Genetic disorders of HDL metabolism: from model to mechanism

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Citation for published version (APA):
Chapter 12

Submitted for publication

Patients with low HDL-cholesterol caused by mutations in LCAT have increased arterial stiffness

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Chapter 12

Abstract

A low high-density lipoprotein cholesterol level and increased arterial stiffness are both strong predictors of cardiovascular disease. Carriers of mutations in \textit{LCAT}, encoding lecithin:cholesterol acyl transferase, are exposed to lifelong low high-density lipoprotein cholesterol levels. Because structural imaging studies of atherosclerosis were inconclusive, we investigated functional alterations in large arteries by measuring arterial stiffness by carotid-femoral pulse wave velocity in 45 carriers of \textit{LCAT} mutations (mean age±SD 46±13 yrs) and 45 age-matched controls. Probands referred with established cardiovascular disease were excluded.

In carriers, high-density lipoprotein cholesterol was lower (32±12 vs. 59±16 mg/dl; p<0.0001) and triglycerides higher (median 116 [IQR 80-170] vs. 71 [IQR 53-89] mg/dl; p<0.001) vs. controls. Pulse wave velocity was higher in carriers vs. controls (7.9±2.0 m/s vs. 7.1±1.6 m/s; p<0.01). This difference retained significance in multivariate analysis after adjustment for age, sex, systolic blood pressure and body mass index, and also after exclusion of matched pairs of which carrier or control had cardiovascular disease. Also, pulse wave velocity was correlated with wall thickening of the carotid arteries assessed by 3.0 Tesla magnetic resonance imaging and ultrasound in carriers (Pearson’s R 0.54; p<0.001 and 0.50; p<0.001, respectively), in controls (R 0.58, p<0.001 and 0.36, p<0.04, respectively), and in the groups combined (R 0.56, p<0.001 and 0.47, p<0.001, respectively).

In conclusion, pulse wave velocity is increased in patients with low high-density lipoprotein cholesterol levels due to \textit{LCAT} mutations and is also associated with carotid wall thickening. It may be a useful adjunct to assess cardiovascular risk in these patients.
Introduction

A low plasma level of high-density lipoprotein cholesterol (HDL-c) is a strong and independent predictor of cardiovascular disease (CVD)\(^1\)\(^-\)\(^2\). Carriers of mutations in \(\text{LCAT}\), encoding lecithin:cholesterol acyl transferase, are exposed to lifelong low HDL-c levels. \(\text{LCAT}\) is a crucial enzyme in HDL metabolism produced in the liver and small intestine.\(^3\)\(^\text{-}^4\) Upon secretion into the circulation, it associates predominantly with HDL where it esterifies free cholesterol using apolipoprotein A-I (apoA-I) as a cofactor\(^4\). Carriers of \(\text{LCAT}\) mutations have been reported to suffer from increased atherosclerosis but this is not undisputed. In this respect, two ultrasound studies reached opposite conclusions, one study reporting increased intima media thickness (IMT) in the carotid arteries of carriers as a surrogate outcome for atherosclerosis\(^5\) and the other reporting decreased IMT in carotid arteries of carriers\(^6\). Using 3.0 T magnetic resonance imaging of the carotid arteries, we recently found support for the conclusion of the first study (see Chapter 11 of this thesis). However, whether the observed atherosclerotic structural changes in \(\text{LCAT}\) mutation carriers are associated with functional alterations of large arteries is undetermined. In carriers of \(\text{ABCA1}\) mutations, who also suffer from low HDL-c levels, endothelial function of the brachial artery as assessed by flow mediated dilation was reduced and infusion of reconstituted HDL (rHDL) restored endothelial function in these patients\(^7\). Also in patients with type 2 diabetes and diabetic dyslipidemia, rHDL infusion restored endothelial function\(^8\). This direct temporary improvement in vascular tone by HDL infusion is probably mediated by induction of endothelial nitric oxide synthase via the HDL-receptor scavenger receptor B-1\(^9\).

