Rare coding variants in 35 genes associate with circulating lipid levels – a multi-ancestry analysis of 170,000 exomes

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Abstract

Large-scale gene sequencing studies for complex traits have the potential to identify

causal genes with therapeutic implications. We performed gene-based association

testing of blood lipid levels with rare (minor allele frequency<1%) predicted damaging

coding variation using sequence data from >170,000 individuals from multiple

ancestries: 97,493 European, 30,025 South Asian, 16,507 African, 16,440

Hispanic/Latino, 10,420 East Asian, and 1,182 Samoan. We identified 35 genes

associated with circulating lipid levels. Ten of these: *ALB*, *SRSF2*, *JAK2, CREB3L3*,

TMEM136, *VARS*, *NR1H3*, *PLA2G12A*, *PPARG* and *STAB1* have not been

implicated for lipid levels using rare coding variation in population-based samples.

We prioritize 32 genes identified in array-based genome-wide association study

(GWAS) loci based on gene-based associations, of which three: *EVI5, SH2B3,* and

PLIN1, had no prior evidence of rare coding variant associations. Most of the

associated genes showed evidence of association in multiple ancestries. Also, we

observed an enrichment of gene-based associations for low-density lipoprotein

cholesterol drug target genes, and for genes closest to GWAS index single

nucleotide polymorphisms (SNP). Our results demonstrate that gene-based

associations can be beneficial for drug target development and provide evidence that

the gene closest to the array-based GWAS index SNP is often the functional gene for

blood lipid levels.

Introduction

Blood lipid levels are heritable complex risk factors for atherosclerotic cardiovascular diseases.[1] Array-based genome-wide association studies (GWAS) have identified >400 loci as associated with blood lipid levels, explaining 9-12% of the phenotypic variance of lipid traits.[2-8] These studies have identified mostly common (minor allele frequency (MAF)>1%) noncoding variants with modest effect and helped define the causal roles of different lipid fractions in cardiovascular disease.[9-13] Despite these advances, the mechanisms and causal genes for most of the identified variants and loci have not yet been determined.

Conventional GWAS with array-derived or imputed common variants are unlikely to directly implicate causal genes, while genetic association studies testing rare variants in coding regions have this potential. Advances in next generation sequencing over the last decade have facilitated increasingly larger studies with improved power to detect associations of rare variants with complex diseases and traits.[14; 15] However, most exome sequencing studies to date have been insufficiently powered for rare variant discovery; for example, Flannick et al. estimated that it would require 75,000 to 185,000 sequenced cases of type 2 diabetes (T2D) to detect associations at known drug target genes at exome-wide significance.[15]

Identifying rare variants with impact on protein function has helped elucidate biological pathways underlying dyslipidemia and atherosclerotic diseases such as coronary artery disease (CAD).[14; 16-25] Successes using this approach have led to the development of novel therapeutic targets to modify blood lipid levels and lower risk of atherosclerotic diseases.[26; 27] The vast majority of participants in these studies have

been of European ancestry, highlighting the need for more diverse study sample.

Such diversity can identify associated variants absent or present at very low

frequencies in European populations and help implicate new genes with

generalizability extending to all populations.

We have assembled exome sequence data from >170,000 individuals across

multiple ancestries and systematically tested the association of rare variants in each

gene with six circulating lipid phenotypes: low-density lipoprotein cholesterol (LDL-C),

high density lipoprotein cholesterol (HDL-C), non-HDL-C, total cholesterol (TC),

triglycerides (TG), and the ratio of TG to HDL-C (TG:HDL). We find 35 genes

associated with blood lipid levels, show evidence of gene-based signals in array-

based GWAS loci, show enrichment of lipid gene-based associations in LDL-C drug

targets and genes in close proximity with GWAS index variants, and test lipid genes

for association with CAD, T2D, and liver enzymes.

Subjects and Methods

Study Overview

Our study samples were derived from four major data sources with exome or

genome sequence data and blood lipid levels: CAD case-control studies from the

Myocardial Infarction Genetics Consortium (MIGen, n = 44,208) and a UKB nested

case-control study of CAD (n = 10,689); T2D cases-control studies from the AMP-

T2D-GENES exomes (n = 32,486); population-based studies from the TOPMed

project freeze 6a data (n = 44,101) restricted to the exome, and the UKB first tranche

of exome sequence data (n = 40,586) (see **Supplementary Note**). Informed consent

was obtained from all subjects and committees approving the studies are available in

the supplement.

Within each data source, individuals were excluded if they failed study-specific

sequencing quality metrics, lacked lipid phenotype data, or were duplicated in other

sources. We additionally removed first- and second-degree relatives across data

sources while we kept relatives within each data source since we were able to adjust

for relatedness within each data source using kinship matrices in linear mixed

models. If samples from the same study were present in different data sources, we

used the samples in the data source which has the largest sample size from the

study and removed the overlapping set from the other data source. For instance,

samples from the Atherosclerosis Risk in Communities (ARIC) Study were removed

from TOPMed and kept in MIGen which had more sequenced samples from ARIC.

Similarly, samples from the Jackson Heart Study were kept in TOPMed and removed

from MIGen. To obtain duplicate and kinship information across data sources we

used 14,834 common (MAF>1%) and no more than weakly dependent ($r^2 < 0.2$)

variants using the make-king flag in PLINK v2.0. Single-variant association analyses

were performed within each data source, case-status, and ancestry combination. The

data were sequenced and variant calling performed separately by data source and

this allowed us to look for effects by case-status and genetically-inferred and/or self-

reported ancestry groups. We performed gene-based meta-analyses by combining

single-variant summary statistics and covariance matrices generated from

RVTESTS.[28] We performed ancestry-specific gene-based meta-analyses by

combining single-variant summary data from five major ancestries with >10,000

across all data sources: European, South Asian, African, Hispanic, and East Asian

ancestries.

Phenotypes

We studied six lipid phenotypes; total, LDL-C, HDL-C, non-HDL-C, TG and TG:HDL.

TC was adjusted by dividing the value by 0.8 in individuals reporting lipid lowering

medication use after 1994 or statin use at any time point. If LDL-C levels were not

directly measured, then they were calculated using Friedewald equation for

individuals with TG levels < 400 mg/dl using adjusted TC levels. If LDL-C levels were

directly measured then, their values were divided by 0.7 in individuals reporting lipid

lowering medication use after 1994 or statin use at any time point.[5] TG and TG:HDL

levels were natural logarithm transformed. Non-HDL-C was obtained by subtracting

HDL-C from adjusted TC levels. Residuals for each trait in each cohort, ancestry, and

case status grouping were created after adjustment for age, age^2, sex, principal

components, sequencing platform, and fasting status (when available) in a linear

regression model. Residuals were then inverse-normal transformed and multiplied by

the standard deviation of the trait to scale the effect sizes to the interpretable units.

Sequencing and Quality Control

Myocardial Infarction Genetics Consortium (MIGen)

A set of common variants was extracted for sample quality control including relative

inference, principal component analysis, and estimation of heterozygosity. SNPs on

autosomes and not in low complexity regions or segmental duplications were

extracted. SNPs with quality of depth (QD)> 2, call rate >98%, self-reported-race-

specific Hardy-Weinberg equilibrium (HWE) p-value >1×10^{-8}, Variant Quality Score

Recalibration (VQSR) of PASS and MAF>1% were retained. Sample relatedness

was estimated with KING and duplicate samples removed. Genetically inferred

ancestry was assigned to each individual by calculating principal components jointly

with 1000 Genomes phase 3 version 5 and building a 5-Nearest Neighbor classifier

using the top 6 principal components. Heterozygosity was estimated within each

genetic ancestry group and samples with F statistic above 0.3 were removed.

Genetic sex was inferred based on high quality X-chromosome variation including

variants with call rate >0.95, MAF>2%, a PASS VQSR, QD>3 if the variant is an

insertion or deletion and QD>2 if it is SNP. Samples with discordant phenotypic sex

and genetic sex were removed. Finally, sample quality control metrics were

calculated using Hail and samples with call rate<0.9a mean depth (DP)<30 and

mean genotype quality (GQ)<0.8 were excluded. A total of 44,240 samples with lipid

data measurements were included after further excluding duplicates and relatives

with other data sources (**Table S1**).

Variant quality control was performed amongst remaining samples and a total of

8,716,575 autosomal variants were included after removing those that fail HWE as

calculated by genetic ancestry group (p-value<1×10^{-8}), lie in low complexity regions

or segmental duplications, with inbreeding coefficient< -0.3, are insertions or

deletions with QD ≤ 3 or SNPs with QD ≤ 2 or variants where VQSR does not PASS

with the exception of singletons where variants with VQSRTrancheSNP99.60to99.80

were retained.

Trans-Omics for Precision Medicine (TOPMed)

Whole genome sequencing at 30X mean depth was performed at one of six

sequencing centers: Broad Institute of MIT and Harvard, Northwest Genomics

Center, New York Genome Center, Illumina Laboratory Services, Psomagen, Inc.

(formerly Macrogen USA), Baylor College of Medicine Human Genome Sequencing

Center. For most studies, all individuals in the study were sequenced at the same

center. Sequence reads were aligned to human genome build GRCh37 or GRCh38

at each center using similar, but not identical, processing pipelines. The resulting

sequence data files were transferred from all centers to the TOPMed Informatics

Research Center (IRC), where they were re-aligned to build GRCh38, using a

common pipeline to produce a set of 'harmonized' .cram files. Processing was

coordinated and managed by the 'GotCloud' processing pipeline. The IRC performed

joint genotype calling on all samples. Quality control was performed at each stage of

the process by the Sequencing Centers, the IRC, and the TOPMed Data

Coordinating Center (DCC). Only samples that passed QC were included in the call

set.

