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Lack of association between common genetic variation in endothelial lipase (*LIPG*) and the risk for CAD and DVT

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ABSTRACT

Objectives: Low levels of high-density lipoprotein cholesterol (HDL-C) are a risk factor for coronary artery disease (CAD) and possibly for deep venous thrombosis (DVT). Endothelial lipase is involved in HDL-C metabolism. Common variants in the endothelial lipase gene (*LIPG*) have been reported to be associated with HDL-C levels and atherothrombosis, but these findings were not consistent. We determined whether five tagging single nucleotide polymorphisms (SNP) in *LIPG* were associated with lipid parameters, the risk of CAD and the risk of DVT. *Methods*: We used the prospective case–control study nested in the EPIC-Norfolk cohort (1138 CAD cases, 2237 matched controls) for the initial association study and, subsequently, the ACT study (185 patients with documented DVT, 586 patients in which DVT was ruled out) to replicate our findings regarding DVT risk. *Results*: In EPIC-Norfolk, we found that the minor allele of one SNP, rs2000813 (p.T111I), was associated with moderately higher HDL-C and apolipoprotein A-I levels, higher HDL particle number and larger HDL size. No variants were associated with CAD risk, but three variants were associated with DVT risk (odds ratios 0.60 [95%CI 0.43–0.84], 2.04 [95%CI 1.40–2.98] and 1.67 [95%CI 1.18–2.38] per minor allele for rs2000813, rs6507931 and rs2097055 respectively, $p < 0.005$ for each). However, the association between *LIPG* SNPs and DVT risk could not be replicated in the ACT study. *Conclusions*: Our data support a modest association between the *LIPG* rs2000813 variant and parameters of HDL metabolism, but no association between common genetic variants in *LIPG* and CAD or DVT risk.

1. Introduction

Family studies underline that 40–60% of the variation in high-density lipoprotein cholesterol (HDL-C) levels is explained by genetic factors [1] and many candidate genes involved in human HDL metabolism have been identified [2]. Endothelial lipase constitutes such a candidate and is a member of the lipase family. These lipolytic enzymes are involved in lipid absorption, transport, and metabolism. Endothelial lipase is synthesized in endothelial cells and possesses phospholipase activity preferentially directed at HDL phospholipids [3]. Overexpression of endothelial lipase reduces HDL-C, whereas deficiency of endothelial lipase leads to elevation of HDL-C levels in mice [3–5].

Genetic association studies have yielded conflicting results with respect to associations between common genetic variants in *LIPG* and HDL-C levels [5–13]. However, in several genome-wide association studies single nucleotide polymorphisms (SNPs) near *LIPG* were identified as being associated with HDL-C [14–19]. Furthermore, rare loss-of-function variants in *LIPG* were recently shown to be a cause of elevated HDL-C in humans [20].

Plasma HDL-C levels correlate inversely with the risk of coronary artery disease (CAD) [21]. In humans, high levels of endothelial lipase are significantly associated with features of the metabolic syndrome and with coronary artery calcification [22]. The data regarding associations between *LIPG* gene variants and CAD risk are conflicting, however. The minor allele of a common variant resulting in a Thr to Ile substitution at codon 111 (rs2000813), was found to occur less frequently in patients who had a history of myocar-

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dial infarction compared to those without [8]. Similar findings were reported from two small case–control studies [10,11]. In line, the same SNP has been reported to be associated with atherothrombotic cerebral infarction in Japanese women [23]. However, in a recent large investigation in three independent prospective cohorts by Jensen et al., no association between this SNP and the risk of CAD was found [13].

Plasma HDL-C levels are also suggested to be associated with venous thromboembolism [24–26]. *In vitro* evidence suggests that HDL may protect from thrombosis by scavenging procoagulant anionic phospholipids [27]. Through its HDL-directed phospholipase activity, endothelial lipase may therefore be hypothesized to modulate this process. However, the association between genetic variation in *LIPG* and the occurrence of deep venous thrombosis (DVT) has, to our knowledge, never been specifically addressed.

Since the potential interrelation between endothelial lipase, high-density lipoprotein metabolism and (athero)thrombosis is currently unclear, we aimed to evaluate whether common genetic variants in *LIPG* associate with plasma lipid parameters, CAD or DVT risk.

