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Genetic Variants in *HSD17B3*, *SMAD3*, and *IPO11* Impact Circulating Lipids in Response to Fenofibrate in Individuals With Type 2 Diabetes

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Additional Supporting Information may be found in the online version of this article.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHORSHIP CONTRIBUTION

D.M.R., S.S.P., J.R.J., G.A.G., H.N.G., H.L.M., J.B.B., M.J.W., and A.M.R. wrote the paper. D.M.R., A.P., A.G., G.A.G., A.D., J.C.M., H.L.M., J.B.B., M.J.W., and A.M.R. designed the research. D.M.R., S.S.P., J.R.J., T.M.H., H.S.S., H.G., A.D., M.J.W., and A.M.R. performed the research. D.M.R., S.W.M., S.S.P., A.P., A.S., H.S.S., M.L.M., and J.C.M. analyzed the data.

Abstract

Individuals with type 2 diabetes (T2D) and dyslipidemia are at an increased risk of cardiovascular disease. Fibrates are a class of drugs prescribed to treat dyslipidemia, but variation in response has been observed. To evaluate common and rare genetic variants that impact lipid responses to fenofibrate in statin-treated patients with T2D, we examined lipid changes in response to fenofibrate therapy using a genomewide association study (GWAS). Associations were followed-up using gene expression studies in mice. Common variants in *SMAD3* and *IPO11* were marginally associated with lipid changes in black subjects ($P < 5 \times 10^{-6}$). Rare variant and gene expression changes were assessed using a false discovery rate approach. *AKR7A3* and *HSD17B13* were associated with lipid changes in white subjects ($q < 0.2$). Mice fed fenofibrate displayed reductions in *Hsd17b13* gene expression ($q < 0.1$). Associations of variants in *SMAD3*, *IPO11*, and *HSD17B13*, with gene expression changes in mice indicate that transforming growth factor-beta (TGF- β) and NRF2 signaling pathways may influence fenofibrate effects on dyslipidemia in patients with T2D.

Dyslipidemia is a significant risk factor for cardiovascular disease (CVD), which is the leading cause of death worldwide.¹ In the United States, it is estimated that 33.5% of adults have high low-density lipoprotein (LDL), and only 48.1% of those individuals are currently being treated.² Individuals with type 2 diabetes (T2D) commonly express a dyslipidemia, characterized by high triglycerides, low high-density lipoprotein (HDL), and an increase in cholesterol poor, small LDL, and are 2–4 times more likely to develop heart disease than nondiabetic individuals.²

Fibrates, a class of medications used to treat individuals with dyslipidemia by activating the peroxisome proliferator-activated receptor-alpha (PPAR α), increases HDL and lowers triglycerides and LDL. Meta-analyses of several large clinical trials indicated that treatment with fibrates decrease the number of nonfatal myocardial infarctions, although they did not decrease all-cause mortality.^{3,4} Statins are the first-line treatment to lower LDL to prevent CVD.⁵ Fibrates are generally not recommended to reduce CVD because of a lack of demonstrated benefit, although they are recommended for the management of hypertriglyceridemia.⁶ Specifically, fenofibrate is recommended in the context of statin therapy because of lower risk of interference with statin metabolism and myopathy.^{5,6}

One goal of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial was to compare the benefits and risks of treatment strategies for intensively managing dyslipidemia with a combined statin and fenofibrate therapy vs. treatment with statin alone, whereas simultaneously targeting normal glycemia and blood pressure vs. standard targets in individuals with T2D at high risk for CVD.^{7,8} The ACCORD trial followed 10,251 participants for up to 8 years at 77 clinical centers in the United States and Canada. Overall, no statistically significant benefits were observed for patients on the combined CVD endpoint of time to first heart attack, stroke, or CVD mortality. In addition, there was an increase in mortality in participants receiving intensive glycemia control.^{9–12} Despite the lack of overall positive findings, interindividual variation in response to the different treatments in ACCORD was observed. Such variation to fibrate lipid response has been

observed in a number of studies^{13–15} and suggests that genetic markers of drug response may be important biomarkers for more targeted and personalized treatment strategies.

Previous studies have also investigated the role of genetic variation in fibrate lipid response with the majority of studies focused on candidate gene approaches to identify common or rare variants associated with differential responses.^{16–19} Here, we performed genomewide and exome-wide genotyping on all consenting individuals in the ACCORD study prescribed fenofibrate ($n = 1,264$). We previously reported a genomewide association study (GWAS) of fenofibrate drug response from a meta-analysis of subjects of European ancestry in ACCORD and the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.¹⁴ Here, we expand the analysis of ACCORD participants to include other ethnicities, using combined data from a genomewide single nucleotide polymorphism (SNP) array, and an exome chip array with >1.2 million combined genotyped SNPs and rare variant analysis for SNPs with minor allele frequencies (MAFs) <3% for changes in LDL, HDL, triglycerides (TGs), and total cholesterol (TC). Our results indicate interesting and potentially impactful associations, and we use mouse studies to test the hypotheses generated by the gene-mapping analysis. To our knowledge, this is the first GWAS of fenofibrate lipid response in multiple ethnicities, and the first exome-wide interrogation of fibrate drug response.

Although the results of the association mapping experiment can point to interesting biology, functional follow-up is a crucial step to support association analyses. In the current study, we considered the GWAS to be a hypothesis generating exercise to prioritize genes for further functional interrogation. We subsequently evaluated the genes identified through association analysis in two mouse gene expression studies, and demonstrate that many of the genes discovered in the association analysis play a significant role in fibrate drug response.

RESULTS

A total of 1,264, 781, and 138 subjects were included in the common and rare variant analyses for all races combined, white, and black cohorts, respectively. Variation in response was observed for all phenotypes: HDL, LDL, TC, and TG (Figure 1). Distributions of response variation for white and black cohorts individually can be found in Supplementary Figures S1 and S2. The mean change in HDL was 3.16 mg/dL (95% confidence interval [CI] = 2.78–3.53) for all races combined, 3.11 mg/dL (95% CI = 2.62–3.60) for white subjects only, and 2.80 mg/dL (95% CI = 1.73–3.86) for black subjects only. The mean change in LDL was –10.64 mg/dL (95% CI = –12.48 to –8.79) for all races combined, –9.31 mg/dL (95% CI = –11.68 to –6.95) for white subjects only, and –10.83 mg/dL (95% CI = –16.73 to –4.94) for black subjects only. The mean change in TG was –45.23 mg/dL (95% CI = –50.27 to –40.28) for all races combined, –52.04 mg/dL (95% CI = –58.31 to –45.78) for white subjects only, and –34.43 mg/dL (95% CI = –46.96 to –21.91) for black subjects only. The mean change in TC was –16.10 mg/dL (95% CI = –18.27 to –13.92) for all races combined, –16.14 mg/dL (95% CI = –18.93 to –13.35) for white subjects only, and –14.51 mg/dL (95% CI = –21.13 to –7.88) for black subjects only.