The two sequence quality criteria that were used to pass sequence data on for joint

variant discovery and genotyping are: estimated DNA sample contamination below

3%, and fraction of the genome covered at least 10x 95% or above. DNA sample

contamination was estimated from the sequencing center read mapping using

software verifyBamId.[29]

The genotype used for analysis are from "freeze 6a" of the variant calling pipeline

performed by the TOPMed Informatics Research Center (Center for Statistical

Genetics, University of Michigan, Hyun Min Kang, Tom Blackwell and Gonçalo

Abecasis). Variant detection (SNPs and indels) from each sequenced (and aligned)

genome was performed by the vt discover2 software tool. The variant calling

software tools are under active development; updated versions can be accessed at

http://github.com/atks/vt, http://github.com/hyunminkang/apigenome, and

https://github.com/statgen/topmed_variant_calling.

One individual from duplicate pairs identified by the DCC was removed, retaining the

individual with lipid levels available when one did not have lipid levels. If both

individuals had lipid levels, one individual was randomly selected. Individuals were

excluded when their genotype determined sex did not match phenotype reported sex

(n=6) and individuals <18 years old were excluded (n=865). Ancestry was defined as

self-reported ancestry.

AMP-T2D-GENES

Sequencing and quality control were performed as previously described.[15] Following

sequencing and variant calling, we measured samples and variants according to

several sequence quality metrics and excluded those that were outliers relative to the

global distribution. These exclusions produced an "analysis" dataset of 45,231

individuals and 6.33M variants. We then estimated, within each ancestry, pairwise

IBD values, genetic relatedness matrices (GRMs), and PCs for use in downstream

association analysis. We used the IBD values to generate lists of unrelated

individuals within each ancestry, excluding 2,157 individuals from an "unrelated

analysis" set of 43,090 individuals (19,828 cases and 23,262 controls) and 6.29M

non-monomorphic variants.

UK Biobank

We used two UKB datasets with exome sequence data. The first is a CAD case control study with 12,938 individuals. 29 samples were removed as they had discordant genotypes with genotyping array data, 17 showed mismatch between the reported and genetically inferred sex, 4 had excess heterozygosity and 6 had a call rate <95%. To perform the sex-mismatch analyses, variants on the X-chromosome were selected after filtering out low quality genotypes, call rate<95%, MAF<2%, low QD score (3 for INDELs and 2 for SNPs), low confidence regions and segmental duplications and those that do not have PASS VQSR. A set of high quality common autosomal variants were extracted for relative inference, principal component analysis, and estimation of heterozygosity after removing low confidence regions and segmental duplications, low quality genotypes, QD<2, call rate<98%, self-reported ancestry-specific HWE $p>1x10^{-6}$ among controls, MAF<1% and do not have PASS VQSR. Heterozygosity was estimated within each ancestry and samples with F statistic>2 were removed. Genetically inferred ancestry was obtained using the 1000 Genomes as reference. Sample QC metrics were then calculated in HAIL using autosomal variants after filtering out low-quality genotypes, variants with ancestry-specific HWE $p<1\times10^{-6}$, low confidence regions and segmental duplications, low QD score (3 for INDELs and 2 for SNPs) and those that do not have PASS VQSR. Samples with call rate below 95%, mean DP below 30 and mean GQ below 80 were removed. Variant QC was done through filtering out monomorphic variants, call rate below 95%, those with HWE ($p < 1 \times 10^{-6}$), lie in low confidence regions or segmental duplications, are insertions or deletions with QD <= 3 or SNPs with QD <= 2 or variants where VQSR does not PASS unless singleton in which case retain those with VQSRTrancheSNP99.60to99.80. A total of 11,216 PC-identified European

ancestry participants were included after additional removal of duplicates and

relatives across data sources. A total of 2,734,519 variants were included.

The second UKB data set is a population-based dataset. Samples were filtered out if

they showed mismatch between genetically determined and reported sex, high rates

of heterozygosity or contamination (D-stat > 0.4), low sequence coverage (<85% of

targeted bases achieving >20X coverage), duplicates, and exome sequence variants

discordant with genotyping chip. More details are described elsewhere.[30] The

"Functionally Equivalent" (FE) call set was used.[31] A total of 43,243 PC-identified

European ancestry individuals were included after additional removal of duplicates

and relatives across data sources.

Variant Annotation

We compiled autosomal variants with call rate>95% within each case and ancestry

specific analysis dataset with MAC≥1 (across the combined data). Variants were

annotated using the Ensembl Variant Effect Predictor[32] and its associated Loss-of-

Function Transcript Effect Estimator (LOFTEE)[33] and the dbNSFP[34] version 3.5a

plugins. We limited our annotations to the canonical transcripts. The LOFTEE plugin

assesses stop-gained, frameshift, and splice site disrupting variants. Loss-of-function

variants are classified as either high confidence or low confidence. The dbNSFP is a

database that provides functional prediction data and scores for non-synonymous

variants using multiple algorithms.[34] This database was used to classify missense

variants as damaging using two different definitions based on bioinformatic prediction

algorithms. The first is based on MetaSVM[35] which is derived from 10 different

component scores (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, GERP++,

MutationTaster, Mutation Assessor, FATHMM, LRT, SiPhy, PhyloP). The second is

based on 5 variant prediction algorithms including SIFT, PolyPhen-2 HumVar,

PolyPhen-2 HumDiv, MutationTaster and LRT score. Additionally, we ran a deep

neural network analysis (Splice AI) to predict splice-site altering variants.[36] Multi-

ancestry and ancestry-specific variant descriptive analyses were performed using

variant-specific statistics obtained from the largest sample size out of the 6

phenotypes.

Single-Variant Association Analysis

Each data source was sub-categorized based on ancestry and CAD or T2D case

status in the studies ascertained by disease status. Subgrouping data sources

yielded a total of 23 distinct sample sub-categories. As relatives were kept within

each sub-group, we performed generalized linear mixed models to analyze the

association of single autosomal variants with standard-deviation corrected-inverse-

normal transformed traits using RVTESTS.[28] RVTESTS was used to generate

summary statistics and covariance matrices using 500 kilobase sliding windows. To

obtain the single-variant associations, we performed a fixed-effects inverse-variance

weighted meta-analysis for multi-ancestry and within each of the five major

ancestries. An exome-wide significance threshold of $P<7.2\times10^{-8}$ (Bonferroni

correction for six traits and using previously recommended threshold for coding

variants $P<4.3\times10^{-7}$)[37] was used to determine significant coding variants.

Gene-Based Association Analysis

We used summary level score statistics and covariance matrices from autosomal

single-variant association results to perform gene-based meta-analyses among all

individuals and within each ancestry using RAREMETALS version 7.2.[38] Samoan

individuals only contributed to the overall analysis. Gene-based association testing

aggregates variants within each gene unit using burden tests and SKAT which allows

variable variant effect direction and size.[39] The "rareMETALS.range.group" function

was used with MAF<1%, which filters out all variants with combined MAF>1% in all

meta-analytic datasets. All variants with call rates<95% and not annotated as LOF

using LOFTEE, splice-site variants or damaging missense as defined by MetaSVM or

by all SIFT, PolyPhen-2 HumVar, PolyPhen-2 HumDiv, MutationTaster and LRT

prediction algorithms (Damaging 5 out of 5) were excluded in the gene-based meta-

analyses.

We used 6 different variant groupings to determine the set of damaging variants

within each gene, 1) high-confidence LOF using LOFTEE, 2) LOF and predicted

splice-site altering variants, 3) LOF and MetaSVM missense variants, 4) LOF,

MetaSVM missense and predicted splice-site altering variants, 5) LOF and damaging

5 out 5 missense variants, and 6) LOF, damaging 5 out 5 missense and predicted

splice-site altering variants. An exome-wide significance threshold of $P<4.3\times10^{-7}$,

Bonferroni corrected for the maximum number of annotated genes (n=19,540) and

six lipid traits, was used to determine significant coding variants. Two gene

transcripts, *DOCK6* and *DOCK7*, that overlap with two well-studied lipid genes,

ANGPTL8 and *ANGPTL3*, respectively, met our exome-wide significance threshold.

After excluding variation observed in *ANGPTL8* and *ANGPTL3*, *DOCK6* and *DOCK7,*

respectively, were no longer significant and have been excluded as associated

genes.

We performed a series of sensitivity analyses for our results. We repeated the multi-

ancestry gene-based analyses using a MAF<0.1%, and compared our exome-wide

significant gene-based results using a MAF<1% to using a MAF<0.1%. We

compared the single variants in our top gene-based associations with respective

traits using GWAS summary data.[8] Gene-based tests were repeated excluding

variants identified in GWAS using $P<5\times10^{-8}$. Furthermore, all single variants included

in each of the top gene-based association were analyzed in relation to the respective

trait. To determine the variants that were contributing mostly to the gene-based

signal, counts and proportions were obtained at different P value thresholds.

To understand whether variants contributing to top gene-based signals were similar

or different across different ancestries, we determined the degree of overlap across

ancestries for all variants incorporated and then for those with P<0.05. Finally, we

checked for overlap across the most significant (lowest P value) variant from each of

the gene-based signals.

Heterogeneity of gene-based estimates in all gene-trait-variant grouping

combinations passing exome-wide significant levels was assessed across the five

main ancestries (European, South Asian, African, Hispanic and East Asian) and

between T2D and CAD cases and controls using Cochran's Q.

Replication of gene-based associations

We performed replication of our top gene-based associations with blood lipid levels in

the Penn Medicine BioBank (PMBB) and UK Biobank samples that did not contribute

to the discovery analysis.

The PMBB is a repository of genotype and phenotype data for 43,731 patients at the

University of Pennsylvania Perelman School of Medicine. All individuals recruited for

PMBB are patients of clinical practice sites of the University of Pennsylvania Health

System. Appropriate consent was obtained from each participant regarding storage

of biological specimens, genetic sequencing, and access to all available EHR data.

The study was approved by the Institutional Review Board of the University of

Pennsylvania and complied with the principles set out in the Declaration of Helsinki.

