Chapter 10

LDL receptor genotype and response to pravastatin in children with familial hypercholesterolemia: substudy of an intima-media thickness trial

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Abstract

**Background** The lipid lowering effects of statin therapy show considerable interindividual variation in patients with familial hypercholesterolemia (FH). In adults, it is not yet established whether or not the type of low-density lipoprotein (LDL) receptor mutation predicts the response to statin treatment. We analyzed the relationship between LDL receptor genotype and response to pravastatin treatment in children with FH using carotid intima-media thickness (IMT) to measure efficacy.

**Methods and Results** In a randomized, placebo-controlled, double-blind, two-year trial with pravastatin, 193 children had genetically confirmed FH and were included in the present substudy. At baseline, children with null alleles had higher LDL cholesterol levels (difference, 0.94 ± (SEM) 0.19 mmol/L; p<0.001) and a greater carotid IMT (difference, 0.019 ± 0.01 mm; p=0.02) compared to children with receptor-defective mutations. The decrease in carotid IMT during the trial was not significantly different in children with null alleles and receptor-defective mutations (0.018 ± 0.012 mm and 0.012 ± 0.010 mm; two-way covariance analysis p=0.7). However, after two-year treatment, the children with null alleles consistently had greater carotid IMT than children with receptor-defective mutations (difference, 0.016 ± 0.01 mm; p=0.02). Moreover, the reduction of LDL cholesterol during the trial tended to be less in carriers of null alleles compared to carriers of receptor-defective mutations (1.30 ± 0.25 mmol/L compared to 1.85 ± 0.20 mmol/L; two-way covariance analysis p=0.08).

**Conclusions** We found in FH children that null allele genotype was associated with a greater carotid IMT, higher LDL cholesterol levels and attenuated efficacy of pravastatin regarding LDL cholesterol reduction, when compared to receptor-defective mutations. Null alleles identify FH patients at the highest cardiovascular disease risk who may benefit from more aggressive treatment, possibly initiated already in childhood.
Introduction

Familial hypercholesterolemia (FH) is a common metabolic disorder caused by mutations in the low-density lipoprotein (LDL) receptor gene. The disorder is characterized by severely elevated LDL cholesterol (LDL-C) levels from birth onwards. Consequently, FH patients have an increased risk of cardiovascular disease. After diagnosis, heterozygous patients are treated lifelong with inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (statins) to prevent premature CVD.

The lipid lowering response to statin therapy, however, shows considerable interindividual variation. Several investigators have assessed whether specific LDL receptor mutations affect the lipid lowering response to statin therapy. Understanding this relationship could result in a more individual approach of the treatment of FH patients. However, these studies have yielded conflicting results. Selection on specific founder mutations, limited numbers of patients, different classifications of the LDL receptor mutation types, and a variety of treatment strategies without randomization made it difficult to compare the results of these studies. In the present subgroup of a randomized, placebo-controlled clinical trial with pravastatin in FH children, we analyzed a large number of different LDL receptor mutations. Children are exposed to a more homogeneous environment than adults and are therefore better suited for genotype-phenotype analyses. The influence of confounding factors on our observation was further reduced by randomization. In addition, since this was a placebo-controlled trial, the natural course of the specific LDL receptor mutations could be determined during the two-year follow-up.

At present, genotype-phenotype studies have focussed on the lipid lowering response to statin therapy instead of analyzing the effects of statins on the atherosclerotic process. In adults, carotid intima-media thickness (IMT) has been accepted as a validated marker for atherosclerosis and future cardiovascular outcome. There are clear indications that carotid IMT is a marker of the increased atherosclerotic burden in childhood also. In a randomized statin trial, we measured both carotid IMT and lipid concentrations and the purpose of this subgroup analysis was to determine whether LDL receptor genotype influenced response to pravastatin treatment in children with heterozygous FH.
Methods

Study Design
The FH children in the present subgroup analysis were participants in a single center clinical trial carried out in The Netherlands. The study has been described in detail elsewhere. In brief, it was a prospective, randomized, placebo-controlled, double-blind trial to assess the effect of two years of treatment with pravastatin on the carotid IMT in 214 children with heterozygous FH, aged between 8 and 18 years. After consenting, we randomized children to receive pravastatin once daily or matching placebo. In the active treatment group, children younger than 14 years of age received 20 mg pravastatin and in those 14 years and older pravastatin 40 mg was given. We monitored study drug compliance by tablet counting. In the present genotype-phenotype substudy, we included all 193 children whose LDL receptor mutation had been identified (Figure 1). The Institutional Review Board approved the study protocol, and informed consent was obtained from all children and parents.

