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

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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; some of these genes have not been previously associated with lipid levels when using rare coding variation from population-based samples. We prioritize 32 genes in array-based genome-wide association study (GWAS) loci based on aggregations of rare coding variants; three (*EVI5, SH2B3,* and *PLIN1*) had no prior association of rare coding variants with lipid levels. Most of our associated genes showed evidence of association among multiple ancestries. Finally, 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 sizes and have been instrumental in defining the causal roles of lipid fractions on cardiovascular disease.[9-13] Despite these advances, the mechanisms and causal genes for most of the identified variants and loci can be difficult to determine.

Genetic association studies testing rare coding variants have potential to directly implicate causal genes. 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 previous 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[28; 29] (MIGen, n = 44,208) and a UKB nested case-control study of CAD[28] (n = 10,689); T2D cases-control studies from the AMP-T2D-GENES exomes[15] (n = 32,486); population-based studies from the TOPMed project [30; 31] freeze 6a data (n = 44,101) restricted to the exome, and the UKB first tranche of exome sequence data[32; 33] (n = 40,586) (see **Supplemental**

374 **Study Participant Descriptions**). Informed consent was obtained from all subjects
375 and committees approving the studies are available in the supplement.
376
377 Within each data source, individuals were excluded if they failed study-specific
378 sequencing quality metrics, lacked lipid phenotype data, or were duplicated in other
379 sources. Sequencing and quality control performed in each study is available in the
380 **Supplemental Methods**. We additionally removed first- and second-degree relatives
381 across data sources while we kept relatives within each data source since we were
382 able to adjust for relatedness within each data source using kinship matrices in linear
383 mixed models. If samples from the same study were present in different data
384 sources, we used the samples in the data source which has the largest sample size
385 from the study and removed the overlapping set from the other data source. For
386 instance, samples from the Atherosclerosis Risk in Communities (ARIC) Study were
387 removed from TOPMed and kept in MIGen which had more sequenced samples from
388 ARIC. Similarly, samples from the Jackson Heart Study were kept in TOPMed and
389 removed from MIGen. To obtain duplicate and kinship information across data
390 sources we used 14,834 common (MAF>1%) and no more than weakly dependent
391 ($r^2 < 0.2$) variants using the make-king flag in PLINK v2.0.
392
393 Single-variant association analyses were performed within each data source, case-
394 status, and ancestry combination. The data were sequenced and variant calling
395 performed separately by data source and this allowed us to look for effects by case-
396 status and genetically-inferred and/or reported ancestry groups. We performed gene-
397 based meta-analyses by combining single-variant summary statistics and covariance
398 matrices generated from RVTESTS.[34] 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 cholesterol (TC), LDL-C, HDL-C, non-HDL-C, triglycerides (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.

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[35] and its associated Loss-of-Function Transcript Effect Estimator (LOFTEE)[36] and the dbNSFP[37] 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.[37] 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[38] 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.[39] Variant descriptive analysis was performed using a maximal set of variants that were used to analyze the lipid phenotype with the largest sample size. The counts and proportions of variants – annotated according to the different predicted consequences described above – were obtained out of an overall set of variants.

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.[34] 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 $P<4.3\times10^{-7}$)[40] 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.[41] 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.[42] 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. For each exome-wide significant gene-based association, we obtained the association of each single variant within the gene-specific variant groups with the respective phenotype. Then we determined – out of each gene's overall set of variants – those that had p-values at different significance thresholds to identify the percentages of variants contributing in order to each gene-based signal. To assess whether the most significant variant within each gene was driving the association, gene-based analyses were repeated after removing the respective top single variant from gene-specific variant groups.

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.

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 (see **Supplemental Methods**).

Gene-Based Analysis of GWAS Loci and Drug Targets

We obtained variants associated with LDL-C, HDL-C, and TG from a recent GWAS in the Million Veterans Program[8]. Then we identified genes within ± 200kb of each GWAS index variant and performed gene-based analysis for each of those genes using the six variant groups. 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[43] 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

