Presence and type of low density lipoprotein receptor (LDLR) mutation influences the lipid profile and response to lipid-lowering therapy in Brazilian patients with heterozygous familial hypercholesterolemia

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ABSTRACT

Objectives: Familial hypercholesterolemia (FH) is an autosomal dominant disease caused mainly by LDLR mutations. This study assessed the influence of the presence and type of LDLR mutation on lipid profile and the response to lipid-lowering therapy in Brazilian patients with heterozygous FH.

Methods: For 14 ± 3 months, 156 patients with heterozygous FH receiving atorvastatin were followed. Coding sequences of the LDLR gene were bidirectionally sequenced, and the type of LDLR mutations were classified according to their probable functional class.

Results: The frequencies of the types of LDLR mutations were: null-mutation (n = 40, 25.6%), defective-mutation (n = 59, 37.8%), and without an identified mutation (n = 57, 36.6%). Baseline total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were higher in patients carrying a null mutation (9.9 ± 1.9 mmol/L, 7.9 ± 1.7 mmol/L), compared to those with a defective (8.9 ± 2.2 mmol/L, 7.0 ± 2.0 mmol/L), or no mutation (7.9 ± 1.9 mmol/L, 5.8 ± 1.9 mmol/L) (p < 0.001). After treatment, the proportion of patients attaining an LDL-C < 3.4 mmol/L was significantly different among groups: null (22.5%), defective (27.1%), and without mutations (47.4%) (p = 0.02). The presence of LDLR mutations was independently associated with higher odds of not achieving the LDL-C cut-off (OR 9.07, 95% CI 1.41 –58.16, p = 0.02).

Conclusions: Our findings indicate that the presence and type of LDLR mutations influence lipid profile and response to lipid-lowering therapy in Brazilian patients with heterozygous FH. Thus, more intensive care with pharmacological therapeutics should be performed in patients who have a molecular analysis indicating the presence of a LDLR mutation.

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1. Introduction

Familial hypercholesterolemia (FH; OMIM 143890) is an autosomal dominant disease mainly caused by mutations in the low-density lipoprotein receptor (LDLR) gene. This common disorder is characterized by severely elevated LDL-C (low-density lipoprotein cholesterol), xanthomas, and the development of premature cardiovascular disease (CVD). To date, more than 1000 mutations in the gene that encodes LDL receptors have been described worldwide [1–3].

The LDLR gene (606945) consists of 18 exons, with correlation between exons and the functional domains of the LDL receptor protein. Regarding the type of LDLR mutations, five classes based on phenotypic effects have been proposed. Class I mutations usually are nonsense mutations, large deletions and promoter mutations resulting in no detectable LDL receptor protein. Class II mutations (transport defective alleles) cause either complete (class II a) or partial (class II b) block of the transport of the LDL receptor from the endoplasmic reticulum to the Golgi apparatus. Class III mutations result in defective LDL binding; class IV mutations cause a deficiency in the internalization of LDL and class V mutations fail to recycle effectively [3–6].
Statins are the first choice of treatment to reduce cardiovascular morbidity and mortality in FH, a condition that is considerably undertreated [7–10]. Furthermore, the influence of the type of LDLR mutations on response to treatment is not completely clear, and few studies have been performed with controversial findings [4,6,11–18]. The aim of this study was to assess the influence of the type of LDLR mutation on lipid profile and the response to lipid-lowering therapy in Brazilian patients with heterozygous FH.

2. Materials and methods

2.1. Patients clinical and laboratory evaluation

This study included 156 index Brazilian patients with a heterozygous FH phenotype from the Lipid Clinic, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil. These patients were diagnosed using biochemical and clinical data [19,20] and were followed for at least 12 months from January 2006 to December 2012 while receiving lipid-lowering therapy. Treatment was administered without any knowledge of the kind of genetic defect causing the FH phenotype by the attending physician. Atorvastatin dosage and the association of ezetimibe, and adjuvant cholesterol lowering medication, were set according to the discretion of the physician, to attain the greatest LDL-C lowering effect possible. All patients were assessed at least 3 times during follow-up. The study protocol was approved by the Institutional Ethics Committee (CAPPesq numbers 022/11 and 191/04), and written informed consent was obtained from all participants prior to entering the study.

