Familial hypercholesterolemia in childhood: diagnostics, therapeutical options and risk stratification

Rodenburg, J.

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Family history and cardiovascular risk in familial hypercholesterolemia: data in more than 1000 children

Albert Wiegman, MD; Jessica Rodenburg, MD; Saskia de Jongh, MD; Joep C. Defesche, PhD; Henk D. Bakker, MD, PhD; John J.P. Kastelein, MD, PhD; Eric J.G. Sijbrands, MD, PhD

From the Departments of Pediatrics and Vascular Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands (A.W., J.R., S.d.J., J.C.D., H.D.B., J.J.P.K); and the Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands (E.J.G.S.).

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Abstract

Background
Elevated LDL cholesterol (LDL-C) levels in childhood predict cardiovascular disease (CVD) later in life. Familial hypercholesterolemia (FH) represents the paradigm of this relation.

Methods and Results
The objectives of this study were to (1) establish the LDL-C level that provides the most accurate diagnosis of FH in children from families with known FH and (2) assess whether lipoprotein variation in these children is associated with premature CVD in relatives. Foremost, however, it was our objective to identify children with FH who are at high risk and in need of early intervention. A total of 1034 consecutive children from FH kindreds were investigated. First, LDL-C levels >3.50 mmol/L had a 0.98 post-test probability (95% CI, 0.96 to 0.99) of predicting the presence of an LDL receptor mutation. Second, children with FH in the highest LDL-C tertile (≥6.23 mmol/L) had a 1.7-times higher incidence (95% CI, 1.24 to 2.36) of having a parent with FH suffering from premature CVD (P=0.001). In addition, such a parent was found 1.8 times more often (95% CI, 1.20 to 2.59) among children with FH who had HDL-C <1.00 mmol/L (P=0.004). Last, children with FH whose lipoprotein(a) was >300 mg/L had a 1.45-times higher incidence (95% CI, 0.99 to 2.13) of having a parent with FH suffering from premature CVD (P=0.05).

Conclusions
In FH families, LDL-C levels allow accurate diagnosis of FH in childhood. Moreover, increased LDL-C and lipoprotein(a) and decreased HDL-C levels in children identify FH kindreds with the highest CVD risk.
Introduction

The incidence of familial hypercholesterolemia (FH) among Dutch children is 1 in every 400 births,\(^1\) and a plethora of mutations in the LDL receptor gene underlie this disorder in our country\(^2\). In children with FH, increased LDL cholesterol (LDL-C) deteriorates endothelial function at very young age\(^3,4\). Along with these functional changes, accumulation of cholesteryl esters changes the vascular morphology, and the intima-media thickness of peripheral arteries increases more rapidly in children with FH\(^5,6\). These findings support the notion of taking preventive measures when children are young instead of waiting until FH heterozygotes reach adulthood. In particular, it has been suggested that lifestyle changes in these children can influence plasma LDL-C\(^7\): The largest and longest placebo-controlled trial with statin therapy showed excellent safety and efficacy in children with FH\(^8\). These results suggest that children with FH receive significant benefit from lifestyle modification as well as from pharmacological intervention to reduce the burden of increased LDL-C.

Analyses of mortality rates show a large variation of the consequences of FH\(^9,10\); specifically, the risk of atherosclerosis varies significantly between families\(^10\). Identification of children who have severely increased familial risk of cardiovascular disease (CVD) could assist in the selection of targeted intervention. In the present study, we performed analyses in a pediatric FH cohort of unparalleled size. First, we sought to determine specific LDL-C levels for the most accurate diagnosis of FH in these children. Subsequently, we addressed whether or not lipoprotein variation was associated with the occurrence of CVD in relatives. Foremost, however, our objective was to identify children with FH at high risk of CVD and in need of early intervention.

Methods

Study Population

Between July 1989 and July 2001, 1034 children from parents with a diagnosis of heterozygous FH were referred to our Pediatric Lipid Clinic. A diagnosis of heterozygous FH in the parent was based on (1) a documented LDL receptor mutation or (2) plasma LDL-C levels above the 95th percentile for age and gender in a family...
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with a history of premature CVD in conjunction with (3) tendon xanthomata. The study protocol was approved by our review board, and analyses were performed with informed consent of the children and both parents.

