Phenotype of Heterozygotes for Low-Density Lipoprotein Receptor Mutations Identified in Different Background Populations

Anne Tybjærg-Hansen, Henrik Kjærrulf Jensen, Marianne Benn, Rolf Steffensen, Gorm Jensen, Børge G. Nordestgaard

Background.—The effect of mutations on phenotype is often overestimated because of ascertainment bias. We determined the effect of background population on cholesterol phenotype associated with specific mutations in the low-density lipoprotein (LDL) receptor and the relative importance of background population and type of mutation (LDL receptor [LDLR] or APOB R3500Q) for cholesterol phenotype.

Methods and Results.—We studied 9255 individuals from the general population, 948 patients with ischemic heart disease (IHD), and 63 patients with clinical familial hypercholesterolemia (FH) for 3 common LDL receptor mutations. Average increase in cholesterol in LDL receptor heterozygotes identified in the general population or among patients with IHD or FH compared with noncarriers was 2.9 mmol/L, 4.1 mmol/L, and 4.9 mmol/L, respectively (P<0.02). Background population and type of mutation determined cholesterol phenotype; average increase in LDL cholesterol from carriers in the general population to carriers with clinical FH was 1.6 mmol/L (P=0.03). The average increase for carriers of LDLR mutations compared with carriers of APOB R3500Q was 1.2 mmol/L (P=0.05).

Conclusion.—The phenotype associated with a given mutation should not be determined in patients, but rather in unselected individuals in the general population. (Arterioscler Thromb Vasc Biol. 2005;25:211-215.)

Key Words: atherosclerosis ■ epidemiology ■ genetics ■ cardiovascular disease ■ hypercholesterolemia

Clinical familial hypercholesterolemia (FH) is most often caused by mutations either in the low-density lipoprotein (LDL) receptor or in its ligand apolipoprotein B-100 (apoB), the sole protein constituent of LDL. The phenotype of either type of mutation is characterized by elevated levels of total and LDL cholesterol, and by an increased risk of ischemic heart disease (IHD).

However, the phenotype of LDL receptor (LDLR) mutation carriers identified in the general population is unknown. We have previously shown that ascertainment bias accounts for 1.4 to 1.9 mmol/L of the increase in cholesterol levels associated with APOB R3500Q in patients with IHD or FH, and that only 2.6 mmol/L is explained by the mutation itself. It is therefore likely that a substantial part of the 4.5 to 7.0 mmol/L increase thought to be caused by LDLR mutations likewise could be explained by ascertainment bias.

So far, the importance of LDLR mutations for cholesterol levels mainly has been studied in patients with clinical FH and/or IHD, and in the families of these patients. The main reason for this is that in most countries, clinical FH is caused by many different LDL receptor mutations, making it difficult to screen a large fraction of the general population for these mutations. In Denmark, however, 3 LDL receptor mutations, W23X, W66G, and W556S, explain >40% of all clinical FH.

This allowed us to ask 3 hitherto unanswered questions by screening the entire Copenhagen City Heart Study cohort, as well as 948 patients with IHD and 63 patients with clinical FH, for these 3 LDLR mutations. What is the phenotype of LDLR mutation carriers identified in the general population? How much additional LDL cholesterol elevation is explained by ascertainment bias in patients with IHD or clinical FH? What is the relative importance of background population and type of mutation (LDLR or APOB R3500Q) for cholesterol phenotype when mutation carriers are identified in identical background populations?

Methods

Subjects
The Copenhagen City Heart Study is a prospective population study of individuals selected randomly within 10-year age groups and by
gender based on the Central Population Register Code to reflect the adult Danish general population aged 20 to 80 years or older. At the third examination, 1991 to 1994, 9259 participants (55% women) gave blood for DNA analyses, of which 9255 were genotyped for the present study.2 3 Thus, participants were completely unselected with respect to lipid levels and IHD but were screened for manifestations of IHD by reviewing all hospital admissions and diagnoses entered in the Danish National Hospital Discharge Register and the Danish National Register of Causes of Death. The diagnoses were based on the International Classification of Diseases, eighth and tenth revisions (IHD codes 410 through 414 and I20 through I25, respectively).

