Multi-ancestry genome-wide gene-smoking interaction study of 387,272 individuals identifies new loci associated with serum lipids

A full list of authors and affiliations appears at the end of the article.

Abstract

The concentrations of high- and low-density lipoprotein cholesterol and triglycerides are influenced by smoking, but it is unknown whether genetic associations with lipids may be modified by smoking. We conducted a multi-ancestry genome-wide gene-smoking interaction study in 133,805 individuals with follow-up in an additional 253,467 individuals. Combined meta-analyses identified 13 novel loci, some of which were detected only because the association differed by smoking status. Additionally, we demonstrated the importance of including diverse populations, particularly in studies of interactions with lifestyle factors, where genomic and lifestyle differences by ancestry may contribute to novel findings.

Editorial summary:

A multi-ancestry genome-wide gene-smoking interaction study identifies 13 new loci associated with serum lipids.

Serum lipids, such as triglycerides and high- and low-density lipoprotein cholesterol (HDL and LDL), are influenced by both genetic and lifestyle factors. Over 250 lipid loci have been identified, yet, it is unclear to what extent lifestyle factors modify the effects of these lipids.
variants, or those yet to be identified. Smoking is associated with an unfavorable lipid profile,\(^7,8\) warranting its investigation as a lifestyle factor that potentially modifies genetic associations with lipids. Identifying interactions using traditional 1 degree of freedom (1df) tests of SNP x smoking terms may have low power, except in very large sample sizes. To enhance power, a 2 degree of freedom (2df) test that jointly evaluates the interaction and main effects was developed.\(^9\)

The Gene-Lifestyle Interactions Working Group, under the aegis of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium\(^10\), was formed to conduct analyses of lifestyle interactions in the genetic basis of cardiovascular traits. As both genetic and lifestyle factors differ across populations with different ancestry backgrounds, and to address the underrepresentation of non-European populations in genomic research, great effort went into creating a large, multi-ancestry resource for these investigations.\(^11\)

Here, we report a genome-wide interaction study that uses both the 1df test of interaction and the 2df joint test of main and interaction effects to test the hypothesis that genetic associations of serum lipids differ by smoking status.

**Results**

**Novel Loci**

We conducted genome-wide interaction meta-analyses for current and ever-smoking status in up to 133,805 individuals of European (EUR), African (AFR), Asian (ASN) and Hispanic (HISP) ancestries (Supplementary Tables 1–3), with follow-up of 17,921 variants with \(p \leq 10^{-6}\) (not pruned for linkage disequilibrium [LD]) in an additional 253,467 individuals of EUR, AFR, ASN, HISP, and Brazilian (BR) ancestries (Supplementary Tables 4–6), as described in Figure 1. Of these, 16,389 variants (487 loci, defined by +/- 1 MB) passed filters and were included in stage 2 analyses. Ninety percent of variants (14,733) and 22% of loci (109) replicated in stage 2 (variants: \(p < 0.05/16,389\), loci: \(p < 0.05/487\)). We conducted meta-analyses of stage 1 and 2 results (Manhattan Plots Supplementary Figure 1; QQ Plots, Supplementary Figure 2) and identified 13 novel loci with \(p < 5 \times 10^{-8}\) that were at least 1 MB away from previously reported lipid loci (Table 1; results by stage: Supplementary Table 7; forest plots: Supplementary Figures 3 and 4; regional association plots: Supplementary Figure 5). These loci had low false discovery rate (FDR) q-values (all \(q < 3 \times 10^{-4}\); Supplementary Table 8). We report novel loci with \(p < 5 \times 10^{-8}\) as well as those passing a more stringent threshold (\(p < 6.25 \times 10^{-9}\)), adjusting for 2 smoking exposures, 2 interaction tests, and ancestry-specific and trans-ancestry tests. The patterns observed in these results are described below and illustrated using output from stage 1 meta-analyses, where results from a main effect model (in all and stratified by smoking exposure) and a smoking-adjusted main effect model were also available (Figure 1; Supplementary Table 9).

Notably, many novel loci were statistically significant only in AFR meta-analyses. For 7 of the 13 novel loci, the minor allele frequencies (MAF) of the index variants were highest in AFR, and inter-ancestry differences in MAF and/or LD may explain the failure to detect similar associations in other ancestries. However, some AFR-only associations were unlikely to be due to diminished power in non-AFR meta-analyses. For instance, the effect of rs12740061 (NC_000001.10:g.69407810C>T; **LOC105378783**) on HDL was significantly
modified by current smoking status among AFR ($p_{1df} = 7.4 \times 10^{-9}$; Figure 2, Table 1), such that the genetic effect was stronger among current smokers than non-smokers (Supplementary Table 9). In contrast, there was virtually no evidence for association in any other ancestry, despite higher MAF (Figure 2). The potential influence of under-adjustment for principal components (PCs) on these results was evaluated by excluding the 6 studies adjusting for only 1 PC (the average number of PCs among AFR studies was 4.2); effect estimates were similar and p-values were increased or similar, consistent with a ~20% reduction in sample size (Supplementary Table 10).

We observed interactions where notable associations were only found among current or ever-smokers, with effect sizes close to zero among non- or never-smokers, including a statistically significant association for the 2df joint test of main and interaction effects for rs7364132 (NC_000022.10:g.20096172G>A; DGC8R8) × ever-smoking on triglycerides ($p_{2df} = 2.5 \times 10^{-8}$; Table 1). Main effect models stratified by smoking status showed a strong genetic association with triglycerides among ever-smokers (difference in mean ln triglycerides per A allele $\beta = -0.05$, $p = 7.9 \times 10^{-5}$), with a negligible association among never-smokers ($\beta = 0.01$, $p = 0.19$; Figure 3a). This association was not significant in a non-stratified main effect model (Table 1; Supplementary Table 9), and was only detectable when modeling permitted a different association across smoking strata. Similar results were observed for rs79950627 (NC_000011.9:g.2233790G>A; MIR4686) × current smoking on LDL (Figure 3b), and rs56167574 (NC_000007.13:g.151245975G>A; PRKAG2) × ever-smoking on LDL (Figure 3c, Supplementary Table 9).

We also observed interactions where the association was in opposite directions in the exposed vs. unexposed stratum, with a larger, more statistically significant association among smokers. For instance, current smoking modified the association between rs73453125 (NC_000007.13:g.146084573G>A; CNTNAP2) and LDL (Table 1). In stratified main effect models, the A allele was associated with lower LDL among current smokers ($\beta = -8.1$ mg/dL, $p = 2.2 \times 10^{-7}$), but higher LDL among non-smokers ($\beta = 2.18$ mg/dL, $p = 0.01$; Figure 4a, Supplementary Table 9). In a non-stratified smoking-adjusted main effects model, no association between rs73453125 and LDL was detected ($\beta = 0.3$ mg/dL, $p = 0.98$). Similar results were observed for rs12740061 (LOC105378783) (Supplementary Table 9).

Although many interactions manifested as associations significant only, or more strongly, in smokers, for rs10937241 (NC_000003.11:g.185822774A>G; ETV5), rs34311866 (NC_00004.11:g.951947T>C; TMEM175), rs10101067 (NC_000008.10:g.72407374G>C; EYA1), and rs77810251 (NC_000007.13:g.121504149G>A; PTPRZ1), the associations observed among non- or never-smokers were more statistically significant. Notably, in stratified main effect models, rs77810251 was associated with increased HDL among never-smokers ($\beta = 0.05$ lnHDL, $p = 6.3 \times 10^{-11}$) with no significant association among ever-smokers ($\beta = -0.005$ lnHDL, $p = 0.56$; Figure 3d; Supplementary Table 9). In a smoking-adjusted main effect model of never- and ever-smokers together, the association was markedly reduced ($\beta = 0.02$ lnHDL, $p = 1.6 \times 10^{-4}$).
The 2df joint test simultaneously evaluates main and smoking interaction effects; some of our results appear to capture a main effect of the variant. For instance, the 2df test for rs12144063 \((EYA3)\) detected an association \((p = 1.3 \times 10^{-10})\), while the 1df test of interaction does not \((p = 0.75)\). The minor alleles for this and three other variants (rs10937241 \([ETV5]\), rs34311866 \([TMEM175]\), and rs10101067 \([EYA4]\)) were common across populations, and their effects were small in magnitude and yet reached genome-wide statistical significance \((p = 0.01)\). For novel main effect loci in well-studied populations, there are two findings, however, for which the relatively large sample size in the AFR meta-analyses appeared to facilitate detection. The MAF for rs73729083 \((NC_000007.13:g.137559799T>C; CREB3L2)\) was much greater among AFR than in HISP and ASN (not present among EUR), and the variant effect estimates were large and consistent across ancestries, while the interaction effect estimates were inconsistent, with wide confidence intervals (Supplementary Figure 3f). The minor allele for rs4758675 \((NC_000012.11:g.122691738C>A; B3GNT4)\) was only present in AFR (Supplementary Figure 3k), but variant effect estimates were consistent across AFR studies, with interaction effect estimates approaching the null (Supplementary Figure 4e). In total, 6 of the 13 novel loci that we identified appear to be driven by main effects of the variant while the remainder show some evidence of interaction.

There were 16 additional novel loci identified in stage 1 meta-analyses \((p_{1df} < 5 \times 10^{-8})\) for which the variants were unavailable for analysis in stage 2 cohorts. These loci were identified only in AFR meta-analyses (many were AFR-specific variants; Table 2). Due to the relatively small number and size of available AFR cohorts in stage 2 (total n = 7,217; n < 2,000 per cohort), these relatively low frequency variants did not pass filters for minor allele count within exposure groups. Nevertheless, these associations had low FDR q-values \((all q < 2.4 \times 10^{-4})\) in stage 1, and some appear worthy of further investigation. One particularly interesting candidate is rs17150980 \((NC_000007.13:g.78173734T>C; MAGI2)\) × ever-smoking on triglycerides \((p_{2df} = 1.4 \times 10^{-9})\), for which consistent effects for both the variant and the interaction were observed across AFR studies, but not in other ancestries (Supplementary Figure 6).

As we ran analyses for both current and ever-smoking status, we evaluated novel associations across smoking exposures to further characterize those loci (Supplementary Table 11). For the 6 probable main effect loci \((EYA3, ETV5, TMEM175, CREB3L2, EYA1, B3GNT4)\), an association of similar statistical significance was observed across smoking status definitions for the 2df joint test, with similar lack of effect for the 1df test of the interaction, consistent with the interpretation that smoking status was unimportant, with the main effect driving the association. For the locus in which a stronger association was observed among non-smokers \((PTPRZ1)\), the 1df interaction p value was dramatically reduced \((from 9.5 \times 10^{-7} for ever-smoking to 0.011 for current smoking)\), consistent with any smoke exposure altering the association between this variant and HDL, and including former smokers with the never smokers (as in the current smoking analysis) diluting the observed association among never smokers. For the reported interactions with current smoking, all the effect estimates were greatly reduced in the ever-smoking analysis, suggesting that active smoking is the relevant exposure. For the reported interactions with ever-smoking, markedly reduced statistical significance was observed in the current smoking...
analysis, likely reflecting a drop in power from excluding former smokers from the exposed group.

We conducted a secondary analysis of smoking dose in two of our AFR cohorts with measured cigarettes per day for four interaction loci (see methods for selection criteria): rs12740061 (LOC105378783), rs73453125 (CNTNAP2), rs79950627 (MIR4686), and rs7364132 (DGCR8). For each of these variants, a stronger association was observed with increasing smoking dose (Supplementary Table 12), and the interaction was statistically significant for all variants but rs7364132, which was just over our threshold for statistical significance (p = 0.0035 vs. p < 0.0021).

Conditional analysis showed no evidence that the novel associations were driven by variants at known lipids loci (Supplementary Table 13). Imputation quality for novel variants was high (minimum 0.75), with sample-size weighted average imputation quality of 0.90 and minor allele frequencies that match publicly-available datasets (Supplementary Table 14).

