A Common Substitution (Asn291Ser) in Lipoprotein Lipase Is Associated with Increased Risk of Ischemic Heart Disease

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Abstract

Lipoprotein lipase degrades triglycerides in plasma and as a byproduct produces HDL particles. Genetic variation in lipoprotein lipase may therefore affect cardiovascular risk.

We tested 9,214 men and women from a general population sample and 948 patients with ischemic heart disease for the Asn291Ser substitution in lipoprotein lipase.

The allele frequency in the general population was 0.024 and 0.026 for women and men, respectively. In comparison with noncarriers, female heterozygous probands had increased plasma triglycerides (Δ = 0.23 mmol/liter), while HDL cholesterol was reduced in both female and male carriers (Δ = 0.18 mmol/liter and Δ = 0.11 mmol/liter, respectively). A similar phenotype was found in six homozygous carriers. On multiple logistic regression analysis, plasma triglycerides and HDL cholesterol were independent predictors of ischemic heart disease in both genders. On univariate analysis, odds ratios for ischemic heart disease in probands were 1.89 in women (95% CI: 1.19–3.01) and 0.90 in men (95% CI: 0.62–1.31), and on multivariate analysis were 1.98 in women (95% CI: 1.11–3.53) and 1.02 in men (95% CI: 0.65–1.60).

This study demonstrates that a single common mutation in the *lipoprotein lipase* gene is associated with elevated plasma triglycerides and reduced HDL cholesterol levels, whereby carriers, in particular women, seem to be predisposed to ischemic heart disease. It cannot be excluded, however, that male carriers of this substitution may represent a subset of low-HDL individuals without raised triglycerides not predisposed to ischemic heart disease. (*J. Clin. Invest.* 1997. 99:1606–1613.) Key words: atherosclerosis • HDL • triglycerides • polymorphism • molecular epidemiology

Introduction

Lipoprotein lipase hydrolyses triglycerides contained in the core of either chylomicrons or VLDL, causing these particles to be transformed into chylomicron remnants and intermediate density lipoproteins (IDL)[1] and LDL, respectively; excess surface molecules are transferred to the HDL fraction (1, 2).

More than 40 different structural mutations in the *lipoprotein lipase* gene have been described in either the homozygote or compound heterozygote state in patients with the chylomicronemia syndrome. As a result of such mutations, the enzyme is either not produced or becomes catalytically ineffective, and these patients typically exhibit severe hypertriglyceridemia, hepatosplenomegaly, episodes of abdominal pain, pancreatitis, and eruptive xanthomas, but in most cases not ischemic heart disease (1). Recently it has been shown that ischemic heart disease may be seen in some patients with catalytic defects in the lipoprotein lipase protein (3).

The majority of these mutations have been located to exons 4, 5, and 6 in the *lipoprotein lipase* gene and are considered to be rare. The Asn291Ser substitution in exon 6, however, has been described in several kindreds of European ancestry and seems to be common (4–6). In the heterozygous state this substitution appears to reduce plasma HDL cholesterol levels, and to increase plasma triglycerides, although these findings have not been consistently significant (6–8). The exact phenotype associated with this substitution therefore remains somewhat unclear and available information on carriers identified in the general population is scarce. Nevertheless, because reduced levels of HDL cholesterol and increased plasma triglycerides are both associated with an increased risk of ischemic heart disease (9, 10), it is possible that the Asn291Ser substitution in lipoprotein lipase could predispose carriers to ischemic heart disease, although no data exist to directly support this hypothesis (11).

The aims of this study were to examine the frequency of this substitution in the general population, to test if the substitution affects plasma triglyceride and HDL cholesterol levels in individuals in the general population, and to examine whether carriers of this substitution are at an increased risk of ischemic heart disease. To answer these questions, we examined a sample of 9,214 men and women from the Danish general population (the Copenhagen City Heart Study) and 948 patients with verified ischemic heart disease (also from the Danish population) for the presence of the Asn291Ser substitution in lipoprotein lipase. This furthermore gave us the opportunity to describe the phenotypic characteristics of six homozygous individuals identified in the general population sample.

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1. *Abbreviation used in this paper:* IDL, intermediate density lipoproteins.

Methods

Subjects. A general population sample (the Copenhagen City Heart Study) including an almost equal number of men and women (55%) stratified into 10-yr age groups from 20 to 80 yr and above was drawn from the Copenhagen Central Population Register, the aim to obtain a representative sample of the general population. The Copenhagen City Heart Study is a prospective cardiovascular population study; a detailed description of the first (1976–1978) and second (1981–1983) examinations has previously been published (12). We studied cross-sectionally individuals who participated in the third examination of this study from 1991 through 1994. The original cohort from 1976–1978 supplemented with 500 20–24-yr olds at the second examination in 1981–1983 and 500 individuals in each of the age groups 20–24, 25–29, 30–34, 35–39, 40–44, and 45–49 yr at the third examination in 1991–1994 were all invited to participate in the third examination: of the 17,180 individuals invited, 10,049 participated, 9,259 gave blood, and of these 9,214 individuals were genotyped. Less than 1% were non-Caucasians and 98.8% had Danish citizenship, i.e., were of Danish descent. Plasma lipids and lipoproteins were measured in the non-fasting state. Plasma triglycerides were measured at the first and third examination, HDL cholesterol at the second and third, plasma cholesterol at all three examinations, and apolipoprotein AI, apolipoprotein B, and lipoprotein (a) only at the third examination.

