Effect of ezetimibe coadministered with statins in genotype-confirmed heterozygous FH patients

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Abstract

We investigated the effect of statins and statins plus ezetimibe in 65 FH heterozygotes carrying LDLR-defective or LDLR-negative mutations as well as the effect of ezetimibe monotherapy in 50 hypercholesterolemic (HCH) patients intolerant to statins. PCSK9 and NPC1L1 genes were analysed to assess the role of genetic variants in response to therapy. In FH patients combined therapy reduced LDL-C by 57%, irrespective of the type of LDLR mutation. The additional decrease of plasma LDL-C induced by ezetimibe showed wide inter-individual variability (from −39% to −4.7%) and was negatively correlated with percent LDL-C decrease due to statin alone (r = −0.713, P < 0.001). The variable response to statins was not due to PCSK9 gene variants associated with statin hyper-sensitivity. The highest response to ezetimibe was observed in a carrier of R174H substitution in NPC1L1, which had been found to be associated with high cholesterol absorption.

In HCH patients, ezetimibe monotherapy induced a variable decrease of plasma LDL-C (from −47.7% to −13.4%). To investigate this variability, we sequenced NPC1L1 gene in patients with the highest and the lowest response to ezetimibe. This analysis showed a higher prevalence of the G allele of the c.816 C>G polymorphism (L272L) in hyper-responders, an observation confirmed also in FH patients hyper-responders to ezetimibe. In both FH and HCH patients, the G allele carriers tended to have a higher LDL-C reduction in response to ezetimibe.

These observations suggest that in FH heterozygotes LDL-C reduction following combined therapy reflects a complex interplay between hepatic synthesis and intestinal absorption of cholesterol.

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Keywords: Familial hypercholesterolemia; LDLR mutations; Statins; Ezetimibe; NPC1L1 gene; PCSK9 gene

1. Introduction

Familial hypercholesterolemia (FH), caused by mutations in the gene encoding the LDL receptor (LDLR), is the most frequent form of autosomal co-dominant hypercholesterolemia. The lack of functioning LDLRs results in decreased catabolism of LDL and the accumulation of this lipoprotein in plasma, a condition, which predisposes to the development of premature coronary artery disease (CAD) [1]. In homozygous and heterozygous FH individuals the increase of plasma LDL as well as the severity of the clinical phenotype are more pronounced in the presence of mutations abolishing the receptor function (receptor-negative mutations) than in mutations resulting in defective receptors (receptor-defective mutations) [1–3].
HMG-CoA reductase inhibitors (statins) have been very effective in lowering plasma LDL cholesterol (LDL-C) [4] and reducing the risk for coronary mortality [5] in heterozygous FH patients. However, FH patients show wide variations in inter-individual plasma LDL-C responses to statins, which are independent of the type of statin and the dose used. It has been suggested that the type of LDL receptor mutation could influence the response to statins. This issue, however, is still controversial as some studies provided evidence in favour of [6,7] and others against [8,9] this hypothesis. Irrespective of the large inter-individual variability in the response to statins, many FH patients are not achieving their LDL-C goal for an effective reduction of CAD risk. To achieve desirable LDL-C levels in FH patients statins have been combined with inhibitors of cholesterol absorption, such as plant stanol esters, bile salt sequestrant resins or ezetimibe [10,11].

Ezetimibe is a new hypolipidaemic drug, which selectively inhibits cholesterol absorption in enterocyte. Recent data suggest that this drug binds to the Niemann-Pick C1 Like 1 (NPC1L1) protein, which is located in the brush-border membrane of the enterocyte where it plays a major role in the intestinal uptake of cholesterol and plant sterols [12–14]. In patients with the clinical diagnosis of primary hypercholesterolaemia ezetimibe has been combined with simvastatin [15], pravastatin [16], lovastatin [17] or atorvastatin [18]. This combined therapy, regardless of the type of statins and their doses, substantially enhanced the LDL-C lowering effect of statins by 12–19% [15–18]. Ezetimibe co-administered with simvastatin or atorvastatin has also been used successfully in homozygous FH [19,20]. However, at present there are no data concerning the effect of the combined therapy in genotype-confirmed heterozygous FH patients.

