Lipoprotein Lipase Mutations, Plasma Lipids and Lipoproteins, and Risk of Ischemic Heart Disease: A Meta-Analysis

Hans H. Wittrup, MD, PhD; Anne Tybjærg-Hansen, MD, DMSc; Børge G. Nordestgaard, MD, DMSc

Background—We assessed in meta-analyses the effect of the Gly188Glu, Asp9Asn, Asn291Ser, and Ser447Ter substitutions in lipoprotein lipase in the heterozygous state on lipid metabolism and risk of ischemic heart disease (same order used below).

Methods and Results—In 29 separate studies, 20,903 white subjects were screened for ≥1 of these substitutions; each meta-analysis included only some of these individuals. In population-based studies, heterozygote frequencies ranged from 0.04% to 0.2%, 2% to 4%, 1% to 7%, and 17% to 22% for the respective substitutions. Postheparin plasma lipoprotein lipase activity decreased 53% (95% CI, 31% to 75%) (only 1 study), 30% (22% to 37%), and 22% (8% to 35%) and was unchanged at 4% (±10% to 19%), respectively. Plasma triglycerides increased 78% (95% CI, 64% to 92%), 20% (9% to 33%), and 31% (20% to 43%) and decreased 8% (4% to 11%), respectively. HDL cholesterol decreased 0.25 mmol/L (0.18 to 0.32), 0.08 mmol/L (0.04 to 0.12), and 0.12 mmol/L (0.10 to 0.15) and increased 0.04 mmol/L (0.02 to 0.06), respectively. Odds ratios for ischemic heart disease were 4.9 (95% CI, 1.2 to 20) (only 1 study), 1.4 (0.8 to 2.4), 1.2 (0.9 to 1.5), and 0.8 (0.7 to 1.0), respectively. Subgroup analysis indicated that women with the Asn291Ser substitution may have an increased risk of ischemic heart disease.

Conclusions—These meta-analyses suggest that compared with noncarriers, carriers of the Gly188Glu, Asp9Asn, and Asn291Ser substitutions have an atherogenic lipoprotein profile, whereas carriers of the Ser447Ter substitution have a protective lipoprotein profile. Accordingly, risk of ischemic heart disease in heterozygous carriers is increased for Gly188Glu carriers; at most, the increase is borderline for Asp9Asn and Asn291Ser carriers; and risk is possibly decreased for Ser447Ter carriers. (Circulation. 1999;99:2901-2907.)

Key Words: lipoproteins ■ genetics ■ cholesterol ■ heart diseases ■ meta-analysis ■ enzymes

In the homozygous form, amino acid–changing mutations in lipoprotein lipase may lead to the chylomicronemia syndrome,1 characterized by severe hypertriglyceridemia and extremely low HDL levels and possibly by ischemic heart disease.2 Heterozygosity for amino acid–changing mutations in this enzyme may therefore lead to intermediate levels of triglycerides and HDL and consequently to increased risk of ischemic heart disease. We explored this possibility.

Most amino acid–changing mutations in lipoprotein lipase are rare, either restricted to single families or isolated geographic regions1; however, those leading to the Gly188Glu, Asp9Asn, Asn291Ser, and Ser447Ter substitutions have been identified widely.3-5 The Gly188Glu, Asp9Asn, and Asn291Ser substitutions are all located in the N-terminal end and may influence the catalytic activity of lipoprotein lipase, whereas the location of the Ser447Ter substitution in the C-terminal end may influence the enzyme-mediated uptake of lipoproteins by receptors on the cell surface.3 The Ser447Ter substitution could therefore have effects quite different from those of the 3 other substitutions. In the homozygous form, the Gly188Glu substitution has been associated with chylomicronemia syndrome,1,6,7 whereas homozygosity for the other 3 substitutions has at most a moderate effect on lipids and lipoproteins.8-14 We assessed in meta-analyses the effects of these 4 amino acid–changing substitutions in the heterozygous state on lipoprotein lipase activity, plasma lipids and lipoproteins, and risk of ischemic heart disease. Mutations that do not change amino acids in lipoprotein lipase were not considered. When possible, subgroup analyses were performed for population-based studies as well as for men separately; only a few studies reported on women separately. These meta-analyses should be viewed as hypothesis generating and not hypothesis testing.

Methods

Study Selection

The selection criteria for studies to be considered for these meta-analyses were as follows: (1) the Gly188Glu, Asp9Asn, Asn291Ser,
or Ser447Ter substitutions in lipoprotein lipase were studied; (2) participants were white; (3) family, cross-sectional, case-control, or case-referent studies were included that identified both heterozygous carriers and noncarriers; (4) data were reported on at least 1 of the following variables: plasma levels of postheparin lipoprotein lipase activity, triglycerides, HDL cholesterol, apolipoprotein AI, total cholesterol, or apolipoprotein B or on the risk of ischemic heart disease; and (5) only data published as part of full articles in peer-reviewed journals were considered.