In the present study, we examined the effect of reduced \(\text{LCAT}\) function on long-term functional alterations of large arteries by assessment of arterial stiffness, another strong and independent predictor of cardiovascular disease (CVD)\(^10\)\(^-\)\(^13\). We studied carotid-femoral PWV, the gold standard of non-invasive measurement of arterial stiffness\(^14\), in patients with \(\text{LCAT}\) gene mutations and age-matched controls. In addition to our primary study objective, we also examined the association between arterial stiffness and structural changes in large arteries by assessment of wall thickening of the carotid arteries using B-mode ultrasound (intima media thickness [IMT]) and 3.0 Tesla magnetic resonance imaging (MRI).

Methods

Study design and participants

The design of this study has been described in detail in Chapter 11. In brief, the study was conducted at the Academic Medical Center in Amsterdam, The Netherlands from October 2008 to October 2009. The study protocol was approved by the local institutional review board and all subjects provided written informed consent. Patients molecularly diagnosed with \(\text{LCAT}\) mutations were enrolled in this study, irrespective of their age and
sex. In order to limit referral bias, we excluded family probands who were referred to our outpatient clinic with clinically manifest CVD. For the control group, unaffected family members of the included carriers were asked to participate in the study, comprising first, second or third degree family members or spouses. These controls were included if they could be individually matched for age to carriers. As insufficient numbers of family controls volunteered, the control group was complemented with unrelated controls recruited by advertisement. Family history of CVD, presence of cardiovascular risk factors, use of medication and alcohol were assessed. 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.

**Blood pressure and arterial stiffness**

Participants visited the hospital after an overnight fast and were asked to refrain from smoking (if applicable) at least three hours before the visit. All measurements were carried out in supine position after 15 minutes rest in a quiet, temperature-controlled room. All hemodynamic measurements were performed by a single investigator (BvdB) who was blinded for the genetic status of the participants. Brachial blood pressure was measured 3 times at 1-minute intervals in supine position at the right arm after 15 minutes rest using a validated oscillometric device (Omron 705IT) in a quiet and temperature controlled room. The mean of the last 2 measurements was used for analysis. Measurements of carotid-femoral pulse wave velocity (PWV) were performed with the SphygmoCor system (Atcor Medical Pty Ltd, West Ryde, Australia). Pulse waveforms were recorded at the right carotid and femoral artery sequentially. Wave travel distance was calculated by subtracting carotid artery – suprasternal notch distance from suprasternal notch – femoral artery distance. Measurements were conducted in duplicate and means were used for analysis.

**Carotid ultrasound and magnetic resonance imaging**

Ultrasound scans of the carotid arterial wall were assessed as surrogate outcome for atherosclerosis according to a standardized protocol\textsuperscript{15}. Values given are means of left and right common carotid artery, carotid bulb and internal carotid. Bilateral 3.0 T MRI scans of the carotid arteries were obtained as previously in Chapter 11. Normalized wall index (NWI) represents mean vessel wall area normalized for the transverse size of the vessel, measured as the outer wall area. Carotid IMT and NWI were assessed during the same hospital visit as PWV.

**Plasma lipids**

Blood was obtained after overnight fasting and stored using standardized protocols. Plasma total cholesterol, HDL-c and triglyceride levels were analyzed using a commercially available enzymatic method (Westburg, USA) on a Cobas Mira autoanalyzer (Roche,
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Switzerland). Low density lipoprotein cholesterol (LDL-c) levels were calculated using the Friedewald equation.

Statistical analysis

Data are expressed as means±standard deviations (SD), median (interquartile range [IQR]) or numbers and percentages where appropriate. Differences between carriers and controls were assessed by comparison of continuous data: independent t-tests for parametric data and Mann-Whitney for non-parametric data; chi-square test was applied to compare categorical data. Missing data were imputed with the mean values of the group. Correlations of PWV, cIMT and NWI are expressed as Pearson’s correlation coefficient. A multivariate model was used with generalized estimating equations in the SAS procedure GENMOD to account for potential confounders, i.e. age, gender, SBP and body mass index (BMI) and correlations within families due to clustering of genetic and/or environmental factors, using stepwise backward elimination. Statistical analyses were done using SPSS (Statistical Package for the Social Sciences) version 16.0 and SAS package version 9.1 (SAS Institute Inc., Cary, NC USA). The authors had full access to the raw data and take responsibility for its integrity.