Common variant analysis

A total of 852,426 genotyped and 7,277,412 imputed variants had MAFs >3% and were included in the common variant analysis. When all races were combined or white subjects were analyzed, no SNPs met the threshold for genomewide significance ($P < 5 \times 10^{-8}$). When all races were combined, 8 SNPs associated with TG (genes: *BEST3*, *LOC105371270*, and *RPGRIP1L*; Supplementary Figure S3), 34 SNPs associated with TC (genes: *FGF14*, *PRRX1*, *MRPL12*, *ZNF775*, and *FBXL7*; Supplementary Figure S4), 1 SNP associated with HDL (no known genes; Supplementary Figure S5), and 2 SNP associated with LDL (genes: *FGF14* and *MRPL12*; Supplementary Figure S6) reached the threshold for suggestive significance ($P < 1 \times 10^{-6}$). For white subjects only, 11 SNPs associated with TG (genes: *LOC105371270* and *RPGRIP1L*; Supplementary Figure S7), 11 SNPs associated with TC (genes: *MAU2* and *PBX4*; Supplementary Figure S8), 0 SNPs associated with HDL (Supplementary Figure S9), and 8 SNP associated with LDL (genes: *PBX4*, *SNX7*, and *MAU2*; Figure 2) reached the threshold for suggestive significance ($P < 1 \times 10^{-6}$). When black subjects were analyzed separately, 6 SNPs were significantly associated ($P < 5 \times 10^{-8}$) with TG (genes: *LRFN2*, *LINC00333*, and *BCL9*), and 55 SNPs reached suggestive significance ($P < 1 \times 10^{-6}$) with TG (genes: *GLIS3*, *CCDC149*, *LINGO2*, *SNHG17*, *LOC105370782*, *TLL8*, *RHOBTB1*, and *LOC101927866*; Supplementary Figure S10). Seven SNPs were significantly associated ($P < 5 \times 10^{-8}$) with TC (genes: *CLN8* and *BICC1*), and 108 SNPs reached suggestive significance ($P < 1 \times 10^{-6}$) with TC (genes: *ST6GALNAC3*, *LOC105372744*, *SCGN*, *LOC102724378*, *NRXN1*, *LCT*, *LOC102724680*, *RBM19*, *MCM6*, *DARS*, *TRIOBP*, *DARS-AS1*, *PEX5L*, *LOC102724680*, and *NLGN1*; Supplementary Figure S11). One SNP was significantly associated ($P < 5 \times 10^{-8}$) with HDL (gene: *MSH3*), and 8 SNPs reached suggestive significance ($P < 1 \times 10^{-6}$) with HDL (genes: *TGFBR3*, *LOC105373670*, *STX8*, and *CELSR1*; Supplementary Figure S12). Last, 5 SNPs associated with LDL (genes: *BICC1*, *FOXPI*, and *PEX5L*; Supplementary Figure S13) reached the threshold for suggestive significance ($P < 1 \times 10^{-6}$). Lead SNPs associated with HDL, LDL, TC, and TG ($P < 1 \times 10^{-6}$) are presented in Tables 1 and 2 and Supplementary Tables S14 and S15. None of the associations reported above were associated with placebo treatment.

Rare variant analysis

A total of 17,081 genes were tested for association with TG, TC, HDL, or LDL in all races combined, white subjects only and black subjects only with rare variants (MAF 0.03). When all races were combined, *DCUNID4* and *DUSP3* were significantly associated ($q < 0.2$) with TG. Additionally, when only white subjects were included, *HARS2* was significantly associated with TG and HDL, *HSD17B13* was significantly associated with TG and HDL, *AKR7A3* was significantly associated with LDL and TC, and *MARCH3* was significantly associated with TG ($q < 0.2$). Last, *POGZ* was significantly associated with TG in black subjects only ($q < 0.2$; Table 3). None of the genes reported above were associated with subjects treated with placebo ($q = 1$; Table 3).

Functional validation

SNPs in genes in the common variant analysis with $P < 1 \times 10^{-5}$ or $q < 0.2$ in the rare variant analysis were compared to gene expression results in wild-type C57BL/6J mice fed vehicle control or fenofibrate for replication²⁰ (REP1). Fifty-seven genes overlapped between the two studies, and 10 were significant in REP1 ($q < 0.3$; *Mcm6*, *Smad3*, *Dcun1d4*, *Hecw2*, *Mipep*, *Ipo11*, *R3hdm1*, *Foxp1*, *Stx8*, and *Mapk10*; Table 4). These genes and 3 additional genes that met our threshold for inclusion in the replication study but were not available in the previously published REP1 study (*Hsd17b13*, *Pbx4*, and *Cyp4f39*) were subsequently tested in REP2, a follow-up gene expression study in mice to confirm the changes in response to fenofibrate vs. vehicle control in liver, adipose, and skeletal muscle. *Cyp4f39* was tested in REP2 because it is the murine homologue of *CYP4F22* in humans, which was marginally significant for change in HDL in ACCORD ($P < 1 \times 10^{-5}$). All genes, except *Mipep* and *Cyp4f39*, were significantly changed in liver tissue in REP2 ($q < 0.3$), however, the direction of the effect was not always consistent with REP1 (Table 4). Genes *Smad3*, *Ipo11*, and *Foxp1* were significantly decreased in both REP1 and REP2 and were considered to have successfully replicated the GWAS findings. *Hsd17b13* and *Pbx4* were significantly decreased in liver tissue in REP2 ($q < 0.3$; Table 4). REP1 was published previously and did not include *Hsd17b13* or *Pbx4*, so these genes were not available for replication in REP1. However, both of these genes were tested in REP2. There were no significant results for gene expression tested in adipose or skeletal muscle (Supplementary Table S16).

