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Associations of circulating protein levels with lipid fractions in the general population

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Abstract

Objective: Revealing patterns of associations between circulating protein and lipid levels could improve biological understanding of cardiovascular disease. In this study, we investigated associations between proteins related to cardiovascular disease, and triglyceride (TG), total cholesterol (TC), LDL and HDL cholesterol (LDL-C and HDL-C) levels in individuals from the general population.

Approach and Results: We measured plasma protein levels using the Olink ProSeek CVD I or II+III arrays and analyzed 57 proteins available in three population-based cohorts: EpiHealth (n=2,029 52% women, median age 61 years), PIVUS (n=790 51% women, all aged 70 years) and

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DISCLOSURES

E. Ingelsson has received consulting fees from Olink Proteomics for work unrelated to the present project. The company had no influence over design, analysis or interpretation of data in the present study, and did not provide any funding for the study.

ULSAM (n=551, all men aged 77 years). A discovery analysis was performed in EpiHealth in a regression framework (adjusted for sex, age, BMI, smoking, glucose levels, systolic blood pressure, blood pressure medication, diabetes medication and CVD history) and associations with $FDR < 0.05$ were further tested in PIVUS and ULSAM, where p-value of 0.05 was considered a successful replication (validation FDR of 0.1%). We used summary statistics from a genome-wide association study (GWAS) on each protein biomarker (meta-analysis of EpiHealth, PIVUS, ULSAM and IMPROVE) and publicly available data from Global Lipids Genetics Consortium (GLGC) to perform Mendelian randomization (MR) analyses to address possible causality of protein levels. Out of 57 tested proteins, 42 demonstrated an association with at least one lipid fraction; 35 were associated with TG, 15 with TC, 9 with LDL-C, and 24 with HDL-C. Among these associations, we found kidney injury molecule (KIM-1), tumor necrosis factor receptor 1 and 2 (TNF-R1 and TNF-R2), TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) and resistin (RETN) to be associated with all four lipid fractions. Further, 15 proteins were related to both TG and HDL-C in a consistent and biologically expected manner, i.e. higher TG and lower HDL-C or vice versa. Another common pattern of associations was concomitantly higher TG, TC and LDL-C, which is associated with higher CVD risk. We did not find evidence of causal links for protein levels.

Conclusions: Our comprehensive analysis of plasma proteins and lipid fractions of 3,370 individuals from the general population provides new information about lipid metabolism.

Keywords

Total cholesterol; HDL; LDL; triglycerides; protein biomarker; general population; mendelian randomization; Biomarkers; Lipids and Cholesterol; Proteomics; Epidemiology

INTRODUCTION

Triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), important lipid fractions, attract clinical attention when present in abnormal concentrations. This is not only due to their strong association with coronary heart disease, the leading cause of death worldwide, but increased TG and decreased HDL-C are also key metabolic abnormalities in patients with obesity, insulin resistance (IR) and type 2 diabetes (T2D),¹ increasing public health burdens.

Central roles of lipids in physiology and disease, and their metabolic and signaling functions often arise from interactions with proteins.² For instance, fatty acid-binding protein 4 (FABP4) acts as a cytoplasmic lipid chaperone, suppressing adipose tissue lipogenesis and promoting lipolysis, with direct effects on the composition of the local and circulating free fatty acid pool.^{3, 4} Similarly growth hormone (GH), in addition to its primary function to control and promote skeletal growth, also stimulates lipolysis in adipose tissue, resulting in increased fatty free acids levels in the blood.⁵ Kidney injury molecule (KIM-1), is a phosphatidylserine receptor that recognizes apoptotic cells and directs them to lysosomes, but also serves as a receptor for oxidized lipoproteins.⁶ Recent advances in highly multiplexed immunoassays allow measuring simultaneously a wide range of plasma proteins selected for their role in cardiovascular disease; thus facilitating studies of the broad picture

of lipid-protein associations in the circulatory system, which may provide valid nodes for drug action to treat human diseases.

Observational epidemiological studies provide a wealth of information on associations between disease exposures and outcomes, but these relations should not be interpreted as causal, owing to limitations introduced by confounding and reverse causality.⁷ Mendelian randomization (MR) is a method using genetic variants as instrumental variables to assess causal relationships from observational data.⁸ Thus, this design excludes possibility of reverse causation and under certain assumptions, MR methods also avoid confounding.⁸

The primary aim of this study was to evaluate associations of circulating proteins with lipid fractions in several population-based cohorts. In addition, we investigated causal effects of protein levels on lipid levels using MR methods, as well as used different orthogonal data sources to increase biological understanding of associations between circulating plasma proteins and lipids.

METHODS

Data are made available to researchers who meet the criteria for confidential data access as stipulated by participant informed consent and institutional review board. Data access in ULSAM is granted through the Interdisciplinary Collaboration Team on Uppsala Longitudinal Studies (ICTUS; <http://www.pubcare.uu.se/ulsam/Research/Proposals>). Data from the PIVUS study are available after application to the PIVUS steering committee (<http://www.medsci.uu.se/pvus/>). Data from the EpiHealth study are granted after application to the EpiHealth steering committee (<https://www.epihealth.se/For-scientists/Research-Application-/>).

Study samples

EpiHealth—The EpiHealth study was initiated in 2011 with the goal of recruiting 300,000 men and women aged 45–75 years in the Swedish towns of Uppsala and Malmö.⁹ The study consists of three parts: a collection of self-reported data on lifestyle by internet-based questionnaires; a clinical visit where blood samples are collected and physiological parameters recorded; and follow-up for occurrence of outcomes using nationwide medical registers. The present study is based on a random sample of 2,467 individuals with measured protein profile. Of this sample, seven individuals were excluded due to unsuccessful protein profiling (not passing the quality control), 141 due to fasting time < six hrs, 240 due to being on lipid-lowering medication, and 50 due to missing information on covariates used in the main analysis; thus, leaving 2,029 individuals for the main analysis (Table 1).

Prospective Study of the Vasculature in Uppsala Seniors (PIVUS)—The PIVUS study has been described previously¹⁰ and on the Internet (www.medsci.uu.se/pivus/pivus.htm). All 70-year old individuals residing in Uppsala, Sweden, in 2001–2005 were eligible for the study, and 2,025 individuals were invited in a randomized order within two months of their 70th birthday. Of these, 1,016 (50.2%) participated and 510 (50.2%) of them were women. We excluded 25 individuals due to unsuccessful protein profiling, 21 individuals due to C-reactive protein (CRP) levels above 20 mg/L (to exclude individuals

with possible ongoing infection and other inflammatory processes), 154 due to being on lipid-lowering medication and 26 individuals due to missing information on covariates in the main analysis. Hence, the eligible sample size for the present study was 790 individuals (Table 1).

Uppsala Longitudinal Study of Adult Men (ULSAM)—The ULSAM study has been described previously¹¹ and on the Internet (<http://www.pubcare.uu.se/ulsam>). The study was initiated in the period 1970–1973 by inviting all men living in Uppsala, Sweden, born between 1920 and 1924. The invitation letter was sent to 2,841 men and 2,322 (81.7%) of them participated in the initial examination. The participants have been re-examined at multiple time points. The present study was based on the re-examination at age 77, when 748 of the original participants had died, and another 176 men were not eligible for other reasons. From 1997 to 2001, the remaining 1,398 individuals were re-invited, and 839 (60.0%) of them participated. We excluded 77 individuals due to unsuccessful protein profiling, 21 individuals due to CRP levels above 20 mg/L, 139 due to lipid lowering medication and 51 individuals due to missing information on covariates in the main analysis. Hence, the eligible sample size for the present study was 551 men.

The EpiHealth, PIVUS and ULSAM studies were approved by the Ethics Committee of Uppsala University in agreement with the Helsinki Declaration, and all subjects gave their informed written consent.

Lipids measurement—In EpiHealth, at the test center, 10ml of the blood sample were used for determinations of fasting glucose, LDL-C and HDL-C, and TG at the hospital laboratory using an Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL, USA)⁹. In PIVUS, lipid variables were measured by standard laboratory techniques at the Uppsala University hospital¹⁰. In ULSAM, cholesterol and triglyceride concentrations were analyzed in serum and isolated lipoprotein fractions by enzymatic techniques using IL Test Cholesterol Trinder's Method 181618–10 and IL Test Triglyceride Enzymatic-colorimetric Method 181610–60 for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA). HDL particles were separated by precipitation with magnesium chloride and phosphotungstate. LDL cholesterol was calculated using Friedewald's formula.¹²

Other covariates—Anthropometric measures (height and weight) of PIVUS, ULSAM and EpiHealth participants were recorded at the time of blood draw used for proteomic analysis and were measured by trained staff. For PIVUS and ULSAM, medical history, smoking habits, and regular medication were collected at time of blood draw by self-administered questionnaires or interviews by a study nurse and national registries. The same data for EpiHealth were collected prior to the clinical visit by Internet-based questionnaires or by national registries.