Patients with IHD were identified among 992 consecutive patients from the greater Copenhagen area who were referred for coronary angiography from 1991 through 1993. All patients were evaluated by experienced cardiologists at Rigshospitalet, Copenhagen University Hospital. Among these, 948 patients (26% women) had IHD with characteristic symptoms of stable angina according to the guidelines of the European Society of Cardiology on the basis of the location, character, and duration of pain and the relation of pain to exercise, plus at least one of the following: severe stenosis on coronary angiography (≥70% stenosis of at least 1 coronary artery or ≥50% stenosis of the left main coronary artery), a previous myocardial infarction, or a positive exercise electrocardiography test.

Sixty-three unrelated index patients with a clinical diagnosis of FH were recruited from the lipid clinic at Aarhus University Hospital. The diagnosis was based on the following criteria used at Aarhus University Hospital: a total cholesterol ≥8.0 mmol/L and LDL cholesterol ≥6.0 mmol/L before treatment with statins or other lipid-lowering drugs, tendinous xanthomas in the patient or a first-degree relative, and a family history of hypercholesterolemia. This corresponds to a definite diagnosis of FH according to the Simon Broome criteria but is somewhat more restrictive than these. Patients with a clinical diagnosis of FH caused by the R3500Q mutation in LDLR were excluded as previously described.2

All lipid and lipoprotein values for carriers of LDLR mutations in the present study were pretreatment values, ie, before treatment with statins or other lipid-lowering drugs. In all groups, >99% were white and of Danish descent. Participants gave informed consent. The studies were approved by the relevant ethics committees in Copenhagen and Aarhus, Denmark.

DNA Analyses

The W23X, W66G, and W556S mutations are caused by the substitution of adenine for guanine at position 131 of cDNA, guanine for thymidine at position 259, and cytosine for guanine at position 1730, respectively, in exons 2, 3, and 12 of the LDLR gene.4–6 Three primers were used in an asymmetrical, allele-specific polymerase chain reaction for the detection of each of the 3 mutations as previously described.2 Primers and polymerase chain reaction conditions are available from the authors.

Other Analyses

Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, high-density lipoprotein cholesterol, triglycerides, and apolipoprotein B (all Boehringer Mannheim, Mannheim, Germany). LDL cholesterol levels were calculated with the equation of Friedewald et al.10

Statistical Analysis

We used Mann–Whitney U test and Fisher exact test. The absolute increase in a given variable in heterozygotes was calculated by subtracting the 50th percentile value for age and sex in the general population from the value for the individual subject. Median increases were compared by Kruskal–Wallis analysis of variance, with the Mann–Whitney U test as a post-hoc test. Relative importance of background population and type of mutation for lipid and lipoprotein levels was evaluated using 2-way analysis of variance, with Student t test as post-hoc test. The ability of mutations to predict IHD and clinical FH was expressed as an odds ratio. P<0.05 on a 2-sided test was considered significant. Probability values are unadjusted for multiple comparisons, but Bonferroni corrected probability values are given in the legends to the Tables and the Figure.

Results

Phenotype of Heterozygous Carriers of LDLR Mutations Identified in the General Population

The W23X, W66G, and W556S mutations were identified in 6 of 9255 subjects in the general population (carrier frequency, 0.06%), in 3 of 948 patients with IHD (0.32%), and in 28 of 63 patients with clinical FH (44%). All 37 subjects were heterozygous for the mutations. The characteristics are shown in Table 1. For age- and sex-adjusted percentiles of lipid, lipoprotein, and apolipoprotein B values for heterozygous subjects identified in the general population or among patients with IHD or clinical FH, please see http://atvb.ahajournals.org.

The average increase in cholesterol, LDL cholesterol, and apolipoprotein B in heterozygous carriers of LDLR mutations in the general population compared with noncarriers of the same age and sex was 2.9 mmol/L, 3.8 mmol/L, and 52 mg/dL, respectively (P<0.001 for all comparisons) (Table 2).

Effect of Background Population on Phenotype: Importance of Ascertainment Bias

Heterozygous carriers of LDLR mutations identified among patients with either IHD or clinical FH had significantly higher plasma cholesterol levels, more often had a family history of premature IHD or hypercholesterolemia, and were more often treated for hypercholesterolemia than carriers in the general population (P=0.05, P=0.003, P<0.001, respectively) (Table 1). Body mass index did not differ significantly between carriers identified among patients with either IHD or clinical FH and carriers in the general population.

When lipid values were adjusted for age and sex, carriers of LDLR mutations identified among patients with either IHD or clinical FH had a greater average increase in cholesterol of 4.1 mmol/L and 4.9 mmol/L, respectively (P=0.02; post-hoc tests: general population versus FH, P=0.007), and in LDL cholesterol of 4.2 mmol/L and 5.3 mmol/L, respectively (P=0.05; post-hoc tests: general population versus FH, P=0.026), than carriers in the general population (Table 2).

