Low-Density Lipoprotein Receptor Genotype and Response to Pravastatin in Children With Familial Hypercholesterolemia
Substudy of an Intima-Media Thickness Trial

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**Background**—The lipid-lowering effects of statin therapy show considerable interindividual variation in patients with familial hypercholesterolemia (FH). Whether the type of LDL receptor mutation predicts the response to statin treatment is not yet established. 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, 2-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 ± 0.19 mmol/L; SEM; *P*<0.001) and a greater carotid IMT (difference, 0.019 ± 0.01 mm; *P*=0.02) compared with 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 and 0.012 ± 0.010 mm; 2-way ANCOVA, *P*=0.7). After 2 years of treatment, the children with null alleles continued to have greater carotid IMT than children with receptor-defective mutations (difference, 0.016 ± 0.01 mm; *P*=0.02). LDL cholesterol lowering tended to be less in carriers of null alleles compared with carriers of receptor-defective mutations (1.30 ± 0.25 and 1.85 ± 0.20 mmol/L; 2-way ANCOVA, *P*=0.08).

**Conclusions**—In FH children, we found that the null allele genotype was associated with a greater carotid IMT, higher LDL cholesterol levels, and a nonsignificant tendency to attenuated LDL cholesterol lowering compared with receptor-defective mutations. Null alleles identify FH patients at the highest cardiovascular disease risk who may benefit from more aggressive treatment started in childhood.

**Key Words:** drugs ■ genetics ■ hypercholesterolemia, familial ■ carotid arteries ■ pediatrics

Familial hypercholesterolemia (FH) is a common metabolic disorder caused by mutations in the LDL receptor gene. The disorder is characterized by severely elevated LDL cholesterol (LDL-C) levels. 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 cardiovascular disease.

The lipid-lowering response to statin therapy, however, shows considerable interindividual variation. In clinical practice, noncompliance with prescribed medication is an important cause of variation in statin response. In addition, 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 of 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. Recently, we have found that studying children with FH provides more accurate information on genotype-phenotype interactions than studying adults, probably because of a lower chronic exposure to known environmental factors that alter lipid levels. The randomization was used to reduce the influence of confounding factors. In addition, our
observations were controlled for placebo effects, which enabled us to determine the natural course of the specific LDL receptor mutations during the 2-year follow-up.

At present, genotype-phenotype studies have focused 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.13–16 There are clear indications that carotid IMT also is a marker of the increased atherosclerotic burden in childhood.17 In a randomized statin trial, we measured both carotid IMT and lipid concentrations, and the purpose of the present subgroup analysis was to determine whether LDL receptor genotype influenced the 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.17,18 In brief, it was a prospective, randomized, placebo-controlled, double-blind trial to assess the effect of 2 years of treatment with pravastatin on the carotid IMT in 214 children with heterozygous FH who were between 8 and 18 years of age. After obtaining consent, we randomized children to receive pravastatin once daily or matching placebo. In the active treatment group, children 14 years of age received 20 mg pravastatin; those ≥14 years were given pravastatin 40 mg. 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: (1) the receptor-negative mutations or null alleles contained all class 1 mutations, class 2A mutations, large rearrangements (except the 2.5-kb deletion, which is a class 3 and 5 mutation), mutations resulting in a deletion of the translation initiation signal, and early stop codons; (2) the receptor-defective mutations contained class 2B to 6 mutations; and (3) the undetermined-receptor-activity mutations contained all remaining mutations with undetermined mutational class. The identified LDL receptor mutations are listed in Table S1 (found in the Data Supplement at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.565507/DC1). 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 groups at 2 years of follow-up compared between null alleles and receptor-defective mutations. A single experienced sonographer performed all B-mode ultrasound examinations. The far walls of the left and right common carotid arteries (CCAs), carotid bulb (BULB), and internal carotid artery (ICA) were imaged. The digital images were analyzed offline by an 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 elsewhere.17,18

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 formula.19 All children had plasma TG concentrations consistently <4.0 mmol/L. Mutational analyses were performed with standard methods as described previously.20

