Chromosome Xq23 is associated with lower atherogenic lipid concentrations and favorable cardiometabolic indices

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Chromosome Xq23 is associated with lower atherogenic lipid concentrations and favorable cardiometabolic indices

Autosomal genetic analyses of blood lipids have yielded key insights for coronary heart disease (CHD). However, X chromosome genetic variation is understudied for blood lipids in large sample sizes. We now analyze genetic and blood lipid data in a high-coverage whole X chromosome sequencing study of 65,322 multi-ancestry participants and perform replication among 456,893 European participants. Common alleles on chromosome Xq23 are strongly associated with reduced total cholesterol, LDL cholesterol, and triglycerides ($P = 8.5 \times 10^{-72}$), with similar effects for males and females. Chromosome Xq23 lipid-lowering alleles are associated with reduced odds for CHD among 42,545 cases and 591,247 controls ($P = 1.7 \times 10^{-4}$), and reduced odds for diabetes mellitus type 2 among 54,095 cases and 573,885 controls ($P = 1.4 \times 10^{-5}$). Although we observe an association with increased BMI, waist-to-hip ratio adjusted for BMI is reduced, bioimpedance analyses indicate increased gluteofemoral fat, and abdominal MRI analyses indicate reduced visceral adiposity. Co-localization analyses strongly correlate increased CHRDL1 gene expression, particularly in adipose tissue, with reduced concentrations of blood lipids.
 Mendelian, population, and functional genetic analyses of blood lipids (total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and triglycerides) have yielded important fundamental insights regarding the root causes of coronary heart disease (CHD)\(^1\). For example, rare and common autosomal genomic variation influencing LDL-C, correspondingly influence CHD risk\(^2\)-\(^6\). Such observations buttress clinical recommendations and bolster efforts to discover and validate lipid-related drug targets for CHD risk reduction\(^7\)-\(^9\).

Although the X chromosome comprises 5% of the genome, it has only been studied in a few genome-wide association analyses for blood lipids and coronary disease\(^10\)-\(^13\). Major reasons for exclusion include incomplete coverage on genotyping arrays, potential discrepancies in genotyping quality on arrays due to haplotype insufficiency in men, imputation and analytic challenges, and somatic X inactivation across tissues in women. Deep-coverage whole-genome sequencing (WGS) and analysis of the X chromosome now offers the promise for uniform coverage and high-fidelity genotyping for both sexes\(^14\).

While differences in lipid levels and CHD risk by sex are well established\(^15\),\(^16\), X chromosome dosage is also linked to lipid differences. Monosomy X (45X, Turner syndrome) is linked to dyslipidemia and premature CHD\(^17\)-\(^19\). While obesity and gonadal deficiency was long believed to be the primary contributor to these phenotypes, women with Turner syndrome have higher total cholesterol, LDL-C, and triglyceride concentrations than age- and body composition-matched 46XX women with premature ovarian failure\(^15\),\(^20\). Men with an additional X chromosome (47XXX, Klinefelter syndrome) also suffer from infertility with higher rates of obesity, dyslipidemia, and CHD\(^21\),\(^22\).

Furthermore, adult gonadectomized mice with XY, XX, and XXY chromosomes, regardless of gonadal sex, demonstrate dose-dependent changes in lipid levels\(^23\). Such observations, suggest that apparent sexual dimorphism in lipid levels may be explained by the sex chromosomes themselves.

Our study aims to discover X chromosome genomic variation associated with blood lipid levels among 65,322 multi-ancestry individuals with high-coverage whole X chromosome sequencing and available lipids in the NHLBI Trans-Omics for Precision Medicine (TOPMed) program\(^24\). Independent serial replication is performed in up to 390,606 and 66,287 individuals with GWAS array and lipids available in the UK Biobank and Nord-Trøndelag Health (HUNT) study, respectively\(^25\),\(^26\). We further evaluate the phenotypic consequences of lipid-associated variation in the X chromosome now offers the promise for uniform coverage and high-fidelity genotyping for both sexes.