A second population included 992 consecutive patients from the greater Copenhagen area referred for coronary angiography in the period 1991 through 1993. Among these, 948 (26% women) had ischemic heart disease with a characteristic symptomatology plus at least one of the following characteristics: severe stenosis on coronary angiography (i.e., $\geq$ 70% stenosis of at least one coronary artery or $\geq$ 50% stenosis of the left main coronary artery, $n = 767$), a previous myocardial infarction or a positive exercise electrocardiography test. Less than 1% were non-Caucasians and > 98% had Danish citizenship, i.e., were of Danish descent. Plasma lipids and lipoproteins were all measured in the fasting state.

This study was approved by Danish ethical committees: No. 100.2039/91 Copenhagen and Frederiksberg committee, and No. KA 93125 Copenhagen County committee.

Screening for the Asn291Ser substitution. DNA was isolated from blood as described (13). The codon 291 substitution (AAT→AGT) (4) was initially diagnosed in all 10,162 (9,214 + 948) individuals with an allele-specific PCR assay using the following primers: 1 μmol/liter of the 5′ primer (5′ AGGTGCAGTTCCAAGGAAGCCTTT 3′), 0.1 μmol/liter of the 3′ primer (5′ CTTTGTAGGGCATCTGAGAACGAG 3′) and 1 μmol/liter of the mutation specific primer (5′ CTTCTTTTGGCTCTGACTGTAC 3′, extra mismatch underlined). This PCR yielded a 150-bp product for the *A*/*A* genotype and both a 150- and 106-bp product for the *A*/*G* and *G*/*G* genotypes. Positive controls were kindly provided by Dr. Anne Minnich (Clinical Research Institute, Montreal, Canada). To distinguish heterozygous from homozygous carriers an RsaI restriction enzyme-based mismatch PCR assay was used, as described previously (4, 14). As an internal control of restriction enzyme digestion, part of exon 10 of the *lipoprotein lipase* gene was amplified in the same PCR. One potential methodological problem is misclassification. The risk of a faulty diagnosis of the Asn291Ser substitution was, however, minimized through the above described use of two different PCR-assays.

Other analyses. Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, HDL cholesterol, triglycerides, glucose, apolipoprotein B, apolipoprotein AI, fibrinogen (CHOD-PAP, precipitation of apolipoprotein B-containing lipoproteins followed by CHOD-PAP, GPO-PAP, hexokinase method, sheep anti–human apolipoprotein B, sheep anti–human apolipoprotein AI, and fibrinogen kinetic, respectively; all from Boehringer Mannheim, Mannheim, Germany) and lipoprotein (a) (rabbit anti–human lipoprotein (a); DAKO A/S, Glostrup, Denmark). Body mass index and blood pressure was determined as described previously (12). Waist–hip ratio was the body circumference measured midway between the lower rib margin and the iliac crest, divided by the maximum circumference over the buttocks.

Analysis of results. Data on women and men were analyzed separately using the SPSS program (15). Means of continuous variables unadjusted as well as adjusted (by analysis of covariance) were compared using Student's t test. The homogeneity of the association of genotype with triglycerides, HDL cholesterol, and apolipoprotein AI levels between tertiles of continuous covariates (age, cholesterol, apolipoprotein B, lipoprotein (a), body mass index, waist–hip ratio, glucose, systolic blood pressure, and diastolic blood pressure), or between the presence or absence of categorical covariates (smoking, diabetes mellitus; in women, menopausal status, and in postmenopausal women, hormonal replacement therapy) was tested using interaction terms in an analysis of variance, including genotype and the covariate in question. There was no evidence for interaction in any of these analyses, most notably no interaction of body mass index and genotype on plasma triglycerides (7), examined in each of the 10-yr age groups of the general population cohort (age 20 to 80+). Odds ratios were calculated; Yates' modified chi-square test was used as a test of independence.

Multivariate logistic regression analysis with forced entry was performed to investigate the role of either genotype, plasma triglycerides, HDL cholesterol, or apolipoprotein AI levels in predicting ischemic heart disease, after allowing for other cardiovascular risk factors (16, 17). Because plasma cholesterol and apolipoprotein B were strongly positively associated, only plasma cholesterol was included in the models. To approach linearity in the logit for the remaining covariates, square-root, logarithmic, or inverse transformations were used for some but not all covariates. Because individuals > 70 yr of age rarely will be referred for coronary angiography in Denmark, only individuals < 70 yr were included in the logistic regression analysis. Results are given as odds ratios (e^{β}) with 95% confidence intervals ($e^{\beta \pm 1.96 \times SE}$); the odds ratios given for continous variables relate to an increase of +1 SD. Overall model fit was tested using the likelihood ratio test.

Results

All results from the Copenhagen City Heart Study are from the third examination in 1991–1994, except that results on plasma triglycerides and HDL cholesterol from the first and second examination in 1976–1978 and 1981–1983, respectively, are also shown (Table I).

Prevalence of mutation. The allele frequency in the general population of the Asn291Ser substitution was 0.024 (95% CI: 0.021–0.027) and 0.026 (95% CI: 0.023–0.030) for women and men, respectively. Genotype and allele frequencies did not differ between 10-yr age groups from either 20 to 80+ yr in the general population sample, or from 30 to 80 yr among patients with ischemic heart disease in women or men (data not shown). Genotype frequencies, both in the general population sample and among patients with ischemic heart disease, were not significantly different from values predicted by the Hardy-Weinberg equilibrium; six and one individuals were homozygous carriers in the two populations, respectively.

Phenotypic characteristics of homozygous carriers. Among female carriers found in the general population sample there was no evidence for differences in phenotypic characteristics between homozygous and heterozygous carriers (Table I). The two homozygous men had lower plasma apolipoprotein AI levels than heterozygous male carriers, however, there was no detectable difference in plasma triglycerides or HDL cholesterol levels.