**Search Strategy**

Literature searches through January 1998 included Medline search, Embase search, reference lists of articles already on file, and the Cochrane Collaboration Library database. A total of 29 studies including 20 903 whites were included,6 –14,16 –34 whereas 25 studies were excluded (tables available from authors).

**Data Collection**

Data were collected as they appeared in the original studies; however, in a few studies,6,11,19,24,26,27 we had to compute means with SDs or ORs with 95% CIs (Prism version 2.0, Graph-Pad Software Inc). For some studies of the Ser447Ter substitution, 10,21,34 it was not possible to separate a small number of homozygous from heterozygous individuals, and therefore these homozygous individuals were included in the present analyses.

**Analysis of Results**

Meta-analyses were performed on Review Manager version 3.0 (the Cochrane Collaboration; ftp://ftp.cochrane.org/pub/handbook).

The measurement of lipoprotein lipase activity is not yet standardized internationally. However, the relative differences between carriers and noncarriers are comparable from study to study. Furthermore, meta-analysis for lipoprotein lipase activity did not show evidence of heterogeneity, indicating that the individual studies were comparable. To concentrate on differences between carriers and noncarriers, the meta-analysis of lipoprotein lipase activity was performed for carriers as a percent of that for noncarriers.

We transformed triglycerides logarithmically14 to approximately fit a normal distribution, an assumption made in the meta-analysis. A post hoc antilogarithm presented triglyceride results as the relative difference (ratio) between carriers and noncarriers.

Meta-analyses using random-effects models were employed. This takes into consideration the within-study comparison between carriers and noncarriers, as well as differences between studies. The nonindependence between family members was not considered. The weighted mean difference was calculated based on weighting of individual results by the inverse variance15,35; this limits the impact of studies with wide CIs. Likewise, ORs were weighted by the inverse variance; Mantel-Haenszel estimates were calculated across individual studies.15,36,37 An approximated normality test (z test) was used to examine the aggregated results.15

We did overall analyses of both sexes combined and subgroup analyses based on population-based studies alone. Only 2 studies reported separate data for women.14,16 Thus, it was only possible to do subgroup analyses for men alone.

**Results**

Twenty-nine family, cross-sectional, case-control, or case-referent studies including 20 903 whites were selected for these meta-analyses. Some studies reported on >1 substitution. Each meta-analysis included only some of the 20 903 individuals. In population-based studies, heterozygote frequencies of the Gly188Glu, Asp9Asn, Asn291Ser, and Ser447Ter substitutions among control individuals ranged from 0.04% to 0.2%, 2% to 4%, 1% to 7%, and 17% to 22%, respectively. Mean plasma levels of postheparin lipoprotein lipase activity, triglycerides, HDL cholesterol, apolipoprotein AI, total cholesterol, and apolipoprotein B in groups of noncarriers in these studies ranged from 50 to 289 mU/mL, 0.80 to 8.46 mmol/L, 0.93 to 1.73 mmol/L, 100 to 173 mg/dL, 4.4 to 8.9 mmol/L, and 72 to 142 mg/dL, respectively.

The inferences drawn concerning the Gly188Glu substitution were based on a limited number of studies, most of which were studies of families. The estimated effects on postheparin plasma lipoprotein lipase activity and risk of ischemic heart disease among these carriers were each based on only 1 study and were therefore not meta-analyses.

**Postheparin Plasma Lipoprotein Lipase Activity**

The Gly188Glu substitution in the heterozygous state decreased postheparin plasma lipoprotein lipase activity by 53% (1 study only), approximately twice the reduction observed for the Asp9Asn and Asn291Ser substitutions, respectively.

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<th>Meta-Analyses of Effects of Lipoprotein Lipase Substitutions</th>
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<td><strong>Substitution in Lipoprotein Lipase</strong></td>
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For the Ser447Ter substitution, lipoprotein lipase activity was statistically unchanged at 4%.

**Plasma Triglycerides**

Gly188Glu, Asp9Asn, and Asn291Ser heterozygous carriers had an average increase in plasma triglycerides of 78%, 20%, and 31%, respectively, whereas carriers of the Ser447Ter substitution had a decrease of 8% compared with noncarriers (Figure 1). In analyses including population-based studies only, the results were similar (Figure 2). This was also the case in the subanalysis of men only (data not shown), except that there was a more discrete effect of the Asn291Ser substitution among men, in whom it increased triglycerides by just 14%.

