INTRODUCTION

Elevated plasma triglyceride (TG), low-density cholesterol lipoprotein (LDL-C) levels and reduced plasma high-density lipoprotein cholesterol (HDL-C) have each been identified as risk factors for cardiovascular disease (CVD).1,2 Current evidence suggests that a number of inflammation markers are also potentially useful predictors of prevalent or incident CVD, and interact with dyslipidemia to mediate CVD risk.3,4 Drug therapy that reduces plasma TG and LDL-C and raises HDL-C has been shown to lower cardiovascular disease (CVD). 1,2 Current evidence suggests that a number of inflammation markers are also potentially useful predictors of prevalent or incident CVD, and interact with dyslipidemia to mediate CVD risk.3,4 Drug therapy that reduces plasma TG and LDL-C and raises HDL-C has been shown to lower adverse cardiovascular outcomes.5,6 Fenofibrate is an efficacious therapeutic agent for hypertriglyceridemia, reducing plasma TG levels by 35–50% while having the collateral benefit of raising HDL-C levels by 10–20%.7 In addition to their effects on TG and cholesterol, fibrates have been shown to modulate the production of inflammation markers.8–12 There is, however, significant inter-individual variation in response to fenofibrate.13 Findings from a number of studies have suggested that lipid, lipoprotein and some inflammation marker responses are partially under genetic influence.

Fibrates are amphipathic carboxylic acids and are agonists of peroxisome proliferator-activated receptor alpha (PPARα). PPARα receptors heterodimerize with the retinoid X receptor and function as transcription factors, regulating the expression of genes. More than 80 genes in humans and mammalian models are known to be regulated by PPARα including apolipoprotein genes (for example, APOA1, APOA2, APOA5 and APOC3), lipoprotein lipase gene, phospholipid transfer protein gene and other genes involved in lipid and lipoprotein metabolism.18,19 PPARα is also known to modulate transcriptional pathways involved in inflammation responses.20

Given that PPARα is the molecular target for fenofibrate and its known, albeit incompletely understood, roles in lipid metabolism and inflammation, we investigated whether genetic variation in the PPARα gene was associated with variation in fasting TG, LDL-C, HDL-C, adiponectin and inflammation marker response to a 3-week fenofibrate trial in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.

MATERIALS AND METHODS

Subjects and Intervention

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.

Subjects were recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.

Materials and Methods

Subjects and Intervention

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.

Subjects and Intervention

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.

Subjects and Intervention

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.

Subjects and Intervention

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.

Subjects and Intervention

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two National Heart, Lung and Blood Institute Family Heart Study field centers: Minneapolis, MN and Salt Lake City, UT. All subjects were Caucasian and nearly all of European ancestry. Eligibility criteria were (1) ≥ 18 years of age; (2) fasting TGs < 1500 mg dl⁻¹; (3) willing to participate in the study and attend the scheduled clinic exams; (4) member of a family with at least two members in a sibship; (5) aspartate aminotransferase and alanine aminotransferase levels < upper limits of normal.
alanine aminotransferase results within normal range; and (6) creatinine ≤ 2.0 mg/dl. Exclusion criteria were (1) history of liver, kidney, pancreas, gall bladder disease or malabsorption; (2) current pregnancy; (3) insulin use; (4) use of lipid lowering drugs (including prescription, over-the-counter and nutraceuticals; volunteers taking these agents were withdrawn from them at least 4 weeks before the study with physician’s approval); (5) use of warfarin; (6) women of childbearing potential not using an acceptable form of contraception; (7) known hypersensitivity to fenofibrate; and (8) history of pancreatitis within 12 months before enrollment. A previous study demonstrated that Caucasians in Utah and Minnesota were homogenous and pooling data across centers would not threaten the validity of this study.21

The details of the GOLDN visits are shown in Figure 1. After granting informed consent, participants underwent a baseline-screening visit. This visit included a fasting blood draw and pregnancy test, if applicable. The day before the first clinical exam, participants came to the clinic for a fasting blood draw. On visits 2 and 4, willing participants underwent a high fat meal (fasting), after 8 h fast which required ingesting 700 kcal m⁻² of body surface area.22 Blood draws were collected before the high fat meal (fasting), 3.5 h after ingestion (uptake) and 6 h after ingestion (clearance). The fenofibrate intervention consisted of a 3-week treatment period, in which participants took fenofibrate (160 mg) daily. Lipids were measured twice on the last 2 days of the treatment period after a minimum 8-h fast.