The present study was designed to assess the effect of the addition of ezetimibe to simvastatin or to atorvastatin in genotyped heterozygous FH patients and to investigate some of the factors underlying the inter-individual variability in the response to this combined therapy. To investigate the inter-individual variability in response to ezetimibe monotherapy we conducted a parallel study in a group of non-genotyped patients with primary hypercholesterolaemia who were intolerant to statins.

2. Subjects and methods

2.1. Molecularly characterized patients with familial hypercholesterolaemia (FH)

Sixty-five heterozygous FH patients who had been treated with statins for at least 2 years were included in the study (Table 1). Fifty-six of them were classified as carriers of receptor-negative or of receptor-defective LDLR mutations, according to the previously described criteria [2]. Nine patients were carriers of unclassified LDLR gene mutations since cultured fibroblasts were not available for LDLR activity assay (Table 2 and Supplementary Table 1).

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Gender</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.4 ± 13.2</td>
<td>57.1 ± 12.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 2.5</td>
<td>24.4 ± 4.2</td>
</tr>
<tr>
<td>T-C (mmol/L)</td>
<td>10.56 ± 1.84</td>
<td>10.94 ± 1.71</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>8.53 ± 1.73</td>
<td>8.80 ± 1.65</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.26 ± 0.39</td>
<td>1.45 ± 0.36</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.71 ± 0.74</td>
<td>1.50 ± 0.71</td>
</tr>
<tr>
<td>Tx (%)</td>
<td>56.7</td>
<td>73.4</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>46.6</td>
<td>34.3</td>
</tr>
<tr>
<td>CA-ATS (%)</td>
<td>56.7</td>
<td>57.1</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. Tx, tendon xanthomas; CAD, coronary artery disease; CA-ATS, carotid atherosclerosis.

Table 2

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Unclassified</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>5/8</td>
<td>19/24</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.6 ± 15.0</td>
<td>55.8 ± 12.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 3.4</td>
<td>24.3 ± 3.7</td>
</tr>
<tr>
<td>T-C (mmol/L)</td>
<td>9.15 ± 1.31*</td>
<td>11.17 ± 1.66</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>7.16 ± 1.17*</td>
<td>9.08 ± 1.58</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.35 ± 0.37</td>
<td>1.39 ± 0.39</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.44 ± 0.70</td>
<td>1.56 ± 0.69</td>
</tr>
<tr>
<td>Tx (%)</td>
<td>15.4*</td>
<td>76.7</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>15.4*</td>
<td>41.8</td>
</tr>
<tr>
<td>CA-ATS (%)</td>
<td>38.4</td>
<td>60.4</td>
</tr>
<tr>
<td>Simvastatin/atorvastatin</td>
<td>7/6</td>
<td>12/31</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. Tx, tendon xanthomas; CAD, coronary artery disease; CA-ATS, carotid atherosclerosis. *Significantly different from the other two groups; †significantly different from receptor-unclassified group.

The distribution of patients in relation to statin treatment was the following: simvastatin 20 mg/day no. 5, simvastatin 40 mg/day no. 18, atorvastatin 20 mg/day no. 14, atorvastatin 40 mg/day no. 28. Four plasma lipid determinations were performed at 2-month interval; their mean value was taken as the reference parameter to evaluate the response to statin treatment. All patients maintained their statin regimen when ezetimibe (10 mg/day) was added. The combined treatment was carried out for 3 months; plasma lipid concentrations were determined monthly. The mean value of the three determinations was used to evaluate the effects of the combined treatment.

2.2. Patients with primary hypercholesterolaemia (HCH) intolerant to statin therapy

Fifty patients with the clinical diagnosis of primary hypercholesterolaemia (19 males and 31 females, 57.3 ± 13.7 years of age, BMI 22.9 ± 4.3 kg/m²) and intolerant to statins (i.e. with a history of adverse events during treatment with statins) were treated with ezetimibe (10 mg/day)
for 3 months. The analysis of LDLR gene was not conducted in this group of patients, which most likely included subjects with heterozygous FH as well as subjects with polygenic hypercholesterolemia. Plasma lipid and apolipoprotein concentrations were determined monthly during ezetimibe treatment and the mean values of the three determinations were compared to the pre-treatment values. In the HCH group overt coronary artery disease was present in eight patients; carotid atherosclerosis with plaques causing >25% stenosis was documented in 48% of the cases.