2. Methods

Studies were approved by Institutional Review Boards and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.1. Data sources

The EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk cohort consists of a prospective population of 25,663 men and women between ages 45 and 79. EPIC-Norfolk is part of the 10-country collaborative EPIC study designed to investigate determinants of cancer. From the outset, additional data were obtained in EPIC-Norfolk to enable the assessment of determinants of other diseases, including self-reported history of DVT at baseline and prospective data on the occurrence of CAD. We performed analyses in a subset of this cohort, a case–control cohort consisting of men and women who developed fatal or nonfatal CAD during 7 years of follow-up ($n = 1138$), and controls, matched for age, sex, and enrollment time ($n = 2237$). CAD was defined as codes 410–414 according to the International Classification of Diseases, Ninth Revision. Participants were identified as having CAD during follow-up if they had had a hospital admission or had died with CAD as the underlying cause. Previous validation studies in our cohort indicate high specificity of such case ascertainment [28]. To replicate the findings from this study, we genotyped all tagSNPs in the Amsterdam Case–control Thrombophilia (ACT) study, consisting of 771 outpatients who were referred to our hospital for evaluation of a suspected deep venous thrombosis (DVT) [29]. In this study, 185 patients with objectively confirmed first DVT were compared to 586 patients in whom this diagnosis was ruled out.

2.2. Laboratory analyses

2.2.1. Genotyping

We used the HAPMAP database and the TAGGER algorithm to capture most of the common variation in the *LIPG* locus (NM_006033). For an in-depth discussion of this method, we refer to reference [30]. Briefly, we selected from HAPMAP all common genetic variants (minor allele frequency > 0.1) in the *LIPG* locus in a population of European ancestry. However, because of high correlations (high r^2) among some pairs of SNPs, genotyping both SNPs would offer only limited extra information. A tagging SNP approach uses the knowledge of associations between genetic variants (linkage disequilibrium [LD] structure) to limit the number of SNPs that needs to be genotyped. TagSNPs are those SNPs which most effectively represent (or 'tag') all the SNPs in a particular locus. We selected tagSNPs using an r^2 cutoff level > 0.8. For the EPIC-Norfolk study genotyping was conducted by KBioscience (http://www.kbioscience.co.uk) using KASPar technology. Genotyping of SNPs in the other cohort was carried out on a Roche Lightcycler 384 wells system, using custom designed primers from Applied Biosystems (Foster City, CA, USA).

2.2.2. Biochemical analyses

Total cholesterol, HDL cholesterol and triglycerides were determined using standard laboratory procedures within 1 h after (non-fasting) blood sampling. Low-density lipoprotein LDL cholesterol levels were calculated with the Friedewald formula. Serum levels of apolipoprotein A-I (apoA-I) and B (apoB) were measured by rate immunonephelometry (Behring Nephelometer BNII, Marburg, Germany) with calibration traceable to the International Federation of Clinical Chemistry primary standards. The interassay coefficient of variation (CV) of the apoA-I and apoB measurements was 5% and 3%, respectively. Serum concentrations of apolipoprotein A-II (apoA-II) were measured with a commercially available immunoturbidimetric assay (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) on a Cobas-Mira autoanalyzer (Roche, Basel, Switzerland). The intra-assay and interassay CVs for this assay were 2.5% and 3.1%, respectively. HDL particle number and HDL size were measured with an automated nuclear magnetic resonance (NMR) spectroscopic assay as described previously [31]. In brief, particle concentrations of lipoprotein subclasses of different sizes were obtained directly from the measured amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Summation of the HDL subclass levels provides total HDL particle concentration. For the present study, we grouped HDL subclasses as follows: small HDL (7.3–8.2 nm), medium HDL (8.2–8.8 nm), and large HDL (8.8–13 nm). NMR spectroscopy-measured HDL size was calculated as the mass-weighted average diameter of the HDL particles in a particular plasma sample. Plasma concentrations of C-reactive protein (CRP) were measured with a sandwich-type enzyme-linked immunosorbent assay as previously described [32]. Samples were analyzed in random order to avoid systematic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

2.3. Statistical analyses

All statistical analyses were performed in cases with complete data, using SPSS version 16.0.2. Two-sided probability values of less than 0.05 were considered statistically significant. Effects of SNPs on continuous variables were examined by ANOVA. To estimate the relative risk of CAD in EPIC-Norfolk, conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals. Conditional logistic regression took into account the matching for sex, age and enrollment time, and was adjusted for Framingham risk score. We furthermore performed a meta-analysis on the relationship between rs2000813 and cardiovascular risk using our own data as well as the published data by Jensen et al., using logistic regression accounting for study [13]. To estimate the relative risk of DVT in EPIC-Norfolk and in the ACT study, differences between cases and controls were analyzed by standard contingency table analysis using two-tailed chi-square test probabilities; linearity of this relationship was assessed using logistic regression analysis. LD plots were created with Haploview, version 4.1 [33].

3. Results

Using HAPMAP, we selected five *LIPG* tagSNPs. These tagSNPs were rs2097055 (c.460-320T > CT > C), rs8093249