While differences in lipid levels and CHD risk by sex are well established\(^15\),\(^16\), X chromosome dosage is also linked to lipid differences. Monosomy X (45X, Turner syndrome) is linked to dyslipidemia and premature CHD\(^17\)-\(^19\). While obesity and gonadal deficiency was long believed to be the primary contributor to these phenotypes, women with Turner syndrome have higher total cholesterol, LDL-C, and triglyceride concentrations than age- and body composition-matched 46XX women with premature ovarian failure\(^15\),\(^20\). Men with an additional X chromosome (47XXX, Klinefelter syndrome) also suffer from infertility with higher rates of obesity, dyslipidemia, and CHD\(^21\),\(^22\).

Furthermore, adult gonadectomized mice with XY, XX, and XXY chromosomes, regardless of gonadal sex, demonstrate dose-dependent changes in lipid levels\(^23\). Such observations, suggest that apparent sexual dimorphism in lipid levels may be explained by the sex chromosomes themselves.

**Results**

**Baseline characteristics, blood lipids, and chromosome X genotypes.** TOPMed sequences were aggregated and aligned, and variants were called by the TOPMed Informatics Research Center. A total of 65,367 out of 140,000 individuals in TOPMed freeze 8 with WGS data, including X chromosome sequence data had harmonized lipid levels available (Supplementary Fig. 1). Forty-five individuals with anomalous X chromosome copy number were excluded, leaving 65,322 individuals for analysis. 40,577 (62.1%) individuals were female and mean (standard deviation [SD]) age was 52.4 (14.9) years. Across all 21 included cohorts, 29,513 (45.2%) were white, 16,431 (25.2%) black, 13,432 (20.6%) Hispanic, 4,714 (7.2%) Asian, 1182 (1.8%) Samoan, and 50 (0.1%) Native American (Supplementary Table 1; Supplementary Fig. 2). The included studies were largely observational cohorts with some variations in ascertainment schemes as described in the Supplementary Note. Blood lipid distributions were generally similar across cohorts with some differences due to differences in study design and ancestry (Supplementary Table 2 and Supplementary Fig. 3). After adjusting for lipid-lowering medicines within each cohort and ancestry, we generated residuals within each cohort and race group adjusted for age, age\(^2\), sex, 11 principal components of ancestry, and cohort-specific covariates. These residuals were inverse rank normalized and multiplied by the standard deviation within each cohort and race group to obtain effects in mg/dl units (see Methods) (Supplementary Fig. 4).

Among 65,322 TOPMed participants with lipid levels and WGS, we identified 19,898,222 total variants on the X chromosome by WGS. Of these variants, 88,008 (0.4%) were nonsynonymous variants and 4632 (0.02%) were rare (MAF < 1%) predicted protein-truncating variants. As expected, participants of African ancestry had the most X chromosome variants (Fig. 1a). Likely due to sample size differences, there were overall more total variants observed in our dataset among white participants compared to other ancestries (Fig. 1b). Within the X chromosome, females had a greater average [SD] number of variants per individual (133,255 [22,455]) than males (90,117 [12,166]), as expected (Supplementary Table 3). Generally, most of the variation observed across individuals was uncommon (i.e., 98.8% of variants had MAF < 5%) (Supplementary Table 4).

**X chromosome single-variant association with lipid levels.** In single-variant discovery analyses in TOPMed, we performed X chromosome-wide association analyses for genetic variants with minor allele count >20 that are not in the pseudautosomal region, yielding 2.2 million analyzed of the 19.8 million detected. To maximize power, all samples (i.e., males and females) were included in the linear mixed model association analyses with SD-adjusted residuals of lipid levels as the outcome, where adjustments included sex (Supplementary Fig. 5).

