Comprehensive Analysis of Established Dyslipidemia-Associated Loci in the Diabetes Prevention Program

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Background—We assessed whether 234 established dyslipidemia-associated loci modify the effects of metformin treatment and lifestyle intervention (versus placebo control) on lipid and lipid subfraction levels in the Diabetes Prevention Program randomized controlled trial.

Methods and Results—We tested gene treatment interactions in relation to baseline-adjusted follow-up blood lipid concentrations (high-density lipoprotein [HDL] and low-density lipoprotein-cholesterol, total cholesterol, and triglycerides) and lipoprotein subfraction particle concentrations and size in 2993 participants with pre–diabetes. Of the previously reported single-nucleotide polymorphism associations, 32.5% replicated at $P<0.05$ with baseline lipid traits. Trait-specific genetic risk scores were robustly associated ($3\times10^{-10}>P>1.1\times10^{-16}$) with their respective baseline traits for all but 2 traits. Lifestyle modified the effect of the genetic risk score for large HDL particle numbers, such that each risk allele of the genetic risk scores was associated with lower concentrations of large HDL particles at follow-up in the lifestyle arm ($\beta=-0.11$ mmol/L per genetic risk scores risk allele; 95% confidence interval, $-0.188$ to $-0.033$; $P=5\times10^{-3}$; $P_{\text{interaction}}=1\times10^{-3}$ for lifestyle versus placebo), but not in the metformin or placebo arms ($P>0.05$). In the lifestyle arm, participants with high genetic risk had more favorable or similar trait levels at 1-year compared with participants at lower genetic risk at baseline for 17 of the 20 traits.

Conclusions—Improvements in large HDL particle concentrations conferred by lifestyle may be diminished by genetic factors. Lifestyle intervention, however, was successful in offsetting unfavorable genetic loading for most lipid traits.

Clinical Trial Registration—URL: https://www.clinicaltrials.gov. Unique Identifier: NCT00004992.

Key Words: clinical trial ▪ genetics ▪ lifestyle ▪ lipids ▪ lipoproteins ▪ molecular epidemiology ▪ polymorphism, genetic

Dyslipidemia is a highly prevalent and heritable risk factor for coronary heart disease. The clinical diagnosis of dyslipidemia includes elevations in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triacylglycerol, and low levels of high-density lipoprotein cholesterol (HDL-C), in addition to other risk factors. Not all of the lipid traits used in the diagnosis of dyslipidemia are causally related to coronary heart disease, and their associations with coronary heart disease in observational studies may be attributable to underlying correlations with lipid and lipoprotein subfractions. Although dyslipidemia has a strong heritable basis, in many patients, it can be effectively managed through lifestyle modification and a range of pharmacotherapies such as statins, bile acid sequestrants, niacin, and fibrates. Of these treatment options, lifestyle modification, dietary changes, regular moderate intensity exercise, smoking cessation, and weight reduction are the frontline therapy for the prevention and treatment of the condition.

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lipoprotein subfraction concentrations that underlie the relatively high heritability estimates (≈0.5 to 1 kg/wk). The principal end point of the DPP interventions were followed by further caloric restriction to induce weight loss, exercise, and weight loss, potentially guiding targeted treatment decisions.

The overarching aim of this study was to examine whether comprehensive sets of lipid- and lipoprotein-associated genetic variants modulate the effects of lifestyle and metformin interventions on lipids and lipoproteins concentrations in prediabetic, overweight adults from the Diabetes Prevention Program (DPP). The specific aims of this study were to (1) validate established genetic associations with lipid traits at baseline; (2) assess established genetic associations in relation to traits correlated to primary lipid traits; (3) assess genotype × treatment interactions in relation to baseline-adjusted 1-year lipid trait levels; (4) assess whether unfavorable genetic predisposition to dyslipidemia can be overcome by intensive lifestyle intervention.

Methods

Ethics Statement
Each participant provided written informed consent, and institutional review board approval was obtained by each of the 27 DPP study centers before the study protocol was initiated.

Participants
The DPP is a multicenter, randomized controlled trial of metformin or intensive lifestyle modification for diabetes mellitus prevention, as described in detail elsewhere. Briefly, individuals with diabetes mellitus incidence, confirmed by a semianual fasting plasma glucose or annual 75-g oral glucose tolerance test. Of those interventions were followed by further caloric restriction to induce a weight loss of ≥0.5 to 1 kg/wk. The principal end point of the DPP was diabetes mellitus incidence, confirmed by a semianual fasting plasma glucose or annual 75-g oral glucose tolerance test. Of those interventions were followed by further caloric restriction to induce a weight loss of ≥0.5 to 1 kg/wk. The principal end point of the DPP was diabetes mellitus incidence, confirmed by a semianual fasting plasma glucose or annual 75-g oral glucose tolerance test. Of those interventions were followed by further caloric restriction to induce a weight loss of ≥0.5 to 1 kg/wk. The principal end point of the DPP was diabetes mellitus incidence, confirmed by a semianual fasting plasma glucose or annual 75-g oral glucose tolerance test. Of those interventions were followed by further caloric restriction to induce a weight loss of ≥0.5 to 1 kg/wk.

