Pediatric implications of heterozygous familial hypercholesterolemia
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Chapter 6

Arterial Intima-Media Thickness in Childhood
A study in familial hypercholesterolemia heterozygotes and their siblings

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Abstract

Background and Rationale

Familial Hypercholesterolemia (FH) patients suffer from severe coronary artery disease (CAD). Myocardial ischemia is often observed in early adulthood in these individuals. Therefore, a crucial remaining question regarding this disorder is whether lipid lowering therapy should be restricted to adult patients or already initiated in childhood.

Objectives

We assessed at what age morphological arterial wall changes can be observed in FH children and determined which factors contribute to this process.

Methods

First, a cross-sectional study of a large cohort of heterozygous FH children and unaffected siblings was performed using B-mode ultrasound assessment of the carotid arterial wall. Second, independent predictors of carotid wall thickness were identified with multivariate regression analysis.

Results

Mean carotid IMT in FH children (n=201) was significantly greater than in controls (n=80); 0.494 ± 0.051 vs 0.472 ± 0.049 mm; p=0.002. These cross-sectional data suggest at least a 5-fold more rapid progression during childhood in FH children versus their normolipidemic siblings: 0.005 versus <0.001 mm/year. The different progression rates in FH children led to a statistically significant IMT deviation from normal around the age of 12 years. Multivariate analysis revealed LDL-C, age, gender as strong and independent predictors of IMT.

Relevance and Conclusion

The structure of the arterial wall starts to deviate from normal in FH children well before puberty. Age, gender, but most important, LDL-C levels contribute to this process. Elevated LDL-C levels can be lowered efficiently by statin therapy and therefore, clinical studies are urgently needed to investigate long-term safety and efficacy of lipid lowering medication in FH children.
Introduction
Although the clinical sequelae of atherosclerosis manifest in adult life, atherogenesis starts in early childhood.\textsuperscript{14} In particular, prospective studies have shown a strong association between elevated low-density lipoprotein cholesterol (LDL-C) levels in young adults and the risk of subsequent coronary artery disease (CAD) later in life.\textsuperscript{5-8} Familial hypercholesterolemia (FH) is the paradigm of this relationship between LDL-C and CAD. This is underlined by the presence of myocardial ischemia and even angiographic coronary artery stenoses in asymptomatic young FH adults.\textsuperscript{9-10}

Non-invasive methods are available that can reliably assess intima-media thickness (IMT) of the arterial wall with high resolution B-mode ultrasound.\textsuperscript{11-13} Increased carotid IMT is correlated with the presence of cardiovascular risk factors.\textsuperscript{14-17} In particular, adults with FH were shown to have significantly increased IMT of the carotid and femoral arteries compared with normocholesterolemic controls.\textsuperscript{18} In a few small studies, pathological carotid scans were also observed in a proportion of younger FH patients.\textsuperscript{19-21} Therefore, we hypothesized that carotid IMT might serve as a surrogate marker that would represent the atherosclerotic burden in FH children and might assist in the decision whether or not to initiate lipid lowering therapy in this high risk group, as was recently stressed in a special report from the Task Force on Research in Pediatric Cardiovascular Disease.\textsuperscript{22}

In the present study, we compared carotid IMT between FH children and controls by assessing a large paediatric FH cohort and matched normolipidemic siblings. Moreover, we estimated the influence of age, gender, LDL-C and other characteristics on this arterial wall parameter and report the results here.

Material and methods
Familial Hypercholesterolemia Heterozygotes and Unaffected Siblings
Eligible were all consecutive children between 8 and 18 years, referred to our outpatient clinic over a two year period, who had one parent with a definite molecular diagnosis of heterozygous FH.\textsuperscript{23} Siblings in whom FH was definitely excluded by DNA analysis served as controls. The study protocol was approved by the Institutional Review Board and analyses were performed with informed consent of both the children and their parents.

