Lipoprotein lipase gene polymorphism, cholesterol subfractions and myocardial infarction in large samples of the general population

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Abstract

\textbf{Objective:} Genetic variants of the lipoprotein lipase gene have been associated with dyslipidemia and coronary artery disease. However, data have been inconsistent and are mainly based on selected predominantly male patient groups. \textbf{Methods:} We evaluated the influence of the HindIII restriction fragment length polymorphism on lipid levels in the general population (1361 participants of a large population-based survey from Augsburg, Germany; 50\% women) as well as the association of this polymorphism with the risk of myocardial infarction (MI; genotype frequencies in 1159 patients with documented MI under 60 years of age). \textbf{Results:} In the population-based survey, a highly significant association between the frequent H2H2 genotype and unfavorable cholesterol subfraction levels was observed in men and in postmenopausal women whereas no significant association was observed in premenopausal women (uni- and multivariate analysis). Such unfavorable lipid levels in homozygotes for the H2 allele may be expected to be associated with a 19–25\% increased risk to suffer from myocardial infarction (MI). Nevertheless, genotype and allele frequencies in the general population were not different from those in patients with previous MI (H2H2 genotype frequency 51.3\% vs. 53.2\%, respectively; \(P=0.63\)). \textbf{Conclusion:} This large study shows that the H2H2 genotype of the lipoprotein lipase gene polymorphism is associated with unfavorable lipid levels. Estrogen status may modulate this association in women. The effects of the genotype on lipid levels were apparently not strong enough to reveal a significant association with MI. © 2000 Elsevier Science B.V. All rights reserved.

\textbf{Keywords:} Lipoproteins; Infarction; Gene expression; Lipid metabolism; Cholesterol

1. Introduction

The genetic background — in interaction with diet and other behavioral factors — determines serum lipid levels and thereby modulates the overall cardiovascular risk [1,2]. Specifically, mutations as well as genomic polymorphisms of genes involved in lipid metabolism have been described to elevate cholesterol and/or triglyceride levels as well as the risk of myocardial infarction. Some of these genomic polymorphisms have been located on chromosome 8p22 in the lipoprotein lipase gene, an enzyme that plays a key role in metabolizing triglyceride-containing lipoproteins and, thereby, indirectly affects HDL\textsubscript{2} and LDL cholesterol generation [3,4]. Of several variants within this gene, the presence of a HindIII restriction site (H2 allele) in intron 8 has been associated with unfavorable lipid levels and coronary artery disease [5]. Since the intronic HindIII polymorphism has uncertain molecular effects on lipoprotein lipase activity, it has been postulated that this variant is in linkage disequilibrium with another functionally relevant mutation [3,4,6–8]. In particular, strong linkage
disequilibrium has been demonstrated for the Serine447Stop variant [6]. However, this polymorphism could not consistently be associated with altered lipid profile [8] or coronary artery disease [9].

Individuals carrying the allele in which the HindIII restriction site is present (H+ or H2 allele) repeatedly displayed an association with hypertriglyceridemia [7,9–11], higher LDL cholesterol levels [7,9] and/or lower HDL cholesterol levels [11,12]. Some previous studies reported also an association with myocardial infarction [13], and positive family history of myocardial infarction [6] as well as an early age of onset or enhanced severity of coronary artery disease [3,8,9,14]. However, some of these positive studies were limited by a rather small population size (<500 individuals) [9,10,12,13], thus, being susceptible to a type I error (i.e. detecting a false positive association). Furthermore, most studies were carried out on preselected in-hospital patients, a design which may confer some risk for selection bias, specifically, with respect to the cardiovascular risk profile including lipid levels. Finally, some studies did not show an association of the H2H2 genotype with adverse lipid levels or coronary artery disease [8,15,16].

Therefore, we re-evaluated the association between the lipoprotein lipase Hind III polymorphism and serum cholesterol as well as cholesterol subfractions in 1361 individuals who participated in an age and sex-stratified random survey of the general population. In addition, the association of this polymorphism with myocardial infarction was investigated in a total of 1159 patients with early myocardial infarction from the same geographical region.