Across variants assessed, we found 21 variants showing suggestive evidence (P < 1 x 10\(^{-6}\)) of association with lipids in TOPMed (Supplementary Table 5 and Supplementary Fig. 6). We evaluated these associations for replication, serially, in the UK Biobank (N = 390,606) (Supplementary Table 6) and HUNT (66,635) (Supplementary Table 7). Three variants showed evidence of replication (P < 0.05/21 = 0.002) in UK Biobank and in HUNT and additionally met a stringent threshold for statistical significance in the meta-analysis (alpha = 0.05/2.2 M variants/4 traits = 5.7 x 10\(^{-9}\)) (Table 1).

The three variants occurred on chrXq23 and were all in at least moderate linkage disequilibrium across all included TOPMed participants (Supplementary Fig. 7 and Supplementary Table 8). They were also in moderate linkage disequilibrium with a previously described nearby variant, rs598547112, (r\(^2\) 0.61–0.76). All three associated variants in our dataset have similar nonreference allele frequency (0.34–0.43), which was also similar between males and females. We observed similar associations for both males and females within TOPMed except male rs5985504-T carriers had greater decrease in triglycerides compared to female rs5985504-T carriers (P\(_{interaction} = 0.001\) (Supplementary Table 9).

The minor alleles for these variants are common in all TOPMed ancestries except for Asian Americans (MAF 0.02) and Samoans (MAF 0.01). Nevertheless, effect estimates were largely of similar magnitude across ancestries in TOPMed for total
cholesterol (Supplementary Table 10) with no evidence of heterogeneity (P_{heterogeneity} > 0.05).

The three chrXq23 variants were associated with reduced atherogenic lipoproteins (i.e., total cholesterol, triglycerides, and LDL-C) (Table 1). The rs5942634-T allele is an intergenic variant and is 8 kb downstream from RTL9 (also referred to as RGAG1 in the literature), and was the top variant for total cholesterol, associated with 1.95 mg/dl lower concentration (P = 2 × 10^{-16}). The rs5942648-A allele occurs 81 kb downstream, is intergenic between RTL9 and CHRDL1, and was the top variant for LDL-C, associated with 1.53 mg/dl lower concentration (P = 1 × 10^{-12}). The rs5985504-T allele resides 60 kb further downstream and is 68 kb from CHRDL1 and was the top variant for log(triglycerides) leading to 2% lower triglycerides concentration (P = 4 × 10^{-11}). Overall, the associated variants reside within a ~0.22 Mb linkage disequilibrium block spanning RTL9 and CHRDL1 (Supplementary Fig. 7). Within this block, variants within predicted active adult liver enhancers are in proximity to both the RTL9 and CHRDL1 genes (Supplementary Fig. 8). Only two variants reside within both an adult liver enhancer and DNase hypersensitivity site—rs2883091 in an intron of RTL9, and rs2143760 residing 4 kb from CHRDL1 but 214 kb from RTL9. These variants are in at least moderate linkage disequilibrium (r^2 > 0.60) with the top associated variants in the locus. Virtual 4C data additionally demonstrate a contact between the rs5985504 site and upstream of CHRDL1 (Supplementary Fig. 9).

To determine whether our signal was independent of previously reported variants in the region, we performed conditional analysis for the associated between total cholesterol and rs5942634 for the individual previously reported variants in the region (results not shown). This indicates that rs5942634 is only partially explained by the three reported variants.

Phenome-wide association of Chrxq23 variants. Given prior genetic associations of LDL-C-lowering and triglyceride-lowering autosomal variants with lower risk for CHD, we hypothesized that sex chromosome variants lowering LDL-C or triglycerides would also lower risk for CHD. In HUNT, UK Biobank, and FinnGen (Supplementary Table 12), we observed that the top lipid-lowering alleles at this locus showed a reduced risk for CHD (Fig. 2). We found a 0.98 (95% CI 0.96, 0.99; P = 1.7 × 10^{-4}) odds of CHD for each rs5942634-T allele, the lead cholesterol-lowering variant (alpha = 0.05 for the single haplotype assessment), and a correlation between the effect sizes of variants on total cholesterol in the chrXq23 locus and the effect sizes of these variants on CAD (r = 0.25), T2D (r = 0.33), and BMI (r = −0.34) (Supplementary Fig. 10).

