Association of Specific LDL Receptor Gene Mutations With Differential Plasma Lipoprotein Response to Simvastatin in Young French Canadians With Heterozygous Familial Hypercholesterolemia

Patrick Couture, Louis D. Brun, François Szots, Michel Lelièvre, Daniel Gaudet, Jean-Pierre Després, Jacques Simard, Paul J. Lupien, Claude Gagné

Abstract—In familial hypercholesterolemia (FH), the efficacy of the inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase shows considerable interindividual variation, and several genetic and environmental factors can contribute to explaining this variability. A randomized, double-blind, placebo-controlled clinical trial with simvastatin, an HMG-CoA reductase inhibitor, was conducted in 63 children and adolescents with heterozygous FH. The patients were grouped according to known LDL receptor genotype. After 6 weeks of treatment with 20 mg/d simvastatin, the mean reduction in plasma LDL cholesterol in patients with the W66G mutation (n = 14) was 31%, whereas in the deletion >15 kb (n = 23) and the C646Y mutation groups (n = 10), it was 38% and 42%, respectively (P < 0.05). After treatment with simvastatin, HDL cholesterol levels were increased in all groups, and triglyceride concentrations were significantly reduced. Multiple regression analyses suggested that 42% of the variation of the LDL cholesterol response to simvastatin can be attributed to variation in the mutant LDL receptor locus, apolipoprotein E genotype, and body mass index, while 35% of the variation in HDL cholesterol response was explained by sex and baseline HDL cholesterol. These results show that simvastatin was an effective and well-tolerated therapy for FH in the pediatric population for all LDL receptor gene mutations. Moreover, the nature of LDL receptor gene mutations and other genetic and constitutional factors play a significant role in predicting the efficacy of simvastatin in the treatment of FH in children and adolescents. (Arterioscler Thromb Vasc Biol. 1998;18:1007-1012.)

Key Words: LDL receptor gene mutation • simvastatin • French Canadian • familial hypercholesterolemia • children

Familial hypercholesterolemia is a common autosomal-dominant disorder caused by mutations in the LDL receptor gene.1 Characteristic phenotypic features of the heterozygous FH form are raised plasma LDL cholesterol concentrations, tendinous xanthomatosis, and premature atherosclerotic coronary artery disease, usually occurring between the ages of 35 and 55 years. Homozygous or compound heterozygous patients show a 6-fold to 8-fold increase in plasma LDL cholesterol concentration and typically present manifestations of coronary artery disease before the age of 20 years.

FH is also one of the most common inherited metabolic disorders, with a worldwide frequency of 1 in 500 for heterozygotes and 1 per million for homozygotes. In the province of Québec, the prevalence of homozygous FH is approximately 6-fold higher and the minimal estimated frequency of heterozygotes ranges from 1:81 to 1:154 in northeastern Québec.2 Eleven mutations in the LDL receptor gene are responsible for more than 90% of the heterozygous FH in French Canadian patients, defined on the basis of clinical and biochemical criteria.3,4 Three of those mutations, a deletion >15 kb (Δ >15 kb) at the 5′ end of the gene and 2 missense mutations in exons 3 (W66G) and 14 (C646Y), are present in approximately 56%, 18%, and 6%, respectively, of FH patients who attend our lipid clinic in Québec city. The Δ >15 kb is a class I mutation and fails to produce immunoprecipitable LDL receptor protein.6,7 The C646Y mutation causes the mutant receptor to be rapidly degraded (class IIa) and results in very low receptor activity (<2% of normal receptor activity), while the W66G mutation exhibits decreased affinity for lipoprotein ligands (class III) and expresses about 25% of normal receptor activity.6,8 The founder basis for the high prevalence of these 3 common mutations in the French Canadian population offers the opportunity to correlate the presence of a single gene defect in the LDL receptor gene with the variation in plasma cholesterol and the
LDLR Gene Mutation Responses to Simvastatin in FH

expression of coronary artery disease in homozygous and heterozygous FH patients.9–11

The present study was designed to determine whether the nature of the LDL receptor mutation affects the response to simvastatin, a potent inhibitor of HMG-CoA reductase. Since environmental factors cause variation in the plasma lipoprotein profile among adults, the study of children and adolescents with heterozygous FH provided an opportunity to examine lipoprotein level variation at a time when environmental factors may be a less important determinant of plasma lipoproteins. In this study, we describe the different responses to simvastatin of plasma lipids, lipoproteins, and apoprotein levels among 3 genetically differentiated groups of heterozygous FH children and adolescents.