Biochemistry
Venous blood samples were collected from all children after a 12 hours overnight fast. Plasma levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), LDL-C and apolipoproteins were determined as previously published.\textsuperscript{24,27}
IMT

All B-mode ultrasound examinations were performed by one well-trained sonographer (J.G.), and were read by one independent image analyst blinded to all information on the child. The within sonographer measurement variability was calculated based on repeated IMT measurements in 20 children. Time between first and second measurement was at least 3 days. The standard deviation of the means of the difference of the paired repeated carotid IMT measurements was 0.04 mm. An Acuson 128XP/10v (Acuson Corporation, Mountain View, CA) ultrasound instrument equipped with a 7 MHz Linear Array transducer (Acuson L7) and 5-10 MHz Extended Frequency (EF) software was used. Separate IMT measurements were obtained at both the left and the right side for 3 individual segments, i.e., the common carotid artery (CCA), the carotid bulb (BULB), and the internal carotid artery (ICA). The posterior (far) wall was magnified at highest possible resolution; e.g., Regional Expansion Selection 2x2cm (RES2) and acoustically focussed upon. All procedures were previously published.

Statistical analyses

Differences in baseline parameters between groups (cases and controls or males and females) were evaluated with logistic regression analysis adjusted for family using generalized estimating equations (GEE) in SAS procedure GENMOD. Variables with a skewed distribution were log-transformed. The same SAS procedure was used to explore, first univariately, the relation between mean carotid IMT and baseline variables using linear regression analysis corrected for family. Independent predictors were identified using multivariate models following stepwise backward selection. We also performed a classical sib-pair analysis on 69 available sib-pairs. Each case was preferentially matched with an unaffected sibling of identical gender and the smallest available difference in age. For statistical analyses the SAS package (release 8.02 SAS Institute Inc, Cary, NC, USA) was used.

Results

General characteristics

From 148 families with definite FH, all 281 children between 8 and 18 years were recruited; of these, 201 FH children with a molecular diagnosis (definite mutation in the LDL-receptor gene) and their 80 unaffected (established by molecular means) siblings were studied. As shown in table 1, FH children and unaffected siblings were well matched for age, gender, smoking, body mass index (BMI) and blood pressure.

Lipids and lipoproteins

As expected, FH children had severely increased LDL-C and decreased HDL-C levels.
compared to unaffected siblings. In line, the levels of apoB were also severely elevated in FH children, while the levels of apo AI were reduced. FH girls had similar mean LDL-C levels as boys (p=0.30), whereas TG levels in FH girls were slightly higher (p=0.05). HDL-C levels were not significantly different between FH girls and boys.

| Table 1. Characteristics of heterozygous FH children and their unaffected siblings |
|-----------------|-----------------|-----------------|
|                 | FH children     | Unaffected siblings |
|                 | (n=201)         | (n=80)           |
| Age, yr [range] | 13.0 [8.0-18.5] | 12.9 [8.0-18.9]  |
| Male, n (%)      | 98 (49%)        | 40 (50%)         |
| Smoking, n (%)   | 23 (11%)        | 9 (11%)          |
| Body Mass Index (kg/m²) | | |
| Male             | 18.6 ± 3.0      | 18.4 ± 2.8       |
| Female           | 20.1 ± 3.7      | 19.7 ± 4.0       |
| Blood pressure (mm Hg) | | |
| Systolic         | 110.9 ± 12.5    | 109.5 ± 11.8     |
| Diastolic        | 61.2 ± 8.6      | 62.4 ± 8.4       |
| Plasma lipoproteins | | |
| Total cholesterol (mmol/L) | 7.80 ± 1.35 | 4.30 ± 0.68 |
| LDL-cholesterol (mmol/L)   | 6.17 ± 1.31 | 2.49 ± 0.57 |
| HDL-cholesterol (mmol/L)   | 1.25 ± 0.27 | 1.46 ± 0.35 |
| Triglycerides (mmol/L)     | 0.73 [0.54-1.05] | 0.63 [0.50-1.05] |
| Lipoprotein (a) (g/L)      | 120 [46-261]  | 81 [43-238]      |
| Plasma Apolipoproteins    |                |                  |
| Apolipoprotein A1 (g/L)    | 1.05 ± 0.14    | 1.41 ± 0.25      |
| Apolipoprotein B100 (g/L)  | 1.40 ± 0.31    | 0.81 ± 0.19      |
| Carotid IMT (mm)           |                 |                  |
| CCA + BULB + ICA          | 0.494 ± 0.051  | 0.472 ± 0.049    |