The six lipid phenotypes studied were HDL-C (n=21,247), LDL-C (n=21,040), non-

HDL-C (n=21,087), TC (n=21,153), TG (n=21,418), and TG:HDL (n=21,213). All

available lipid trait measurements up to July 2020 were included. HDL-C, LDL-C, TC,

and TG levels were measured directly and accessible via PMBB. Non-HDL-C levels

were obtained by subtracting HDL-C from TC levels. TG and TG:HDL levels were

logarithmically transformed to normalize their distribution for association testing. Due

to the clinical nature of the biobank, samples often had multiple phenotype values

corresponding to a patient's various clinical appointments. Gene-based associations

were performed on the minimum, median, and maximum phenotype values to

account for both potentially protective and pathogenic effects. For the gene-based

association analysis, 10 different variant groupings were used to determine the set of

damaging variants within each gene including the six groupings used in the initial

study. The additional four groupings used predicted loss-of-function (pLOF) variants

that included frameshift, stop gain, and splicing variants as annotated by RefGene.

Missense variants were annotated using Rare Exome Variant Ensemble Learner

(REVEL) and filtered for those with a pathogenicity score>0.5. The four additional

groupings consisted of, 1) pLOF, MAF≤0.1%, 2) pLOF, MAF≤0.1%, REVEL

missense, 3) pLOF, MAF≤1%, and 4) pLOF, MAF≤1%, and REVEL missense. Each

of the 10 groupings were used in a gene-based association test with the minimum,

median, and maximum values of the 6 lipid phenotypes. Furthermore, ancestry-

specific associations were also performed to elucidate any potential ancestry-specific

effects. This included associations among African and European ancestries

separately, and then the two populations meta-analyzed. All associations were

adjusted for sex, age, and principal components. The first 5 principal components

were used for African ancestry associations, and the first 10 principal components

were used for European ancestry associations.

In UK Biobank, we analyzed the association of rare variant aggregates from the 10

genes against four lipid phenotypes in the UK biobank whole exome sequencing

(WES) data. Variant aggregates were obtained for the following four categories 1)

LOFTEE – HC 2) LOFTEE - HC & predicted splice site altering 3) LOFTEE - HC &

deleterious-METAsvm 4) LOFTEE - HC & deleterious-METAsvm & predicted splice

site altering. We removed UK Biobank individuals used in the discovery analysis,

resulting in 150,694 individuals for replication. The phenotypes were adjusted for lipid

lowering medications, where total cholesterol was adjusted by dividing by 0.8 and

LDL-C by dividing by 0.7. Triglycerides were natural log transformed for analysis. The

phenotypes were inverse rank normalized and scaled by the standard deviation of

the trait and adjusted for covariates (sex, age, age2, PC1-PC10, if British ancestry).

Rare variant aggregate test was conducted using STAAR[40] with a MAF of 0.01 for

the four lipids. Effect estimates were calculated using glmm.wald burden test. As a

sensitivity analysis to determine the effects of statin treatment on the results, a

similar analysis was carried out for a subset of individuals where samples with statins

were removed, resulting in 127,459 individuals without statin treatment. For our top

associations in our discovery, we found the effect sizes and p-value in the analysis

including and excluding individuals on statin treatment remained similar (**Figure S1**).

Gene-Based Analysis of GWAS Loci and Drug Targets

We performed gene-based analysis using the six variant groups for genes in GWAS

loci. A locus was defined as the region around each GWAS index variant ± 200kb.

Top GWAS signals were obtained from a recent meta-analysis of >300,000

individuals in the Million Veterans Program.[8] In-silico lookup of gene-based

associations for respective lipid traits were then performed for all genes within

defined GWAS loci. Drug target genes were obtained from the drug bank database[41]

using the following search categories: "Hypolipidemic Agents, Lipid Regulating

Agents, Anticholesteremic Agents, Lipid Modifying Agents and

Hypercholesterolemia". A liberal definition for drug targets was used – drugs with any

number of targets and targets targeted by any number of drugs – and then in-silico

lookups were performed for gene-based associations.

Gene-set Enrichment Analysis

Gene-set enrichment analyses were performed for sets of Mendelian-, protein-

altering- and non-protein altering GWAS, and drug target genes with LDL-C, HDL-C

and TG. 21 Mendelian genes were included based on previous literature[2]: *LDLR*,

APOB, *PCSK9*, *LDLRAP1*, *ABCG5*, *ABCG8*, *CETP*, *LIPC*, *LIPG*, *APOC3*, *ABCA1*,

APOA1, *LCAT*, *APOA5*, *APOE*, *LPL*, *APOC2*, *GPIHBP1*, *LMF1*, *ANGPTL3*, and

ANGPTL4. We analyzed GWAS gene sets based on their coding status and their

proximity to the most significant signal in the GWAS. Coding variants were defined as

missense, frameshift, or stop gained variants. Gene sets for coding or non-coding

variants were then stratified into three categories based on proximity to the most

significant variant within each locus – closest-, second closest- and greater than

second closest gene. For each gene within each set, we obtained the most

significant association in the multi-ancestry or ancestry specific meta-analysis set

using any of the six different variant groups. Then each gene within each gene set

was matched to 10 other genes based on sample size, total number of variants,

cumulative MAC, and variant grouping nearest neighbors using the matchit R

function. Then we compared the proportions using Fisher's exact test between the

main and matched gene sets by applying different P-value thresholds.

Association of Lipid Genes with CAD and T2D data and liver fat/markers

We determined the associations of 40 genes identified in the main and GWAS loci

analyses with CAD, T2D, and glycemic and liver enzyme blood measurements. The

association with T2D was obtained from the latest gene-based exome association

data from the AMP-T2D-GENES consortium.[15] The reported associations were

obtained from different variant groups based on their previous analyses. We

additionally performed gene-based association analyses with CAD using the MIGen

case-control, UKB case-control, and UKB cohort samples using the variant groups

described above. Further, six traits including fasting plasma glucose, HbA1c, alanine

aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and

albumin were analyzed in the UKB dataset. Single variant association analyses were

performed with RVTESTS. Linear mixed models incorporating kinship matrices were

used to adjust for relatedness within each study. Covariance matrices were

generated using 500 kilobase sliding windows. RAREMETALS was used to assess

associations between aggregated variants (MAF<1%) in burden and SKAT tests with

CAD and each of the six quantitative traits. We used 6 different variant groupings to

determine the set of damaging variants within each gene, 1) high-confidence LOF

using LOFTEE, 2) LOF and predicted splice-site altering variants, 3) LOF and

MetaSVM missense variants, 4) LOF, MetaSVM missense and predicted splice-site

altering variants, 5) LOF and damaging 5 out 5 missense variants, and 6) LOF,

damaging 5 out 5 missense and predicted splice-site altering variants.

Results

Sample and variant characteristics

Individual-level, quality-controlled data were obtained from four sequenced study

sources with circulating lipid data for individuals of multiple ancestries (**Figure 1**).

Characteristics of the study samples are detailed in **Table S1.** We analyzed data on

up to 172,000 individuals with LDL-C, non-HDL-C (a calculated measure of TC minus

HDL-C), TC, HDL-C, TG, and TG:HDL ratio (a proxy for insulin resistance).[42; 43]

56.7% (n=97,493) of the sample are of European ancestry, 17.4% (n=30,025) South

Asian, 9.6% (n=16,507) African American, 9.6% (n=16,440) Hispanic, 6.1%

(n=10,420) East Asian, and 0.7% (n=1,182) Samoan, based on genetically-estimated

and/or self-reported ancestry.

After sequencing, we observed 15.6 M variants across all studies; 5.0 M (32.6%) we

classified as transcript-altering coding variants based on an annotation of frameshift,

missense, nonsense, or splice site acceptor/donor using the Variant Effect Predictor

(VEP).[32] A total of 340,214 (6.7%) of the coding variants were annotated as high

confidence loss-of-function (LOF) using the LOFTEE VEP plugin,[33] 238,646 (4.7%)

as splice site altering identified by Splice AI,[36] 729,098 (14.3%) as damaging

missense as predicted by the MetaSVM algorithm[35], and 1,106,309 (21.8%) as

damaging missense as predicted by consensus in all five prediction algorithms (SIFT,

PolyPhen-2 HumVar, PolyPhen-2 HumDiv, MutationTaster and LRT).[34] As expected,

we observed a trend of decreasing proportions of putatively deleterious variants with

increasing allele count (**Figure S2, Table S3**).

Single-variant association

We performed inverse-variance weighted fixed-effects meta-analyses of single-

variant association results of LDL-C, non-HDL-C, TC, HDL-C, TG and TG:HDL ratio

from each consortium and ancestry group. Meta-analysis results were well controlled

with genomic inflation factors ranging between 1.01 and 1.04 (**Table S4**). Single-

variant results were limited to the 425,912 protein-altering coding variants with a total

minor allele count (MAC) > 20 across all 172,000 individuals. We defined significant

associations by a previously established exome-wide significance threshold for

coding variants ($P<4.3\times10^{-7}$)[37] which was additionally corrected for testing six traits

($P=4.3\times10^{-7}$ divided by 6) within all study samples or within each of the five major

ancestries (**Tables S5-S10**); this yielded in each analysis a significance threshold of

$P<7.2\times10^{-8}$. A total of 104 rare coding variants in 57 genes were associated with

LDL-C, 95 in 54 genes with non-HDL-C, 109 in 65 genes with TC, 92 in 56 genes

with HDL-C, 61 in 36 genes with TG, and 68 in 42 genes with TG:HDL. We identified

six missense variants in six genes (*TRIM5* p.Val112Phe, *ADH1B* p.His48Arg, *CHUK*

p.Val268Ile, *ERLIN1* p.Ile291Val, *TMEM136* p.Gly77Asp, *PPARA* p.Val227Ala) >1Mb

away from any index variant previously associated with a lipid phenotype (LDL-C,

HDL-C, TC, or TG) in previous genetic discovery efforts (**Tables S5-S10**).[3; 7; 8]

PPARA p.Val227Ala has previously been associated with blood lipids at a nominal

significance level in East Asians (P < 0.05), where it is more common than in other

ancestries.[44] Both *TRIM5* and *ADH1B* LDL-C increasing alleles have been

associated with higher risk of CAD in a recent GWAS from CARDIOGRAM (OR:

1.08, P=2×10^{-9}; OR=1.08, P=4×10^{-4}).[45] Single variant associations were further

performed in each of the five main ancestries (**Table S11**).