Relative Importance of Type of Mutation and Background Population for Phenotype

Background population was a significant overall determinant of average increase in both cholesterol (P=0.01; post-hoc tests: general population versus FH and IHD versus FH, P<0.001 and P=0.018, respectively) and LDL cholesterol (P=0.03; post-hoc tests: general population versus FH or IHD versus FH, P<0.001 and P=0.01, respectively) (Figure). Average increase in cholesterol and LDL cholesterol from carriers in the general population to carriers with clinical FH was 1.9 mmol/L and 1.6 mmol/L, respectively. Type of mutation was also a significant overall determinant of average increase in LDL cholesterol (P=0.05; cholesterol, P=0.16). Average increase in LDL cholesterol for carriers of LDLR mutations compared with carriers of APOB R3500Q was 1.2 mmol/L and for cholesterol was 0.8 mmol/L.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>General Population (N=6)</th>
<th>Patients With IHD (N=3)</th>
<th>Patients With FH (N=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>1 2 3 4 5 6</td>
<td>7 8 9</td>
<td></td>
</tr>
<tr>
<td>Sex, F/M</td>
<td>M F F M F F</td>
<td>M F F</td>
<td>2/1 19/9</td>
</tr>
<tr>
<td>Age, y</td>
<td>35 40 53 53 61 70</td>
<td>53 (35–69)</td>
<td>47 49 60</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>7.2 8.4 9.0 9.2 14.2 9.6</td>
<td>9.1 (7.2–14.2)**</td>
<td>9.9 10.4 9.7</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>5.4 6.7 6.2 7.7 12.2 8.0</td>
<td>7.2 (5.4–12.2)</td>
<td>8.1 ND 7.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.3 1.4 1.7 0.7 1.5 1.1</td>
<td>1.4 (0.7–1.7)</td>
<td>1.0 ND 1.5</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.0 0.7 2.5 1.8 1.2 1.1</td>
<td>1.2 (0.7–2.5)</td>
<td>1.7 1.8 1.2</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>106 119 138 146 210 ND</td>
<td>138 (106–210)</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>APOE genotype</td>
<td>ε3 ε3 ε3 ε4 ε4 ε3</td>
<td>ε3 ε3 ε3 ε3 ε3</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.7 25.9 25.5 27.9 22.9 22.4</td>
<td>24.2 (22.4–27.9)</td>
<td>29.4 19.3 24.8</td>
</tr>
<tr>
<td>Symptoms of IHD (N of subjects)</td>
<td>No No No No Yes 1</td>
<td>Yes Yes Yes Yes</td>
<td>3 8</td>
</tr>
<tr>
<td>Age at onset of symptoms of IHD, y</td>
<td>— — — — — 69 69 45 44 57 45 (44–57)</td>
<td>49 (41–78)</td>
<td>23.6 (19.3–37.7)</td>
</tr>
<tr>
<td>Family history of IHD younger than age 60 years, HC, or both (N of subjects)</td>
<td>No HC IHD No No IHD/HC 3§</td>
<td>IHD/HC IHD/HC IHD/HC HC</td>
<td>3 28</td>
</tr>
<tr>
<td>Treatment for HC (N of subjects)</td>
<td>No No No No Yes 1</td>
<td>Yes Yes Yes Yes</td>
<td>3 8</td>
</tr>
</tbody>
</table>

*Continuous variables are median and range. All lipids and lipoproteins are pretreatment values, ie, before treatment with statins or other lipid-lowering drugs.

P values are for comparisons between heterozygotes for the 3 mutations identified among patients with clinical familial hypercholesterolemia (FH) or ischemic heart disease (IHD) combined vs heterozygotes identified in the general population by the Mann–Whitney U test and Fisher exact test. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; HC, hypercholesterolemia; ND, not determined; F, female; M, male.

LW23X/W66G/W556S.

Heterozygotes identified in the general population vs heterozygotes identified among patients with IHD or FH: ‡P<0.05, §P<0.003, ¶P<0.001. P<0.006 is significant when adjusted for 9 simultaneous comparisons using the Bonferroni method.