Measurements
Blood was drawn from an antecubital vein after an overnight fast (≥12 hours). Measurements of triacylglycerol, TC, and HDL-C were made at the DPP central biochemistry laboratory using enzymatic methods standardized to the Centers for Disease Control and Prevention reference methods. HDL-C concentrations were obtained by precipitation of apolipoprotein B–containing lipoproteins by the dextran sulfate Mg2+ treatment. The Friedewald equation was used to calculate LDL-C. Where triacylglycerol levels exceeded 4.5 mmol/L, the lipoprotein fractions were separated using preparative ultracentrifugation of plasma by β quantification. Nuclear magnetic resonance spectroscopy (LipoScience Inc., Raleigh, NC) was used to quantify IDL-C and ApoB concentration, VLDL particle numbers (total, small, medium, and large subfractions), LDL particle numbers (total, small, medium, and large subfractions), and HDL particle numbers (total, small, medium, and large subfractions), as well as their average total particle sizes.

Genotyping
Standard methods were used to extract DNA from peripheral blood leukocytes. The DPP was genotyped using the MetaboChip genotyping array (Illumina Inc.). From the MetaboChip array, we selected 71 TC-associated, 37 triacylglycerol-associated, 68 HDL-C-associated, and 54 LDL-C–associated SNPs (with overlaps, 150 individual SNPs for the 4 main lipid traits) and 91 lipoprotein subfraction–associated SNPs that had been identified through recent GWAS meta-analyses. All together, we extracted 234 SNPs from the MetaboChip array. To ensure quality control, study participants with failed genotyping (n=1), sex inconsistency (n=14), or cryptic familial relatedness (n=47) were excluded. From the 234 SNPs, none deviated from Hardy–Weinberg equilibrium (P<10−5) in any ethnic groups. The SNPs associated with the various lipoprotein traits are listed in Table I in the Data Supplement. Where the index SNPs were not available on the MetaboChip array (eg, they had dropped out during the quality control stage), suitable HapMap proxies (r2≥0.80) were identified, and these variants were used in place of the index SNPs. The genotyping success rate for the 234 SNPs was 99.6%.

Statistical Analysis
Analyses were performed using STATA (version 13.1; StataCorp LP, TX) and PLINK (v1.07). We conducted 2 parallel sets of analyses. First, dependent variables were analyzed in their native distribution. Second, all analyses were performed with inverse normalized (mean=0, SD=1) variables as outcomes. In the first case, effect sizes and SEs are reported in the outcome traits’ native unit. In the second case, effect sizes are reported in SD units to facilitate comparisons across traits.

Pairwise Pearson correlations between traits were determined (Table II in the Data Supplement). As the 4 primary lipid traits strongly correlate with multiple subfractions, we hypothesized that some genetic variants identified for the primary lipid traits might also associate with lipoprotein subfractions. Thus, SNPs from the Global Lipids Genetics Consortium meta-analysis were evaluated (for marginal and treatment interaction effects) for their respective standard lipid traits and any subfraction that was correlated (P≥0.5 with the associated traits. Thus, guided by the results in Table II in the Data Supplement, triacylglycerol-associated SNPs were also evaluated for association with LDL particle size, large VLDL, medium VLDL, small LDL; total VLDL; TC; and LDL-C–associated SNPs were also evaluated for association with ApoB and total LDL; HDL-C–associated SNPs were also evaluated for association with large HDL, large LDL, small LDL, LDL particle size, and total HDL. In addition, SNPs associated with lipoprotein subfractions were evaluated for associations and treatment interactions with those respective traits.

In analyses seeking to replicate the previously reported genetic association results, we used the baseline DPP data. Additive genetic effects were assumed for each SNP, with a value of 0, 1, or 2 being assigned based on the number of minor allele copies. In these analyses, baseline traits were adjusted for age, age2, sex, and principal components for genetic markers of ancestry (to minimize confounding by population stratification). Individual SNP
analyses that focused on that SNP’s primary lipid trait(s) at baseline (i.e., the trait for which it was established at a genome-wide level of significance to be associated with in published literature) were not corrected for multiple comparisons, as the previous probability for association is high in these cases given existing replication data. Bonferroni correction, however, was applied in cases where we investigated associations between correlated lipid traits, as described above.