Informed consent was obtained from all subjects investigated. The study protocol was approved by the institutional human investigation committee of each participating institution.

2.3. Plasma cholesterol (T-C) and triglycerides (TG) were measured enzymatically and apolipoproteins AI and B by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Germany) using an automated analyzer (Hitachi model 912, Hitachi Ltd., Tokyo). High-density lipoprotein cholesterol (HDL-C) was measured in plasma supernatant after precipitation of apo B-containing lipoproteins by phosphotungstate-MgCl₂. LDL-C was calculated by Friedewald’s formula.

2.4. Patient genotyping

The sequence of LDLR and PCSK9 genes was performed as previously described [2,3,21]. NPC1L1 gene was amplified using appropriately designed primers and PCR conditions specified in Supplementary Table 2. Patients were also genotyped for APOE polymorphism [22]. The complete sequence of PCSK9 gene was performed in FH patients hyper-responders (with percent LDL-C decrease above the 85th percentile of the distribution) and in FH patients hypo-responders (with percent LDL-C decrease below the 15th percentile) to statin.

Since it has been reported that some sequence variants in the coding region of NPC1L1 gene influence cholesterol absorption and plasma LDL-C [23], we screened all FH and HCH patients for four rare non conservative amino acid variants reported to be associated with low (c.916 C>T, R306C; c.1249 C>T, R417W) or high (c.521 G>A, R174H; c.845 C>T, P282L) cholesterol absorption in Caucasian subjects [23]. The R174H, P282L and R306C mutations were digested using appropriately designed primers and PCR conditions. The sequence of LDLR and PCSK9 genes was performed in FH patients, which most likely included subjects with polygenic hypercholesterolemia. Plasma lipid and apolipoprotein concentrations were determined monthly during ezetimibe treatment and the mean values of the three determinations were compared to the pre-treatment values. In the HCH group overt coronary artery disease was present in eight patients; carotid atherosclerosis with plaques causing >25% stenosis was documented in 48% of the cases.

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Informed consent was obtained from all subjects investigated. The study protocol was approved by the institutional human investigation committee of each participating institution.

The gene sequence variants were designated according to the Human Genome Variation Society (www.hgvs.org/mutnomen); the numerical series of codons includes the sequence of the signal peptide. For genomic DNA and cDNA, the A of the ATG of the initiator methionine codon is denoted nucleotide +1. Nucleotide 5’ to +1 is numbered −1.

2.5. Statistical analysis

Statistical analysis was performed by using the SPSS 13.0 (SPSS Inc., Chicago, IL) program. Differences in the distribution of categorical variables were assessed by χ² or Fisher’s exact tests. Statistically significant differences between lipid values before and after treatment were evaluated by ANOVA or by Student’s t-test for paired data. Triglyceride values which were not distributed normally were logarithmically transformed before analysis. Multiple comparisons among pairs of means were performed by t-tests with Bonferroni’s correction. Least squares mean ± S.E. percent changes from baseline are reported. Median percentage change from baseline was calculated for triglycerides, since this parameter is asymmetrically distributed.

3. Results

3.1. Response of FH patients to combined treatment

Table 3 shows the changes of lipid parameters during statin monotherapy and after 3 months of combined therapy with statin plus ezetimibe in the whole group of FH patients. There were no significant differences in the response of LDL-C to statin therapy among patients with receptor-defective (−32.5%), receptor-negative (−38.2%) or unclassified mutations (−35.6%). The same was true for the combined therapy (−54.5%, −56.9% and −58.7%, respectively). Statins (simvastatin 20/40 mg day⁻¹; atorvastatin 20/40 mg day⁻¹) induced a substantial decrease of T-C and LDL-C (mean decrease −29.7% and −36.7%, respectively) and a moderate decrease of TG (median decrease −14.4%). The addition of ezetimibe (10 mg/day) resulted in a further reduction of T-C and LDL-C (mean decrease −46.4% and −56.7%, respectively) and a more significant reduction of plasma TG (median decrease −25.9%). During statin treatment none of the patients achieved the recom-
The values are mean±S.D. Statistical analysis was performed by ANOVA and Bonferroni’s test for multiple comparisons; \(^bP<0.0001\) vs. baseline values; \(^cP<0.001\) vs. statin values; \(^dP<0.05\) vs. baseline value; \(^eP<0.001\) vs. baseline values. Least squares mean ± S.E. percent changes from baseline are reported in brackets. Median percent change is reported for TG.