**Interactions at Known Loci**

We examined interactions at known lipid loci. Since results for the 2df test at known lipid loci are expected to predominantly reflect previously identified main effects, we exclusively evaluated the 1df test of interaction. No interactions within known loci were statistically significant (p_{1df} < 0.05/269 known loci in our data). To evaluate whether the proportion of known variants with p_{1df} < 0.05 was higher than would be expected by chance (5%), we conducted binomial tests for each trait-exposure combination (p-values Bonferroni-corrected for multiple tests). There was significant enrichment of known variants with 1df interaction p < 0.05: HDL-current smoking p = 9.6 × 10^{-12}, HDL-ever smoking p = 5.9 × 10^{-7}, LDL-current smoking p = 8.4 × 10^{-15}, LDL-ever smoking p = 3.1 × 10^{-5}, triglycerides-current smoking p = 4.0 × 10^{-3}, triglycerides-ever smoking p = 3.1 × 10^{-4}. We conducted power calculations under different interaction scenarios to determine the conditions under which an interaction analysis and a main effect analysis would both be sufficiently powered to detect the same locus (i.e. when an interaction could be detected in a locus previously identified in a main effect analysis; Supplementary Table 15). At current trans-ancestry meta-analyses sample sizes and assuming a large effect size, there was limited power to detect either a main effect or an interaction when an association was larger or only present among smokers (main effect <1%; interaction 77%), or when associations differed in magnitude but not direction (main effect >99%; interaction <1%); thus, making it unlikely to detect an interaction at a known locus. We were well-powered for both interaction and main effect analyses to detect smoking interactions for which smoking eliminates or drastically reduces a large association among non- or never-smokers. We identified one such interaction in our data, for PTPRZ1 in AFR only, which may not have been previously identified in a main effect analysis because of limited power of AFR main effect analyses thus far.

**Proportion Variance Explained by Identified Loci**

Ten studies from four ancestries were used to calculate the proportion of the variance in lipid traits explained by the genome-wide statistically significant novel loci: 13 loci from stage 1 and 2 combined meta-analyses (Table 1), and 16 loci from stage 1 that were not available in
stage 2 analyses (Table 2). Two different methods were used (Online Methods), and the range of findings across these methods are presented (Supplementary Table 16). In AFR, novel variants and their interactions explained 1.0–2.7% of HDL, 0.7–2.6% of LDL, and 1.3–3.2% of triglycerides. The proportion explained was smaller among EUR (0.06–0.14% of HDL, 0.01–0.07% of LDL, and 0.10–0.19% of triglycerides), ASN (0.27–0.86% of HDL, 0.09–0.82% of LDL, and 0.8–1.5% of triglycerides), and HISP (0.2–0.4% of HDL, 0.2–0.5% of LDL, and 0.2–0.4% of triglycerides). These results should be considered in the context of the inter-ancestry MAF differences: the proportion of novel variants that could be evaluated varied by ancestry, with 94–97% among the AFR cohorts, but only 32–39% among the EUR and ASN cohorts, and 55% in the HISP cohort. In contrast, each of the cohorts investigated had similar proportions of the requested known variants (83–96%).

Reproducing Known Lipids Associations

We evaluated the degree to which our data reproduce previously reported lipid loci. Given that approximately 81% of cohorts in stage 1 were included both in this and in previous efforts, this analysis is not a formal replication. For comparability with traditional GWAS, we evaluated results from stage 1 main effect models. Of the 356 previously reported associations for 279 variants (compiled from1–6,12), there were 236 associations for 189 variants that were confirmed in our data (consistent direction and p < 0.05/356), for a 66.3% concordance rate (Supplementary Table 17).

Bioinformatics

To characterize the potential impact of our novel associations for chronic disease risk and to investigate biological mechanisms, we conducted a series of follow-up analyses and annotations. We performed extensive bioinformatics annotation on variants within the 29 novel loci (Tables 1 and 2). These loci included 78 associated variants that were in or near 33 unique genes (Supplementary Table 18). We conducted look-up of these variants in previously conducted GWAS for related traits (Supplementary Tables 19–24), the Genotype-Tissue Expression (GTEx v7.0) portal and Regulome DB (Supplementary Table 25), HaploReg v4.1 (Supplementary Table 26), and an analysis of cis- and trans- expression quantitative trait loci (eQTL) in whole blood from Framingham Heart Study participants (Supplementary Table 27). Additionally, for each trait we performed DEPICT gene prioritization (Supplementary Tables 28–30), gene set enrichment (Supplementary Tables 31–33), and tissue or cell type enrichment analyses13 (Supplementary Tables 34–37), using both novel and known loci. Notable findings from these follow-up analyses are summarized below by locus.

Consistent with our observations of an association of the C allele for rs10101067 (EYAI) with higher triglycerides, this allele was associated with increased risk of coronary artery disease ($\beta = 0.036, p = 0.03$; Supplementary Table 19), ischemic stroke ($\beta = 0.11, p = 0.04$; Supplementary Table 20), and higher waist to hip ratio adjusted for BMI ($\beta = 0.029$ units, $p = 6.5 \times 10^{-4}$, with similar results observed for waist circumference adjusted for BMI; Supplementary Table 21).
We found an association of the T allele of rs12144063 (NC_000001.10:g.28406047G>T; EYA3) with lower HDL. This allele was associated with increased risk of all stroke types ($\beta = 0.05$, $p = 0.04$), as well as stroke subtypes (Supplementary Table 20). rs7529792 (NC_000001.10:g.28306250C>T), a variant in LD with rs12144063 ($r^2 = 0.97$) regulates gene expression of EYA3 and has a high Regulome DB score (1b; Supplementary Table 25). Haploreg also shows regulatory features for rs12144063, including being in a promoter location expressed in liver and brain, in enhancer histone marks, and at DNAse marks for EYA3 (Supplementary Table 26). DEPICT predicted a role for these variants in regulating EYA3 and XKR8 (Supplementary Table 28), which encodes a phospholipid scramblase important in apoptotic signaling.

We report an interaction between smoking and rs77810251 (PTPRZ1) with the minor allele associated with higher HDL only among never-smokers. While this variant was not available in look-up data for GIANT, a variant in this locus with a similar association, rs740965 (NC_000007.13:g.121513561T>G), was associated with lower BMI among EUR ($\beta = -0.01$ kg/m$^2$, $p = 0.01$, similar results for trans-ancestry analysis). This variant was also associated with lower waist circumference adjusted for BMI among EUR women ($\beta = -0.016$, $p = 0.04$; Supplementary Table 21). PTPRZ1 was shown to be downregulated in cells treated with an acute dose of nicotine, which supports our observation of a lack of an association of PTPRZ1 variants among ever-smokers.

We report a main effect of rs34311866 on HDL and triglycerides. rs34311866 is a missense variant in TMEM175, which has been associated with Parkinson’s disease and type 2 diabetes. This variant contributes to the regulation of DGKQ ($p = 5.3 \times 10^{-21}$) and is an eQTL of DGKQ in adipose, artery, lung, nerve and thyroid tissue (Supplementary Table 25). The expression of DGKQ is more strongly regulated by another significantly associated variant in this locus, rs4690220 (NC_000004.11:g.980464A>G), which is located upstream of IDUA and in an intron of SLC26A1. This variant had a high score in the RegulomeDB (1f), supporting a potential functional effect (Supplementary Table 25). Importantly, DGKQ has been implicated in studies of cholesterol metabolism, bile acid signaling, glucose homoeostasis in hepatocytes, primary biliary cirrhosis, and Parkinson’s disease. DGKQ interacts with the key lipid enzymes LPL, LIPG, and PNPLA3 (Supplementary Figure 7). These results suggest that the observed association with HDL and triglycerides could act on cholesterol metabolism through regulation of DGKQ. Also, rs34311866 is a trans-eQTL for GNPDA1 (Supplementary Table 27); expression of this gene has been associated with a set of traits, including hyperlipidemia.

In our data, there was a significant rs12740061 (LOC105378783) × smoking interaction, such that the minor allele was associated with decreased HDL only among current smokers. This variant is a trans-eQTL for TAS1R1 (Supplementary Table 27). Variants in this gene have been found to influence taste receptors, notably affecting cigarette smoking habits.

**Discussion**

In this study, we evaluated gene-smoking interactions in large, multi-ancestry, meta-analyses of serum lipids, using varying associations among smoking subgroups to improve the ability...
to detect novel lipid loci. We report 13 novel loci for serum lipids from stage 1 and 2 meta-analyses. Sixteen additional statistically significant novel loci were found in stage 1 but were unavailable in stage 2. All 29 novel associations had a low q-value (p < 3 × 10^{-4}). Using both the 1df test of interaction and the 2df joint test of main and interaction effects in this study allowed us to improve our inferences based on the results: the 2df test bolstered the power to detect interactions, while the 1df test could discriminate between associations that predominantly reflected main effects vs. interactions.

Our results provide support for future efforts to evaluate lifestyle interactions with complex traits. We identified loci for which an association with serum lipids was only observed in one smoking stratum. In main effect models at these loci, the signal from one subgroup was not detected when all individuals were evaluated together (regardless of adjusting for smoking). These loci could only be observed by an analysis that was either smoking-stratified or contained an interaction term, highlighting the importance of considering potential effect modification in association studies. Additionally, through use of the joint 2df test, we identified six loci that appear to show novel main effects. Consistent with this characterization, five of these loci were within 500 KB of variants identified in recent large-scale association studies using main effect models: \textit{ETV}^{27–29}, \textit{TMEM175}^{28}, \textit{EYA1}^{28}, \textit{EYA3}^{28}, and \textit{B3GNT4}^{28}.

With 23,753 AFR individuals in the Stage 1 analyses and 30,970 AFR individuals overall, this work represents one of the largest studies of serum lipids in AFR. It is therefore unsurprising that two of our novel lipid loci (\textit{CREB3L2} and \textit{B3GNT4}) appear to be driven primarily by genetic main effects. Importantly, these associations could not have been detected in EUR, as the tested allele for both rs4758675 (\textit{B3GNT4}) and rs73729083 (\textit{CREB3L2}) are absent in EUR.

In addition to these probable main effect loci, the prominence of novel loci that were statistically significant only in AFR meta-analyses deserves further discussion. Some findings could not be effectively evaluated in other ancestry groups because of inter-ancestry MAF differences: the minor alleles for half of the variants were much more frequent in AFR. More puzzling, however, is the discovery of loci with evidence of strong interactions in AFR but not in meta-analyses in other ancestries, despite comparable or higher allele frequencies, such as were observed with rs12740061 (\textit{LOC105378783}, Figure 2) or rs17150980 (\textit{MAGI2}, Supplementary Figure 6). This phenomenon suggests inter-ancestry differences in either genomic or environmental context. There are variants in LD (r^2 > 0.2) among AFR for rs12740061 (\textit{LOC105378783}) and rs17150980 (\textit{MAGI2}) that are not in LD with these variants in other ancestries, but these variants were directly tested in our study with no evidence of an association in non-AFR analyses. Thus, it is unlikely that inter-ancestry LD differences explain these results, although unmeasured causal variants are a possibility. Inter-ancestry differences in smoking are also a potential explanation. In addition to known differences in smoking patterns\cite{31}, there are pronounced ancestry differences in preferred cigarette type, with over 85% of AFR smokers using menthol cigarettes compared to 29% of EUR smokers (in the US)\cite{32}. Menthol cigarettes are thought to facilitate greater absorption of harmful chemicals because of deeper inhalation\cite{31,33} through desensitization of nicotinic acetylcholine receptors that cause nicotine-induced irritation\cite{34}. Evidence for an
excess risk of cardiovascular disease associated with mentholated cigarettes, however, is equivocal\textsuperscript{35–39}. Ancestry differences in smoking-related metabolites and carcinogens have been reported\textsuperscript{40–43}, and differential metabolism of key compounds may underlie observed differences by ancestry. Some behaviors/conditions that co-occur with smoking may also differ by ancestry, and this additional factor may modify the observed genetic associations with serum lipids.