DISCUSSION

Dyslipidemia continues to be a widespread disorder with significant health impacts worldwide. Although recent studies, including ACCORD, have raised questions concerning the role of additional lipid-lowering therapy in the context of statin to reduce cardiovascular events,^{9,21} dyslipidemia remains a significant risk factor for CVD and fibrates are commonly prescribed.²² In addition, it is important to understand the variation in response to fenofibrate in people with T2D who are at an especially high risk of developing an adverse cardiovascular event (e.g., stroke and myocardial infarction). We previously published a meta-analysis combining the results of the fenofibrate lipid response in the GOLDN cohort with white subjects in ACCORD, and found significant associations with SNPs in *PBX4* and change in LDL in response to fenofibrate treatment.¹⁴ Here, we expand the previous study to include all races in ACCORD and black subjects only. In addition, we conduct a rare variant analysis for changes in HDL, LDL, TC, and TG and follow-up both common and rare variant GWAS findings in two studies of mice exposed to fenofibrate. Importantly, these functional validation studies highlight novel common and rare variants that contribute to variation in fenofibrate lipid response in individuals with T2D.

Combining all subjects that met our inclusion criteria in ACCORD resulted in 1,264 subjects available for analysis. Cytochrome P450 family 4 subfamily F member 22 (*CYP4F22*), was marginally associated with changes in HDL ($P = 2.50 \times 10^{-6}$, $\beta = -0.023$). *CYP4F22* is part of the 12(R)-lipoxygenase pathway, and has been shown to produce potent PPAR α agonists,^{23,24} which makes SNPs in *CYP4F22* biologically plausible for causing variation in HDL,

because PPAR α is the therapeutic target of fenofibrate. Although the biological role of *CYP4F22* is a compelling candidate for fibrate drug response, *Cyp4f39*, the murine homologue of *CYP4F22*, did not replicate in REP2, suggesting that the GWAS finding may be a false-positive, the replication may have failed due to species differences or gene expression may not be the appropriate test for *CYP4F22* response to fenofibrate exposure.

In black subjects only, lead SNP rs142923802, located in importin 11 (*IPO11*) was also marginally associated with change in LDL ($P = 1.52 \times 10^{-6}$, $\beta = 0.095$). Gene expression of *Ipo11* was significantly decreased in both REP1 ($q = 0.24$) and in the liver in REP2 ($q = 0.15$). *IPO11* codes the nuclear import receptor, importin 11, and in conjunction with ubiquitin-conjugating enzyme, UBE2E3, restricts KEAP1, which is a major suppressor of Nrf2.²⁵ Notably, Nrf2 in mice has been shown to interact with lipogenic genes and to regulate hepatic lipid homeostasis.²⁶ Moreover, Nrf2-*null* mice displayed reduced liver weight, decreased fatty acid content of hepatic triacylglycerol, and increases in serum HDL, and very low-density lipoprotein triglyceride. Finally, PPAR γ and other genes were found to be direct targets of Nrf2 activation, demonstrating that Nrf2, regulated by KEAP1 and IPO11 in humans, modulate lipid homeostasis. Rare variants in Aldo-keto reductase 7A3, *AKR7A3*, were significantly associated with LDL in white subjects only ($q = 0.08$). The AKR family of enzymes catalyze a wide range of endogenous and exogenous chemicals, including glucose, steroid hormones, and lipids. *Akr7a3* is transcriptionally regulated by Nrf2 in mice, which, in addition to IPO11 results discussed above, further supports the implication of Nrf2 signaling in regulating fenofibrate drug response.^{27,28} Previous studies have demonstrated that Nrf2 signaling is activated by fenofibrate through *Keap1* in mice, and may be responsible for the protective effect of fenofibrate for oxidative stress.²⁹ Here, we present evidence that SNPs located in genes in the Nrf2 signaling pathway may play an important role in regulating the change in LDL upon fenofibrate treatment.

In black subjects, rs12912310, located in the gene, mothers against decapentaplegic-3 (*SMAD3*), was marginally associated with LDL ($P = 5.75 \times 10^{-6}$) and TC ($P = 1.88 \times 10^{-6}$). *Smad3* expression was significantly decreased in response to fenofibrate in both REP1 ($q = 0.09$) and REP2 ($q = 0.19$). SMAD3 is a member of the SMAD family of genes and is an intracellular signal transducer and transcriptional modulator activated by transforming growth factor-beta (TGF- β), and binds to the promoter region of many genes regulated by TGF- β and activates them by forming a SMAD3/SMAD4 complex.³⁰⁻³² In a study by Tan *et al.*,³¹ SMAD3 knockout mice had lower plasma free fatty acid and glycerol, and reduced adiposity. The same study demonstrated that SMAD3 knockout mice had altered regulation of PPAR γ and PPAR β . Furthermore, PPAR α , the therapeutic target of fenofibrate, has been shown to inhibit TGF- β , which regulates SMAD2, SMAD3, and SMAD4 transcription factors.³³ Pathways involving PPAR α , TGF- β , and SMAD transcription factors are clearly convoluted and more research is needed to elucidate these relationships, and these results suggest that *SMAD3* may play a role in fenofibrate lipid response. Furthermore, in black subjects, SNP rs1653969 located in *FOXP1*, was associated with a poorer LDL response to fenofibrate ($P = 9.18 \times 10^{-7}$). *FOXP1* is a member of the forkhead box class of genes, which is a large family of transcription factors. Little is known about the role of FOXP1, but other FOX transcription factors (e.g., FOXO3a) have been shown to be impacted by fenofibrate treatment.³⁴ Importantly, expression of *Foxp1* was significantly decreased in both REP1 and

REP2 analysis, and additional research is needed to further elucidate the role of *FOXP1* in fenofibrate lipid response.

When the cohort was limited to white subjects only, there was a significant association between LDL and the lead SNP, rs140229040, which is located in the PBX homeobox 4 (*PBX4*) gene ($P = 3.66 \times 10^{-7}$). SNPs in this gene are part of a large region in linkage disequilibrium, and this region has been previously identified as being associated with LDL cholesterol.^{35–37} We reported this finding previously with a meta-analysis using the GOLDN cohort.¹² This gene was not available for follow-up in REP1 but was significantly decreased in liver tissue of mice exposed to fenofibrate in REP2 ($q = 0.12$). Interestingly, functional validation in the study by Holmen *et al.*³⁸ identified TM6SF2, which is in high linkage disequilibrium with *PBX4*, as being the gene functionally responsible for regulating LDL.

