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

Anne Tybjærg-Hansen, Henrik Kjærulf 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
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, 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<0.001, respectively) (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>
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<tr>
<td>Sex, F/M</td>
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<td></td>
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<tr>
<td>LDL receptor mutation (N of subjects)</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Cholesterol, mmol/L</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td></td>
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<tr>
<td>Triglycerides, mmol/L</td>
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<tr>
<td>Apolipoprotein B, mg/dL</td>
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<tr>
<td>APOE genotype</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms of IHD (N of subjects)</td>
<td></td>
<td></td>
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<tr>
<td>Age at onset of symptoms of IHD, y</td>
<td></td>
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<tr>
<td>Family history of IHD younger than age 60 years, HC, or both (N of subjects)</td>
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<tr>
<td>Treatment for HC (N of subjects)</td>
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</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.

†W23X/W66G/W556S.

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>
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</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 ischemic heart disease (IHD), and among patients with clinical familial hypercholesterolemia (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<0.005, ‡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, 8.3–16.8) than in the general population (Table 3). Risk of IHD was not significantly different in carriers of mutations compared with carriers of LDLR R3500Q (odds ratio, 14; 95% CI, 3 to 62).

Discussion

Among Danes, 3 mutations in the LDLR predominate (W23X, W66G, W556S) and account for 40% to 45% of mutations in patients with clinical FH. 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 increasingly higher 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 important as determinants of cholesterol levels.
Effect of background population: cholesterol, cholesterol, and LDL cholesterol levels were made with 2-way
LDLR
±emia (FH)) and type of mutation (IHD versus patients with familial hypercholesterol-
disease (IHD) versus patients with familial hypercholesterol-
Means. Comparisons of effects of background population
of background population. Values are estimated marginal
W556S) and
hoc tests: GP versus FH and IHD versus FH,

P
0.018, respectively. LDL cholesterol,
P
0.01, respectively. Effect of type of mutation: cholesterol,
P
0.016. LDL cholesterol, P
0.05; post-hoc tests: NS.

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.

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 from carriers identified in the general
population to carriers identified in 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, inde-
dependent of differences in specific mutations, age, and other
confounding factors. In heterozygotes identified in the general
population, a different genetic makeup or environmental factors
could counteract the effect of LDLR mutations by reducing
synthesis or increasing breakdown rates of LDL, resulting in
lower cholesterol levels. However, differences in cholesterol
levels between probands identified in the general population or
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 or among patients with IHD or FH is the fact that we found the exact same mutations and carriers of APOB or in the distribution of single nucleotide polymorphisms in APOB when identified in similar background populations.

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 for factor V Leiden and venous thrombosis.

The odds ratio for IHD did not differ between 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 Apolipoprotein B 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.