Values are mean ± standard deviation, except for triglycerides and lipoprotein (a) given as median [interquartile range], statistical analyses for TG and Lp(a) after log conversion; LDL=low-density lipoprotein, HDL=high-density lipoprotein, CCA=common carotid artery, BULB=carotid bulb; ICA=internal carotid artery; Carotid IMT = average IMT of combined CCA + BULB + ICA far wall segments.

**IMT**

The mean combined IMT (CCA plus BULB plus ICA) was 0.494 ± 0.051 mm in FH children, compared to 0.472 ± 0.049 mm in the unaffected siblings (p=0.002) (Table 1). This finding remained highly significant after adjustment for family history of premature CVD, HDL-C or TG in a multivariate model. Within the present cohort, 69 sib-pairs were available for analysis; results showed that the mean difference of the
carotid IMT between FH children and their unaffected siblings was 0.022 mm (95% CI 0.005 to 0.031 mm, p=0.006), which constitutes a similar outcome as obtained for the whole study cohort. Adjustments for differences in the distributions of age and gender within the pairs in a matched multiple regression model did not change these results. When the difference between FH children and unaffected siblings in terms of carotid IMT (Δ IMT) is plotted against age (Figure 1), it is clear that when FH children have reached 18 years, Δ IMT differs very significantly from unaffected siblings, suggesting a strikingly more rapid IMT progression during childhood (0.005 mm/yr), possibly 5 times more rapid than in controls (<0.001 mm/yr). In addition, it might be deduced from this figure that carotid IMT in FH starts to deviate from normal long before puberty, and that a statistically significant difference between FH and controls is reached around the age of 12.

Figure 1 Difference in mean carotid intima-media thickness (Δ IMT: thick line) and 95% confidence interval (CI: thin lines) between FH children and unaffected siblings plotted versus age, adjusted for family relations.

Contributors to IMT
The effect of the individual baseline variables on carotid IMT and the results of the multivariate analyses for the entire group are given in table 2. Age, LDL-C, and were identified as independent predictors of carotid IMT. Although HDL-C levels reached significance in univariate analysis, this was lost in multivariate analysis. Analyses were performed separately in boys and girls: LDL-C and age were the main contributors to IMT differences in boys, and only LDL-C in girls (data not shown). In analyses restricted to FH children, age was significantly associated with IMT increase (0.005 mm/year; 95% CI 0.003 to 0.008; p<0.0001). Moreover, FH boys had a significantly thicker mean carotid artery wall than girls (0.017 mm; 95% CI 0.003 to 0.031: p=0.02).
Table 2. Determinants of carotid IMT of FH children and unaffected siblings

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th>Multivariate</th>
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<tbody>
<tr>
<td></td>
<td>Regression</td>
<td>p-value</td>
<td>Regression</td>
<td>p-value</td>
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<tr>
<td></td>
<td>coefficient (se)</td>
<td></td>
<td>coefficient (se)</td>
<td></td>
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<tr>
<td>Age (y)</td>
<td>0.0035 (0.0011)</td>
<td>0.002</td>
<td>0.0038 (0.0011)</td>
<td>0.0007</td>
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<td>Male gender</td>
<td>0.0116 (0.0060)</td>
<td>0.05</td>
<td>0.0148 (0.0062)</td>
<td>0.02</td>
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<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.0052 (0.0014)</td>
<td>0.0003</td>
<td>0.0052 (0.0014)</td>
<td>0.0002</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>-0.0237 (0.0116)</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Triglycerides</td>
<td>-0.0025 (0.0077)</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>0.0002 (0.0004)</td>
<td>0.6</td>
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<td>-</td>
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<tr>
<td>Body Mass Index (kg/m2)</td>
<td>0.0018 (0.0011)</td>
<td>0.08</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Premature CVD in first degree</td>
<td>0.0102 (0.0077)</td>
<td>0.2</td>
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</table>