Type of LDL Receptor Mutation
We classified the LDL receptor mutations into mutation groups based on their functional class as reported in the literature: (a) the receptor-negative mutations or null alleles contained all class 1 mutations, class 2A mutations, large rearrangements (except the 2.5kb deletion which is a class 3 and 5 mutation), mutations resulting in a deletion of the translation initiation signal, early stop-codons, and nonsense mutations, although the latter had often undetermined residual function; (b) the receptor-defective mutations contained class 2B to 6 mutations; (c) the undetermined-receptor-activity mutations contained all remaining mutations with undetermined mutational class. The identified LDL receptor mutations are listed in the online Addendum. A total of 49 different mutations were detected in 193 children with heterozygous FH. We found 17 null alleles in 75 children, 14 receptor-defective mutations in 80 children, and 18 mutations with undetermined residual function in 38 children (Figure 1).

Intima-Media Thickness
The primary efficacy outcome of this substudy was defined as the difference in change from baseline in mean carotid IMT between the placebo and pravastatin group at two years of follow-up compared between null alleles and receptor-defective mutations. A
Figure 1. Flow of study participants in the genotype-phenotype substudy.

214 Randomized Children With Untreated Familial Hypercholesterolemia

3 Lost To Follow-Up

211 Children With Familial Hypercholesterolemia Completed Trial

18 Excluded
9 Apolipoprotein B Mutations
9 Without LDL Receptor Mutation

193 Children With Genetically Confirmed, Heterozygous Familial Hypercholesterolemia

75 Null Alleles
37 Received Pravastatin
38 Received Placebo

80 Receptor-Defective Mutations
41 Received Pravastatin
39 Received Placebo

38 Undetermined Receptor Activity Mutations
21 Received Pravastatin
17 Received Placebo

single experienced sonographer performed all B-mode ultrasound examinations. The far walls of the left and right common carotid artery (CCA), carotid bulb (BULB), and internal carotid artery (ICA) were imaged. The digital images were analyzed off-line by one image analyst. For a given segment, IMT was defined as the average of the left and right IMT measurements. Mean carotid IMT was defined as the mean of the CCA, BULB, and ICA far wall segments. The quantitative IMT measurements have been described in detail elsewhere16,17.

Laboratory Methods
Plasma concentrations of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using standard (automated) methods after a 12-hour overnight fast. LDL-C concentrations were calculated by the Friedewald formula18. All children had plasma TG concentrations consistently below 4.0 mmol/L. Mutational analyses were performed with standard methods as described previously19.
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Statistical Analysis
All statistical analyses were carried out with SPSS software (version 11.5, SPSS). We compared children with null alleles and receptor-defective mutations for relevant clinical characteristics, lipid concentrations, and carotid IMT. Differences among LDL receptor genotype groups were analyzed with Students’ t-test for continuous data. Since TG concentrations had a skewed distribution, the analyses were based on log-transformed data. In a multivariate linear regression analysis, we analyzed the relationship between LDL receptor genotypes and carotid IMT adjusted for lipid levels. Changes in carotid IMT and lipid concentrations during the trial were calculated between the placebo and pravastatin group, and the differences within the two LDL receptor genotype groups were analyzed with multivariate linear regression analysis. The interaction between genotype (null alleles or receptor-defective mutations) and treatment (placebo or pravastatin) was tested with the interaction test of two-way covariance analysis. Since LDL receptor genotype was associated with baseline lipid levels and carotid IMT, we did not adjust for baseline values in multivariate linear regression analysis and two-way covariance analysis. Nonetheless, when we adjusted for baseline values, similar trends were found between LDL receptor genotype and response to treatment (data not shown). Throughout, a two-tailed P value of less than 0.05 was interpreted as indicating a statistical significant difference.

