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The T111I variant in the endothelial lipase gene and risk of coronary heart disease in three independent populations

Majken K. Jensen[1,2,3]*, Eric B. Rimm[2,4,5], Kenneth J. Mukamal[6], Andrew C. Edmondson[7], Daniel J. Rader[7], Ulla Vogel[8,9,10], Anne Tjønneland[11], Thorkild I.A. Sørensen[12], Erik B. Schmidt[3,13], and Kim Overvad[1,3,13]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aalborg, Denmark; [2]Department of Nutrition, Harvard School of Public Health, Boston, MA, USA; [3]Center for Cardiovascular Research, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [4]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA; [5]Department of Medicine, Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [6]Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA; [7]Department of Medicine, University of Pennsylvania, PA, USA; [8]Research Centre for the Working Environment, Copenhagen, Denmark; [9]National Food Institute, Technical University of Denmark, Soborg, Denmark; [10]Institute of Science, Systems and Models, Roskilde University, Roskilde, Denmark; [11]Danish Cancer Society, Copenhagen, Denmark; [12]Institute of Preventive Medicine, Center for Health and Society, Copenhagen, Denmark; and [13]Department of Cardiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

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Aims Endothelial lipase (*LIPG*) is implicated in the metabolism of high-density lipoprotein cholesterol (HDL-C). Small studies in selected populations have reported higher HDL-C levels among carriers of the common T111I variant in *LIPG*, but whether this variant is associated with plasma lipids and risk of coronary heart disease (CHD) in the general population is unclear. The objective of this study was to address the associations of the T111I variant with plasma lipids and risk of CHD in three independent prospective studies of generally healthy men and women.

Methods and results The T111I variant was genotyped in case–control studies of CHD nested within the Diet, Cancer, and Health study with 998 cases, Nurses' Health Study with 241 cases, and Health Professionals Follow-up Study with 262 cases. The minor allele frequency in the combined pool of controls was 0.29. The T111I variant was not associated with HDL-C or any other lipid and lipoprotein measures. Compared with wildtype homozygotes, the pooled estimate for risk of CHD was 0.95 (0.85–1.06) per T111I allele.

Conclusion Our analysis among healthy Caucasian men and women from three independent studies does not support an association between the T111I variant and HDL-C, other plasma lipids, or risk of CHD.

Keywords Genetic epidemiology • Endothelial lipase • HDL-cholesterol • CHD-risk

Introduction

High-density lipoprotein cholesterol (HDL-C) is strongly associated with a lower risk of coronary heart disease (CHD), but the complex metabolism of HDL particles has limited efforts to develop pharmacologic therapies.[1] The recently identified endothelial lipase (EL) is considered unique because it is synthesized by endothelial cells and it functions mostly as a phospholipase with HDL-C as its preferred substrate.[2,3] In animal models, overexpression of EL reduces and its inhibition raises HDL-C levels.[2,4,5] Evidence that EL plays a role in human lipid metabolism comes from two cross-sectional studies, where an inverse association was observed between plasma EL concentration and HDL-C levels.[6,7]

Sequencing of the endothelial lipase gene (*LIPG*) has revealed several non-synonymous variants,[8,9] most of which are rare and difficult to study in population samples. The 584C/T polymorphism in exon 3 (resulting in a Thr to Ile substitution at codon 111) is an exception, as it occurs with a high frequency in the general population (minor allele frequency ≈ 0.30). Several studies have

* Corresponding author. Tel: +1 617 432 6893, Fax: +1 617 432 2435, Email: mkjensen@hsph.harvard.edu

explored the association between the T111I variant and HDL-C, but the findings have been inconsistent. Some studies have found higher HDL-C levels among variant allele carriers,[5,9–11] but others have not confirmed a difference between genotype groups.[12–14]

So far, the association of the T111I variant with risk of incident CHD has not been examined among Caucasians. Previous reports include two US studies among CAD patients; one reporting the same minor allele frequency between survivors of myocardial infarction (MI) and controls and another reporting a lower frequency of the variant allele among participants with a history of MI.[5,9]

Our aim was to examine the associations of the T111I variant with plasma lipids and lipoproteins in three independent studies of generally healthy Caucasians. Further, we investigated the risk of CHD associated with the T111I variant in a prospective setting.