Phenotypic characteristics of heterozygous carriers. In comparison with noncarriers, female and male heterozygous carri-

Table I. Impact of the Asn291Ser Substitution in Lipoprotein Lipase on Phenotypic Characteristics in Individuals from the General Population Sample

	Amino acids at position 291 in lipoprotein lipase			Student's *t* test	
	Asn/Asn	Ser/Asn	Ser/Ser	Asn/Asn vs. Ser/Asn	Ser/Asn vs. Ser/Ser
Women					
No. of individuals*§	4654–4860	225–234	4		
Age (yr)§	58.3 (57.9–58.7)	58.1 (56.2–60.1)	46.3 (16.4–76.1)	NS	NS
Plasma cholesterol (mmol/liter)§	6.29 (6.26–6.33)	6.23 (6.05–6.41)	6.70 (4.49–8.91)	NS	NS
Plasma apolipoprotein B (mg/dl)§	86.2 (85.6–86.9)	87.2 (84.1–90.4)	92.3 (49.5–135.0)	NS	NS
Plasma lipoprotein (a) (mg/liter)‡§	323 (312–335)	329 (281–377)	293 (123–463)	NS	NS
Plasma HDL cholesterol (mmol/liter)§	1.73 (1.72–1.74)	1.56 (1.50–1.62)	1.65 (1.27–2.03)	$P < 0.001$	NS
Plasma HDL cholesterol (mmol/liter)‖	1.26 (1.25–1.27)	1.14 (1.09–1.18)	1.65, 0.92	$P < 0.001$	NS
Plasma apolipoprotein AI (mg/dl)§	151.4 (150.6–152.2)	143.3 (139.9–146.7)	150.0 (122.9–177.1)	$P < 0.001$	NS
Plasma triglycerides (mmol/liter)‡§	1.68 (1.65–1.71)	1.90 (1.76–2.04)	1.78 (0.81–2.75)	$P < 0.001$	NS
Plasma triglycerides (mmol/liter)‡¶	1.36 (1.33–1.39)	1.52 (1.39–1.64)	1.04	$P = 0.003$	—
Men					
No. of individuals*§	3654–3902	199–212	2		
Age (yr)	56.7 (56.2–57.1)	58.0 (55.9–60.0)	57, 72	NS	NS
Plasma cholesterol (mmol/liter)§	5.97 (5.93–6.00)	5.94 (5.77–6.12)	4.6, 5.5	NS	NS
Plasma apolipoprotein B (mg/dl)§	86.4 (85.7–87.1)	86.5 (83.5–89.6)	79, 89	NS	NS
Plasma lipoprotein (a) (mg/liter)‡§	292 (281–304)	281 (234–329)	61, 117	NS	NS
Plasma HDL cholesterol (mmol/liter)§	1.39 (1.37–1.40)	1.30 (1.25–1.36)	0.9, 0.9	$P = 0.006$	NS
Plasma HDL cholesterol (mmol/liter)‖	1.05 (1.04–1.06)	0.97 (0.93–1.01)	0.85	$P = 0.001$	—
Plasma apolipoprotein AI (mg/dl)§	130.1 (129.3–130.8)	125.6 (122.4–128.7)	86, 87	$P = 0.008$	$P = 0.02$
Plasma triglycerides (mmol/liter)‡§	2.12 (2.05–2.18)	2.41 (1.97–2.85)	1.9, 1.1	NS	NS
Plasma triglycerides (mmol/liter)‡¶	2.03 (1.97–2.09)	2.09 (1.91–2.28)	2.2	NS	—

Values are shown as means and 95% confidence intervals. *Because some of the characteristics in this table were not determined for all individuals, the number of individuals vary between characteristics. ‡To approach normal distribution the values were transformed logarithmically before statistical test, but mean values and 95% confidence intervals are shown for nontransformed values. §Values from the third examination of the Copenhagen City Heart Study (1991–1994). ‖Values from the second examination of the Copenhagen City Heart Study (1981–1983); numbers of women and men were 3,542 and 2,692 for Asn/Asn, 169 and 151 for Ser/Asn, and 2 and 1 for Ser/Ser, respectively. ¶Values from the first examination of the Copenhagen City Heart Study (1976–1978); numbers of women and men were 3,464 and 2,572 for Asn/Asn, 169 and 145 for Ser/Asn, and 1 and 1 for Ser/Ser, respectively. The difference in HDL cholesterol levels between the second and third examination and in triglyceride levels between the first and third examination most likely represent differences in calibrators used at these different timepoints, or to some extent also changes in these levels in the Danish population over this time period; importantly, however, the comparison between carriers and noncarriers are unaffected by these differences and are still valid at both timepoints. NS, non significant. ($P > 0.05$).

ers of the Asn291Ser substitution had lower plasma HDL cholesterol and apolipoprotein AI levels, as well as higher plasma levels of triglycerides, although the latter effect only reached statistical significance in women (Table I). These results were confirmed when triglycerides and HDL cholesterol values measured in 1976–1978 and 1981–1983 were examined, including the fact that plasma triglycerides were significantly increased in female carriers only. In women the largest effect of genotype on plasma triglycerides was seen in postmenopausal women, both on or without hormonal replacement therapy, while the effect in premenopausal women was nominally smaller (Table II). This difference in magnitude of effects between pre- and postmenopausal women was similar with respect to the effect on HDL cholesterol and apolipoprotein AI levels.