Proteomic analysis—Proteomic profiling in EpiHealth was performed using the Olink Proseek® Multiplex cardiovascular disease (CVD) II and III^{96×96} kits (<http://www.olink.com/products/cvd-ii-panel/> and <http://www.olink.com/products/cvd-iii-panel/>) measuring 92 selected cardiovascular disease-related proteins simultaneously. The kits are based on the proximity extension assay (PEA) technology, where 92 oligonucleotide-

labelled antibody probe pairs are allowed to bind to their respective target present in the sample. The PEA technique is exceptionally high specific and very sensitive^{13, 14}. The platform provides normalized protein expression (NPX) data where a high protein value corresponds to a high protein concentration, but not an absolute quantification. Samples that (i) failed for technical reasons on both chips; or (ii) a sample call rate <0.95 on either chip were excluded. Proteins with 10% missingness were also excluded. Each protein was normalized by plate (by setting the mean = 0 and standard deviation = 1 within each plate).

Details on PIVUS and ULSAM blood samples have been described previously.^{15, 16} Analyses were performed using the Olink Proseek® Multiplex CVD I^{96×96} kit (<http://www.olink.com/products/document-download-center/>). All samples and runs for PIVUS and ULSAM passed technical quality control. Twelve proteins with a call rate <85% (i.e. <85% of the individuals had a valid measurement of that specific protein) in PIVUS and/or ULSAM were removed from further analysis, including IL-4, melusin, BNP, Beta-NGF, SIRT2, NEMO, mAmP, PTX3, NT-pro-BNP, MMP-7, cystatin-B and heat shock 27 kDa protein. Individuals with excess missingness based on visual inspection of a histogram were excluded (>5% and >3% in PIVUS and ULSAM, respectively). Each protein was normalized by plate and further by storage time (correction based on the observed values and predicted values from a spline model). We analyzed 57 proteins that overlapped between the CVD I, II and III arrays and that passed quality control in all three cohorts. The full lists of analyzed proteins are presented in Supplementary Table I.

Statistical Analysis

Primary Analysis—We evaluated the distribution of protein values and clinical covariates using the Shapiro-Wilk normality test and qq-plots. All protein and lipid variables were normalized by rank transformation. The EpiHealth study was *a priori* designated as the discovery cohort as it was largest, and had equal proportions of males and females. Linear regression models were used for all analyses. Lipids levels were used as the independent variables and the 57 proteins were used as dependent variables in separate models (one per lipid fraction and protein marker). The analysis of the protein profiling data was performed in an age- and sex-adjusted model (only age in ULSAM as all participants were males), and a multivariable-adjusted model. We considered the multivariable-adjusted models as our main models, both when deciding which protein markers to pursue for replication, and which to annotate as significantly associated. The multivariable-adjusted model included age, sex, body mass index (BMI), smoking (a factor variable with three levels: never, former and current smoker), glucose levels, systolic blood pressure, antihypertensive medication, diabetes medication and prior myocardial infarction, angina, stroke, heart failure and atrial fibrillation. Prior cardiovascular diseases were defined by self-report (EpiHealth and ULSAM) or the International Classification of Diseases (ICD) codes (ULSAM). Protein markers with a false discovery rate (FDR; estimated using the Benjamini & Hochberg method)¹⁷ < 5% in EpiHealth, were selected for further replication in PIVUS and ULSAM. Study-specific estimates from PIVUS and ULSAM were combined using an inverse variance-weighted mixed-effects meta-analysis, and associations with the same direction as in EpiHealth at p-value < 0.05 were considered successful replication. We have previously shown that this replication strategy is conservative and indeed, the risk of false positive

findings in the replication stage (vFDR) was calculated to 0.1% for each lipid fraction separately (<http://fafner.meb.ki.se/personal/yudpaw/rdr/18>). The study design is depicted in Figure 1.

Orthogonal data sources

We queried Search Tool for the Retrieval of Interacting Genes/Proteins (STRING)¹⁹ for protein-protein interaction networks, filtering on an interaction score of strong evidence (0.7). Then, we used data from the Genotype-Tissue Expression (GTEx) project²⁰ to depict expression patterns in physiologically relevant tissues, specifically whole blood (as the protein biomarkers in our study were measured in blood samples); coronary artery, aorta, tibial artery, heart left ventricle and heart atrial appendage (representing cardiovascular system since the protein biomarkers were selected based on their relation to cardiovascular disease; and subcutaneous adipose tissue, visceral adipose tissue, liver and skeletal muscle (tissues involved in lipid metabolism and insulin resistance). Finally, we searched Catalog of published genome-wide association studies (GWAS Catalog)²¹ inquiring gene names corresponding to protein biomarkers. This allowed us to find associations in GWAS ($P < 5 \times 10^{-8}$) if variants were mapped to the inquired genes or the inquired genes were in the neighborhood of associated loci.

Mendelian Randomization

In MR analyses, an association between a genetic instrument and the outcome is indicative of a causal effect of the risk factor on the outcome.²² In this study, we performed two-sample MR analyses using genetic summary statistics where we tested the hypothesis that different protein levels affect lipid fractions. The analyses were performed for all lipid-protein associations that were replicated in the observational analyses.

Summarized data used in MR—Genome-wide association studies (GWAS), which typically report regression coefficients summarizing the associations of many genetic variants with various traits, are potentially a powerful source of data for MR investigations.²³ We used summary statistics from GWAS performed on each protein biomarker, as described in Folkersen et al.,²⁴ but in the present study, we obtained results from a meta-analysis of EpiHealth, PIVUS, ULSAM and IMPROVE (total number of subjects $n=7,390$). The IMPROVE study is a multicentre, observational study, which recruited 3,711 men and women aged between 55 to 79 years with at least three cardiovascular risk factors but without symptoms of CVD.²⁵ The study was conducted in accordance with the declaration of Helsinki and all participants gave written informed consent. The ethics and sampling of this cohort have been further documented in prior publications, e.g.²⁶

For lipid fractions we used summary statistics data from Global Lipids Genetics Consortium (GLGC), the largest ($n=188,577$), publicly available GWAS of lipid traits.²⁷

As instrumental variables, we selected all independent variants ($r^2 < 0.01$) associated with protein levels at genome-wide significant level ($P < 5 \times 10^{-8}$) and having *cis* effects on protein-coding gene expression, i.e. variants in the same locus as the protein coding gene (Supplementary Table III). Out of the 42 proteins associated with at least one lipid fraction,

we were able to create genetic instruments for 30 protein biomarkers. There were no genome-wide significant variants associated with GH and LEP; no cis single nucleotide polymorphisms (SNPs) associated with levels of FGF23, IL-27, IL-6, MCP-1, PDGF subunit B, SCF, tPA, VEGF-D; and no proxies in the GWAS data on lipids in GLGC for variants associated with CD40L and FABP4 (presumably due to low minor allele frequency, 1.2% for the single variants associated with these proteins). For outcomes, we used data from GLGC.²⁷ We used the Wald method when only one SNP was used as an instrument, while the inverse-variance weighted method was used for combinations of more than one variant.²⁸

All MR analyses were performed with the TwoSampleMR package in R, which allows evaluating the causal effect of an exposure on an outcome using only summary statistics from GWAS.²⁹ To minimize risk of false positive findings due to high number of tests, we applied Bonferroni correction of the nominal alpha threshold of 0.05. Using an online tool (<https://sb452.shinyapps.io/power/>), we estimated statistical power for the different MR analyses using the outcome sample sizes, effect sizes observed using traditional regression, variance explained for the instrumental variable and the Bonferroni-corrected P-value, all specific for each analysis.

RESULTS

The clinical characteristics of the study samples are presented in Table 1. Compared to the EpiHealth (discovery cohort), the PIVUS and ULSAM populations were older, and more likely to be taking antihypertensive and diabetes medication and have prior CVD. Also, importantly, ULSAM consisted exclusively of males.

