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A genome-wide study of lipid response to fenofibrate in Caucasians: A joint analysis of the GOLDN and ACCORD studies

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Abstract

Background—Fibrates are commonly prescribed for hypertriglyceridemia but also lower low-density lipoprotein cholesterol (LDL-C) and raise high-density lipoprotein cholesterol (HDL-C).

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Conflicts of interest: None declared

Large inter-individual variation in lipid response suggests that some persons may benefit more than others and genetic studies could help identify those persons.

Methods—We conducted the first genome-wide association study (GWAS) of lipid response to fenofibrate using data from two well characterized clinical trials, the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Study. GWAS data from both studies were imputed to the 1000 Genomes CEU reference panel (phase 1). Lipid response was modeled as the log ratio of the post-treatment lipid level to the pre-treatment level. Linear mixed models (GOLDN, N=813 from 173 families) and linear regression models (ACCORD, N=781) adjusted for pre-treatment lipid level, demographic variables, clinical covariates, and ancestry were used to evaluate the association of genetic markers with lipid response. Among Caucasians, results were combined using inverse-variance weighted fixed-effects meta-analyses. Top findings from the meta-analyses were examined in other ethnic groups from the HyperTG study (N=267 Hispanics) and ACCORD (N= 83 Hispanics, 138 African Americans).

Results—A known lipid locus harboring the pre-B-cell leukemia homeobox 4 (*PBX4*) gene on chromosome 19 is important for LDL-C response to fenofibrate (smallest p-value = 1.5×10^{-8}). Top results replicated with nominal statistical significance in Hispanics from ACCORD (p-value <0.05).

Conclusions—Future research should evaluate the usefulness of this locus to refine clinical strategies for lipid lowering care.

Keywords

genome-wide association study; GWAS; fenofibrate; triglyceride; cholesterol; lipid; lipoprotein; dyslipidemia

Introduction

Hypertriglyceridemia (defined as triglycerides (TG) ≥150 mg/dL) affects 30% of adults over the age of 20 years in the United States [1]. Many prospective studies implicate TGs as a risk factor for coronary heart disease (CHD) [2]. Despite the evidence, the etiological role of TGs in CHD risk has been a topic of considerable debate because of substantial inter-correlation between lipids, in particular high TGs and low high density lipoprotein cholesterol (HDL-C), challenging the premise that the observed TG-CHD risk accurately reflects the underlying biological processes [3]. However, recent insights from genetic studies convincingly relate lipid metabolism gene variants with TG levels and CHD risk [4, 5]. These studies add further support for a causal relationship of TG levels to CHD, fueling a resurging clinical interest in TG lowering therapies [6, 7].

Fibrates are a class of drugs commonly used to treat hypertriglyceridemia and mixed dyslipidemia. They activate nuclear transcription factor peroxisome proliferator-activated receptor alpha (PPARα) which is predominantly expressed in tissues that metabolize fatty acids, such as the liver, kidney, heart and muscle [8]. Upon activation, PPARα binds with retinoid X receptor (RXR) and the resulting dimer anneals to specific PPARα response elements in the genome, modulating expression of target lipid metabolism genes. Activated

PPARα ultimately upregulates plasma lipoprotein lipase (LPL) activity, raises apolipoprotein A-I and apolipoprotein A-II levels, and reduces apolipoprotein CIII, all of which enhance the clearance of circulating triglyceride-rich lipoproteins (TRLs). In addition, fibrates promote a shift in the density of low density lipoprotein cholesterol (LDL-C) particles towards larger, more buoyant particles that are less susceptible to oxidation and have increased affinity for the low-density lipoprotein receptor (LDL-R) [9-12]. Among the fibrate class, fenofibrate has demonstrated TG and LDL-C lowering effects in the majority of subjects, though the magnitude of response is highly variable (24-55% lowering for TGs and 6-35% lowering for LDL-C), whereas its effects on HDL-C are typically positive (8-38%).

Despite effective lipid lowering, studies have demonstrated both positive [13, 14] and mixed [15] results of fibrates in terms of cardiovascular disease (CVD) protection. The beneficial effects of fibrates to reduce the incidence of major cardiovascular events were particularly pronounced within the Veterans Affairs High-Density Lipoprotein Intervention Trial population, especially for patients with type 2 diabetes [16]. In contrast, evaluation of fenofibrate in patients with type 2 diabetes in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study [15] failed to demonstrate a positive outcome for the primary endpoint (CHD death or non-fatal myocardial infarction) and produced mixed effects for several of the secondary endpoints. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial of fenofibrate, which added fenofibrate to baseline simvastatin therapy among persons with type 2 diabetes, reported a statistically insignificant 8% reduction in cardiovascular events [17]. Reasons for the mixed conclusions are still being debated [18]; however, it is apparent that there are large inter-individual variations in TG and other lipid fraction responses to fibrate therapy [15]. Response to fenofibrate is heritable (39% and 29% for TG and LDL-C response, respectively in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN)) and several candidate gene studies of fenofibrate response have been conducted [19-23]. However, there are few data from genome-wide scans of lipid response to fenofibrate. In the current study, we conducted genome-wide association meta-analyses of ≈7 million variants in a combined sample of 813 Caucasians from GOLDN as well as 781 Caucasians from the ACCORD study for lipid response to fenofibrate.

