SHORT COMMUNICATION

Carotid Intima-Media Thickness in Children with Familial Hypercholesterolemia

D. Meeike Kusters1,2, A. Wiegman2, John J. Kastelein1, Barbara A. Hutten3

Departments of 1Vascular Medicine; 2Pediatrics, and; 3Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, the Netherlands.

Running title: Carotid Intima-Media Thickness in Children with FH

Subject codes:
[90] Lipid and lipoprotein metabolism
[150] Imaging

Address correspondence to:
Dr. Barbara A. Hutten
Academic Medical Center
Department of Clinical Epidemiology
Biostatistics and Bioinformatics
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Tel: 00 31 20 566 6881
Fax: 00 31 20 6912683
b.a.hutten@amc.uva.nl

In September 2013, the average time from submission to first decision for all original research papers submitted to Circulation Research was 13.2 days.
ABSTRACT

**Rationale:** Familial hypercholesterolemia (FH) predisposes patients to premature cardiovascular disease, with the process of atherosclerosis initiated in early childhood.

**Objective:** In part of an ongoing trial to assess the efficacy and safety of rosuvastatin in FH children aged 6 to 17, we report the differences in carotid intima-media thickness (cIMT) at baseline between FH children and their unaffected siblings.

**Methods and Results:** B-mode ultrasound measurements of the carotid artery were made in 196 children with FH and 64 of their siblings. Mean (±standard error) cIMT in children with FH was significantly greater than that of unaffected siblings (0.398 ± 0.052 mm vs 0.377 ± 0.045 mm, \( P < 0.001 \)). A significantly greater cIMT value was observed before the age of 8 years. Multivariable analyses showed that age, male sex and presence of FH were independent predictors of cIMT.

**Conclusions:** The difference in mean cIMT between children with FH and their unaffected siblings may be significant as early as age 8 years. This study confirms the need for early cholesterol-lowering in this high risk population. These patients participating in a carefully monitored study will help assess the long-term efficacy on cIMT and safety of statin therapy in young children.

**Keywords:** Familial hypercholesterolemia, pediatrics, statins, carotid intima-media thickness

**Nonstandard Abbreviations and Acronyms**
- cIMT: carotid intima-media thickness
- CVD: cardiovascular disease
- FH: familial hypercholesterolemia
- LDL-C: low-density lipoprotein cholesterol

INTRODUCTION

In children with familial hypercholesterolemia (FH), functional and morphological changes of the arterial wall become apparent at early age, indicating that the atherosclerotic process begins shortly after birth.\(^1,2\) These findings have led to the hypothesis that effective low-density lipoprotein cholesterol (LDL-C) lowering therapy, usually statin treatment, should be initiated in childhood, generally from the age of 10\(^3\) or even 8\(^4\) years, to reduce the incidence of cardiovascular disease (CVD) in later years. Carotid intima-media thickness (cIMT), as assessed by B-mode ultrasound, is considered to be a valid surrogate marker of cardiovascular disease.\(^5\) Previously it was shown that age, sex and LDL-C were strong and independent predictors of cIMT in children. Furthermore, children with FH exhibit a much more rapid increase in cIMT with age than their healthy siblings, with a statistically significant deviation in cIMT from the age of 12.\(^2\) We hypothesize, however, that with improvements in ultrasound technology, these earlier findings could be much improved perhaps to detect even earlier changes in cIMT in FH children. Therefore, as part of an ongoing clinical trial (NCT01078675) to evaluate the efficacy and safety of rosuvastatin in FH children, we report the baseline cIMT of these FH children compared with that of their unaffected siblings.
METHODS

Between January 2010 and January 2011, children aged 6 to less than 18 years were recruited from 14 Lipid Clinics in the Netherlands, Belgium, Norway, Canada and the United States. Children were eligible if they had heterozygous FH and fasting LDL-C >4.92 mmol/L or LDL-C >4.10 mmol/L if there was a family history in a first or second degree relative of premature CVD. FH was defined by a documented genetic defect or documented evidence of FH in a first-degree relative (LDL-C>4.9 mmol/L in an adult; LDL-C >4.1 mmol/L in a child <18 years of age). Siblings were eligible if they had a documented absence of the genetic defect or a documented LDL-C of <3.00 mmol/L, without lipid-lowering medication. The protocol was reviewed and approved by each participating site’s Institutional Review Board, and written informed consent was obtained from participants from the age of 12 years and all parents. The trial was registered with NCT as NCT01078675.

In all FH subjects, a full physical examination was done and venous blood samples were taken. Plasma lipid concentrations were measured with CDC standardized assays. Ultrasound measurements on all participants were made using the Acuson Sequoia 512 instrument (Siemens AG, Malvern, Pa, and Erlangen, Germany) equipped with an 85 Mhz linear array transducer. All sonographers were trained and certified before their participation in the study. B-mode scans of the right and left common carotid artery, carotid bulbs and internal carotid artery were obtained according to strict protocol specifications. The image readings were randomly assigned to image analysts for qualitative and quantitative evaluation. Image analysts were blinded to subject. Mean carotid IMT was defined as the mean IMT of the right and left common carotid, the carotid bulb, and the internal carotid far wall segments. For the subjects of whom the scan of one of the segments had failed, mean IMT was calculated as the mean of the other two segments.