The biological mechanisms through which smoking influences the observed genetic associations will require further investigation, as the myriad components of cigarette smoke and their downstream consequences (including oxidative stress and inflammation) affect pathways throughout the body\textsuperscript{44}. However, there is evidence for differential expression of $PTPRZ1$\textsuperscript{15}, $LPL$\textsuperscript{15} and $LDLR$\textsuperscript{45} in cells exposed to an acute dose of nicotine. Also, concentrations of CETP\textsuperscript{46}, ApoB\textsuperscript{47}, and LPL\textsuperscript{48} are associated with smoking status.

The sample size attained for diverse ancestries is a key strength of our study, particularly among AFR. As a result, we were able to identify loci that had not been previously detected in meta-analyses of ancestries that are better represented in genomic research. Additionally, our use of nested models in our stage 1 analyses allowed us to more fully characterize loci. Despite these strengths, however, a smaller number of AFR studies were available for stage 2, resulting in an inability to follow up on some of our stage 1 low frequency findings.

In conclusion, this large, multi-ancestry genome-wide study of gene-smoking interactions on serum lipids identified 13 novel loci based on combined analysis of stages 1 and 2, and an additional 16 novel loci based on stage 1 that were unavailable in stage 2. Some loci were detected only in analyses stratified by smoking status or with a smoking interaction term, thus motivating further study of gene $\times$ environment interactions with other lifestyle factors to identify new loci for lipids and other complex traits. We demonstrate the importance of including diverse populations, reaching a sufficient sample size in these analyses for discovery of novel main effect lipid loci for AFR. Careful consideration of ancestry may be of particular importance for gene $\times$ environment interactions, as ancestry may be a proxy for both genomic and environmental context.

**URLs**

1000 Genomes Project: http://www.internationalgenome.org/

dbGaP: https://www.ncbi.nlm.nih.gov/gap

dbSNP: http://ncbi.nlm.nih.gov/snp/

DEPICT: http://data.broadinstitute.org/mpg/depict/

EasyQC: http://www.genepli-regensburg.de/easyqc

EasyStrata: http://www.genepi-regensburg.de/easystrata

ENCODE: https://www.encodeproject.org/
Online Methods

Details regarding motivation and methodology of this and other projects of the CHARGE Gene-Lifestyle Interactions Working Group are available in our recently published methods paper\(^1\), and detailed information on study design can be found in the Life Sciences Reporting Summary.

Participants

Analyses included men and women between 18 and 80 years of age of European (EUR), African (AFR), Asian (ASN), Hispanic (HISP), and (in stage 2 only) Brazilian (BR) ancestry. Participating studies are described in Supplementary Materials, with further details of sample sizes, trait distribution, and data preparation available in Supplementary Tables 1–6. Considerable effort was expended to engage as many studies of diverse ancestry as possible. This work was approved by the Washington University in St. Louis Institutional Review Board and complies with all relevant ethical regulations. Each study obtained
informed consent from participants and received approval from the appropriate institutional review boards.

**Phenotypes**

Analyses evaluated the concentrations of high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides. LDL could be either directly assayed or derived using the Friedewald equation (if triglycerides ≤400 mg/dL and individuals were fasting for at least 8 hours). Lipid-lowering drug use was defined as any use of a statin drug or any unspecified lipid-lowering drug after 1994 (when statin use became common). If LDL was directly assayed, adjustment for lipid-lowering drug was performed by dividing the LDL value by 0.7. If LDL was derived using the Friedewald equation, total cholesterol was first adjusted for lipid-lowering drug use (total cholesterol/0.8) before calculation of LDL by the Friedewald equation. No adjustments were made for any other lipid medication, nor were adjustments made to HDL or triglycerides for medication use. If samples were from individuals who were non-fasting (fasting ≤8 hours), then neither triglycerides nor calculated LDL were used. Both HDL and triglycerides were natural log-transformed, while LDL remained untransformed. In the event that multiple measurements of lipids were available (i.e. in a longitudinal study), analysts selected the visit for which data were available for the largest number of participants, and the measurement from that visit was included in analyses.

**Environmental Exposure Status**

Smoking variables evaluated were current smoking status (yes/no) and ever smoking status (yes/no). Current smokers were included in the exposed group for both of these variables, and never smokers were included in the unexposed group for both of these variables. Former smokers were included in the unexposed group for the current smoking variable and the exposed group for the ever-smoking variable. Smoking variables were coded as 0/1 for unexposed/exposed groups.

**Genotype Data**

Genotyping was performed by each participating study using genotyping arrays from either Illumina (San Diego, CA, USA) or Affymetrix (Santa Clara, CA, USA). Each study conducted imputation using various software. The cosmopolitan reference panel from the 1000 Genomes Project Phase I Integrated Release Version 3 Haplotypes (2010–11 data freeze, 2012–03-14 haplotypes) was specified for imputation and used by most studies, with some using the HapMap Phase II reference panel instead. Only variants on the autosome and with MAF of at least 0.01 were considered. Specific details of each participating study’s genotyping platform and imputation software are described (Supplementary Tables 3 and 6). Genotype was coded as the dosage of the imputed genetic variant, coded additively (0,1,2).

**Stage 1 Analysis**

Stage 1 genome-wide interaction analyses included 29 cohorts contributing data from 51 study/ancestry groups and up to 133,805 individuals of EUR, AFR, ASN, and HISP ancestry (Supplementary Tables 1–3). All cohorts ran three models in all individuals: a main effect...
model, a model adjusted for smoking, and an interaction model that included a multiplicative interaction term between the variant and smoking status (Figure 1). Additionally, the main effect model was run stratified by smoking exposure. All models were run for 3 lipids traits (HDL, LDL, and triglycerides) and 2 smoking exposures (current smoking and ever smoking). Thus, each study/ancestry group completed 30 GWAS (using 5 models × 3 traits × 2 exposures).

All models were adjusted for age, sex, and field center (as appropriate). Principal components derived using genotyped SNPs were included based on the study analyst’s discretion. All AFR cohorts were requested to include at least the first principal component, and 71% of AFR cohorts used multiple PCs (with 25% using 10 PCs). The average number of PCs used was 4.2. Additional cohort-specific covariates could be included if necessary to control for other potential confounding factors. Studies including participants from multiple ancestry groups conducted and reported analyses separately by ancestry. Participating studies provided the estimated genetic main effect and robust estimates of standard error for all requested models. In addition, for the models with an interaction term, studies also reported the interaction effects and robust estimates of their standard errors, and a robust estimate of the corresponding covariance matrix between the main and interaction effects. To obtain robust estimates of covariance matrices and robust standard errors, studies with only unrelated participants used R packages; either sandwich or ProbABEL. If the study included related individuals, either generalized estimating equations (R package geepack) or linear mixed models (GenABEL, MMAP, or R) were used. Sample code provided to studies to generate these data has been previously published (see Supplementary Materials 11).

Extensive quality control (QC) was performed using EasyQC on study-level (examining the results of each study individually), and then on ancestry-level (examining all studies within each ancestry group together). Study-level QC consisted of exclusion of all variants with MAF < 0.01, extensive harmonization of alleles, and comparison of allele frequencies with ancestry-appropriate 1000 Genomes reference data. Ancestry-level QC included the compilation of summary statistics on all effect estimates, standard errors and p-values across studies to identify potential outliers, and production of SE-N and QQ plots to identify analytical problems (such as improper trait transformations)50. Variants were excluded from ancestry-specific meta-analyses for an imputation score < 0.5; the same threshold was implemented regardless of imputation software, as imputation quality measures are shown to be similar across software51. Additionally, variants were excluded if the minimum of the minor allele count in the exposed or unexposed groups × imputation score was less than 20. To be included in meta-analyses, each variant had to be available from at least 3 studies or 5,000 individuals contributing data.

Meta-analyses were conducted for all models using the inverse variance-weighted fixed effects method as implemented in METAL. We evaluated both a 1 degree of freedom test of interaction effect (1df) and a 2 degree of freedom joint test of main and interaction effects (2df), following previously published methods52. A 1df Wald test was used to evaluate the 1df interaction, as well as the main effect and the smoking-adjusted main effect in models without an interaction term. A 2df Wald test was used to jointly test the effects of both the variant and the variant x smoking interaction52. Meta-analyses were conducted within each
ancestry separately, and then trans-ancestry meta-analyses were conducted on all ancestry-specific meta-analyses. Genomic control correction was applied before all meta-analyses.

Variants that were associated in any analysis at $p \leq 10^{-6}$ were carried forward for analysis in Stage 2. A total of 17,921 variants from 519 loci (defined by physical distance +/- 1 MB) were selected for Stage 2 analyses.

**Stage 2 Analysis**

Variants selected for Stage 2 were evaluated in 50 cohorts, with data from 75 separate ancestry/study groups totaling up to 253,467 individuals (Supplementary Tables 4–6). In addition to the 4 ancestry groups listed above, stage 2 analyses also included studies of Brazilian (BR) individuals. BR were considered only in the trans-ancestry meta-analyses, since there were no stage 1 BR results for meta-analysis. In stage 2, variants were evaluated only in a model with the interaction term (Figure 1).

Study- and ancestry-level QC was carried out as in stage 1. In contrast to stage 1, no additional filters were included for the number of studies or individuals contributing data to stage 2 meta-analyses, as these filters were implemented to reduce the probability of false positives, and were less relevant in stage 2. Stage 2 variants were evaluated in all ancestry groups and for all traits, no matter what specific meta-analysis met the $p$-value threshold in the stage 1 analysis. Genomic control was not applied to stage 2 meta-analyses, given the expectation of association. To ensure quality of analyses, all quality control and meta-analyses of replication data were completed independently by analysts at two different institutions (ARB and JLB [NIH], EL, XD, and CTL [Boston University]), with differences resolved through consultation.

**Meta-Analyses of Stages 1 and 2**

Given the increased power of combined meta-analysis of stage 1 and 2 results compared with a discovery and replication strategy, combined stage 1 and 2 meta-analyses were carried out for all the selected variants. We report variants significant at $5 \times 10^{-8}$ as well as those significant at Bonferroni correction for 2 smoking traits, 2 interaction tests, and ancestry-specific and trans-ancestry testing, with $p$-value of $6.25 \times 10^{-9}$ ($5 \times 10^{-8}/8$). Loci that are significant at the stricter $p$-value are identified in main tables. Loci were defined based on physical distance (+/- 1 MB) and are described by the index variant (i.e. the most statistically significant variant within each locus). Novelty was determined by physical distance (+/- 1 MB) from known lipids loci compiled from large meta-analyses, False Discovery Rate $q$ values were determined using EasyStrata to implement the Benjamini-Hochberg method of calculation. Results were visualized using R 3.1.0, including the package ‘forestplot’ (Supplementary Figures 3 and 4), and LocusZoom v1.4 (Supplementary Figure 5) for regional association plots.

**Smoking Dose Analysis**

To further characterize these associations, we evaluated an interaction between smoking dose and a few of the observed novel loci. While smoking dose data was not available for many of the included studies, we conducted secondary analysis on smoking dose interaction

*Nat Genet.* Author manuscript; available in PMC 2019 September 29.
in a subset of loci in our two largest AFR studies: WHI-SHARE and ARIC. We identified 4 loci from our main results (LOC105378783, CNTNAP2, MIR4686, DGCR8) for follow-up based on the following criteria: an interaction locus (as opposed to a probable main effect), stronger association observed among smokers compared to non-/never-smokers, the presence of contributing cohort(s) with smoking dose variables available and with p < 0.05 for reported result (to ensure sufficient power for analysis). We investigated these 4 loci using 3 methods of characterizing cigarettes per day: a quantitative variable, a categorical variable based on meaningful dose levels (less than a half a pack, between a half a pack and a pack, and more than a pack per day), and binary variable defined by the median of cigarettes per day in that cohort. Dose variables were defined separately by smoking status, such that cigarettes per day for former smokers were set to 0 for variables defined for current smokers, while the cigarettes per day for both current and former smokers were quantified when defined for ever smokers. Statistical significance was set at p < 0.0021, Bonferroni correction for investigation of 4 loci, 3 smoking dose variables, and 2 smoking status exposures.