Methods

Study populations

The Diet, Cancer, and Health (DCH) study was initiated in 1993–1997 when 57 053 Danish born residents, aged 50–64 years and free of cancer, participated in a clinical examination and detailed lifestyle survey. Blood was sampled at baseline in the study clinic and stored as plasma, serum, lymphocytes, and erythrocytes at −150°C. The Nurses' Health Study (NHS) enrolled 121 701 female nurses aged 35–55 who returned a mailed questionnaire in 1976 regarding lifestyle and medical history. The Health Professionals' Follow-up Study (HPFS) enrolled 51 529 males aged 40–75 who returned a similar questionnaire in 1986. Participants of both cohorts have received follow-up questionnaires biennially to record newly diagnosed illnesses and to update lifestyle and dietary information. Detailed descriptions of the study cohorts have been published previously.[15–17]

Endpoint and study designs

In the DCH, a case-cohort study was designed using incident validated cases of acute coronary syndrome (including unstable angina, non-fatal, and fatal MI) as the outcome. Information on the disease endpoint was obtained by linking the participants (via the unique identification number assigned to all Danish citizens) with central Danish registries of hospital discharge diagnoses and causes of death (ICD-8 codes 410-410.99, 427.27, and ICD-10 codes I20.0, I21.x, I46.x). In total, 1150 incident cases were verified between baseline and 1 January 2004, the date of the last available update from the hospital discharge register.[18] Consistent with the case-cohort design,[19] 1800 participants were selected from the entire DCH study at random (subcohort members will also be referred to as controls throughout the paper, although this group includes 33 participants who later became cases, as expected in a case-cohort design).

A blood sample was requested from all active participants in 1989–1990 in NHS and 1993–1995 in the HPFS. A total of 32 826 female participants in NHS returned samples and 18 224 men in the HPFS did the same. Nested case–control studies were designed using incident CHD, with non-fatal MI, and fatal CHD as the outcome. Cases were identified primarily through self-reporting in questionnaires and subsequent review of medical records, as previously described.[20] Among participants who provided blood samples and who were without cardiovascular disease or cancer at blood draw, 249 women sustained an incident CHD between blood draw and 30 June 1998, and 266 cases occurred prior to 31 January 2000 in HPFS. Using risk-set sampling,[21] controls were selected randomly and matched in a 2:1 ratio on age, smoking, and month of blood return.

Laboratory analysis methods

Plasma lipids, lipoproteins, and apolipoproteins were assessed using standard methods with reagents from Roche Diagnostics (Indianapolis, IN, USA) and Bayer Diagnostics (New York, NY, USA).[22] Genotypes were determined by Taqman allelic discrimination (ABI 7500/7900HT, Applied Biosystems) using rs2000813. The case status of the samples was blinded when analysing the DNA in the laboratory.[23] Controls were included in each run and repeated genotyping of a random 10% subset yielded 100% identical genotypes.

Statistical analysis

Conformity with Hardy–Weinberg equilibrium was tested with an exact test in the controls. An additive model of inheritance was assumed for multivariable regression analyses where study-specific z-scores were created for each lipid phenotype within the controls of each study (separately by sex in the DCH). The regression coefficients from these models represent the incremental effect of one copy of the minor allele on a given biomarker in increments of one study-specific standard deviation. The association between the T111I variant and HDL-C has previously been reported for the HPFS,[24] but was included here for completeness of a new meta-analysis with the NHS and DCH data. Relative rate ratios and 95% confidence intervals for the association between genotype and CHD were estimated using Cox proportional hazard regression with Kalbfleisch and Lawless weights and robust variance suitable for the DCH case-cohort data,[25] and conditional logistic regression for the NHS and HPFS nested case–control data.[21] Analyses in DCH were performed separately for men and women to allow for direct comparisons with the all female NHS and all male HPFS cohorts. All analyses included adjustment for age, smoking, alcohol, and BMI, and menopausal status and hormone replacement therapy among women. We tested for statistical interaction between the T111I variant and these cardiovascular risk factors by comparing −2 log likelihood ratios in models with and without their joint effects. In total, numbers with information available on plasma lipids, genotype, and covariates were: 2608 (998 cases) in DCH; 241 cases, 477 controls in NHS; 262 cases, 519 controls in HPFS.