Laboratory Analysis

Plasma total cholesterol, HDL-C, and triglycerides (TG) were determined with the use of commercially available kits (Boehringer Mannheim). LDL-C concentrations were calculated by means of the Friedewald formula. Apolipoproteins A1 and B100 were determined on a Behring nephelometer (BN100, Behring). Lipoprotein(a) [Lp(a)] concentrations were determined with the use of the Apo-Tek ELISA (Organon Teknika). Mutations in the LDL receptor gene were assessed by conventional sequencing of the coding regions, as previously published.11

Statistical Analysis

To select the LDL-C level that minimizes the proportion of false-negative and false-positive values, a receiver operating characteristic (ROC) curve was constructed according to Altman and Bland.12 The diagnostic value of a given LDL-C level was analyzed by considering pretest and post-test probabilities and was expressed as the post-test likelihood of having FH (odds). ANOVA and $\chi^2$ analysis were used to compare subgroups. Statistical testing of TG and Lp(a) was performed after logarithmic transformation. To study the relation between the lipid and lipoprotein levels and family history, children with FH were divided into 3 groups: (1) those with a family history of premature CVD in first-degree relatives, (2) those with such a history in second- or third-degree relatives, and (3) those without such a history in any relative. Trends were analyzed by multiple linear regression with concomitant inclusion of covariables. Relative risks were analyzed by Cox regression, and cumulative event-free survival was illustrated with the Kaplan-Meier method. Statistical significance was assessed at the 5% level of probability.
Results

Diagnosis of Familial Hypercholesterolemia

A total of 1034 children from 591 families with a certain diagnosis of heterozygous FH were seen in our clinic. Until this time, a molecular diagnosis was obtained in 806 children: 6 FH homozygotes and 611 FH heterozygotes, whereas 189 brothers and sisters did not carry that specific LDL receptor gene mutation. Seventy-four different mutations were found in the 611 heterozygous children with FH.

For these 611 heterozygotes and their 189 normal siblings, ROC curves of plasma LDL-C, age- and gender-specific LDL-C percentiles and apoB levels are shown in Figure 1. The largest area was found under the curve of plasma LDL-C. The best LDL-C value for the diagnosis of FH in children in these families was 3.50 mmol/L.

![Figure 1. Receiver operating characteristic curves of plasma LDL-C, age- and gender-specific LDL-C percentiles, and apoB levels. Area under the LDL-C concentration curve is larger than areas under the other curves. Best cutoff level for LDL-C is 3.50 mmol/L.](image)

(135 mg/dL). Levels below this concentration were found in only 4.3% of children with a mutated LDL receptor (false-negative; 95% CI, 2.6% to 6.1%). In contrast, children with LDL-C >3.50 mmol/L (135 mg/dL) had 0.98 (95% CI, 0.96 to 0.99) post-test probability of FH. It is important to note that this ROC curve and LDL-C cutoff is only valid against the background of a family investigation with a definite diagnosis of FH established. These data do not apply to the general population or to
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children with other dyslipidemias. The remaining children numbered 228 from families in which an LDL receptor gene mutation has not yet been identified (they are still in the cue for sequencing). However, when we apply the cutoff LDL-C level of 3.50 mmol/L (135 mg/dL) to these remaining children, 131 of them will have a 98% chance of having heterozygous FH.

This brings the total of heterozygous children with FH to 611 (DNA diagnosis) plus 131 (LDL-C level and parental diagnosis), which equals 742 children. According to the ROC analysis, the expected number of false-positive diagnoses is therefore <3 children (95% CI, 1 to 5) of these 742.

In contrast, a total of 286 siblings (189 with DNA diagnosis and 97 according to LDL-C levels) were normolipidemic. This ratio is not the expected 0.5 probability in autosomal dominant inheritance. The reason for this is that siblings with very low levels of LDL-C were often not referred to our center.

However, for the exact comparison between FH heterozygotes and children without FH, we have used the 189 normolipidemic siblings, because they are, by molecular means, certainly non-FH (Table 1).