To further describe the association of the Asn291Ser substitution with plasma triglycerides, HDL cholesterol, and apolipoprotein AI, these variables were adjusted by analysis of covariance for variation due to age, body mass index, diabetes mellitus, antihypertensive, and diuretic medication, alcohol consumption (beer, wine, and liquor), smoking habits, physical activity (at work and leisure) and, in women, for menopausal status and hormonal replacement therapy (Table III). In women 16, 12, and 12% of the variation in triglycerides, HDL cholesterol, and apolipoprotein AI, respectively, was explained by the above mentioned covariates. In men, the equivalent values were 9, 13, and 11%, respectively. The effect of the Asn291Ser substitution on unadjusted or adjusted levels of HDL cholesterol, triglycerides, and apolipoprotein AI was similar (compare Tables I and III): for adjusted values, female carriers compared with noncarriers had elevated plasma triglycerides of †0.23 mmol/liter (95% confidence interval: +0.10–+0.36 mmol/liter), decreased HDL cholesterol of −0.18 mmol/liter (95% confidence interval: −0.13–−0.23 mmol/liter), and decreased apolipoprotein AI of −8.2 mg/dl (−4.7–−11.7 mg/dliter). In male carriers compared with noncarriers the effect on these variables was +0.33 mmol/liter (95% CI: −0.11–+0.77 mmol/liter), −0.11 mmol/liter (95% CI: −0.06–−0.16 mmol/liter) and −5.9 mg/dl (−2.8–−8.9 mg/dl), respectively. Plasma fibrinogen, body mass index, waist–hip ratio, plasma glucose, and systolic and diastolic blood pressure did not differ between carriers and noncarriers (data not shown). The fre-

Table II. Impact of the Asn291Ser Substitution in Lipoprotein Lipase on Phenotypic Characteristics in Women from the General Population Sample

	Amino acids at position 291 in lipoprotein lipase			Student's *t* test	
	Asn/Asn	Ser/Asn	Ser/Ser	Asn/Asn vs. Ser/Asn	Ser/Asn vs. Ser/Ser
Premenopausal, *n* =	1376	70	2		
Plasma HDL cholesterol (mmol/liter)	1.71 (1.68–1.73)	1.59 (1.49–1.68)	1.8, 1.7	$P = 0.03$	NS
Plasma apolipoprotein AI (mg/dl)	144.9 (143.5–146.2)	140.1 (133.9–146.3)	146, 167	NS	NS
Plasma triglycerides (mmol/liter)*	1.31 (1.26–1.37)	1.47 (1.30–1.63)	1.09, 1.64	$P = 0.02$	NS
Postmenopausal without treatment, *n* =	2772	130	0		
Plasma HDL cholesterol (mmol/liter)	1.72 (1.70–1.74)	1.55 (1.47–1.62)	—	$P < 0.001$	—
Plasma apolipoprotein AI (mg/dl)	152.0 (150.9–153.0)	144.5 (140.1–148.9)	—	$P = 0.002$	—
Plasma triglycerides (mmol/liter)*	1.85 (1.81–1.89)	2.08 (1.88–2.28)	—	$P = 0.007$	—
On hormonal replacement therapy, *n* =	652	31	2		
Plasma HDL cholesterol (mmol/liter)	1.86 (1.82–1.91)	1.55 (1.38–1.73)	1.80, 1.30	$P = 0.004$	NS
Plasma apolipoprotein AI (mg/dl)	162.6 (160.0–165.2)	145.7 (134.1–157.1)	159, 128	$P = 0.006$	NS
Plasma triglycerides (mmol/liter)*	1.73 (1.66–1.80)	2.12 (1.64–2.61)	2.57, 1.81	$P = 0.06$	NS

Values are shown as means and 95% confidence intervals, and are from the third examination of the Copenhagen City Heart Study in 1991–1994. *To approach normal distribution the values were transformed logarithmically before statistical test, but mean values and 95% confidence intervals are shown for nontransformed values. NS, non significant. ($P > 0.05$).

quencies of treatment for hypertension and for ischemic heart disease, as well as smoking habits, also did not differ between carriers and noncarriers (data not shown).

HDL cholesterol, triglycerides, and risk of ischemic heart disease. HDL cholesterol was decreased and plasma triglycerides increased in both women and men with ischemic heart disease, while apolipoprotein AI was not significantly different between patients with ischemic heart disease and individuals in the general population sample (Table IV). These results were similar when the patients with known severe stenosis on coronary angiography only were compared with the general population sample (data not shown). Because values were measured in the fasting state for patients with ischemic heart disease, but in the nonfasting state for individuals in the general population sample, this would tend to underestimate the differences in plasma triglycerides mentioned above.

Among both women and men with ischemic heart disease most individuals were in the age range of 40–70 yr (Table IV). The age distribution in the general population sample was somewhat similar, except that this sample also included a num-

Table III. Impact of the Asn291Ser Substitution on Adjusted Plasma Levels of HDL Cholesterol, Apolipoprotein AI, and Triglycerides in Individuals from the General Population Sample

	Amino acids at position 291 in lipoprotein lipase			Student's *t* test	
	Asn/Asn	Ser/Asn	Ser/Ser	Asn/Asn vs. Ser/Asn	Ser/Asn vs. Ser/Ser
Women					
No. of individuals*	4689–4712	226–229	4		
Plasma HDL cholesterol (mmol/liter)	1.74 (1.72–1.75)	1.56 (1.50–1.61)	1.58 (1.26–1.91)	$P < 0.001$	NS
Plasma apolipoprotein AI (mg/dl)	151.5 (150.8–152.3)	143.4 (140.0–146.7)	146.6 (117.0–176.3)	$P < 0.001$	NS
Plasma triglycerides (mmol/liter)‡	1.69 (1.66–1.71)	1.92 (1.79–2.04)	1.99 (1.08–2.89)	$P < 0.001$	NS
Men					
No. of individuals*	3732–3805	205–209	1–2		
Plasma HDL cholesterol (mmol/liter)	1.39 (1.38–1.40)	1.28 (1.23–1.33)	1.0, 1.1	$P < 0.001$	NS
Plasma apolipoprotein AI (mg/dl)	130.3 (129.5–131.0)	124.4 (121.4–127.4)	86, 94	$P < 0.001$	$P = 0.03$
Plasma triglycerides (mmol/liter)‡	2.16 (2.10–2.23)	2.49 (2.06–2.93)	0.7	NS	—