549 association with T2D was obtained from the latest gene-based exome association
550 data from the AMP-T2D-GENES consortium.[15] The reported associations were
551 obtained from different variant groups based on their previous analyses. We
552 additionally performed gene-based association analyses with CAD using the MIGen
553 case-control, UKB case-control, and UKB cohort samples using the variant groups
554 described above. Further, six traits including fasting plasma glucose, HbA1c, alanine
555 aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and
556 albumin were analyzed in the UKB dataset. Single variant association analyses were
557 performed with RVTESTS. Linear mixed models incorporating kinship matrices were
558 used to adjust for relatedness within each study. Covariance matrices were
559 generated using 500 kilobase sliding windows. RAREMETALS was used to assess
560 associations between aggregated variants (MAF<1%) in burden and SKAT tests with
561 CAD and each of the six quantitative traits. We used 6 different variant groupings to
562 determine the set of damaging variants within each gene, 1) high-confidence LOF
563 using LOFTEE, 2) LOF and predicted splice-site altering variants, 3) LOF and
564 MetaSVM missense variants, 4) LOF, MetaSVM missense and predicted splice-site
565 altering variants, 5) LOF and damaging 5 out 5 missense variants, and 6) LOF,
566 damaging 5 out 5 missense and predicted splice-site altering variants.
567

568 ## Results

569 ### Sample and variant characteristics

570 Individual-level, quality-controlled data were obtained from four sequenced study
571 sources with circulating lipid data for individuals of multiple ancestries (**Figure 1**).
572 Characteristics of the study samples are detailed in **Table S1.** We analyzed data on
573 up to 172,000 individuals with LDL-C, non-HDL-C (a calculated measure of TC minus

574 HDL-C), TC, HDL-C, TG, and TG:HDL ratio (a proxy for insulin resistance).[44; 45]
575 56.7% (n=97,493) of the sample are of European ancestry, 17.4% (n=30,025) South
576 Asian, 9.6% (n=16,507) African American, 9.6% (n=16,440) Hispanic, 6.1%
577 (n=10,420) East Asian, and 0.7% (n=1,182) Samoan, based on genetically-estimated
578 and/or self-reported ancestry.
579
580 After sequencing, we observed 15.6 M variants across all studies; 5.0 M (32.6%) we
581 classified as transcript-altering coding variants based on an annotation of frameshift,
582 missense, nonsense, or splice site acceptor/donor using the Variant Effect Predictor
583 (VEP).[35] A total of 340,214 (6.7%) of the coding variants were annotated as high
584 confidence loss-of-function (LOF) using the LOFTEE VEP plugin,[36] 238,646 (4.7%)
585 as splice site altering identified by Splice AI,[39] 729,098 (14.3%) as damaging
586 missense as predicted by the MetaSVM algorithm[38], and 1,106,309 (21.8%) as
587 damaging missense as predicted by consensus in all five prediction algorithms (SIFT,
588 PolyPhen-2 HumVar, PolyPhen-2 HumDiv, MutationTaster and LRT).[37] As expected,
589 we observed a trend of decreasing proportions of putatively deleterious variants with
590 increasing allele count (**Figure S2, Table S3**).
591
592 **Single-variant association**
593 We performed inverse-variance weighted fixed-effects meta-analyses of single-
594 variant association results of LDL-C, non-HDL-C, TC, HDL-C, TG and TG:HDL ratio
595 from each consortium and ancestry group. Meta-analysis results were well controlled
596 with genomic inflation factors ranging between 1.01 and 1.04 (**Table S4**). Single-
597 variant results were limited to the 425,912 protein-altering coding variants with a total
598 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 ($P<4.3\times10^{-7}$)[40] 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 [rs2862954], *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.[46] 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, $P=2\times10^{-9}$; OR=1.08, $P=4\times10^{-4}$).[47] 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)[48] 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

624 altering variants using Splice AI, 3) LOF and MetaSVM missense variants, 4) LOF,
625 MetaSVM missense and predicted splice-site altering variants, 5) LOF and damaging
626 5 out 5 missense variants, and 6) LOF, damaging 5 out 5 missense and predicted
627 splice-site altering variants. Meta-analyses results were well controlled (**Table S12**).
628
629 We identified 35 genes reaching exome-wide significance ($P=4.3\times10^{-7}$) for at least
630 one of the six variant groupings (**Tables S13-S19**). Most of the significant results
631 were from the multi-ancestry analysis, with multiple ancestries contributing to the top
632 signals (**Figure 2A**) and most of the 35 genes were associated with more than one
633 lipid phenotype (**Figure 2B**). Ten of the 35 genes did not have prior evidence of
634 gene-based links with blood lipid phenotypes (**Table 1**), and seven genes, including
635 *ALB*, *SRSF2*, *CREB3L3*, *NR1H3*, *PLA2G12A*, *PPARG,* and *STAB1* have evidence
636 for a biological connection to circulating lipid levels (**Box 1**).
637
638 We performed a series of sensitivity analyses on our results. To determine whether
639 low frequency variants between 0.1%-1% frequency were driving our gene-based
640 association results, we performed the gene-based multi-ancestry meta-analyses
641 using a maximum MAF threshold of 0.1% instead of 1%. We observed exome-wide
642 significant associations ($P<4.3\times10^{-7}$) for 29 genes using a 0.1% MAF threshold, all
643 observed in our primary analyses using a MAF threshold of 1% (**Table S20**). We then
644 intersected our 35 lipid associated genes from 85 gene-based associations observed
645 in the primary analysis with our results using a MAF threshold of 0.1%. All genes
646 remained at least nominally significant ($P < 0.05$) using a 0.1% MAF threshold,
647 except the *A1CF* and *TMEM136* associations (**Table S21**). Furthermore, we
648 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)[49] 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 observed that after removal of the most significant variant in *JAK2* (p.V617F; rs77375493), a somatic variant, there is no association between *JAK2* and total cholesterol (p =0.10, **Table S13**).