To pool the estimates from the three study populations, we used the weighted average of regression estimates using the DerSimonian and Laird random-effects model.[26] Analyses were performed using SAS 9 (SAS Institute Inc., Cary, NC, USA) and STATA 9.1 (STATA Corp., College Station, TX, USA).

Results

Table 1 shows the baseline characteristics of cases and controls in the three study populations. The DCH participants were slightly younger, consumed more alcohol, smoked more, and had slightly higher HDL-C than the US participants.

T111I genotype was in Hardy–Weinberg equilibrium in the control study populations, except in the HPFS controls, where there was borderline evidence of disequilibrium ($P = 0.03$). Allele frequencies were very similar in the three studies. In the combined samples, the minor allele frequency among controls was 0.29.

The T111I variant was not associated with HDL-C (*Table 2*). Mean HDL-C levels differed by 0.01 to 1 mg/dL in carriers vs. non-

Table I Characteristics of cases and controls in the Diet, Cancer, and Health (DCH) study, Nurses' Health Study (NHS), and the Health Professionals Follow-up Study (HPFS)[a]

Variable	DCH				NHS		HPFS	
	Women		Men					
	Cases (n = 235)	Controls[b] (n = 763)	Cases (n = 763)	Controls[b] (n = 880)	Cases (n = 241)	Controls (n = 477)	Cases (n = 262)	Controls (n = 519)
Age (years)	60 (52; 64)	56 (51; 63)	58 (52; 64)	56 (51; 63)	62 (47; 68)	62 (47; 68)	66 (50; 78)	66 (50; 78)
BMI (kg/m^2)	26.3 (21.4; 32.8)	24.6 (21.0; 30.5)	26.9 (23.3; 32.4)	26.4 (22.6; 31.1)	24.8 (18.4; 36.2)	23.3 (18.8; 33.0)	25.7 (21.0; 31.9)	25.1 (20.8; 31.8)
Diabetes (%)	5.3	1.1	5.6	2.8	19.5	6.5	9.2	4.4
Hypercholesterolaemia[c] (%)	17.0	5.8	12.3	9.8	52.3	40.3	49.2	40.5
Hypertension (%)	43.9	17.4	26.3	16.0	56.9	28.9	42.4	30.1
Postmenopausal(%)	72.5	59	N/A	N/A	86.0	84.1	N/A	N/A
Current smoker (%)	60.6	36.4	58.6	39.0	32.4	31.7	17.6	16.0
Alcohol (g/day)	5.7 (0.7; 33)	8.5 (1.1; 34)	17 (2; 61)	20 (3; 62)	0.9 (0; 24)	1.8 (0; 28)	5.6 (0; 45)	7.0 (0; 48)
Plasma lipids (mg/dL)								
Triglycerides	181 (88; 314)	137 (94; 182)	209 (93; 361)	180 (83; 307)	135 (57; 357)	108 (42; 263)	147 (57; 472)	113 (47; 313)
Total cholesterol	256 (203; 311)	234 (188; 284)	244 (193; 297)	231 (186; 280)	235 (171; 304)	222 (168; 294)	215 (157; 277)	203 (150; 273)
LDL-C	153 (110; 208)	136 (94; 185)	154 (110; 195)	139 (101; 185)	145 (84; 204)	132 (77; 192)	136 (80; 193)	124 (78; 183)
HDL-C	62.5 (45.2; 80.4)	69.8 (50.3; 93.6)	51.7 (39.1; 66.5)	56.5 (41.8; 74.6)	49.5 (31.1; 78.9)	58.4 (36.6; 93.2)	40.8 (27.5; 62.3)	44.1 (28.2; 69.6)

[a]Medians (5th and 95th percentiles) of continuous covariates.
[b]Random sample of cohort at baseline (includes 33 that later became cases).
[c]Diagnosed with hypercholesterolaemia or reporting to use cholesterol-lowering medication.