Methods

Patients

A total of 63 heterozygous FH patients aged 8 to 17 and weighing 27 kg or more were enrolled at the Laval University Lipid Research Clinic in Québec City, Canada. Eligibility criteria for the study were designed to select heterozygous patients with plasma LDL cholesterol levels persistently above the 95th percentile for age and sex12 while maintaining a lipid-lowering diet. Of those 63 heterozygous children selected, 31 had the Δ>15 kb mutation, 13 had the C646Y mutation, and 19 had the W66G mutation. Patients with concomitant conditions, such as diabetes mellitus; anorexia nervosa; kidney, liver, or thyroid disorder; and constitutional or pathologically delayed puberty, were not eligible for participation. None of the 63 subjects selected were excluded. The study was approved by a local ethical review committee and the Minister of Health of the Province of Québec, and informed consent was obtained from each of the patients and their parents as required.

Study Design

This was a randomized, double-blind, placebo-controlled clinical trial. All potential patients were individually screened approximately 6 weeks before entering this clinical research project to verify inclusion and exclusion criteria and explain the different study phases to patients and parents and obtain their informed consent. This prestudy visit included a complete medical history and physical examination, an interview with a dietitian, and blood sampling for laboratory tests. The laboratory tests comprised lipid and apolipoprotein determinations, including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total apoA-I, and total apoB, as well as routine hematologic and blood chemistry tests. All lipid-lowering medications were discontinued at least 6 weeks before the start of the study.

All eligible participants received a placebo for 4 weeks (weeks -4 to 0), after which they were randomized to double-blind active treatment and enrolled to receive 20 mg/d simvastatin or placebo for 6 weeks (weeks 0 to 6). The randomization process has been designed to obtain a treated/placebo ratio of 3:1. Patient compliance was verified by tablet counts at weeks 0, 2, 4, and 6. At all clinic follow-up visits (weeks -4, 0, 2, 4, and 6), patients were questioned about any adverse or unusual signs or symptoms, but none were suggested. Routine hematologic and blood chemistry test results were obtained at weeks -6 and 6, whereas plasma lipids and apolipoprotein concentrations were determined at every visit (-6, -4, 0, 2, 4, and 6) after fasting for 12 hours.

Throughout the trial, patients were counseled by the dietitian to follow a standard cholesterol-lowering diet (American Heart Association phase I diet) with focus on unrestricted daily caloric intake depending on age and physical activity.

Plasma Lipids, Lipoproteins, and Apoproteins

Twelve-hour fasting venous blood samples were obtained from an antecubital vein into evacuated tubes (Vacutainer) containing K2EDTA (1 mg/mL final concentration). Plasma was separated from blood cells by centrifugation and immediately used for the measurement of lipids and apoA-I and apoB. Plasma cholesterol and triglyceride concentrations were determined with an AutoAnalyzer RA-1000 (Technicon Instruments Corporation), as previously described.13 HDL cholesterol was measured in the supernatant after precipitation of apoB– containing lipoproteins with heparin-manganese chloride.14 LDL cholesterol concentrations were estimated with the equation of Friedewald et al.15 Plasma apoA-I and apoB were measured by the rocket-immunoelectrophoresis method of Laurell,16 as previously described.13 Serum standards for the apoprotein assays were prepared in our laboratory and calibrated against serum samples from the Centers for Disease Control and Prevention. The coefficients of variation for total cholesterol, HDL cholesterol, triglyceride, and apoprotein measurements were each <3%.