Biochemical analyses
Protocols for measuring TG, HDL-C, LDL-C and fenofibric acid have been previously described.23-24 All lipids were measured using the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA). TGs were measured using a glycerol blanked enzymatic method, cholesterol was measured using a cholesterol esterase, cholesterol oxidase reaction, and LDL-C was measured by a homogeneous direct method (LDL Direct Liquid Select Cholesterol Reagent; Equal Diagnostics, Exton, PA, USA). HDL-C was calculated after precipitation of non-HDL-C with dextran sulfate. The interlaboratory coefficients of variation in a pooled plasma control were 2.6, 1.9, 3.9 and 1.8% for TGs, cholesterol, HDL-C and LDL-C, respectively.

Inflammatory markers were measured using the following enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, MN, USA): Quantikine High Sensitivity Human IL-6; Quantikine Human IL-2 RFL, Quantikine Human MCP-1 and Quantiglo Human TNF-α. The interlaboratory coefficients of variation on a pooled plasma control were 12.3, 5.7, 7.5 and 9.7% for interleukin-6 (IL-6), IL-2, monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-α), respectively.

Single-nucleotide polymorphism selection and genotyping
Single-nucleotide polymorphisms (SNPs) were identified through searching public databases such as dbSNP (http://www.ncbi.nlm.nih.gov/SNP). We selected 10 SNPs at the PPARα gene on the basis of the following criteria, in order of importance in our selection scheme: (1) validation status, that is, experimentally validated in Caucasians; (2) functional relevance and importance, namely the potential ‘functional’ SNPs residing within the transcription factor binding sites in the 5’ promoter region, in the mRNA (messenger RNA) stability regulatory protein binding sites in 3’UTR, in exons that change amino-acid sequences or in exon-intron boundaries that alter mRNA splicing; (3) degree of heterozygosity, that is, minor allele frequencies ≥ 0.05; and (4) previous evidence of association with lipid measurements. SNP genotyping methods have been described elsewhere.25 The overall genotyping error and missing rate was ~1%. We used the GRR software to detect pedigree errors via graphically inspecting the distribution for marker allele sharing among pairs of family members on all pairs of individuals. SNP allele frequencies were estimated via a maximum-likelihood method.26 For each SNP, a χ²-test was used to examine the deviation of SNP genotypes from Hardy–Weinberg equilibrium.

Statistical analyses
Responses to fenofibrate. Lipid and inflammatory responses to fenofibrate were calculated using growth curve models. Eight lipid and inflammatory marker measures were analyzed: TG, HDL-C, LDL-C, adiponectin, IL-2, IL-6, MCP and TNF-α. At time point 1 (fasting), TG, HDL-C, adiponectin, IL-2, IL-6, MCP and TNF-α concentrations were measured. Post-prandial lipids were measured at time points 2 (3.5 h post ingestion or uptake) and 3 (6 h post ingestion or clearance), TG concentrations were log transformed to normalize the distribution. The normalized data were used for the growth curve models. Fasting data were used from visits 1 and 2 (pre-fenofibrate) and 3 and 4 (post-fenofibrate; Figure 1). For each condition (pre/post), the two draws were treated as repeated measures to reduce error variance in the slope estimation. Growth curve slopes were adjusted for age, age², sex and data collection center. Compared with a simple delta phenotype, SOLAR estimated heritabilities were much higher for growth curve phenotypes (> 50%). In addition to analyzing genotype associations with lipid, lipoprotein and inflammation marker response phenotypes, we analyzed genotype associations with serum fenofibric acid concentrations. For the fenofibric acid phenotype, we used the partial area under the fenofibric acid concentration-time curve (FA AUC₀₋₆) from serum concentrations measured over 6 h (at 0, 3.5 and 6 h after dosing) on the final day of the intervention. To test whether there were significant differences in lipid and inflammatory marker concentrations before and after the fenofibrate trial, t-tests were conducted, stratified by sex, on data that had been transformed where necessary, as above.