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 $P<0.05$. Finally, gene-based associations in *A1CF, BUD13, JAK2* and *TMEM136* were lost after removal of the respective most significant single variant from the group of variants aggregated in each gene-based association (**Table S13**).

Comparison of gene-based associations across ancestries

We determined the overlap between single variants included in gene-based signals, which additionally were nominally significant ($P<0.05$) in each of 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 4 genes (*CETP*, *ABCA1*, *CD36*, and *LCAT*) were observed to have significant gene-based associations with HDL-C in multi-ancestry meta-analyses. A total 68% of variants from European ancestry samples that contributed to HDL-C gene-based associations did not overlap with any other ancestry, as was 62% in South Asian-, 44% in African-, 41% in Hispanic- and

699 59% in East Asian ancestry. When restricted to variants with P<0.05 in the multi-
700 ancestry meta-analysis, the overlap among ancestries increased (**Figure S4**). A total
701 of 61% of variants from European ancestry did not overlap with any other ancestry,
702 as was 46% in South Asian-, 27% in African-, 27% in Hispanic- and 32% in East
703 Asian ancestry. Finally, we determined the top single variant contributing to each
704 gene-based association (**Figure S5**). Out of the 4 HDL-C or the 3 LDL-C genes,
705 none of the top variants overlapped among any of the ancestries, and at least 1 out
706 of 3 variants from the TG genes was shared between 2 ancestries. .
707
708 But, the gene-based associations were mostly consistent across the six ancestry
709 groupings: European, South Asian, African, Hispanic, and East Asian. Three of the
710 17 HDL-C genes showed association in at least two different ancestries at exome-
711 wide significance level ($P=4.3\times10^{-7}$). Similarly, 3/9 LDL-C, 4/10 non-HDL-C, 5/14 TC,
712 2/18 TG and 2/17 TG:HDL genes showed association in at least two difference
713 ancestries at an exome-wide significance level. Using a less stringent significance
714 level (P<0.01), across the six lipid traits, 59-89% of associated genes from the joint
715 analysis were associated in at least two different ancestries.
716
717 We tested the top 35 genes for heterogeneity across all 303 gene-trait-variant
718 grouping combinations passing the exome-wide significance threshold ($P<4.3\times10^{-7}$).
719 We observed heterogeneity in effect estimates ($P_{Het}<1.7\times10^{-4}$, accounting for 303
720 combinations) in 19 (6%) different gene-trait-variant grouping combinations and in six
721 different genes: *LIPC*, *LPL*, *LCAT*, *ANGPTL3*, *APOB*, and *LDLR* (**Table S27**).
722 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}=3×10^{-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=3×10^{-4}) and TG:HDL (**β**=-20%, P=6×10^{-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=2×10^{-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 4 out of 10 genes without prior evidence of gene-based links with blood lipid phenotypes to have a p< 0.005 (Bonferroni correction for testing 10 genes) and in the same direction as the discovery s (*SRSF2, CREB3L3, PLA2G12A, PPARG*) with their respective blood lipids with an additional two genes that met a nominal significance level (p<0.05; *JAK2* and *NR1H3*). 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 6 of the 10 genes were associated at a P <0.005 and in the same direction of effect as the discovery analysis (ALB*, CREB3L3, NR1H3, PLA2G12A, PPARG, STAB1*) (**Table S29**) with *JAK2* reaching a nominal significance threshold (p<0.05). The only two genes that did not show any 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 ($P_{Het} < 6\times10^{-4}$) (**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 ($P<3.4\times10^{-5}$, 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 ($P_{SKAT}=2\times10^{-5}$). 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, $P=1\times10^{-6}$) among Europeans and variants that were annotated as LOF in *PLIN1* were associated with higher HDL-C (**β**=3.9 mg/dL, $P=1\times10^{-5}$) (**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.[50] The gene encodes perilipin, the most abundant protein that coats adipocyte lipid droplets and is critical for optimal TG storage.[51] 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,[52] 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-