To test whether the SNPs modified response to the DPP interventions, multiple linear regression was used to model the product of the SNP and the treatment condition (lifestyle versus placebo and metformin versus placebo) against the value of the lipid or lipoprotein trait measured 1 year after baseline (dependent variables). In the regression models, we fitted the 1-year (follow-up) trait levels as dependent variables, the SNP×treatment interaction term as the independent variable, and SNP, treatment condition, the corresponding baseline trait, baseline age, baseline age2, sex, and genetic principal components as covariates. As there was no difference in lipid medication use (P>0.05) by treatment arm at baseline or 1-year follow-up, we did not adjust for lipid-lowering medication use. In total, we ran 1101 interaction tests. As these genetextact interaction tests aim to test different biological associations than the regressions testing baseline associations, we corrected for multiple testing in this set of results. The Bonferroni-corrected α type 1 error rate was set to 0.05/1101=4.5x10^{-5}.

Aggregated genetic risk was assessed by constructing trait-specific genetic risk scores (GRS). All SNPs previously associated in published GWAS for a given trait (Table I in the Data Supplement) were used to create the respective trait’s GRS. GRSs were calculated in 2 ways. In the first instance, we assumed an equal magnitude of effect for each risk allele (unweighted GRS) by adding the number of risk alleles (0, 1, or 2) that a participant carried for each SNP associated with the trait of interest. In the second instance, we followed the same principle, but assigned weights to the allele counts based on published effect sizes reported by large-scale GWAS for each SNP and constructed a weighted GRS (wGRS). Regardless of the GRS approach used, and with the exception of HDL-associated SNPs, alleles at each SNP locus were designated risk alleles if, within published meta-analyses, they were related with elevated concentrations of the respective lipid or lipoprotein subfractions. Risk alleles for HDL-associated SNPs were those associated with lower HDL-related trait concentrations in published meta-analyses. In the event that, for a given participant, SNP data were missing (up to 4 SNPs of those required to construct a given GRS) and we were unable to replace it with an appropriate proxy variant, genotypes were imputed within each of the 5 DPP ethnic groups, as previously described.22 GRS and wGRS descriptives are shown in Table III in the Data Supplement. The GRSs were modeled as continuous independent variables in multiple regression analyses; dependent variables were the lipid or lipoprotein traits (at baseline or follow-up, depending on the model), and they were adjusted in the same way as the individual SNP analyses outlined above. In interaction analyses, the Bonferroni-corrected α type 1 error rate was set to 0.05/34=0.0015. For figurative purposes, we dichotomized the GRSs based on their median values.

To assess the public health impact of lifestyle and metformin interventions across participants with low- and high-risk genotypes, we stratified the cohort by above and below the median GRS value and compared the groups’ phenotype levels for each trait at baseline and follow-up in the metformin and lifestyle arms separately. For these analyses, we used independent samples t tests to determine the statistical significance of any differences between groups over time. Our purpose with these analyses was to determine whether the relevant genetic effects can be offset by metformin or lifestyle interventions.

As DPP is a multiethnic study, we further assessed potential confounding by population stratification by repeating all GRS analyses in the subgroup of self-reported white participants only (n=1408, the largest ethnic group in the DPP) and compared effect estimates with the overall DPP results.

Detailed a priori power calculations and graphical illustrations are shown in Text II in the Data Supplement.

Functional Annotation and Pathway Analysis
We assessed whether SNPs demonstrate liver-specific expression quantitative trait loci (eQTL) evidence using The Genotype-Tissue Expression (GTEx) project database,29 as many of the lipids and lipoprotein subfractions studied here are synthesized in the liver. These SNPs were incorporated in eQTL GRSs in a trait-specific fashion, and the analyses described above were repeated using these GRSs.

We conducted detailed functional annotation of the 234 SNPs analyzed in this study using the ANNOVAR software tool.29 Pathway enrichment analysis for the 20 GRSs were performed using the REACTOME platform.29,30 As these analyses are not the main scope of this project, we present these results in the Data Supplement.

Results
Thirty-two of the 234 SNPs included in the current analyses have been studied previously in the DPP.32 Participant characteristics for the DPP study population used in the current analyses are described elsewhere,32 as are the effects of the DPP interventions on 1-year changes in the lipid and lipoprotein traits studied here.

Phenotypic Variation Explained by Genetic Factors
Table IV in the Data Supplement reports the phenotypic variance explained by the GRSs and wGRSs (adjusted models). The average variance explained by the trait-specific GRSs was 1.7%. The trait-specific wGRSs explained on average 2.4% of the phenotypic variance of the traits. In further analyses, all GRSs (for 20 traits) cumulatively explained 5% of the phenotypic variance on average. All wGRSs explained 6% of the phenotypic variance on average (ranging from 2.7% for HDL-C to 10% for large VLDL particles).