Methods

Study Populations

The GOLDN study (N=1,327, clinicaltrials.gov-NCT00083369) was designed to identify genes that determine response of lipids to two interventions, one to raise (ingestion of high-fat meal) and one to lower lipids (fenofibrate treatment). The GOLDN study has been previously described in Irvin et al [24]. Briefly, the study ascertained and recruited families from the NHLBI Family Heart Study at two centers, Minneapolis, MN and Salt Lake City, UT. All of participants were self-reported to be white. Only families with at least two siblings were recruited and only participants who did not take lipid-lowering agents (pharmaceuticals or nutraceuticals) for at least 4 weeks prior to the initial visit were

included. A total of 861 GOLDN participants received open label, once daily 160 mg micronized fenofibrate for three weeks and were followed for treatment response.

The ACCORD Trial (N=10,251, clinicaltrials.gov-NCT00000620) was designed to determine the effects of intensive treatment of blood glucose vs. standard treatment (with a hemoglobin A1c goal of <6% vs. a hemoglobin A1c goal of 7% to 7.9%), and either blood pressure (ACCORD Blood Pressure) or plasma lipids (ACCORD Lipid), on atherosclerotic CVD outcomes in high risk patients with type 2 diabetes [25]. The ACCORD Lipid trial tested the hypothesis that the addition of fenofibrate to background statin treatment in patients with type 2 diabetes would further reduce CVD risk compared with statin treatment alone by decreasing TG and increasing HDL-C [26]. Patients were eligible to participate in the lipid trial if they had the following: LDL-C of 60 to 180 mg per deciliter (1.55 to 4.65 mmol per liter), HDL-C below 55 mg per deciliter (1.42 mmol per liter) for women and African Americans or below 50 mg per deciliter (1.29 mmol per liter) for all other groups, and TG levels below 750 mg per deciliter (8.5 mmol per liter) if they were not receiving lipid therapy or below 400 mg per deciliter (4.5 mmol per liter) otherwise. All patients provided written informed consent. A total of 5,518 men and women with type 2 diabetes were enrolled in the ACCORD Lipid trial. All participants received simvastatin (20–40 mg/day) and were randomly assigned to masked fenofibrate (160 or 54 mg/day, depending on renal function) (N=2,765) or placebo (N=2,753) one month after initiation of simvastatin. Of the 2765 participants randomized to fenofibrate in the ACCORD lipid trial, genotype information was available for 2229 participants. Additional exclusions for the discovery analysis included self-reported race other than white (N=730), treatment with fenofibrate at baseline (N=77), self-reported fenofibrate compliance criteria not met (described below) (N=516), missing lipid levels or other clinical measurements (N=21), and missing concomitant medication information (N=104); 781 eligible participants were included in the discovery analysis. For a post hoc sensitivity analysis, we further stratified the sample to only include patients that were on statins at baseline (~60%) so that we could test to make sure that the genetic associations held in the absence of concomitant statin initiation.

Clinical Data and Lipid Measurements

Clinical lipid measurements (TG, HDL-C, LDL-C, and total cholesterol) pre and post 3 weeks of fenofibrate treatment in GOLDN have been described [27]. In ACCORD, clinical lipid levels were evaluated at a central laboratory at baseline, and on average, 4 months later using standardized protocols. Extensive demographic, lifestyle, medical history and clinical data collected during the trial by trained staff have been described [25].

Fenofibrate response

In GOLDN, fenofibrate response was defined as post-treatment/pre-treatment ratios of plasma concentrations for HDL-C, LDL-C, total cholesterol and TGs. The number of pills taken over the three week treatment period was recorded and the average number of pills taken per day was derived as the number of pills taken / number of days and used as an indicator of compliance. Subjects in the ACCORD lipid trial that were not already treated with a statin were started on simvistatin at baseline. Subjects in the fenofibrate arm of the ACCORD lipid trial started fenofibrate approximately one month into the trial. Pre-

treatment lipid levels were recorded at baseline (no more than 60 days prior to the start of fenofibrate). Post-treatment lipid levels were acquired after at least 90 days and no more than 120 days from the start of fenofibrate treatment. Similarly to GOLDN, fenofibrate response was calculated as the ratio of the pre-treatment and post-treatment lipid measurements. Only compliant subjects were included (i.e., participants maintained compliance on fenofibrate for 90 (+/- 15) consecutive days, and maintained 100% compliance ≥ 80% of the recorded visits). Any record of complete non-compliance during the 90-day time frame excluded the subject from the selection. If compliance was not recorded during the post-treatment visit, the first compliance recorded post-treatment was carried backward to fill the missing compliance record.