To explore the range of phenotypes associated with the chrXq23 locus, we evaluated the associations of each of these three variants with 80 manually curated diverse clinical traits and conditions in the UK Biobank (Supplementary Table 13). Given the high degree of correlation among these variants, phenome-wide association results were similar (Supplementary Tables 14-16). As expected, the strongest associations were for reduced odds of hypercholesterolemia. Associations reaching a P < 6.3 × 10^{-4} (P < 0.05/80 traits) included reduced odds for diabetes mellitus type 2 (T2D), hypertension, and glaucoma, but increased odds for ever smoking as well as increased body-mass index (BMI) and body fat percentage. Notably, we observed lower odds of T2D for rs5942648 (OR = 0.97; 95% CI 0.96, 0.99; P = 1.4 × 10^{-5}) (Fig. 2).

We additionally explored the association between each of these three variants with lipoprotein subspecies identified through nuclear magnetic resonance spectroscopy (NMR) within the Framingham Heart Study and Multi-Ethnic Study of Atherosclerosis cohorts (up to 6356 individuals). While we did not find any associations that passed a Bonferroni-corrected significance threshold (0.05/(3 SNPs × 16 lipoprotein subspecies) = 0.001; Supplementary Table 17), we found two lipoprotein subspecies
associated with suggestive evidence (p < 0.05), including greater concentration of medium HDL particles (but no effect on small or large HDL particles) and greater LDL size. We assessed for evidence of replication for indices related to LDL size (alpha 0.05) since the chrX variants associated with LDL-C. Among 6443 participants of the Atherosclerosis Risk in Communities cohort, we concordantly observed a −0.034 SD (P = 0.022) lower concentration of small dense LDL for rs5942648-A. Among 365,365 participants of the UK Biobank, when using LDL-C/apolipoprotein B ratio as a proxy for LDL particle size, we observed a nominal increase in LDL size even with adjusting for both LDL-C and apolipoprotein B (Beta = 1.1 × 10^{-5}, P = 0.048).

To better characterize effects on adiposity given the aforementioned clinical phenotype associations, we evaluated the association between rs5942634-T and body composition measurements in the UK Biobank. Although rs5942634-T was associated with increased BMI, it was associated with slightly reduced waist-to-hip ratio adjusted for BMI (Beta = −6.3 × 10^{-4}, SE = 1.1 × 10^{-4}, P = 1.3 × 10^{-8}). rs5942634-T is associated with both increased truncal fat mass (Beta = 63 g, SE = 10 g, P = 4.0 × 10^{-10}) as well as increased total peripheral fat mass, with increase of 21 g (P = 3.6 × 10^{-12}) of the right leg, 20 g (P = 3.4 × 10^{-12}) of the left leg, 7 g (P = 4.1 × 10^{-7}) of the right arm, and 8 g (P = 1.7 × 10^{-9}) of the left arm (Supplementary Table 18). Additionally, among 4750 unrelated UK Biobank participants with abdominal MRI measures available, rs5942634-T was associated with log-transformed inverse rank standardized increased abdominal subcutaneous adipose tissue (Beta = +0.43, SE = 0.15, P = 5.9 × 10^{-5}) but decreased visceral adipose fat (Beta = −1.12, SE = 0.14, P = 1.1 × 10^{-15}) to a greater degree. Given nine adiposity traits assessed, Bonferroni-corrected significance was assigned at 0.05/9 = 5.6 × 10^{-3}.

Rare pathogenic variants in CHRD1 were previously linked to X-linked recessive megalocornea, a condition characterized by enlarged corneal diameters with associated complications, including reduced visual acuity. Given these prior observations, we asked whether common variants associated with cholesterol at the CHRD1 locus were associated with differences in visual acuity. Among 112,842 UK Biobank participants (46.5% women; median age at assessment 58.5 years), we observed no association of lipid-associated chrXq23 alleles with altered visual acuity (P > 0.05; Supplementary Table 19). Given our sample size of 112,842 and SNP frequency of 34.4%, we had >99% power to detect effects >1/10th of a standard deviation unit of visual acuity at an alpha of 0.05.