Results

Patient Characteristics
The baseline characteristics according to the type of LDL receptor mutation are presented in Table 1. Study drug compliance was similar among the LDL receptor genotype groups. Children with null alleles and receptor-defective mutations were evenly distributed over placebo-treated and pravastatin-treated groups. As expected, children with null alleles had significantly more elevated mean TC levels (difference (± SEM), 0.94 ± 0.20 mmol/L; p<0.001) as well as mean LDL-C levels (difference, 0.97 ± 0.20 mmol/L; p<0.001) compared to children with receptor-defective mutations. Moreover, carriers of null alleles had a greater mean carotid IMT (difference, 0.020 ± 0.01 mm; p=0.01) and mean IMT of the ICA segment (difference, 0.022 ± 0.01 mm;
Table 1. Baseline Characteristics of Children with Null Alleles, Receptor-Defective Mutations, and Mutations with Undetermined Receptor Activity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Undetermined receptor activity (n=38)</th>
<th>Null alleles (n=75)</th>
<th>Receptor-defective mutations (n=80)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>12.4 (8.0-18.5)</td>
<td>13.4 (8.4-18.0)</td>
<td>12.6 (8.1-18.5)</td>
<td>0.1</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>16 / 22</td>
<td>33 / 42</td>
<td>44 / 36</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.3 ± 0.6</td>
<td>19.5 ± 0.4</td>
<td>18.9 ± 0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Study drug compliance, %</td>
<td>88.5 ± 2.3</td>
<td>89.4 ± 1.8</td>
<td>90.2 ± 1.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Lipids in mmol/L**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Undetermined receptor activity (n=38)</th>
<th>Null alleles (n=75)</th>
<th>Receptor-defective mutations (n=80)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>7.98 ± 0.22</td>
<td>8.30 ± 0.16</td>
<td>7.36 ± 0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>6.42 ± 0.21</td>
<td>6.66 ± 0.15</td>
<td>5.70 ± 0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.21 ± 0.04</td>
<td>1.23 ± 0.03</td>
<td>1.26 ± 0.03</td>
<td>0.5†</td>
</tr>
<tr>
<td>TG</td>
<td>0.78 ± 0.07</td>
<td>0.92 ± 0.06</td>
<td>0.90 ± 0.06</td>
<td>0.9‡</td>
</tr>
</tbody>
</table>

**IMT in mm**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Undetermined receptor activity (n=38)</th>
<th>Null alleles (n=75)</th>
<th>Receptor-defective mutations (n=80)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA</td>
<td>0.520 ± 0.01</td>
<td>0.521 ± 0.01</td>
<td>0.501 ± 0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>BULB</td>
<td>0.529 ± 0.08</td>
<td>0.532 ± 0.01</td>
<td>0.515 ± 0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>ICA</td>
<td>0.429 ± 0.01</td>
<td>0.455 ± 0.01</td>
<td>0.433 ± 0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>mean carotid</td>
<td>0.492 ± 0.01</td>
<td>0.503 ± 0.01</td>
<td>0.483 ± 0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

BMI=body mass index. TC=total cholesterol. LDL=low-density lipoprotein. HDL=high-density lipoprotein. TG=triglyceride. IMT=intima-media thickness. CCA=common carotid artery. BULB=carotid bulb. ICA=internal carotid artery. mean carotid=mean of the CCA, BULB, and ICA. All values are given as mean ± standard error of the mean (SEM). * Comparison between null alleles and receptor-defective mutations. † Statistical analysis adjusted for individual serum triglyceride levels did not change the result (data not shown). ‡ Statistical testing after logarithmic transformation.

p=0.03) compared to carriers of receptor-defective mutations. Mean IMT of the CCA and BULB segments also tended to be higher in children with null alleles but this was not significant (difference, 0.021 ± 0.01 mm; p=0.06 and difference, 0.017 ± 0.01 mm; p=0.08, respectively). Furthermore, after adjustment for baseline LDL-C levels, the difference in mean carotid IMT between carriers of null alleles and receptor-defective mutations was 0.018 ± 0.01 mm (p=0.04).