798 threshold level of significant association in this study using burden or SKAT tests (p <
799 0.005), we determined the enrichment of i) Mendelian dyslipidemia (N=21 genes)-;[2]
800 ii) lipid GWAS (N=487 for LDL-C, N=531 for HDL-C and N=471 for TG)[8]; and iii) drug
801 target genes (N=53).[43] We stratified genes in GWAS loci according to coding status
802 of the index SNP and proximity to the index SNP (nearest gene, second nearest
803 gene, and genes further away). We tested for enrichment of gene-based signals
804 (P<0.005) in the gene sets compared to matched genes (**Figure 3**). For each gene
805 within each gene set, the most significant association in the multi-ancestry or an
806 ancestry specific analysis was obtained and then matched to 10 genes based on
807 sample size, total number of variants, cumulative MAC, and variant grouping. The
808 strongest enrichment was observed for Mendelian dyslipidemia genes within the
809 genes that reached P < 0.005 in our study. For example, 52% of the HDL-C
810 Mendelian genes versus 1.4% of the matched set reached P < 0.005 (OR:71, 95%
811 CI: 16-455). We also observed that 45.5% of the set of genes closest to an HDL-C
812 protein-altering GWAS variant reached P < 0.005 versus 1.4% in the matched gene
813 set (OR:57, 95% CI: 13-362). Results were significant but much less striking for
814 genes at non-coding index variants. We observed that 8.9% of the set of genes
815 closest to an HDL-C non-protein altering GWAS variant reached P < 0.005 versus
816 2.3% in the matched set (OR:4.1, 95% CI: 1.8-8.7). While 8% of the set of genes in
817 the second closest to an HDL-C non-protein altering GWAS variant reached P
818 <0.005 versus 2.6% in the matched set (OR: 3, 95% CI: 1.1-8.3). There was no
819 significant enrichment in second closest or >= third closest genes to protein altering
820 GWAS signals and in >= third closest genes to non-protein altering GWAS signals.
821 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, $P=7\times10^{-24}$), *APOB* ($P_{SKAT}=4\times10^{-5}$), *PCSK9* (OR: 0.5, $P=2\times10^{-4}$) 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. However, JAK2 was no longer associated with Hba1c after removal of the p.V617F missense variant that is known to frequently occur as a somatic mutation (beta=0.22, se=0.40, p= 0.47).

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**).

Discussion

We conducted a large multi-ancestry 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.

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.[53] 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.[54] 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

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.[55] This suggests PLIN1 as a putative target for CAD prevention; however, replication of the CAD association would be needed to confirm those signals.

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.[56] 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.[41]

In summary, we demonstrated association between rare protein-altering variants with circulating lipid levels in >170,000 individuals of diverse ancestries. We identified 35 genes associated with blood lipids, including ten genes not previously shown to have gene-based signals. Our results support the hypothesis that genes closest to a GWAS index SNP are enriched for evidence of association.

Supplemental data

Supplemental data includes in 8 figures, 49 tables, Supplemental Methods, 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 advisor to Apple, Blackstone Life Sciences, and Novartis, and spousal employment at Vertex, all unrelated to the present work. A.V.K. has served as a scientific advisor to Sanofi, Medicines Company, Maze Pharmaceuticals, Navitor Pharmaceuticals, Verve Therapeutics, Amgen, and Color; received speaking fees from Illumina, MedGenome, Amgen, and the Novartis Institute for Biomedical Research; received sponsored research agreements from the Novartis Institute for Biomedical Research and IBM Research, and reports a patent related to a genetic risk predictor (20190017119). CJW spouse employed at Regeneron. Dr. Emery is currently an employee of Celgene/Bristol Myers Squibb. Celgene/Bristol Myers Squibb had no role in the funding, design, conduct, and interpretation of this study. MEM receives funding from Regeneron unrelated to this work. EEK has received speaker honoraria from Illumina, Inc and Regeneron Pharmaceuticals. BMP serves on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. LAC has consulted with the Dyslipidemia Foundation on lipid projects in the Framingham Heart Study. PTE is supported by a grant from Bayer AG to the Broad Institute focused on the genetics and therapeutics of cardiovascular disease. PTE has consulted for Bayer AG, Novartis, MyoKardia and Quest Diagnostics. SAL receives sponsored research support from Bristol Myers Squibb / Pfizer, Bayer AG,