Associations of SNPs With Baseline Lipid Traits
Of the 150 SNPs tested for individual SNP associations with standard lipid traits, 71 were previously associated with TC, 37 with triglycerides, 68 with HDL-C, and 54 with LDL-C. As some SNPs were associated with multiple traits, a total of 230 replication analyses of these standard traits were performed. Fifty-nine (25.7%) of these associations replicated at the nominal α=0.05 level. Collectively, 113 SNPs have been previously associated with the lipoprotein subfractions that are available in the DPP. For these lipoprotein subfractions, 207 trait-specific associations and 673 associations based on highly correlated traits (in total, 880 association tests), 180 (20.5%) replicated at the nominal α=0.05 level, whereas 24 (2.7%) replicated at the Bonferroni-adjusted level of P<5.7x10^{-5}. Table V in the Data Supplement reports the association of each SNP with each of the baseline lipid and lipoprotein traits. In all, 227/1110 (20.5%) of these association tests were statistically significant at a critical α=0.05, with 28 (2.5%) replicating at a Bonferroni-adjusted level of P<4.5x10^{-3}. Three SNPs previously only associated with the main lipid traits (TC, LDL-C, or HDL-C), survived Bonferroni correction for a lipoprotein particle measure or ApoB. These are rs629301 for ApoB (β=0.05 g/L per copy of the risk allele; SE=0.008; P=4.3x10^{-12}), rs3764261 for LDL-C and rs2836123 for HDL-C.
LDL particle size ($\beta=-0.4$ nm per copy of the risk allele; SE=0.08; $P=1.8 \times 10^{-6}$), and rs1532085 for large HDL ($\beta=-0.43$ mmol/L per copy of the risk allele; SE=0.08; $P=3.7 \times 10^{-7}$).

**Associations of GRSs With Baseline Lipid Traits**

Table 1 reports all GRS/wGRS trait associations. In the majority of cases (32/34), these tests of association were statistically significant at baseline ($P$ values ranging from $1.3 \times 10^{-4}$ for total LDL to $1.1 \times 10^{-16}$ for TC), with $P<0.05$ for tests of association for medium HDL and IDL-C with their respective GRSs. Repeating these models using the inverse normalized traits did not change the results ($P$ values ranging from $1.1 \times 10^{-4}$ for total LDL to $1.1 \times 10^{-16}$ for triacylglycerol, with associations for medium HDL and IDL-C $P>0.05$). Analyses conducted only in self-reported white DPP participants (to help reassure the absence of confounding by population stratification) yielded results that were largely consistent with those observed in the full DPP cohort. Using the wGRS strengthened the results for the majority of the traits (28/34 associations). The GRS was positively correlated with baseline concentrations of triacylglycerol; TC; LDL-C; small, large, and total LDL particle numbers; small, medium, large, and total VLDL particle numbers; ApoB; and LDL and VLDL particle sizes. The GRS was negatively correlated with IDL-C, HDL-C, HDL particle size and small, medium, large, and total HDL particle numbers.

**Interactions Between Interventions and SNPs on 1-Year Lipid Traits**

Results for all SNPs are shown in Tables VI and VII in the Data Supplement, for lifestyle and metformin interactions, respectively. One interaction test passed the Bonferroni-corrected critical $\alpha$ level ($\alpha=0.05/1101=4.5 \times 10^{-5}$). The rs581080 variant in tetratricopeptide repeat domain 39B (TTC39B) showed evidence for lifestyle treatment modification with large HDL particle numbers ($P_{\text{interaction}}=2.8 \times 10^{-4}$ for lifestyle versus placebo). The treatment interaction effect for this SNP was less statistically significant when assessed using the inverse normalized large HDL particle numbers variable ($P_{\text{interaction}}=1.7 \times 10^{-4}$ for lifestyle versus placebo). The interaction for rs581080 was no longer statistically significant when assessed only in European ancestry participants ($P_{\text{interaction}}=0.12$ for lifestyle versus placebo), which may reflect lower statistical power owing to the smaller sample size of this subcohort.