Changes in Carotid IMT

Figure 2 shows mean differences of the changes in carotid IMT during the trial between the placebo and pravastatin group according to the LDL receptor genotype. The decrease of mean carotid IMT as well as of mean IMT in the CCA, the BULB, and the ICA segments between children who received placebo and pravastatin treatment was not significantly different in carriers of null alleles compared to carriers of receptor-
Figure 2. Mean differences of the changes in carotid IMT during the trial between the placebo and pravastatin group in children with null alleles and receptor-defective mutations.

- Null alleles (n=75) □ Receptor-defective mutations (n=80)

IMT=intima-media thickness, CCA=common carotid artery, BULB=carotid bulb, ICA=internal carotid artery, mean carotid=mean of the CCA, BULB, and ICA. Error bars indicate standard error of the mean difference. P values for the interaction between genotype (null alleles or receptor-defective mutations) and treatment (placebo or pravastatin) were calculated using two-way covariance analysis.

defective mutations (two-way covariance analysis; p=0.7, p=0.4, p=0.3, and p=0.7, respectively). Adjustment for changes in LDL cholesterol levels during the trial also did not change the decrease of mean carotid IMT among carriers of null alleles and carriers of receptor-defective mutations: both 0.014 ± 0.01 mm (two-way covariance analysis; p=0.6). However, after two-year treatment, children with null alleles had a consistently greater mean carotid IMT (difference, 0.016 ± 0.01 mm; p=0.02) and mean IMT of the CCA segment (difference, 0.019 ± 0.01 mm; p=0.04) compared to children with receptor-defective mutations. Mean IMT of the BULB and the ICA segments after the treatment period tended to be higher in children with null alleles but this did not reach significance (difference, 0.017 ± 0.01 mm; p=0.07 for both segments).
Changes in Lipid Concentrations
Mean differences of the changes in lipid concentrations during the trial between the placebo and pravastatin group according to the LDL receptor genotype are presented in Figure 3. Children with null alleles tended to less reduction of TC and LDL-C levels during the trial compared to children with receptor-defective mutations, but this did not reach statistical significance (two-way covariance analysis; both p=0.08). After two-year treatment, mean TC in carriers of null alleles was 7.62 ± 0.20 mmol/L and mean LDL-C was 5.94 ± 0.19 mmol/L, and remained significantly more elevated than the 6.60 ± 0.17 mmol/L and 4.90 ± 0.17 mmol/L in carriers of receptor-defective mutations (both p<0.001).
Pravastatin increased HDL-C levels and reduced TG levels to a similar extent in both LDL receptor genotype groups (two-way covariance analysis; p=0.9 and p=0.7, respectively).

Figure 3. Mean differences of the changes in lipid concentrations during the trial between the placebo and pravastatin group in children with null alleles and receptor-defective mutations.

- Null alleles (n=75) ■ Receptor-defective mutations (n=80)

TC = total cholesterol, LDL = low-density lipoprotein, HDL = high-density lipoprotein, TG = triglyceride. Error bars indicate standard error of the mean difference. P values for the interaction between genotype (null alleles or receptor-defective mutations) and treatment (placebo or pravastatin) were calculated using two-way covariance analysis.
Discussion

In this subgroup study of a randomized, placebo-controlled, two-year trial with pravastatin in heterozygous FH children, we showed that LDL receptor genotype was significantly associated with the carotid IMT, independent of LDL-C levels. Although the reduction of LDL-C levels by pravastatin treatment tended to be less in carriers of null alleles, we observed no significant difference in change of carotid IMT during the trial between the two LDL receptor genotype groups. However, at baseline and after two-year treatment, carotid IMT and lipid profile were more unfavorable in children with null alleles compared to children with receptor-defective mutations.