Gene-based association

Next we performed gene-based testing of transcript-altering variants in aggregated

burden and sequence kernel association tests (SKAT)[46] tests in all study participants

and within each of the six main ancestries for six lipid traits: LDL-C, HDL-C, non-

HDL-C, TC, TG, and TG:HDL. We excluded the Samoans from the single-ancestry

analysis given the small number of individuals. We limited attention to variants with

MAF≤1% for each of six variant groups: 1) LOF, 2) LOF and predicted splice-site

altering variants using Splice AI, 3) LOF and MetaSVM missense variants, 4) LOF,

MetaSVM missense and predicted splice-site altering variants, 5) LOF and damaging

5 out 5 missense variants, and 6) LOF, damaging 5 out 5 missense and predicted

splice-site altering variants. Meta-analyses results were well controlled (**Table S12**).

We identified 35 genes reaching exome-wide significance (P=4.3×10^{-7}) for at least

one of the six variant groupings (**Tables S13-S19**). Most of the significant results

were from the multi-ancestry analysis, with multiple ancestries contributing to the top

signals (**Figure 2A**) and most of the 35 genes were associated with more than one

lipid phenotype (**Figure 2B**). Ten of the 35 genes did not have prior evidence of

gene-based links with blood lipid phenotypes (**Table 1**), and seven genes, including

ALB, *SRSF2*, *CREB3L3*, *NR1H3*, *PLA2G12A*, *PPARG,* and *STAB1* have evidence

for a biological connection to circulating lipid levels (**Box 1**).

We performed a series of sensitivity analyses on our results. To determine whether

low frequency variants between 0.1%-1% frequency were driving our gene-based

association results, we performed the gene-based multi-ancestry meta-analyses

using a maximum MAF threshold of 0.1% instead of 1%. We observed exome-wide

significant associations (P<4.3×10^{-7}) for 29 genes using a 0.1% MAF threshold, all

observed in our primary analyses using a MAF threshold of 1% (**Table S20**). We then

intersected our 35 lipid associated genes from 85 gene-based associations observed

in the primary analysis with our results using a MAF threshold of 0.1%. All genes

remained at least nominally significant (P < 0.05) using a 0.1% MAF threshold,

except the *A1CF* and *TMEM136* associations (**Table S21**). Furthermore, we

determined whether those signals were driven by previously reported GWAS hits. We

identified a total of 7 HDL-C associated variants in 6 genes, 7 LDL-C variants in 3

genes, 3 TC variants in 1 gene and 7 TG variants in 6 genes that were previously

found to be genome-wide significant in MVP (**Table S22**).[8] Respective gene-based

analyses were repeated without those variants. Gene-based signals at *A1CF* and

BUD13 were lost after removal of 1 variant in each of those genes (**Table S23**).

The *JAK2* signal was further investigated after splitting the 136 contributing variants

into those annotated as somatic using the Catalogue Of Somatic Mutations In Cancer

(COSMIC) database and not annotated as a somatic variant. We observed an

association only among a set of 26 variants annotated as somatic while no

association was observed using the remaining 110 variants (**Table S24**).

We also determined which of the 35 genes were outside GWAS regions defined as

those within ±200kb flanking regions of GWAS indexed Single nucleotide

polymorphisms (SNPs) for TC (487 SNPs), LDL-C (531 SNPs), HDL-C, and TG (471

SNPs).[8] We identified 1,295 unique genes included in these lipid GWAS regions.

Eight out of the 35 associated genes (23%) were not within a GWAS region (**Table

S13**).

To understand whether the gene-based signals were driven by variants that could be

identified through single variant analyses, we looked at the proportion of the 35

genes that were associated with each trait that have at least one single contributing

variant that passed the genome-wide significance threshold of 5×10^{-8}. Seventeen

genes were associated with HDL-C at exome-wide significance (**Table S13**); eight

genes had at least one variant with $P<5\times10^{-8}$ (**Table S8**). Similarly, we observed 4/9

for LDL-C, 4/10 non-HDL-C, 4/14 TC, 7/18 TG, and 6/17 TG:HDL genes with at least

one genome-wide significant variant (**Tables S5-S10**).

For genes with both gene-based and single variant signals, we determined the

variants were driving these signals, and determined the single variant associations

for all variants contributing to the top 35 genes (**Table S25**). From a total of 85 gene-

based associations, 33 had at least one and 19 had only one single variant with

$P<5\times10^{-8}$ (**Tables S25 and S26**). All of the 19 had at least 2 variants passing

nominal significance (P<0.05) and 13 had at least 10 variants with P <0.05.

Comparison of gene-based associations across ancestries

Comparison of gene-based associations across ancestries

We determined the overlap between single variants included in gene-based signals

across the five main ancestries. A large proportion of variants from each ancestry did

not overlap with any other ancestry (**Figure S3**). For example, a total of 15 genes

were observed to have significant gene-based associations with HDL-C in multi-

ancestry meta-analyses. A total 69% of variants from European ancestry samples

that contributed to HDL-C gene-based associations did not overlap with any other

ancestry, and was 60% in South Asian-, 44% in African-, 40% in Hispanic- and 59%

in East Asian ancestry. When restricted to variants with $P<0.05$ in the multi-ancestry

meta-analysis, the overlap among ancestries increased (**Figure S4**). A total 57% of

variants from European ancestry did not overlap with any other ancestry, and was

42% in South Asian-, 20% in African-, 24% in Hispanic- and 34% in East Asian

ancestry. Finally, we determined the top single variant contributing to each gene-

based association (**Figure S5**). None of the top variants overlapped with all

ancestries and 80% of EUR variants did not overlap with any other ancestry, and was

87% in South Asian-, 93% in African-, 80% in Hispanic- and 93% in East Asian

ancestry.

But, the gene-based associations were mostly consistent across the six ancestry

groupings: European, South Asian, African, Hispanic, and East Asian. Three of the

17 HDL-C genes showed association in at least two different ancestries at exome-

wide significance level ($P=4.3\times10^{-7}$). Similarly, 3/9 LDL-C, 4/10 non-HDL-C, 5/14 TC,

2/18 TG and 2/17 TG:HDL genes showed association in at least two difference

ancestries at a exome-wide significance level. Using a less stringent significance

level (P<0.01), across the six lipid traits, 59-89% of associated genes from the joint

analysis were associated in at least two different ancestries.

We tested the top 35 genes for heterogeneity across all 303 gene-trait-variant

grouping combinations passing the exome-wide significance threshold ($P<4.3\times10^{-7}$).

We observed heterogeneity in effect estimates ($P_{Het}<1.7\times10^{-4}$, accounting for 303

combinations) in 19 (6%) different gene-trait-variant grouping combinations and in six

different genes: *LIPC*, *LPL*, *LCAT*, *ANGPTL3*, *APOB*, and *LDLR* (**Table S27**).

Although the LOF gene-based effect sizes were largely consistent across ancestries,

there were differences in the cumulative frequencies of LOF variants for several

genes including *PCSK9*, *NPC1L1*, *HBB* and *ABCG5* (**Figures S6-S8**).

We observed LOF and predicted damaging variants in the *TMEM136* gene

associated with TG and TG:HDL only among individuals of South Asian ancestry

($P_{SKAT}=3\times10^{-9}$ and 2×10^{-11}, respectively) (**Table 1**, **Figure 2A**). With the same variant

grouping and ancestry, we observed associations with reduced TG by burden tests

(β=-15%, $P=3\times10^{-4}$) and TG:HDL (β=-20%, $P=6\times10^{-5}$) (**Tables S18 and S19**).

Additionally, a single missense variant was associated only among South Asians

(rs760568794,11:120327605-G/A, p.Gly77Asp) with TG (β=-36.9%, $P=2\times10^{-8}$)

(**Table S9**). This variant was present only among South Asian (MAC=24) and

Hispanics (MAC=8), but showed no association among Hispanics (P=0.86). This

gene encodes a transmembrane protein of unknown function.

Replication of gene-based associations

We performed replication using the Penn Medicine BioBank (PMBB) and UK Biobank

samples that did not contribute to the initial analysis. In PMBB, we observed

associations at a nominal significance level (p<0.05) and in the same direction as the

discovery for 6 out of the 10 genes without prior evidence of gene-based links with

blood lipid phenotypes (*SRSF2, JAK2, CREB3L3, NR1H3, PLA2G12A, PPARG*) with

their respective blood lipids. For the gene *TMEM136*, we found an association of

nominal significance for TG and TG:HDL as well, but with a beta in the opposite and

positive direction. For the other 3 genes, *ALB, VARS, and STAB1*, we did not find

associations at a nominal significance level for their respective blood lipid traits

(**Table S28**). In UK Biobank, we found 7 of the 10 genes were associated at a

nominal significance level and in the same direction of effect as the discovery

analysis (ALB*, JAK2, CREB3L3, NR1H3, PLA2G12A, PPARG, STAB1*) (**Table S29**).

The only two genes that did not show evidence of replication in at least one of the

replication studies were *TMEM136* and *VARS*. This may indicate these associations

are false positives or that we lack power for replication for these associations. Our

replication studies did not include individuals of South Asian ancestry and we

observed that our association of *TMEM136* with TG and TG:HDL is driven by

individuals of South Asian ancestry.

Comparison of gene-based associations by case-status

We analyzed heterogeneity by CAD or T2D case status for the top 35 genes. The top

85 signals presented in **Table S13** determined in case-status specific meta-analyses

for CAD and T2D. Out of the 85 different gene-based associations, we observed

minimal heterogeneity in the results by case status. *LDLR*, *LCAT* and *LPL* showed

significant heterogeneity by CAD case status and *LCAT* and *ANGPTL4* by T2D

status ($P_{Het} < 6×10^{-4}$) (**Tables S30 and S31**).