Results

Population characteristics

We studied 45 carriers of LCAT gene mutations (from 15 families of Dutch descent) and 45 age-matched controls of which 19 were family members and 26 were unrelated individuals. Of the carriers, 43 had one mutant LCAT allele, while 1 was homozygous for T147I and one was compound heterozygous for T147I and V333M. Both the homozygote and the compound heterozygote had previously presented with corneal opacification and HDL deficiency, without the presence of proteinuria, and had thus been diagnosed with fish eye disease (FED).

Table 1 summarizes the demographic, lifestyle, and clinical characteristics of carriers and controls. Proper matching for age was achieved for carriers of an LCAT mutation and controls. The percentage of males did not differ between the two groups. On average, carriers had a 1.9 kg/m2 higher BMI (p<0.02). More carriers had experienced cardiovascular events and received statin treatment than controls, and carriers tended to receive antihypertensive treatment more frequently than controls. Systolic/diastolic blood pressures were 135±15 / 79±9 mmHg for LCAT mutation carriers and 131±13 / 77±9 mmHg for controls (p=0.12 / p=0.19). HDL-c levels in carriers of LCAT mutations were lower compared to controls (32±12 vs. 59±16 mmol/l, p<0.0001), while LDL-c levels were identical (125±35 vs. 125±31 mmol/l, p=0.65). Triglycerides were higher in carriers of LCAT mutations (116 [IQR 80-170] vs. 71 [IQR 53-89] mmol/l, p<0.001).
Pulse wave velocity in carriers of LCAT mutations and age-matched controls

PWV was higher in carriers of a mutation in LCAT compared to controls, 7.9±2.0 vs. 7.1±1.6 m/s \((p<0.01)\), see Figure 1. In a multivariate regression model that adjusted for age, sex, SBP, BMI and family clustering, this difference retained statistical significance \((p<0.01)\). After exclusion of matched pairs of which the carrier (n=6) and/or control (n=1) had suffered from CVD, the PWV of the 38 remaining carriers remained significantly higher compared to the respective matched controls (7.7±2.0 vs. 6.9±1.6 m/s, \(p<0.05\)). Again, this difference retained significance after adjustment for age, sex, SBP and family clustering in the multivariate regression model \((p<0.01)\).

PWV and carotid wall thickening assessed by ultrasound and 3.0 T MRI

In a random set of 36 carriers and 36 controls individually matched for age, carotid wall thickening had also been assessed by ultrasound (cIMT) and by using 3.0 T MRI scanning. (see Chapter 11), cIMT tended to be higher in carriers (0.85±0.08 mm) compared to controls (0.70±0.04 mm, \(p = 0.07\)). NWI was significantly higher in carriers (0.34±0.08) compared to controls (0.31±0.04, \(p = 0.04\)). Table 2 and Figure 2 show that PWV correlated well with both cIMT and carotid NWI obtained by MRI. We observed strong correlations in carriers: Pearson’s R for PWV to cIMT: 0.50, \(p<0.001\), Pearson’s R for PWV to NWI: 0.54, \(p<0.001\). In controls PWV was also correlated to cIMT and NWI: Pearson’s R for PWV to cIMT: 0.36, \(p<0.04\), and for PWV to NWI: Pearson’s R 0.58, \(p<0.001\).