Statistical Analysis

All statistical analyses were carried out with SPSS software (version 11.5, SPSS Inc). We compared children with null alleles and
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*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range), y</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, M/F</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>
<tr>
<td>Lipids, mmol/L</td>
<td>TC 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></td>
<td>LDL-C 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></td>
<td>HDL-C 1.21±0.04</td>
<td>1.23±0.03</td>
<td>1.26±0.03</td>
<td>0.5†</td>
</tr>
<tr>
<td></td>
<td>TG 0.78±0.07</td>
<td>0.92±0.06</td>
<td>0.90±0.06</td>
<td>0.9‡</td>
</tr>
<tr>
<td>IMT, mm§</td>
<td>CCA 0.520±0.01</td>
<td>0.521±0.01</td>
<td>0.501±0.01</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>BULB 0.529±0.08</td>
<td>0.532±0.01</td>
<td>0.515±0.01</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>ICA 0.429±0.01</td>
<td>0.455±0.01</td>
<td>0.433±0.01</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Mean carotid 0.492±0.01</td>
<td>0.503±0.01</td>
<td>0.483±0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>IMT, mm§</td>
<td>CCA 0.486±0.07</td>
<td>0.510±0.05</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>BULB 0.549±0.07</td>
<td>0.513±0.05</td>
<td>0.511±0.04</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>ICA 0.430±0.07</td>
<td>0.431±0.05</td>
<td>0.421±0.04</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Mean carotid 0.489±0.05</td>
<td>0.485±0.04</td>
<td>0.479±0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. All values are given as mean±SEM.

*Comparison between null alleles and receptor-defective mutations.
†Statistical analysis adjusted for individual serum TG levels did not change the result (data not shown).
‡Statistical testing after logarithmic transformation.
§Carotid IMT adjusted for initial LDL-C levels.

Receptor-defective mutations for relevant clinical characteristics, lipid concentrations, and carotid IMT. Differences among LDL receptor genotype groups were analyzed by Students’ t test for continuous data. Because TG concentrations had a skewed distribution, statistical testing was performed after logarithmic transformation. To examine the relationship between LDL receptor genotype and baseline carotid IMT independently of lipid concentrations, we used a multivariate linear regression analysis adjusted for initial LDL-C levels.

We have performed a placebo-controlled subgroup analysis to determine the effect of genotype on efficacy of pravastatin treatment. First, we calculated for each participant the change from baseline in carotid IMT and lipid concentrations during the trial. Second, we used a linear regression model to analyze the difference between the placebo and pravastatin groups in carotid IMT and lipids change during the trial for each genotype group separately (IMT/lipids change during the trial = β0 + βgenotype group × treatment group). The βgenotype group is the difference in IMT/lipids change during the trial. As a result of randomization for the trial and of randomization by nature of the genotypes, we did not expect differences in age and sex distributions between the genotype groups. Nonetheless, we did perform multivariate linear regression analysis to adjust for age and sex and found similar results (data not shown). Third, the interaction between genotype (null alleles or receptor-defective mutations) and treatment (placebo or pravastatin) was statistically tested with 2-way ANCOVA. Because 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 2-way ANCOVA. Nonetheless, when we adjusted for baseline values, similar trends were found between LDL receptor genotype and response to treatment (data not shown). Throughout, a 2-tailed value of P<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 the Table. Study drug compliance was similar among the LDL receptor genotype groups. Children with null alleles and receptor-defective mutations were evenly distributed between the placebo-treated and pravastatin-treated groups. As expected, children with null alleles had significantly more elevated mean TC levels (difference, 0.94±0.20 mmol/L [SEM]; P<0.001) and mean LDL-C levels (difference, 0.97±0.20 mmol/L; P<0.001) compared with children with receptor-defective mutations. Moreover, carriers of null alleles had a greater mean carotid IMT (difference, 0.02±0.01 mm; P=0.01) and mean IMT of the ICA segment (difference, 0.022±0.01 mm; P=0.03) compared with 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 difference was not significant (difference, 0.021±0.01 mm, P=0.06; 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 in the changes in carotid IMT during the trial between the placebo and pravastatin...
groups according to the LDL receptor genotype. The separate effects in the placebo and pravastatin groups per genotype are available in Figure S1 of the Data Supplement. The decrease in mean carotid IMT and mean IMT in the CCA, BULB, and ICA segments between children who received placebo and pravastatin treatment was not significantly different in carriers of null alleles compared with carriers of receptor-defective mutations (2-way ANCOVA, $P=0.7$, $P=0.4$, $P=0.3$, and $P=0.7$, respectively). The changes in carotid IMT in Figure 2 were not adjusted for the changes in LDL-C concentrations. Adjustment for changes in LDL-C levels during the trial did not influence the decrease in mean carotid IMT among carriers of null alleles and carriers of receptor-defective mutations (both $0.014 \pm 0.01$ mm; 2-way ANCOVA, $P=0.6$). However, after 2 years of treatment, children with null alleles had a consistently greater mean carotid IMT (difference, $0.016 \pm 0.01$ mm; $P=0.02$) and mean IMT of the CCA segment (difference, $0.019 \pm 0.01$ mm; $P=0.04$) compared with 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, $0.017 \pm 0.01$ mm; $P=0.07$ for both segments).