Boehringer Ingelheim, Fitbit, and IBM, and has consulted for Bristol Myers Squibb / Pfizer, Bayer AG, and Blackstone Life Sciences. The views expressed in this article are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health. MMcC has served on advisory panels for Pfizer, NovoNordisk and Zoe Global, has received honoraria from Merck, Pfizer, Novo Nordisk and Eli Lilly, and research funding from Abbvie, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, NovoNordisk, Pfizer, Roche, Sanofi Aventis, Servier, and Takeda. As of June 2019, MMcC is an employee of Genentech, and a holder of Roche stock. MEJ holds shares in Novo Nordisk A/S. H.M.K. is an employee of Regeneron Pharmaceuticals; he owns stock and stock options for Regeneron Pharmaceuticals. MEJ has received research grants form Astra Zeneca, Boehringer Ingelheim, Amgen, Sanofi. SK is founder of Verve Therapeutics.

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Data and code availability

Controlled access of the individual-level data is available through dbGAP (please refer to the Supplementary Information), and the individual-level UK Biobank data are available upon application to the UK Biobank. Summary association results are available on the Downloads page of the Cardiovascular Disease Knowledge Portal (broadcvdi.org).

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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. Beta (effect size) is obtained from the corresponding burden test for SKAT results. 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.

Figure 3. Enrichment of Mendelian, GWAS, and drug target genes in the gene-based lipid associations

Enrichment of gene sets of Mendelian genes (n=21), GWAS loci for LDL-C (n=487), HDL-C (n=531), and triglycerides (TG) (n=471) genes and drug target genes (n=53).

Table 1. Novel Genes Associated with Blood Lipids

Gene	Name	Trait	N	cMAC	nVAR	beta	se	P	Mask	Test	Ancestry	UKBB Replication	PMBB Replication
ALB	Albumin	**LDL-C**	165,003	51	18	29.51	5.11	7.76E-09	LOF	Burden	Multi-ancestry	<0.005	N/A
ALB	Albumin	**Non-HDL-C**	166,327	50	17	33.91	6.07	2.27E-08	LOF	Burden	Multi-ancestry	N/A	N/A
ALB	Albumin	**TC**	172,103	54	18	33.37	5.89	1.48E-08	LOF	Burden	Multi-ancestry	N/A	N/A
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	N/A	<0.005
JAK2	Janus Kinase 2	**TC**	975,33	441	136	-7.10	1.98	1.71E-07	LOF/DAM5of5/SPLICE AI	SKAT	EUR	<0.05	<0.05
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	<0.005	<0.005
CREB3L3	CAMP Responsive Element Binding Protein 3 Like 3	**TG/HDL-C**	165,380	855	69	0.14	0.02	5.76E-13	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry	N/A	N/A
TMEM136	Transmembrane Protein 136	**TG**	29,571	157	24	-0.15	0.04	3.39E-09	LOF/DAM5of5/SPLICE AI	SKAT	SAS	N/A	N/A
TMEM136	Transmembrane Protein 136	**TG/HDL-C**	29,517	157	24	-0.20	0.05	1.76E-11	LOF/DAM5of5/SPLICE AI	SKAT	SAS	N/A	N/A
VARS	Valyl-TRNA Synthetase 1	**TG**	56,140	67	51	0.32	0.06	4.30E-07	LOF/MetaSVM	Burden	EUR	N/A	N/A
NR1H3	Nuclear Receptor Subfamily 1 Group H Member 3	**HDL-C**	93,044	521	111	3.47	0.60	1.45E-11	LOF/MetaSVM/SPLICE AI	SKAT	EUR	<0.005	<0.05
PLA2G12A	Phospholipase A2 Group XIIA	**HDL-C**	166,441	1975	47	-2.28	0.31	8.12E-14	LOF/DAM5of5	Burden	Multi-ancestry	<0.005	<0.005
PLA2G12A	Phospholipase A2 Group XIIA	**TG**	170,239	2047	47	0.06	0.01	1.17E-08	LOF/DAM5of5	Burden	Multi-ancestry	N/A	N/A
PLA2G12A	Phospholipase A2 Group XIIA	**TG/HDL-C**	165,380	1969	46	0.11	0.01	7.56E-13	LOF/DAM5of5	Burden	Multi-ancestry	N/A	N/A
PPARG	Peroxisome Proliferator Activated Receptor Gamma	**HDL-C**	166,441	147	72	-6.24	1.07	4.71E-09	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry	<0.005	<0.005
STAB1	Stabilin 1	**HDL-C**	166,441	6550	804	0.83	0.16	2.58E-07	LOF/MetaSVM/SPLICE AI	Burden	Multi-ancestry	<0.005	N/A