**Plasma HDL Cholesterol and Apolipoprotein AI**

Gly188Glu, Asp9Asn, and Asn291Ser heterozygous carriers had an average decrease in HDL cholesterol of 0.25, 0.08, and 0.12 mmol/L, respectively, whereas Ser447Ter carriers had an increase of 0.04 mmol/L compared with noncarriers (Figure 3). In analyses restricted to population-based studies, the effects were similar (Figure 4). This also applied to subanalysis on men only (data not shown), except that the effect of the Asn291Ser substitution in men was slightly less than in the overall analysis, with HDL cholesterol decreased by 0.09 mmol/L (0.06 to 0.13 mmol/L).

Apolipoprotein AI levels among heterozygous carriers showed a similar pattern as that observed for HDL cholesterol (Table).

**Plasma Cholesterol and Apolipoprotein B**

There was no effect of the Gly188Glu, Asp9Asn, and Asn291Ser substitutions on plasma cholesterol, but surprisingly, the Ser447Ter substitution appeared to slightly decrease plasma cholesterol (0.1 mmol/L; Table), an effect also found for men alone (data not shown).

Apolipoprotein B levels among heterozygous carriers showed a similar pattern as that observed for HDL cholesterol (Table).

**Risk of Ischemic Heart Disease**

ORs for ischemic heart disease in heterozygous carriers of the Gly188Glu, Asp9Asn, Asn291Ser, and Ser447Ter substitutions were 4.9 (1.2 to 19.6) (1 study only), 1.4 (0.8 to 2.4), 1.2 (0.9 to 1.5), and 0.8 (0.7 to 1.0), respectively (Figure 5). In analysis of men only, ORs for Asp9Asn, Asn291Ser, and Ser447Ter carriers were similar to those in the overall analysis (data not shown). The single study examining only women found a highly significant increase in risk of ischemic heart disease among carriers of the Asn291Ser substitution.14
Discussion

The present meta-analyses suggest that risk of ischemic heart disease is increased in Gly188Glu carriers, that the increase is borderline at most for Asp9Asn and Asn291Ser carriers, and that risk is possibly decreased for Ser447Ter carriers. The result for the Gly188Glu substitution is based on 1 study only and is therefore not based on a meta-analysis. The rare Gly188Glu substitution (carrier frequency 0.06%) has a relatively large effect on decreasing enzyme activity (1 study only), increasing plasma triglycerides, and decreasing plasma HDL cholesterol. Furthermore, the more common Asp9Asn and Asn291Ser substitutions (carrier frequencies 3% and 5%, respectively) have more moderate effects on these variables, whereas the very common Ser447Ter substitution (carrier frequency 20%) appears to have the smallest and opposite effect on these variables.

Whether the Asn291Ser substitution doubles the risk of ischemic heart disease in women remains to be confirmed, because thus far only 1 study has demonstrated this effect in women. In support of this effect occurring specifically in women, the aggregated effect of the Asn291Ser substitution on plasma triglycerides, apolipoprotein AI, and HDL cholesterol was smaller in the analyses of men alone than in the overall analysis, which suggests that this substitution has a more pronounced effect in women than in men.

Methodological Quality of Individual Studies

Although all of the individual studies selected for these meta-analyses were of high quality and were published in peer-reviewed journals, several problems related to the individual studies may have influenced the summarized results. (1) Some studies measured plasma lipids and lipoproteins in the nonfasting state; however, an association between a more severe phenotype in carriers of any of these amino acid substitutions and the postprandial state has never been established. (2) Interaction with other potentially important variables such as age, diabetes mellitus, alcohol consumption, smoking habits, body mass index, digestion of saturated versus unsaturated fat, exercise, certain medication, and for women, menopausal status and use of hormonal replacement therapy may be important for the results regarding lipids and lipoproteins. Some of the studies included in the present meta-analyses presented plasma lipid and lipoprotein values...
adjusted for some or all of these variables; however, although the consequence of such adjustment may have been to remove the effect of the amino acid substitution observed for unadjusted values, aggregated data nevertheless suggested that there was indeed a detectable effect of these 4 amino acid substitutions. (3) Ischemic heart disease status was not reported with identical diagnostic criteria but either specifically as coronary artery disease (indicating that angiography was performed) or nonfatal myocardial infarction, or more broadly as ischemic heart disease. This difference in reporting disease status, however, is unlikely to present a major problem because in every study, carriers and noncarriers were diagnosed by use of identical criteria. (4) It was not possible to exclude homozygous individuals from all the reported data; however, although these homozygous carriers may have experienced greater effects on triglycerides and HDL cholesterol than heterozygous carriers, it is likely that the effect of this small number of individuals was diluted among the many heterozygous individuals. (5) We included both family studies and population-based studies. It therefore can be questioned how these 2 types of samples can be combined in a meaningful analysis and for what population of inference the results are relevant, if any. However, we found only minimal evidence of heterogeneity in analyses in which the 2 types of studies were combined. Furthermore, in subanalysis of population-based studies only, the results were similar to those in which the 2 types of studies were combined. (6) Finally, effects of these substitutions may be context dependent and thus vary from study to study.