Plasma HDL cholesterol, apolipoprotein AI, and triglycerides are adjusted for age, body mass index, diabetes mellitus, antihypertensive and diuretic medication, alcohol consumption (beer, wine, and liquor), smoking habits, physical activity (at work and leisure), and among women in addition for menopausal status and hormonal-replacement therapy. Values are shown as means and 95% confidence intervals and represent values from the third examination of the Copenhagen City Heart Study in 1991–1994. *Because some of the characteristics in this table were not determined for all individuals, the number of individuals vary between characteristics. ‡To approach normal distribution the values were transformed logarithmically before statistical test, but mean values and 95% confidence intervals are shown for nontransformed values. NS, non significant. ($P > 0.05$).

Table IV. Age and Plasma Lipid Levels in Study Populations

	General population sample	Patients with ischemic heart disease
Women		
No. of individuals*	5078–5098	223–248
20–29 yr	5%	0%
30–39 yr	10%	4%
40–49 yr	12%	19%
50–59 yr	20%	32%
60–69 yr	26%	36%
70–79 yr	23%	9%
80 yr and above	5%	0%
Age (median, yr)	61	58
Age (mean, yr)	58.3 (57.9–58.7)	57.2 (56.0–58.4)
Plasma HDL cholesterol (mmol/liter)	1.73 (1.71–1.74)	1.33 (1.27–1.39)§
Plasma apolipoprotein AI (mg/dl)	151.0 (150.3–151.8)	149.1 (145.5–152.6)
Plasma triglycerides (mmol/liter)‡	1.69 (1.66–1.72)	2.19 (1.82–2.56)§
Men		
No. of individuals*	4102–4116	634–700
20–29 yr	5%	0%
30–39 yr	12%	3%
40–49 yr	14%	15%
50–59 yr	22%	36%
60–69 yr	24%	33%
70–79 yr	19%	13%
80 yr and above	4%	0%
Age (median, yr)	58	59
Age (mean, yr)	56.7 (56.3–57.2)	58.6 (57.9–59.3)§
Plasma HDL cholesterol (mmol/liter)	1.38 (1.37–1.39)	1.07 (1.04–1.11)§
Plasma apolipoprotein AI (mg/dl)	129.8 (129.0–130.5)	129.3 (127.4–131.1)
Plasma triglycerides (mmol/liter)‡	2.13 (2.07–2.20)	2.38 (2.25–2.51)§

Values in the lower part of the table for both women and men are shown as mean and 95% confidence intervals. For the general population sample, values represent measurements obtained in 1991–1994 at the third examination of the Copenhagen City Heart Study. *Because some of the characteristics in this table were not determined for all individuals, the number of individuals varies between characteristics. ‡To approach normal distribution, the values were transformed logarithmically before statistical test, but mean values and 95% confidence intervals are shown for nontransformed values; §$P < 0.001$ for patients vs. individuals in the general population sample using Student's *t* test.

ber of individuals < 40 yr and > 70 yr of age. For multiple logistic regression analysis only individuals < 70 yr of age were therefore included: when age, plasma cholesterol, lipoprotein (a), body mass index, diabetes mellitus, smoking, and hypertension were allowed for, the HDL cholesterol and plasma triglycerides were independent predictors of ischemic heart disease in both women and men (Table V).

The Asn291Ser substitution and risk of ischemic heart disease. As described above, this substitution is associated with a decrease in HDL levels in both genders and an increase in plasma triglycerides, significant only in women (Table I). Plasma HDL cholesterol and triglycerides were predictors of ischemic heart disease on univariate (Table IV) as well as on multivariate analysis (Table V). Furthermore, female carriers of the Asn291Ser substitution (homozygous or heterozygous) were found more commonly among patients with ischemic heart disease than among individuals in the general population sample (odds ratio 1.89; Table VI). When the comparison was restricted to patients known to have severe stenosis on coronary angiography the difference was even more pronounced (odds ratio 2.27). This increase in frequency of the substitution among patients was mainly seen in women > 60 yr: odds ratio for ischemic heart disease was 3.02 (95% CI of 1.68–5.44, $P < 0.0003$) and for severe stenosis on coronary angiography 2.98 (95% CI of 1.50-5.92, $P < 0.003$). In men, however, there was no evidence that the Asn291Ser substitution was more common among patients with ischemic heart disease or severe stenosis on coronary angiography than among individuals in the general population sample. When HDL cholesterol, triglycerides, and apolipoprotein AI were excluded from the logistic regression analysis and when individuals < 70 yr old only were examined, the Asn291Ser substitution alone was an independent predictor of ischemic heart disease in women but not in men (Table V).

Discussion

In the majority of individuals ischemic heart disease has a multifactorial etiology: a combination of multiple susceptibility genes, each with a minor effect, but together with a cumulative effect on disease progression, will in certain environmental contexts lead to the development of disease (18). The present data suggest that the common mutation causing the Asn291Ser substitution in lipoprotein lipase (allele frequency ~ 0.025 in the general population) leads to small increases in plasma triglycerides mainly in women, and reductions in HDL levels in both genders. Because of this and because these two cardiovascular risk factors have been found to be independent predictors of ischemic heart disease, in the present and former studies (9, 10), the present data support the notion that the Asn291Ser substitution in lipoprotein lipase may represent a susceptibility mutation for ischemic heart disease, at least in the Danish population and in women in particular.