Table 1. Characteristics of Heterozygous Children with FH and Nonaffected Siblings.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FH (n=742)</th>
<th>Siblings (n=189)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (range)</td>
<td>11.0 2.0-18.7</td>
<td>11.0 3.1-19.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>345/397</td>
<td>96/93</td>
<td>0.29</td>
</tr>
<tr>
<td>Menses, n (%)</td>
<td>144 36.3%</td>
<td>29 31.2%</td>
<td>0.35</td>
</tr>
<tr>
<td>Fat-restricted diet, n (%)</td>
<td>625 84.9%</td>
<td>-</td>
<td>n.a.</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>47 6.3%</td>
<td>8 4.5%</td>
<td>0.35</td>
</tr>
<tr>
<td>Stigmata, n (%)</td>
<td>35 4.8%</td>
<td>-</td>
<td>n.a.</td>
</tr>
<tr>
<td>BM, kg/m² (range)</td>
<td>18.5 12.2-41.1</td>
<td>18.0 12.9-29.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>7.26 ± 0.06</td>
<td>4.30 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>5.62 ± 0.06</td>
<td>2.55 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C mmol/L</td>
<td>1.25 ± 0.01</td>
<td>1.42 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>6.09 ± 0.07</td>
<td>3.17 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.73 [0.53-1.03]</td>
<td>0.66 [0.44-0.89]</td>
<td>0.001</td>
</tr>
<tr>
<td>Lp(a) mg/L</td>
<td>119 [50-294]</td>
<td>85 [33-273]</td>
<td>0.05</td>
</tr>
<tr>
<td>ApoA1 g/l</td>
<td>1.27 ± 0.01</td>
<td>1.37 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB100 g/L</td>
<td>1.59 ± 0.02</td>
<td>0.83 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. *except for TG and Lp(a), which are given as median±IR and statistical testing after logarithmic transformation.

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General Characteristics

On the basis of the above-mentioned diagnostic criteria, 742 children (397 girls and 345 boys) from 508 families were heterozygous for FH (Table 1). Their mean age was 11 years (range, 2 to 19 years). Typical physical characteristics of FH (xanthomas, xanthelasmas, or arcus cornealis) were only found in 35 children (5%; 95% CI, 3% to 7%). Of the children with FH, 85% were on a fat-restricted diet. A total of 47 (6%; 95% CI, 5% to 8%) children were cigarette smokers. Age, height, and body mass index (BMI) were not significantly different between the children with and those without FH. As expected, children with FH had severely increased LDL-C and decreased HDL-C levels compared with nonaffected siblings (Table 1). LDL-C and apoB-100 levels were highly correlated (r=0.95; P<0.001), as were HDL-C and apoA1 levels (r=0.76; P<0.001). Girls with FH had mean LDL-C of 5.80 mmol/L (95% CI, 5.64 to 5.96 mmol/L) versus 5.42 mmol/L (95% CI, 5.27 to 5.57 mmol/L; P=0.001) for boys with FH. The median TG level in girls with FH was 0.76 mmol/L (interquartile range [IR]: 0.59 to 1.06 mmol/L) versus 0.67 mmol/L (IR, 0.49 to 0.97 mmol/L) in boys (after logarithmic transformation, P<0.001). Mean BMI, 18.8 kg/m², was significantly higher in girls with FH (95% CI, 18.4 to 19.2 kg/m²) than the 18.1 kg/m² in boys with FH (95% CI, 17.8 to 18.4 kg/m²; P=0.005). No significant differences were found with regard to HDL-C and apoA1 between girls and boys.

Lifestyle and Plasma Lipoprotein Levels

Heterozygous children with FH (742) grouped by LDL-C tertiles had similar distributions of age, diet, smoking, and BMI (data not shown). Also, LDL-C levels of children with FH not on a diet versus children with FH on a diet were similar. HDL-C <1.00 mmol/L was found in 132 children. They had a similar distribution of age, gender, and smokers compared with 610 children with FH with normal HDL-C levels (data not shown). However, in the low HDL-C group, 79% were on a fat-restricted diet compared with 86% in the normal HDL-C group (χ²=4.93, df=1; P=0.03). In addition, the mean BMI of children with FH with low HDL-C was 19.3 kg/m² (95% CI, 18.5 to 20.0 kg/m²) versus 18.3 kg/m² (95% CI, 18.1 to 18.6 kg/m²; P=0.007) in the normal HDL-C group. The median TG level of the low HDL-C group was 0.99 mmol/L (IR, 0.70 to 1.33 mmol/L) versus 0.69 mmol/L (IR, 0.51 to 0.94 mmol/L) in the normal HDL-C group (after logarithmic transformation, P<0.001).
Adjustment for age and gender did not change the diagnostic value of LDL-C levels as shown in the ROC curve, and no influence of age on lipoproteins became evident in our cohort of children with FH.