### Table 1. Clinical characteristics of LCAT mutation carriers compared to unaffected controls

<table>
<thead>
<tr>
<th></th>
<th>LCAT mutation carriers</th>
<th>Matched controls</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>45</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>46 ± 13</td>
<td>45 ± 14</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Male sex, n (%)</strong></td>
<td>35 (78%)</td>
<td>29 (64%)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>26.3±4.1</td>
<td>24.6±3.0</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Smoking, n (%)</strong></td>
<td>7 (16%)</td>
<td>5 (11%)</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>History of CVD, n (%)</strong></td>
<td>6 (13%)</td>
<td>1 (2%)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>17 (38%)</td>
<td>13 (29%)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td>135 ± 15</td>
<td>131 ± 13</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>79 ± 9</td>
<td>77 ± 9</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Statin users, n (%)</strong></td>
<td>16 (36%)</td>
<td>1 (2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total Cholesterol, mg/dl</strong></td>
<td>172 ± 47</td>
<td>191 ± 35</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>LDL-c, mg/dl</strong></td>
<td>125 ± 35</td>
<td>125 ± 35</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>HDL-c, mg/dl</strong></td>
<td>32 ± 12</td>
<td>59 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dl</strong></td>
<td>116 [IQR 80-170]</td>
<td>71 [IQR 53-89]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are indicated as means ± SD or median [IQR] unless otherwise indicated.
Arterial stiffness is increased in LCAT deficiency

Figure 1. Carotid-femoral pulse wave velocity in carriers of an LCAT mutation and matched controls

Boxed values are means ± SD for PWV.
*Difference retained significance in multivariate analysis, independent of age, sex, systolic blood pressure and BMI and correlations within families due to clustering of genetic and/or environmental factors, and also after exclusion of 6 matched pairs with members with a history of CVD.

Figure 2. Scatter plot of PWV to IMT (carotid ultrasound) and NWI (carotid MRI)

2a: Carotid-femoral PWV plotted to carotid IMT. Continuous line indicates correlation between PWV and cIMT in controls (open symbols; n=36), Pearson’s R = 0.36 (p < 0.04); dashed line indicates correlation between PWV and cIMT in LCAT mutation carriers (closed symbols; n = 36), Pearson’s R = 0.50 (p < 0.001) (see Table 2 for combined analysis).

2b: Carotid-femoral PWV plotted to carotid NWI, assessed by 3.0 T MRI. Continuous line indicates correlation between PWV and NWI in controls (open symbols; n=36), Pearson’s R = 0.58 (p<0.001); dashed line indicates correlation between PWV and NWI in LCAT mutation carriers (closed symbols; n=36), Pearson’s R = 0.54 (P < 0.001) (see Table 2 for combined analysis).
Discussion

In this well matched case-control study, we demonstrate that aortic pulse wave velocity is increased in LCAT mutation carriers compared to controls, indicative of increased arterial stiffness in these patients. This difference retained significance in multivariate analysis and after exclusion of patients with CVD. In addition to our primary study objective, this study also reveals a strong correlation between arterial stiffness and thickness of the carotid arterial wall as assessed by ultrasound (IMT) and 3.0 T MRI.

Relevant to the question whether LCAT is a feasible target for HDL-enhancing strategies is the matter of atherogenesis in carriers of LCAT mutations. Two ultrasound studies have reached opposite conclusions.\(^5\)\(^6\).

In order to more dynamically investigate the condition of large arteries in carriers of LCAT mutations in our center, we assessed carotid-femoral pulse wave velocity, considered the gold standard of non-invasive arterial stiffness measurements.\(^14\) The observed increase in arterial stiffness in LCAT mutation carriers might result from accelerated atherosclerosis in these patients, which in turn might be caused by decreased reverse cholesterol transport from the vascular wall due to the impaired maturation of HDL. We did, however, not observe a correlation of PWV with HDL-c (not in carriers, nor in controls nor in the groups combined; data not shown), although this might be explained by large standard deviations in both parameters. Previously, PWV was found to be inversely related to HDL-c levels.\(^16\)-\(^18\). In a population-based study, 122 middle-aged subjects with low HDL-c levels had significantly higher PWV independent of age, sex, physical activity and smoking status, compared to 795 subjects with normal HDL-c levels.\(^16\) In a cross-sectional study among postmenopausal women, HDL-c was also reported to be inversely and independently related to PWV.\(^17\) However, neither of these two studies adjusted for SBP, an important determinant of PWV.\(^19\).