Gene-based associations in GWAS loci

We determined whether genes near lipid array-based GWAS signals[8] were

associated with the corresponding lipid measure using gene-based tests of rare

variants with the same traits. We obtained genes from 200 Kb flanking regions on

both sides of each GWAS signal; 487 annotated to LDL-C GWAS signals, 531 to

HDL-C signals, and 471 to TG signals. We analyzed genes within these three sets

for gene-based associations with their associated traits. A total of 13, 19, and 13

genes were associated ($P<3.4×10^{-5}$, corrected for the number of genes tested for the

three traits) with LDL-C, HDL-C or TG, with 32 unique genes identified in the GWAS

loci (**Tables S32-S37)**.

Three of the 32 genes had no prior aggregate rare variant evidence of blood lipid

association. Variants annotated as LOF or predicted damaging in *EVI5* were

associated with LDL-C ($P_{SKAT}=2×10^{-5}$). The burden test showed association with

higher LDL-C levels (**β**=1.9 mg/dL, P=0.008) (**Table S32)**. Variants annotated as

LOF or predicted damaging in *SH2B3* were associated with lower HDL-C (**β**=-2.5

mg/dL, $P=1×10^{-6}$) among Europeans and variants that were annotated as LOF in

PLIN1 were associated with higher HDL-C (**β**=3.9 mg/dL, $P=1×10^{-5}$) (**Table S33**).

Other genes in the regions of *EVI5*, *SH2B3*, and *PLIN1* did not show an association

with the corresponding lipid traits (P>0.05) in multi-ancestry analyses. A previous

report implicated two heterozygous frameshift mutations in *PLIN1* in three families

with partial lipodystrophy.[47] The gene encodes perilipin, the most abundant protein

that coats adipocyte lipid droplets and is critical for optimal TG storage.[48] We

observed a nominal associations of *PLIN1* with TG (β=-7.0%, P=0.02). Our finding is

contrary to what would be expected with hypertriglyceridemia in a lipodystrophy

phenotype given the association with lower TG. This gene has an additional role

where silencing in cow adipocytes has been shown to inhibit TG synthesis and

promote lipolysis,[49] which may explain those contradictions.

Enrichment of Mendelian-, GWAS-, and drug targets genes

We next sought to test the utility of genes that showed some evidence for association

but did not reach exome-wide significance. Within the genes that reached a sub-

threshold level of significant association in this study using burden or SKAT tests (p <

0.005), we determined the enrichment of i) Mendelian dyslipidemia (N=21 genes)-;[2]

ii) lipid GWAS (N=487 for LDL-C, N=531 for HDL-C and N=471 for TG)[8]; and iii) drug

target genes (N=53).[41] We stratified genes in GWAS loci according to coding status

of the index SNP and proximity to the index SNP (nearest gene, second nearest

gene, and genes further away). We tested for enrichment of gene-based signals

(P<0.005) in the gene sets compared to matched genes (**Figure 3**). For each gene

within each gene set, the most significant association in the multi-ancestry or an

ancestry specific analysis was obtained and then matched to 10 genes based on

sample size, total number of variants, cumulative MAC, and variant grouping. The

strongest enrichment was observed for Mendelian dyslipidemia genes within the

genes that reached P < 0.005 in our study. For example, 52% of the HDL-C

Mendelian genes versus 1.4% of the matched set reached P < 0.005 (OR:71, 95%

CI: 16-455). We also observed that 45.5% of the set of genes closest to an HDL-C

protein-altering GWAS variant reached P < 0.005 versus 1.4% in the matched gene

set (OR:57, 95% CI: 13-362). Results were significant but much less striking for

genes at non-coding index variants. We observed that 8.9% of the set of genes

closest to an HDL-C non-protein altering GWAS variant reached P < 0.005 versus

2.3% in the matched set (OR:4.1, 95% CI: 1.8-8.7). While 8% of the set of genes in

the second closest to an HDL-C non-protein altering GWAS variant reached P

<0.005 versus 2.6% in the matched set (OR: 3, 95% CI: 1.1-8.3). There was no

significant enrichment in second closest or >= third closest genes to protein altering

GWAS signals and in >= third closest genes to non-protein altering GWAS signals.

Drug target genes were significantly enriched in LDL-C gene-based associations

(OR: 5.3, 95% CI: 1.4-17.8) but not in TG (OR: 2.2, 95% CI: 0.2-11.2) or HDL-C (OR:

1.0, 95% CI: 0.1-4.3) (**Figure 3 and Tables S38-S41**).

Association of lipid genes with CAD, T2D, glycemic traits, and liver enzymes

We tested the genes identified through our main (35 genes) and GWAS loci (32

genes) for associations with CAD or T2D in our gene-based analyses (40 genes

across the two sets). The CAD analyses were restricted to a subset of the overall

exome sequence data with information on CAD status which included the MIGen

CAD case-control, UK Biobank (UKB) CAD nested case-control, and the UKB cohort

with a total of 32,981 cases and 79,879 controls. We observed four genes

significantly associated with CAD (P_{CAD}<0.00125, corrected for 40 genes). The four

genes associated with lipids and CAD were all primarily associated with LDL-C:

LDLR (OR: 2.97, P=7×10^{-24}), *APOB* (P_{SKAT}=4×10^{-5}), *PCSK9* (OR: 0.5, P=2×10^{-4}) and

JAK2 (P_{SKAT}=0.001). Several other known CAD associated genes (*NPC1L1*, *CETP*,

APOC3, and *LPL*) showed nominal significance for association with lipids (P<0.05).

We observed nominal associations with CAD for two of the newly-identified lipid

genes: *PLIN1* (P_{SKAT}=0.002) and *EVI5* (OR: 1.29, P=0.002; **Table S42**). None of the

40 lipid genes reached significance for association with T2D in the latest AMP-T2D

exome sequence results. We observed nominal associations of T2D with *STAB1*

(OR: 1.05, P_{T2D}=0.002) and *APOB* (OR: 1.08, P_{T2D}=0.005) (**Table S43**).[15]

We additionally tested the 40 genes for association with six glycemic and liver

biomarkers in the UKB: blood glucose, HbA1c, alanine aminotransferase (ALT),

aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and albumin

(**Tables S44-S49**). Using an exome-wide significance threshold of P=0.0012, we

found associations between *PDE3B* and elevated blood glucose, *JAK2* and *SH2B3*

and lower HbA1c, and *APOC3* and higher HbA1c. We found associations between

CREB3L3 and lower ALT, ALB, and higher AST, and between *A1CF* and higher

GGT. *ALB* and *SRSF2* were associated with lower and higher albumin levels,

respectively (**Tables S44-S49**).

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Discussion

We conducted a large multi-ethnic study to identify genes in which protein-altering

variants demonstrated association with blood lipid levels. First, we confirm previous

associations of genes with blood lipid levels and show that we detect associations

across multiple ancestries. Second, we identified gene-based associations that were

not observed previously. Third, we show that along with Mendelian lipid genes, the

genes closest to both protein altering and non-protein altering GWAS signals, and

LDL-C drug target genes have the highest enrichment of gene-based associations.

Fourth, of the new gene-based lipid associations, *PLIN1* and *EVI5* showed

suggestive evidence of an association with CAD.

1012

Our study found that evidence of gene-based associations for the same gene in

multiple ancestries. The heterogeneity in genetic association with common traits and

complex diseases has been discussed extensively. A recent study has shown

significant heterogeneity across different ancestries in the effect sizes of multiple

GWAS identified variants.[50] However, our study shows that gene-based signals are

detected in multiple ancestries with limited heterogeneity in the effect sizes.

Our study highlights enrichment of gene-based associations for Mendelian

dyslipidemia genes, genes with protein-altering variants identified by GWAS, and

genes that are closest to non-protein altering GWAS index variants. A previous

transcriptome-wide Mendelian randomization study of eQTL variants indicated that

most of the genes closest to top GWAS signals (>71%) do not show significant

association with the respective phenotype.[51] In contrast, our study provides evidence

from sequence data that the closest gene to each top non-coding GWAS signal is

most likely to be the causal one, indicating an allelic series in associated loci. This

has implications for GWAS results, suggesting the prioritization of the closest genes

for follow-up studies. We also observed enrichment of drug target genes only among

LDL-C gene-based associations and not for HDL-C and TG gene-based

associations, consistent with the fact that most approved therapeutics for

cardiovascular disease targeting LDL-C

1032

The gene-based analyses of lipid genes with CAD confirmed previously reported and

known associations (*LDLR*, *APOB,* and *PCSK9).* Using a nominal P threshold of 0.05

we also confirmed associations with *NPC1L1*, *CETP*, *APOC3*, and *LPL*. Of the novel

lipid genes, we observed borderline significant signals with *EVI5* and higher risk of

CAD and between *PLIN1* and lower risk of CAD. The putative cardio-protective role

of PLIN1 deficiency is supported by previous evidence in mice which has indicated

reduced atherosclerotic lesions with Plin1 deficiency in bone marrow derived cells.[52]

This suggests PLIN1 as a putative target for CAD prevention; however, replication of

the CAD association would be needed to confirm those signals.

1042

There are limitations to our results. First, we had lower sample sizes for the non-

European ancestries, limiting our power to detect ancestry-specific associations, and

detect replication for *TMEM136* that was driven by a variant in South Asians.

However, we find consistency of results across ancestries, and when we relax our

significance threshold, the majority of associations (59-89%) are observed in more

than one ancestry. Second, it has been reported that there was an issue with the

UKB functionally equivalent WES calling.[53] This mapping issue may have resulted in

under-calling alternative alleles and therefore should not increase false positive

findings. Third, we relied on a meta-analysis approach using summary statistics to

perform our gene-based testing due to differences in sequencing platforms and

genotyping calling within the multiple consortia contributing to the results. This

approach has been shown to be equivalent to a pooled approach for continuous

outcomes.[38]

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1057 In summary, we demonstrated association between rare protein-altering variants with

1058 circulating lipid levels in >170,000 individuals of diverse ancestries. We identified 35

1059 genes associated with blood lipids, including ten genes not previously shown to have

1060 gene-based signals. Our results support the hypothesis that genes closest to a

1061 GWAS index SNP are enriched for evidence of association.

Supplemental data

Supplemental data includes in 8 figures, 49 tables, Study Descriptions, and Banner

Authors.