Plasma total cholesterol and LDL-C values were measured by enzymatic methods obtained at least in the following periods: at baseline (first value), at the initiation of atorvastatin use (immediately before), and on average after 1-year of treatment onset (the two first measured values were not necessarily the same). For cholesterol values, to convert from the SI unit (mmol/L) to the conventional unit (mg/dL), divide by the conversion factor 0.0259. Laboratory assessment of plasma creatine kinase (CK) concentrations was performed at least 3 times during follow-up by a kinetic method. Musculoskeletal treatment side effects considered were: myalgia (atorvastatin-induced muscle pain, irrespective of CK values, at onset of treatment or in dose up-titration until the first year of follow-up), CK elevations of more than 3 times the upper limit of the normal range (irrespective of symptoms), and rhabdomyolysis, as previously described [21].

2.2. Sequencing

Genomic DNA was extracted from peripheral blood following a standard salting-out procedure. Coding sequences of the LDLR gene (18 exons) were amplified by polymerase chain reaction (PCR) using primers described in the Supplementary Table 1. PCR products were purified using ExoSAP-IT® reagent (GE Healthcare, NJ, USA) and were bidirectionally sequenced using the ABI Terminator sequencing kit (Applied Biosystems, Foster City, CA, USA). In addition, sequencing for the APOB and PCSK9 genes and multiplex ligation-dependent probe amplification (MLPA) technique for the identification of LDLR gene deletions/insertions were performed.

LDLR gene mutations were classified according to their probable functional class as previously reported in the literature [5,6,22–26] and through the use of a curated database (www.jojogenetics.nl/wp/database/). Null-mutations included classes 1 and 2A, and large rearrangements or deletions leading to a frameshift and a premature stop codon. Defective-mutation included classes 2B, 3, 4, and 5, and non-frameshift small deletions or insertions. There are also patients without an FH-related mutation who were classified in the “without identification mutation”.

2.3. Statistical analysis

Continuous variables data are presented as mean and standard deviation and categorical variables as frequencies. Chi-square or Fisher exact tests were performed for comparative analysis of the categorical variables according to gender or the type of LDLR mutation. The Student t test or analysis of variance (ANOVA) was used for comparing age, body mass index, CK values, cholesterol data, and variation of LDL-C means according to the type of the LDLR mutation. Variation data were calculated as 1-year treatment value minus baseline value divided by baseline value, expressed as a percentage. Biochemical data were adjusted for age, gender, and selfREFERRED ethnicity. Variation data and 1-year treatment data were also adjusted for baseline values. Atorvastatin doses and use of ezetimibe, were also inserted in the model as covariates. Tukey’s post hoc test was performed for identifying statistically different groups. In addition, logistic regression univariate and multivariate analyses were performed to evaluate predictors for LDL-C<3.4 mmol/L after 1 year. All statistical analyses were carried out using SPSS software (version 16.0, IBM, New York, NV), with the level of significance set at p ≤ 0.05.

3. Results

3.1. General characteristics of FH patients receiving lipid-lowering therapy

Baseline characteristics of study subjects according to gender are shown in Table 1. Of the 156 patients (mean age 52.5 ± 14.5 years), 106 (67.9%) were female and 50 (32.1%) were male. All patients received atorvastatin for at least 12 months (mean and standard deviation 14 ± 3 months of follow-up of therapy). Initial daily doses were 20 mg (n = 14, 9.0%), 40 mg (n = 51, 32.7%), 60 mg (n = 2, 1.3%) and 80 mg (n = 89, 57.0%); and only 10 (6.4%) had their values, at onset of treatment or in dose up-titration until the first year of follow-up).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Females</th>
<th>Males</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>54.3 ± 15.3</td>
<td>48.8 ± 12.0</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI, Kg/m²</td>
<td>27.5 ± 5.3</td>
<td>26.7 ± 5.5</td>
<td>0.46</td>
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<tr>
<td>Race/color, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>73.6</td>
<td>74.0</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>19.8</td>
<td>14.0</td>
<td>0.40</td>
</tr>
<tr>
<td>Black</td>
<td>6.6</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Smoking, %</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>73.6</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>13.2</td>
<td>44.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>7.6</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>8.8 ± 2.3</td>
<td>8.7 ± 1.8</td>
<td>0.88</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>6.8 ± 2.2</td>
<td>6.8 ± 1.9</td>
<td>0.85</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.6 ± 0.8</td>
<td>2.0 ± 1.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>67 ± 73</td>
<td>55 ± 43</td>
<td>0.59</td>
</tr>
<tr>
<td>Type of the LDLR gene mutation, % (null/defective)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient without identified mutation</td>
<td>40.5</td>
<td>28.0</td>
<td></td>
</tr>
</tbody>
</table>

