CCIMT (ultrasound), with an intraclass correlation coefficient of 0.91 (95%CI 0.86 – 0.94, p<0.001), and a mean paired difference of 0.01 (SD 0.11) mm.

Discussion

The present study shows that carriers of LCAT gene mutations exhibit increased carotid artery wall thickening as assessed by 3.0 Tesla MRI compared to age-matched controls. This finding has clinical relevance since carotid artery wall thickening is associated with an increased risk of cardiovascular events\textsuperscript{17,18}. Whereas previous carotid ultrasound studies were unable to bring consensus on the impact of LCAT gene mutations on carotid atherosclerosis\textsuperscript{10,11}, the current MRI data lend support to the concept that decreased LCAT function, as a result of LCAT gene mutations, is associated with accelerated atherogenesis.

The aim of our study was to test the hypothesis that decreased LCAT function is associated with accelerated atherogenesis. To this purpose, we investigated two parameters of atherosclerosis with MRI: arterial wall thickening, and presence of plaque components. First, the data show that the carriers of LCAT gene mutations have thickened carotid artery walls compared to controls with significant increases of NWI, MWA and TWV. These differences remained statistically significant after adjustments for age, gender, hypertension, LDL-c, BMI, smoking, family history of cardiovascular disease (CVD) and clustering of genetic and/or environmental factors in families. Second, carriers presented with a 32% increased prevalence of PC and 16.5 times larger total PC volume compared to controls. These are features of carotid artery plaques which have been associated with increased CV event rates\textsuperscript{17,18}. Combined, these findings point towards accelerated atherogenesis in individuals with reduced LCAT function.

We also assessed atherogenesis by means of carotid ultrasound IMT measurements. Whereas IMT parameters tended towards an increase in carriers, the differences did not reach statistical significance. The latter most likely pertains to lack of power, as attested to by the significant MRI findings. In fact, we previously showed that the measurement variability of carotid 3.0 Tesla MRI is less compared to that of ultrasound IMT\textsuperscript{12}.

Various studies have attempted to unravel the relation between LCAT function and CVD in humans. Recent genome wide-association studies (GWAS) revealed that LCAT
correlates to HDL-c levels, but not to CVD risk\textsuperscript{19,20}. The prevalence of single nucleotide polymorphisms (SNPs) in the population, however, is low and it is unknown if these SNPs actually affect LCAT function. Moreover, total variation in HDL-c explained by LCAT SNPs was very small. Therefore, GWAS may not be the most sensitive technique to detect a relation between LCAT and CVD. In cross-sectional studies in patients with either angiographically documented CAD or acute myocardial infarction, both decreased and increased LCAT activity have been observed\textsuperscript{21-23}. A recent prospective nested case-control study studied LCAT concentration in 2785 healthy subjects with a follow-up of 6 years\textsuperscript{24}. In this study, LCAT levels did not differ between cases and controls. However, since the variation of LCAT concentration in this population was small, a potential contribution of LCAT to CVD risk may have been overcome by other risk factors, such as diabetes mellitus, smoking, blood pressure, BMI and LDL-c.

Two prior imaging studies have addressed the relation between LCAT and atherosclerosis in carriers of LCAT gene mutations using carotid IMT. Hovingh \textit{et al.} showed that carotid IMT was significantly increased in the carriers\textsuperscript{10}, while Calabresi \textit{et al.} showed the opposite with carotid IMT being significantly decreased in carriers\textsuperscript{11}. This apparent discrepancy may be explained by differences in the populations. The carriers in the current study and in Hovingh's study predominantly had a different type of LCAT mutation than those in the study of Calabresi. Both in Hovingh's as well as the present study, the vast majority of carriers of LCAT gene mutations exhibited loss of $\alpha$-activity (LCAT activity on HDL), whereas Calabresi \textit{et al.} predominantly investigated individuals with loss of function mutations of $\alpha$- and $\beta$-activity (LCAT activity on HDL and LDL). Accordingly, LDL-c levels in the current study were 23\% higher compared to those in Calabresi's study (127 mg/dl vs 103 mg/dl respectively). In fact, the average LDL-c of the patients studied by Calabresi was on target of the National Cholesterol Education Program (NCEP) ATPIII guidelines. In fact, the absence of the primary trigger of atherosclerosis, that is increased LDL-c, in Calabresi's study may be an important explanation why they did not observe increased atherogenesis in their familial LCAT deficiency patients.

To date, it has been unclear how to monitor and treat carriers of LCAT gene mutations. Whereas ideally a prospective randomized controlled trial is required to settle this issue, this type of evidence is unlikely to become available given the rareness of the disease. Considering the present data combined with the fact that decreased levels of HDL-c are strongly associated with CVD risk, we propose to closely monitor as well as treat CVD risk factors in both heterozygous as well as homozygous patients with LCAT gene mutations. The current study results support a distinct role of LCAT in atherogenesis. Whether this effect relates to the effects on HDL-c or to e.g. the anti-inflammatory properties\textsuperscript{25,26} that are attributed to LCAT, cannot be determined by the current study.
Limitations
A limitation inherent to this type of small cohort studies is referral bias of the examined individuals. Carriers and family controls were recruited with the same method, while unrelated controls were recruited by advertisement. Nonetheless, related and unrelated controls were similar in terms of NWI, so it is unlikely that differences in recruitment methods introduced bias. Furthermore, we have attempted to minimize this effect by excluding patients with pre-existent CVD and included only carriers identified in families of which the probands were asymptomatic for CVD. These probands presented either with marked corneal clouding, or low HDL-c levels identified through (random) screening for CVD risk factors.

Conclusions
The present study shows that carriers of LCAT gene mutations have increased carotid atherosclerosis compared to controls. Our data have two clinical implications. First, as carriers of LCAT gene mutations have experienced lifelong exposure to marked dyslipidemia and the current data suggest that they are at increased risk of developing atherosclerosis, close monitoring and treatment of CVD risk factors is advocated. Second, based on these data it is tempting to speculate that increasing LCAT activity is an interesting target to reduce cardiovascular risk27,28.

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