**Interactions Between Interventions and GRSs on 1-Year Lipid Traits**

The lifestyle intervention modified the effect of the GRS for large HDL particle numbers, such that a higher GRS$_{\text{HDL-large}}$ was associated with lower 1-year baseline-adjusted large HDL particle numbers in the lifestyle group ($\beta=-0.11$ mmol/L per GRS risk allele; 95% confidence interval [CI], $-0.188$ to $-0.033$; $P=5 \times 10^{-3}$; $P_{\text{interaction}}=1 \times 10^{-4}$ for lifestyle versus placebo), but not in the metformin group ($\beta=-0.08$ mmol/L per GRS risk allele; 95% CI, $-0.141$ to $-0.008$; $P=0.027$; $P_{\text{interaction}}=0.07$ for metformin versus placebo) or the placebo group ($\beta=-0.02$ mmol/L per GRS risk allele; 95% CI, $-0.086$ to $0.042$; $P=0.50$; Figure). Using the wGRS attenuated this result, such that the interaction between lifestyle intervention and GRS$_{\text{HDL-large}}$ on large HDL particle number ($P_{\text{interaction}}=6 \times 10^{-3}$ for lifestyle versus placebo) became nominally statistically significant. Repeating the analyses with inverse normalized large HDL particle number did not materially change the results ($P_{\text{interaction}}=5 \times 10^{-3}$). The exclusion of those individuals initiated on lipid-lowering medication (n=226) between baseline and follow-up did not materially impact the results ($P_{\text{interaction}}=6 \times 10^{-3}$). GRS results for large HDL particle numbers per treatment arm are shown in Table 2, whereas all GRS and wGRS/lifestyle and metformin interactions are shown in Tables VIII and IX in the Data Supplement, respectively. Repeating analyses only in European ancestry DPP participants (n$_{\text{max}}=1408$) attenuated the statistical significance of the interactions observed in all participants although the pattern of the interaction effects remained the same for large HDL ($\beta=-0.16$ mmol/L per GRS risk allele; 95% CI, $-0.283$ to $-0.047$; $P=6 \times 10^{-3}$; $P_{\text{interaction}}=0.054$ for lifestyle versus placebo).

**Lipid Profile Change From Baseline to 1 Year**

In the lifestyle arm, participants at higher genetic risk (GRS above median) had more favorable ($P<0.05$ or similar ($P<0.05$) trait levels at 1 year than participants with lower genetic risk (GRS below median) at baseline (Figure) for all traits, except for large LDL, small VLDL particle numbers, and LDL size (3 of 20 traits). In the metformin arm, participants at higher genetic risk had more favorable trait levels at 1 year than participants at lower genetic risk at baseline for triacylglycerol, LDL-C, HDL-C, IDL-C, ApoB; small, medium, large, and total HDL; small and total LDL; medium and large VLDL particle numbers; and HDL size. No difference was observed for TC; large LDL; small and total VL DL particle number nor LDL or VLDL size.

**Functional Annotation and Pathway Analysis**

Two SNPs for TC (rs10893499 and rs4530754), LDL-C (rs10893499 and rs4530754), and total VLDL (rs10889353 and rs646776) demonstrated liver-specific eQTL evidence in GTEx; therefore, we repeated our interaction analyses with 3 trait-specific eQTL GRSs comprised with these SNPs for TC, LDL-C, and total VLDL, respectively. Although all 3 GRSs demonstrated nominal statistical significance ($\beta=-0.268$ mmol/L per GRS risk allele; 95% CI, $-0.530$ to $-0.005$; $P_{\text{interaction}}=0.050$ for lifestyle versus placebo for LDL-C; $\beta=-0.341$ mmol/L per GRS risk allele; 95% CI, $-0.644$ to $-0.037$; $P_{\text{interaction}}=0.028$ for lifestyle versus placebo for TC; $\beta=4.120$ mmol/L per GRS risk allele; 95% CI, 0.539–7.700; $P_{\text{interaction}}=0.024$ for lifestyle versus placebo for total VLDL), none of these associations remained significant after correction for multiple testing.

Detailed functional annotation of all SNPs is shown in Table X in the Data Supplement, whereas results from trait-specific pathway enrichment analyses are shown in Table XI in the Data Supplement.
### Table 1. Unweighted and Weighted Genetic Risk Scores–Trait Associations at Baseline (n max=2585)