SNP-phenotype associations. We used a linear mixed model implemented in SAS (version 9.1, SAS Institute, Cary, NC, USA). The effects of SNP genotypes (categorical variable with three classes) were treated as fixed effects and the dependencies among members within each family were treated as random effects. Field center, age, age², sex and gender were included in the model as covariates. To control for multiple testing, false discovery rate (FDR)27 procedure was applied to the data, which gives rise to q-values that represent P-values corrected for the number tests run, which seeks to maximize power while correcting for the family-wise error rate in multiple tests. Hardy-Weinberg equilibrium was calculated using Haploview,24 and all SNPs were in Hardy–Weinberg equilibrium with an α value of P > 0.05. The slopes for IL-2, MCP and TNF-α were log transformed to normality for the genetic analysis. The square root was taken of the FA AUC₀₋₆ values in the genetic models, again, to approximate normality.

Haplotypic analysis. For all traits with more than one significant fasting SNP-phenotype association within a haplotype block (Figure 2) we conducted haplotype analysis. Haplotypic frequencies were calculated, and included in the association analysis were haplotype frequencies ≥ 0.01. Tests of association, controlling for pedigree were conducted using the haplotype association module hbat within the FBAT program.28
**RESULTS**

**Study sample characteristics**

The number of subjects having screened for eligibility was 1327 (639 men and 688 women) from 148 families. Of these, 861 subjects (427 men and 434 women) underwent the fenofibrate intervention, had all genotyoe data, and had all lipidd, lipoprotein and inflammation marker phenotype measures. Table 1 shows the baseline characteristics of this sample. The mean age of men was 50.6 years, and that of women was 51.1 years. Mean phenotype data are shown stratified by sex in Table 1.

For both men and women, TG and LDL-C concentrations were significantly lower after fenofibrate treatment than those before treatment (all \( P \leq 0.001 \)). HDL-C increased significantly after fenofibrate treatment (\( P < 0.001 \)). Adiponectin was the only marker which was significantly lower in both men (\( P < 0.0001 \)) and women (\( P = 0.0022 \); concentrations of IL-2, TNF-\( \alpha \) and MCP increased in both men and women (\( P < 0.0001 \)) with IL-6 higher in men (\( P = 0.0499 \)) and not in women (\( P = 0.1651 \)).

**Single-SNP associations**

Supplementary Figure 1 shows the location of our SNPs in the PPAR\( \alpha \) gene and Figure 2 gives a linkage disequilibrium plot from haploview for our 10 SNPs. Table 2 summarizes the significant results of the association analyses between each of the single PPAR\( \alpha \) SNPs and response to fenofibrate (that is, growth curve slopes) for all phenotypes. For TG, evidence for association was found for rs4253701 (\( P = 0.025 \)) but this did not survive the FDR correction for multiple testing (\( q = 0.321 \)). For LDL-C, there were significant associations for rs135550 (\( P = 0.001 \)) and rs135543 (\( P = 0.001 \); both \( q = 0.300 \)). For inflammation marker response phenotypes, only IL-2 and TNF response to fenofibrate showed associations with any SNP. rs9626730 showed an association with IL-2 (\( P = 0.0022 \); \( q = 0.181 \)), and rs13550, rs15522, rs135543 and rs4253701 showed suggestive associations with TNF response to fenofibrate (\( P < 0.05 \)) that did not survive the FDR correction (\( q > 0.05 \)). Significant associations were not observed for FA AUC\( _{0-6} \) phenotype, although rs135550 showed a trend association with FA AUC\( _{0-6} \) (\( P = 0.024 \); \( q = 0.321 \)).