Observational analyses

Out of 57 investigated protein biomarkers, 42 demonstrated a replicated association with at least one of the lipid fractions; 35 were associated with TG, 15 with TC, 10 with LDL-C, and 22 with HDL-C (Figure 2 and 3; Table 2). Among these associations, we found kidney injury molecule (KIM-1), tumor necrosis factor receptor 1 and 2 (TNF-R1 and TNF-R2), TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) and resistin (RETN) to be associated with all four lipid fractions. Further, 15 proteins were related to both TG and HDL-C in a consistent and biologically expected manner, i.e. higher TG and lower HDL-C or vice versa. Out of these, stem cell factor (SCF) and GH represent a pattern generally associated with lower insulin resistance and CVD risk, as they were associated with lower TG and higher HDL-C. FABP4, tissue-type plasminogen activator (t-PA), interleukin-1 receptor antagonist protein (IL-1RA), C-C motif chemokine 3 (CCL3), fibroblast growth factor 23 (FGF-23), interleukin 16 (IL-16), interleukin 18 (IL-18), matrix metalloproteinase-12 (MMP-12), placenta growth factor (PIGF), RETN, TNF-R1, TNF-R2 and TRAIL-R2 were associated with higher TG and lower HDL-C, thus a pattern consistent with insulin resistance. Another common pattern of associations was concomitantly higher TG, TC and LDL-C, which often is associated with higher CVD risk. This was observed for higher C-X-C motif chemokine 16 (CXCL16), Gal3 (Galectin-3) and monocyte chemoattractant protein 1 (MCP-1). Moreover, there was a set of proteins that were associated only with TG, all of them in a positive fashion (Figure 2). Results from the age- and sex-adjusted analyses

are presented in Supplementary Table I. Overall, some associations from age- and sex-adjusted model were no longer significant in the fully adjusted analyses (Supplementary Table II).

We performed additional bioinformatics analyses of the five proteins associated with all lipid fractions. Four of them, TNF-R1, TNF-R2, TRAIL-R2 and RETN, displayed protein-protein interactions with high confidence (probability of 0.7) according to the STRING database. These interactions included interactions from curated databases of biological pathway knowledge such as Kyoto Encyclopedia of Genes and Genomes (KEGG), interactions determined in experiments, co-expression and protein homology (Figure 4). TNF-R1, TNF-R2 and TRAIL-R2 share some of the functional partners including TRADD (TNFRSF1A Associated Via Death Domain) which is involved in apoptosis modulation and signaling; RIPK-1 (Receptor Interacting Serine/Threonine Kinase 1) that plays a role in inflammation and cell death in response to tissue damage; and TRAF2 (TNF Receptor Associated Factor 2) which plays a central role in the regulation of cell survival and apoptosis. The only partner for RETN identified by STRING was NR3C1, a glucocorticoid receptor; whereas there were no functional partners identified for KIM-1. *TNFRSF1A*, *TNFRSF1B* and *TNFRSF10B*, encoding for TNF-R1, TNF-R2 and TRAIL-R2 respectively, are expressed in whole blood, coronary artery, aorta, tibial artery, heart left ventricle, heart atrial appendage, subcutaneous and visceral adipose tissue, liver and skeletal muscle; whereas *RETN* is detected mostly in the whole blood (Figure 5). There is no expression of *HAVCR1* encoding for KIM-1 in the examined tissues. GWAS results from previous studies show that intronic variant rs1553318 in *HAVCR1*, as well intergenic SNPs in the *HAVCR1* locus (rs6882076 and rs1501908) have been associated with TG, TC and LDL-C levels, which is consistent with our findings from observational analyses. Intronic variants in *TNFRSF1A* (rs1800693, rs4149576, rs2284344, rs4149577, rs1860545) have been associated with autoimmune and inflammatory diseases, such as multiple sclerosis, primary biliary cirrhosis or ankylosing spondylitis. A variant mapped to the chromosome region of *TNFRSF1B* has been associated with tuberculosis, and a variant mapped to the *TNFRSF10B* locus (encoding for TRAIL-R2) has been associated with renal cell carcinoma. A variant rs1423096 in 3'-region of *RETN* which is also in high linkage disequilibrium with variation in the promoter of *RETN*, as well as variant rs3219175 in 5' near *RETN* have been associated with RETN levels (Supplementary Table IV).

Mendelian Randomization

The number of SNPs included in each instrument is indicated in Table 3. In total, we performed 59 tests in these MR analyses (many of the 30 proteins were associated with several lipid fractions) resulting in an alpha threshold of 0.0008 (after Bonferroni correction). We had at least 80% statistical power to detect an effect size of 0.07 standard deviation (SD) change in protein level per one SD change in lipid level with variance explained for the instrumental variable of 1.5%, or 100% statistical power for an effect size of 0.06 standard deviation (SD) change in protein level per one SD change in lipid level with variance explained for the instrumental variable of 15%. For 45 of the 59 analyses, we had >80% power to detect the effect size from the observational analyses (Table 3).

DISCUSSION

The aim of this study was to investigate the associations between circulating cardiovascular-related protein biomarkers and triglycerides, total cholesterol, LDL and HDL cholesterol. The data obtained from three Swedish populations provide a broad picture of lipid-protein associations in the circulatory system, showing amongst other things that kidney injury molecule (KIM-1), tumor necrosis factor receptor 1 and 2 (TNF-R1 and TNF-R2), TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) and resistin (RETN) were associated with all four lipid fractions. To the best of our knowledge, this study represents the most comprehensive analysis of the circulating lipid fractions and protein biomarkers. We studied a broad set of different proteins such as cytokines, enzymes, growth factors, hormones and receptors in healthy, general population. This is of great importance, since it could provide ideas for possible strategies of intervention before cardiovascular disease develop. Moreover, it is well-established that alterations in glucose metabolism are strongly associated with cardiovascular disease.³⁰ In the current study, we found 15 of the cardiovascular-related protein biomarkers to be associated with higher TG and lower HDL-C (or vice versa), a phenotype reflecting insulin resistance.

Potential biological explanations to our observations

TNF- α , a proinflammatory cytokine produced mainly by macrophages, has the capacity to induce dyslipoproteinemia.^{31–33} TNF- α , exerts its action through two receptors, soluble TNF receptor 1 (sTNFR1) and sTNFR2, both of which are expressed in membrane-bound form on the surface of most cells.³⁴ From the cell surface, both receptors are shed into the circulation, partly as a response to TNF. In inflammatory states, the levels of soluble TNFRs usually parallel the circulating levels of TNF.^{34, 35} It has been shown that TNF- α decreases HDL cholesterol levels,³⁶ which is in line with our results showing negative associations between HDL and TNFR1 and TNFR2. TG levels were associated with the activities of TNF- α , sTNFR1 and sTNFR2 in patients with systemic lupus erythematosus (SLE).³⁷ Thus, enhanced activity in the TNF α /TNFR system may be one underlying factor contributing to both dyslipoproteinemia and promotion of chronic inflammation not only in SLE patients,³⁷ but also in healthy individuals.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a death ligand, a cytokine that activates apoptosis through cell surface death receptors. The levels of circulating TRAIL correlate with total body fat and serum lipid levels.³⁸ Its receptor, TRAIL-R2 is expressed on the cellular surface of human preadipocytes and is still present in significant amounts on lipid-laden adipocytes.³⁹ In the previous study, activation of TRAIL-R2 with recombinant human TRAIL resulted in a robust inhibition of insulin-stimulated glucose uptake and insulin-stimulated *de novo* lipogenesis.³⁹ By binding to TRAIL-R2, TRAIL activates the cleavage of caspase-8 and caspase-3, which in turn cleaves and inactivates peroxisome proliferator activated receptor gamma (PPAR γ). This causes changes in gene expression of lipogenic genes and finally leads to the inhibition of insulin-stimulated glucose uptake and lipogenesis. In the current study TRAIL-R2 levels were associated with higher TG levels, but lower TC, LDL-C and HDL-C levels.

KIM-1 is a phosphatidylserine receptor that recognizes apoptotic cells directing them to lysosomes. It also serves as a receptor for oxidized lipoproteins and hence is adept at recognizing apoptotic cell 'eat me' signals.⁴⁰ In previous studies, higher urinary KIM-1 levels have been associated with increased risk for heart failure, lower insulin sensitivity and higher risk of cardiovascular mortality.^{41–43} In our study, circulating KIM-1 was associated with high levels of all four lipid fractions.