| Trait, Units | GRS | n   | \(\beta\) | SE  | P Value | 95% CI LL  | 95% CI UL  | \(P_{\text{wGRS}}\) Value | \(\beta_{\text{inv}}\) | SE inv | P inv Value | \(P_{\text{wGRSinv}}\) Value |
|--------------|-----|-----|-----------|-----|---------|------------|------------|---------------------------|-------------------|--------|-------------|---------------------------|
| ApoB, g/L    | ApoB GRS | 2567 | 0.014     | 0.002 | 1.9E−10 | 0.01  | 0.018   | 2.2E−25                  | 0.058             | 0.009  | 8.0E−11     | 4.3E−26                   |
| ApoB, g/L    | LDL-C GRS | 2567 | 0.007     | 0.001 | 2.8E−11 | 0.005 | 0.009  | 1.4E−14                  | 0.03              | 0.004  | 1.9E−11     | 4.0E−15                   |
| ApoB, g/L    | TC GRS | 2567 | 0.006     | 0.001 | 4.2E−12 | 0.005 | 0.008  | 1.6E−18                  | 0.027             | 0.004  | 2.1E−12     | 6.5E−19                   |
| HDL size, nm | HDL size GRS | 1714 | −0.018   | 0.004 | 1.3E−05 | −0.026 | −0.01  | 5.7E−08                  | −0.04             | 0.01   | 8.6E−05     | 6.4E−07                   |
| HDL-C, mmol/L | HDL-C GRS | 2584 | −0.007   | 0.001 | 1.9E−12 | −0.01 | −0.005 | 4.2E−31                  | −0.027            | 0.003  | 1.6E−14     | 1.8E−36                   |
| IDL-C, nmol/L | IDL-C GRS | 2567 | 1.568    | 1.409 | 2.7E−01 | −4.332 | 1.197  | 4.6E−01                  | −0.011            | 0.013  | 4.1E−01     | 4.4E−01                   |
| Large HDL, µmol/L | HDL-C GRS | 2584 | −0.052   | 0.01  | 5.6E−07 | −0.032 | 6.6E−15 | 0.023                      | 0.004             | 0.004  | 4.8E−08     | 4.4E−16                   |
| Large HDL, µmol/L | Large HDL GRS | 1712 | 0.151    | 0.026 | 5.7E−09 | −0.202 | −0.1   | 4.9E−13                  | −0.06             | 0.01   | 6.2E−09     | 1.2E−12                   |
| Large LDL, nmol/L | Large LDL GRS | 1632 | −4.903   | 1.152 | 2.2E−05 | −7.162 | −2.644 | 4.2E−12                  | −0.011            | 0.005  | 7.2E−06     | 4.4E−13                   |
| Large VLDL, nmol/L | Large HDL GRS | 1714 | 0.353    | 0.088 | 6.9E−05 | 0.179  | 0.526  | 1.5E−04                  | 0.049             | 0.012  | 2.4E−05     | 1.7E−05                   |
| Medium HDL, µmol/L | Medium HDL GRS | 1714 | 0.261    | 0.045 | 9.3E−09 | 0.172  | 0.349  | 2.0E−17                  | 0.035             | 0.006  | 3.5E−09     | 2.4E−18                   |
| Medium VLDL, nmol/L | Medium VLDL GRS | 1707 | 1.113    | 0.236 | 2.5E−06 | 0.651  | 1.575  | 6.5E−03                  | 0.056             | 0.012  | 1.3E−06     | 7.3E−03                   |
| Small HDL, µmol/L | Small HDL GRS | 1713 | −0.366   | 0.056 | 6.2E−11 | −0.475 | −0.257 | 6.8E−19                  | −0.076            | 0.012  | 7.9E−11     | 9.6E−19                   |
| Small LDL, nmol/L | Small HDL GRS | 1714 | 8.772    | 1.793 | 1.1E−06 | 5.256  | 12.289 | 5.0E−08                  | 0.022             | 0.004  | 6.3E−07     | 6.4E−12                   |
| Small VLDL, nmol/L | Small VLDL GRS | 1708 | 0.83     | 0.182 | 5.6E−06 | 0.473  | 1.187  | 6.3E−08                  | 0.045             | 0.01   | 5.4E−01     | 4.0E−01                   |
| Total HDL, nmol/L | Total HDL GRS | 1714 | −0.051   | 0.027 | 5.7E−02 | −0.103 | 0.002  | 1.2E−04                  | −0.01             | 0.004  | 2.8E−02     | 4.4E−05                   |
| Total HDL, µmol/L | Total HDL GRS | 1711 | −0.517   | 0.094 | 4.7E−08 | −0.702 | −0.332 | 1.6E−10                  | 0.015             | 0.015  | 1.9E−08     | 7.0E−11                   |
| Total LDL, nmol/L | Total LDL GRS | 1714 | 9.782    | 2.064 | 2.3E−06 | 5.734  | 13.829 | 5.0E−08                  | 0.027             | 0.005  | 9.9E−07     | 3.0E−08                   |
| Total LDL, µmol/L | Total LDL GRS | 1714 | 8.442    | 1.786 | 2.5E−06 | 4.94   | 11.945 | 3.9E−09                  | 0.023             | 0.005  | 1.1E−06     | 1.7E−09                   |
| Total VLDL, nmol/L | Total VLDL GRS | 1714 | 14.083   | 3.67  | 1.3E−04 | 6.884  | 21.281 | 3.1E−08                  | 0.037             | 0.01   | 1.1E−04     | 2.9E−08                   |
| VLDL size, nm  | VLDL size GRS | 1648 | 0.579    | 0.131 | 1.0E−05 | 0.323  | 0.835  | 9.5E−08                  | 0.066             | 0.015  | 1.9E−05     | 1.6E−07                   |