Our study shows increased levels of plasma triglycerides in carriers compared to controls, a finding that has also been reported by others\(^20\) and which is possibly caused...
Arterial stiffness is increased in LCAT deficiency by increased de novo lipogenesis in the liver\textsuperscript{21}. However, in a recent population-based study, PWV was not associated with triglycerides\textsuperscript{18}, making it unlikely that the increase in triglycerides contributes to the increased PWV in carriers. Lastly, we identified a slight increase in BMI in carriers. However in multivariate analysis, BMI did not affect the relation between LCAT genotype and PWV. This is in accordance with a systematic review, indicating that risk factors other than age and blood pressure contribute only modestly to arterial stiffness\textsuperscript{19}.

Arterial stiffness predicts CVD independently of traditional cardiovascular (CV) risk factors\textsuperscript{10-13}. In a Danish population-based study of 1968 participants, PWV significantly improved CV risk prediction, especially in subjects with low estimated CVD risk\textsuperscript{11}. In a recent analysis of the Framingham Heart Study, CVD risk was increased by 48\% per standard deviation increase in PWV\textsuperscript{10}.

In a recent editorial, Wilkinson et al postulate three hypotheses concerning the nature of the relation between arterial stiffness and CVD\textsuperscript{22}. First, the relationship might be causal: arterial stiffness might promote CVD independent of other CV risk factors, by causing: 1) increased wave reflection, leading to elevated central aortic systolic pressure and pulse pressure which in turn increases left ventricular work load and (2) altered hemodynamics and shear stress, stimulating the formation of atherosclerotic plaques. Secondly, PWV could reflect the integrated consequences of established risk factors over time. Thirdly, PWV might simply reflect the burden of atherosclerotic plaque in the arterial wall. In this respect, we observed strong relationships between arterial stiffness and carotid wall thickness assessed by ultrasound and MRI, in line with previous observations in the general population\textsuperscript{12} and in patients with type 2 diabetes mellitus\textsuperscript{23}. To study the basis of the relationship between PWV and CVD, large prospective studies are called for\textsuperscript{22}.

Two aspects of our study merit closer consideration. First, to minimize potential referral bias in our case control study, we excluded \textit{LCAT} gene carriers who were referred to our outpatient clinic with a history of CVD. We only included carriers identified in families of which the probands were asymptomatic for cardiovascular disease. The use of statins and antihypertensive medication is considerably higher in \textit{LCAT} mutation carriers, and since both statins\textsuperscript{24-27} and antihypertensive drugs\textsuperscript{28} have shown to decrease PWV, the actual difference in PWV might even be larger between carriers and controls.

\textbf{Perspectives}

In addition to statins and antihypertensive medication, increasing HDL-c through lifestyle modifications or pharmacological treatment might also reduce arterial stiffness by improvement of cholesterol efflux, reduction of inflammation or improvement of endothelial function\textsuperscript{16, 29, 30}. In future studies, it would be of interest to examine whether treatment specifically aimed at increasing HDL-c will affect arterial stiffness.
This first report of a strong relation between PWV and arterial wall thickness assessed by MRI underscores the interrelation between arterial stiffness and arterial wall thickening in atherogenesis and provides a solid basis for large prospective studies designed to characterize the role of arterial stiffness in atherogenesis.

In conclusion, carriers of \textit{LCAT} mutations characterized by low HDL-c levels have increased arterial stiffness compared to unaffected controls. This study supports close clinical monitoring of cardiovascular risk factors in carriers of \textit{LCAT} mutations. Because PWV is a reproducible, non-invasive and readily applicable functional measure of arterial stiffness, it may be an useful method to assess and monitor the increased CVD risk in these patients. Lastly, our data bolsters the notion that LCAT might be an interesting target to reduce cardiovascular risk and supports LCAT enhancing strategies currently evaluated in preclinical studies\textsuperscript{31-33}.

\textbf{Funding sources}

Part of the study was sponsored with an educational research grant by Merck Sharp and Dohme (MSD, USA) and by a grant from the Dutch Heart Foundation (2008B070). A.G. Holleboom is supported by a grant of the Netherlands Organisation for Scientific Research (NWO; project number 021.001.035). Dr. Kastelein is a recipient of the Lifetime Achievement Award (2010) of The Dutch Heart Foundation (2010 T082).
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