Genotype Data

In GOLDN, a total of 906,600 single nucleotide polymorphisms (SNPs) were genotyped using the Affymetrix Genome-Wide Human 6.0 array and the Birdseed calling algorithm [28]. After quality control exclusions, 584,029 genotyped SNPs remained as described by Aslibekyan et al. [28]. A two-stage procedure for imputation was used during which data was first prephased using MACH and subsequently imputed using MINIMAC with the 1000G Phase I v3 Shapeit2 Reference (2010-11 data freeze, 2013-09 haplotypes) panel (http://www.sph.umich.edu/csg/abecasis/MACH/download/1000G.2013-09.html) with singletons and monomorphic sites removed [29]. The imputation yielded, a genotype dataset consisting of 27.5 million variants. Markers with R^2<0.1 and minor allele frequency (MAF)<3% were removed for a final count of 9,003,514 million variants (of which 777,279 were indels). GOLDN participants were excluded from the analysis if they were missing outcome data or genotype data, yielding N= 813 for the single marker analyses. In ACCORD, initial genotypes were subjected to quality control to account for duplicate concordance, Mendelian segregation (in HapMap trios included on the genotyping plates), Hardy-Weinberg Equilibrium, and predicted gender. Cryptic relatedness was identified using KING (v1.4), and one member of each pair with a kinship coefficient $>\left(\frac{1}{2}\right)^{\frac{5}{2}}=0.1768$ was removed from the analysis data set [30]. The cleaned dataset consisted 386,212 probes after excluding variants with MAF<3%. Probes significantly deviating from HWE (χ^2 > 19.51, p-value < 10^{-5}) in at least two of the three main ethnic subgroups were excluded from the imputation process. The remaining untyped genotypes were prephased using SHAPEIT2 (v2.r778) [31, 32] and imputed using IMPUTE2 [33] to the same 1000G reference panel as described for GOLDN for a total of 26,862,499 imputed variants (of which 1,335,851 were indels). ACCORD markers with R^2<0.1 and MAF<3% were removed for a final count 7,052,236 million variants (of which 656,466 were indels).

Genome Wide Association Study (GWAS) Analysis

Log-transformations were carried out for each outcome in GOLDN to achieve normality of residuals. The SNP associations of interest were assessed using linear mixed models, adjusted for the pre-treatment lipid level, sex, age, and center as fixed effects, and a kinship coefficient considered as a random effect to adjust for family relatedness using the *lmekin* function in R. The additive assumption was used to model genotypic effects. Population

substructure was assessed using principal components (PCs) generated using EIGENSOFT 3.0 and found to be limited in the GOLDN data. Any of 10 PCs from EIGENSOFT and/or a variable called pills per day (to adjust for compliance) were included in the model for a particular phenotype if p-value <0.05 after backward selection. The pills per day variable was included in the model for both TG and HDL-C response. The third PC was included in the model for LDL-C.

Each lipid ratio outcome was log transformed in ACCORD. Covariates including pre-treatment lipid level (i.e. LDL-C, HDL-C, TG, or total cholesterol), and study arm (intensive/standard glycemia treatment) were forced into the models. Additional covariates were made available via backwards-selection into the linear model. These covariates included: baseline age, gender, BMI, PC1-10, number of years with diabetes, number of years with dyslipidemia, smoking, education, glomerular filtration rate, diastolic blood pressure, systolic blood pressure, waist size (cm), network, fasting plasma glucose, alcohol consumption, and many concomitant medications. Concomitant medications (antihypertensive, glycemia and other lipid lowering) were scored according to exposure timing in relation to the pre and post fenofibrate lipid measurement (eg. exposed pre-fenofibrate but stopped medication prior to the post-fenofibrate lipid measure). For a complete description of the concomitant medication score as well as scores retained in lipid response models see the Supplemental Methods (Supplemental Digital Content 1). PCs based on the genotype data were computed using EIGENSTRAT (v4.2), similar to the methods described for GOLDN, and were used to control population stratification [34].

Backwards selection by Bayesian information criteria retained the following covariates when white subjects were analyzed: TG- statin concomitant medication score and smoking (never vs. former vs. current); LDL-C- statin concomitant medication score, duration of diabetes in years, duration of dyslipidemia in years, and gender; HDL-C- PC3; total cholesterol- statin concomitant medication score, duration of diabetes in years, and duration of dyslipidemia in years. Analysis for genotyped SNPs was carried out using PLINK v1.07, and R using *lm* for imputed variants, under an additive genetic model.

Meta-Analysis

Inverse-variance weighted fixed-effects meta-analyses were carried out on the GOLDN and ACCORD cohort data using METAL (www.sph.umich.edu/csg/abecasis/metal/). After meta-analyses, results were filtered to remove SNPs and indels that were missing in either dataset for a total of 6,982,258 variants in common between the two studies (of those, 650,503 were indels). Statistical heterogeneity was evaluated using Cochrane's χ^2 test (Q-test). P-values $<5\times10^{-8}$ indicated genome-wide significant results.

Pathway Analysis and other functional annotation analysis

Functional annotation analysis using QIAGEN's Ingenuity® Pathway Analysis tool (IPA® , QIAGEN Redwood City, www.qiagen.com/ingenuity) was carried out for each lipid response phenotype. We annotated the entire meta-analysis results set for each phenotype to a gene where possible (all extra-genic variants were annotated to the nearest gene within 200 kb; variants >200 kb from a gene were not included in the pathway analysis). The top 200K

genes were uploaded into the tool for each lipid. We also used the UCSC genome browser (https://genome.ucsc.edu) and the Broad Institute's HaploReg tool v4.1 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) to provide further functional evidence from ENCODE and other sources in the region of our statistically significant findings. Finally, we compared our top results with those published by the Global Lipids Genetics Consortium (http://csg.sph.umich.edu//abecasis/public/lipids2013/) [35].

Replication Cohorts

HyperTG recruited 350 Hispanic-American participants from Los Angeles, CA for a pharmacogenetic study of the response to fenofibrate (160 mg per day for 8 weeks). Of the 350 subjects, 267 had available clinical lipid and genotype data (Illumina Metabochip) relevant for the replication phase of this study. Similarly to GOLDN, fenofibrate response after 4 weeks of treatment was defined as the log ratio of post-treatment/pre-treatment lipid level. Linear mixed models were fit to evaluate the associations between variants (under an additive genetic model) identified in GOLDN/ACCORD and the outcomes, adjusted for sex, age, PCs and pedigree as a random effect.