**Gene expression analyses at chromosome Xq23.** We leveraged the GTEx eQTL data to better understand the gene or genes in the region that are influencing atherogenic lipid levels. Our most significant SNP, rs5942634, was associated with reduced expression of CHRD1 in skeletal muscle (beta = −0.17, P = 1.2 × 10^{-11}), subcutaneous adipose (beta = −0.16, P = 8.6 × 10^{-8}), visceral adipose (beta = −0.17, P = 4.3 × 10^{-6}), and liver (beta = −0.25, P = 5.9 × 10^{-5}). Additionally, rs5942634 was associated with increased expression of RTL9 in skeletal muscle (beta = 0.18, P = 2.7 × 10^{-5}; Supplementary Table 20).

Interrogating eQTL data for a single variant may lead to biased interpretations for causal gene inference. Therefore, we colocalized eQTL results for 8 genes (i.e., ACSL4, TMEM164, AMMECR1, RTL9, CHRD1, PAK3, CAPN6, DCX) within the ChrXq23 region across prespecified lipid-related tissues (i.e., subcutaneous adipose, terminal ileum, visceral omentum adipose, whole blood, liver, and skeletal muscle) to relate aggregate blood lipid-association data with gene expression data. We observe that increased gene expression of CHRD1 shows consistent colocalization with decreased cholesterol

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**Table 1 Discovery and replication of chromosome Xq23 variants associated with lipid levels in TOPMed, UK Biobank, and HUNT.**
Fig. 2 Association of lead cholesterol-lowering chrXq23 variant rs5942634-T with reduced odds of coronary heart disease and diabetes mellitus type 2. The lead cholesterol-lowering allele at chrXq23 (i.e., rs5942634-T) and evidence of association with coronary heart disease and diabetes mellitus type 2 in each of three datasets in black, UK Biobank, HUNT, and FinnGEN, as well as meta-analysis in blue are shown. Odds ratios (OR) and 95% confidence intervals around the odds ratios are displayed.

Fig. 3 Colocalization of expression of genes at chrXq23 in subcutaneous adipose tissue with blood cholesterol effects strongly implicates CHRLDL1. The x-axis represents eight genes in the chrXq23 locus and y-axis represents standardized gene expression effect estimates in subcutaneous adipose tissues with 95% confidence intervals. Accounting for linkage disequilibrium, standardized effects and evidence of associations of cholesterol-lowering alleles were correlated with gene expression of genes at chrXq23 (ACSL4, TMEM164, AMMCCRI, RTL9, CHRLDL1, PAK3, CAPN6, and DCX). Observations allow us to draw several conclusions about X chromosome genetic variation with blood lipid levels, as well as related cardiometabolic effects.

First, bioinformatic analyses implicate CHRLDL1 as a candidate causal gene for the association of chrXq23 variants with lipids. Based on genomic proximity of the strongest signal, RTL9 was assigned as the likely causal gene in prior work. However, colocalization analyses strongly prioritize increased CHRLDL1 gene expression in lipid-related, particularly adipose, tissues with reduced lipoprotein measures over other genes in the region. CHRLDL1 is not a previously known Mendelian lipid gene. In our study, disruptive rare coding variants in CHRLDL1 were not significantly associated with lipids, nor was any gene in the region.

Second, despite observing an association with increased BMI, chrXq23 lipid-associated alleles may lead to favorable effects on adiposity. We observed that chrXq23 lipid-lowering alleles were associated with increased gluteofemoral adipose tissue. Autosomal alleles similarly linked to expansion of gluteofemoral adipose tissue are associated with favorable risk for CHD and T2D. Autosomal alleles associated with body fat distribution are also associated with various functional adipose measures, including morphology, lipolysis, and lipogenesis. Recent gene expression analyses of human adipose-derived stromal cells showed persistent upregulation of CHRLDL1 after inducing adipogenesis. CHRLDL1 is believed to influence adipogenic differentiation in human isolated preadipocytes. Comparative gene expression analyses suggest relatively greater CHRLDL1 expression in subcutaneous versus visceral fat. In our analyses, the chrXq23 lipid-lowering alleles were associated with an increase in abdominal subcutaneous adipose tissue but a decrease in visceral adipose tissue. A proteomic discovery analysis showed that increasing circulating CHRLDL1 concentrations were associated with increased birth weight but decreased triglycerides and homeostatic model assessment of insulin resistance.