2. Methods

2.1. Study population

The individuals examined here participated in the echocardiographic sub-study of the third MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) survey of the general population of Augsburg in 1994/95 that originates from a sex-age-stratified cluster sample of all German residents of the Augsburg study area (participating n=1674) [17,18]. All subjects responded to a questionnaire on medical history, physical activity, medication, and personal habits. Body height and weight were recorded in light clothing, and body mass index was calculated as weight in kg divided by height in meters squared (kg/m²). Resting blood pressure was measured after subjects had been in the sitting position for a minimum of 30 min. Using a mercury sphygmomanometer (Hawksley random zero sphygmomanometer), blood pressure was read three times at the right arm.

Individuals with myocardial infarction participated either in a follow-up examination of a population-based MI register (4976 non-fatal myocardial infarctions from 1985 to 1995) [19] that is located in the Augsburg region (participating n=609; examination in 1996/1997) or were recruited in post MI rehabilitation centers from the same area (n=559; examination in 1997/1998). All patients had had their first myocardial infarction before 60 years of age. MI patients were examined either at a visit to a study center or at a home visit by a physician. All patients were studied by a standardized interview, clinical examination, and biochemical as well as molecular analyses. Myocardial infarction had been verified according to standard criteria described elsewhere [19]. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Serum lipoprotein levels

Asservation of serum was carried out in a sitting position from non-fasting individuals. Serum total cholesterol and HDL cholesterol were measured with a standard enzymatic method (CHOD-PAP). HDL cholesterol was determined after precipitation with phosphotungstate/Mg²⁺. LDL cholesterol was determined after precipitation with dextrane sulfate in the supernatant.

2.3. DNA analysis

Genotyping of the lipoprotein lipase HindIII restriction fragment length polymorphism was carried out as described by Mattu et al. [9] After DNA purification from whole frozen blood according to a standard protocol (Puregene, Biozym, Hessisch Oldendorf, Germany) 80 ng of DNA were subjected to polymerase chain reaction (Taq polymerase was purchased from PAN Systems GmbH, Eidenbach, Germany) for 30 cycles at 55 and 72°C and a final extension for 7 min using a sense primer (5’-GATGTCCTACCTGGATAATCAAAG-3’) and an antisense primer (5’-CTTCAGCTAGACATTGCTAGTGT-3’). After restriction of the amplification product with HindIII (Boehringer Mannheim, Mannheim, Germany) at 37°C for 1 h, bands were separated on a 2.5% agarose gel and visualized under UV illumination. The restricted DNA (representing the H2 allele) is characterized by bands of 160 and 205 base pairs whereas the undigested fragment (H1 allele) shows a band of 365 base pairs. Genotyping was successfully performed in 1361 individuals from the general population and 1159 MI patients.

2.4. Statistical analysis

Lipid levels in the general population were compared in groups according to lipoprotein lipase HindIII genotypes by ANOVA for comparison of independent samples and post hoc analysis using Fisher’s least-significant difference test. Significance was accepted at P<0.05. Multiple regression analyses were calculated with lipid levels as dependent and lipoprotein lipase H2H2 genotype as independent
variables including age, body mass index, intake of alcohol or lipid lowering drugs, cigarette smoking, and, in women, hormone replacement therapy and status of menopause in the models.

Assuming a theoretical increase of relative risk of 22% to experience MI in carriers of the H2H2 genotype (please see Results section for calculation of this theoretical risk), the power to detect an association of the H2H2 genotype with MI in the study sample of 1172 cases and 1361 controls at a \( P \) of 0.05 is 0.7. Allele and genotype frequencies between the general population and MI patients were compared using the \( \chi^2 \) test. Comparison between groups with respect to demographic data was done using Student’s \( t \)-test for independent samples. The statistical softwares SPSS/PC 7.5 and SAS (multiple regression analysis; power calculation) were used for these calculations.

3. Results

Anthropometric data and prevalence of risk factors of the sample of the general population and patients with MI are shown in Table 1. Lipoprotein lipase genotype groups were homogeneous with respect to gender distribution, age, systolic blood pressure, body mass index, smoking status and prevalence of diabetes mellitus.