FH: familial hypercholesterolemia; BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol. Race/color was categorized in White, Intermediate ("Mulatto or Pardo") in Portuguese, person with admixture between White and Black) and Black. Biochemical data were adjusted for age and race.
doses modified during follow-up. None of study subjects discontinued the drug during follow-up. In addition to atorvastatin treatment, 89 (57.1%) patients also received ezetimibe 10 mg/day. The most frequently used drugs were: acetylsalicylic acid (37.8%), angiotensin converting enzyme inhibitors (33.3%), beta-blockers (30.8%), hydrochlorothiazide (25.0%), and metformin (13.5%). Frequent comorbidities were: hypertension (58.3%), diabetes mellitus (14.7%), and acute myocardial infarction (9.6%). During the one-year follow-up period, 17 patients (11.6%) developed a myalgia clinical phenotype, and no patient developed rhabdomyolysis. No difference regarding the frequency of myalgia (p = 0.45) or CK values (p = 0.70) according to the type of the LDLR mutation was observed (Table 2).

The frequencies of the type of LDLR mutations were null-mutation (n = 40, 25.6%), defective-mutation (n = 59, 37.8%), and without identified mutation (n = 57, 36.6%). Patients carrying the p.(Glu317Glyfs*15), p.(His388Profs*53), p.(Phe629Tyrfs*16), and exons 15 + 16 deletion new mutations were classified in the null-mutation group. The p.Ala391Thr and c.1706-10G > A genetic alterations were considered non-pathogenic based on in silico programs. Thus, we included patients carrying both alterations (n = 2) in the without FH-related mutation group. Neither patient presented APOB or PCSK9 mutations. Supplementary Table 2 shows the LDLR gene mutations according to their probable functional class.

The frequencies of ezetimibe use and atorvastatin doses were differently distributed according to the type of the mutation (p = 0.002, p = 0.04, respectively). The frequency of ezetimibe use was 67.5%, 67.8% and 38.6% respectively in those with null, defective and no identified mutations. The frequency of atorvastatin dose of 80 mg/day was 70% in those with null-mutation, 61% in those with defective-mutation, and 45.6% in those without identified mutation.

Table 2 shows baseline and 1-year treatment lipid levels. Baseline total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were higher in patients carrying null compared to the defective and without a mutation group (p < 0.001, for both parameters). In addition, LDL-C values were significantly different among groups in the initial time of lipid-lowering therapy and in the 1-year treatment period (Supplementary Fig. 1).

After treatment, patients carrying null-mutations had higher TC and LDL-C values, while those without identified mutations had lower TC and LDL-C values (p = 0.02, p = 0.003, respectively). The variations of TC and LDL-C, expressed in percentage considering baseline values or expressed in mmol/L, were higher in the null-mutation group compared to other groups, adjusted for covariates (Table 2). Even adjusting for ezetimibe use and atorvastatin dose as additional covariates, 1-year treatment and variation data remained significantly different. Supplementary Table 3 shows stratified analysis for biochemical data and response to lipid-lowering therapy according to type of LDLR mutation.

The proportion of patients with LDL-C< 3.4 mmol/L after 1 year treatment was significantly different among groups: null-mutation (22.5%), defective-mutation (27.1%), and without mutation (47.4%), p = 0.02. The analysis of predictors for achieving LDL-C< 3.4 mmol/L is shown in Table 3 and Supplementary Table 4. The presence of LDLR mutation was independently associated with higher odds of not achieving the LDL-C cutoff in an adjusted model (OR 9.07, 95% CI 1.41–58.16, p = 0.02) (Table 3).

4. Discussion

The main findings of this study indicate the influence of the presence and type of LDLR mutation found in Brazilian patients with heterozygous FH phenotype on the lipid profile and response to lipid-lowering treatment. Thus, focusing on reducing CVD morbidity and mortality in FH, more intensive care with pharmacological therapeutics should be performed in patients who have a molecular analysis indicating the presence of a LDLR mutation.

Corroborating our findings, some studies have previously shown positive associations between the type of LDLR mutation and some phenotypes: higher TC and LDL-C levels, different response to statins, and higher frequencies of premature CVD (myocardial infarction, angina pectoris, percutaneous coronary intervention or other invasive procedures and coronary artery bypass grafting) [4,6,11,14–17,22,27–29]. The present study extends these analyses to the 1-year follow-up period in patients receiving lipid-lowering treatment. Thus, the higher TC and LDL-C values identified in the baseline and 1-year periods in the null-mutation patient group...
support the idea that the relative reduction in plasma cholesterol concentrations can be more apparent in null-mutation carriers than in other mutation groups. On the other hand, the proportion of patients achieving the therapeutic target is lower in null-mutation carriers. Consequently, more aggressive treatment and attention should be practiced on these patients.