LDL=low-density lipoprotein; HDL=high-density lipoprotein; CVD=cardiovascular disease; se=standard error; triglycerides are log-transformed; Mean arterial blood pressure = (systolic blood pressure + 2x diastolic blood pressure)/3.

Discussion

We have studied a large cohort of children with a definite molecular diagnosis of FH and their unaffected siblings. As expected, FH children were characterized by severely increased total and LDL-C levels, while HDL-C levels were decreased, similar to observations made three decades ago.\(^{29}\) In contrast, novel and striking findings were that carotid arterial wall IMT was already significantly greater in these FH children than in unaffected siblings, which suggests that this surrogate marker for vascular disease exhibits a more rapid progression during childhood. Moreover, and importantly, we could show that LDL-C, age and gender contributed significantly to carotid IMT progression in childhood.

High resolution B-mode ultrasonography provides an accurate non-invasive measurement of carotid IMT and is widely used as an indicator for subclinical atherosclerotic arterial wall changes. Several studies have shown significant associations between carotid IMT and cardiovascular events.\(^{30-33}\) In the present study, measurements of IMT revealed that, even at young age, FH children have atherosclerotic arterial wall abnormalities.

Furthermore, LDL-C was a prominent contributor to the carotid IMT in our paediatric cohort. This highlights the pivotal role of this lipoprotein for the development of premature vascular disease. Herein lies the importance of our observations. When FH heterozygotes have reached adulthood, their atherosclerotic burden may have increased significantly as the result of a single factor, elevated LDL-C. As a consequence, in FH a nearly 100-fold increase in CAD risk has been observed in the age group between 20 and 40 years.\(^{34,35}\) Therefore, it now remains to be determined at what age lipid lowering
intervention needs to be initiated before vascular lesions become inevitable and what the consequences of such therapy are in terms of safety and efficacy.

The strength of our study is partly based on a cohort of unparalleled size, in which a molecular diagnosis of FH was made and from a meticulous IMT procedure. The combination of a single sonographer, a single reader and high-resolution digital image acquisition and image analysis equipment produced very accurate measurements.

A possible limitation of our study includes the "prospective" interpretation of cross-sectional data. However, in patients who inherited a disorder with expression from birth onwards it is unlikely that prospective follow-up and case control studies yield different results, especially in children in whom additional exposure to environmental risk factors is likely small.

Moreover, we were able to confirm the results of our multivariate analysis with a classical sib-pair analysis. This particular study design was chosen to analyse the effect of the mutated LDL receptor on lipoprotein levels and on the carotid IMT without additional environmental influences.

Another possible limitation is that our data might not apply to other FH cohorts due to clinical sampling or referral bias, lack of genetic heterogeneity and non-representative life-style characteristics or environment. This is highly unlikely, however, because our FH children were referred from different regions, the Dutch population is molecularly very heterogeneous and life-style and diets are similar to most western societies.

In conclusion, the mean carotid IMT was significantly greater in FH children than in their normcholesterolemic siblings, and age and LDL-C levels were important contributors to this surrogate marker of CAD. Clinical research in these individuals should focus on the treatment of this disorder, initiated possibly before puberty in order to preserve normal arterial wall composition in FH children who are at such high risk for future premature CAD.

Prof. dr. J.J.P. Kastelein is an established investigator of the Netherlands Heart Foundation (grantnr: 2000D039).

References