Resistin (RETN), named after its insulin resistance effect observed in mice after resistin injection, is produced and released from adipose tissue to serve endocrine functions.⁴⁴ In our study it was associated with IR phenotype, i.e. high TG and low HDL. RETN increases the production of LDL-C and also degrades LDL receptors in the liver, thus decreases liver's ability to clear LDL cholesterol from circulation.⁴⁵ Moreover, resistin accelerates the accumulation of LDL in arteries, increasing the risk of heart disease. Interestingly in our study, RETN was associated with lower LDL-C and this needs more attention in future studies to evaluate the effect of RETN on circulating lipids in healthy individuals, as most of the previous studies was performed in obese or diabetics subjects.

TNF-R1, TNF-R2 and TRAIL-2 represent members of the tumor necrosis factor (TNF) family of cytokines that induce apoptosis in a wide variety of cells, and according to STRING their share some functional partners. For instance, TRADD (TNFRSF1A Associated Via Death Domain), RIPK-1 (Receptor Interacting Serine/Threonine Kinase 1) and TRAF2 (TNF Receptor Associated Factor 2) are all involved in response to tissue damage, regulation of cell survival and apoptosis. Further, a predicted partner for RETN is glucocorticoid receptor (NR3C1) and glucocorticoids are potent inducers of apoptosis in many cell types and tissues. There were no functional partners identified by STRING for KIM-1, however KIM-1 is a phosphatidylserine receptor that recognizes apoptotic cells and directs them to lysosomes. Taken together, it seems like proteins associated with all four lipid fractions in our study are involved in apoptosis. Whereas apoptosis is a normal mechanism for cellular turnover, it may be accelerated by cellular stresses caused by lipid accumulation. Lipotoxicity is an increased accumulation of lipid intermediates into non-adipose tissue, notably, skeletal muscle, liver, and heart, leading to cellular dysfunction and cell death, i.e. lipoapoptosis.⁴⁶ Using orthogonal data, we indeed showed that genes encoding for TNF-R1, TNF-R2 and TRAIL-2 are expressed in heart, skeletal muscle and liver.

Among proteins associated with higher TG and lower HDL-C; FABP4, t-PA and IL-1RA have been previously linked to insulin resistance measured by homeostasis model assessment (HOMA) in two community-based cohorts.⁴⁷ For the remaining 12 proteins showing this pattern, less is known about their relation to insulin resistance and dyslipidemia. That said, several proteins have known biology that could imply a role in insulin resistance. For example, IL-16 plays a role in initiating and/or sustaining an inflammatory response via stimulation of IL-1B and IL-6 expression in human monocytes.⁴⁸ Neutralization of IL-16 protects non-obese diabetic mice from autoimmune type 1 diabetes,⁴⁹ but more studies are needed to explain the association between TG, HDL-C and IL-6 in general human population.

In MR analyses, we used genetic variants to explore possible causal effects of protein levels on lipid levels and we had at least 80% power to detect most of the possible effects. None of MR analyses revealed causal relationships, but there may still exist smaller causal effects than what we had no power to test for. We cannot rule out the possibility that lipid levels might be causal for protein levels, as we were unable to test it due to lack of statistical power.

Strengths & Limitations

A major strength of the study is the design in which findings from the discovery cohort were confirmed in other general population-based cohorts. Moreover, with strict multiple testing correction the risk of false positive findings in the replication stage was calculated to 0.1%. Another strength is the large number of available protein biomarkers in all three cohorts (57 proteins) which allowed us for a comprehensive analysis. Moreover, in this study we present not only an observational analysis, but we also attempted to investigate causality between the observed associations. However, as a limitation of the MR analyses, there is a possibility that a non-synonymous variant may affect binding of the protein-detecting antibodies, thus affecting the quantification. This could result in an invalid instrument since the instrument will not really reflect the protein levels, but occurs as synthetic association due to a technical artefact. Although this risk is probably very small, it has been reported for a genetic association study of adiponectin analyzed with enzyme-linked immunosorbent assay (ELISA).⁵⁰ We also want to acknowledge that in contrast to mass spectrometry methods (MS), the Olink protein immunoassay does not allow profiling of different protein isoforms or posttranslational modifications. However, MS methods have the limitation of lower throughput making it difficult to analyze samples from 3,370 individuals. Finally, our study samples were middle-aged to elderly and of Northern European descent, so the generalizability to other ethnicities and younger individuals is unknown.

CONCLUSION

In summary, we report a comprehensive lipid-protein association map, rich in cytokines, enzymes, receptors and growth factors. Some of these proteins, such as KIM-1, RETN, TNFR1, TNFR2 and TRAIL-2, could be good candidates to follow up in longitudinal studies to determine their ability to predict changes to lipids levels. Further, future studies should address lipoapoptotic properties of these proteins, as well as potential causality of lipid metabolism on protein levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

BMI	body mass index
CCL3	C-C motif chemokine 3
CXCL16	C-X-C motif chemokine 16
CRP	C-reactive protein
CVD	cardiovascular disease
ELISA	enzyme-linked immunosorbent assay
FABP4	fatty acid-binding protein 4
FDR	false discovery rate
FGF-23	fibroblast growth factor 23
Gal3	Galectin-3
GH	growth hormone
GTE_x	genotype-tissue expression
GWAS	genome-wide association study
ICD	International Classification of Diseases
IL-6, 8 and 18	interleukin 6, 8 and 18
HDL-C	high-density lipoprotein cholesterol
HOMA	homeostasis model assessment
IL-1RA	interleukin-1 receptor antagonist protein
IR	insulin resistance
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIM-1	(HAVCR1) kidney injury molecule
LDL-C	low-density lipoprotein cholesterol
MCP-1	monocyte chemotactic protein 1
MMP-12	matrix metalloproteinase 12

MR	mendelian randomization
MS	mass spectrometry
NPX	normalized protein expression
NR3C1	nuclear receptor subfamily 3 group C member 1, glucocorticoid receptor
PEA	proximity extension assay
PIVUS	Prospective Study of the Vasculature in Uppsala Seniors
PPARγ	peroxisome proliferator activated receptor gamma
RETN	resistin
SCF	stem cell factor
SLE	systemic lupus erythematosus
STRING	search tool for the retrieval of interacting genes/proteins
TC	total cholesterol
TG	triglycerides
T2D	type 2 diabetes
TNF-R1	(TNFRSF1A) tumor necrosis factor receptor 1
TNF-R2	(TNFRSF1B) tumor necrosis factor receptor 2
t-PA	tissue-type plasminogen activator
TRADD	TNFRSF1A associated via death domain
TRAF2	TNF receptor associated factor 2
TRAIL-R2	(TNFRSF10B) TNF-related apoptosis-inducing ligand receptor 2
ULSAM	Uppsala Longitudinal Study of Adult Men

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HIGHLIGHTS:

- In our comprehensive study of circulating protein biomarkers, we found 42 proteins to be associated with at least one lipid fraction.
- We found kidney injury molecule (KIM-1), tumor necrosis factor receptor 1 and 2 (TNF-R1 and TNF-R2), TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) and resistin (RETN) to be associated with all four lipid fractions.
- Comprehensive studies of lipid-protein associations may provide novel nodes for drug action to treat human diseases.
- Our studies reveal new insights to the pathophysiology underlying dyslipidemia.