Regarding this issue some studies have reported controversial results [4,6,12,13,16,17,27]. Sun et al. examined 42 patients with heterozygous FH receiving simvastatin and concluded that there were no differences in the response to treatment in terms of either relative reduction or absolute decrease in LDL-C concentration between patients with different LDL-receptor defects [12]. Sijbrands et al. compared the response to simvastatin, 20 mg daily for 9 weeks, in 27 FH patients. They observed that the percentage LDL lowering response to simvastatin treatment was similar in patients with negative or positive mutations [13]. Chaves et al. observed that 22 FH patients with null-mutations showed a poorer response to simvastatin treatment than in 20 patients with defective-mutations [17]. On the other hand, Miltiadous et al. studied 49 patients receiving atorvastatin 20 mg/day and followed for 12 weeks. They showed that patients with a class V mutation exhibited higher percentage decrease in LDL-C after statin therapy compared to patients carrying class II mutations [6]. Couture et al. evaluated 63 children and adolescents with heterozygous FH and receiving simvastatin 20 mg/day for 6 weeks. They concluded that the nature of LDLR gene mutations and other genetic and constitutional factors play a significant role in predicting the efficacy [16]. However, some aspects should explain part of these controversial results, such as small sample size and power of analysis, different types of statins or drug combinations, the use of different end-points (such as decrease of LDL-C or achievement of LDL-C goal), mutation heterogeneity within the same predicted class, presence of other pharmacogenetic markers, ethnicity, and different follow-up times. Thus, a robust meta-analysis, performed with previous studies but with similar characteristics, might indicate a resolution to the conflicting conclusions currently available in the literature.

Here, our casuistic was formed with over two-fold more female than male patients. In addition, the male group was younger and presented a higher proportion of null mutations. A hypothesis for these differences is that females, as previously described, are characteristically more aware of their health problems and are more able to seek specialized care. It is known that women are more prevalent in population-wide screening and tend to be more adherent to primary care programs. Men with null mutations have more severe dyslipidemia and this may have triggered an overall increased recall in this specific substrata. The gender variable was significant in a multivariate analysis of factors associated with LDL-C < 3.4 mmol/L. However, this finding on FH treatment scenario needs further investigation. Also, even though the null-mutation patient group had higher frequencies of ezetimibe and atorvastatin 80 mg/day use, they had a lower frequency of attaining LDL-C values < 3.4 mmol/L and higher risk of not achieving the therapeutic target. Our data are similar to previous studies that report the necessity of improving FH management [2,22,28].

In this context, and for a genetic disease with more than 1000 known mutations, a randomized prospective study plus a large follow-up of patients receiving pharmacological treatment will allow better assessment of the effects consequent to the type of LDLR mutation on the outcomes of lipid-lowering treatment and to understand what is the best way to prevent CVD in this particular population of high-risk individuals. It is possible that patients carrying different functional LDLR mutations may respond differently to drugs, because HMG-CoA reductase may respond differently according to pathological status.

There are limitations in our study. First, our sample size is relatively small. Second, it is not possible to completely exclude the influence of ezetimibe use and atorvastatin dose according to mutation, but even with this difference, null-mutation carriers had worse LDL-C status. Third, we supported FH patients in whom no mutation was found by biochemical and clinical phenotypes, but we were not able to sequence LDLR non-coding regions or to conduct functional tests. Finally, other variables could still be evaluated in terms of management of FH according to the type of LDLR mutation, for examples: cost-effectiveness, other markers associated with the pharmacogenomics of statins, use of concomitant medications, comorbidities, age, gender, and ethnicity [30–33].

In conclusion, our findings indicate that the presence and type of LDLR mutation influences the lipid profile and the response to lipid-lowering therapy in Brazilian patients with FH. Patients carrying null-mutations have higher LDL-C values at baseline and after 1-year and, although having a higher nominal decrease in LDL-C, the frequency of patients achieving a therapeutic target is lower. Thus, more intensive care with pharmacological therapeutics should be performed in patients who have a molecular analysis indicating the presence of a LDLR mutation.

**Conflict of interest**

RDS has the following conflicts of interest to declare: honoraria or consulting fees from Astra Zeneca, Aegerion, Amgen, Biolab, Bristol Myers Squibb, Biolab, Merck, Pfizer, Sanofi, Regeneron, Genzyme, Novo-Nordisk, Lilly, Boehringer Ingelheim, Novartis and, Nestle.

The other authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2013.12.028.

**References**