Declaration of Interests

The authors declare no competing interests for the present work. PN reports

investigator-initiated grants from Amgen, Apple, and Boston Scientific, is a scientific

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1173

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1189   **Data and code availability**

1190   Controlled access of the individual-level data are available through dbGAP (please

1191   refer to the Supplementary Information), and the individual-level UK Biobank data are

1192   available upon application to the UK Biobank

References

References

1. Emerging Risk Factors Collaboration, Di Angelantonio, E., Sarwar, N., Perry, P.,

Kaptoge, S., Ray, K.K., Thompson, A., Wood, A.M., Lewington, S., Sattar, N., et al.

(2009). Major lipids, apolipoproteins, and risk of vascular disease. JAMA 302, 1993-

2000.

2. Teslovich, T.M., Musunuru, K., Smith, A.V., Edmondson, A.C., Stylianou, I.M.,

Koseki, M., Pirruccello, J.P., Ripatti, S., Chasman, D.I., Willer, C.J., et al. (2010).

Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466,

707-713.

3. Willer, C.J., Schmidt, E.M., Sengupta, S., Peloso, G.M., Gustafsson, S., Kanoni,

S., Ganna, A., Chen, J., Buchkovich, M.L., Mora, S., et al. (2013). Discovery and

refinement of loci associated with lipid levels. Nat Genet 45, 1274-1283.

4. Chasman, D.I., Pare, G., Mora, S., Hopewell, J.C., Peloso, G., Clarke, R.,

Cupples, L.A., Hamsten, A., Kathiresan, S., Malarstig, A., et al. (2009). Forty-three

loci associated with plasma lipoprotein size, concentration, and cholesterol content in

genome-wide analysis. PLoS genetics 5, e1000730.

5. Peloso, G.M., Auer, P.L., Bis, J.C., Voorman, A., Morrison, A.C., Stitziel, N.O.,

Brody, J.A., Khetarpal, S.A., Crosby, J.R., Fornage, M., et al. (2014). Association of

low-frequency and rare coding-sequence variants with blood lipids and coronary

heart disease in 56,000 whites and blacks. Am J Hum Genet 94, 223-232.

6. Asselbergs, F.W., Guo, Y., van Iperen, E.P., Sivapalaratnam, S., Tragante, V.,

Lanktree, M.B., Lange, L.A., Almoguera, B., Appelman, Y.E., Barnard, J., et al.

(2012). Large-scale gene-centric meta-analysis across 32 studies identifies multiple

lipid loci. Am J Hum Genet 91, 823-838.

7. Liu, D.J., Peloso, G.M., Yu, H., Butterworth, A.S., Wang, X., Mahajan, A.,

Saleheen, D., Emdin, C., Alam, D., Alves, A.C., et al. (2017). Exome-wide

association study of plasma lipids in >300,000 individuals. Nat Genet 49, 1758-1766.

8. Klarin, D., Damrauer, S.M., Cho, K., Sun, Y.V., Teslovich, T.M., Honerlaw, J.,

Gagnon, D.R., DuVall, S.L., Li, J., Peloso, G.M., et al. (2018). Genetics of blood lipids

among ~300,000 multi-ethnic participants of the Million Veteran Program. Nat Genet

50, 1514-1523.

9. Voight, B.F., Peloso, G.M., Orho-Melander, M., Frikke-Schmidt, R., Barbalic, M.,

Jensen, M.K., Hindy, G., Holm, H., Ding, E.L., Johnson, T., et al. (2012). Plasma

HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study.

Lancet 380, 572-580.

10. Do, R., Willer, C.J., Schmidt, E.M., Sengupta, S., Gao, C., Peloso, G.M.,

Gustafsson, S., Kanoni, S., Ganna, A., Chen, J., et al. (2013). Common variants

associated with plasma triglycerides and risk for coronary artery disease. Nature

genetics 45, 1345-1352.

11. Hindy, G., Engstrom, G., Larsson, S.C., Traylor, M., Markus, H.S., Melander, O.,

Orho-Melander, M., and Stroke Genetics, N. (2018). Role of Blood Lipids in the

Development of Ischemic Stroke and its Subtypes: A Mendelian Randomization

Study. Stroke 49, 820-827.

12. Smith, J.G., Luk, K., Schulz, C.A., Engert, J.C., Do, R., Hindy, G., Rukh, G.,

Dufresne, L., Almgren, P., Owens, D.S., et al. (2014). Association of low-density

lipoprotein cholesterol-related genetic variants with aortic valve calcium and incident

aortic stenosis. JAMA 312, 1764-1771.

13. Afshar, M., Luk, K., Do, R., Dufresne, L., Owens, D.S., Harris, T.B., Peloso, G.M.,

Kerr, K.F., Wong, Q., Smith, A.V., et al. (2017). Association of Triglyceride-Related

Genetic Variants With Mitral Annular Calcification. J Am Coll Cardiol 69, 2941-2948.

14. Dewey, F.E., Murray, M.F., Overton, J.D., Habegger, L., Leader, J.B., Fetterolf,

S.N., O'Dushlaine, C., Van Hout, C.V., Staples, J., Gonzaga-Jauregui, C., et al.

(2016). Distribution and clinical impact of functional variants in 50,726 whole-exome

sequences from the DiscovEHR study. Science 354.

15. Flannick, J., Mercader, J.M., Fuchsberger, C., Udler, M.S., Mahajan, A., Wessel,

J., Teslovich, T.M., Caulkins, L., Koesterer, R., Barajas-Olmos, F., et al. (2019).

Exome sequencing of 20,791 cases of type 2 diabetes and 24,440 controls. Nature

570, 71-76.

16. Do, R., Stitziel, N.O., Won, H.H., Jorgensen, A.B., Duga, S., Angelica Merlini, P.,

Kiezun, A., Farrall, M., Goel, A., Zuk, O., et al. (2015). Exome sequencing identifies

rare LDLR and APOA5 alleles conferring risk for myocardial infarction. Nature 518,

102-106.

17. Pollin, T.I., Damcott, C.M., Shen, H., Ott, S.H., Shelton, J., Horenstein, R.B.,

Post, W., McLenithan, J.C., Bielak, L.F., Peyser, P.A., et al. (2008). A null mutation in

human APOC3 confers a favorable plasma lipid profile and apparent

cardioprotection. Science 322, 1702-1705.

18. TG and HDL Working Group of the Exome Sequencing Project, National Heart,

Lung, and Blood Institute, Crosby, J., Peloso, G.M., Auer, P.L., Crosslin, D.R.,

Stitziel, N.O., Lange, L.A., Lu, Y., et al. (2014). Loss-of-function mutations in APOC3,

triglycerides, and coronary disease. N Engl J Med 371, 22-31.

19. Jorgensen, A.B., Frikke-Schmidt, R., Nordestgaard, B.G., and Tybjaerg-Hansen,

A. (2014). Loss-of-function mutations in APOC3 and risk of ischemic vascular

disease. The New England journal of medicine 371, 32-41.

20. Musunuru, K., Pirruccello, J.P., Do, R., Peloso, G.M., Guiducci, C., Sougnez, C.,

Garimella, K.V., Fisher, S., Abreu, J., Barry, A.J., et al. (2010). Exome sequencing,

ANGPTL3 mutations, and familial combined hypolipidemia. The New England journal

of medicine 363, 2220-2227.

21. Dewey, F.E., Gusarova, V., Dunbar, R.L., O'Dushlaine, C., Schurmann, C.,

Gottesman, O., McCarthy, S., Van Hout, C.V., Bruse, S., Dansky, H.M., et al. (2017).

Genetic and Pharmacologic Inactivation of ANGPTL3 and Cardiovascular Disease. N

Engl J Med 377, 211-221.

22. Dewey, F.E., Gusarova, V., O'Dushlaine, C., Gottesman, O., Trejos, J., Hunt, C.,

Van Hout, C.V., Habegger, L., Buckler, D., Lai, K.M., et al. (2016). Inactivating

Variants in ANGPTL4 and Risk of Coronary Artery Disease. N Engl J Med 374, 1123-

1133.

23. Cohen, J., Pertsemlidis, A., Kotowski, I.K., Graham, R., Garcia, C.K., and Hobbs,

H.H. (2005). Low LDL cholesterol in individuals of African descent resulting from

frequent nonsense mutations in PCSK9. Nature genetics 37, 161-165.

24. Cohen, J.C., Boerwinkle, E., Mosley, T.H., Jr., and Hobbs, H.H. (2006).

Sequence variations in PCSK9, low LDL, and protection against coronary heart

disease. The New England journal of medicine 354, 1264-1272.

25. Kathiresan, S., and Myocardial Infarction Genetics, C. (2008). A PCSK9

missense variant associated with a reduced risk of early-onset myocardial infarction.

The New England journal of medicine 358, 2299-2300.

26. Sabatine, M.S., Giugliano, R.P., Keech, A.C., Honarpour, N., Wiviott, S.D.,

Murphy, S.A., Kuder, J.F., Wang, H., Liu, T., Wasserman, S.M., et al. (2017).

Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. The

New England journal of medicine 376, 1713-1722.

27. Gaudet, D., Alexander, V.J., Baker, B.F., Brisson, D., Tremblay, K., Singleton,

W., Geary, R.S., Hughes, S.G., Viney, N.J., Graham, M.J., et al. (2015). Antisense

Inhibition of Apolipoprotein C-III in Patients with Hypertriglyceridemia. N Engl J Med

373, 438-447.

28. Zhan, X., Hu, Y., Li, B., Abecasis, G.R., and Liu, D.J. (2016). RVTESTS: an

efficient and comprehensive tool for rare variant association analysis using sequence

data. Bioinformatics 32, 1423-1426.

29. Jun, G., Flickinger, M., Hetrick, K.N., Romm, J.M., Doheny, K.F., Abecasis, G.R.,

Boehnke, M., and Kang, H.M. (2012). Detecting and estimating contamination of

human DNA samples in sequencing and array-based genotype data. Am J Hum

Genet 91, 839-848.