Only ACCORD participants with self-reported Caucasian race were included in the discovery meta-analysis constituting 70% of the ACCORD population. African American and Hispanic participants comprised the majority of the remaining subjects and were used as an additional replication arm for this study. Using the same criteria described for Caucasians a total of 83 Hispanics and 138 African Americans qualified for this analysis. Parallel statistical methods were used to evaluate the association of top findings separately in each ethnic group. Information regarding covariates selected into the models for Hispanic and African American strata are available in the Supplemental Methods (Supplemental Digital Content 1).

Results

Baseline characteristics and lipid fenofibrate response for GOLDN and ACCORD are presented in Table 1. On average, the ACCORD cohort was older and had fewer female participants than GOLDN. By design, the ACCORD cohort was diabetic, while the prevalence of diabetes in GOLDN was representative of the US population at large (~10%) [36]. At baseline, ACCORD participants had higher mean TG levels and lower HDL-C levels compared to GOLDN. Mean LDL-C and total cholesterol concentrations at baseline were higher in GOLDN as compared to ACCORD, likely reflecting the use of statins in ACCORD at baseline among a substantial portion of participants (~60% in ACCORD vs. 0% in GOLDN). Overall, following 3 weeks of fenofibrate treatment in GOLDN and on average 3 months of fenofibrate treatment in ACCORD clinically meaningful lipid changes were observed in both intervention studies.

The most significant findings from the discovery meta-analyses of GOLDN and ACCORD are listed in Table 2. The Manhattan plots summarizing the results of the genome-wide meta-analysis for TG, LDL-C, HDL-C and total cholesterol are shown in Figure 1 panels a-d, respectively. No SNP or indel was significantly associated with TG, HDL-C or total cholesterol. Six unique variants on chromosome 19 in a cluster of genes including ATPase

type 13A1 (*ATP13A1*), pre-B-cell leukemia homeobox 4 (*PBX4*) and nearby MAU2 chromatid cohesion factor homolog (*MAU2*) (~250 kb upstream of *PBX4*) were statistically significantly associated with LDL-C response to fenofibrate (p-value $<5.0*10^{-8}$). A regional plot for this ~300kb region is shown in Figure 2. The top finding for TG response (rs73199626) was on chromosome 3 in between small ILF3/NF90-associated RNA I (*SNAR-I*) and osteocrin (*OSTN*) (p-value $=8.5*10^{-7}$). The top finding for HDL-C (rs62041965) was located on chromosome 16 between WW domain containing oxidoreductase (*WWOX*) and v-maf musculoaponeurotic fibrosarcoma oncogene (*MAF*) with p-value $=2.6*10^{-7}$. We also observed a dense peak for HDL-C response on chromosome 15 in or near tumor necrosis factor, alpha-induced protein 8-like 3 (*TNFAIP8L3*) with the smallest p-value $=4.5*10^{-7}$ for rs148486743. Another peak for HDL-C response was on chromosome 18 near mitochondrial protein NADH dehydrogenase (ubiquinone) flavoprotein 2 (*NDUFV2*) with smallest p-value$=7.4*10^{-7}$. The top findings for total cholesterol were in the G protein-coupled receptor 20 (*GPR20*) with smallest p-value $1.1*10^{-6}$. For a complete list of results from METAL with p-value$<1*10^{-6}$ for each lipid response please see Supplemental Spreadsheet (Supplemental Digital Content 2). The results of the sensitivity analysis among the statin stratified samples from ACCORD Caucasians further confirmed the stability of the results. *P*-values were consistent in their order of magnitude and the estimated beta values were identical out to two decimal places (data not shown).

One SNP from Table 2 was found on the Metabochip and passed QC in the HyperTG study (rs73004962). However, that SNP did not replicate for LDL-C response (p-value>0.05). Three results presented in Table 2 approached statistical significance (p-value ~0.05) in African Americans from ACCORD for LDL-C (rs150268548, rs73001065, and rs140868651) in the region of *MAU2* (see the Supplemental Table, Supplemental Digital Content 3). However, for each variant the direction of effect was opposite of that reported for Caucasians and the frequency of the effect allele was smaller (~3.5% vs. ~8%). Variants in *PBX4* (rs73004959, rs73004962, rs57504626) were marginally associated with LDL-C response in Hispanics from ACCORD ($0.02< p$-value <0.045) with the same direction of effect and similar effect allele frequency (see the Supplemental Table, Supplemental Digital Content 3). No SNPs for TG, HDL-C or total cholesterol response from Table 2 were replicated even with nominal p-value in other ethnic groups from ACCORD. We also searched for additional replication signals located within 500 kb of index variants listed in Table 2 for LDL-C response in ACCORD African Americans and Hispanics, filtering out variants with imputation quality <0.3, R^2 with the index variant in Table 2 <0.5, and MAF<1%. Results for ACCORD Hispanics and African Americans are presented in two Supplemental Tables (Supplemental Digital Content 4 and 5, respectively). Two SNPs in the region of *MAU2-PBX4* (rs76244467 and rs74756308) were associated with LDL-C response to fenofibrate in Hispanics with p-value $<7.0*10^{-3}$. One SNP (rs145535422) meeting the described criteria was marginally associated with LDL-C response among blacks. Those SNPs were rarer than the index variants identified in the meta-analysis between ACCORD and GOLDN Caucasians.