We additionally conducted SNP-phenotype associations with the post-prandial data at both time points (Figure 1). This confirmed our findings with the same significant SNP-lipid associations, in the expected direction (Supplementary Table 1). There were no additional significant SNP-trait associations.

**Haplotype analysis**

Only LDL-C and TNF-\( \alpha \) were associated with SNPs within a haplotype block (Table 2; Figure 2) at \( P < 0.05 \).

**Association within haplotype block 1.** This consisted of SNPs rs135550 and rs135549 (Figure 2). FBAT identified four haplotypes, of which three had frequencies of \( > 0.01 \). Haplotypes 1 (T-C) and 2 (C-T) were associated with fasting LDL-C responses to fenofibrate (\( P = 0.01 \) and \( P = 0.03 \); Table 3), but were not associated with fasting TNF-\( \alpha \) (\( P = 0.48 \) and \( P = 0.44 \); Table 3).

**Association within haplotype block 2.** This consisted of SNPs rs135550, rs135549, rs135543 and rs9626730 (Figure 2). FBAT identified 14 haplotypes, of which 7 had a frequency of \( > 0.01 \).

---

**Table 1.** Characteristics of study subjects

|                      | Men (n = 427) | P-value | Women (n = 434) | P-value |
|----------------------|--------------|---------|----------------|---------|
|                      | Pre-fenofibrate | Post-fenofibrate | Pre-fenofibrate | Post-fenofibrate |
| Age, year            | 50.6 ± 15.9   | 51.1 ± 15.8 | 28.72 ± 4.86   | 28.48 ± 3.63   |
| BMI, kg m\(^{-2}\)    | 103.41 ± 14.01| 99.18 ± 59.83| 41.57 ± 9.84   | 43.67 ± 9.99   |
| HDL-C, mg dl\(^{-1}\) | 123.17 ± 30.32| 111.45 ± 32.79| 216.67 ± 69.79 | 224.26 ± 75.71 |
| LDL-C, mg dl\(^{-1}\) | 3.68 ± 7.08   | 3.81 ± 5.29  | 200.86 ± 58.45 | 221.04 ± 74.98 |
| TGF-\( \beta \), pg ml\(^{-1}\) | 1042.60 ± 390.66 | 1132.51 ± 494.82 | 1023.72 ± 342.21 | 1191.78 ± 561.40 |
| IL-6, pg ml\(^{-1}\) | 2.07 ± 4.13   | 2.31 ± 4.26  | 1.89 ± 1.63    | 2.06 ± 2.15    |
| FA AUC (mg L\(^{-1}\)) hour\(^{-1}\) | 643.26 ± 351.42 | 584.01 ± 319.56 | 10 216.32 ± 506.67 | 9681.73 ± 4672.38 |

Abbreviations: BMI, body mass index; FA AUC\( _{0-6} \), fenofibric acid serum concentration area under the 0–6 h curve; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor.

\( P \)-value for pre- to post-fenofibrate treatment reported in the text.

\( a \) FA AUC measured on final day of fenofibrate trial on a sample of 364 men and 355 women.

Values are listed as mean ± s.d.
RESULTS
A total of four markers within the haplotype block showed a significant association with LDL-C responses to fenofibrate, and although one SNP (rs135550) was associated with fasting TNF-α (all P > 0.05).

DISCUSSION
This study demonstrates that people who carry different PPARα variants responded differently to fenofibrate intervention with respect to reductions in LDL-C and changes in inflammation marker plasma concentrations. Although PPARα has been studied in this context before, this is the first study of its size and scope investigating these associations in a healthy population, and the first study to include the effects of PPARα variants on inflammation responses to fenofibrate.