*\(P\) values are based on linear regression models. SNP associations were tested by fitting the genetic risk scores as the independent variables with the different lipoprotein subfractions at baseline as dependent variables. \(\beta\) coefficients reflect the association of one genetic risk score unit (effect allele) with the trait (expressed in native units and inverse normalized units). Age, age², sex, and genomic principal components were used as covariates in all models. 95% CI LL indicates 95% confidence interval lower limit; 95% CI UL, 95% confidence interval upper limit; ApoB, apolipoprotein B; \(\beta\), beta effect estimate; \(\beta_{\text{inv}}\), beta effect estimate for inverse normalized traits; GRS, genetic risk score; HDL, high-density lipoprotein; LDL, low-density lipoprotein; n, sample size; \(P_{\text{wGRS}}\), \(P\) value for weighted GRS associations; \(P_{\text{wGRSinv}}\), \(P\) value for weighted GRS associations undertaken with the inverse normalized traits; SE inv, SE for inverse normalized traits; TC, total cholesterol; and VLDL, very-low-density lipoprotein.
Discussion

This is to our knowledge the most comprehensive assessment to date of established lipid- and lipoprotein-associated loci in the context of human diabetes prevention interventions. It is also the first study to our knowledge to examine the effects of these loci on changes in lipid and lipoprotein concentrations over time.

The major finding of this study is that genetic predisposition to a higher large HDL particle number modifies the response to lifestyle intervention. Although in GRS analyses, lifestyle intervention robustly increased the number of large HDL particles at 1-year DPP participants, the intervention was less effective in participants at higher genetic risk. The participants at higher genetic risk also had fewer large HDL particles at baseline than those at lower genetic risk. Nevertheless, lifestyle intervention generally improved lipoprotein values in people at higher genetic risk to a level that was similar or more favorable than observed in participants with lower genetic burden assigned to the control arm (17 of the 20 traits), suggesting that lifestyle intervention can overcome genetic risk for dyslipidemia. Of note, analyzing treatment interactions with GRSs constructed exclusively from SNPs demonstrating eQTL evidence in the liver did not yield clinically relevant results.

In SNP analyses, 1 SNP (TTC39B rs581080)×lifestyle interaction passed the predefined conservative threshold for multiple test-corrected statistical significance for large HDL particle numbers; no such interaction with metformin was observed. However, we believe this interaction with lifestyle to be spurious, as the interaction is driven by differences in the genetic effect on large HDL particle numbers by treatment arm before randomization, and not by the joint effect of the interventions and genotypes (which was apparent when the data were visualized).

The minor C allele of the TTC39B rs581080 variant was originally associated with lower HDL-C and TC concentrations, and in vivo knockdown of its mouse homolog correlates with higher HDL-C concentrations. The function of the TTC39B gene in humans is presently unknown. No human studies of gene lifestyle interaction for this locus have been reported to our knowledge.

Table 2. Statistically Significant GRS Interactions With Baseline-Adjusted 1-Year Traits (n max=1374)

| Trait         | GRS        | Intervention Arm | n  | β   | SE  | P Value | 95% CI LL | 95% CI UL | P interaction Value |
|---------------|------------|------------------|----|-----|-----|---------|-----------|------------|---------------------|
| Large HDL, µmol/L | Large HDL GRS | Placebo         | 463 | −0.022 | 0.032 | 0.499 | −0.086 | 0.042 | Not available |
| Large HDL, µmol/L | Large HDL GRS | Metformin       | 461 | −0.075 | 0.034 | 0.499 | −0.141 | −0.008 | 0.07 |
| Large HDL, µmol/L | Large HDL GRS | Lifestyle       | 450 | −0.111 | 0.039 | 5×10⁻³ | −0.188 | −0.033 | 1×10⁻³ |

P values are based on linear regression models. GRS associations were modeled by fitting the GRSs as the independent variables with the different lipoprotein subfractions as dependent variables. P interaction values are based on linear regression models. GRS associations were tested by fitting the GRS×lifestyle vs placebo intervention and GRS×metformin vs placebo intervention interaction terms as the independent variables with the different lipoprotein subfractions at follow-up as dependent variables. β coefficients reflect the association of 1 GRS unit (effect allele) with the trait (expressed in native units and inverse normalized units). Age, age², sex, baseline lipoprotein subfraction values, and genomic principal components were used as covariates in all models. 95% CI LL indicates 95% confidence interval lower limit; 95% CI UL, 95% confidence interval upper limit; β, beta effect estimate per GRS risk allele; GRS, genetic risk score; HDL, high-density lipoprotein; and n, sample size.
Lifestyle modification is the frontline therapy to combat dyslipidemia; our data help understand better why some people are more responsive than others to lifestyle interventions. In addition, lifestyle and other therapies that target specific lipoprotein subfractions might be clinically more relevant than only modifying the major fractions, such as LDL-C, HDL-C, or triacylglycerol levels. 