Conditional Analyses

To assess independence of novel loci from established lipids loci, we conducted conditional analyses using GCTA. GCTA’s conditional and joint analysis option (COJO) calculates approximate conditional and joint association analyses based on summary statistics from a GWAS meta-analysis and individual genotype data from an ancestry-appropriate reference sample (for LD estimation). For novel loci from predominantly AFR meta-analyses, the LD reference set included unrelated AFR from HUFS, CFS, JHS, ARIC, and MESA (total N = 8,425). For novel loci from predominantly EUR meta-analyses, the LD reference set included unrelated EUR from ARIC (total N = 9,770). Excluding HUFS, these data were accessed through dbGaP (ARIC phs000280.v2.p1, phs000090.v2.p1; CFS phs000284.v1.p1; JHS phs000286.v4.p1, phs000499.v2.p1; and MESA phs000209.v13.p1, phs000420.v6.p3) and imputed to 1000 Genomes phase 1 v. 3 using the Michigan Imputation Server. For loci with a p < 5 × 10^{-8} for the 1df test of interaction, results from stage 1 and 2 meta-analyses were adjusted for all known lipids loci. A method for running conditional analyses for 2df tests has not been implemented within GCTA, therefore we evaluated loci with a p < 5 × 10^{-8} for the 2df joint test of main and interaction effects by conditioning stage 1 stratified analyses on known lipids loci (stratified analyses were not conducted in stage 2 studies). The conditioned 2df joint test of main and interaction effects was then calculated using EasyStrata on the conditioned stratified results.

Power Calculations for Detecting Interactions at Known Lipids Loci

To better contextualize our lack of detection of an interaction at a known locus, we conducted power calculations under a variety of scenarios. We explored the power to detect both an interaction and a main effect, making assumptions based on our data, as the sample sizes achieved in this project are comparable to the largest main effect GWAS for lipids. Using previously developed analytical power formulas, we evaluated three interaction scenarios: a pure interaction effect (no effect in non-smokers and a positive effect in current smokers), a quantitative interaction (effects in the same direction across strata, but of different magnitude), and a qualitative interaction (effects in opposite directions and of
different magnitude). We assumed stage 1 + 2 sample sizes and 19% prevalence of smoking (as in our data). For the purposes of illustration, we assumed relatively large effects which explain 0.06% of the variance in the lipid trait; the median variance explained from known lipid loci, as estimated from a previous publication (their Supplemental Table 1)\(^2\), is 0.04%.

**Proportion of Variance Explained**

To evaluate the proportion of the variance explained by our novel associations, we conducted additional analyses of our variants of interest in cohorts of diverse ancestries (Supplementary Table 16). In each of 10 studies from 4 ancestries (EUR, AFR, ASN, and HISP), we ran a series of nested regression models to determine the relative contribution of each set of additional variables. The first model included only standard covariates (age, sex, center, principal components, etc.). The second model additionally included smoking status (both current and ever smoking). The third added known variants\(^1\)–\(^5\),\(^12\). The fourth model added all novel variants, and the last model also included interaction terms for novel variants. For the purposes of this analysis, novel variants included the lead variant for each genome-wide significant locus in the meta-analyses of stages 1 and 2 (Table 1) and that were significant but only available in stage 1 meta-analyses (Table 2). By subtracting the \(r^2\) values from each of these nested regression models, the proportion of variance explained by the additional set of variables was determined. We conducted these analyses using two approaches. In Approach 1, all variants with MAF ≥ 0.01 and imputation quality ≥ 0.3 were included in regression models. While the imputation quality threshold used for the main analyses (≥ 0.5) was higher in order to reduce the risk of spurious associations, we selected a lower threshold for this secondary analysis to maximize the number of variants of interest included. In Approach 2, to avoid possible overfitting, stepwise regression was used for variant selection, such that only variants that were associated (p < 0.05) were retained in the model. All variants were considered in models for each trait and ancestry, regardless of the trait or ancestry in which the association was identified.

**Reproducing Previously Reported Lipids Associations**

To evaluate the degree to which our data confirmed previous associations, we evaluated statistically significant associations reported from recent large meta-analyses\(^1\)–\(^5\),\(^12\). In the event of overlap between reports, the most statistically significant variant-trait association was considered, for a total of 346 unique associations for 269 variants. Output from our main effect models (stage 1) was extracted for all ancestries for each previously reported variant-trait combination. Reproducibility was determined by p < 0.05 in any ancestry and a consistent direction of effect (Supplementary Table 17).

**Functional Inference**

To evaluate the degree to which our novel variants might influence other cardiometabolic traits, we extracted our novel variants (Tables 1 and 2) from previous studies. Supplementary Tables 19–24 present the association of these variants with coronary artery disease and myocardial infarction, using data from the CARDIoGRAM consortium\(^56\); neurological traits, using data from the Neurology Working Group of the CHARGE Consortium; anthropometry, using data from the GIANT consortium\(^57\)–\(^59\); adoptive smoking interaction, using data from the GIANT consortium\(^60\); diabetes and related traits, using data from...
MAGIC\textsuperscript{61}, AAIGLE\textsuperscript{62}, and DIAGRAM\textsuperscript{63, 64}; and kidney outcomes, using data from the COGENT-Kidney consortium\textsuperscript{65}.

To conduct functional annotation of our novel variants (Supplementary Tables 18, 25-27), we used NCBI Entrez gene (see URLs) for gene information, dbSNP to translate positions to human genome build 38, HaploReg (v4.1) and RegulomeDB for gene expression and regulation data from ENCODE and RoadMap projects, and GTEx v7.0 for additional gene expression information. We also investigated our novel variants in cis- and trans-eQTL data based on analysis of the whole blood of Framingham Heart Study participants\textsuperscript{66}.

**Pathway and Gene Set Enrichment Analyses**

We conducted DEPICT analyses\textsuperscript{13} based on genome-wide significant (p < 5 \times 10^{-8}) variants separately for the three traits HDL, LDL and triglycerides (Supplementary Tables 28-37). To obtain input for the prioritization and enrichment analyses, DEPICT first created a list of non-overlapping loci by applying a combined distance and LD based threshold (500 KB flanking regions and LD r^2 > 0.1) between the associated variants and the 1000 Genomes reference data. DEPICT then obtained lists of overlapping genes by applying an LD based threshold (r^2 > 0.5) between the non-overlapping variants and known functional coding or cis-acting regulatory variants for the respective genes. Finally, the major histocompatibility complex region on chromosome 6 (base position 25,000,000 – 35,000,000) was removed from further analyses. DEPICT prioritized genes at associated regions by comparing functional similarity of genes across associated loci using a gene score that was adjusted for several confounders, such as gene length. Utilizing lead variants from 500 pre-compiled null GWAS the scoring step was repeated 50 times to obtain an experiment-wide FDR for the gene prioritization. Second, DEPICT conducted gene-set enrichment analyses based on a total of 14,461 pre-compiled reconstituted gene sets. The reconstituted gene sets involve 737 Reactome database pathways, 2,473 phenotypic gene sets (derived from the Mouse Genetics Initiative\textsuperscript{67}, 184 Kyoto Encyclopedia of Genes and Genomes (KEGG) database pathways, 5,083 Gene Ontology database terms, and 5,984 protein molecular pathways (derived from protein-protein interactions\textsuperscript{68}). Third, DEPICT conducted tissue and cell type enrichment analyses based on expression data in any of the 209 MeSH annotations for 37,427 microarrays of the Affymetrix U133 Plus 2.0 Array platform. In addition, we used STRING database for identifying protein x protein interactions.

**Data Availability**

All summary results will be made available in dbGaP (phs000930.v7.p1).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Authors**