Parameters such as diet, BMI, plasma TG, and HDL-C were correlated. Therefore, we analyzed these relations with different logistic regression models with subsequent inclusion of diet and BMI and of diet, BMI, and plasma TG. Diet and BMI were weakly correlated ($R=-0.11, P=0.005$). In the regression model, diet did not change the influence of BMI on HDL-C levels (data not shown). However, TG levels included in the model showed a strong inverse relation with HDL-C levels (OR, 0.25; 95% CI, 0.16 to 0.39; $P<0.001$) and fully explained the effect of BMI on HDL-C (OR, 1.01; 95% CI, 0.96 to 1.07; $P=0.7$) and partly that of diet (OR, 1.49; 95% CI, 0.90 to 2.48; $P=0.1$) (Table 2).

In brief, children with FH with lower HDL-C levels were heavier and had higher TG levels. In contrast, LDL-C levels were mostly independent of lifestyle characteristics or anthropomorphic measures.

### Table 2. Relationships Between HDL-C > 1.00 mmol/L, Diet, BMI and Plasma TG in Heterozygous Children with FH.

<table>
<thead>
<tr>
<th>Determinants</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1.71</td>
<td>1.06-2.76</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI</td>
<td>0.93</td>
<td>0.89-0.98</td>
<td>0.006</td>
</tr>
<tr>
<td>TG</td>
<td>0.25</td>
<td>0.16-0.38</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Model 1:**

- Diet: 1.63; 95% CI 1.00-2.65; $P=0.05$
- BMI: 0.94; 95% CI 0.89-0.99; $P=0.01$

**Model 2:**

- Diet: 1.49; 95% CI 0.90-2.48; $P=0.12$
- BMI: 1.01; 95% CI 0.96-1.07; $P=0.67$
- TG: 0.25; 95% CI 0.16-0.39; <0.001

Logistic regression analyses were performed with 1, 2, and 3 covariables, respectively.

### Family History of Premature CVD

The analyses of the relation between premature CVD and lipoprotein levels in children were restricted to 1 child per family (508 index children with FH). Their general characteristics, including lipids and lipoproteins, are shown in Table 3. A positive family history for premature CVD in first-degree relatives was found in 155 (31%) children.
A total of 290 (57%) children had a positive family history of premature CVD in a second- and/or third-degree relative. No family history of premature CVD was found in 63 (12%) children. Gender and age of the index children was equally distributed among these three groups. Strikingly, children (1) with premature CVD among first-degree relatives, (2) with premature CVD among second-degree relatives, and (3) without premature CVD in any relative showed, respectively, higher, intermediate, and lower LDL levels ($P_{\text{for trend}}=0.002$). ApoB100 levels showed a similar trend ($P_{\text{for trend}}=0.01$).

### LDL and HDL Cholesterol of the Index Child in Relation to CVD in the Parent With FH

In support of these findings, children with FH with LDL-C levels $6.23 \text{ mmol/L}$ (the highest tertile) were 1.7 times more likely (95% CI, 1.24 to 2.36; $P=0.001$) to have a parent with FH with premature onset of CVD than those with LDL-C <6.23 mmol/L. This analysis is shown in Figure 2; parents of children with these high LDL-C levels had shorter event-free survival than parents of children with lower LDL-C levels (log rank $=10.35$; $df=1$; $P=0.001$). When adjusted for parental gender and calendar period with Cox regression analysis, children with FH with HDL-C levels <1.00 mmol/L
were 1.8 times more likely (95% CI, 1.20 to 2.59; \( P=0.004 \)) to have a parent with FH with premature onset of CVD (Figure 3). In agreement with the Cox regression analysis, parents of children with low HDL-C levels had shorter event-free survival compared with parents of children with normal HDL-C levels (log rank=3.93; \( df=1; P=0.048 \)). Moreover, adjusted for parental gender and calendar period, the upper quartile of children with FH whose Lp(a) was 300 mg/L were 1.45 times more likely (95% CI, 0.00 to 2.13) to have a parent with FH with premature CVD compared with the other quartiles with low Lp(a) levels (\( P=0.05 \)).