LDL receptor mutations were significantly more frequent among patients with IHD (odds ratio, 4.9; 95% CI, 1.2 to 20) and among patients with clinical FH (odds ratio, 1233; 95% CI, 481 to 3164) than in the general population (Table 3). Risk of IHD was not significantly different in carriers of LDLR mutations with carriers of W23X, W66G, and W556S and account for 40% to 45% of mutations in patients with clinical FH.4–6 Our data indicate that these 3 mutations occur at a frequency of 0.06% in the general population, and even when identified in this background population are associated with considerably higher than normal plasma cholesterol, LDL cholesterol, and apolipoprotein B levels. Furthermore, background population was a significant determinant of the apparent phenotype associated with these mutations: carriers identified among patients with IHD or clinical FH had increasing levels of cholesterol compared with carriers in the general population. Finally, when comparing carriers of LDLR mutations with carriers of APOB R3500Q identified in similar background populations, background population and type of mutation were equally as important determinants of cholesterol levels.

**TABLE 2. Increase in Cholesterol, LDL Cholesterol, and Apolipoprotein B Levels in Heterozygotes for W23X, W66G, and W556S Identified in Different Background Populations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>General Population (N=6)</th>
<th>Patients With IHD (N=3)</th>
<th>Patients With FH (N=28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td>2.9 (1.9 to 7.5)</td>
<td>4.1 (3.0 to 4.9)</td>
<td>4.9 (3.1 to 11.0)**</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.8 (2.3 to 8.1)</td>
<td>4.2 (3.9 to 4.6)</td>
<td>5.3 (3.8 to 8.7)**</td>
<td>0.05</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>52 (33 to 118)</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*Values are median differences between values for heterozygotes and the 50th percentile values for persons of the same age and sex in the general population.

P values are for the comparison of heterozygotes identified in the general population with heterozygotes identified among patients with IHD or clinical FH by Kruskal–Wallis analysis of variance. Post hoc tests by the Mann–Whitney U test for heterozygotes in the general population vs heterozygotes identified among patients with clinical FH: †P=0.007, ‡P=0.026. P<0.025 significant when adjusted for 2 simultaneous comparisons using the Bonferroni method.
Effect of background population: cholesterol, analysis of variance and post hoc tests with Student lesterol and LDL cholesterol levels were made with 2-way anlysis of variance and post hoc tests with Student t test. Effect of background population: cholesterol, P<0.01; post-hoc tests: GP versus FH and IHD versus FH, P<0.001 and P=0.018, respectively. LDL cholesterol, P<0.01; post-hoc tests: GP versus FH and IHD versus FH, P<0.001 and P=0.016. LDL cholesterol, P=0.05; post-hoc tests: NS.

Relative Importance of Background Population and Type of Mutation for Phenotype
Background population and type of mutation were equally important in determining cholesterol and LDL cholesterol levels. Cholesterol and LDL cholesterol increased 1.9 mmol/L and 1.6 mmol/L, respectively, as a function of background population in carriers identified among patients with clinical FH, with IHD carriers in between. Thus, ≈40% of the apparent increase in cholesterol in carriers identified among patients with clinical FH was caused by ascertainment bias. Within each background population, carriers of LDLR mutations had cholesterol and LDL cholesterol levels that were on average 0.8 mmol/L and 1.2 mmol/L higher than carriers of APOB R3500Q, similar in magnitude to the increase caused by ascertainment bias between carriers identified in the general population and among patients with IHD.

Limitations
It can be argued that it is difficult to conclude with confidence that the mean cholesterol value of 6 individuals ascertained from the population is systematically lower than that of 3 patients with IHD and 28 FH patients ascertained from a lipid clinic, independent of differences in specific mutations, age, and other confounding factors. The additional cholesterol elevation observed in mutation carriers in the patient groups was a consequence of the background populations in which cholesterol was either a risk factor (IHD) or used as a cutoff point for the diagnosis (FH) and was therefore caused by ascertainment bias.

Phenotype of Carriers of LDLR Mutations Identified in the General Population
Even in probands in the general population, the average effect of W23X, W66G, and W556S on median increase in plasma cholesterol and LDL cholesterol levels was substantial: 2.9 and 3.8 mmol/L, respectively. However, these values were considerably lower than the average increase in cholesterol of 4.5 to 7.0 mmol/L previously reported in individuals older than 20 years of age with a clinical diagnosis of FH, suggesting an overestimation of cholesterol levels in patients.