Amy R Bentley\textsuperscript{1,2,\ast}, Yun J Sung\textsuperscript{2,213,\ast}, Michael R Brown\textsuperscript{3,213,\ast}, Thomas W Winkler\textsuperscript{4,213,\ast}, Aldi T Kraja\textsuperscript{5,213,\ast}, Ioanna Ntalla\textsuperscript{6,213,\ast}, Karen Schwander\textsuperscript{2,213}, Daniel I Chasman\textsuperscript{7,8}, Elise Lim\textsuperscript{9}, Xuan Deng\textsuperscript{9}, Xiuqing Guo\textsuperscript{10}, Jingmin Liu\textsuperscript{11}, Yingchang
Lu12, Ching-Yu Cheng13,14,15, Xueling Sim16, Dina Vojinovic17, Jennifer E Huffman18, Solomon K Musani19, Changwei Li20, Mary F Feitosa5, Melissa A Richard21, Raymond Noordam22, Jenna Baker1, Guanjie Chen1, Hugues Aschard23,24, Traci M Bartz25, Jingzhong Ding26, Rajkumar Dorajoo27, Alisa K Manning28,29, Tuomo Rankinen30, Albert V Smith31,32, Salman M Tajuddin33, Wei Zhao34, Mariaelisa Graff35, Maris Alver36, Mathilde Boisset37, Jin Fang Chai16, Xu Chen38, Jasmin Divers39, Evangelos Evangelou40,41, Chuan Gao42, Anuj Goel43,44, Yanick Hagemeijer45, Sarah E Harris46,47, Fernando P Hartwig48,49, Meinan He50, Andrea RVR Horimoto51, Fang-Chi Hsu39, Yi-Jen Hung52,53, Anne U Jackson54, Anuradhani Kasturiratne55, Pirjo Komulainen56, Brigitte Kühnel57,58, Karin Leander59, Keng-Hung Lin60, Jian’an Luan61, Leo-Pekka Lyytikäinen62,63, Nana Matoba64, Ilja M Nolte65, Maik Pietzner66,67, Bram Prins68, Muhammad Riaz69,70, Antonietta Robino71, M Abdullah Said45, Nicole Schupf72, Robert A Scott61, Tamar Sofer29,73, Alena Stančáková74, Fumihiro Takeuchi75, Bamidele O Tayo76, Peter J van der Most65, Tibor V Varga77, Tzung-Dau Wang76,77, Yajuan Wang80, Erin B Ware81, Wanqing Wen82, Yong-Bing Xiang83, Lisa R Yanek84, Weihua Zhang85,86, Jing Hua Zhao85, Adebowale Adeyemo1, Saima Afaq85, Najaf Amin17, Marzeyh Amini65, Dan E Arking67, Zorayr Arzumanyan10, Tin Aung13,15,88, Christie Ballantyne89,90, R Graham Barr91, Lawrence F Bielak34, Eric Boerwinkle3,92, Erwin P Bottinger12, Ulrich Broeckel83, Morris Brown6,94, Brian E Cade73, Archie Campbell95, Mickaël Canouil37, Sabanayagam Charumathi13,14, Yi-Der Ida Chen10, Kaare Christensen96, COGENT-Kidney Consortium97, Maria Pina Concas71, John M Connell98, Lisa de las Fuentes2,99, H Janaka de Silva100, Paul S de Vries3, Ayo Doumatay1, Qing Duan101, Charles B Eaton102, Ruben N Eppinga46, Jessica D Fau81, James S Floyd103, Nita G Forouhi81, Terrence Forrester104, Yechiel Friedlander105, Ilaria Gandin106, He Gao40, Mohsen Ghanbari17,107, Sina A Gharib108, Bruna Gigante59, Franco Giuliani7, Hans J Grabe109, C Charles Gu2, Tamara B Harris110, Sami Heikkilä111,74, Chew-Kiat Heng112,113, Makoto Hirata114, James E Hixson3, M Afar Ikram17,115,116, EPIC-InterAct Consortium97, Yucheng Jia10, Roby Joehanes117,118, Craig Johnson119, Jost Bruno Jonas120,121, Anne E Justice35, Tomohiro Katuya122,123, Chiea Chuen Khor27, Tuomas O Kilpeläinen124,125, Woon-Puay Koh16,126, Ivana Kolcic127, Charles Kooperberg128, Jose E Krieger51, Stephen B Kritchevsky129, Michiaki Kubo130, Johanna Kuusisto74, Timo A Lakka56,111,131, Carl D Langefeld39, Claudia Langenberg61, Lenore J Launer110, Benjamin Lehne132, Cora E Lewis133, Yize Li2, Jingjing Liang80, Shiow Lin5, Ching-Ti Liu9, Jianjun Liu27,134, Kiang Liu135, Marie Loh85,136,137,138, Kurt Lohman39, Tin Louie139, Anna Luzzi10, Reedik Mägi36, Anubha Mahajan44, Ani W Manichaikul140, Colin A McKenzie141, Thomas Meitinger142,143,144, Andres Metspalu36, Yuri Milanesci145, Lili Milano36, Karen L Mohlke101, Yukihide Momozawa146, Andrew P Morris34,147, Alison D Murray148, Mike A Nalls149,150, Matthias Nauck66,67, Christopher P Nelson69,70, Kari E North35, Jeffrey R O’Connell151,152, Nicholette D Palmer153, George J Papanicolaou154, Nancy L Pedersen38, Annette Peters58,155, Patricia A Peyser34, Ozren Polasek127,156,157, Neil Poulter158, Olli T Raitakari159,160, Alex P Reiner128, Frida Renström17,161, Treva K Rice2, Stephen S Rich140, Jennifer G Robinson162, Lynda M Rose7, Frits R
Rosendaal163, Igor Rudan164, Carsten O Schmidt165, Pamela J Schreiner166, William R Scott132,167, Peter Sever167, Yuan Shi13, Stephen Sidney168, Mario Sims19, Jennifer A Smith34,81, Harold Snieder65, John M Starr46,169, Konstantin Strauch170,171, Heather M Stringham54, Nicholas YQ Tan13, Hua Tang172, Kent D Taylor10, Yik Ying Teo16,27,173,174,175, Yih Chung Tham13, Henning Tiemeier17,176, Stephen T Turner177, André G Utterlinden17,178,17, Understanding Society Scientific Group97, Diana van Heemst22, Melanie Waldenberger45,58, Heming Wang29,73, Lan Wang9, Lihua Wang5, Wen Bin Wei179, Christine A Williams5, Gregory Wilson Sr180, Mary K Wojczynski5, Jie Yao10, Kristin Young35, Caizheng Yu50, Jian-Min Yuan181,182, Jie Zhou1, Alan B Zonderman183, Diane M Becker84, Michael Boehnke54, Donald W Bowden153, John C Chambers40,86,85,138,184, Richard S Cooper76, Ulf de Faire59, Ian J Deary46,185, Paul Elliott85, Tõnu Esko36,186, Martin Farrall43,44, Paul W Franks77,187,188,189, Barry I Freedman190, Philippe Foguel1,191, Paolo Gasparini71,106, Christian Gieger58,192, Bernardo L Horta48, Jyh-Ming Jimmy Juang193,79, Yoichiro Kamatani64, Candace M Kammerer194, Norihiro Kato75, Jaspal S Kooner86,85,167,184, Markku Laakso74, Cathy C Laurie139, I-Te Lee195,196,197, Terho Lehtimäki62,63, Lifelines Cohort Study97, Patrik KE Magnusson38, Albertine J Oldehinkel198, Brenda WJH Penninx145, Alexandre C Pereira81, Rainer Rauramaa56, Susan Redline73, Nilesh J Samani69,70, James Scott167, Xiao-Ou Shu82, Pim van der Harst45,199, Lynne E Wagenknecht200, Jun-Sing Wang195,197, Ya Xing Wang121, Nicholas J Wareham61, Hugh Watkins43,44, David R Weir81, Ananda R Wickremasinghe55, Tangchun Wu50, Eleftheria Zeggini68,201, Wei Zheng82, Claude Bouchard30, Michele K Evans33, Vilmundur Gudnason31,32, Sharon LR Kardia34, Yongmei Liu202, Bruce M Psaty203,204, Paul M Ridker7,8, Rob M van Dam16,134, Dennis O Mook-Kanamori163,205, Myriam Fornage206,3, Michael A Province5, Tanika N Kelly207, Ervin R Fox208, Caroline Hayward18, Cornelia M van Duijn17,209, E Shyoung Tai16,126,134, Tien Yin Wong13,15,88, Ruth JF Loos12,210, Nora Franceschini35, Jerome I Rotter10, Xiaofeng Zhu80,213, Laura J Bierut211,213, W James Gauderman212,213, Kenneth Rice139,213,*, Patricia B Munroe9,34,213,*, Alanna C Morrison3,213,*, Dabeeru C Rao2,213,*, Charles N Rotimi1,213,*, and L Adrienne Cupples9,118,213,*

Affiliations

1 Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA.
2 Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri, USA.
3 Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas, USA.
4 Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany.
5 Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, Missouri, USA.
6 Clinical Pharmacology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK.
7 Division of Preventive Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA.
8 Harvard Medical School,
Boston, Massachusetts, USA. 9 Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA. 10 The Institute for Translational Genomics and Population Sciences, Department of Pediatrics, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, USA. 11 WHI CCC, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. 12 The Charles Bronfman Institute for Personalized Medicine, The Icahn School of Medicine at Mount Sinai, New York, New York, USA. 13 Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore, Singapore. 14 Centre for Quantitative Medicine, Academic Medicine Research Institute, Ophthalmology & Visual Sciences Academic Clinical Program (Eye ACP), Duke-NUS Medical School, Singapore, Singapore, Singapore. 15 Department of Ophthalmology, Hong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, Singapore. 16 Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore, Singapore. 17 Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. 18 Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom. 19 Jackson Heart Study, Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA. 20 Epidemiology and Biostatistics, University of Georgia at Athens College of Public Health, Athens, Georgia, USA. 21 Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, Texas, US. 22 Internal Medicine, Gerontology and Geriatrics, Leiden University Medical Center, Leiden, Netherlands. 23 Centre de Bioinformatique, Biostatistique et Biologie Intégrative (C3BI), Institut Pasteur, Paris, France. 24 Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts, USA. 25 Cardiovascular Health Research Unit, Biostatistics and Medicine, University of Washington, Seattle, Washington, USA. 26 Center on Diabetes, Obesity, and Metabolism, Gerontology and Geriatric Medicine, Wake Forest University Health Sciences, Winston-Salem, North Carolina, USA. 27 Genome Institute of Singapore, Agency for Science Technology and Research, Singapore, Singapore. 28 Clinical and Translational Epidemiology Unit, Massachusetts General Hospital, Boston, Massachusetts, USA. 29 Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA. 30 Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA. 31 Icelandic Heart Association, Kopavogur, Iceland. 32 Faculty of Medicine, University of Iceland, Reykjavik, Iceland. 33 Health Disparities Research Section, Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA. 34 Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA. 35 Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina, USA. 36 Estonian Genome Center, Institute of Genomics, University of Tartu, Tartu, Estonia. 37 CNRS UMR 8199, European Genomic Institute for Diabetes (EGID), Institut Pasteur de Lille, University of Lille, Lille, France. 38 Department of Medical
Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Stockholm, Sweden. 39 Department of Biostatistics and Data Science, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA. 40 Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK. 41 Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece. 42 Molecular Genetics and Genomics Program, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA. 43 Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, Oxfordshire, UK. 44 Wellcome Centre for Human Genetics, University of Oxford, Oxford, Oxfordshire, UK. 45 University of Groningen, University Medical Center Groningen, Department of Cardiology, Groningen, The Netherlands. 46 Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, Edinburgh, UK. 47 Medical Genetics Section, University of Edinburgh Centre for Genomic and Experimental Medicine and MRC Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, UK. 48 Postgraduate Programme in Epidemiology, Federal University of Pelotas, Pelotas, RS, Brazil. 49 Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, UK. 50 Department of Occupational and Environmental Health and State Key Laboratory of Environmental Health for Incubating, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. 51 Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, SP, Brazil. 52 Endocrinology and Metabolism, Tri-Service General Hospital, Taipei, Taiwan. 53 School of Medicine, National Defense Medical Center, Taipei, Taiwan. 54 Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA. 55 Department of Public Health, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka. 56 Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland. 57 Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. 58 Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. 59 Unit of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. 60 Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan. 61 MRC Epidemiology Unit, University of Cambridge, Cambridge, UK. 62 Department of Clinical Chemistry, Fimlab Laboratories, Tampere, Finland. 63 Department of Clinical Chemistry, Finnish Cardiovascular Research Center - Tampere, Faculty of Medicine and Technology, Tampere University, Tampere, Finland. 64 Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. 65 University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands. 66 DZHK (German Centre for Cardiovascular Health), Partner Site Greifswald, Greifswald, Germany. 67 Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany. 68 Human Genetics,
69. Department of Cardiovascular Sciences, University of Leicester, Leicester, UK. 70. NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, UK. 71. Institute for Maternal and Child Health - IRCCS “Burlo Garofolo”, Trieste, Italy. 72. Taub Institute for Research on Alzheimer’s Disease and the Aging Brain, Columbia University Medical Center, New York, New York, USA. 73. Division of Sleep and Circadian Disorders, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 74. Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland. 75. Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan. 76. Department of Public Health Sciences, Loyola University Chicago, Maywood, Illinois, USA. 77. Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University Diabetes Centre, Skåne University Hospital, Malmö, Sweden. 78. Cardiovascular Center and Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan. 79. National Taiwan University College of Medicine, Taipei, Taiwan. 80. Department of Population Quantitative and Health Sciences, Case Western Reserve University, Cleveland, Ohio, USA. 81. Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, Michigan, USA. 82. Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA. 83. SKLORG & Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. 84. Division of General Internal Medicine, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. 85. MRC-PHE Centre for Environment and Health, School of Public Health, Imperial College London, London, UK. 86. Department of Cardiology, Ealing Hospital, Middlesex, UK. 87. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. 88. Ophthalmology & Visual Sciences Academic Clinical Program (Eye ACP), Duke-NUS Medical School, Singapore, Singapore, Singapore. 89. Section of Cardiovascular Research, Baylor College of Medicine, Houston, Texas, USA. 90. Houston Methodist DeBakey Heart and Vascular Center, Houston, Texas, USA. 91. Departments of Medicine and Epidemiology, Columbia University Medical Center, New York, New York, USA. 92. Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, USA. 93. Section of Genomic Pediatrics, Department of Pediatrics, Medicine and Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA. 94. NIHR Barts Cardiovascular Biomedical Research Centre, Queen Mary University of London, London, London, UK. 95. Centre for Genomic & Experimental Medicine, Institute of Genetics & Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom. 96. The Danish Aging Research Center, Institute of Public Health, University of Southern Denmark, Odense, Denmark. 97. A full list of authors can be found in the Supplementary Note. 98. Ninewells Hospital & Medical School, University of Dundee, Dundee, Scotland, UK. 99. Cardiovascular Division, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA. 100. Department of Medicine, Faculty of...
Medicine, University of Kelaniya, Ragama, Sri Lanka. 101. Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA. 102. Department of Family Medicine and Epidemiology, Alpert Medical School of Brown University, Providence, Rhode Island, USA. 103. Cardiovascular Health Research Unit, Medicine and Epidemiology, University of Washington, Seattle, Washington, USA. 104. UWI Solutions for Developing Countries, University of the West Indies, Kingston, Jamaica. 105. Braun School of Public Health, Hebrew University-Hadassah Medical Center, Jerusalem, Israel. 106. Department of Medical Sciences, University of Trieste, Trieste, Italy. 107. Department of Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. 108. Computational Medicine Core, Center for Lung Biology, UW Medicine Sleep Center, Medicine, University of Washington, Seattle, Washington, USA. 109. Department Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany. 110. Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA. 111. Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio Campus, Finland. 112. Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore. 113. Kho Teck Puat – National University Children’s Medical Institute, National University Health System, Singapore, Singapore. 114. Laboratory of Genome Technology, Human Genome Center, Institute of Medical Science, The University of Tokyo, Minato-ku, Japan. 115. Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands. 116. Department of Neurology, Erasmus University Medical Center, Rotterdam, The Netherlands. 117. Hebrew SeniorLife, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA. 118. Framingham Heart Study, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA. 119. Collaborative Health Studies Coordinating Center, University of Washington, Seattle, Washington, USA. 120. Department of Ophthalmology, Medical Faculty Mannheim, University Heidelberg, Mannheim, Germany, Germany. 121. Beijing Institute of Ophthalmology, Beijing Ophthalmology and Visual Science Key Lab, Beijing Tongren Eye Center, Capital Medical University, Beijing, China. 122. Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Suita, Japan. 123. Department of Geriatric and General Medicine, Osaka University Graduate School of Medicine, Suita, Japan. 124. Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2200, Denmark. 125. Department of Environmental Medicine and Public Health, The Icahn School of Medicine at Mount Sinai, New York, New York, USA. 126. Duke-NUS Medical School, Singapore, Singapore. 127. Department of Public Health, Department of Medicine, University of Split, Split, Croatia. 128. Fred Hutchinson Cancer Research Center, University of Washington School of Public Health, Seattle, Washington, USA. 129. Sticht Center for Healthy Aging and Alzheimer’s Prevention, Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA. 130. RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. 131. Department of Clinical
Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland.  
132. Institute of Clinical Sciences, Department of Molecular Sciences, Imperial College London, London, UK.  
133. Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA.  
134. Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.  
135. Epidemiology, Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA.  
136. Translational Laboratory in Genetic Medicine, Agency for Science, Technology and Research, Singapore.  
137. Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.  
Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore.  
139. Department of Biostatistics, University of Washington, Seattle, Washington, USA.  
140. Center for Public Health Genomics, University of Virginia, Charlottesville, Virginia, USA.  
141. Tropical Metabolism Research Unit, Caribbean Institute for Health Research, University of the West Indies, Mona, Jamaica.  
142. Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.  
143. Institute of Human Genetics, Technische Universität München, Munich, Germany.  
144. Technische Universität München, Munich, Germany.  
145. Department of Psychiatry, Amsterdam Neuroscience and Amsterdam Public Health Research Institute, Amsterdam UMC, Vrije Universiteit, Amsterdam, The Netherlands.  
146. Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan.  
147. Department of Biostatistics, University of Liverpool, Liverpool, UK.  
148. The Institute of Medical Sciences, Aberdeen Biomedical Imaging Centre, University of Aberdeen, Aberdeen, United Kingdom.  
149. Data Tecnica International, Glen Echo, MD, USA.  
150. Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD, USA.  
151. Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland, USA.  
152. Program for Personalized and Genomic Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA.  
153. Biochemistry, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA.  
154. Epidemiology Branch, Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland.  
155. Chair of Epidemiology, Faculty of Medicine, IBE, LMU, Munich, Germany.  
156. Psychiatric Hospital "Sveti Ivan", Zagreb, Croatia.  
157. Gen-info Ltd, Zagreb, Croatia.  
158. School of Public Health, Imperial College London, London, UK.  
159. Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland.  
160. Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland.  
161. Department of Biobank Research, Umeå University, Umeå, Västerbotten, Sweden.  
162. Department of Epidemiology and Medicine, University of Iowa, Iowa City, Iowa, USA.  
163. Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands.  
164. Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, United Kingdom.  
165. Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany.
166. Division of Epidemiology & Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA. 167. National Heart and Lung Institute, Imperial College London, London, UK. 168. Division of Research, Kaiser Permanente Northern California, Oakland, California, USA. 169. Alzheimer Scotland Dementia Research Centre, The University of Edinburgh, Edinburgh, UK. 170. Chair of Genetic Epidemiology, IBE, Faculty of Medicine, LMU, Munich, Germany. 171. Institute of Genetic Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. 172. Department of Genetics, Stanford University, Stanford, California, USA. 173. Department of Statistics and Applied Probability, National University of Singapore, Singapore, Singapore. 174. Life Sciences Institute, National University of Singapore, Singapore, Singapore. 175. NUS Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore, Singapore. 176. Department of Social and Behavioral Sciences, Harvard TH Chan School of Public Health, Boston, Massachusetts, USA. 177. Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA. 178. Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands. 179. Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing, China, China. 180. Jackson Heart Study, School of Public Health, Jackson State University, Jackson, Mississippi, USA. 181. Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. 182. Division of Cancer Control and Population Sciences, UPMC Hillman Cancer, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. 183. Behavioral Epidemiology Section, Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA. 184. Imperial College Healthcare NHS Trust, London, UK. 185. Psychology, The University of Edinburgh, Edinburgh, UK. 186. Broad Institute of the Massachusetts Institute of Technology and Harvard University, Boston, Massachusetts, USA. 187. Harvard T. H. Chan School of Public Health, Department of Nutrition, Harvard University, Boston, Massachusetts, USA. 188. Department of Public Health & Clinical Medicine, Umeå University, Umeå, Västerbotten, Sweden. 189. OCDEM, Radcliffe Department of Medicine, University of Oxford, Oxford, UK. 190. Nephrology, Internal Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA. 191. Department of Genomics of Common Disease, Imperial College London, London, United Kingdom. 192. German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany. 193. Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan. 194. Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. 195. Endocrinology and Metabolism, Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan. 196. School of Medicine, Chung Shan Medical University, Taichung, Taiwan. 197. School of Medicine, National Yang-Ming University, Taipei, Taiwan. 198. University of Groningen, University Medical Center Groningen, Department of Psychiatry, Groningen, The Netherlands. 199. University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, The Netherlands. 200. Public Health
Acknowledgments