Top pathways from the Qiagen's Ingenuity Pathway Analysis tool for each response phenotype are provided in Supplemental Digital Content 6. We found the top pathway for 3

of the 4 phenotypes is a neurological pathway. The second most significant pathway for LDL-C response was PPAR/RXR activation and, importantly, fenofibrate activates this gene dimer. This PPAR/RXR pathway was also statistically significant for cholesterol and TG response ($\leq 3.76 \times 10^{-3}$). Supplemental Figure 1 (Supplemental Digital Content 7) summarizes annotation results on chromosome 19 in the region of *PBX4* output from the genome browser. There are several transcription factor binding sites in this gene-rich region as evidenced by Transcription factor CHIP-Seq analysis and HMR Conserved Transcription Factor Binding Sites. Importantly, a binding site for PPARA can be found in the 3′ region of *GATAD2A*. Two clinically associated SNPS are also (rs137852869 and rs267605377, both for cancer types). Examination of this region using the Broad Institute HaploReg tool v4.1 http://www.broadinstitute.org/mammals/haploreg/haploreg.php) provided further evidence of protein binding sites, motif changes, NHGRI GWAS hits and expression QTLs. We have provided the results for each of the statistically significant LDL-C variants in Supplemental Digital Content 8. Notably, a SNP (rs58434384) in tight LD with our top finding changes a motif for PPAR binding with *ZNF101*.

Discussion

Whether fenofibrate treatment prevents CVD in high risk populations remains a topic of debate in the medical community. It is suspected that large inter-individual variation in lipid fraction response to fenofibrate has contributed to mixed results for CVD prevention reported by clinical studies. Since there is evidence that fenofibrate treatment may offer cardioprotective effects for some individuals, research focused on identifying treatment responsive persons is a worthy pursuit. To the best of our knowledge, the role of genetic factors in fenofibrate response has only been considered at the candidate gene level, making this the first genome-wide study of treatment response using data collected on 1600 participants from two well characterized clinical trials. Overall, our findings support a role for a known lipid locus on chromosome 19 in the region of *PBX4*. Further validation is needed to help confirm the importance of this region for fenofibrate response across racial groups in order to determine whether markers in the region could be useful for lipid lowering treatment strategies in the future.

Several SNPs and indels in or near *MAU2*, *PBX4*, and *ATP13A1* are listed in Table 2. Three of the SNPs replicated with marginal significance in a small group of Hispanics from ACCORD. No markers from Table 2 replicated in African Americans from ACCORD. Expansion of replication in the region surrounding the index SNPs listed in Table 2 among ACCORD Hispanics further suggests this locus may be important for LDL-C fenofibrate response (see Supplemental Table, Supplemental Digital Content 4). SNPs in or near *PBX4* and nearby *ZNF101* have been associated with fasting lipids and adiposity traits in the GWAS literature [37-42]. Three of the statistically significant results for LDL-C from Table 2 were significantly associated with fasting LDL-C according to the latest published results from the Global Lipids Genetics Consortium (rs17217098, rs73004967, rs73001065 $P \leq 3.6*10^{-24}$) [35]. Other published work in GOLDN has suggested variants that are associated with fasting lipids in large meta-analyses may be important for lipid response to fenofibrate [19]. This region covers ~ 13 genes and ~300kb (*SUGP1, TM6SF2, MAU2, GATAD2A, TSSK6, NDUFA13, YJEFN3, CILP2, PBX4, LPAR2, GMIP, ATP13A1,*

ZNF101) and finding a causal variant remains challenging (Figure 2). One recent study reported a nonsynonymous variant in *TM6SF2* influences total cholesterol levels and is associated with myocardial infarction. The authors also reported both *TM6SF2* overexpression and knockdown in mice altered serum lipid profiles [43]. There are far fewer pharmacogenetic studies of this locus. To our knowledge, only one study examined the association of this region with LDL-C response to statin and reported no association among 895 men with dyslipidemia and 672 normolipidemic controls [44] This locus was also not associated with LDL-C response to statin among Caucasians from the placebo arm of ACCORD (i.e. participants not treated concomitantly with fenofibrate, data not shown). A SNP (rs10401969) in this region in the cartilage intermediate layer protein 2 (*CILP2*) gene (located between *MAU2* and *PBX4*) was marginally linked to LDL-C and total cholesterol fenofibrate response in a candidate gene study in GOLDN (with the smallest p-value = 0.03) [19]. Given the strength of association of variants in the region with LDL-C response this locus is promising, supporting further validation and regional sequencing efforts in search of a causal locus. Annotation of this region using freely available bioinformatic tools point to PPAR binding sites that may help prioritize follow-up studies.

All of our statistically significant findings were for LDL-C response to fenofibrate. This may be due to the heterogenous effect fenofibrate has on TG lowering and HDL raising mechanisms such as increased TRL lipolysis, induction of hepatic fatty acid uptake, reduction of hepatic TG production, increased production of HDL-C precursors (Apo A-I and Apo A-II), and stimulation of reverse cholesterol transport making an effect of individual genetic factors difficult to dissect [45]. Nonetheless, we observed at least one interesting, marginally significant peak for TG response between *SNAR-I* and *OSTN* on chromosome 3. *OSTN* is associated with ostioblastic differentiation, but it is also expressed in skeletal muscle and fat and has been linked to glucose and lipid metabolism [46]. We found markers near genes linked to inflammation (*TNFAIP8L3*) and mitochondrial function (*NDUFV2)* marginally associated with HDL-C response to fenofibrate. Pathway analysis did not provide substantial further insight into our results. Still many of the top findings could be biologically plausibly linked to lipid fenofibrate response and should be considered in further studies.