We also tested rare variants for associations with LDL, HDL, TG, and TC. Six unique genes (*POGZ*, *HSD17B13*, *HARS2*, *DCUN1D4*, *DUSP3*, and *MARCH3*) were significantly associated with TG. Gene expression changes for *DCUN1D4* was significantly altered in both REP1 and REP2, but with opposing directions ($q < 0.3$). Very little is known about the function of *DCUN1D4*, with studies mostly conducted in *C. elegans* and *S. cerevisiae*.³⁹ In addition to TG, *HSD17B13* was also associated with change in HDL in white subjects only ($q < 0.2$). Importantly, rare genetic variants in hydroxysteroid 17-beta dehydrogenase 3 (*HSD17B13*) were significantly associated with TG and HDL in white subjects ($q < 0.05$) and mice fed fenofibrate displayed a significant reduction in *Hsd17b13* gene expression when administered fenofibrate vs. vehicle control in REP2 ($q = 5.93 \times 10^{-4}$; Supplementary Table S6). *HSD17B13*, an isoform of 17 beta-hydroxysteroid dehydrogenase (17 β HSD), is highly expressed in the testis, and is also expressed in the liver.⁴⁰ However, other isoforms of 17 β HSD are expressed in many tissues. Unlike many of the other isoforms of 17 β HSD, only recently has the role of 17 β HSD13 become clear. Human fatty liver samples have shown that 17 β HSD13 is upregulated in lipid droplet fractions.⁴¹ Furthermore, 17 β HSD13 was significantly upregulated in the livers of both diabetic mice and mice fed high-fat diets, suggesting that 17 β HSD13 may play an important role in the pathogenesis of fatty liver in both mice and in humans and may also be relevant in diabetes. In the same study, overexpression of *Hsd17b13* in C57BL/6 mouse livers increased lipogenesis and lipid accumulation and overexpression of *17 β HSD13* increased lipid droplet formation in human cell lines.⁴¹ Interestingly, in the mouse model, overexpression of *Hsd17b13* did not increase plasma TG or TC levels.⁴¹ Although the results presented by Su *et al.*⁴¹ demonstrate a clear role of 17 β HSD13 in nonalcoholic fatty liver disease, the results presented here mark the first time that rare variant SNPs in 17 β HSD13 have been shown to impact the lipid lowering effects of fenofibrate.⁴¹ Additional research is needed to fully elucidate the relationship between 17 β HSD13 and fenofibrate lipid response. It is possible that *17 β HSD13* may become an important biomarker in precision medicine initiatives for more targeted treatment of fenofibrate.

These findings occurred in subjects with T2D treated with statins, which is more clinically representative than fenofibrate monotherapy, because fibrates are commonly prescribed with statin therapy.⁴² Furthermore, these associations were not observed in subjects treated with placebo and statin, lending support for these associations with fenofibrate drug response.

Although several GWAS findings were replicated in two functional studies, those studies were conducted using a mouse model that may not be applicable to human subjects, and not all genes with GWAS associations here were available for gene expression follow-up. Additionally, gene expression may not be the most relevant mechanism, as numerous ways exist for SNPs to impact drug response. Future studies will require larger cohorts and further functional work in relevant tissues to elucidate the pathways in which fenofibrate and PPAR α alter lipid concentrations.

We have identified novel common variants in black subjects located in several genes (e.g., *SMAD3* and *IPO11*) and rare variants (e.g., *HSD17B13*) that explain lipid variation in response to fenofibrate treatment in individuals with T2D treated with statins. These findings were further supported by changes in gene expression in mice and provide novel findings that explain variation in fenofibrate lipid response in individuals with T2D.

MATERIALS AND METHODS

Study participants

The ACCORD trial (clinicaltrials.gov-NCT00000620) was a double 2×2 factorial design, consisting of 10,251 recruited subjects with T2D and either a history of CVD or at least two known risk factors for CVD, such as documented atherosclerosis, albuminuria, dyslipidemia, hypertension, smoking, or obesity.⁷ Subjects were randomized to either intensive or standard glycemia treatment strategies (targeting HbA1c <6.0 vs. HbA1c between 7.0 and 7.9). Over 80% of subjects in the ACCORD study consented to being genotyped. There were 5,518 subjects who were further randomized to intensive vs. standard lipid management (fenofibrate vs. placebo, with all subjects on simvastatin). Each ACCORD participant provided written informed consent using procedures reviewed and approved by each clinical site's local institutional review board and based on a template provided by the study group that was approved and subsequently centrally monitored by the Coordinating Center and the National Heart, Lung, and Blood Institute (IRB: FWA00003429). Entry criteria and additional information about the lipid subtrial and patient selection are described in the Supplementary Material online. As in the prior ACCORD and GOLDN meta-analyses, fenofibrate lipid response was calculated as:

$$phenotype = \log_{10} \left(\frac{a}{b} \right)$$

where a is the pretreatment measurement of HDL, LDL, TC, or TG, and b is the on-treatment measurement of HDL, LDL, TC, or TG. After subsetting patients from the lipid subtrial based on consent, genotyping, drug response criteria, and quality control (see below) of DNAs extracted from these samples, the population for the current study included 1,264 subjects. These subjects included individuals that self-identified as white, black, Hispanic, Asian, and other.

Genotyping and quality control

Genomic DNA extraction and cell preparations are described in the Supplementary Material. Genomewide genotyping was performed in two independent laboratories on different platforms: 6,085 unique samples, composed of those ACCORD participants who consented to genetic studies conducted by any investigator, were genotyped at the University of Virginia on Illumina HumanOmniExpressExome-8 version 1.0 chips (set 1)⁴³; 8,174 unique samples, including the above 6,085 samples plus 2,089 samples from ACCORD participants who consented to genetic studies only if conducted by ACCORD investigators were genotyped at the University of North Carolina on Affymetrix Axiom Biobank1 chips (set 2). Additional information regarding the merging of set 1 and set 2, imputation, and quality control can be found in the Supplementary Material.

Covariate selection

Here, we take a combined approach to variable selection to address potential confounding variables. A substantial proportion of the cohort was taking lipid-lowering medications at the time baseline lipid measurements were taken (e.g., 63% were on a statin prior to entering the trial). Statin, additional concomitant medications, and nondrug covariates (e.g., age, gender, body mass index, and smoking status) were incorporated into the model, as previously described in Graham & Rotroff *et al.*⁴⁴ and is described in the Supplementary Material. A full list of covariate names and descriptions can be found in Supplementary Table S1.