Table 2 Estimated proportion of 1 standard deviation change in standardized residual of lipid markers (*P*-value) per copy of the T111I variant among controls from the Diet, Cancer, and Health (DCH) study, the Nurses' Health Study (NHS), and the Health Professionals Follow-up Study (HPFS)[a]

T111I	HDL-C	Total cholesterol	Triglycerides	LDL-C	ApoA1	ApoB
DCH women	0.010 (0.85)	0.047 (0.40)	0.081 (0.14)	0.013 (0.82)	0.040 (0.46)	0.031 (0.57)
DCH men	−0.001 (0.99)	0.054 (0.31)	0.029 (0.56)	0.029 (0.59)	−0.001 (0.98)	0.051 (0.33)
NHS	−0.004 (0.96)	0.038 (0.59)	0.040 (0.58)	0.040 (0.58)	−0.0764 (0.58)[b]	0.023 (0.74)
HPFS	0.029 (0.65)	−0.032 (0.65)	−0.001 (0.99)	−0.036 (0.61)	NA	−0.005 (0.84)
Pooled[c]	−0.012 (0.41)	0.034 (0.40)	0.041 (0.41)	−0.010 (0.32)	0.019 (0.61)	0.031 (0.43)

Study specific z-score = (lipid marker -mean lipid in the study)/standard deviation within study (sex-specific in the DCH).
All models adjusted for age, smoking, alcohol intake, body mass index, and postmenopausal status and use of HRT among women.
Participants in DCH were non-fasting. Majority of participants in the NHS and HPFS were fasting.
[a]β's estimated in multivariable regression models predicting the study-specific z-scores of lipids according to additive effects of the minor allele.
[b]Only available in 117 controls from the NHS study.
[c]Meta-analysis using random effects model.

carriers in the three study populations, but were not statistically different in any of the studies. Because absolute HDL-C levels differed between the studies and between men and women, we standardized the cohorts by estimating the incremental effect of one copy of the minor allele on the lipids in increments of one study-specific standard deviation and used these estimates in our pooled analysis. We also did not detect any associations for the other measures of lipids and lipoproteins (*Table 2*).

The frequency of the T111I variant allele did not differ between cases and controls in the three study populations (*Table 3*). There was no evidence that the association between T111I and CHD differed between the three study populations or between men and women. We also did not detect any significant statistical interaction between the T111I variant and age, smoking, BMI and alcohol on HDL-C, or risk of CHD (data not shown).

Discussion

The present findings consistently demonstrate that the T111I variant in the gene encoding EL is not associated with plasma lipids or lipoprotein measures. In addition, we found no consistent evidence that the T111I variant affects the risk of developing CHD during 7–10 years of follow-up.

We conducted our analyses in three independent cohorts of Caucasian men and women who were free from cardiovascular disease at baseline. Previously, the T111I variant allele has been associated with higher HDL-C among US patients with cardiovascular disease and among Japanese American and Chinese control samples.[5,10,11] In contrast, T111I was not associated with HDL-C level in a healthy Canadian population and in a small Japanese sample.[12,14] Overall, previous investigations were limited by small samples and already established cardiovascular disease among study participants. Because underlying cardiovascular disease and the treatment for this condition may affect plasma lipids, we believe it is important to investigate the association between genetic variants and plasma lipids in generally healthy population samples. Although our population cohorts were without CHD at baseline, a high proportion of especially the US participants reported a diagnosis of hypercholesterolaemia at baseline. In a sub-analysis, we did not find materially different results when we excluded these participants (data not shown).