Changes in Lipid Concentrations
Mean differences in the changes in lipid concentrations during the trial between the placebo and pravastatin groups according to the LDL receptor genotype are presented in Figure 3. The separate effects in the placebo and pravastatin groups per genotype are available in Figure S2 of the Data Supplement. Children with null alleles tended to have a smaller reduction in TC and LDL-C levels during the trial compared with children with receptor-defective mutations, but this did not reach statistical significance (2-way ANCOVA, both $P=0.08$). After the 2-year treatment, mean TC in carriers of null alleles was $7.62 \pm 0.20$ mmol/L and mean LDL-C was $5.94 \pm 0.19$ mmol/L, which remained significantly more elevated than the $6.60 \pm 0.17$ and $4.90 \pm 0.17$ mmol/L in carriers of receptor-defective mutations (both $P<0.001$).

Pravastatin increased HDL cholesterol levels and reduced TG levels to a similar extent in both LDL receptor genotype groups (2-way ANCOVA, $P=0.9$ and $P=0.7$, respectively).

Discussion
In this subgroup study of a randomized, placebo-controlled, 2-year trial with pravastatin in heterozygous FH children, we showed that LDL receptor genotype was significantly associated with the carotid IMT independently of LDL-C levels. Although the reduction in LDL-C levels by pravastatin treatment tended to be smaller in carriers of null alleles, we observed no significant difference in change of carotid IMT during the trial between the 2 LDL receptor genotype groups. However, at baseline and after 2 years of treatment, carotid IMT and lipid profile were more unfavorable in children with null alleles compared with children with receptor-defective mutations.

Our present analysis is the first genotype-phenotype study in FH to demonstrate 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 as a result of drug treatment is associated with a decrease in the incidence of vascular events.\textsuperscript{13–16} 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 also is a marker for atherosclerosis in childhood. Children with FH have a 5-fold-more-rapid increase in carotid arterial wall IMT during childhood than their unaffected siblings.\textsuperscript{13} This increase led to a significant deviation in terms of IMT values beginning at 12 years of age. Therefore, it might be suggested to measure carotid IMT as a marker of the increased atherosclerotic burden in FH children.

In this placebo-controlled trial, the response to pravastatin treatment on carotid IMT was not significantly different between the 2 genotype groups. Nonetheless, carriers of null
alleles had a greater carotid IMT than carriers of receptor-defective mutations at baseline, and this unfavorable difference was largely maintained during treatment. After correction for LDL-C levels, the differences in carotid IMT between the LDL receptor genotype groups became smaller and less significant. The lower significance may be based partly on loss of statistical power as a result of an additional covariable (LDL-C) in the multivariate analysis. The small decrease in the difference in carotid IMT between the genotype groups after correction for LDL-C levels, however, suggests that the greater carotid IMT in carriers of null alleles was partly but not solely the result of higher LDL-C levels. Knowledge of LDL receptor genotype may therefore improve clinical decision-making; untreated and treated children carrying null alleles exhibit a more increased risk of cardiovascular disease that may be partially independent of their increased LDL-C levels. Carriers of null alleles may have, to a certain extent, irreversible atherosclerosis, but we hope that they just need more aggressive statin treatment. Clearly, the carotid IMT of children with null alleles was reduced during pravastatin treatment. Unfortunately, we could not assess the relationship between LDL receptor genotype and responses to increasing doses of statins in our substudy. In future research, the effect of more aggressive and earlier statin treatment in children with null alleles should be investigated. Some studies on the efficacy and safety of stronger statins and high dosages in FH children are starting, and some are ongoing. In addition to statin treatment, a healthy lifestyle should be advised because our results suggest that cholesterol-independent mechanisms affect the carotid IMT and more aggressive lipid lowering may have a disappointing effect, especially in carriers of null alleles.

In the present study, the reductions in TC and LDL-C levels during pravastatin treatment were not significantly different in the 2 genotype groups. Previous genotype-phenotype studies in adult FH patients have yielded conflicting results.3,5–11 In a recent study, we showed that children with FH are better suited for genotype-phenotype analysis than adults.12 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 (intraclass 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. Children probably have a lower chronic exposure to known environmental factors that alter lipid levels. Moreover, because we used placebo-controlled data, information was available about the natural course of the specific LDL receptor mutation on carotid IMT and lipid profile during the 2-year follow-up. Adjusting for the natural course reduces bias in analyses of the relationship between LDL receptor genotype and treatment response; placebo effect and secular trends did not influence our observations.

Our subgroup analysis had not enough power to observe small differences in the carotid IMT responses between the 2 LDL receptor genotype groups with significance. The question arises as to whether we have made a type II error, that a difference was not observed with statistical significance because of a lack of power owing to small numbers. However, we did not find a difference between the point estimates. Recently, Schultz and Grimes32 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 allow such analyses to ultimately be combined in a meta-analysis. We have estimated that a meta-analysis to test the results of this hypothesis-generating study should be considered when ≥2600 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 independently of LDL-C levels. Selection of null alleles identifies children with the highest cardiovascular disease risk who may benefit by more aggressive and earlier lipid-lowering treatment.

References


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