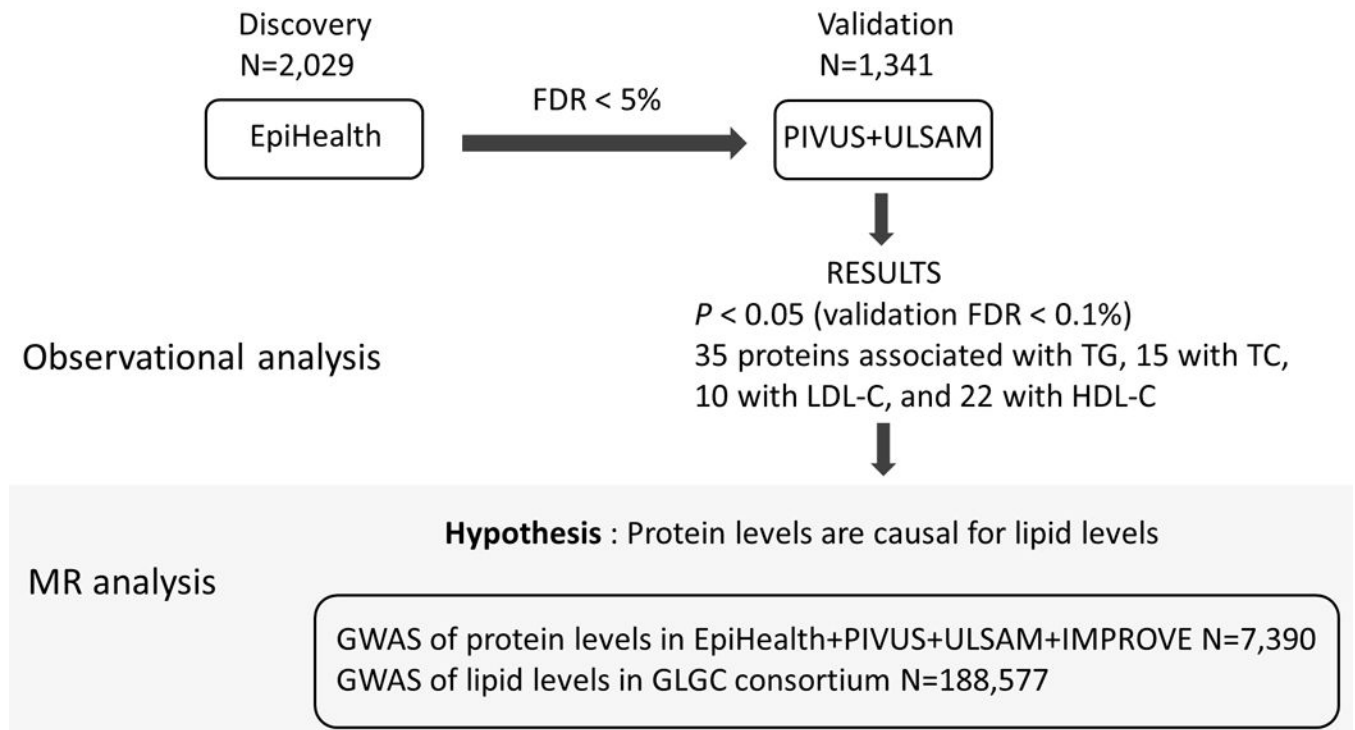


Figure 1.
Study design.

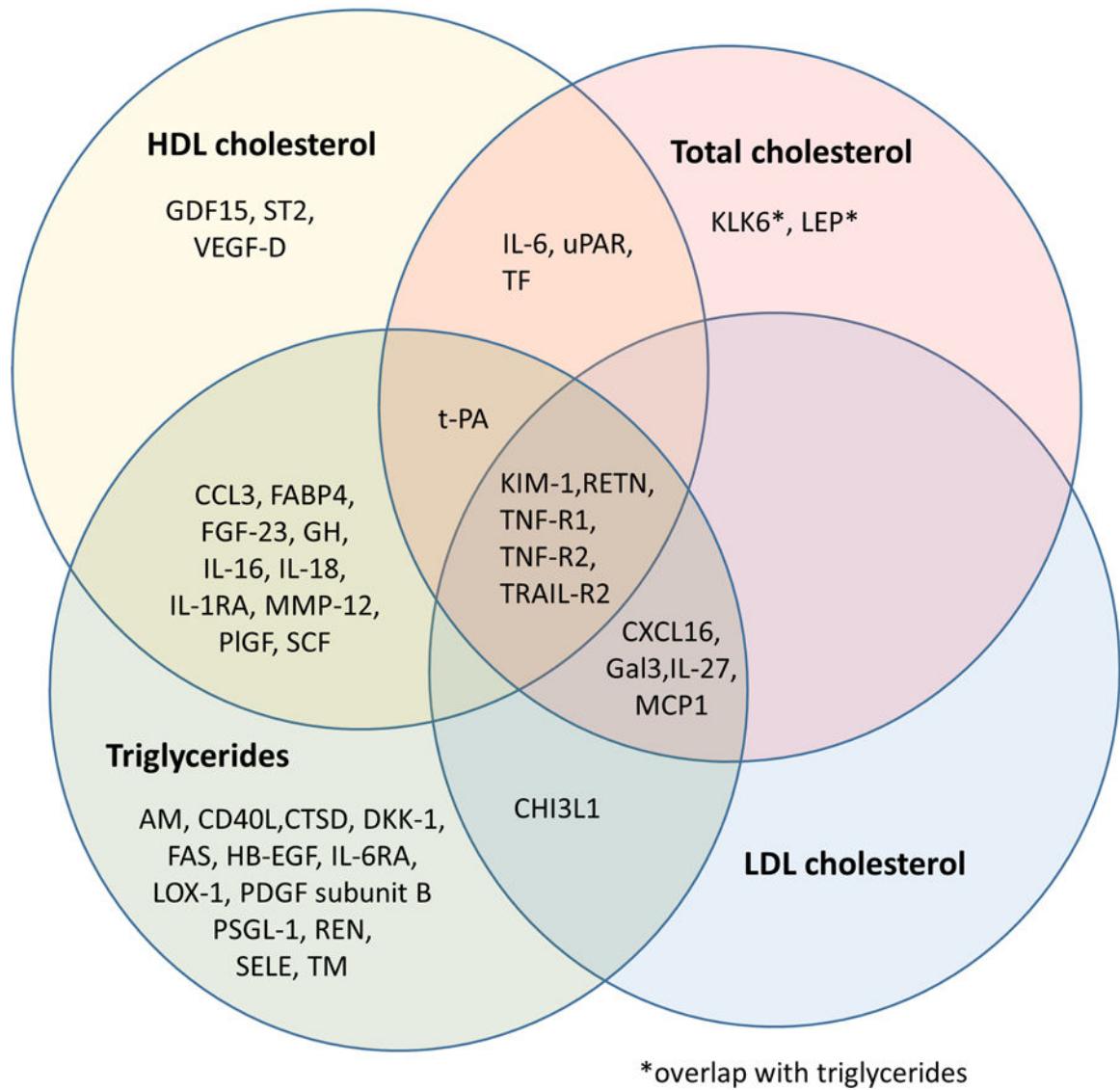


Figure 2.
Overlap of protein biomarkers associated with lipid fractions.