30. Van Hout, C.V., Tachmazidou, I., Backman, J.D., Hoffman, J.X., Ye, B., Pandey,

A.K., Gonzaga-Jauregui, C., Khalid, S., Liu, D., Banerjee, N., et al. (2019). Whole

exome sequencing and characterization of coding variation in 49,960 individuals in

the UK Biobank. bioRxiv, 572347.

31. Regier, A.A., Farjoun, Y., Larson, D.E., Krasheninina, O., Kang, H.M., Howrigan,

D.P., Chen, B.J., Kher, M., Banks, E., Ames, D.C., et al. (2018). Functional

equivalence of genome sequencing analysis pipelines enables harmonized variant

calling across human genetics projects. Nat Commun 9, 4038.

32. McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R., Thormann, A., Flicek,

P., and Cunningham, F. (2016). The Ensembl Variant Effect Predictor. Genome Biol

17, 122.

33. Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alfoldi, J., Wang, Q.,

Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2020). The

mutational constraint spectrum quantified from variation in 141,456 humans. Nature

581, 434-443.

34. Liu, X., Wu, C., Li, C., and Boerwinkle, E. (2016). dbNSFP v3.0: A One-Stop

Database of Functional Predictions and Annotations for Human Nonsynonymous and

Splice-Site SNVs. Hum Mutat 37, 235-241.

35. Dong, C., Wei, P., Jian, X., Gibbs, R., Boerwinkle, E., Wang, K., and Liu, X.

(2015). Comparison and integration of deleteriousness prediction methods for

nonsynonymous SNVs in whole exome sequencing studies. Hum Mol Genet 24,

2125-2137.

36. Jaganathan, K., Kyriazopoulou Panagiotopoulou, S., McRae, J.F., Darbandi,

S.F., Knowles, D., Li, Y.I., Kosmicki, J.A., Arbelaez, J., Cui, W., Schwartz, G.B., et al.

(2019). Predicting Splicing from Primary Sequence with Deep Learning. Cell 176,

535-548 e524.

37. Sveinbjornsson, G., Albrechtsen, A., Zink, F., Gudjonsson, S.A., Oddson, A.,

Masson, G., Holm, H., Kong, A., Thorsteinsdottir, U., Sulem, P., et al. (2016).

Weighting sequence variants based on their annotation increases power of whole-

genome association studies. Nat Genet 48, 314-317.

38. Liu, D.J., Peloso, G.M., Zhan, X., Holmen, O.L., Zawistowski, M., Feng, S.,

Nikpay, M., Auer, P.L., Goel, A., Zhang, H., et al. (2014). Meta-analysis of gene-level

tests for rare variant association. Nat Genet 46, 200-204.

39. Wu, M.C., Lee, S., Cai, T., Li, Y., Boehnke, M., and Lin, X. (2011). Rare-variant

association testing for sequencing data with the sequence kernel association test.

Am J Hum Genet 89, 82-93.

40. Li, X., Li, Z., Zhou, H., Gaynor, S.M., Liu, Y., Chen, H., Sun, R., Dey, R., Arnett,

D.K., Aslibekyan, S., et al. (2020). Dynamic incorporation of multiple in silico

functional annotations empowers rare variant association analysis of large whole-

genome sequencing studies at scale. Nat Genet 52, 969-983.

41. Wishart, D.S., Feunang, Y.D., Guo, A.C., Lo, E.J., Marcu, A., Grant, J.R., Sajed,

T., Johnson, D., Li, C., Sayeeda, Z., et al. (2018). DrugBank 5.0: a major update to

the DrugBank database for 2018. Nucleic Acids Res 46, D1074-D1082.

42. McLaughlin, T., Abbasi, F., Cheal, K., Chu, J., Lamendola, C., and Reaven, G.

(2003). Use of metabolic markers to identify overweight individuals who are insulin

resistant. Ann Intern Med 139, 802-809.

43. Li, C., Ford, E.S., Meng, Y.X., Mokdad, A.H., and Reaven, G.M. (2008). Does the

association of the triglyceride to high-density lipoprotein cholesterol ratio with fasting

serum insulin differ by race/ethnicity? Cardiovasc Diabetol 7, 4.

44. Chan, E., Tan, C.S., Deurenberg-Yap, M., Chia, K.S., Chew, S.K., and Tai, E.S.

(2006). The V227A polymorphism at the PPARA locus is associated with serum lipid

concentrations and modulates the association between dietary polyunsaturated fatty

acid intake and serum high density lipoprotein concentrations in Chinese women.

Atherosclerosis 187, 309-315.

45. van der Harst, P., and Verweij, N. (2018). Identification of 64 Novel Genetic Loci

Provides an Expanded View on the Genetic Architecture of Coronary Artery Disease.

Circ Res 122, 433-443.

46. Wu, M.C., Lee, S., Cai, T.X., Li, Y., Boehnke, M., and Lin, X.H. (2011). Rare-

Variant Association Testing for Sequencing Data with the Sequence Kernel

Association Test. American Journal of Human Genetics 89, 82-93.

47. Gandotra, S., Le Dour, C., Bottomley, W., Cervera, P., Giral, P., Reznik, Y.,

Charpentier, G., Auclair, M., Delepine, M., Barroso, I., et al. (2011). Perilipin

deficiency and autosomal dominant partial lipodystrophy. N Engl J Med 364, 740-

748.

48. Brasaemle, D.L., Subramanian, V., Garcia, A., Marcinkiewicz, A., and

Rothenberg, A. (2009). Perilipin A and the control of triacylglycerol metabolism. Mol

Cell Biochem 326, 15-21.

49. Zhang, S., Liu, G., Xu, C., Liu, L., Zhang, Q., Xu, Q., Jia, H., Li, X., and Li, X.

(2018). Perilipin 1 Mediates Lipid Metabolism Homeostasis and Inhibits Inflammatory

Cytokine Synthesis in Bovine Adipocytes. Front Immunol 9, 467.

50. Wojcik, G.L., Graff, M., Nishimura, K.K., Tao, R., Haessler, J., Gignoux, C.R.,

Highland, H.M., Patel, Y.M., Sorokin, E.P., Avery, C.L., et al. (2019). Genetic

analyses of diverse populations improves discovery for complex traits. Nature 570,

514-518.

51. Porcu, E., Rüeger, S., Lepik, K., Agbessi, M., Ahsan, H., Alves, I., Andiappan, A.,

Arindrarto, W., Awadalla, P., Battle, A., et al. (2019). Mendelian randomization

integrating GWAS and eQTL data reveals genetic determinants of complex and

clinical traits. Nature Communications 10, 3300.

52. Zhao, X., Gao, M., He, J., Zou, L., Lyu, Y., Zhang, L., Geng, B., Liu, G., and Xu,

G. (2015). Perilipin1 deficiency in whole body or bone marrow-derived cells

attenuates lesions in atherosclerosis-prone mice. PLoS One 10, e0123738.

1383 53. Jia, T.Q., Munson, B., Allen, H.L., Ideker, T., and Majithia, A.R. (2020).

1384 Thousands of missing variants in the UK Biobank are recoverable by genome

1385 realignment. Ann Hum Genet 84, 214-220.

1386 54. Minchiotti, L., Galliano, M., Caridi, G., Kragh-Hansen, U., and Peters, T., Jr.

1387 (2013). Congenital analbuminaemia: molecular defects and biochemical and clinical

1388 aspects. Biochim Biophys Acta 1830, 5494-5502.

1389 55. Koot, B.G., Houwen, R., Pot, D.J., and Nauta, J. (2004). Congenital

1390 analbuminaemia: biochemical and clinical implications. A case report and literature

1391 review. Eur J Pediatr 163, 664-670.

1392 56. Cheng, Y., Luo, C., Wu, W., Xie, Z., Fu, X., and Feng, Y. (2016). Liver-Specific

1393 Deletion of SRSF2 Caused Acute Liver Failure and Early Death in Mice. Mol Cell Biol

1394 36, 1628-1638.

1395 57. Cefalu, A.B., Spina, R., Noto, D., Valenti, V., Ingrassia, V., Giammanco, A.,

1396 Panno, M.D., Ganci, A., Barbagallo, C.M., and Averna, M.R. (2015). Novel CREB3L3

1397 Nonsense Mutation in a Family With Dominant Hypertriglyceridemia. Arterioscler

1398 Thromb Vasc Biol 35, 2694-2699.

1399 58. Lee, J.H., Giannikopoulos, P., Duncan, S.A., Wang, J., Johansen, C.T., Brown,

1400 J.D., Plutzky, J., Hegele, R.A., Glimcher, L.H., and Lee, A.H. (2011). The

1401 transcription factor cyclic AMP-responsive element-binding protein H regulates

1402 triglyceride metabolism. Nature medicine 17, 812-815.

1403 59. Dron, J.S., Dilliott, A.A., Lawson, A., McIntyre, A.D., Davis, B.D., Wang, J., Cao,

1404 H., Movsesyan, I., Malloy, M.J., Pullinger, C.R., et al. (2020). Loss-of-Function

1405 CREB3L3 Variants in Patients With Severe Hypertriglyceridemia. Arterioscler

1406 Thromb Vasc Biol 40, 1935-1941.

60. Becares, N., Gage, M.C., Voisin, M., Shrestha, E., Martin-Gutierrez, L., Liang, N.,

Louie, R., Pourcet, B., Pello, O.M., Luong, T.V., et al. (2019). Impaired LXRalpha

Phosphorylation Attenuates Progression of Fatty Liver Disease. Cell Rep 26, 984-

995 e986.

61. Zhao, C., and Dahlman-Wright, K. (2010). Liver X receptor in cholesterol

metabolism. J Endocrinol 204, 233-240.

62. Hong, C., and Tontonoz, P. (2014). Liver X receptors in lipid metabolism:

opportunities for drug discovery. Nat Rev Drug Discov 13, 433-444.