The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Human Genome Research Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services. This project was largely supported by a grant from the US National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (R01HL118305) and by the Intramural Research Program of the National Human Genome Research Institute of the National Institutes of Health through the Center for Research on Genomics and Global Health (CRGGH). The CRGGH is supported by the National Human Genome Research Institute, the National Institute of Diabetes and Digestive and Kidney Diseases, the Center for Information Technology, and the Office of the Director at the National Institutes of Health (Z01HG200362). Additional and study-specific acknowledgments appear in the Supplementary Note.

Appendix

Author Contributions

All authors reviewed and approved the manuscript. Study concept and design: A.B.Z., A.C.M., A.C.P., A.J.O., A.R., A.R.B., A.R.W., B.I.F., B.L.H., C.A.M.K., C.Ballantyne, C.Bouchard, C.C.K., C.C.L., C.D.L., C.H., C.Langenberg, C.M.van D., C.M.K., C.N.R., C-T.L., C.Y., C-Y.C., D.C.R., D.I.C., D.M.B., D.R.W., D.W.B., E.B., E.P.B., E.R.F., E.S.T., F.R.R., G.W., H.A., H.J.de S., H.Watkins, I.G., I.J.D., I.K., I.B.J., I.Ding, I.Divers, I.D.F., J.E.Hixson, J.E.K., J.I.R., J.K., Jianjun Liu, J.M.C., J.M.S., J-M.Y., K.C., K.K.L., K.L.M., L.A.C., Lifelines Cohort Study, L.E.W., L.J.L., M.A.I., M.A.P., M.Brown, M.Boehnke, M.Farrall, M.Fornage, M.He, M.K., M.K.E., M.Laakso, M.S., N.G.F., N.J.S., N.J.W., N.K., N.L.P., N.P., N.S., O.P., O.T.R., P.F., P.G., P.H., P.K., P.K.E.M., P.M.R., P.S., R.A.S., R.M.D., R.R., R.S.C., S.C., S.K.M., S.L.R.K., S.R., S.T.T., T.A., T.A.L., T.B.H., T.F., T.K.R., T.Lehtimäki, T.N.K., T.R., T.W., T.Y.W., U.de F., V.G., W.B.W., W.P.K., X.G., Y.K., Y.Liu, Y.W., Y.X.W., and Y.Y.T. Phenotype data acquisition and/or quality control:
References

1. Willer CJ et al. Discovery and refinement of loci associated with lipid levels. Nature genetics 45, 1274–83 (2013). [PubMed: 24097068]

Nat Genet. Author manuscript; available in PMC 2019 September 29.
2. Do R et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nature genetics 45, 1345–52 (2013). [PubMed: 24097064]

3. Peloso GM et al. Association of low-frequency and rare coding-sequence variants with blood lipids and coronary heart disease in 56,000 whites and blacks. American journal of human genetics 94, 223–32 (2014). [PubMed: 24507774]

4. Spracklen CN et al. Association analyses of East Asian individuals and trans-ancestry analyses with European individuals reveal new loci associated with cholesterol and triglyceride levels. Human Molecular Genetics 26, 1770–1784 (2017). [PubMed: 28334899]

5. Teslovich TM et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466, 707–13 (2010). [PubMed: 20686565]

6. Kathiresan S et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 358, 1240–9 (2008). [PubMed: 18354102]

7. Kar D et al. Relationship of cardiometabolic parameters in non-smokers, current smokers, and quitters in diabetes: a systematic review and meta-analysis. Cardiovascular Diabetology 15, 158 (2016). [PubMed: 27881170]

8. Zong C et al. Cigarette smoke exposure impairs reverse cholesterol transport which can be minimized by treatment of hydrogen-saturated saline. Lipids in Health and Disease 14, 159 (2015). [PubMed: 26634341]

9. Manning AK et al. Meta-analysis of Gene-Environment interaction: joint estimation of SNP and SNP×Environment regression coefficients. Genetic Epidemiology 35, 11–18 (2011). [PubMed: 21181894]

10. Psaty BM et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from five cohorts. Circulation. Cardiovascular genetics 2, 73–80 (2009). [PubMed: 20031568]

11. Rao DC et al. Multiancestry Study of Gene–Lifestyle Interactions for Cardiovascular Traits in 610 475 Individuals From 124 Cohorts. Design and Rationale 10(2017).

12. Lanktree MB et al. Genetic meta-analysis of 15,901 African Americans identifies variation in EXOC3L1 is associated with HDL concentration. Journal of Lipid Research 56, 1781–6 (2015). [PubMed: 26199122]

13. Pers TH et al. Biological interpretation of genome-wide association studies using predicted gene functions. Nature Communications 6, 5890–5890 (2015).

14. Suzuki J, Imanishi E & Nagata S Xkr8 phospholipid scrambling complex in apoptotic phosphatidylserine exposure. Proceedings of the National Academy of Sciences of the United States of America 113, 9509–9514 (2016). [PubMed: 27503893]

15. Wang J et al. Genome-Wide Expression Analysis Reveals Diverse Effects of Acute Nicotine Exposure on Neuronal Function-Related Genes and Pathways. Frontiers in Psychiatry 2, 5 (2011). [PubMed: 21556275]

16. International Parkinson Disease Genomics, C. Imputation of sequence variants for identification of genetic risks for Parkinson’s disease: a meta-analysis of genome-wide association studies. Lancet 377, 641–649 (2011). [PubMed: 21292315]

17. Ng MCY et al. Meta-Analysis of Genome-Wide Association Studies in African Americans Provides Insights into the Genetic Architecture of Type 2 Diabetes. PLOS Genetics 10, e1004517 (2014). [PubMed: 25102180]

18. Cai K, Lucki NC & Sewer MB Silencing diacylglycerol kinase-theta expression reduces steroid hormone biosynthesis and cholesterol metabolism in human adrenocortical cells(). Biochimica et biophysica acta 1841, 552–562 (2014). [PubMed: 24369117]

19. Cai K & Sewer MB Diacylglycerol kinase θ couples farnesoid X receptor-dependent bile acid signalling to Akt activation and glucose homeostasis in hepatocytes. The Biochemical journal 454, 267–274 (2013). [PubMed: 23767959]

20. Cordell HJ et al. International genome-wide meta-analysis identifies new primary biliary cirrhosis risk loci and targetable pathogenic pathways. Nature Communications 6, 8019 (2015).