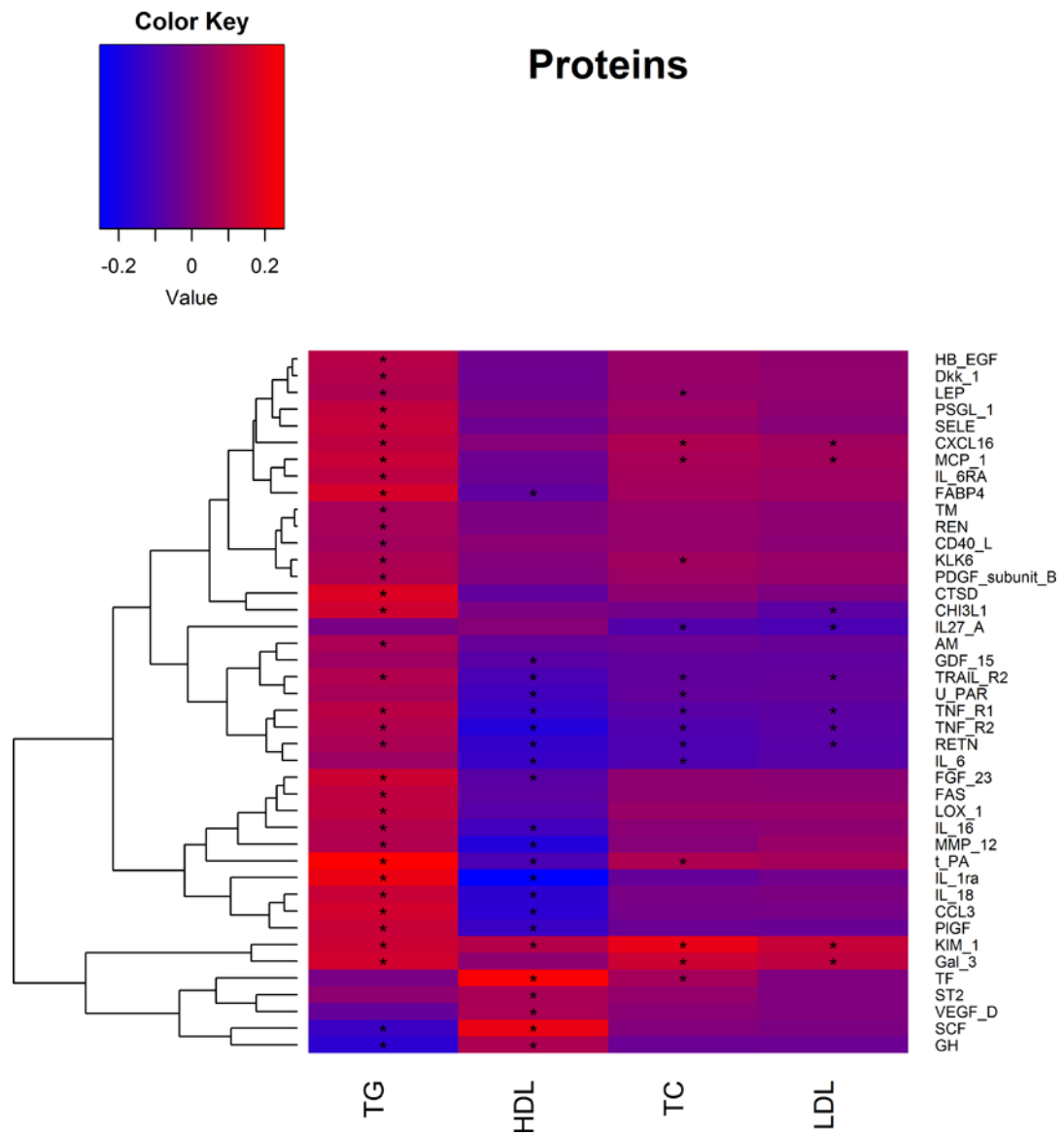


Figure 3. Directions of the associations between protein biomarkers and lipid levels. Asterisks indicate significant associations. Effects for protein levels are given for a 1-SD increase in lipid levels.

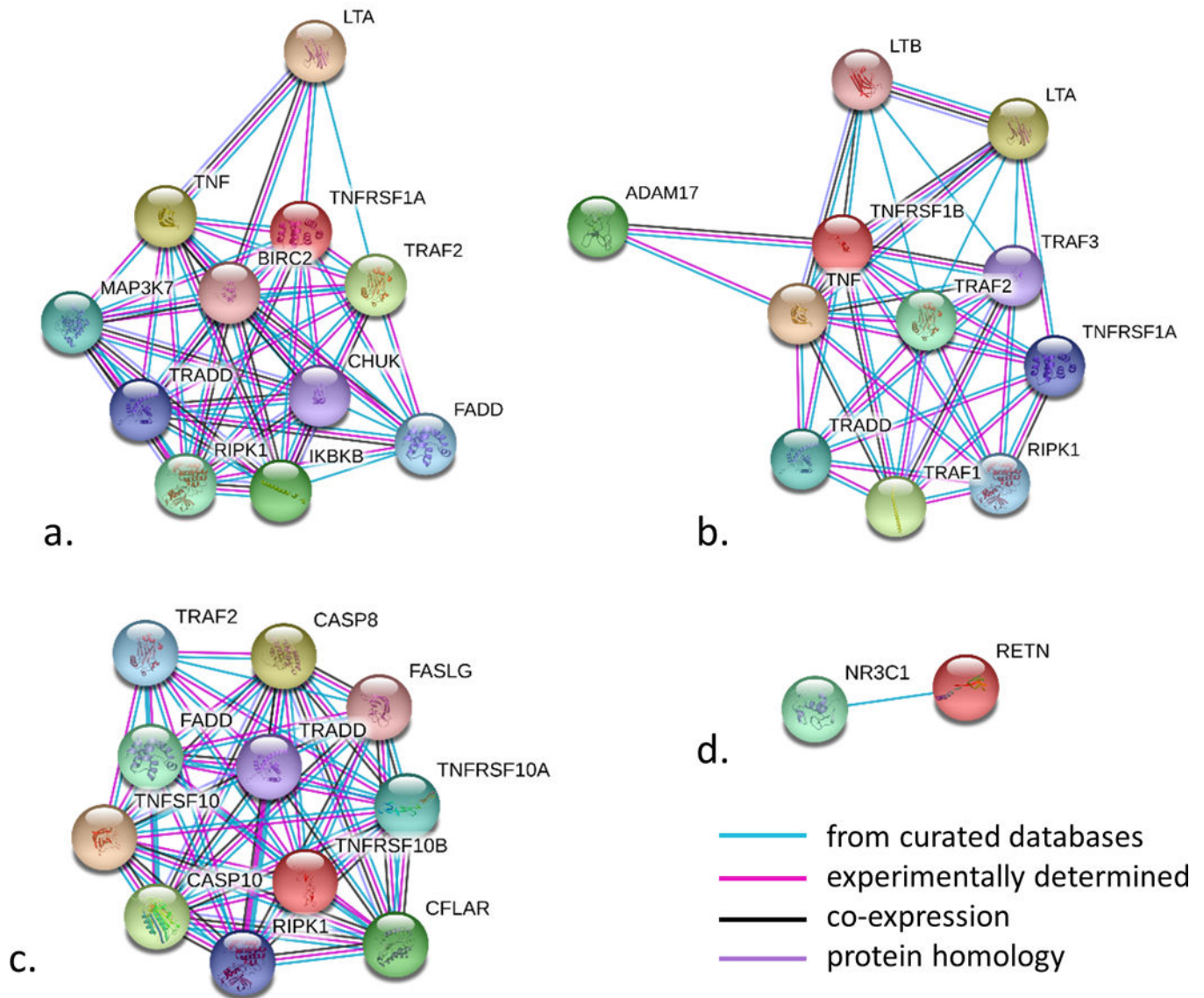


Figure 4. STRING networks for proteins associated with all four lipid fractions a. TNF-R1 (UniProt symbol TNFRSF1A), b. TNF-R2 (UniProt symbol TNFRSF1B), c. TRAIL-R2 (UniProt symbol TNFRSF10B) and d. RETN. Inquired proteins are depicted in red and interactions are shown for scores of strong evidence (the estimated likelihood of a given interaction being biologically meaningful, specific and reproducible, given the supporting evidence is 0.7).

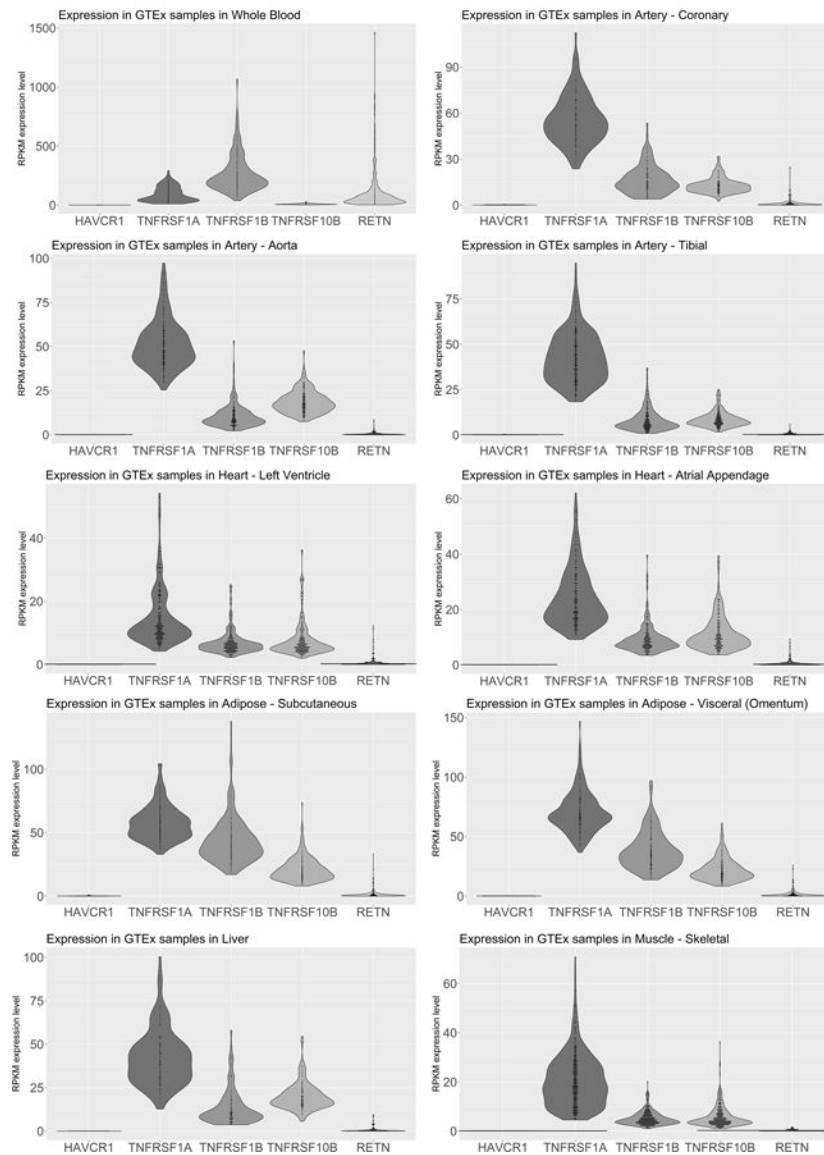


Figure 5. Expression of genes encoding proteins associated with four lipid fractions in relevant tissues based on GTEx data. *HAVCR1* is a gene encoding for KIM-1, *TNFRSF1A* encoding for TNF-R1, *TNFRSF1B* encoding for TNF-R2 and *TNFRSF10B* encoding for TRAIL-R2.