### Biological Mechanism

Lipoprotein lipase is believed to be organized in an N-domain (residues 1 to 312), which is important for the catalytic function of the enzyme, and a C-domain (residues 313 to 448), which is important for the lipoprotein lipase–mediated uptake of lipoproteins by receptors on the cell surface. This may help explain the effects of the 4 amino acid substitutions. The Gly188Glu substitution is located in the lipid-binding region: reduced binding and thus degradation of triglycerides may affect plasma levels of triglycerides and HDL cholesterol. The Asp9Asn substitution at the N-terminal end is not in a region with a known function but is situated near a glycosylation site that may influence overall catalytic activity. The Asn291Ser substitution is located in a heparin-binding cluster and may thus affect the interaction of lipoprotein lipase with the cell wall glycosaminoglycans. These 3 amino acid substitutions are located in the N-domain and therefore may reduce enzyme activity and consequently increase triglyceride levels, whereas the Ser447Ter substitution is located in the C-domain and thus may cause increased binding affinity of the shortened lipoprotein lipase to receptors or may affect its subunit interaction, either facilitating or otherwise affecting the formation of dimers, which would explain the opposite effect of this substitution compared with the other 3.

Mechanistically, it seems plausible that lipoprotein lipase substitutions leading to decreased enzyme activity and consequently elevated triglyceride levels and reduced HDL cholesterol levels should increase an individual’s risk of ischemic heart disease. Both increased plasma triglycerides and reduced HDL cholesterol levels are well-known cardiovascular risk fac-
Elevated triglycerides indicate that IDLs, VLDLs, and/or chylomicron remnants are present in plasma, and these particles may be selectively retained in the intima and consequently promote atherosclerosis. The exact mechanism by which a mutation in lipoprotein lipase should result in remnant accumulation, however, is not clear. Reduced HDL cholesterol may result in reduced reverse cholesterol transport, indirectly promoting atherosclerosis. Mutations in lipoprotein lipase resulting in increased triglyceride levels could even promote atherosclerosis by still other mechanisms (for example, by their association with small, dense LDL particles; by particularly promoting postprandial triglyceride-rich lipoproteins; or by an influence on hemostasis).

Potential Confounding

Linkage disequilibrium with other causative mutations nearby cannot be excluded completely. For the Gly188Glu and Asn291Ser substitutions, however, none have ever been identified. In contrast, the HindIII polymorphism in intron 8 of the lipoprotein lipase gene (strongly associated with altered lipid levels) seems to be in almost complete linkage disequilibrium with the Ser447Ter substitution; however, the Ser447Ter substitution is believed to be more important for the observed effects simply because it truncates 2 amino acids, whereas the HindIII polymorphism is an intron variant. Nevertheless, it is also possible that the HindIII polymorphism is in linkage disequilibrium with another hitherto-undescribed variant in lipoprotein lipase or another nearby locus. Recently, a promoter mutation (T-93G) was described in a patient with familial combined hyperlipidemia and the Asp9Asn substitution, but at this point no evidence supports a causative role of the promoter mutation.

Future Research Efforts

Although there is substantial evidence pointing toward these amino acid substitutions in lipoprotein lipase as important susceptibility mutations for small to moderate changes in plasma triglycerides, HDL cholesterol, and apolipoprotein AI, the evidence for the Gly188Glu substitution is only supported by data from ~75 carriers and that for the Asp9Asn by data from ~200 carriers, both considerably less than the number of carriers included in the comparisons for the Asn291Ser and Ser447Ter substitutions. Therefore, more population-based research to support the role of the Gly188Glu and Asp9Asn substitutions is required. Furthermore, the evidence for the effect of these substitutions on postheparin plasma levels of lipoprotein lipase mass and activity is very limited, and the effects on plasma cholesterol and apolipoprotein B need to be explored, in particular to strengthen the evidence for a possible explanation of a subset of patients with familial combined hyperlipidemia. Although some evidence exists to support a direct association with ischemic heart disease, it is not strong, and within the area of a related disease, namely, ischemic cerebrovascular disease, it is almost absent. In addition, the potential differences related to sex and different ethnic groups, whether the effects of the substitutions are postprandial, and interactions with other common polymorphisms, eg, apolipoprotein E, need to be explored further.


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