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.[57] 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.[58] 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.[59] 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).[60-62] 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*.[61]

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.[63] 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.[64; 65] It has previously been shown that disrupting the LXRα phosphorylation at Ser196 in mice prevents non-alcoholic fatty liver disease.[63]

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.[66; 67] 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.[68-70]

STAB1

The *STAB1* gene is a scavenger receptor that has been shown to mediate uptake of oxidized LDL-C.[71; 72] There was a suggestive association between LOF variants and higher LDL-C (β = 4.3 mg/dL, $P = 2 \times 10^{-3}$) consistent with its role in LDL-C uptake.

Figure 1

Click here to access/download;Figure;Fig1.tiff

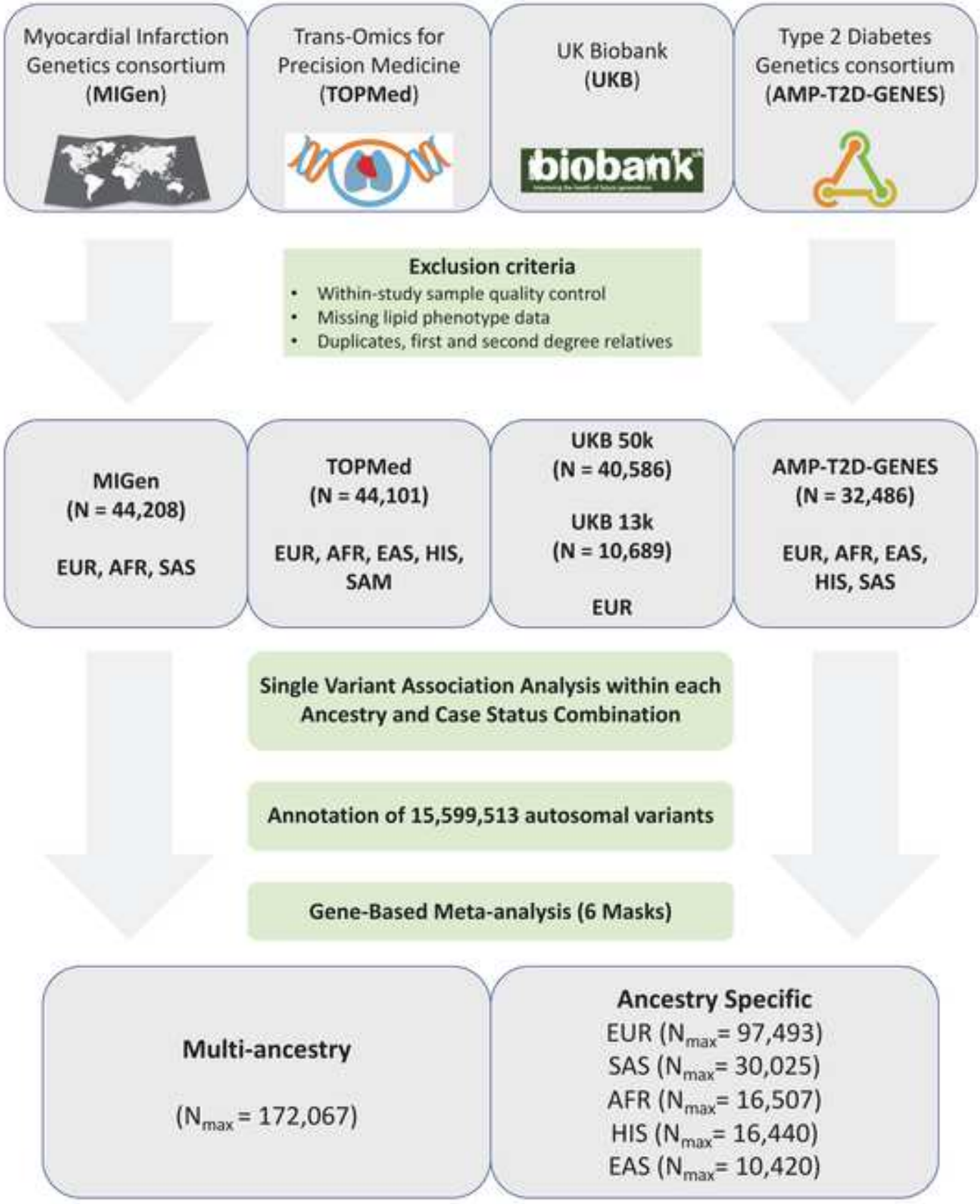


Figure 2

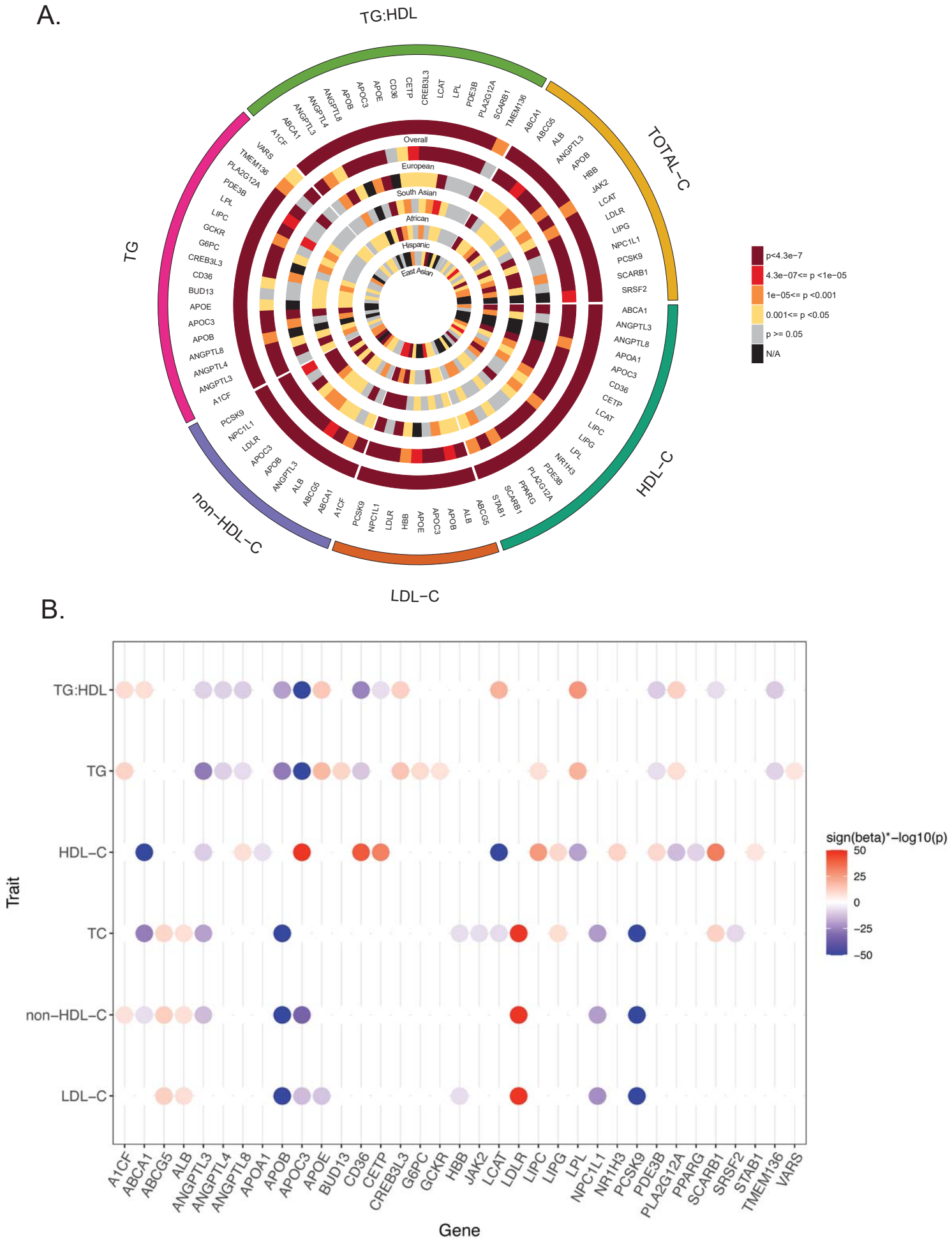
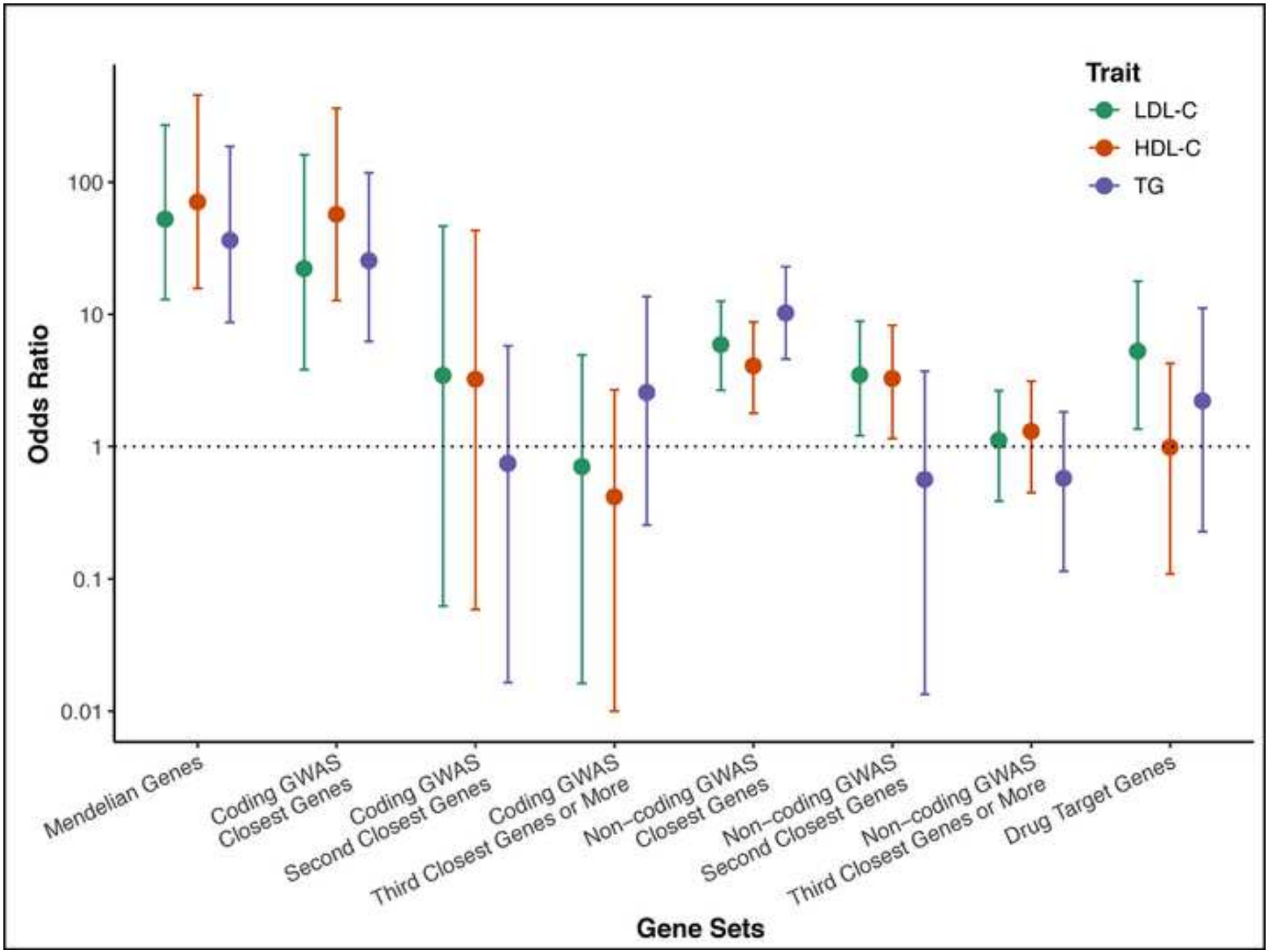

A.
TG:HDL
TOTAL−C
HDL−C
LDL−C
non−HDL−C
TG
Overall
European
South Asian
African
Hispanic
East Asian
p<4.3e−7
4.3e−07<= p <1e−05
1e−05<= p <0.001
0.001<= p <0.05
p >= 0.05
N/A
B.
Trait
TG:HDL
TG
HDL−C
TC
non−HDL−C
LDL−C
Gene
sign(beta)*−log10(p)
50
25
0
−25
−50

Figure 3

Click here to access/download;Figure;Fig3.tiff



Supplement

Supplemental Tables