Effect of Background Population on Phenotype: Importance of Ascertainment Bias
Heterozygous carriers of LDLR mutations with IHD or FH had cholesterol levels that were on average 1.2 and 2.0 mmol/L higher than in carriers identified in the general population. Because the type of LDLR mutations were the same in carriers identified in the 3 different background populations, the increase in cholesterol levels in the patient groups was not caused by an effect of the LDLR mutations, but could be attributed to both “environmental factors,” such as dietary intake and obesity, and to other minor mutations that modulate the cholesterol phenotype in the IHD and FH populations in general. Unfortunately, dietary intake was not available in the present study. However, body mass index did not differ significantly between carriers identified among patients with IHD or FH and carriers in the general population, and thus could not explain the impact of background population on cholesterol phenotype. Carriers identified among patients with IHD or FH more often had a family history of hypercholesterolemia or IHD than carriers in the general population, perhaps suggesting that other genetic factors impacted on the lipid phenotype in these families. The most obvious candidate would be the APOE polymorphism. APOE genotype was not available for the FH patients but did not differ between carriers in the general population and carriers with IHD.

Thus, the “minimal” phenotype of a given mutation is best identified in an unselected sample of the general population. The additional cholesterol elevation observed in mutation carriers in the patient groups was a consequence of the background populations in which cholesterol was either a risk factor (IHD) or used as a cutoff point for the diagnosis (FH) and was therefore caused by ascertainment bias.
among patients with IHD or FH could not be explained by differences in type of LDLR mutation, because these were the same and could also not be explained by differences in the most obvious confounders: age, gender, and body mass index. In addition, differences in cholesterol levels between probands identified in the general population or among patients with IHD could also not be explained by differences in APOE genotypes or in the distribution of single nucleotide polymorphisms in APOB at the following positions: codons 2488, 2712, 3611, 4154, and 4311. Probands identified in the general population did not have secondary hypolipidemia caused by hyperthyroidism, liver disease, or low body mass index. Thus, on this basis, we believe it is rather unlikely that nonascertainment bias or a lowering of cholesterol level in probands in the general population could explain the differences in cholesterol levels, but we cannot completely rule this out.

In further support of our conclusion that cholesterol levels associated with a given mutation are overestimated among patients with IHD or FH is the fact that we found the exact same results for APOB R3500Q when identified in similar background populations. Finally, that phenotype associated with a given mutation is overestimated in patients has also clearly been demonstrated by us and others for hemochromatosis mutations and carriers of LDLR mutations and carriers of APOB R3500Q. Because cholesterol levels were higher in carriers of LDLR mutations, the odds ratio for IHD for LDLR mutation carriers may be underestimated in our study. A possible explanation for this is that carriers of LDLR mutations identified in the general population were more often treated for hypercholesterolemia than carriers of APOB R3500Q, thus preventing or postponing the development of IHD. In contrast, the higher cholesterol levels in carriers of LDLR mutations were clearly demonstrated by the 14-fold over-representation among patients with clinical FH compared with carriers of APOB R3500Q.

In more general terms, effects of mutations on phenotype are overestimated when mutation carriers are identified—as is most often the case—among patients when the same phenotype is either a risk factor for the disease or one of the clinical criteria used for the diagnosis. A consequence of this is that determination of relative effects of different mutations on the same phenotype also requires that carriers be identified in the same background population.

In conclusion, our results suggest that the phenotype associated with a given mutation should not be determined in patients, but rather in unselected individuals in the general population.

References

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Mean percentiles for Plasma Lipids, Lipoproteins, and ApolipoproteinB in Heterozygotes for LDL Receptor Mutations (*LDLR: W23X, W66G, and W556S*) identified in the General Population (GP), among Patients with Ischemic Heart Disease (IHD), or among Patients with Clinical Familial Hypercholesterolemia (FH).

Mean percentiles and 95% confidence intervals are given relative to age-matched and sex-matched subjects in the total general population sample. Comparisons of heterozygotes identified in the GP, among patients with IHD, or among patients with clinical FH with the total general population sample were made with the z-test (“Carriers vs. general population”) \(^1,2\). Comparisons between heterozygotes identified in the GP, among patients with IHD, or among patients with clinical FH
were made with Kruskal Wallis analysis of variance (“Carriers: GP vs. IHD vs. FH”), and post hoc tests with the Mann-Whitney U test: *General population versus patients with IHD, cholesterol: P=0.048, LDL cholesterol: NS; general population versus patients with FH, cholesterol: P=0.002, LDL cholesterol: P=0.022.

LDL=low-density lipoprotein, HDL=high-density lipoprotein.

References.