Our present analysis is the first genotype-phenotype study in FH that demonstrated the influence of statin therapy on carotid IMT. In adults, numerous studies have shown that an increase in IMT of the carotid artery is associated with an increased risk of myocardial infarction and stroke, and that a decrease in carotid IMT due to drug treatment is associated with a decrease in the incidence of vascular events\textsuperscript{12-15}. Therefore, noninvasive B-mode carotid IMT has now been accepted as a validated marker for the process of atherosclerosis in adults. There are clear indications that carotid IMT is also a marker for atherosclerosis in childhood. Children with FH have a 5-fold more rapid increase of carotid arterial wall IMT during childhood than their unaffected siblings\textsuperscript{16}. This increase led to a significant deviation in terms of IMT values from the age of 12 years onwards. Therefore, it might be suggested to measure carotid IMT as a marker of the increased atherosclerotic burden in children.

In this placebo-controlled trial, the response to pravastatin treatment on carotid IMT was not significantly different between carriers of null alleles and receptor-defective mutations. Nonetheless, independent of LDL-C levels, the carriers of null alleles had a greater carotid IMT than carriers of receptor-defective mutations at baseline and this unfavorable difference persisted during treatment. Hence, untreated and treated children carrying null alleles exhibit a more increased risk of cardiovascular disease compared to children carrying receptor-defective mutations. We could not assess the relationship between LDL receptor genotype and responses to increasing doses of statins in the present substudy. In future research, the effect of more aggressive and earlier statin treatment in children with null alleles should be investigated.

Previous studies in adult FH patients analyzed whether the nature of the LDL receptor
LDL receptor genotype

mutation influenced lipid lowering response to statin therapy, but have yielded conflicting results\(^4-10\). In a recent study, we showed that children with FH are better suited for genotype-phenotype analysis than adults\(^11\). In a linear mixed model, we calculated the contribution of familial factors to the variance of LDL-C levels in a pediatric and an adult FH cohort (intra-class correlation). Familial factors explained 50.4% of the variance in LDL-C levels among the FH children and only 9.5% in adult FH patients. Hence, the LDL-C levels showed a much stronger correlation among related children than in adult siblings. Likely, children refrain from unfavorable lifestyles and share their environment to a greater extent than adults. The consequences of LDL receptor genotype are, therefore, estimated against a more homogeneous background in children with FH. Moreover, we used placebo-controlled data and, therefore, information was available about the natural course of the specific LDL receptor mutation on carotid IMT and lipid profile during the two-year follow-up. Adjusting for the natural course reduces bias in the analyses of the relationship between LDL receptor genotype and treatment response: placebo effect and secular trends do not influence our observations.

We found that the reduction of TC and LDL-C levels during pravastatin treatment tended to be less in null alleles compared to receptor-defective mutations in this placebo-controlled trial. Most likely, this finding was not significant as a result of small numbers of children. In FH children, only one other study has been reported that investigated the relationship between the LDL receptor genotype and lipid levels. In contrast to our findings, two types of null mutations associated with significantly larger LDL-C reductions compared to one receptor-defective mutation\(^10\). However, these children were selected on specific founder mutations and the results might have been influenced by additional familial factors. Moreover, placebo-treated children were excluded from these analyses, thereby loosing control for the natural course of the LDL receptor genotype.

Our subgroup analysis had not enough power to observe small differences in the carotid IMT responses between the two LDL receptor genotype groups with significance. However, we had enough children in each genotype group to make a valid estimate of the mean difference of the change in carotid IMT between the placebo and pravastatin group of the subgroups and we observed similar point-estimates. The question arises whether or not we have made a type II error: a difference is not observed with statistical significance because of lack of power due to small numbers. We did not find, however, a difference between the point-estimates. Recently,
Schultz and Grimes emphasized that methodological rigor to eliminate bias, properly report to avoid misinterpretation, and always publish results to avert publication bias is more important than insufficient sample size. Moreover, the methodological advantages enable that such analyses could ultimately be combined in meta-analysis. We have estimated that a meta-analysis to test the results of this hypothesis-generating study should be considered when 2600 or more children have been included in statin IMT trials. Future studies on genotype effects should also maintain the placebo-controlled data of the trial to enable such a meta-analysis.

In summary, we conclude that LDL receptor genotype was significantly associated with the carotid IMT before and during treatment with pravastatin in heterozygous FH children, independent of LDL-C levels. Selection of null alleles identifies children with the highest cardiovascular disease risk who may benefit by more aggressive as well as earlier lipid lowering treatment.

References