Common variant analysis

Association between a phenotype, selected, and forced covariates, and a single common variant (MAF >3%) was tested with an additive genetic model using linear regression in the PLINK software for genotyped variants.⁴⁵ Imputed variants were tested using a linear regression model in the statistical programming language, R, where $g = p(Aa) + 2p(aa)$ is the dosage score computed from the posterior probabilities for genotypes *Aa* and *aa*.^{45,46} For SNPs that were only genotyped in set 1 subjects and were imputed in set 2 subjects, association tests results were combined by meta-analysis using PLINK.⁴⁵ Tables and figures specify whether each SNP association was genotyped, imputed, or meta-analyzed. The results from the common variant tests were considered statistically significant based on a $P < 5 \times 10^{-8}$ and $P < 1 \times 10^{-6}$ was considered the threshold for suggestive significance. To maximize the likelihood of finding genes expression altered by fenofibrate exposure, a more liberal threshold of $P < 1 \times 10^{-5}$ was used only for functional validation, as described below. Additional information regarding the common variant analysis can be found in the Supplementary Material.

Rare variant analysis

We implemented a suite of five rare variant tests that can be divided into two classes, burden and nonburden approaches, as previously described.⁴⁷ Burden tests collapse a set of rare variants from a gene into a single variable, which is then tested for association with a phenotype. However, simple burden tests do not account for the direction (positive or negative association) of a rare variant effect.⁴⁸ One nonburden rare variant test that allows for different directions and magnitudes of effects for each variant is the sequence kernel

association test (SKAT).⁴⁸ The balance between SKAT and burden tests was addressed using the optimal test, SKAT-O, which aims to optimize the combination of the two approaches. Gene annotations were performed using Ensemble (GRCh37.p13), which mapped the 232,678 rare variants (MAF \leq 3%) genotyped in set 1 subjects to 17,081 total genes. Subsequently, the combined *P* value was corrected for multiple comparisons with an false discovery rate (FDR) approach using the R package, *qvalue* (version 1.36.0) and $q < 0.2$ was considered to be statistically significant.⁴⁹ Additional details regarding the rare variant analysis implemented here can be found in Marvel & Rotroff *et al.*⁴⁷ and the Supplementary Material.

Placebo analysis

Study protocols for those receiving placebo in the lipid subtrial of ACCORD were the same as the fibrate arm of the trial, except that placebo was administered instead of fenofibrate. To confirm that the results were associated with fibrate and not placebo or statin, we conducted common and rare variant associations using the same analysis workflow, covariates, and models as described above for all races combined ($n = 1,336$), white ($n = 908$), and black subjects ($n = 186$). The results from the placebo analysis are included along with the fenofibrate results in Tables (1–3) and Supplementary Tables S14 and S15.

Mouse gene expression validation

We investigated gene expression changes in wild-type C57BL/6J mice administered fenofibrate compared with mice administered vehicle control, as described by Liu *et al.*²⁰ to provide additional validation for common and rare variant associations with fenofibrate lipid response identified (REP1). To maximize the likelihood of finding genes with expression changes due to fenofibrate exposure, we expanded the genes chosen for evaluation to include those with common variant associations $P < 1 \times 10^{-5}$, where the variants were annotated as being in genes according to the National Center for Biotechnology Information database using the *rsnps* package,⁵⁰ and genes with $q < 0.2$ in the rare variant tests. An additional follow-up replication study was conducted at the University of Kentucky (REP2) to try and further validate the findings in REP1 and include additional genes identified in the ACCORD analysis that were not available in the previously published data in REP1. Additional details regarding the mouse gene expression methods can be found in the Supplementary Material. Furthermore, the gene expression results of *Rab27b*, a gene identified in an interim analysis of only set 2 data, and was not significant after merging set 1 and set 2 data ($q > 0.2$) is presented in the Supplementary Material.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Fibrates are a class of drugs commonly prescribed to lower serum lipid levels; however, individual variation in response to fenofibrates has been observed and drivers of this variation are not well understood.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Here, we evaluate the association of common and rare genetic variants with variation in response to fenofibrate treatment in individuals with T2D.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ We demonstrate novel associations of common genetic variants in *SMAD3* and *IPO11* genes in black subjects, and rare variants in *AKR7A3* and *HSD17B13* in white subjects were associated with variation in fibrate lipid response. We then support these findings using gene expression in a mouse model.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

☑ Our findings highlight genetic variants in TGF- β and NRF2 signaling pathways that may influence fenofibrate effects on dyslipidemia in individuals with T2D. This insight could help to identify patients for more targeted treatment strategies or elucidate novel therapeutic targets.