Third, chrXq23 lipid-lowering alleles have favorable cardiometabolic effects that appear to reduce risk for CHD and T2D. Our results for CHD at chrXq23 are consistent with prior work at this locus, and extend to prior observational epidemiology, genetic, functional, and clinical trial evidence implying a causal relationship between reduced LDL-C and reduced CHD risk. In aggregate, autosomal LDL-C-reducing alleles are associated with increased T2D risk but the effects are inconsistent across individual variants. In meta-analyses of randomized controlled trials, statins are associated with a modestly increased risk of...
incident T2D. The effects of triglyceride-lowering alleles and T2D risk are generally inconsistent. Common triglyceride-lowering variants at LPL and ANGPTL4 p.E40K in the lipoprotein lipase pathway are associated with reduced triglyceride concentrations, CHD odds, and T2D risk. Rare loss-of-function variants in ANGPTL3, also implicated in the lipoprotein lipase pathway, are associated with reduced LDL-C and triglyceride concentrations as well as reduced CHD odds but favorable effects on T2D risk have not yet been observed. However, we uniquely describe a genetic locus associated with reduced LDL-C concentrations, reduced triglyceride concentrations, lower CHD risk, and lower T2D risk. Similarly, we observed that lipid-lowering chrXq23 alleles were independently associated with increased LDL-C/apoB ratio, which has been independently associated with reduced CHD and T2D risk in observational epidemiologic studies. These data imply that therapeutic modulation of the causal pathway may lead diverse favorable cardiometabolic indices. Whether implicated variants influence the lipoprotein lipase pathway or represent a novel lipid-related pathway for combined CHD and T2D should be addressed by future research.

Fourth, common lipid-associated variants linked to increased CHRD1L expression are not associated with visual acuity measures. Ventrino, the product of CHRD1L, was first described as a bone morphogenic protein 4 inhibitor and a regulator of retinal development. Pathogenic disruptive variants in CHRD1L are implied in X-linked megalocornea. However, common lipid-associated variants linked to increased CHRD1L expression in the present study do not associate with measures of visual acuity in the UK Biobank. This data imply that therapeutic modulation to recapitulate the protective effects associated with chrXq23 lipid-lowering alleles is not anticipated to lead to on-target adverse visual acuity effects.

Important limitations should be considered in the interpretation of our findings. First, our genetic association analyses of the X chromosome do not account for random X inactivation. Accounting for random X inactivation is expected to modestly improve power and thus our approach biases our findings toward the null. We found that there was slightly higher variance of total cholesterol in heterozygous females (sd = 44.1 mg/dl) compared to homozygous females (homozygous ref sd = 43.0, homozygous alt sd = 43.9) of rs5942634 using the Levene’s Test for Homogeneity of variance (P = 0.002), indicating this locus may be subject to random X inactivation. Second, while our in silico analyses and prior literature strongly implicate CHRD1L as the causal gene, additional analyses including perturbational experiments in model systems are necessary to confirm our hypotheses. Furthermore, whether additional cis-acting or trans-acting gene expression for other genes are additionally relevant for the observed effects on blood lipids are currently unknown. Third, chrXq23 lipid-lowering alleles were associated with both increased truncal and gluteofemoral adiposity indices, and whether the former associations result in adverse clinical consequences is not known. Nevertheless, phenome-wide association analyses did not reveal concerning clinical phenotype associations related to modest increases in BMI. Notably, chrXq23 lipid-lowering alleles were associated with decreased visceral adipose tissue.