21. Edwards TL et al. Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. Annals of human genetics 74, 97–109 (2010). [PubMed: 20070850]
22. Lill CM et al. Comprehensive Research Synopsis and Systematic Meta-Analyses in Parkinson’s Disease Genetics: The PDGene Database. PLOS Genetics 8, e1002548 (2012). [PubMed: 22438815]

23. Nalls MA et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson’s disease. Nature genetics 46, 989–993 (2014). [PubMed: 25064009]

24. Pankratz N et al. Meta-analysis of Parkinson disease: Identification of a novel locus, RIT2. Annals of Neurology 71, 370–384 (2012). [PubMed: 22451204]

25. Wang J et al. Phlegm-Dampness Constitution: Genomics, Susceptibility, Adjustment and Treatment with Traditional Chinese Medicine. The American Journal of Chinese Medicine 41, 253–262 (2013). [PubMed: 23548117]

26. Choi J-H et al. Variations in TAS1R taste receptor gene family modify food intake and gastric cancer risk in a Korean population. Molecular Nutrition & Food Research 60, 2433–2445 (2016). [PubMed: 27321875]

27. Hoffmann TJ et al. A large electronic-health-record-based genome-wide study of serum lipids. Nature Genetics 50, 401–413 (2018). [PubMed: 29074222]

28. Klarin D et al. Genetics of blood lipids among ~300,000 multi-ethnic participants of the Million Veteran Program. Nature Genetics (2018).

29. Liu DJ et al. Exome-wide association study of plasma lipids in ~300,000 individuals. Nature Genetics 49, 1758 (2017). [PubMed: 29083408]

30. Ward LD & Kellis M HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Research 40, D930–D934 (2012). [PubMed: 22064851]

31. U.S. Department of Health and Human Services. Tobacco Use Among U.S. Racial/Ethnic Minority Groups—African Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, and Hispanics: A Report of the Surgeon General (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, GA, 1998).

32. Villanti AC et al. Changes in the prevalence and correlates of menthol cigarette use in the USA, 2004–2014. Tobacco Control 25, i14 (2016). [PubMed: 27729565]

33. Ross KC, Dempsey DA, Helen G St., Delucchi K & Benowitz NL The influence of puff characteristics, nicotine dependence, and rate of nicotine metabolism on daily nicotine exposure in African American smokers. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 25, 936–943 (2016).

34. Ton HT et al. Menthol Enhances the Desensitization of Human α3β4 Nicotinic Acetylcholine Receptors. Molecular Pharmacology 88, 256–264 (2015). [PubMed: 25964258]

35. Alexander LA et al. Why We Must Continue to Investigate Menthol’s Role in the African American Smoking Paradox. Nicotine & Tobacco Research 18, S91–S101 (2016). [PubMed: 26980870]

36. Jones MR, Tellez-Plaza M & Navas-Acien A Smoking, Menthol Cigarettes and All-Cause, Cancer and Cardiovascular Mortality: Evidence from the National Health and Nutrition Examination Survey (NHANES) and a Meta-Analysis. PLoS ONE 8, e77941 (2013). [PubMed: 24205038]

37. Munro HM, Tarone RE, Wang TJ & Blot WJ Menthol and Nonmenthol Cigarette Smoking: All-Cause Deaths, Cardiovascular Disease Deaths, and Other Causes of Death Among Blacks and Whites. Circulation 133, 1861–1866 (2016). [PubMed: 27022064]

38. Murray RP, Connett JE, Skeans MA & Tashkin DP Menthol Cigarettes and Health Risks in Lung Health Study Data. Nicotine & Tobacco Research 9, 101–107 (2007). [PubMed: 17365741]

39. Vozoris NT, Mhc, Md & Frcpc. Mentholated cigarettes and cardiovascular and pulmonary diseases: A population-based study. Archives of Internal Medicine 172, 590–593 (2012). [PubMed: 22493467]

40. Pérez-Stable EJ, Herrera B, Jacob IP & Benowitz NL Nicotine metabolism and intake in black and white smokers. JAMA 280, 152–156 (1998). [PubMed: 9669788]
41. Khariwala SS et al. Cotinine and Tobacco-Specific Carcinogen Exposure Among Nondaily Smokers in a Multiethnic Sample. Nicotine & Tobacco Research 16, 600–605 (2014). [PubMed: 24297808]

42. Jain RB Distributions of selected urinary metabolites of volatile organic compounds by age, gender, race/ethnicity, and smoking status in a representative sample of U.S. adults. Environmental Toxicology and Pharmacology 40, 471–479 (2015). [PubMed: 26282484]

43. Benowitz NL, Dains KM, Dempsey D, Wilson M & Jacob P Racial Differences in the Relationship Between Number of Cigarettes Smoked and Nicotine and Carcinogen Exposure. Nicotine & Tobacco Research 13, 772–783 (2011). [PubMed: 21546441]

44. U.S. Department of Health and Human Services. The Health Consequences of Smoking: 50 Years of Progress A Report of the Surgeon General. ( U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, GA, 2014).

45. Ito S et al. Nicotine-Induced Expression of Low-Density Lipoprotein Receptor in Oral Epithelial Cells. PLoS ONE 8, e82563 (2013). [PubMed: 24358207]

46. Dullaart RP, Hoogenberg K, Dikkeschei BD & van Tol A Higher plasma lipid transfer protein activities and unfavorable lipoprotein changes in cigarette-smoking men. Arteriosclerosis, Thrombosis, and Vascular Biology 14, 1581–1585 (1994).

47. Frondelius K et al. Lifestyle and Dietary Determinants of Serum Apolipoprotein A1 and Apolipoprotein B Concentrations: Cross-Sectional Analyses within a Swedish Cohort of 24,984 Individuals. Nutrients 9, 211 (2017).

48. Onat A et al. Preheparin serum lipoprotein lipase mass interacts with gender, gene polymorphism and, positively, with smoking. in Clinical Chemistry and Laboratory Medicine Vol. 47 208 (2009). [PubMed: 19191728]

49. Winkler TW et al. Quality control and conduct of genome-wide association meta-analyses. Nature protocols 9, 1192–212 (2014). [PubMed: 24762786]

50. Winkler TW et al. EasyStrata: evaluation and visualization of stratified genome-wide association meta-analysis data. Bioinformatics 31, 259–261 (2015). [PubMed: 25260999]

51. Marchini J & Howie B Genotype imputation for genome-wide association studies. Nat Rev Genet 11, 499–511 (2010). [PubMed: 20517342]

52. Kraft P, Yen YC, Stram DO, Morrison J & Gauderman WJ Exploiting Gene-Environment Interaction to Detect Genetic Associations. Human Heredity 63, 111–119 (2007). [PubMed: 17283440]

53. Skol AD, Scott LJ, Abecasis GR & Boehnke M Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38, 209–213 (2006). [PubMed: 16415888]

54. Das S et al. Next-generation genotype imputation service and methods. Nature Genetics 48, 1284 (2016). [PubMed: 27571263]

55. Winkler TW et al. Approaches to detect genetic effects that differ between two strata in genome-wide meta-analyses: Recommendations based on a systematic evaluation. PLoS One 12, e0181038 (2017). [PubMed: 28749953]

56. Nikpay M et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet 47, 1121–1130 (2015). [PubMed: 26343387]

57. Wood AR et al. Defining the role of common variation in the genomic and biological architecture of adult human height. Nature genetics 46, 1173–1186 (2014). [PubMed: 25282103]

58. Locke AE et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 518, 197 (2015). [PubMed: 25673413]

59. Shungin D et al. New genetic loci link adipose and insulin biology to body fat distribution. Nature 518, 187 (2015). [PubMed: 25673412]

60. Justice AE et al. Genome-wide meta-analysis of 241,258 adults accounting for smoking behaviour identifies novel loci for obesity traits. Nature Communications 8, 14977 (2017).

61. Manning AK et al. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nature genetics 44, 659–669 (2012). [PubMed: 22581228]
62. Liu CT et al. Trans-ethnic Meta-analysis and Functional Annotation Illuminates the Genetic Architecture of Fasting Glucose and Insulin. Am J Hum Genet 99, 56–75 (2016). [PubMed: 27321945]

63. Gaulton KJ et al. Genetic fine-mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. Nature genetics 47, 1415–1425 (2015). [PubMed: 26551672]

64. DIAbetes Genetics Replication Meta-analysis Consortium, Asian Genetic Epidemiology Network Type Diabetes Consortium, South Asian Type Diabetes Consortium Mexican American Type Diabetes Consortium & Type Diabetes Genetic Exploration by Next-generation sequencing in multi-Ethnic Samples Consortium. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nature genetics 46, 234–244 (2014). [PubMed: 24509480]

65. Mahajan A et al. Trans-ethnic Fine Mapping Highlights Kidney-Function Genes Linked to Salt Sensitivity. American Journal of Human Genetics 99, 636–646 (2016). [PubMed: 27588450]

66. Joehanes R et al. Integrated genome-wide analysis of expression quantitative trait loci aids interpretation of genomic association studies. Genome Biology 18, 16 (2017). [PubMed: 28122634]

67. Blake JA et al. The Mouse Genome Database: integration of and access to knowledge about the laboratory mouse. Nucleic Acids Res 42, D810–7 (2014). [PubMed: 24285300]

68. Lage K et al. A human phenome-interactome network of protein complexes implicated in genetic disorders. Nat Biotechnol 25, 309–16 (2007). [PubMed: 17344885]
Figure 1. Study Overview:
Summary of data included in this study. 1 16,389 variants passed filtering criteria and were included in stage 2 analyses. 2 Trans-ancestry (TRANS) stage 1 and 2 combined meta-analyses were meta-analyses of stage 1 TRANS and stage 2 TRANS meta-analyses, and not meta-analyses of ancestry-specific stage 1 and stage 2 combined meta-analyses.
Figure 2.
Interaction of rs12740061 (LOC105378783) and Current Smoking (1df). A forest plot showing the betas (95% confidence intervals) and p values (1df) for the rs12740061 × Current Smoking interaction term in linear regression models of HDL adjusted for age, sex, study-specific covariates (if applicable), smoking status, and principal components. Results for each AFR study are shown, as well as the ancestry-specific combined stage 1 and 2 meta-analyses.
Figure 3.
Associations Observed Primarily Among One Smoking Stratum. For selected variants for which an association was primarily observed only in one smoking stratum, a comparison of the p values for stage 1 linear association models, including a main effect model adjusted for age, sex, principal components, and study-specific covariates (as appropriate) in all individuals and stratified by smoking exposure; a model additionally adjusted for smoking exposure; and a model that also includes a smoking exposure × SNP interaction term, from which a 1df test of interaction and a 2df joint test of main effect and interaction were calculated. a.) rs7364132 (DGCR8) × ever-smoking on triglycerides (n = 21,834 [11,113 never smokers; 10,725 ever-smokers]), b.) rs79950627 (MIR4686) × current smoking on LDL (n = 23,348 [18,384 non-smokers; 4,973 current smokers]), c.) rs56167574 (PRKAG2) × ever smoking on LDL (n = 23,353 [11,700 never smokers; 11,649 ever-smokers]), and d.) rs77810251 (PTPRZ1) × ever smoking on HDL (n = 23,146 [11,560 never smokers; 11,592 ever-smokers]).
Figure 4.
Forest Plots of Selected Associations. (a.) Plot showing the association between rs73453125 and LDL among AFR in stage 1 (where a series of models were available). Variant betas (95% confidence intervals) and p values are drawn from main effect linear regression models of Non-Smokers, Smokers, all individuals, and all individuals with adjustment for smoking status. (b.) Plot showing the association between rs10101067 (EYA1) and triglycerides in ancestry-specific and combined analysis from stages 1 and 2. Variant main and interaction betas (95% confidence intervals) are drawn from linear regression models that include a current smoking × SNP term and p values are for the 2df joint test of main effect and interaction.
Table 1: Statistically Significant (p < 5×10<sup>-8</sup>) Results in Stage 1 and 2 Meta-Analysis