Fig. 1. Individual LDL-C response to ezetimibe (% decrease) in 65 genotype confirmed heterozygous FH patients treated with simvastatin 20/40 mg day\(^{-1}\) or atorvastatin 20/40 mg day\(^{-1}\). Grey columns indicate patients considered high- or low-responders to ezetimibe (percent reduction of LDL-C due to ezetimibe above the 90th and below the 10th percentile, respectively). R174H (boxed) indicates the subject carrying this rare amino acid substitution of NPC1L1 protein reported to be associated with high cholesterol absorption (Ref. [23]).

Fig. 2. Pearson’s correlation between percent decrease of LDL-C induced by statins and by ezetimibe in genotype confirmed heterozygous FH patients. 

Table 3

Plasma lipid concentrations in heterozygous FH patients after treatment with statin and statin plus ezetimibe

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Statin</th>
<th>Statin + ezetimibe</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-C (mmol/L)</td>
<td>10.76 ± 1.76</td>
<td>7.50 ± 1.24 (−29.7 ± 1.1%)(^d)</td>
<td>5.70 ± 0.85 (−46.4 ± 0.9%)(^e)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>8.68 ± 1.68</td>
<td>5.42 ± 1.13 (−36.7 ± 1.3%)(^d)</td>
<td>3.69 ± 0.72 (−36.7 ± 0.9%)(^e)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.36 ± 0.38</td>
<td>1.47 ± 0.39</td>
<td>1.47 ± 0.39</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.60 ± 0.73</td>
<td>1.34 ± 0.48 (−14.4%)(^d)</td>
<td>1.18 ± 0.47 (−25.9%)(^d)</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. Statistical analysis was performed by ANOVA and Bonferroni’s test for multiple comparisons; \(^dP<0.0001\) vs. baseline values; \(^eP<0.001\) vs. baseline values. Least squares mean ± S.E. percent changes from baseline are reported in brackets. Median percent change is reported for TG.

Table 4

Effect of ezetimibe treatment in patients with primary hypercholesterolemia (HCH) intolerant to statin therapy