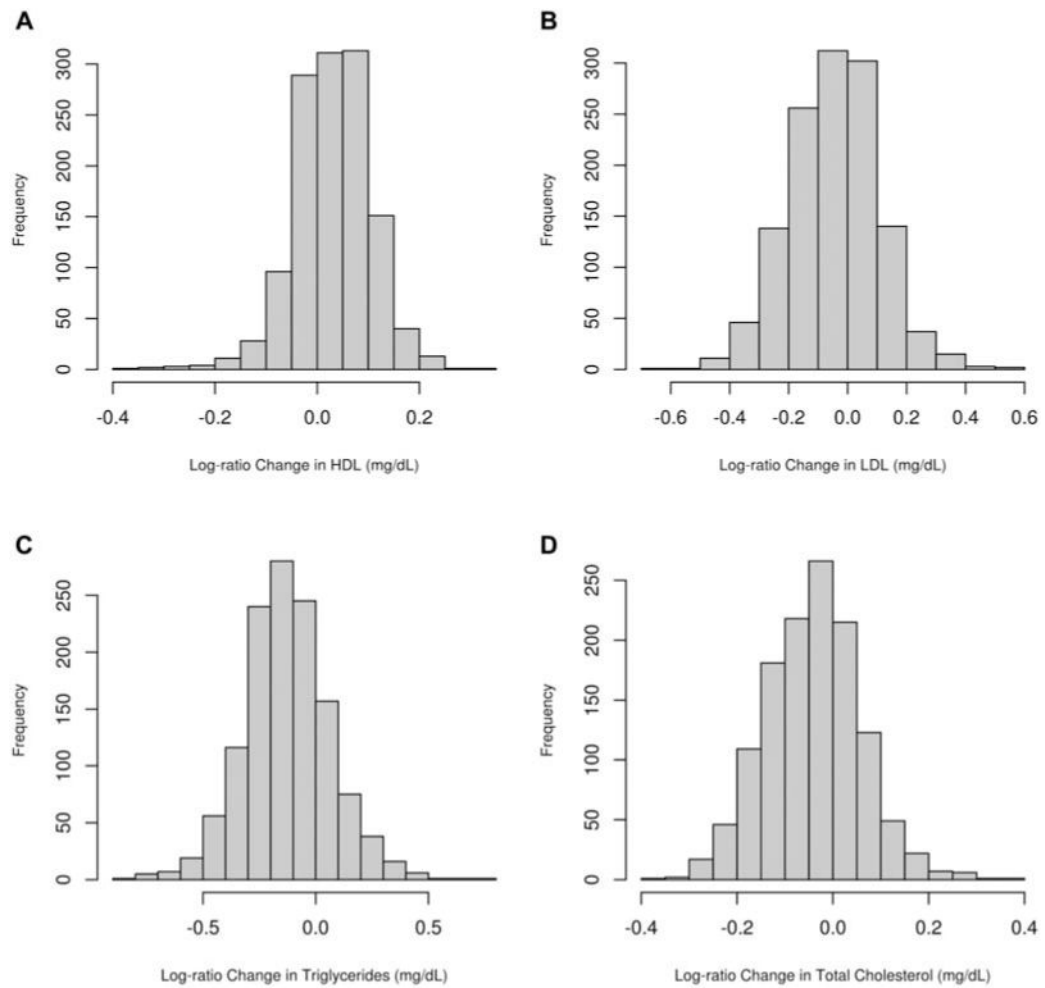


Figure 1. Distributions of fenofibrate response on lipid measurements in subjects of all combined races ($N=1264$). (a) Log-ratio of the change in high-density lipoprotein (HDL; mg/dL). (b) Log-ratio of the change in low-density lipoprotein (LDL; mg/dL). (c) Log-ratio of the change in triglycerides (mg/dL). (d) Log-ratio of the change in total cholesterol (mg/dL).

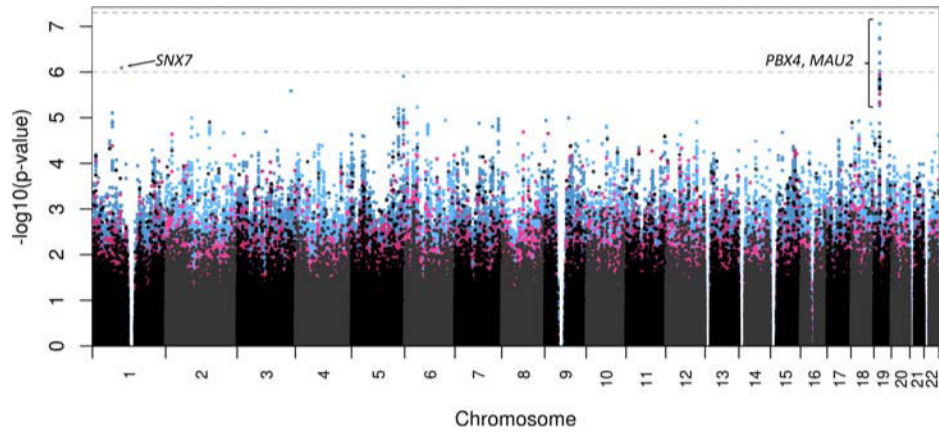


Figure 2. Manhattan plot of single-nucleotide polymorphism associations with change in low-density lipoprotein in white subjects only. [Color figure can be viewed at wileyonlinelibrary.com]

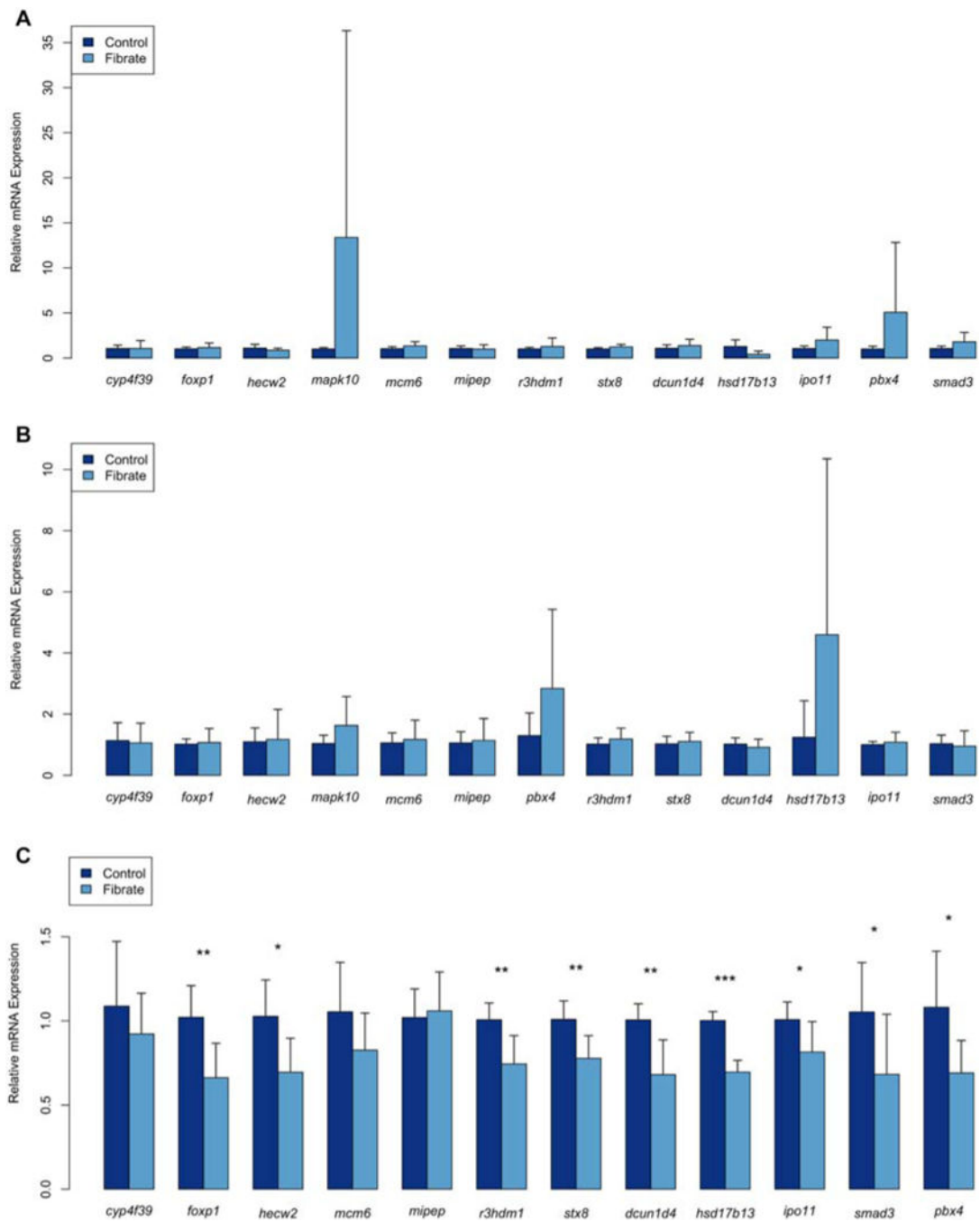


Figure 3. Gene expression in mice exposed to fenofibrate vs. vehicle control in (a) adipose tissue, (b) skeletal muscle, and (c) liver tissue. ***False discovery rate (FDR) P value < 0.01; **FDR P value < 0.1; *FDR P value < 0.2. [Color figure can be viewed at wileyonlinelibrary.com]