In conclusion, these data indicate that both severely elevated LDL-C and Lp(a) levels and decreased HDL-C levels point to a subgroup of FH families exposed to severe CVD risk.
In this large cohort of FH families, we showed that LDL-C levels <3.50 mmol/L (135 mg/dL) are only found in 4.3% of children with a mutation in the LDL receptor gene. Elevated LDL-C levels in childhood suggest a diagnosis of classic FH, and in seminal studies by the NIH group, it was shown that this diagnosis could be made on the basis of cord blood LDL-C levels. However, the same authors also showed that cholesterol levels overlap to a certain extent between affected and normal children. The demonstration of a defect in the LDL receptor gene is more accurate for the diagnosis of FH than an LDL-C measurement, but DNA sequencing is only available to a limited number of physicians, and our data support the use of an LDL-C cutoff level at minimal loss of specificity and sensitivity. However, it should be stated explicitly that the ROC curves and LDL-C cutoff levels in our study only apply to families in which the diagnosis of FH is certain. They cannot be extrapolated to other dyslipidemias or to the general population.

We could also show, as has been known in the past three decades, that these children have severely elevated total cholesterol, LDL-C and apoB levels, in conjunction with decreased HDL-C and apoA1 levels. In the early 1970s, Kwiterovich and colleagues established by investigating cord blood that HDL-C levels were significantly lower in children with FH than in nonaffected siblings. This finding was subsequently confirmed in older children with FH by the same authors. The reason(s) for low HDL-C in heterozygous FH have not been fully elucidated. They could be related to increased very-low-density lipoprotein synthesis as seen in FH or due to the fact that intermediate-density lipoprotein, also cleared by the LDL receptor, accumulates in this disorder. These metabolic alterations of TG-rich lipoproteins could cause increased cholesteryl ester transfer protein (CETP) activity, with subsequent depletion of cholesterol in the HDL particle, as has indeed been suggested by Imazu and colleagues. Kinetic studies have suggested both increased fractional catabolic rate and decreased synthesis of HDL-apoA1. Schaefer and colleagues suggested that deficiency of the LDL receptor leads to an increased pool size of apoE, which in turn could lead to apoE-enriched HDL particles and subsequent increased clearance of these particles in FH. Taken together, increased CETP activity in conjunction with increased HDL-C clearance could be hypothesized to underlie the lower HDL-C levels in children with FH.
LDL-C levels showed a wide range in our cohort. In healthy twin children, the variation of cholesterol levels was attributed for 24% to genetic influences and for a stunning 76% to environmental influences\textsuperscript{19}. This is in sharp contrast to our findings; age, diet, BMI, and smoking were essentially similar across all LDL tertiles in children with FH. The loss of half of LDL receptor function might be an overriding force and may overwhelm any subtle environmental or other genetic influence on LDL-C levels, as was shown previously for apoE genotype and diet in relation to FH\textsuperscript{20,21}.

At the present time, the recommended therapeutic regimen for children with FH is restricted to bile acid–binding resins, which are only slightly more effective than diet alone\textsuperscript{22–26}. In contrast, Stein and colleagues\textsuperscript{8} reported on the long-term efficacy and safety of lovastatin in children and adolescents with FH, showing excellent tolerability and lack of serious side effects in this age cohort. However, not all children with FH have the dire consequences of accelerated atherosclerosis but, in fact, may have a normal life expectancy\textsuperscript{9,10}. In our opinion, targeted intervention of children with FH should take family history and notably the severity of parental coronary disease into account. Event-free survival of the affected parent with FH showed in our study a strong relation with both LDL-C and HDL-C levels in children. Indeed, a positive family history is a strong and independent risk factor for both genders, and its effect is synergistic with other CVD risk factors as well, also for individuals without FH\textsuperscript{27}. In addition, children with FH with Lp(a) levels >300 mg/L more often had a parent with FH with premature CVD than those with lower Lp(a) levels.

Our observations suggest that a high familial risk of CVD may be identified in a child with FH before it becomes family history by analyzing the lipid profile. In conclusion, when the diagnosis of FH is certain in the family, simple measurement of the most important lipoproteins, LDL-C, HDL-C, and Lp(a), allows an accurate diagnosis of FH in childhood and leads to identification of FH families with the highest risk of CVD. It would therefore follow to study efficacy and safety of long-term statin use in children with FH with a family history of premature CVD.
# References


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