This is supported by data, showing that particle numbers, lipoprotein-associated protein levels (such as ApoA1 or ApoB), and their relative amounts predict cardiovascular risk and other hard clinical outcomes with higher accuracy than the major lipids. 

Metformin treatment, unlike lifestyle intervention, seems to act independently on changes in VLDL, LDL, and HDL, suggesting that the 2 interventions influence these traits through different mechanisms. In support of this, we found that the GRS intervention interactions were only apparent for lifestyle and not for metformin. All of these changes are thought to favorably impact CVD risk. For example, pharmacologically increased small HDL particle numbers (with fibrates) reduces CVD risk in some studies.

In previous analyses within the DPP, we observed interactions between a GRS and lifestyle intervention for LDL-C and small LDL particle numbers. A key distinction between these analyses and the ones reported here is that the GRS used in the former analysis was not trait specific, but included a set of 32 SNPs with heterogeneous roles in lipid biology, whereas the GRSs studied here were fitted to the specific lipid traits. Elsewhere in the DPP, Goldberg et al examined the lipid and lipoprotein traits examined here for their relationships with various cardiometabolic outcomes. Compared with placebo intervention, the DPP lifestyle intervention lowered VLDL particle numbers, especially large VLDL particles, which are prominent in diabetic dyslipidemia, and VLDL particle size. Possibly as a consequence of the DPP lifestyle intervention’s effects on VLDL, the intervention also lowered LDL particle numbers, especially for small LDL particles, increased average LDL particle size (which associate with fasting insulin, hepatic lipase, and cholesteryl ester transfer protein [CETP] concentrations), and increased large HDL particle numbers by ≈1 μmol/L and size by ≈1.5 nm. By contrast, metformin did not affect VLDL particle numbers or size in the DPP. Metformin did, however, lower LDL subfraction concentrations and increased small and total HDL particle numbers. Despite the robust and wide-ranging effects of the DPP lifestyle and metformin interventions on lipoprotein subfractions reported by Goldberg et al., only one of these traits (large HDL particle numbers) seems to be influenced by genetreatment interactions in the current analyses. Although recent evidence suggests that HDL-C is not causal in the development of cardiovascular disease, the findings of this analysis might represent underlying causal effects of HDL-C or its correlates through geneenvironment interactions.

The major strength of this analysis is that it was conducted in a tightly controlled randomized clinical trial, which limits the extent to which confounding, reverse causality and some other sources of bias are likely to underlie our findings. As the DPP is a multiethnic trial, we dealt with potential confounding by population stratification using genomic control and ethnic-specific quality control. We also conducted subgroup analyses in self-reported white participants, but we did not observe major differences between these set of results and the ones we obtained from analyzing the whole study. Although DPP is one of the largest clinical trials investigating the effects of metformin and lifestyle, our a priori power calculations indicate that some of our apparently negative findings are likely to be false negatives owing to insufficient statistical power to detect small interaction effects. However, the objective of this study was to determine if established dyslipidemia-associated loci are likely to be of clinical relevance, and the small effects that this study is underpowered to detect are unlikely to be clinically useful.

We have replicated the effects of genetic variants previously associated with lipid and lipoprotein subfraction traits. We provide evidence that the deleterious effects of some established lipid- and lipid subfraction–associated loci modify the effects of intensive lifestyle interventions. Specifically, individuals genetically predisposed to low large HDL particle concentrations are less responsive to the ability of these interventions to increase these levels. Nonetheless, participants at higher genetic risk assigned to lifestyle intervention had comparable lipid profiles at 1 year post randomization to those at lower genetic risk at baseline, indicating that these interventions are of value to individuals with high-risk genetic profiles. Although this study provides some evidence of gene lifestyle interactions at a few loci and for specific lipid traits, most tests yielded no compelling evidence of gene lifestyle interactions, indicating that most GWAS-derived loci do not affect response to lifestyle interventions to a clinically relevant degree.

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Appendix
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In our study in the Diabetes Prevention Program randomized clinical trial, we aimed to detect gene environment interactions of known lipid and lipoprotein subfraction loci (individually and amalgamated in genetic risk scores) and metformin/lifestyle intervention versus the placebo arm. We detected statistically significant interactions between the genetic risk score of large high-density lipoprotein (HDL) particle concentrations and the lifestyle arm (versus placebo) in relation to large HDL particle concentrations. Those at higher genetic risk fewer large HDL particles at baseline than those at lower genetic risk and lifestyle intervention elevated the number of large HDL particles at 1 year, but the intervention was less effective in people at higher genetic risk. The clinical relevance of our study is that participants at higher genetic risk assigned to lifestyle intervention had comparable lipid profiles for most traits (including large HDL particle concentrations and HDL size) at 1 year post randomization to those at lower genetic risk who had been assigned to the placebo-control intervention, indicating that these interventions are of value to individuals with high-risk genetic profiles.