Unexpectedly we found that the effect of the Asn291Ser substitution on plasma triglycerides, HDL cholesterol, and apolipoprotein AI levels was more pronounced in women than in men, which could explain the observed association found between this substitution and risk of ischemic heart disease in women, but not in men. The present data suggest that the postmenopausal state exacerbates the effect of the Asn291Ser substitution on especially triglycerides but also on HDL cholesterol levels. Effects such as this may help explain that women after menopause approach risk rates for ischemic heart disease similar to men. The finding also emphasizes that effects of susceptibility mutations are context dependent. One alternative explanation for the gender difference in risk of ischemic heart disease could be that male, but not female carriers of the Asn291Ser substitution represent a subset of low HDL individuals who do not have an increased risk of ischemic heart disease due to the low HDL level. A third possible explanation for the gender difference could be that the triglyceride raising effect of this substitution rather than the HDL lowering effect

Table V. Role of Either Plasma Triglycerides, HDL Cholesterol, Apolipoprotein AI, or Genotype in the Prediction of Ischemic Heart Disease Using Multiple Logistic Regression Analysis

	Odds ratio (95% CI)	Likelihood ratio test
Women (n = 3922)*		
HDL cholesterol	0.43 (0.36–0.51)‡	$P < 0.0005$
Apolipoprotein AI	1.05 (0.90–1.23)‡	$P < 0.30$
Triglycerides	1.14 (1.00–1.31)‡	$P < 0.05$
Asn/Ser or Ser/Ser substitution	1.98 (1.11–3.53)§	$P < 0.05$
Men (n = 3771)*		
HDL cholesterol	0.37 (0.33–0.43)‡	$P < 0.0005$
Apolipoprotein AI	0.97 (0.87–1.07)‡	$P < 0.20$
Triglycerides	1.13 (1.04–1.23)‡	$P < 0.0005$
Asn/Ser or Ser/Ser substitution	1.02 (0.65–1.60)§	$P < 0.95$

*Only individuals ≤ 70 yr were included (see Methods). ‡For plasma triglycerides, HDL cholesterol, and apolipoprotein AI odds ratios express change in the odds when the variable changes plus one SD. §For genotype the odds ratio expresses the change in odds in individuals carrying the mutation compared to noncarriers. The models allowed for age, plasma cholesterol, lipoprotein (a), body mass index, diabetes mellitus, smoking, and hypertension. *CI*, confidence interval.

is important in promoting atherosclerosis and ischemic heart disease (HDL was reduced in both genders, but triglycerides was mainly raised in women). Age differences between male and female patients with ischemic heart disease do not appear to explain the difference in risk of ischemic heart disease because the age distribution of male and female patients was similar, and because even on multiple logistic regression analysis, performed only for individuals less than 70 yr of age and with age differences allowed for, the Asn291Ser substitution was an independent predictor of ischemic heart disease in women but not in men. Nevertheless, the exact explanation for the observed gender difference remains unclear.

At least three different mechanisms, or combinations thereof, could account for a defective lipoprotein lipase function leading to ischemic heart disease. (*a*) Slower conversion of chylomicrons and VLDL particles due to reduced triglyceride hydrolysis could lead to a longer half-life of the intermediate sized lipoproteins in plasma (chylomicron remnants, small VLDL and IDL), as also suggested by the observed slightly elevated plasma triglyceride levels in carriers; such particles appear to be trapped in the vessel wall, and may therefore promote atherogenesis (19). (*b*) A decreased triglyceride catabolism could decrease transfer of excess surface material from chylomicrons and VLDL particles to HDL particles, whereby fewer of such particles would be available for reverse cholesterol transport, i.e., the removal of cholesterol from peripheral cells and from the arterial wall, eventually followed by uptake in the liver (20). Because the clearest gender difference in the present study was the lack of a triglyceride raising effect among male carriers (Table I) while HDL cholesterol was reduced in both genders, and because only women had an increased risk of ischemic heart disease the present data suggest that elevated levels of triglyceride-rich lipoproteins may be more important for the development of atherosclerosis than a low HDL level. (*c*) Elevated plasma triglycerides have been shown to be associated with a subclass of small, dense LDL particles that by themselves may promote atherogenesis (10). At present, however, it is unclear which of these three mechanisms may be the most important in promoting atherogenesis. Nevertheless, they all support the point that mutations in the *lipoprotein lipase* gene, which lead to moderate elevations in plasma triglycerides and reductions in HDL levels, most likely also increase susceptibility of the carriers to ischemic heart disease.

The Asn291Ser substitution in the heterozygous state was first described in a patient with pregnancy-induced chylomicronemia and low plasma HDL cholesterol (4). Since then, this substitution has been described in groups of patients with type III hyperlipoproteinemia (14), in patients with familial combined hyperlipidemia (21, 22), and in hypertriglyceridemic patients (5). Plasma HDL cholesterol levels have been found to be decreased and triglyceride levels to be increased in probands identified among patients with familial combined hyperlipidemia (21, 22). Furthermore, a decrease in plasma HDL cholesterol levels and a nonsignificant trend towards elevated plasma triglyceride levels was observed in probands identified among patients with coronary artery disease, or controls (6), whereas in another study no effect on plasma HDL cholesterol but still a nonsignificant increase in triglycerides was observed in probands identified among either patients with myocardial infarction or controls (8). This apparent discrepancy, when compared with the present results, could be explained by the fact that the two former studies (6, 8) examined men only, the

Table VI. Susceptibility to Ischemic Heart Disease in Probands Heterozygous or Homozygous for the Asn291Ser Substitution in Lipoprotein Lipase