Table 1

Lead SNPs associated with LDL in common variant analysis ($P < 1 \times 10^{-6}$)

| Race | SNP | Chromosome | Position | Gene | Type | MAF | Beta | Fibrate P value | Placebo P value |
|-----------|--------------------------|------------|-----------|-------------------|------|-------|--------|-----------------------|-------------------|
| All races | rs73354145 | 17 | 81706392 | <i>MRPL12</i> | IMPU | 0.037 | 0.032 | 2.39×10^{-7} | 0.699 |
| All races | rs2607653 | 13 | 102066313 | <i>FGF14</i> | IMPU | 0.031 | 0.038 | 4.28×10^{-7} | 0.262 |
| Black | rs72804453 | 10 | 58827231 | <i>BICC1</i> | IMPU | 0.043 | 0.111 | 3.74×10^{-7} | 0.950 |
| Black | rs112284299 | 3 | 179850307 | <i>PEX5L</i> | IMPU | 0.036 | 0.107 | 7.02×10^{-7} | 0.197 |
| Black | rs1653969 | 3 | 71282212 | <i>FOXP1</i> | IMPU | 0.492 | 0.037 | 9.18×10^{-7} | 0.232 |
| White | rs150268548 ^a | 19 | 19383673 | <i>PBX4, MAU2</i> | IMPU | 0.075 | -0.028 | 8.73×10^{-8} | 0.567 |
| White | rs9285630 | 1 | 98677990 | <i>SXX7</i> | IMPU | 0.173 | -0.022 | 8.09×10^{-7} | 0.419 |

MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

^a rs150268548 is not located in a gene, but SNPs in the same peak ($P < 1 \times 10^{-6}$) are located in *PBX4* and *MAU2*.

Table 2

Lead SNPs associated with total cholesterol in common variant analysis ($P < 1 \times 10^{-6}$)

| SNP | Chromosome | Position | Gene | Type | MAF | Beta | Fibrate P value | Placebo P value |
|-------------|------------|-----------|---------------------|------|-------|--------|------------------------|-----------------|
| rs6693796 | 1 | 170674136 | <i>PRRX1</i> | IMPU | 0.044 | 0.019 | 6.23×10^{-8} | 0.040 |
| rs2607653 | 13 | 102066313 | <i>FGF14</i> | IMPU | 0.031 | 0.023 | 9.51×10^{-8} | 0.150 |
| rs7810240 | 7 | 150387371 | <i>ZNF775</i> | META | 0.461 | -0.015 | 1.99×10^{-7} | 0.711 |
| rs73354145 | 17 | 81706392 | <i>MRPL12</i> | IMPU | 0.037 | 0.018 | 2.66×10^{-7} | 0.801 |
| rs80333777 | 5 | 15524360 | <i>FBXL7</i> | IMPU | 0.032 | 0.020 | 4.76×10^{-7} | 0.021 |
| rs9472719 | 6 | 46183648 | | IMPU | 0.264 | 0.008 | 8.98×10^{-7} | 0.553 |
| rs72804453 | 10 | 58827231 | <i>BICC1</i> | IMPU | 0.043 | 0.078 | 7.61×10^{-11} | 0.648 |
| rs80147136 | 16 | 47050041 | | IMPU | 0.033 | 0.071 | 2.38×10^{-8} | 0.344 |
| rs34030778 | 8 | 1771327 | <i>CLN8</i> | GENO | 0.047 | 0.124 | 4.10×10^{-8} | 0.374 |
| rs181126208 | 1 | 236495209 | | IMPU | 0.051 | 0.066 | 6.88×10^{-8} | 0.016 |
| rs112284299 | 3 | 179850307 | <i>PEX5L</i> | IMPU | 0.036 | 0.065 | 1.09×10^{-7} | 0.544 |
| rs250567 | 16 | 23383077 | | META | 0.107 | 0.072 | 1.36×10^{-7} | 0.347 |
| rs141864436 | 2 | 50971651 | <i>NRXN1</i> | IMPU | 0.032 | 0.062 | 1.37×10^{-7} | 0.294 |
| rs10034465 | 4 | 34193881 | <i>LOC105374394</i> | META | 0.357 | 0.053 | 1.47×10^{-7} | 0.711 |
| rs113816795 | 5 | 99159793 | | IMPU | 0.032 | 0.072 | 1.71×10^{-7} | 0.487 |
| rs112209655 | 12 | 84947357 | <i>LOC102724680</i> | IMPU | 0.040 | 0.066 | 1.89×10^{-7} | 0.008 |
| rs117168171 | 15 | 101854598 | | IMPU | 0.033 | 0.066 | 2.43×10^{-7} | 0.615 |
| rs2823310 | 21 | 15497528 | | IMPU | 0.092 | 0.043 | 2.72×10^{-7} | 0.664 |
| rs143838781 | 12 | 113940538 | <i>RBM19</i> | IMPU | 0.035 | 0.061 | 3.56×10^{-7} | 0.571 |
| rs75298135 | 5 | 28184371 | | IMPU | 0.030 | 0.065 | 3.74×10^{-7} | 0.899 |
| rs75639901 | 6 | 25667802 | <i>SCGN</i> | IMPU | 0.077 | 0.039 | 5.08×10^{-7} | 0.617 |
| rs115990514 | 3 | 19055897 | | IMPU | 0.033 | 0.065 | 5.43×10^{-7} | 0.118 |
| rs138270994 | 13 | 22791996 | | IMPU | 0.055 | 0.045 | 6.23×10^{-7} | 0.387 |
| rs76043556 | 15 | 30060225 | | IMPU | 0.044 | 0.058 | 6.66×10^{-7} | 0.156 |
| rs58847779 | 22 | 37736643 | <i>TRIOBP</i> | IMPU | 0.077 | 0.039 | 6.77×10^{-7} | 0.503 |