| Index Variant (Nearest Gene) | Chr:Position | 1000 Genomes Freq<sup>1</sup> | Tested Allele: Freq | Ancestry | Trait/ Exposure<sup>2</sup> | n | Effect | SE | Int. Effect | SE | 1df Int. P-value<sup>3</sup> | 2df Joint P-value<sup>4</sup> | Adj. Main Effect P-value<sup>5</sup> |
|-----------------------------|--------------|-------------------------------|---------------------|----------|-----------------|---|--------|----|------------|----|-------------------------|-------------------------|-----------------------------|
| rs1740061 (LOC105378783)   | 1:69407810   | 0.01/0.170/0.02/22.00       | T: 0.05            | AFR      | HDL/CS         | 16,606 | 0.02   | 0.0082 | −0.11       | 0.019 | 7.40E-09               | 2.4E-08                 | 15,499                      |
| rs77810251 (PTPZR2)        | 7:121504149  | 0.02/0.220/340.11           | A: 0.04            | AFR      | HDL/ES         | 24,253 | 0.052  | 0.0083 | −0.06       | 0.012 | 9.50E-07               | 1.2E-09                  | 23,146                      |
| rs73453125 (CNTNAP2)       | 7:146084573  | 0.09/0.020/0.00             | A: 0.07            | TRANS, AFR| LDL/CS        | 40,566 | 0.19   | 0.69   | −8.3        | 1.4   | 1.70E-09               | 2.0E-08                  | 23,146                      |
| rs56167574 (PRKAG2)        | 7:151245975  | 0.02/0.220/0.22             | A: 0.04            | AFR      | HDL/ES         | 25,778 | 0.19   | 0.8     | −6.1        | 1.1   | 1.50E-08               | 8.4E-08                  | 23,353                      |
| rs79950627 (MIR4686)       | 11:2233790   | 0.06/0.10/0.00              | A: 0.05            | TRANS, AFR| LDL/ES        | 38,272 | −0.1   | 0.79   | −8.4        | 1.6   | 1.40E-06               | 7.2E-09                  | 23,348                      |
| rs60293995 (ZNF729)        | 19:22446748  | 0.15/0.01/0.03/0.00         | A: 0.13            | AFR      | TRIG/CS       | 19,048 | 0.041  | 0.0092  | −0.097      | 0.018 | 3.00E-08               | 8.2E-08                  | 15,747                      |
| rs7364132 (DGCR8)          | 22:20096172  | 0.19/0.020/0.00             | A: 0.16            | AFR, TRANS| TRIG/ES      | 23,935 | 0.012  | 0.0091  | −0.066      | 0.013 | 8.80E-07               | 2.5E-08                  | 21,834                      |
| rs12144063 (ETYA)          | 1:28406047   | 0.35/0.280/530.30           | T: 0.37            | TRANS    | HDL/CS, ES    | 375,418 | −0.004 | 0.00069 | −0.00033   | 0.0016 | 1.3E-10                | 131,057                  | 4.70E-07                    |
| rs10937241 (ETV5)          | 3:185822774  | 0.30/0.310/580.19           | A: 0.17            | EA, TRANS| HDL/CS, ES    | 230,919 | −0.008 | 0.0012  | 0.0021      | 0.0026 | 4.2E-12                | 90,266                   | 4.50E-07                    |
| rs34313866 (TMEM175)       | 4:951947     | 0.01/0.070/120.20           | C: 0.17            | TRANS, EA| HDL, TRIG/CS  | 351,489 | −0.006 | 0.00097 | 0.0014      | 0.0022 | 6.60E-07               | 1.6E-09                  | 115,640                     |
| rs73259083 (CREBL2)        | 7:13759799   | 0.110/0.04/0.02/0.00        | C: 0.05            | TRANS, AFR| LDL/CS, ES    | 84,091 | −3.7   | 0.66   | −0.37       | 0.95  | 0.53                   | 1.3E-14                  | 35,909                      |
| rs10101067 (ETYA)          | 8:72407374   | 0.04/0.070/130.06           | C: 0.08            | TRANS    | TRIG/CS      | 317,809 | 0.014  | 0.0025  | −0.0092     | 0.0053 | 4.1E-08                | 102,263                  | 2.10E-06                    |
| rs7068757 (B3GNT4)         | 12:122691738 | 0.02/0.020/0.00             | C: 0.02            | AFR      | TRIG/CS      | 12,982 | −0.13  | 0.025   | −0.029      | 0.057  | 8.50E-08               | 1.3E-08                  | 11,875                      |

Abbreviations: African ancestry (AFR), Current Smoking (CS), European ancestry (EUR), Ever-Smoking (ES), Trans-ancestry (TRANS), Triglycerides (TRIG).

<sup>1</sup>Listed variants represent the lead associations within 1 MB region for the 2 and 1 degree of freedom tests of the variant x smoking interaction after excluding variants within 1 MB of known lipids loci. If variant is in/within 2 KB of a gene, that gene name is listed;

<sup>2</sup>Frequency of the tested allele in 1000 Genomes data by ancestry: Asian (ASN), Americas (AMR), African (AFR), and European (EUR).
3. If the region was associated with the trait in more than one meta-analysis, the most statistically significant result is listed first and described in table;

4. Bolding indicates genome-wide statistical significance;

5. P-values in this column come from a smoking-adjusted main effect model (available in Stage 1 cohorts only, see Figure 1);

* Findings with an asterisk are statistically significant using a stricter p-value threshold, after Bonferroni correction for 2 smoking traits, 2 interaction tests, and ethnic and trans-ethnic testing ($p < 5 \times 10^{-8}/8 = 6.25 \times 10^{-9}$).
Table 2:

Statistically Significant (p < 5×10^{-8}) Results in Stage 1 Meta-Analysis Unavailable in Stage 2

| **Index Variant** (Nearest Gene)^2 | **Bld 37 Chr:Position** | **1000 Genomes Freq**^3 | **Tested Allele: Freq** | **Ancestry** | **Trait/Exposure** | **Stage 1** | **Stage 2** |
|----------------------------------|-------------------------|--------------------------|------------------------|--------------|-------------------|------------|------------|
| **n** | **Effect** | **SE** | **Int. Effect** | **SE** | **P-value** | **1df Interaction P-value**^4 | **2df Joint P-value**^4 | **Adj. Main Effect P-value**^5 |
| rs140602625 (EXOC6B) | 2:72849325 | 0.01/0/0/0 | C: 0.02 | AFR | LDL/CS | 7,755 | −3.4 | 3.1 | −35 | 7.1 | 1.0E-6 | 1.5E-8 | 0.018 |
| rs14138886 (LOC107985905) | 2:84428024 | 0.02/0/0/0 | T: 0.02 | AFR | LDL/CS | 7,755 | 2.4 | 2.9 | −29 | 5.4 | 9.3E-8 | 4.4E-8 | 0.47 |
| rs149776574 (REEP1) | 2:86472455 | 0.01/0.08/0/0 | G: 0.02 | AFR | TRIG/CS | 7,756 | −0.048 | 0.033 | 0.40 | 0.069 | 4.2E-10^* | 5.1E-10^* | 0.88 |
| rs143396479 (LOC105374426/TMEM33) | 4:41911366 | 0.02/0/0/0 | A: 0.01 | AFR | LDL/ES | 10,912 | −16.0 | 2.6 | 15 | 4.5 | 0.022 | 6.8E-9 | 0.0094 |
| rs148187465 (MARCH1) | 4:164639694 | 0.01/0/0/0 | C: 0.01 | AFR | LDL/CS | 7,755 | −2.1 | 3.0 | −32 | 6.2 | 3.7E-7 | 4.8E-9 | 0.032 |
| rs143396479 (LOC105374426/TMEM33) | 5:151189283 | 0.02/0.01/0/0 | G: 0.02 | AFR | TRIG/CS | 7,756 | 0.046 | 0.033 | −0.41 | 0.071 | 8.5E-9 | 3.3E-8 | 0.96 |
| rs15580718 (BMPS9) | 6:7880037 | 0.02/0/0/0 | G: 0.01 | AFR | TRIG/CS | 7,756 | −0.12 | 0.036 | −0.29 | 0.082 | 0.0045 | 1.2E-9 | 1.6E-6 |
| rs17150980 (MAGI2) | 7:78173734 | 0.01/0.12/0.45/0.01 | C: 0.03 | AFR | TRIG/ES | 12,972 | −0.17 | 0.028 | 0.24 | 0.044 | 7.5E-8 | 1.4E-9 | 0.085 |
| rs16592443 (LYZL2) | 10:30884890 | 0.02/0/0/0 | A: 0.01 | AFR | TRIG/CS | 7,756 | 0.073 | 0.038 | −0.46 | 0.081 | 1.8E-8 | 1.2E-7 | 0.76 |
| rs15628664 (UNC5B) | 10:72899880 | 0.03/0/0/0 | G: 0.01 | AFR | TRIG/CS | 7,756 | 0.027 | 0.040 | −0.39 | 0.071 | 4.7E-8 | 6.7E-9 | 0.44 |
| rs183911507 (TP53I11) | 11:44978366 | 0.01/0/0/0 | G: 0.02 | AFR | TRIG/CS | 10,287 | −0.043 | 0.029 | 0.33 | 0.059 | 1.7E-8 | 6.5E-8 | 0.82 |
| rs199771018 (STOML3) | 13:39507838 | 0.02/0/0/0 | T: 0.02 | AFR | HDL/CS | 7,756 | −0.019 | 0.019 | 0.23 | 0.037 | 1.2E-9 | 6.3E-10 | 0.55 |
| rs190976513 (LOC105370255) | 13:71114207 | 0.02/0.01/0/0 | A: 0.02 | AFR | LDL/CS | 10,234 | −5.1 | 2.6 | −20 | 5.2 | 9.3E-5 | 3.2E-8 | 1.1E-4 |
| rs182600360 (LOC105370531) | 14:63607120 | 0.02/0/0/0 | A: 0.02 | AFR | LDL/CS | 7,755 | 6.6 | 3.3 | −39 | 7.1 | 4.4E-8 | 3.3E-7 | 0.56 |
| Index Variant (Nearest Gene) \(^2\) | Bld 37 Chr:Position | 1000 Genomes Freq \(^3\) | Tested Allele: Freq | Ancestry | Trait/Exposure | n | Effect | SE | Int. Effect | SE | 1df Interaction P-value \(^3\) | 2df Joint P-value \(^4\) | Adj. Main Effect P-value \(^5\) |
|-----------------------------------|--------------------|-------------------|-------------------|---------|---------------|---|--------|----|-------------|----|-----------------|-----------------|-----------------|
| rs62064821 (CCT6B)               | 17:33280904        | 0.01/0.04/0/0.06  | T: 0.01           | AFR     | LDL/CS        | 10,234 | 8.5    | 3.3 | −30         | 5.5 | 3.1E-8          | 6.0E-7          | 0.17            |

Abbreviations: African ancestry (AFR), Current Smoking (CS), Ever-Smoking (ES), Triglycerides (TRIG).

1 All loci have some evidence for interaction (p<0.05 for 1df test of interaction); thus, results not categorized into “Loci with Evidence for Interaction” or “Probable Main Effects (without evidence for interaction)”;

2 Listed variants represent the lead associations within 1 MB region for the 2 and 1 degree of freedom tests of the variant × smoking interaction after excluding variants within 1 MB of known lipids loci. If variant is in/within 2 KB of a gene, that gene name is listed;

3 Frequency of the tested allele in 1000 Genomes data by ancestry: Asian (ASN), Americas (AMR), African (AFR), and European (EUR);

4 Bolding indicates genome-wide statistical significance;

5 P-values in this column come from a smoking-adjusted main effect model (available in Stage 1 cohorts only, see Figure 1).

* Findings with an asterisk indicate statistical significance using a stricter p-value threshold, after Bonferroni correction for 2 smoking traits, 2 interaction tests, and ethnic and trans-ethnic testing (5 × 10\(^{-8}\) = 6.25 × 10\(^{-9}\)).