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ezetimibe</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-C (mmol/L)</td>
<td>7.93 ± 1.05</td>
<td>6.19 ± 0.88 (−22.0 ± 0.6%)(^f)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>5.54 ± 0.99</td>
<td>3.88 ± 0.84 (−30.2 ± 1.0%)(^f)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.61 ± 0.40</td>
<td>1.62 ± 0.41</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.73 ± 0.57</td>
<td>1.43 ± 0.49 (−19.5%)(^f)</td>
</tr>
<tr>
<td>Apo A (mg/dl)</td>
<td>167.5 ± 28.6</td>
<td>170.7 ± 29.8</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>142.7 ± 25.0</td>
<td>112.9 ± 20.8 (−20.8 ± 1.1%)(^f)</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. \(^fP<0.0001\) vs. baseline values (paired \(t\)-test). Least squares mean ± S.E. percent changes from baseline are reported in brackets. Median percent change is reported for TG.
variants found in both groups were the following: c.42
response to statin therapy in FH patients [25]. The amino acid
G>A, G106R; c.471 C>A, N157K; c.709 C>T, R237W) are
in the coding region of PCSK9 gene (c.137 G>T, R46L; c.316
to statins
3.5. Sequence variants in PCSK9 gene and the response
had no effect on the response to ezetimibe in HCH patients
not statistically significant. Similarly, the APOE genotype
/H9255
3.4. Relation between APOE genotype and the response
to treatment
When we stratified FH patients according to APOE genotype
we found the following plasma LDL-C decrease after
statin or combined treatment respectively: ε2ε3 (no. 4) −44.4% and −57.4%; ε3ε3 (no. 47) −35.6% and −57.0%;
ε3ε4 (no. 14) −36.7% and −55.6%. These differences were
not statistically significant. Similarly, the APOE genotype
had no effect on the response to ezetimibe in HCH patients
intolerant to statins.
3.5. Sequence variants in PCSK9 gene and the response
to statins
It has been recently reported that some missense mutations
in the coding region of PCSK9 gene (c.137 G>T, R46L; c.316
G>A, G106R; c.471 C>A, N157K; c.709 C>T, R237W) are
associated with hypercholesterolemia and possibly increased
response to statin therapy in FH patients [25]. The amino acid
variants found in both groups were the following: c.42,43ins
CTG (ins L at position 24, previously reported as 15,16
insL [26]), c.158 C>T (A53V) and c.1420 A>G (I474V)
(Supplementary Table 3). No carriers of the missense mutations
associated with hyper-sensitivity to statins were found.
3.6. Sequence variants in NPC1L1 gene and the response
to ezetimibe
The screening of FH and HCH patients for the rare amino
acid variants of NPC1L1 protein, reported to be associated
with low (R306C and R417W) or high (R174H and P282L)
cholesterol absorption [23], revealed that only one patient (a
genotype confirmed heterozygous FH) was a carrier of one
of these variants (R174H); this patient was found to have
the highest response (−39.2%) to ezetimibe (Fig. 1). During
this screening we also genotyped all patients for the silent
mutation c.816 C>G (L272L). The genotype distribution was
as follows: in the FH group 50 patients were CC, 14 were CG
and 1 GG; in the HCH group 39 patients were CC, 9 were
CG and 2 GG.
The sequence of NPC1L1 gene in FH and HCH subjects
hyper- or hypo-responders to ezetimibe showed several variants,
which are reported in Supplementary Table 4. Most of
these variants had been previously reported [24,27]; two of
them (Ex 15 g.22658, c.3201 C>T, L1067L, found in an HCH
hyper-responder, and Ex 17 g.24362, c.3405 CA, I1135I,
found in an HCH hypo-responder) are novel. This analysis
also showed a higher prevalence of the G allele of the
c.816 C>G polymorphism (see above) in hyper-responders
of both groups with respect to hypo-responders (FH: 0.25
versus 0.00; HCH 0.50 versus 0.10) and the presence of two
homozygotes for the rare allele (GG) among the five HCH
hyper-responders. In view of this finding we asked whether
this polymorphism had any effect on the LDL-C response
to ezetimibe. Comparing the LDL-C reduction induced by
ezetimibe in subjects homozygous for the common allele
(CC) with that observed in heterozygous and homozygous
for the rare allele in both groups, we observed that car-
riers of the G allele tended to have a higher response to
ezetimibe. The LDL-C percent decrease (mean ±S.E.M.)
was as follows: −18.9 ±1.0% in CC versus −23.3 ±2.3%
in CG+GG (P <0.07) in FH patients; −29.2 ±1.0% in CC
versus −33.7 ±2.6% in CG+GG (P <0.06) in HCH patients.
4. Discussion
In the present study we documented that in genotype-
confirmed heterozygous FH patients treated with statins: (1)
co-administration of ezetimibe induced a substantial addi-
tional decrease of plasma LDL-C; (2) ezetimibe-dependent
decrease of LDL-C showed a high degree of inter-individual
variability; (3) there was a negative correlation between the
response to statins and the subsequent response to ezetimibe
(patients hyper-responders to statins were poor-responders
to ezetimide and vice versa).
Although many studies have been conducted on the effect
of combined therapy (statin plus ezetimibe) on the lipid
profile in patients with primary hypercholesterolemia, the
present work is the first, which reports the effects of this treat-
ment in a large group of patients with molecularly defined
diagnosis of heterozygous FH. We thought that a well char-
acterized group of FH patients with receptor-negative or
receptor-defective mutations would provide a unique oppor-
tunity to ascertain whether the response to this new treatment
was affected by the type of defect in the LDL receptor. The
mean reduction of LDL-C obtained in our FH patients
(−56.7%) was similar to that observed in patients with
primary hypercholesterolemia, who had been treated with
comparable doses of simvastatin or atorvastatin together with
the standard dose of ezetimibe [15–18]. Our study showed
that the response to statins as well as the response to combined therapy was highly variable among FH subjects. This variability was not affected by the type of LDL receptor defect or by the APOE genotype. We also ruled out that this variability was due to the presence of mutations in PCSK9 gene, reported to be associated with a good response to statin treatment [25]. These negative results suggest that other environmental or genetic factors are involved, which influence cholesterol synthesis or cholesterol absorption.