| Race | SNP | Chromosome | Position | Gene | Type | MAF | Beta | Fibrate <i>P</i> value | Placebo <i>P</i> value |
|-------|-------------|------------|-----------|---------------------|------|-------|--------|------------------------|------------------------|
| Black | rs62210650 | 21 | 19649853 | <i>LOC105372744</i> | IMPU | 0.034 | 0.064 | 6.82×10^{-7} | 0.200 |
| Black | rs146807753 | 6 | 3904004 | | IMPU | 0.030 | 0.060 | 7.37×10^{-7} | 0.380 |
| Black | rs116616455 | 12 | 58041828 | | IMPU | 0.065 | 0.047 | 7.47×10^{-7} | 1.000 |
| Black | rs114007472 | 8 | 63563680 | | IMPU | 0.036 | 0.071 | 7.51×10^{-7} | 0.840 |
| Black | rs145010525 | 2 | 135975205 | <i>DARS</i> | IMPU | 0.033 | 0.058 | 8.20×10^{-7} | 0.968 |
| Black | rs4988198 | 2 | 0.0076 | <i>MCM6</i> | IMPU | 0.033 | 0.058 | 8.74×10^{-7} | 0.964 |
| Black | rs6847878 | 4 | 81419697 | | IMPU | 0.032 | 0.062 | 9.57×10^{-7} | 0.333 |
| Black | rs2631781 | 1 | 76128100 | <i>ST6GALNAC3</i> | IMPU | 0.041 | 0.051 | 9.66×10^{-7} | 0.181 |
| Black | rs115092681 | 3 | 174201322 | <i>NLGN1</i> | IMPU | 0.033 | 0.061 | 9.82×10^{-7} | 0.424 |
| White | rs7504626 | 19 | 19609589 | <i>PBX4</i> | IMPU | 0.092 | -0.014 | 6.22×10^{-7} | 0.641 |

MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

Table 3Results from rare variant analysis ($q < 0.2$)

| Phenotype | Race | Gene | Fibrate Lancaster <i>P</i> value | Fibrate <i>q</i> value | Placebo Lancaster <i>p</i> value | Placebo <i>q</i> value |
|-----------|-----------|-----------------|----------------------------------|------------------------|----------------------------------|------------------------|
| TG | Black | <i>POGZ</i> | 1.00×10^{-6} | 0.017 | 0.113 | 1 |
| HDL | White | <i>HSD17B13</i> | 2.15×10^{-6} | 0.037 | 0.445 | 1 |
| TG | White | <i>HARS2</i> | 3.09×10^{-6} | 0.053 | 0.726 | 1 |
| TG | White | <i>HSD17B13</i> | 4.42×10^{-6} | 0.075 | 0.534 | 1 |
| LDL | White | <i>AKR7A3</i> | 4.80×10^{-6} | 0.082 | 0.271 | 1 |
| HDL | White | <i>HARS2</i> | 7.09×10^{-6} | 0.121 | 0.327 | 1 |
| TG | All races | <i>DCUN1D4</i> | 7.46×10^{-6} | 0.127 | 0.322 | 1 |
| TG | All races | <i>DUSP3</i> | 9.20×10^{-6} | 0.157 | 0.142 | 1 |
| TG | White | <i>MARCH3</i> | 9.80×10^{-6} | 0.167 | 0.040 | 1 |
| TC | White | <i>AKR7A3</i> | 1.17×10^{-5} | 0.199 | 0.241 | 1 |

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.

Table 4
Replication of ACCORD common and rare variant results in REP1 and REP2 replication sets

| Gene symbol | Gene name | REP1 | | | REP2 | | |
|----------------------------|--|-------------|-----------------------|-------------|-------------------|-----------------------|-------------------------|
| | | Fold change | P value | FDR P value | Liver fold change | Liver FDR P value | Replication concordance |
| <i>Mcm6</i> | Minichromosome maintenance complex component 6 | 1.69 | 5.35×10^{-4} | 0.034 | -0.351 | 0.298 | ↔ |
| <i>Smad3</i> | SMAD family member 3 | -2.23 | 2.88×10^{-3} | 0.091 | -0.625 | 0.192 | ↔↔ |
| <i>Dcun1d4</i> | Defective in cullin neddylation 1 domain containing 4 | 1.47 | 0.013 | 0.194 | -0.563 | 0.065 | ↔ |
| <i>Hecw2</i> | HECT, C2, and WW domain containing E3 ubiquitin protein ligase 2 | 2.31 | 0.014 | 0.194 | -0.563 | 0.098 | ↔ |
| <i>Mipep</i> | Mitochondrial intermediate peptidase | 1.72 | 0.015 | 0.194 | 0.055 | 0.791 | ↑- |
| <i>Ipo11</i> | IMP 11 | -1.88 | 0.022 | 0.235 | -0.305 | 0.147 | ↔↔ |
| <i>R3hdm1</i> | R3H domain containing 1 | 1.51 | 0.030 | 0.270 | -0.436 | 0.065 | ↔ |
| <i>Foxp1</i> | Forkhead box P1 | -1.41 | 0.035 | 0.276 | -0.624 | 0.065 | ↔↔ |
| <i>Stx8</i> | Syntaxin 8 | 1.51 | 0.043 | 0.295 | -0.374 | 0.065 | ↔ |
| <i>Mapk10</i> | Mitogen-activated protein kinase 10 | -1.43 | 0.047 | 0.295 | NA | NA | ↓ |
| <i>Hsd17b13</i> | Hydroxysteroid 17-β dehydrogenase 13 | NA | NA | NA | -0.526 | 5.93×10^{-4} | ↓ |
| <i>Pbx4</i> | PBX homeobox 4 | NA | NA | NA | -0.646 | 0.122 | ↓ |
| <i>Cyp4f39^a</i> | Cytochrome P450, family 4, subfamily f, polypeptide 39 | NA | NA | NA | -0.237 | 0.539 | - |

FDR, false discovery rate; IMP-11, importin 11; NA, not applicable.

^a *Cyp4f39* is the murine homologue for human *CYP4F22*; ↑ statistically significant increase (FDR P value < 0.3); ↓ statistically significant decrease (FDR P value < 0.3), fold change not statistically significant (FDR P value = 0).