We assessed differences in demographic and cIMT between FH subjects and siblings by logistic or linear regression analysis with generalised estimating equations in the SAS procedure GENMOD to account for correlations within families. The same procedure was used to univariately explore the association between mean c-IMT (response variable), and demographic and clinical characteristics (explanatory variables). Multivariable analysis was used to identify independent predictors after stepwise backward selection. An equation for difference in cIMT (ΔIMT) was derived by subtracting the equation for children with FH (if GROUP=1): ‘IMTFH = β1AGE + β2GROUP + β3AGE×GROUP = β1AGE + β2 + β3AGE’ from the equation for their siblings (if GROUP=0): ‘IMTSIB = β1AGE’, as described previously2. This calculation resulted in: ΔIMT= β2 + β3AGE. Beta’s and standard errors (SE) were derived from the output of a linear regression analysis for the whole group (n=260). Variables with a skewed distribution were log-transformed. For statistical analyses, we used SAS release 9.2 (SAS Institute, Cary, NC, USA) and SPSS 18.0 software (SPSS Inc, Chicago, IL).

RESULTS

In total, 196 children with FH were enrolled. From 53 families of these children, 64 unaffected siblings were included as controls. Children with FH and their siblings were comparable with respect to age (mean age (SD): 12.1 ± 3.3 years vs. 11.9 ± 3.5, respectively; P=0.32) and gender (males: 44% vs. 52%, respectively; P=0.39) (Table 1). Mean cIMT (± standard error) was 0.398 ± 0.052 mm in children with FH and 0.377 ± 0.045 mm in healthy siblings, which remained significant after adjustment for age, sex and family relations (P<0.001). Associations between baseline variables of FH patients and cIMT are shown in Table 2. After stepwise backward elimination, multivariable regression analysis identified age and sex as independent predictors for cIMT. When siblings were also included in the multivariable model, age (regression coefficient [SE] of 0.004 [0.001], P<0.001), sex (regression coefficient [SE] of
0.022 [0.006] for males, \( P < 0.001 \) and FH status (regression coefficient [SE] of 0.022 [0.007] for FH, \( P = 0.002 \)) revealed to be independent predictors for cIMT.

In Figure 1, the difference in cIMT between children with FH and their siblings was plotted against age. A significant difference in cIMT between FH and controls was observed before the age of 8 years. In fact, FH patients showed an increase of 0.0041 mm/year compared to an increase of 0.0032 mm/year in siblings.

DISCUSSION

In the current study, we show that children with FH have greater mean cIMT values as compared to their unaffected siblings before the age of 8 years and we also report that age, gender and the presence of FH were independent predictors of carotid arterial wall atherosclerosis. These results reaffirm the findings of our previous study, but extend them to the age of 8 years, as compared to 12 years in our previous study.

In our previous study we found that the difference in cIMT increased with age between the FH patients and siblings. We therefore expected a similar increase in the current study. However, our current data were not definitive. Interestingly, although the progression with age is almost similar between the FH children of the previous and the current study (0.005 mm/years vs. 0.0041 mm/year, respectively), the mean progression with age in the siblings is currently greater: \(<0.001\) mm/year in the previous study versus 0.0032 mm/year in the current study (all adjusted for age and family relations). As all non-FH controls were siblings of the FH patients, genetic and environmental variation between the two groups was kept to a minimum. Generally speaking, apart from FH, no major differences in risk factors for CVD would be expected between the two groups. However, as the first study was performed more than 10 years before the current one, one possible explanation could be the rise in childhood obesity during the last decade. This might be more manifest in siblings, as children with FH receive lifestyle advice on a frequent basis. If the children in the current cohort are indeed more obese, with all its metabolic sequelae, this might explain the faster progression in these sibs. However, to further delineate these data in siblings, more imaging studies that include such healthy siblings as controls are needed.

Furthermore, despite the appearance of significant differences in the mean IMT results between FH patients and unaffected siblings, individual children cannot be distinguished on the basis of their cIMT results due to the extensive overlap in the data.

In our current study, in children from aged 6 to 18 years, the difference in cIMT between FH subjects and unaffected siblings may be significant as early as age 8. This finding again underscores the importance of early LDL-C lowering therapy\(^6,7\), usually statins, possibly from a younger age than is currently recommended, as treatment should preferably be started before the process of atherosclerosis is detectable. Clinical trials such as the current rosvustatin long-term cIMT trial, should focus on treatment of these younger children, to explore the efficacy and safety of statin treatment in these FH patients. At the same time, extensive follow-up studies are needed to establish the long-term efficacy, safety and tolerability of statin therapy initiated in childhood and to further address the question of the appropriate age to start statin therapy.
ACKNOWLEDGEMENTS
The authors thank the children and parents who are participating in this study, as well as the investigators and staff at study sites for their significant contributions to this study. The authors thank Dr. Evan Stein for his important contribution to this study and his valuable input for this manuscript.