3.1. Lipoprotein lipase genotype and cholesterol subfraction levels in the general population

In the sample from the general population, men and women were analyzed separately with individuals taking lipid-lowering agents \((n=89)\) being excluded from univariate analysis. Men carrying the H2H2 genotype displayed significantly higher LDL cholesterol levels than heterozygous men (Table 2). Conversely, HDL cholesterol
levels were significantly lower in men with the H2H2 genotype as compared with heterozygous men or men carrying the H1H1 genotype. The ratio of total/HDL cholesterol was also significantly higher in men with the H2H2 genotype. In men, multivariate analysis of HDL cholesterol as well as the ratio of total/HDL cholesterol displayed significant associations with body mass index, alcohol intake, and cigarette smoking (Table 3). Furthermore, significant associations were also found between age and body mass index and LDL cholesterol levels (not shown). The strong association of the H2H2 genotype with HDL cholesterol, total/HDL cholesterol and LDL cholesterol persisted in these multivariate models.

In the entire group of women, no significant association of the H2H2 genotype with unfavorable lipid levels was found (Table 2). Age-stratified multiple regression analysis revealed that specifically younger women (below 45 years of age) did not show an association between the H2H2 genotype and lipid levels (Table 3) whereas alcohol intake, use of hormonal contraception ($P=0.0436, \beta=+4.6 \text{mg/dl}$) and cigarette smoking ($P=0.0009, \beta=-7.6 \text{mg/dl}$) were associated with HDL cholesterol levels in this model. However, an association of the H2H2 genotype with lower HDL cholesterol levels and a higher ratio of total cholesterol/HDL cholesterol was clearly observed in middle-aged women (45–64 years of age, Table 3). In this age-group, no significant effects were detected by the multivariate model for alcohol intake, hormone replacement therapy, and status of menopause. Further stratification of women between 25 and 64 years of age by status of menopause revealed significantly lower HDL cholesterol levels in postmenopausal women carrying the H2H2 genotype compared with those carrying the H1H2 genotype (Table 4) whereas pre-menopausal women did not display any differences with respect to cholesterol subfractions.

### 3.2. Association of lipoprotein lipase H2H2 genotype and myocardial infarction

Before we tested whether the lipoprotein lipase polymorphism affects the risk of myocardial infarction, individual ten year risk estimates were determined based on the lipid levels and other risk factors in the general population. Algorithms derived from the Nordic Risk Assessment (NORA) study, the Multiple Risk Factor Intervention Trial (MRFIT) and the MONICA study predicted a relative risk for the incidence of a coronary

<table>
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<tr>
<th>Table 3</th>
<th>Multiple regression analysis of lipoprotein lipase genotype and covariates that may affect cholesterol levels in the general population</th>
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<tbody>
<tr>
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<td>HDL cholesterol</td>
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<tr>
<td></td>
<td>$\beta$-coefficient</td>
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<td></td>
<td>mg/dl (mmol/l)</td>
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<tr>
<td></td>
<td>$P$</td>
</tr>
<tr>
<td><strong>Men (25–64 years) $n=498$</strong></td>
<td></td>
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<tr>
<td>Body mass index (per 1 kg/m$^2$ increase)</td>
<td>$-1.2 \ (-0.03)$</td>
</tr>
<tr>
<td>Alcohol intake ($&gt;80$ vs. $0$ g/day)</td>
<td>$+8.5 \ (+0.22)$</td>
</tr>
<tr>
<td>Cigarette smoking (yes vs. no)</td>
<td>$-5.0 \ (-0.13)$</td>
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<tr>
<td>Lipoprotein lipase genotype (H2H2 vs. H1H2)</td>
<td>$-3.5 \ (-0.09)$</td>
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<tr>
<td><strong>Women (25–44 years) $n=209$</strong></td>
<td></td>
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<tr>
<td>Body mass index (per 1 kg/m$^2$ increase)</td>
<td>$-1.2 \ (-0.03)$</td>
</tr>
<tr>
<td>Alcohol intake (yes vs. no)</td>
<td>$+7.7 \ (+0.20)$</td>
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<tr>
<td>Cigarette smoking (yes vs. no)</td>
<td>$-7.6 \ (-0.19)$</td>
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<tr>
<td>Lipoprotein lipase genotype (H2H2 vs. H1H2)</td>
<td>$+1.9 \ (+0.05)$</td>
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<tr>
<td><strong>Women (45–64 years) $n=284$</strong></td>
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<tr>
<td>Body mass index (per 1 kg/m$^2$ increase)</td>
<td>$-1.2 \ (-0.03)$</td>
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<tr>
<td>Alcohol intake (yes vs. no)</td>
<td>$-3.5 \ (-0.09)$</td>
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<tr>
<td>Cigarette smoking (yes vs. no)</td>
<td>$-10.4 \ (-0.27)$</td>
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<tr>
<td>Lipoprotein lipase genotype (H2H2 vs. H1H2)</td>
<td>$-4.2 \ (-0.11)$</td>
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Table 4
Adjusted levels of LDL cholesterol, HDL cholesterol and the ratio of total/HDL cholesterol in pre- and post-menopausal women