DNA Analysis

All children in the present study were screened for the 6 previously known French Canadian mutations in the LDL receptor gene.8,37 Genomic DNA was isolated from peripheral blood leukocytes by standard methods.18

Genotyping of apoE was done by polymerase chain reaction amplification of a 244-bp fragment of the exon 4 of the apoE gene with oligonucleotides F4 and F6 and digestion of polymerase chain reaction fragments with the restriction enzyme HhaI.19

Statistical Analysis

The χ2 test was used to analyze associations in contingency tables. Differences in age, weight, height, BMI, mean baseline levels and percentage changes of plasma lipids, lipoproteins and apoprotein concentrations among the different genetic groups were assessed by one-way ANOVA using Fisher post hoc test when a significant group effect was observed. Changes in plasma lipids, lipoproteins, and apoprotein values were tested using the paired t test. In the different analyses, plasma triglyceride data were log transformed to normalize their distribution. Multiple regression analyses were subsequently used to estimate the independent contributions of the different LDL receptor gene mutations, age, sex, BMI, apoE genotype, and mean baseline plasma lipid, lipoprotein, and apoprotein concentrations to the LDL cholesterol and HDL cholesterol responses to simvastatin. The covariates were selected because of their univariate associations with LDL cholesterol and HDL cholesterol responses. These analyses were performed using the JMP statistical software (release 3.2.1, SAS Institute).

Results

Patient Characteristics

A total of 63 French Canadian children and adolescents with heterozygous FH (37 boys and 26 girls) with either the Δ>15 kb, C646Y, or W66G mutation in the LDL receptor gene were recruited in the study. Table 1 shows the phenotypic characteristics of the study participants at the week 0 randomization visit according to the LDL receptor gene mutation groups. The 3 genetic groups were equivalent with respect to age, weight, height, BMI, HDL cholesterol, total apoA-I, total apoB, and triglyceride concentrations. The W66G mutation group showed lower total cholesterol and LDL cholesterol concentrations compared with the Δ>15 kb and C646Y groups.
concentrations than the 2 other groups, but this tendency did not reach statistical significance. There was no difference between the groups in the frequency of alcohol consumption and cigarette smoking. The male-to-female ratio was similar in all mutation groups ($\chi^2 = 1.7$; $P = 0.43$), and there was no significant sex difference in the lipoprotein-lipid profile among or within the study groups. The apoE genotype distribution was similar across the different genetic groups ($\chi^2 = 5.3$; $P = 0.50$), and the characteristics of subjects included in the placebo group were not significantly different from those of patients included in the treatment group.

**Efficacy Measurements**

The total cholesterol, LDL cholesterol, and apoB values obtained from the placebo and simvastatin groups are illustrated in the Figure. Compared with placebo, simvastatin 20 mg/d significantly reduced total cholesterol, LDL cholesterol, and total apoB levels ($P<0.0001$ for all time measurements). The mean percentage difference between the placebo and treatment groups, according to the different time measurements, varied from $-25\%$ to $-28\%$ for total cholesterol values, from $-31\%$ to $-38\%$ for LDL cholesterol, and from $-23\%$ to $-26\%$ for total apoB. As shown in the Figure, marked effects on total cholesterol, LDL cholesterol, and apoB were seen within 2 weeks, and the maximum responses were seen within 4 to 6 weeks. Compliance to the prescribed medication was assessed weekly by tablet counting, and there was no significant difference in compliance among the mutation groups.

**Variability in the Response to Simvastatin**

Of the 63 children and adolescents with heterozygous FH enrolled in the study, 47 received simvastatin 20 mg/d for 6 weeks during the active treatment phase. As shown in Table 2, simvastatin treatment exerted a significant effect on baseline total cholesterol, LDL cholesterol, and total apoB concentrations in all genetic groups. Moreover, analysis with one-way ANOVA indicated a statistically significant difference in the percentage decreases of plasma total and LDL cholesterol among the 3 genetic groups ($P<0.05$). Further analysis with Fisher’s PLSD pairwise comparison procedure revealed that the mean percentage changes in total and LDL cholesterol were significantly smaller in the W66G mutation group than in the 2 other mutation groups, with an overall alpha level of 0.05. In fact, the difference in the mean percentage reductions of LDL cholesterol values between the W66G group and the $D.\ 15\ kb$ group was $7\%$ and reached $11\%$ when the W66G group was compared with the $C.646Y$ group. This discrimination between the 3 mutation groups was also observed in the total cholesterol data ($6\%$ versus $9\%$ for the $\Delta>15\ kb$ and $C.646Y$ groups, respectively), but there was no statistical difference in the mean percentage changes of total apoB, HDL cholesterol, total apoA-I, and triglyceride values between the 3 mutation groups.