Table 1.

Clinical characteristics of the study samples.

Variable	EpiHealth (n=2,029)	PIVUS (n=790)	ULSAM (n=551)
Female sex	1051 (52)	404 (51)	0 (0)
Age (years)	61 (45-75)	70	78 (76-80)
Current smoking	148 (7)	84 (11)	45 (8)
BMI (kg/m ²)	26.0 (16.4-50.7)	26.5 (16.6-49.7)	25.8 (16.4-41.3)
SBP (mmHg)	134 (88-240)	148 (90-230)	150 (102-230)
Triglycerides (mmol/L)	1.1 (0.4-15.0)	1.1 (0.1-4.2)	1.2 (0.3-6.1)
Total cholesterol (mmol/L)	6.0 (3.1-13.0)	5.5 (1.8-9.4)	5.5 (2.8-10.2)
LDL (mmol/L)	4.0 (1.5-9.7)	3.5 (0.8-6.9)	3.6 (1.4-7.6)
HDL (mmol/L)	1.5 (0.7-3.7)	1.4 (0.6-3.8)	1.3 (0.6-2.6)
Blood glucose (mmol/L)	5.8 (4.3-19.7)	5.0 (2.8-19.9)	5.5 (2.9-17.2)
Diabetes treatment	22 (1.1)	37 (4.7)	61 (10.2)
Antihypertensive treatment	365 (18.0)	214 (27.1)	205 (37.2)
Myocardial infarction	18 (0.9)	23 (2.9)	49 (8.9)
Angina	15 (0.8)	31 (3.9)	39 (7.1)
Stroke	11 (0.5)	20 (2.5)	47 (8.5)
Heart failure	11 (0.5)	24 (3.0)	65 (11.8)
Atrial Fibrillation	42 (2.1)	19 (2.4)	52 (9.4)

Data are medians (range) or number (%).

Table 2. Significant associations between protein biomarkers and lipid fractions in the multivariable model.*

TRIGLYCERIDES (TG)	EpiHealth				PIVUS+ULSAM			
	Beta	SE	P		Beta meta	SE meta	P meta	
Marker								
Fatty acid-binding protein 4 (FABP4)	0.153	0.017	9.27E-19		0.193	0.022	5.65E-18	
Tissue-type plasminogen activator (t-PA)	0.263	0.020	7.68E-37		0.216	0.026	2.95E-16	
Interleukin-1 receptor antagonist protein (IL-1RA)	0.220	0.021	3.68E-25		0.198	0.026	2.60E-14	
Cathepsin D (CTSD)	0.171	0.023	2.13E-13		0.197	0.027	4.70E-13	
Galactin-3 (Gal-3)	0.149	0.023	1.30E-10		0.181	0.027	4.32E-11	
C-C motif chemokine 3 (CCL3)	0.159	0.023	1.31E-11		0.169	0.028	3.19E-09	
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	0.057	0.022	8.86E-03		0.162	0.028	7.58E-09	
Matrix metalloproteinase-12 (MMP-12)	0.057	0.023	1.09E-02		0.161	0.028	1.09E-08	
Kidney Injury Molecule-1 (KIM-1)	0.150	0.022	1.20E-11		0.148	0.027	7.82E-08	
Tumor necrosis factor receptor 1 (TNF-R1)	0.091	0.022	4.92E-05		0.148	0.028	9.94E-08	
Chitinase-3-like protein 1 (CH3L1)	0.156	0.022	4.45E-12		0.151	0.028	1.40E-07	
E-selectin (SELE)	0.135	0.023	8.09E-09		0.145	0.028	2.47E-07	
Placenta growth factor (PIGF)	0.140	0.022	3.02E-10		0.142	0.028	4.11E-07	
Interleukin-18 (IL-18)	0.143	0.024	1.62E-09		0.140	0.028	8.39E-07	
Stem cell factor (SCF)	-0.131	0.024	7.65E-08		-0.139	0.028	9.83E-07	
Tumor necrosis factor receptor 2 (TNF-R2)	0.072	0.023	1.96E-03		0.136	0.028	1.75E-06	
Heparin-binding EGF-like growth factor (HB-EGF)	0.093	0.025	1.54E-04		0.135	0.029	3.49E-06	
Leptin (LEP)	0.097	0.014	7.62E-12		0.084	0.018	4.56E-06	
Growth hormone (GH)	-0.184	0.021	6.62E-18		-0.125	0.027	5.35E-06	
Fibroblast growth factor 23 (FGF-23)	0.170	0.024	1.14E-12		0.124	0.028	8.44E-06	
Tumor necrosis factor receptor superfamily member 6 (FAS)	0.124	0.023	5.38E-08		0.127	0.029	9.59E-06	
Monocyte chemoattractant protein 1 (MCP-1)	0.150	0.023	1.74E-10		0.127	0.029	1.08E-05	
Lectin-like oxidized LDL receptor 1 (LOX-1)	0.128	0.024	6.95E-08		0.122	0.029	1.95E-05	
C-X-C motif chemokine 16 (CXCL16)	0.129	0.024	6.79E-08		0.119	0.028	3.14E-05	

TRIGLYCERIDES (TG)	EpiHealth			PIVUS+ULSAM		
	0.123	0.024	5.24E-07	0.115	0.029	6.46E-05
Interleukin-6 receptor subunit alpha (IL-6RA)	0.061	0.024	1.16E-02	0.115	0.029	7.08E-05
Resistin (RETN)	0.077	0.024	1.51E-03	0.106	0.028	1.69E-04
Platelet-derived growth factor subunit B (PDGF subunit B)	0.084	0.025	7.85E-04	0.109	0.029	1.77E-04
Interleukin-16 (IL-16)	0.101	0.024	2.82E-05	0.106	0.029	2.06E-04
Renin (REN)	0.058	0.023	1.13E-02	0.099	0.027	2.34E-04
Dickkopf-related protein 1 (DKK-1)	0.102	0.025	3.79E-05	0.105	0.029	3.25E-04
P-selectin glycoprotein ligand 1 (PSGL-1)	0.148	0.024	1.80E-09	0.100	0.029	5.45E-04
Thrombomodulin (TM)	0.070	0.024	3.73E-03	0.088	0.029	2.17E-03
Adrenomedullin (AM)	0.092	0.021	8.95E-06	0.072	0.028	9.91E-03
CD40 ligand (CD40L)	0.065	0.025	8.86E-03	0.070	0.029	1.62E-02
TOTAL CHOLESTEROL (TC)						
Kidney Injury Molecule-1 (KIM-1)	0.188	0.020	5.79E-20	0.232	0.027	7.44E-18
Galectin-3 (Gal-3)	0.140	0.021	6.03E-11	0.166	0.027	1.02E-09
Tumor necrosis factor receptor 2 (TNF-R2)	-0.080	0.021	2.11E-04	-0.110	0.028	9.12E-05
Tissue factor (TF)	0.060	0.022	7.16E-03	0.092	0.028	1.02E-03
Tumor necrosis factor receptor 1 (TNF-R1)	-0.071	0.021	6.80E-04	-0.086	0.028	1.80E-03
C-X-C motif chemokine 16 (CXCL16)	0.096	0.022	1.46E-05	0.087	0.028	2.04E-03
Resistin (RETN)	-0.109	0.022	1.08E-06	-0.087	0.028	2.30E-03
Interleukin-6 (IL-6)	-0.106	0.021	3.34E-07	-0.077	0.027	5.12E-03
Tissue-type plasminogen activator (t-PA)	0.107	0.019	4.52E-08	0.068	0.026	9.54E-03
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	-0.052	0.020	9.94E-03	-0.068	0.028	1.44E-02
Monocyte chemoattractant protein 1 (MCP-1)	0.089	0.022	4.60E-05	0.065	0.028	2.17E-02
Interleukin-27 subunit alpha (IL27-A)	-0.101	0.023	1.15E-05	-0.063	0.028	2.41E-02
Urokinase plasminogen activator surface receptor (uPAR)	-0.054	0.020	6.89E-03	-0.060	0.028	3.20E-02
Kallikrein-6 (KLK6)	0.067	0.023	3.02E-03	0.059	0.028	3.52E-02
Leptin (LEP)	0.048	0.013	3.03E-04	0.037	0.018	4.28E-02
LDL CHOLESTEROL (LDL-C)						