<table>
<thead>
<tr>
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<th>Premenopausal &lt;45 years (n=203)</th>
<th>Post-menopausal ≥45 years (n=206)</th>
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<tbody>
<tr>
<td></td>
<td>H1H2</td>
<td>H2H2</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>115.0±3.9</td>
<td>119.3±3.5</td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>2.98±0.10</td>
<td>3.09±0.09</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>60.2±1.5</td>
<td>61.4±1.5</td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>1.56±0.04</td>
<td>1.59±0.04</td>
</tr>
<tr>
<td>Total/HDL chol.</td>
<td>3.59±0.10</td>
<td>3.73±0.10</td>
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* No significant difference between H1H2 and H2H2 is indicated by n.s.

...event of 1.199, 1.219, and 1.244, respectively, in individuals carrying the H2H2 genotype compared with those carrying the H1H2 genotype [20].

To test whether such mildly increased relative risk in individuals carrying the H2H2 genotype translates into an association with myocardial infarction genotype frequencies were determined in 1159 patients from the same geographic region who had experienced a myocardial infarction before 60 years of age. Patients with MI were older compared with the general population and displayed typical characteristics of MI patients, i.e. higher prevalence of male gender, diabetes mellitus, lower prevalence of current smoking and higher body mass index (Table 1). The two groups of MI patients showed little, but significant differences with respect to age, blood pressure, total/HDL cholesterol ratio, as well as prevalences of current smoking and diabetes mellitus (Table 1).

The lipoprotein lipase genotype distribution was not different between the general population and the entire group of MI patients (Table 5). Separate analysis of MI patients from the MI register and the rehabilitation centers showed no significant differences in genotype distribution (Table 5). Likewise, frequencies of the H2 allele were similar in MI patients and the general population (men: H2 allele frequency 71.8% in the general population vs. 72.9% in MI patients; women: 72.1% vs. 72.9%). In addition, separate analysis of three different age groups showed similar frequencies of the H2H2 genotype in MI patients and the general population (Table 6). Furthermore, genotype distribution was not associated with the time that had elapsed between MI and DNA sampling (H2H2 genotype 51.6% in patients <3 years after MI, 56.0% patients 3–6 years after MI, 50.6% in patients >6 years after MI). Similarly, age at time of MI was not different in the lipoprotein lipase genotypes (not shown). In conjunction, the...
data indicate that the H2H2 genotype was not significantly associated with myocardial infarction in this population.

4. Discussion

In agreement with previous studies on smaller samples or selected in-hospital patients this study on a large, carefully age- and gender-stratified general population survey corroborates an association of the lipoprotein lipase HindIII polymorphism and lipid levels. Specifically, in men HDL cholesterol was lower by 6% on average and LDL cholesterol was higher by 6% on average in those with the H2H2 genotype. Our study is the first study to examine this relationship in a large number of women, as well. Women of middle ages, particularly those after menopause carrying the H2H2 genotype displayed unfavorable lipid levels whereas younger premenopausal women did not. This finding suggests an interaction of estrogen status and H2 allele status in the modulation of lipid levels. Specifically, in premenopausal women, serum lipid levels may be strongly modulated by estrogen which inhibits hepatic lipase [21] and lipoprotein lipase [22] and increases hepatic LDL-receptor expression [23,24]. In men and postmenopausal women, without a strong influence of estrogen (non-suppressed lipase activity) the lipoprotein lipase HindIII polymorphism appears to be functionally relevant. Our observational study, however, falls short to clarify the exact mechanisms of this interaction.