Fenofibrate binds to and activates PPARα. PPARs are specific transcription factors that mediate the gene regulation effects thought to induce the lipid- and inflammation-lowering effects of fibrates. We thus tested whether variants in PPARα are associated with differences in fenofibrate responses by lipids and inflammatory markers. For lipid phenotypes, two variants (rs135550 and rs135543) showed an association with change in LDL-C of 28 and 43% after fibrate treatment, but receptor-independent catabolism remained unchanged.28 This is the first study, to our knowledge, to report associations between the degree of change in LDL-C concentration after fenofibrate treatment and these two variants. rs135550 is in the untranslated region and has previously been associated with baseline lipids29 and myocardial infarction as part of a three-marker haplotype.30 Our results were confirmed with similar SNP-phenotype associations, in the expected direction, with the post-prandial data.

We did not show any variant-phenotype association with HDL-C response to fenofibrate, and although one SNP (rs4253701) showed an association with TG of P < 0.05, this did not survive the correction for multiple testing (q = 0.321). We are unaware of any previous associations between PPARα variants and fenofibrate response of HDL-C. Brisson et al.31 report no association with TG response to fenofibrate and rs1800206 (commonly referred to as PPARα Leu162Val; not tested here), but Foucher et al.32 report an association between rs4253778 and

| SNP      | Phenotypic mean ± s.e. |        |        | F(df)a | P   | Q   |
|----------|------------------------|--------|--------|--------|-----|-----|
|          | 1/1 | 1/2 | 2/2 |
| TG       |     |     |     |       |     |     |
| rs4253701b | –0.130 (0.085) | –0.042 (0.027) | 0.014 (0.013) | 5.06 (612) | 0.025 | 0.321 |
| LDL-C    |     |     |     |       |     |     |
| rs135550  | 8.349 (2.550) | –1.617 (1.267) | 0.541 (1.155) | 7.77 (612) | 0.001 | 0.300 |
| rs135543  | 0.403 (1.207) | –1.468 (1.237) | 7.898 (2.345) | 6.93 (614) | 0.001 | 0.300 |
| IL-2     |     |     |     |       |     |     |
| rs9626730c | 0.130 (0.042) | –0.037 (0.014) | –0.023 (0.008) | 13.64 (679) | 0.0002 | 0.018 |
| TNF      |     |     |     |       |     |     |
| rs135550  | –0.099 (0.042) | –0.010 (0.010) | –0.068 (0.017) | 3.33 (674) | 0.036 | 0.500 |
| rs15522b  | –0.048 (0.028) | –0.021 (0.018) | –0.091 (0.022) | 3.24 (676) | 0.016 | 0.288 |
| rs135543b | –0.073 (0.018) | –0.016 (0.019) | –0.056 (0.039) | 2.53 (676) | 0.044 | 0.495 |
| rs4253701 | –0.291 (0.112) | 0.005 (0.030) | –0.055 (0.014) | 4.19 (673) | 0.016 | 0.288 |
| FA AUC    |     |     |     |       |     |     |
| rs135550d | 7.547 (0.243) | 8.187 (0.116) | 8.033 (0.104) | 5.12 (601) | 0.024 | 0.321 |

Table 2. Significant genetic variant-phenotype associations with fasting data

| Haplotype | Frequency | LDL-C | TNF-α |
|-----------|-----------|-------|-------|
| Block 1   | rs135550-rs135549 |       |       |
| T-C       | 0.70      | 0.01  | 0.48  |
| T-C       | 0.27      | 0.03  | 0.46  |
| Block 2   | rs135550-rs135549-rs135543-rs9626730 |       |       |
| T-C-A-A   | 0.57      | 0.02  | 0.67  |

Table 3. Significant phenotype associations with haplotype blocks consisting of two (rs135550 and rs135549) and four (rs135550, rs135549, rs135543, rs9626730) marker haplotypes

Abbreviations: LDL-C, low-density lipoprotein cholesterol; TNF, tumor necrosis factor alpha.

Abbreviations: FA, fasting acid concentration area under the 0–6 h curve; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; SNP, single-nucleotide polymorphism; TG, triglyceride; TNF, tumor necrosis factor.