Few studies have attempted to determine whether the T111I variant is associated with cardiovascular endpoints. Two reports in Caucasian patients reported inconsistent results. The T111I variant was not associated with past history of MI or the progression of atherosclerosis during 2.5 years of follow-up in the Lipoprotein and Coronary Atherosclerosis Study of 371 patients with existing coronary artery disease, whereas the T111I variant allele occurred less frequently among participants in the ACCESS statin trial who had a history of MI compared with those without.[9] Recently, the latter finding was supported by two small case–control studies from Japan and China.[11,14] To our knowledge, no prospective studies have evaluated the role of T111I and genetic variation in *LIPG* and risk of incident CHD in healthy Caucasian populations. It is difficult to reconcile discrepant findings when they originate from patients, healthy individuals, and populations of different ethnicities as different pathophysiological circumstances and different distributions of lifestyle characteristics may be of significance. However, by investigating the association between the T111I variant and risk of CHD in a prospective setting, we have avoided the potential survival bias that could be a problem in case-control studies including only survivors of recent cardiovascular events. In addition, with 1500 cases of CHD and a minor allele frequency of 30%, our estimated statistical power was greater than 80% for the detection of relative risk of 1.15 or greater.

Our results do not suggest that the T111I variant in the *LIPG* gene plays a role for plasma lipids and lipoproteins and CHD. We explored the association of this genetic variant with other plasma lipids and lipoproteins because inhibition of EL has been shown to raise plasma levels of not only HDL-C but also apoB-containing lipoproteins.[27] In accordance with our results, Edmondson *et al.*[24] recently found that the lipolytic activity of the T111I variant was similar to the wildtype allele using *in vitro* assays. The exploration of other variants in the EL gene in relation to lipid concentrations remains of great scientific interest. Because high EL

Table 3 Minor allele frequencies (MAF), relative risk, and 95% confidence intervals of CHD according to T111I genotype in the Diet, Cancer, and Health (DCH) study, the Nurses' Health Study (NHS), and the Health Professionals Follow-up Study (HPFS)[a]

SNP	MAF (SE)		*n* (cases/controls)			Relative risk (95% confidence interval)			
	Cases	Control	Wildtype homozygotes	Heterozygotes	Variant homozygotes	Wildtype homozygotes	Heterozygotes	Variant homozygotes	Per variant allele
T111I			TT	TI	II	TT	TI	II	Per I-allele
DCH women	0.29 (0.02)	0.28 (0.01)	116/391	102/312	17/60	1.0 (ref)	1.03 (0.72–1.46)	1.04 (0.53–2.02)	1.02 (0.77–1.35)
DCH men	0.29 (0.01)	0.29 (0.01)	393/446	304/361	66/73	1.0 (ref)	0.96 (0.76–1.20)	0.91 (0.61–1.34)	0.96 (0.81–1.13)
NHS	0.29 (0.02)	0.31 (0.01)	115/224	110/214	16/39	1.0 (ref)	1.04 (0.74–1.45)	0.86 (0.46–1.60)	0.97 (0.75–1.24)
HPFS	0.28 (0.02)	0.31 (0.02)	129/240	117/239	16/40	1.0 (ref)	0.86 (0.63–1.19)	0.73 (0.39–1.35)	0.86 (0.67–1.10)
Pooled[b]	0.29 (0.01)	0.29 (0.01)	753/1301	633/1126	115/212	1.0 (ref)	0.97 (0.83–1.12)	0.87 (0.72–1.06)	0.95 (0.85–1.06)

Thirty-three cases that occurred in the random subcohort of the DCH study were included as both cases and controls in the table.
All models adjusted for age, smoking, alcohol intake, body mass index, and postmenopausal status and use of HRT among women.
[a]Cox proportional hazard regression models in DCH. Conditional logistic regression models were run in NHS and HPFS data.
[b]Meta-analysis using random effects model.

mass has been inversely associated with HDL-C and positively associated with coronary artery calcification in 858 healthy individuals,[6] the investigation of primarily loss-of-function variation in the EL gene, lifelong HDL-C concentrations, and risk of CHD may provide key insight into this protein as a potential new drug target.

We presented the results for women and men separately, although we did not have adequate statistical power to formally test for sex interactions. However, this allows for direct comparison between the estimates for each gender in the three cohorts. We did not observe any statistically significant lifestyle modification of the association between the T111I variant and risk of CHD, but we lacked statistical power to detect such heterogeneity when risk estimates were only modest. In-depth exploration of gene-environment interactions may be of interest for future investigations in larger studies.

In conclusion, our findings suggest that the common non-synonymous T111I variant in the EL gene is not associated with plasma lipids and risk of CHD in Caucasian population-based samples.

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Conflict of interest: none declared.

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