This study has several strengths and limitations, and there are some notable differences between the GOLDN and ACCORD populations. Overall, GOLDN represents a healthy population and the participants were required to discontinue all lipid lowering medications prior to initiating monotherapy with fenofibrate. In contrast, the ACCORD clinical trial aimed to evaluate the additional cardioprotective effect of adding fenofibrate to baseline statin treatment among persons with type 2 diabetes, and the majority of ACCORD participants were on statin prior to starting the trial. We used statistical approaches help overcome the potential for confounding by baseline statin use (including a sensitivity analysis where ACCORD participants were treatment naïve to fenofibrate but not statin at baseline) and other concomitant medication use in ACCORD. The lack of association of the top variants on chromosome 19 with LDL-C response to statin in the absence of fenofibrate in ACCORD helps support our statistical approach. Despite these notable differences the discovery populations were both Caucasian, GWAS data were imputed to the same 1000 genomes reference panel and clinically significant changes in lipids were observed after

initiating fenofibrate treatment. Finally, we observed only marginal replication among Hispanics from ACCORD for index SNPs in Table 2 in or near *MAU2* and *PBX4*. Variants listed in Table 2 did not replicate in Hispanics from the HyperTG study or African Americans from ACCORD. This may be due to small sample size in these other race groups considered. Casting a wider net for variants near (<500 kb away) our index variants (with R^2>0.5 for LD) found additional markers more strongly associated with LDL-C response in ACCORD Hispanics and African Americans lending additional support for our findings and highlighting the continued need for studying ethnic groups separately to discover fenofibrate response variants (see two Supplemental Tables, Supplemental Digital Content 3 and 4).

Clinical data demonstrating the cardiovascular benefit of lipid lowering with the drug fenofibrate have shown mixed results. To date the drug has largely fallen out of clinical favor. However, wide variability in lipid response has been demonstrated, suggesting the drug may provide some benefits in specific persons or subgroups. Genetic factors predicting favorable lipid altering response to fenofibrate represent prime candidates for understanding these individual differences and thus provide an opportunity for possible pre-emptive genotyping to guide clinical decisions related to individualizing drug selection. Herein, we took a genome-wide approach representing data on ~ 1600 people. Overall, our results demonstrate a known lipid locus is associated with LDL-C response to fenofibrate. Future studies should consider whether this well known locus on chromosome 19 is useful to determine the best lipid lowering treatment regimen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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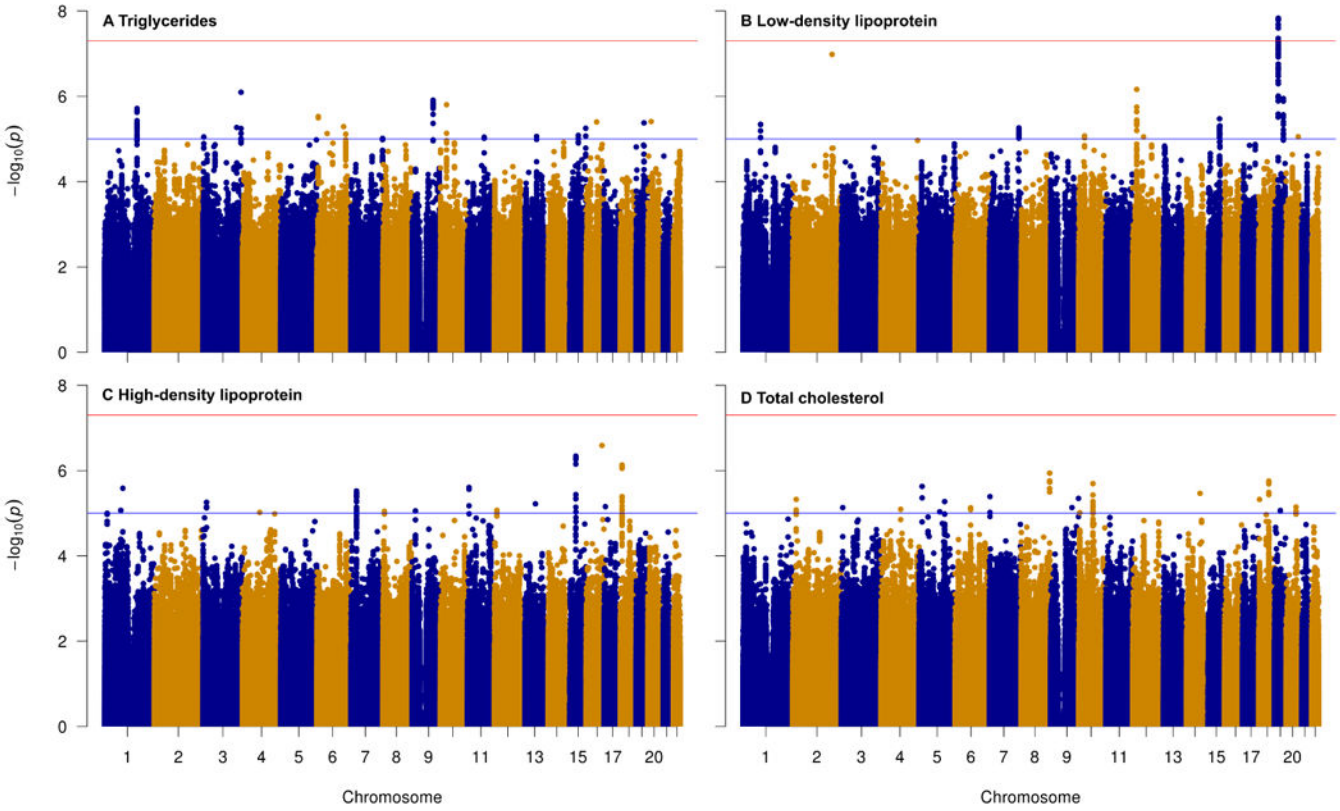