In conclusion, we observe a consistent association of chrXq23 alleles with reduced total cholesterol, LDL-C, triglycerides, CHD, and T2D. Despite an increase in BMI, these alleles were favorably associated with increased gluconeomral and abdominal subcutaneous adiposity, decreased visceral adiposity, and increased LDL-C/apoB ratio. Colocalization analyses strongly implicate increased CHRD1L expression in adipose tissues with these favorable cardiometabolic indices, pointing to CHRD1L as the leading candidate gene in the region.
Blood lipid measurements and phenotypic modeling. Conventionally measured fasting blood lipids, including total cholesterol, LDL-C, HDL-C, and triglycerides, were included for analysis. Harmonization of the lipid values, lipid-lowering medication status, and fasting status at lipid blood draw was performed by the TOPMed Data Coordinating Center (DCC). LDL-C was either calculated by the Friedewald equation when triglycerides were <400 mg/dl or directly measured. Given the available data, the first 10 PCs of ancestry (as recommended by the TOPMed DCC), as well as a number of other covariates (study site or known founder mutations) where created within each study by self-reported race. Effect sizes are reported in mg/dl or log(mg/dl) for TG.

Coronary heart disease and diabetes mellitus type 2 phenotyping. In the UK Biobank, we used the National Health System OPCS-4 (Office of Population, Censuses and Surveys: Classification of Interventions and Procedures, version 4) codes K75.8-75.9 to indicate the presence of coronary heart disease. For diabetes mellitus type 2 classification in the UK Biobank, we used the presence of OPCS-4 codes E10.0-E11.9 or ICD9 code 250.

Coronary heart disease (CHD) in HUNT was defined as individuals with self-reported coronary artery bypass, angioplasty, or stent placement or with diagnosis of acute myocardial infarction. As a result of chronic ischemic diseases, lipid-lowering medication and hormone replacement therapy was occurrence of the following codes: ICD9: 410, 411, 412, 414.0, 414.8, 414.9 or ICD10: 121, 122, 123, 124, 125.1, 125.2, 125.5, 125.6, 125.7, 125.8, 125.9. Individuals with angina were excluded from controls. Type 2 diabetes was defined based on at least 1 occurrence of the following diagnosis codes: ICD9: 250.00, 250.02, 250.10, 250.12, 250.20, 250.22, 250.30, 250.32, 250.40, 250.52, 250.50, 250.60, 250.62, 250.70, 250.72, 250.80, 250.82, 250.90, 250.92 ICD10: E11 or by diagnosis of diabetes during HUNT clinical examinations (nonfasting serum or blood glucose > 200 mg/dl).

For replication in HUNT, a cohort within a founder population, plasma lipids were analyzed using efficient linear mixed models implemented by BOLT-LMM v2.3.169 with covariates for age, sex, age2, batch, and principle components 1–4. CAD was analyzed using SAIGE with birth year, batch, sex, and principle components 1–4 as covariates.

Covariates included in the models of association for each contributing study were based on study characteristics and recommendations from study investigators. We took loci reaching suggestive association with lipid levels (P < 1 x 10−6) in TOPMed onto replication in UKBB and HUNT. For replication, we used a significance threshold of 0.001 (0.0002 M variants) for loci that met a suggestive level of association in TOPMed. We used a fixed effects meta-analysis to combine the association results from TOPMed, UKBB, and HUNT. We set the statistical significance for our meta-analysis to be alpha = 5.7 × 10−8 (0.0022 M variants/4 traits = 5.7 × 10−6), which is more stringent than a standard genome-wide significance level of 5 x 10−8. Heterogeneity across studies was determined through Cochran’s Q and I² test. Additionally, we determined the interaction for rs9355054 T on log triglyceride adjusting for the same covariates as the main analysis.