63. Jaworski, K., Ahmadian, M., Duncan, R.E., Sarkadi-Nagy, E., Varady, K.A.,

Hellerstein, M.K., Lee, H.Y., Samuel, V.T., Shulman, G.I., Kim, K.H., et al. (2009).

AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding

or leptin deficiency. Nat Med 15, 159-168.

64. Quach, N.D., Arnold, R.D., and Cummings, B.S. (2014). Secretory phospholipase

A2 enzymes as pharmacological targets for treatment of disease. Biochem

Pharmacol 90, 338-348.

65. Barroso, I., Gurnell, M., Crowley, V.E., Agostini, M., Schwabe, J.W., Soos, M.A.,

Maslen, G.L., Williams, T.D., Lewis, H., Schafer, A.J., et al. (1999). Dominant

negative mutations in human PPARgamma associated with severe insulin resistance,

diabetes mellitus and hypertension. Nature 402, 880-883.

66. Agostini, M., Schoenmakers, E., Mitchell, C., Szatmari, I., Savage, D., Smith, A.,

Rajanayagam, O., Semple, R., Luan, J., Bath, L., et al. (2006). Non-DNA binding,

dominant-negative, human PPARgamma mutations cause lipodystrophic insulin

resistance. Cell Metab 4, 303-311.

67. Majithia, A.R., Flannick, J., Shahinian, P., Guo, M., Bray, M.A., Fontanillas, P.,

Gabriel, S.B., Go, T.D.C., Project, N.J.F.A.S., Consortium, S.T.D., et al. (2014). Rare

variants in PPARG with decreased activity in adipocyte differentiation are associated

with increased risk of type 2 diabetes. Proc Natl Acad Sci U S A 111, 13127-13132.

68. Goerdt, S., Walsh, L.J., Murphy, G.F., and Pober, J.S. (1991). Identification of a

novel high molecular weight protein preferentially expressed by sinusoidal endothelial

cells in normal human tissues. J Cell Biol 113, 1425-1437.

69. Li, R., Oteiza, A., Sorensen, K.K., McCourt, P., Olsen, R., Smedsrod, B., and

Svistounov, D. (2011). Role of liver sinusoidal endothelial cells and stabilins in

elimination of oxidized low-density lipoproteins. Am J Physiol Gastrointest Liver

Physiol 300, G71-81.

Figure titles and legends

Figure 1. Study samples and design

Flow chart of the different stages of the study. Exome sequence genotypes were derived from four major data sources: The Myocardial Infarction Genetics consortium (MIGen), the Trans-Omics from Precision Medicine (TOPMed), the UK Biobank and the Type 2 Diabetes Genetics (AMP-T2D-GENES) consortium. Single-variant association analyses were performed by ancestry and case-status in case-control studies and meta-analyzed. Single-variant summary estimates and covariance matrices were used in gene-based analyses using 6 different variant groups and in multi-ancestry and each of the five main ancestries. AFR=African ancestry, EAS=East Asian ancestry, EUR=European ancestry, HIS=Hispanic ancestry, SAM=Samoan ancestry, SAS=South Asian ancestry

Figure 2. Exome-wide significant associations with blood lipid phenotypes

A) Circular plot highlighting the evidence of association between the exome-wide significant 35 genes with any of the six different lipid traits ($P < 4.3 \times 10^{-7}$). The most significant associations from any of the six different variant groups are plotted. For almost all of the genes the most significant associations were obtained from the multi-ancestry meta-analysis. **B)** Strength of association of the 35 exome-wide significant genes based on the most significant variant grouping and ancestry across the six lipid phenotypes studied. Most of the genes indicated associations with more than one phenotype. Sign(beta)*-log10(p) displayed for associations that reached a P $< 4.3 \times 10^{-7}$. When the Sign(beta)*-log10(p) > 50, they were trimmed to 50.

1465   **Figure 3. Enrichment of Mendelian, GWAS, and drug target genes in the gene-**

1466   **based lipid associations**

1467   Enrichment of gene sets of Mendelian genes (n=21), GWAS loci for LDL-C (n=487),

1468   HDL-C (n=531), and triglycerides (TG) (n=471) genes and drug target genes (n=53).

1469

Table 1. Novel Genes Associated with Blood Lipids

Gene	Name	Trait	N	cMAC	nVAR	beta	se	P	Mask	Test	Ancestry
ALB	Albumin	LDL-C	165,003	51	18	29.51	5.11	7.76E-09	LOF	Burden	Multi-ancestry
		Non-HDL-C	166,327	50	17	33.91	6.07	2.27E-08	LOF	Burden	Multi-ancestry
		TC	172,103	54	18	33.37	5.89	1.48E-08	LOF	Burden	Multi-ancestry
SRSF2	Serine And Arginine Rich Splicing Factor 2	TC	172,103	59	14	-30.59	5.49	2.46E-08	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
JAK2	Janus Kinase 2	TC	975,33	441	136	-7.10	1.98	1.71E-07	LOF/DAM5of5/SPLICE AI	SKAT	EUR
CREB3L3	CAMP Responsive Element Binding Protein 3 Like 3	TG	1702,39	874	71	0.12	0.02	2.43E-15	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
		TG/HDLC	165,380	855	69	0.14	0.02	5.76E-13	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
TMEM136	Transmembrane Protein 136	TG	29,571	157	24	-0.15	0.04	3.39E-09	LOF/DAM5of5/SPLICE AI	SKAT	SAS
		TG/HDLC	29,517	157	24	-0.20	0.05	1.76E-11	LOF/DAM5of5/SPLICE AI	SKAT	SAS
VARS	Valyl-TRNA Synthetase 1	TG	56,140	67	51	0.32	0.06	4.30E-07	LOF/MetaSVM	Burden	EUR
NR1H3	Nuclear Receptor Subfamily 1 Group H Member 3	HDLC	93,044	521	111	3.47	0.60	1.45E-11	LOF/MetaSVM/SPLICE AI	SKAT	EUR
PLA2G12A	Phospholipase A2 Group XIIA	HDLC	166,441	1975	47	-2.28	0.31	8.12E-14	LOF/DAM5of5	Burden	Multi-ancestry
		TG	170,239	2047	47	0.06	0.01	1.17E-08	LOF/DAM5of5	Burden	Multi-ancestry
		TG/HDLC	165,380	1969	46	0.11	0.01	7.56E-13	LOF/DAM5of5	Burden	Multi-ancestry
PPARG	Peroxisome Proliferator Activated Receptor Gamma	HDLC	166,441	147	72	-6.24	1.07	4.71E-09	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
STAB1	Stabilin 1	HDLC	166,441	6550	804	0.83	0.16	2.58E-07	LOF/MetaSVM/SPLICE AI	Burden	Multi-ancestry

cMAC=cumulative minor allele count; nVAR=number of variants in test; AFR=African ancestry, EAS=East Asian ancestry, EUR=European ancestry, HIS=Hispanic ancestry, SAS=South Asian ancestry.

Box 1. Genes with biological links to lipid metabolism

ALB	The association between mutations in the albumin gene and elevated cholesterol levels has been previously observed in rare cases of congenital analbuminemia.[54] This has been mainly suggested to result from compensatory increases in hepatic production of other non-albumin plasma proteins to maintain colloid osmotic pressure particularly apolipoprotein B-100 leading to elevations in TC and LDL-C but normal HDL-C levels – which is consistent with our findings – although the exact mechanisms remain uncertain.[55] A lipodystrophy-like phenotype has also been linked to analbuminemia which is consistent with the suggestive tendency for increased risk of T2D with LOF and predicted damaging variants in albumin in the population (OR=1.85; P=0.007) (**Supplementary Table 30**).
SRSF2	The *SRSF2* gene encodes a highly conserved serine/arginine-rich splicing factor and has previously been linked to acute liver failure in liver-specific knockout in mice with accumulation of TC in the mutant liver.[56] Thus, this gene could be linked to a non-alcoholic fatty liver phenotype with accumulation of lipids in the liver as observed with other genes as *PNPLA3* and *TM6SF2*.[7] Therefore, we looked at association with liver function markers and we found an association between *SRSF2* and higher albumin levels $(P = 1 \times 10^{-4})$ and a suggestive tendency for higher gamma glutamyl transferase (GGT) $(P = 0.05)$, consistent with potential liver involvement (**Supplementary Table 33-36**).
CREB3L3	The association between *CREB3L3* and higher TG supports previous evidence from a single family and cohorts with severe hypertriglyceridemia but not sufficient evidence to be classified as a Mendelian lipid gene (ref).[57-59] This has been additionally supported by functional studies where *Creb3l3* knockout mice showed hypertriglyceridemia partly due to deficient expression of lipoprotein lipase coactivators (*Apoc2*, *Apoa4*, and *Apoa5*) and increased expression of activator *Apoc3*.[58]
NR1H3	The observed association of *NR1H3* with higher HDL-C and lower TG is supported by previous evidence of a role in non-alcoholic fatty liver disease in mice.[60] This gene encodes a liver X receptor alpha (LXRα) which is a nuclear receptor that acts as a cholesterol sensor and protects from cholesterol overload.[61; 62] It has previously been shown that disrupting the LXRα phosphorylation at Ser196 in mice prevents non-alcoholic fatty liver disease.[60]
PLA2G12A	*PLA2G12A* is in the secretory phospholipase A2 (sPLA$_2$) family, which liberates fatty acids in the -sn2 position of phospholipids. This pattern suggests a previously unreported possible lipolytic role of this phospholipase in a manner similar to another member of the adipose-specific phospholipases, *PLA2G16*, which has been shown to have a lipolytic role in mice.[63; 64] Further studies are needed to confirm whether *PLA2G12A* has a lipolytic role.
PPARG	Rare loss of function mutations in *PPARG* have been previously found to be associated with reduced adipocyte differentiation, lipodystrophy and increased risk of T2D.[65-67]
STAB1	The *STAB1* gene is a scavenger receptor that has been shown to mediate uptake of oxidized LDL-C.[68; 69] There was a suggestive association between LOF variants and higher LDL-C $(\beta = 4.3$ mg/dL, $P = 2 \times 10^{-3})$ consistent with its role in LDL-C uptake.

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