The NORA, MONICA and MRFIT studies as well as interventional trials on cholesterol lowering drugs [25,26] provide consistent data that allow to predict the risk of MI at various serum lipid levels. Based on these risk estimates, we calculated that individuals with the H2H2 genotype may carry a 19–24% higher relative risk of suffering from MI than those carrying the H1H1 or H1H2 genotype. However, our data do not support such risk increment since genotype frequencies were similar in the general population and groups with MI. A potential explanation for this negative finding would be that our study sample was too small. However, the predicted power should have been sufficient to detect an association between MI and the lipoprotein lipase polymorphism. Alternatively, we cannot exclude that mortality (and thus drop-out) after myocardial infarction was different in respective genotype groups. However, the age at the time of myocardial infarction, the time of follow-up after myocardial infarction, and the distribution of other risk factors were similar in respective genotype groups such that we have no indication for selection bias. Moreover, given the presumed mechanism that might link the lipoprotein lipase polymorphism and myocardial infarction, i.e. a mildly unfavorable lipid profile, a substantially different mortality rate after myocardial infarction appears to be unlikely. This is also supported by similar genotype frequencies in individuals below 45 and above 65 years of age. Finally, we selected patients who suffered from myocardial infarction at a relatively young age at which genetic factors are even more prominent [27], thus, increasing the odds of a positive study.

Nevertheless, the potential of selection bias can be resolved with certainty only by a prospective study in the general population. Given the mild theoretical risk increment of 22% associated with the lipid changes in the H2H2 genotype group and an incidence of the disease of 0.4% per year (MONICA) [28], it can be calculated that approximately 300 000 individuals have to be followed prospectively for 10 years to provide statistical assurance for the estimated difference in relative risk to experience an MI. However, given the negative outcome of the present study, it appears difficult to encourage such enormous effort.

An alternative explanation for the lack of association may be that not all sorts of genetic (or environmental) modulations of serum cholesterol confer the same risk at a given cholesterol level. For example, HDL or LDL subfractions may be modulated differentially by various factors. Moreover, interactions of genetic factors with age, estrogen status (as shown above), or diet may interfere with the risk of myocardial infarction such that the risk increment that applies to the general population may not be identical to that in different lipoprotein lipase genotype groups.

Previously described associations between the lipoprotein lipase polymorphism and myocardial infarction could not be reproduced in our study despite larger sample size [8,13]. In fact, most of the previous studies did not have the power to detect the small theoretical risk increment due to different lipid levels in respective genotype groups [7–9,11,13,14]. Thus, some of these studies might have been more susceptible to a type I-error and detection of a false positive association. Furthermore, selection bias resulting from inhomogeneous patient populations or control groups not drawn from the overall population might have affected previous results [6–8,14,15,29]. By contrast, our study confirms the findings of Ukkola et al. and Ye et al. who did not find an association between the lipoprotein lipase polymorphism and coronary artery disease in 440 individuals [10,29]. Discrepant results may also be explained by ethnic differences of the studied populations. For example, dietary fat intake that may differ considerably between ethnic groups modulates the genetic influence on lipid levels since the H2H2 genotype appears to be associated with a good response of lipid levels to diet [7]. Thus, in a population with low fat intake the association of the H2H2 genotype with unfavorable lipid levels or cardiovascular endpoints may be less pronounced.

In conclusion, this study on a large sample from the general population confirms an association between the H2H2 genotype of the HindIII lipoprotein lipase polymorphism and mildly unfavorable lipid levels in men. For the first time it also shows this association in middle-aged postmenopausal women while no association could be detected in premenopausal women. However, the small
Theoretical risk increment associated with the lipoprotein lipase polymorphism was not strong enough to reveal a significant association with myocardial infarction in this large cohort.

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