TRIGLYCERIDES (TG)									
	EpiHealth			PIVUS+ULSAM					
Kidney Injury Molecule-1 (KIM-1)	0.132	0.020	7.77E-11	0.154	0.027	8.95E-09			
Galectin-3 (Gal-3)	0.113	0.021	1.03E-07	0.134	0.027	5.95E-07			
Chitinase-3-like protein 1 (CHI3L1)	-0.055	0.021	8.19E-03	-0.094	0.028	8.24E-04			
Tumor necrosis factor receptor 2 (TNF-R2)	-0.066	0.021	1.81E-03	-0.083	0.028	2.86E-03			
Tumor necrosis factor receptor 1 (TNF-R1)	-0.064	0.021	1.93E-03	-0.077	0.027	4.64E-03			
Interleukin-27 subunit alpha (IL27-A)	-0.128	0.023	1.65E-08	-0.066	0.028	1.73E-02			
Resistin (RETN)	-0.088	0.022	7.55E-05	-0.067	0.028	1.77E-02			
Monocyte chemoattractant protein 1 (MCP-1)	0.076	0.022	4.53E-04	0.062	0.028	2.70E-02			
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	-0.056	0.020	5.33E-03	-0.057	0.027	3.71E-02			
C-X-C motif chemokine 16 (CXCL16)	0.071	0.022	1.35E-03	0.057	0.028	3.99E-02			
HDL CHOLESTEROL (HDL-C)									
Interleukin-1 receptor antagonist protein (IL-1RA)	-0.282	0.023	7.97E-34	-0.208	0.027	3.65E-14			
Matrix metalloproteinase-12 (MMP-12)	-0.160	0.025	8.30E-11	-0.214	0.029	4.98E-13			
Tumor necrosis factor receptor 2 (TNF-R2)	-0.158	0.025	4.74E-10	-0.208	0.030	2.80E-12			
Tissue factor (TF)	0.262	0.025	2.74E-24	0.206	0.030	5.86E-12			
Stem cell factor (SCF)	0.231	0.026	4.62E-18	0.188	0.030	3.19E-10			
Tumor necrosis factor receptor 1 (TNF-R1)	-0.122	0.024	7.08E-07	-0.162	0.029	3.79E-08			
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	-0.068	0.024	4.43E-03	-0.157	0.029	1.09E-07			
C-C motif chemokine 3 (CCL3)	-0.169	0.026	7.02E-11	-0.154	0.030	3.18E-07			
Kidney Injury Molecule-1 (KIM-1)	0.075	0.024	2.29E-03	0.146	0.029	5.47E-07			
Resistin (RETN)	-0.145	0.026	4.02E-08	-0.151	0.030	6.79E-07			
Interleukin-18 (IL-18)	-0.172	0.026	3.85E-11	-0.146	0.030	1.33E-06			
Placenta growth factor (PIGF)	-0.135	0.024	3.43E-08	-0.140	0.029	2.26E-06			
Interleukin-6 (IL-6)	-0.152	0.024	4.39E-10	-0.121	0.029	3.93E-05			
Vascular endothelial growth factor D (VEGF-D)	0.071	0.026	7.01E-03	0.103	0.029	4.26E-04			
Fibroblast growth factor 23 (FGF-23)	-0.062	0.026	1.90E-02	-0.097	0.029	8.80E-04			
Growth/differentiation factor 15 (GDF-15)	-0.061	0.021	3.64E-03	-0.095	0.029	9.48E-04			
Growth hormone (GH)	0.090	0.024	1.35E-04	0.095	0.029	1.06E-03			

TRIGLYCERIDES (TG)	EpiHealth			PIVUS+ULSAM		
Interleukin-16 (IL-16)	-0.136	0.026	2.43E-07	-0.098	0.030	1.20E-03
Fatty acid-binding protein 4 (FABP4)	-0.051	0.019	7.36E-03	-0.076	0.024	1.64E-03
Urokinase plasminogen activator surface receptor (uPAR)	-0.144	0.023	8.14E-10	-0.086	0.030	4.22E-03
ST2 protein (ST2)	0.084	0.025	8.20E-04	0.078	0.030	8.28E-03
Tissue-type plasminogen activator (t-PA)	-0.130	0.023	1.64E-08	-0.064	0.028	2.39E-02

* Adjusted for age, sex, BMI, smoking, glucose levels, systolic blood pressure, antihypertensive medication, diabetes medication and myocardial infarction, angina, stroke, heart failure and atrial fibrillation.

Table 3.

Results of MR analyses, shown if the statistical power was at least 80%.

Exposure	Outcome	N SNPs	B	SE	P
ADM	Triglycerides	2	-0.067	0.025	0.006
CCL3	HDL cholesterol	1	0.005	0.015	0.717
CCL3	Triglycerides	1	0.003	0.014	0.833
CHI3L1	LDL cholesterol	3	0.021	0.019	0.269
CHI3L1	Triglycerides	3	0.003	0.019	0.892
CSTD	Triglycerides	2	0.024	0.015	0.099
CXCL16	LDL cholesterol	2	-0.041	0.052	0.428
CXCL16	Total cholesterol	2	-0.006	0.069	0.926
CXCL16	Triglycerides	2	0.050	0.045	0.269
Dkk-1	Triglycerides	4	-0.065	0.047	0.166
FAS	Triglycerides	2	0.017	0.016	0.267
Gal3	LDL cholesterol	3	-0.015	0.010	0.129
Gal3	Total cholesterol	3	-0.017	0.010	0.070
Gal3	Triglycerides	3	-0.015	0.009	0.090
GDF15	HDL cholesterol	2	-0.056	0.022	0.010
IL-16	HDL cholesterol	2	0.007	0.008	0.384
IL-16	Triglycerides	2	0.005	0.010	0.587
IL-18	HDL cholesterol	4	0.016	0.016	0.321
IL-18	Triglycerides	4	0.003	0.016	0.866
IL-1RA	HDL cholesterol	2	0.013	0.017	0.458
IL-1RA	Triglycerides	2	0.022	0.013	0.099
IL-6RA	Triglycerides	2	-0.024	0.041	0.553
KIM-1	HDL cholesterol	4	0.000	0.008	0.974
KIM-1	LDL cholesterol	4	-0.016	0.020	0.432
KIM-1	Total cholesterol	4	-0.017	0.020	0.400
KIM-1	Triglycerides	4	-0.004	0.013	0.737
LOX-1	Triglycerides	1	0.031	0.034	0.374
MMP-12	HDL cholesterol	3	-0.016	0.015	0.271
MMP-12	Triglycerides	3	-0.001	0.013	0.964
PIGF	HDL cholesterol	1	0.001	0.029	0.967
PIGF	Triglycerides	1	-0.052	0.028	0.064
PSGL-1	Triglycerides	1	-0.017	0.010	0.089
RETN	HDL cholesterol	1	-0.019	0.034	0.564
RETN	LDL cholesterol	1	0.006	0.035	0.876
RETN	Total cholesterol	1	0.014	0.034	0.675
SELE	Triglycerides	1	0.038	0.036	0.289
ST2	HDL cholesterol	5	-0.007	0.011	0.507

Exposure	Outcome	N SNPs	B	SE	P
TF	HDL cholesterol	2	0.012	0.033	0.711
TF	Total cholesterol	2	0.017	0.019	0.373
TM	Triglycerides	2	0.004	0.009	0.613
TRAIL-R2	HDL cholesterol	1	-0.025	0.016	0.109
TRAIL-R2	LDL cholesterol	1	0.004	0.017	0.817
TRAIL-R2	Total cholesterol	1	-0.012	0.017	0.486
TRAIL-R2	Triglycerides	1	0.022	0.016	0.157
uPAR	HDL cholesterol	1	-0.034	0.035	0.337

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