Figure 1.

Manhattan Plot of Meta-Analysis P-values derived from the discovery in GOLDN and ACCORD Caucasians for triglycercide (a), LDL-C (b), HDL-C (c) and total cholesterol (D) response to fenofibrate

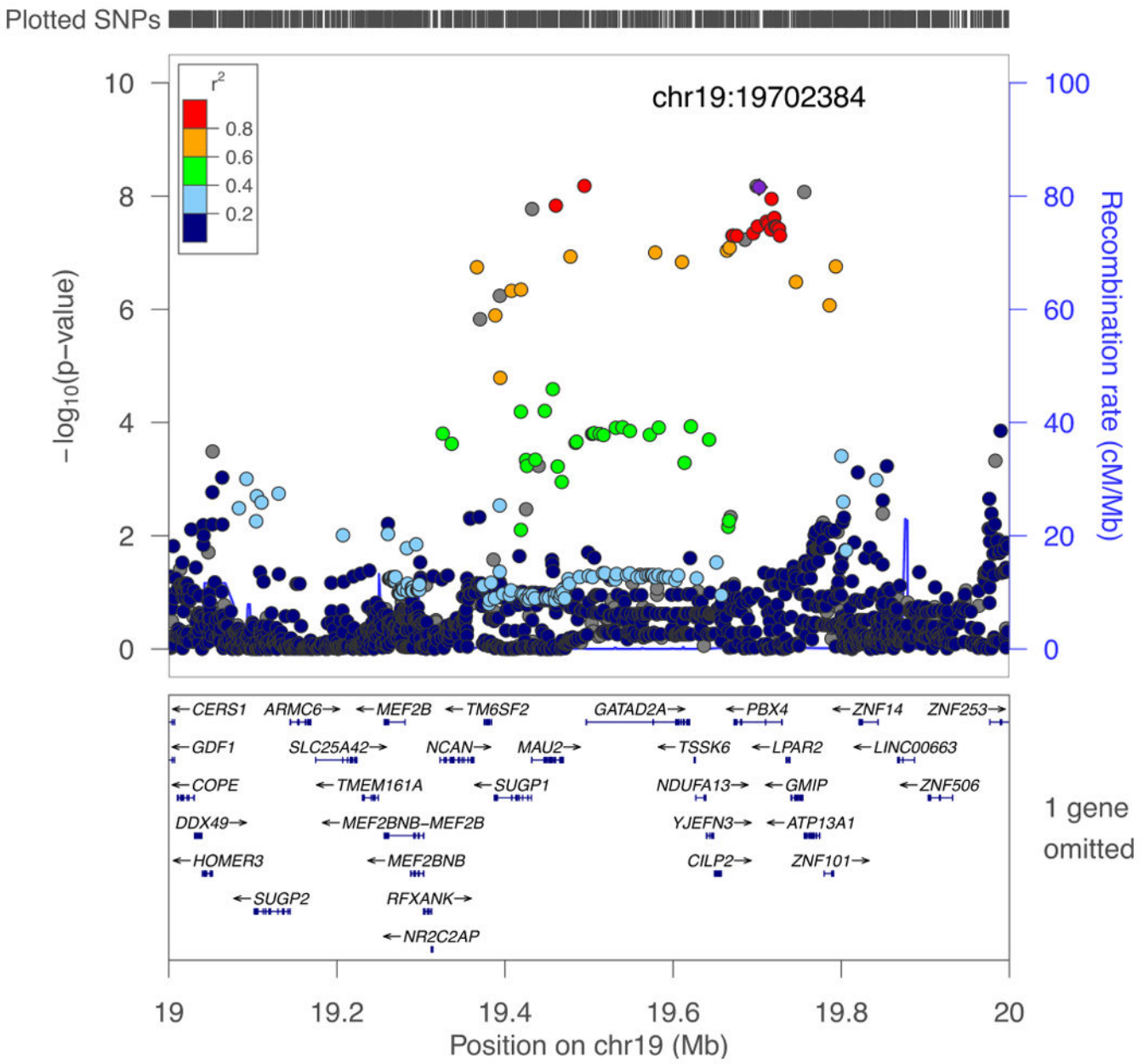

Figure 2. Regional plot of GOLDN/ACCORD top SNVs on chromosome 19

Table 1

Baseline characteristics and lipid response to fenofibrate in GOLDN and ACCORD studies