Table 3 presents the results of the multiple regression analysis of percentage of LDL cholesterol and HDL cholesterol changes with simvastatin treatment of various independent variables. Children and adolescents with the $\Delta>15\ kb$ or

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$\Delta&gt;15\ kb$</th>
<th>C646Y</th>
<th>W66G</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>M/F</td>
<td>19/12</td>
<td>9/4</td>
<td>9/10</td>
</tr>
<tr>
<td>Age, y</td>
<td>12.4±2.3</td>
<td>12.4±2.4</td>
<td>12.9±2.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>45.0±11.3</td>
<td>44.4±12.0</td>
<td>47.4±14.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>153±12</td>
<td>152±13</td>
<td>154±13</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>18.9±2.5</td>
<td>18.7±3.5</td>
<td>19.5±3.3</td>
</tr>
<tr>
<td>Total C, mmol/L</td>
<td>7.75±1.27</td>
<td>7.37±1.28</td>
<td>6.99±1.02</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>6.05±1.17</td>
<td>5.72±1.07</td>
<td>5.37±1.02</td>
</tr>
<tr>
<td>Total apoB, g/L</td>
<td>1.54±0.24</td>
<td>1.48±0.29</td>
<td>1.41±0.22</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.17±0.129</td>
<td>1.12±0.16</td>
<td>1.19±0.20</td>
</tr>
<tr>
<td>Total apoA-I, g/L</td>
<td>1.22±0.18</td>
<td>1.22±0.10</td>
<td>1.21±0.10</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>1.17±0.51</td>
<td>1.19±0.70</td>
<td>0.95±0.25</td>
</tr>
</tbody>
</table>

C indicates cholesterol; TG, triglycerides. Results are listed as mean±SD.
C646Y mutation had a significantly greater mean LDL cholesterol response, as did patients with the apoE3/E2 genotype. Similarly, BMI was inversely correlated with greater LDL cholesterol response to simvastatin. The cumulative \( R^2 \), which represents the proportion of the variation in the percentage changes of LDL cholesterol attributed to the independent variables, reached 42%. The proportion of variability in the relative (percent) change in LDL cholesterol explained by LDL receptor gene mutations reached 13.4% after controlling for covariates such as BMI and apoE genotype.

The results of the multiple regression analysis of percentage HDL cholesterol changes with simvastatin treatment are also presented in Table 3. Female subjects, as well as patients with low baseline HDL cholesterol levels, showed greater responses to treatment. The proportion of variability in HDL cholesterol response attributed to baseline HDL cholesterol and sex reached 35%. Age, BMI, mutations in the LDL receptor gene, and apoE genotype were not associated with significant variations in the relative (percent) HDL cholesterol changes.

**Discussion**

The present study demonstrated that the LDL cholesterol response to the HMG-CoA reductase inhibitor simvastatin in heterozygous FH children and adolescents was related, at least in part, to specific genetic factors. The 3 LDL receptor gene mutations studied alter the LDL receptor function differently. In 2 of these mutations (\( \Delta >15 \) kb and C646Y), the LDL receptor protein is completely absent from the cell surface. The \( \Delta >15 \) kb impairs the production of mRNA due to deletion of the promoter and exon 1 (class 1, null allele), while the C646Y mutation is associated with a defective protein transport between the endoplasmic reticulum and the
Golgi complex (class 2A, transport). On the other hand, in the W66G missense mutation, the LDL receptor protein can be expressed at the cell surface but fails to normally bind LDL particles (class 3, binding-defective allele). Although most patients benefited from a substantial reduction of plasma total and LDL cholesterol concentrations with the administration of simvastatin, children and adolescents with the defective allele W66G missense mutation demonstrated a significantly smaller response than the 2 other genetic groups. Moreover, the LDL cholesterol response to simvastatin was significantly related to other independent variables including ApoE genotype and BMI. Forty-two percent of the variance of LDL cholesterol response to simvastatin could be explained by these variables and as much as 13% of this response was attributed to the variation at the mutant LDL receptor locus alone. Thus, our data support the results of Leitersdorf et al20 showing that 18% of the LDL cholesterol response to an HMG-CoA reductase inhibitor is explained by LDL receptor gene mutations. In addition, our results showed that age, sex, and baseline lipid and lipoprotein levels did not exert a major influence on LDL cholesterol response, although other investigators studying adult subjects have not found similar results.20–22