SOURCES OF FUNDING
This study was funded by AstraZeneca.

DISCLOSURES
J.J.P. Kastelein has received grant support from AstraZeneca, Pfizer, Roche, Novartis, Merck, Merck–Schering-Plough, Isis, Genzyme, and Sanofi-Aventis; lecture fees from AstraZeneca, GlaxoSmithKline, Pfizer, Novartis, Merck–Schering-Plough, Roche, Isis, and Boehringer Ingelheim; and consulting fees from AstraZeneca, Abbott, Pfizer, Isis, Genzyme, Roche, Novartis, Merck, Merck–Schering-Plough, and Sanofi-Aventis.

REFERENCES


FIGURE LEGEND

Figure 1. Panel A: Correlation between age and c-IMT for both children with familial hypercholesterolemia and unaffected siblings. Panel B: Difference in mean carotid intima-media thickness and 95% confidence interval between children with familial hypercholesterolemia and unaffected siblings, plotted against age, taking account for family relations (mean=thick line, 95% confidence interval=dashed lines).
Table 1: Demographic and laboratory data of children with familial hypercholesterolemia and age, gender and race of unaffected siblings

<table>
<thead>
<tr>
<th></th>
<th>FH N=196</th>
<th>nonFH n=64</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>12.1 ± 3.3</td>
<td>11.9 ± 3.5</td>
</tr>
<tr>
<td><strong>Male gender, n (%)</strong></td>
<td>88 (44)</td>
<td>33 (52)</td>
</tr>
<tr>
<td><strong>Caucasian, n (%)</strong></td>
<td>176 (90)</td>
<td>53 (85)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>19.3 ± 4.4</td>
<td>-</td>
</tr>
<tr>
<td><strong>Blood pressure (mmHg)</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systolic</td>
<td>107.5 ± 10.9</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic</td>
<td>64.0 ± 8.1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lipids (mmol/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>7.87 ± 1.34</td>
<td>-</td>
</tr>
<tr>
<td>LDL-C</td>
<td>6.10 ± 1.26</td>
<td>-</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.30 ± 0.33</td>
<td>-</td>
</tr>
<tr>
<td>TG*</td>
<td>0.90 (0.67 – 1.25)</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are given as mean and standard deviation or otherwise indicated
*Given as median (interquartile range). BMI: body-mass index; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides

Table 2: Determinants of carotid intima-media thickness in children with familial hypercholesterolemia at baseline

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (SE)</td>
<td>P</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>0.004 (0.001)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
<td>0.21 (0.006)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>0.002 (0.001)</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong>*</td>
<td>0.001 (&lt;0.001)</td>
<td>0.064</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dL)</strong></td>
<td>&lt;0.001(&lt;0.001)</td>
<td>0.787</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
<td>&lt;0.001 (&lt;0.001)</td>
<td>0.197</td>
</tr>
<tr>
<td><strong>TG (mg/dL)†</strong></td>
<td>0.002 (0.008)</td>
<td>0.841</td>
</tr>
<tr>
<td><strong>Previous statin use</strong></td>
<td>0.19 (0.011)</td>
<td>0.100</td>
</tr>
</tbody>
</table>

*Mean arterial blood pressure = [systolic blood pressure + (2 x diastolic blood pressure)]/3; †Log-transformed. SE: standard error; BMI: body-mass index; MAP: mean arterial bloodpressure; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides.
Novelty and Significance

What Is Known?

- Familial hypercholesterolemia (FH) is a common disorder, characterised by severely elevated cholesterol levels from birth onwards.

- High cholesterol levels lead to atherosclerosis, which can be visualized in an early stage by measuring the intima-media thickness of the carotid artery (cIMT)

What New Information Does This Article Contribute?

- This study shows that young children with FH already have a significant greater cIMT than their siblings without FH.

- This study underlines the importance of treatment of children with FH, to lower cholesterol levels and prevent atherosclerosis and cardiovascular disease.

FH is characterised by severely elevated cholesterol levels from birth onwards. When left untreated, patients are at high risk of premature cardiovascular disease. Subclinical atherosclerosis can be visualized by measuring cIMT. In this study, we found that children with FH may have a significant greater cIMT than their unaffected siblings from about the age of 8 years. Our current results reaffirm the findings of a previous study, and extend them even to a younger age. This study underlines the importance of lipid-lowering treatment in children with FH and future trials should focus on the efficacy and safety of starting treatment before atherosclerosis is detectable.
Carotid Intima-Media Thickness in Children with Familial Hypercholesterolemia
Dorothe M Kusters, Albert Wiegman, John J Kastelein and Barbara A Hutten

Circ Res. published online November 5, 2013;
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/early/2013/11/05/CIRCRESAHA.114.301430

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/