Previous studies showed that FH patients who were good responders to statins had a high basal level of plasma mevalonic acid, an indicator of a higher cholesterol synthesis [10]. Other studies showed that statins reduced serum cholesterol more efficiently in those coronary patients with the lowest plasma level of cholesterol, indicating a less efficient intestinal cholesterol absorption [28]. Indeed the strong negative correlation between the LDL-C response to statins and the subsequent response to ezetimibe found in our FH patients can be explained in terms of variability in cholesterol synthesis and cholesterol absorption. It is likely that the rate of cholesterol absorption and the rate of cholesterol synthesis are biologically linked, in that subjects with low cholesterol absorption capacity presumably have a reduced delivery of intestinal cholesterol to the liver and a higher rate of hepatic cholesterol synthesis (due to a less pronounced down-regulation of HMG-CoA reductase) and vice versa. Patients with low cholesterol absorption are expected to respond more efficiently to statins and less efficiently to ezetimibe, whereas patients with high cholesterol absorption are expected to be poor responders to statins but good responders to ezetimibe. In this context the genetic factors controlling cholesterol absorption might play an important role. NPC1L1 protein is one of the key factors in this process as recent studies have showed that amino acid sequence variants of this protein influence the cholesterol absorption and plasma LDL levels under physiological conditions [23], as well as during ezetimibe treatment [24,27]. For example, some non-conservative amino acid substitutions in NPC1L1 protein, found more frequently in African-Americans from the Dallas-Heart Study, have been shown to be associated with reduced cholesterol absorption and lower plasma LDL-C; conversely, other rare amino acid variants have been found to be associated with high cholesterol absorption [23]. Our FH patients were screened for four rare amino acid variants (R174H, P282L, R306C, R417W) associated with variations in cholesterol absorption in Caucasian subjects from the Dallas-Heart Study population [23]. We found that the FH patient with the highest response to ezetimibe was, in fact, a carrier of the R174H substitution, which has been associated with high cholesterol absorption [23].

To ascertain whether NPC1L1 sequence variants could affect the LDL-C response to ezetimibe we sequenced the whole NPC1L1 gene in FH and HCH patients with the highest (above the 90th percentile) and the lowest (below the 10th percentile) LDL-C response to ezetimibe. We found several sequence variants (Supplementary Table 4), the rare alleles of some of which (g.-18 A, g.1679 G, g.25286 C and g.27621 C) had been previously found to be associated with a higher LDL-C response to ezetimibe [24,27]. In our patients, however, three of these rare alleles (g.1679 G, g.25286 C, g.27621 C) were present in both hyper- and hypo-responders, thus suggesting that their role in response to ezetimibe does not clearly emerge in a small sample of subjects. Nevertheless, among HCH hyper-responders one subject was found to be a carrier of all the above mentioned four rare alleles (g.-18 A, g.1679 G, g.25286 C and g.27621 C) and two subjects were homozygous for the rare G allele of the g.1679 C>G (c.816 C>G) polymorphism. The latter polymorphism, when investigated in all FH and HCH patients, seems to have some influence on response to ezetimibe, as carriers of the rare G allele showed the tendency to have a higher reduction of plasma LDL-C following ezetimibe therapy. It is unlikely that this polymorphism, which results in a silent mutation (L272L), directly affects the function of the NPC1L1 protein. It is possible that it is linked to another mutation outside the coding region, which might affect the expression of NPC1L1 gene and consequently the rate of cholesterol absorption.

The mean reduction of LDL-C induced by the addition of ezetimide to statins therapy (i.e. the difference between percent decrease induced by combined therapy minus that induced by statin) was about —20% in FH patients (Fig. 1). In the group of HCH patients intolerant to statins we observed a higher percent reduction of LDL-C (about 30%) in response to ezetimibe administration (Fig. 3). This difference might be due to the heterogeneity of the metabolic defect in the HCH group, as compared to the group of genotyped FH patients. It is possible that the HCH group includes a high percentage of individuals with a high intestinal absorption of cholesterol, who, for the reasons illustrated above, are expected to be good responders to ezetimibe treatment.

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Appendix A. Supplementary data


References