To determine the correlation of the effect sizes of variants on total cholesterol in the chrXq23 locus and the effect sizes of these variants on CAD, T2D, and BMI, we performed analysis of chrXq23 variation on these three outcomes adjusting for age, age2, sex, genotyping array, and PCs in the UK Biobank, using the main effects model assuming X inactivation. We correlated effect sizes of total cholesterol–variants with effect sizes of variants on CAD, T2D, or BMI limiting to total cholesterol–variants with a MAF > 0.05 and P < 0.05.

Expression quantitative trait analyses. We downloaded the v7 SNP gene association results in tissue-specific files from the GTEx portal (https://gtexportal.org/home/datasets). We limited to six tissues that have been implicated in lipid biology or CHD (Adipose_Subcutaneous, Small_Intestine_Terminal_Ileum, Adipose_visceral, Omentum, Whole_Blood, Liver, Muscle_Skeletal) and looked at expression of eight genes within the ChrXq23 region (ACSL4, TIMEM164, AMMCR1, RASA9, CHDOIL, PAK3, CAPN6, DCK). We set our significance threshold to 0.001 (0.05/6 tissues x 8 genes). First, we determined eQTls of our top association with lipids. Second, we performed correlation of our lipid–variant associations with the association of each of the eight genes expression on the variants using the gene transcripts ± 100 KB. Lastly, we used the ImeKin function in the R package kinship2 to reproduce the TOPMed linear mixed model for lipid-variant test statistic (Z = beta/SE) from the expression–variant test statistic to adjust for the correlation between the variants.
Lipoprotein association analyses. Concentrations of lipoprotein particles were measured at LipoScience, Inc. (Raleigh, NC) using NMR spectroscopy on plasma EDTA specimens. LipoScience has developed validated software for analysis of NMR measured LipoProfile spectra that uses an optimized deconvolution algorithm to quantify lipoprotein subfractions.66,67. MESA was measured with the LipoProfile-III assay while FHS samples were measured with the LipoProfile-I assay, which provides less accuracy for some measurements but is similar to LipoProtein-III. We associated lipoprotein profiles with top associated SNPs within up to 1,802 FHS and 4551 MESA participants adjusting for age, sex, and lipid-lowering therapy.

For individuals who participated in ARIC study visit 4 (1996–1998), a homogenous assay method was used for the direct measurement of sd-LDL-C in plasma (sd-LDL-EX “Seiken”, Denka Seiken, Tokyo, Japan) on a Hitachi 917 automated chemistry analyzer.68. We associated top associated SNPs with ARIC participants adjusting for age, sex, lipid-lowering therapy, race, study center, and the first 11 principal components of ancestry.

Rare variant association analyses. We performed the SKAT test to associate aggregates of rare coding variants with blood lipid levels within TOPMed as implemented by GENESIS v2.14.4 in the CHARGE Analysis Commons.69-70. For this gene-based test, high confidence loss-of-function (HC LOF by LOFTEE71) and missense metaSVM2 damaging variants with MAF ≤ 1% were collapsed into regions based on the gene annotations generated by snpEff 4.3t (https://snp-eff.sourceforge.net/) using the GRCh38.86 database.8

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Controlled access of the individual-level TOPMed data is available through dbGaP, and the individual-level UK Biobank data are available upon application to the UK Biobank (https://www.ukbiobank.ac.uk/). FinnGen summary-level data are fully freely available at https://www.finngen.fi/en/access_results. Individual-level access to FinnGen and HUNT cohorts may be obtained through reasonable request and suitable institutional review board approvals. The dbGaP accessions for TOPMed cohorts are as follows: Atherosclerosis Risk in Communities (ARIC) phs001211 and phs000280; Old Order Amish phs000956 and phs000391; Mi Sinai BioMe Biobank phs001644 and phs000925; Coronary Artery Risk Development in Young Adults (CARDIA) phs001612 and phs000285; Cleveland Family Study (CFS) phs000954 and phs000179; Cardiovascular Health Study (CHS) phs001308; Diabetes Heart Study (DHS) phs001412 and phs000102; Framingham Heart Study (FHS) phs000974 and phs000007; Genetic Epidemiology Network of Atheros Gene expression data


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Competing interests
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