Subjects studied	No. (%) of individuals with Asn/Ser or Ser/Ser	Odds ratio* (95% CI)	Chi-square test‡
Women			
Pts. with verified ischemic heart disease (n = 248)	21 (8.5%)	1.89 (1.19–3.01)	$P = 0.01$
Pts. with severe stenosis on coronary angiography (n = 150)	15 (10.0%)	2.27 (1.31–3.93)	$P = 0.005$
General population sample (n = 5098)	238 (4.7%)		
Men			
Pts. with verified ischemic heart disease (n = 700)	33 (4.7%)	0.90 (0.62–1.31)	$P = 0.66$
Pts. with severe stenosis on coronary angiography (n = 617)	32 (5.2%)	1.00 (0.68–1.46)	$P = 0.99$
General population sample (n = 4116)	214 (5.2%)		

*Comparison between patient groups and total general population sample. ‡Based on Yates' corrected chi-square. *CI*, confidence interval; *Pts.*, patients.

gender where we observed the smallest effects. In support of this view, another large previous study found a small increase in plasma triglycerides in probands among healthy men, but a much larger increase in plasma triglycerides when a group of healthy men and women were jointly examined (7).

In accordance with a previous report on one individual (23), homozygous carriers of the Asn291Ser substitution, in contrast to homozygosity for a number of other known mutations in this enzyme (1) did not present with severe chylomicronemia. This observation is possibly explained by the fact that the previously described mutations cause the enzyme either not to be produced or to be catalytically ineffective, whereas for the Asn291Ser substitution an enzyme with reduced efficiency only is produced (4, 6, 14, 24, 25). It is also conceivable, however, that environmental or other genetic factors are important in precipitating and unmasking chylomicronemia in individuals homozygous for mutations in the *lipoprotein lipase* gene, and that such environmental or genetic factors are not present in the homozygous individuals studied in the present investigation. Because the effect on lipid levels is moderate for carriers of the Asn291Ser substitution, a larger number of homozygous individuals needs to be studied before it can be determined if homozygous carriers have a more severe phenotype than heterozygous carriers.

If the presence of the Asn291Ser substitution influenced referrals for coronary angiography this could be one possible source of selection bias in the present study. It might be that some of those at risk for ischemic heart disease due to the Asn291Ser substitution already had suffered critical events and therefore were unable to participate. If this was the case, however, the present results would represent an underestimation of the true effect of the Asn291Ser substitution on the increased susceptibility to ischemic heart disease in carriers. Therefore, this potentially could be the explanation for the nonsignificant finding in men, especially if men with high risk of ischemic heart disease did not participate in the study. However, the even distribution of genotypes and alleles in men among all age groups in both patients and the general population sample did not indicate a dropout of carriers as a serious problem.

Measurement of plasma lipids and lipoproteins in the nonfasting state in individuals from the general population sample, but in the fasting state in patients with ischemic heart disease, has no direct implication for the results concerning the effect of the Asn291Ser substitution on lipids and lipoproteins presented in this study, because these results only relate to individuals in the general population sample. With respect to plasma triglycerides it is possible, however, that the present results may differ from results obtained in the fasting state. Nevertheless, individuals heterozygous for the Asn291Ser substitution also have raised fasting plasma triglycerides (7). Because nonfasting plasma triglyceride levels are higher than fasting levels, the present study probably underestimated the role of triglycerides in predicting ischemic heart disease (Tables V and VI).

Linkage disequilibrium with another susceptibility mutation nearby is a possible, though unlikely confounder because in vitro expression studies have shown that this substitution alone causes a decrease in lipoprotein lipase activity of 30–50% (4, 6, 14, 24, 25). Furthermore, carriers of the Asn291Ser substitution in the heterozygous state show a decrease in postheparin plasma lipoprotein lipase activity of $\sim$ 30% (6, 25).

Taken together, this study demonstrates that the Asn291Ser substitution in lipoprotein lipase is associated with increased plasma triglycerides in women and decreased plasma HDL levels in both genders. This in turn seems to lead to increased susceptibility to ischemic heart disease in carriers, an effect which is more pronounced in women than in men.

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References

1. Brunzell, J. 1995. Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. *In* The Metabolic and Molecular Bases of Inherited Disease. 7th ed. C. Scriver, A. Beaudet, W. Sly, and D. Valle, editors. McGraw-Hill Inc., New York. 1913–1932.

2. Olivecrona, G., and T. Olivecrona. 1995. Triglyceride lipases and atherosclerosis. *Curr. Opin. Lipidol.* 6:291–305.

3. Benlian, P., J.L.D. Gennes, L. Foubert, H. Zhang, S.E. Gagné, and M. Hayden. 1996. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N. Engl. J. Med.* 335: 848–854.

4. Ma, Y., T.C. Ooi, M.S. Liu, H. Zhang, R. McPherson, A.L. Edwards, I.J. Forsythe, J. Frohlich, J.D. Brunzell, and M.R. Hayden. 1994. High frequency of mutations in the human lipoprotein lipase gene in pregnancy-induced chylomicronemia: possible association with apolipoprotein E2 isoform. *J. Lipid Res.* 35: 1066–1075.

5. Minnich, A., A. Kessling, M. Roy, C. Giry, G. DeLangavant, J. Lavigne, S. Lussier-Cacan, and J. Davignon. 1995. Prevalence of alleles encoding defective lipoprotein lipase in hypertriglyceridemic patients of French Canadian descent. *J. Lipid Res.* 36:117–124.