	GOLDN	ACCORD
Variable (mean ± SD or %)	N=820	N=781
Age	49±16	63.6±6.3
Sex % Female	50	28
Current Smoker %	8	13
BMI, Kg/m2	28±6	33±5
Diabetes (%)	7.5%	100%
Triglycerides, mg/dL		
Baseline	139.9±98.97	196.82±104.57
After fenofibrate treatment	92.91±57.99	144.77±83.68
Low density lipoprotein, mg/dL		
Baseline	118.53±32.57	99.40±29.53
After fenofibrate treatment	104.92±31.34	90.08±24.42
High density lipoprotein, mg/dL		
Baseline	45.02±13.62	37.23±7.41
After fenofibrate treatment	49.16±13.33	40.34±9.63
Total Cholesterol, mg/dL		
Baseline	191.71±39.83	174.97±36.24
After fenofibrate treatment	167.16±34.71	158.83±30.74

Table 2

Top findings from the discovery meta-analysis of lipid response to fenofibrate in GOLDN and ACCORD

Statistically significant findings								GOLDN				ACCORD				Meta
SNP	**Chr**	**BP**	**A1**	**A2**	**Gene**	**Location**	**Trait**	**FRQ**	**Beta**	**SE**	**P**	**FRQ**	**Beta**	**SE**	**P**	**P**
rs3841260	19	19756073	A	AGCC	*ATP13A1*	UTR3	LDL-C	0.08	-0.067	0.02	$1.2*10^{-3}$	0.08	-0.323	0.07	$1.8*10^{-6}$	$1.5*10^{-8}$
rs140229040	19	19699398	G	GA	*PBX4*	intronic	LDL-C	0.07	-0.058	0.02	$3.4*10^{-3}$	0.07	-0.353	0.07	$3.7*10^{-7}$	$1.6*10^{-8}$
rs17217098	19	19702384	A	G	*PBX4*	intronic	LDL-C	0.07	-0.058	0.02	$3.4*10^{-3}$	0.07	-0.352	0.07	$3.8*10^{-7}$	$1.6*10^{-8}$
rs150268548	19	19494483	A	G	*MAU2/GATAD2A*	intergenic	LDL-C	0.08	-0.050	0.02	$9.2*10^{-3}$	0.08	-0.363	0.07	$8.7*10^{-8}$	$2.0*10^{-8}$*
rs73004967	19	19717056	G	A	*PBX4*	intronic	LDL-C	0.07	-0.058	0.01	$3.5*10^{-3}$	0.07	-0.342	0.07	$6.4*10^{-7}$	$2.5*10^{-8}$
rs73001065	19	19460541	C	G	*MAU2*	intronic	LDL-C	0.07	-0.050	0.02	$1.0*10^{-2}$	0.07	-0.367	0.07	$1.8*10^{-7}$	$4.3*10^{-8}$*
Top findings																
rs140868651	19	19432290	A	AG	*MAU2*	intronic	LDL-C	0.07	-0.049	0.01	$1.2*10^{-2}$	0.07	-0.367	0.07	$1.9*10^{-7}$	$5.1*10^{-8}$*
rs57504626	19	19720399	T	C	*PBX4*	intronic	LDL-C	0.09	-0.051	0.02	$5.0*10^{-3}$	0.09	-0.300	0.06	$1.1*10^{-6}$	$5.4*10^{-8}$+
rs73004959	19	19711139	T	C	*PBX4*	intronic	LDL-C	0.09	-0.051	0.02	$5.0*10^{-3}$	0.09	-0.301	0.06	$1.1*10^{-6}$	$6.2*10^{-8}$+
rs73004962	19	19713069	T	A	*PBX4*	intronic	LDL-C	0.09	-0.051	0.02	$5.0*10^{-3}$	0.09	-0.297	0.06	$1.3*10^{-6}$	$6.7*10^{-8}$+
rs73199626	3	190888563	C	T	*SNAR-I/OSTN*	intergenic	TG	0.14	-0.084	0.02	$4.7*10^{-4}$	0.17	-0.220	0.06	$4.9*10^{-4}$	$8.1*10^{-7}$
rs10990032	9	105116201	A	G	*GRIN3A/CYL2*	intergenic	TG	0.06	-0.155	0.04	$5.4*10^{-5}$	0.06	-0.271	0.09	$4.9*10^{-3}$	$1.2*10^{-6}$
rs62041965	16	79551227	A	G	*WWOX/MAF*	intergenic	HDL-C	0.05	0.064	0.01	$1.6*10^{-6}$	0.06	0.298	0.12	0.01	$2.6*10^{-7}$
rs72744354	15	51352875	A	G	*TNFAIP8L3*	intronic	HDL-C	0.04	-0.066	0.01	$2.6*10^{-6}$	0.04	-0.319	0.13	0.02	$4.6*10^{-7}$
rs10090941	8	142370872	G	C	*GPR20*	intronic	TC	0.17	0.018	0.01	$1.1*10^{-6}$	0.21	0.090	0.05	0.04	$1.2*10^{-6}$
rs10090104	8	142370862	A	G	*GPR20*	intronic	TC	0.17	0.018	0.01	$1.1*10^{-6}$	0.21	0.090	0.04	0.04	$1.2*10^{-6}$

A1, allele 1; A2, allele 2 (A2 is the effect allele); BP, base-pair location; Chr, chromosome; FRQ, allele frequency for A1; Meta, meta-analysis result SE, standard error; TC, total cholesterol; SNP, single-nucleotide polymorphism. $0.01 < P < 0.05$ for ACCORD African Americans* or Hispanics+