The differences in LDL cholesterol responses between the various LDL receptor gene mutations are not yet completely understood. One possibility to explain these differences in LDL cholesterol responses to treatment with simvastatin is that upregulation of the wild-type LDL receptor allele is affected by the nature of the mutant allele. In fact, upregulation of the normal allele at the LDL receptor locus would be greater in heterozygotes for the ΔG197 (class IIB) mutation than in heterozygotes for the W66G mutation. This hypothesis is supported by the finding that subjects heterozygous for the Δ>15 kb express only one third of the mean plasma total cholesterol levels of the homozygotes, while the heterozygotes for the W66G mutation have about half of the mean concentration of cholesterol of the corresponding homozygotes.9 Moreover, genetic variability in DNA polymorphisms of the wild-type allele of women heterozygous for the Δ>15 kb contributes to quantitative variation in HDL cholesterol and LDL cholesterol concentrations.21 Another interesting explanation for varying responses between the genetic groups is that the mutant LDL receptor protein, when upregulated, interacts with the normal LDL receptor protein along its intracellular processing and may thus partially inhibit its normal function.20,22 This hypothesis is also supported by observations that heterozygotes for the C660X mutation (class IIA), which leads to the production of a truncated LDL receptor protein, have greater LDL cholesterol responses to fluvastatin than heterozygotes for the D147H (class IIA) or the ΔG197 (class IIB) mutation.23

In the present study, we have demonstrated that patients carrying the apoE3/E2 genotype have significantly greater LDL cholesterol–lowering responses to simvastatin treatment than subjects with the apoE3/E3 or apoE4/E3 genotype; no significant differences were found between the latter 2 groups. Thus, our data confirm the results previously reported by Carmena et al21 that heterozygous FH patients with an e4 allele have a significantly reduced total and LDL cholesterol response to lovastatin compared with patients with an e3 or e2 allele. Our results also confirm the trend observed by O’Malley23 and De Knijff25 that patients with the apoE4/E4 or apoE4/E3 phenotype show a smaller reduction in total and LDL cholesterol levels when treated with lovastatin or simvastatin. It is reasonable to assume that the mechanism by which LDL cholesterol responses are increased in FH patients with the e2 allele may be related to the slower catabolic rate of E2 compared with E3 or E4.26 The resulting decrease in intracellular cholesterol concentrations could increase the upregulation process of LDL receptor expression by HMG-CoA reductase inhibitors.

In this study, we also investigated the effect of simvastatin treatment on HDL cholesterol levels. HDL cholesterol concentrations increased in all genetic groups, but the differences among groups were not statistically significant. As observed with fluvastatin treatment,20 the effects of simvastatin on LDL cholesterol appear to be independent of those on HDL cholesterol. For example, in patients with the W66G mutation, the response of LDL cholesterol to simvastatin was the lowest, whereas the response of HDL cholesterol was the highest. This observation suggests that the mechanisms by which simvastatin acts on LDL and HDL cholesterol are independent of each other. The mechanisms of action of HMG-CoA inhibitors responsible for the increase in HDL cholesterol are not yet well known.

The results of the present study suggest that the nature of LDL receptor gene mutations and other genetic and constitutional factors may play a significant role in determining the efficacy of the HMG-CoA reductase inhibitor simvastatin in the treatment of children and adolescents with heterozygous FH. The question remains as to which other factors were responsible for the rest of the variance in the lipid-lowering response. A number of environmental factors and other candidate genes involved in the lipoprotein metabolism would have to be considered to account for the remainder of the variance. We believe that the factors presented in this study and other as yet uncharacterized parameters may provide useful markers to predict the efficacy of treatment of FH. On the basis of their efficacy and safety profiles,27,28 HMG-CoA reductase inhibitors also seem to be very promising agents for the treatment of FH in childhood, but long-term studies will be needed before this medication can be used.
be recommended for chronic treatment of FH in the pediatric population.

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