6. Reymer, P.W., E. Gagné, B.E. Groenemeyer, H. Zhang, I. Forsyth, H. Jansen, J.C. Seidell, D. Kromhout, K.E. Lie, J. Kastelein, and M.R. Hayden. 1995. A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. *Nat. Genet.* 10:28–34.

7. Fisher, R.M., F. Mailly, R.E. Peacock, A. Hamsten, M. Seed, J.S. Yudkin, U. Beisiegel, G. Feussner, G. Miller, S.E. Humphries, and P.J. Talmud. 1995. Interaction of the lipoprotein lipase asparagine 291→serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: a study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults. *J. Lipid Res.* 36:2104–2112.

8. Jemaa, R., F. Fumeron, O. Poirier, L. Lecerf, A. Evans, D. Arveiler, G. Luc, J.-P. Cambou, J.-M. Bard, J.-C. Fruchart, et al. 1995. Lipoprotein lipase gene polymorphisms: associations with myocardial infarction and lipoprotein levels, the ECTIM study. *J. Lipid Res.* 36:2141–2146.

9. Gordon, D.J., J.L. Probstfield, R.J. Garrison, J.D. Neaton, W.P. Castelli, J.D. Knoke, D.R. Jacobs, Jr., S. Bangdiwala, and H.A. Tyroler. 1989. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* 79:8–15.

10. Austin, M.A. 1991. Plasma triglyceride and coronary heart disease. *Arterioscler. Thromb.* 11:2–14.

11. Goldberg, I.J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* 37:693–707.

12. Appleyard, M., A.T. Hansen, P. Schnohr, G. Jensen, and J. Nyboe. 1989. The Copenhagen City Heart Study, Østerbrundersøgelsen. A book of tables with data from the first examination (1976–78) and a five year follow-up (1981–83). *Scand. J. Soc. Med. Suppl.* 41:1–160.

13. Talmud, P., A. Tybjaerg-Hansen, D. Bhatnagar, A. Mbewu, J.P. Miller, P. Durrington, and S. Humphries. 1991. Rapid screening for specific mutations in patients with a clinical diagnosis of familial hypercholesterolaemia. *Atherosclerosis.* 89:137–141.

14. Zhang, H., P.W.A. Reymer, M.-S. Liu, I.J. Forsythe, B.E. Groenemeyer, J. Frohlich, J.D. Brunzell, J.J.P. Kastelein, M.R. Hayden, and Y. Ma. 1995. Patients with apoE3 deficiency (E2/2, E3/2, and E4/2) who manifest with hyperlipidemia have increased frequency of an Asn 291→Ser mutation in the human LPL gene. *Arterioscler. Thromb. Vasc. Biol.* 15:1695–1703.

15. SPSS for Windows. 1993. Base System User's Guide, release 6.0. Norusis, M. J. and SPSS Inc. 6.0 USA. SPSS Inc.

16. Hosmer, D.W., and S. Lemeshow. 1989. Model-building strategies and methods for logistic regression. *In* Applied Logistic Regression. John Wiley & Sons, New York. 82–134.

17. Menard, S. 1995. An introduction to logistic regression diagnostics. *In* Applied Logistic Regression Analysis. Sage Publications, Inc., Thousand Oaks, CA. 58–79.

18. Beaudet, A.L., C.R. Scriver, W.S. Sly, and D. Valle. 1995. Genetics, biochemistry, and molecular basis of variant human phenotypes. *In* The Metabolic & Molecular Bases of Inherited Disease. 7th ed. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, editors. McGraw-Hill Inc., New York. 53–118.

19. Nordestgaard, B. G., R. Wootton, and B. Lewis. 1995. Selective retention of VLDL, IDL, and LDL in the arterial intima of genetically hyperlipidemic rabbits in vivo. Molecular size as a determinant of fractional loss from the intima-inner media. *Arterioscler. Thromb. Vasc. Biol.* 15:534–542.

20. Barter, P.J., and K.-A. Rye. 1996. High density lipoproteins and coronary heart disease. *Atherosclerosis.* 121:1–12.

21. Reymer, P.W.A., B.E. Groenemeyer, E. Gagné, L. Miao, E.E.G. Appelman, J.C. Seidel, D. Kromhout, S.M. Bijvoet, K. van de Oever, T. Bruin, et al. 1995. A frequently occurring mutation in the lipoprotein lipase gene (Asn291Ser) contributes to the expression of familial combined hyperlipidemia. *Hum. Mol. Genet.* 4:1543–1549.

22. Hoffer, M.J.V., S.J.H. Bredie, D.I. Boomsma, P.W.A. Reymer, J.J. P. Kastelein, P. de Knijff, P.N.M. Demacker, A.F.H. Stalenhoef, L.M. Havekes, and R.R. Frants. 1996. The lipoprotein lipase (Asn291→Ser) mutation is associated with elevated lipid levels in families with familial combined hyperlipidemia. *Atherosclerosis.* 119:159–167.

23. Funke, H., and G. Assmann. 1995. The low down on lipoprotein lipase. *Nat. Genet.* 10:6–7.

24. Busca, R., J. Peinado, E. Vilella, J. Auwerx, S.S. Deeb, S. Vilaro, and M. Reina. 1995. The mutant Asn291→Ser human lipoprotein lipase is associated with reduced catalytic activity and does not influence binding to heparin. *FEBS Lett.* 367:257–262.

25. Syvänne, M., M. Antikainen, S. Ehnholm, H. Tenkanen, S. Lahdenperä, C. Ehnholm, and M.-R. Taskinen. 1996. Heterozygosity for Asn^{291}→Ser mutation in the lipoprotein lipase gene in two Finnish pedigrees: effect of hyperinsulinemia on the expression of hypertriglyceridemia. *J. Lipid Res.* 37:727–738.