Results from mixed linear models which controlled for age and sex, pedigree and study center. SNPs were predictors and changes in fasting triglycerides (TGS), cholesterol and inflammatory markers were outcomes.

Dominant model.

FA AUC measured on final day of fenofibrate trial on a sample of 364 men and 355 women.
TG response to fenofibrate in diabetics. It is not clear why we did not replicate Foucher et al.'s variant-phenotype associations with TG response to fenofibrate on the PPARα. This may relate to the different variants typed in our study, where the closest is 1 700 048 bp downstream of variant rs4253778 (SNP (SNP Annotation and Proxy Search) software tool provided by the Broad Institute (http://www.broadinstitute.org/mpg/snp/ldeSearch.php) which uses HapMap Project data, reports that none of our SNPs is in linkage disequilibrium with an r² > 0.8 with rs4253778). Alternatively, Foucher et al.'s variant-phenotype association between TG response to fenofibrate and SNPs in the PPARα gene could be due to the population used, which was not enriched for diabetics and had a higher baseline TG level. In comparison, GOLDN is a general population sample, with a low percentage (~20%) of diabetics, consequently, baseline TG levels are lower. As baseline TG levels in themselves modulate TG responses to fenofibrate, this may partially explain why we did not replicate Foucher et al.'s variant-response association.

We examined PPARα variants and their effect on inflammatory marker reductions and adiponectin levels with fenofibrate treatment. No variants were associated with change in adiponectin, MCP or IL-6, although concentrations of these inflammatory markers did increase significantly in both men and women before and after the fenofibrate trial. Two previous studies reported on two of the variants used in this study (rs4253701 and rs4253728) but neither demonstrated an association between either SNP and either myocardial infarctions or apoC-III levels.

However, these studies were seeking associations between PPARα variants and select outcomes, in contrast to our pursuits of PPARα variants as modulators of lipid or inflammatory markers in response to fenofibrate as an agonist of PPARα. We did show a significant mediation of the IL-2 increase in response to fenofibrate by the variant rs9626730 (P = 0.0002; q = 0.0018). Given that the increase in IL-2 was surprising, this highlights the importance of genetic variation in PPARα in protecting against this. Although there were four SNPs that showed suggested associations with TNF-α increases (P < 0.05), none of these response-variant associations survived the FDR correction. Fibrates have previously been shown to reduce a number of systemic inflammation markers such as IL-6 and TNF-α,16–38 presumed to act through their effects on PPARα. Other PPARα variants such as PPARα activation may also affect inflammation markers, but its role is more controversial and less well understood.39 PPARα activation has been shown to interact with nuclear factor-κB and activator protein-1 signaling pathways to lead to a reduction in inflammatory markers, such as IL-6.39 It remains unclear whether this decrease is due solely to the known increase in catabolism of inflammatory markers, or because of independent effects of PPARα activation on their production.39 Notably, reductions in IL-6 with fenofibrate have been shown to occur independently of lipid changes in patients with the metabolic syndrome, suggesting the direct effects of fenofibrate and PPARα on inflammation.36 In our study, we show that carriers of the minor allele had reductions in IL-6, yet those homozygous for the major allele had increases in IL-6. This highlights the complexity of this biological pathway and warrants further research. Fenofibric acid is the major metabolite of fenofibrate. After oral fenofibrate administrations, fenofibrate is rapidly hydrolyzed by esterases to form fenofibric acid, the rest being excreted through urine (primary) and feces (secondary).40 Given that serum fenofibric acid concentration has been correlated with reductions in total, and LDL-C, it is of interest to examine genotype-phenotype associations with serum fenofibric acid after a standardized dose. rs135550 was significantly associated with serum fenofibric acid concentrations (P = 0.024), and may be interesting given its significant association with change in LDL-C concentrations after fenofibrate treatment, however, as this variant-phenotype association did not survive the correction for multiple testing, the relationship of this variant to the lipid and inflammatory marker lowering potential of fenofibrate acid, and the role of fenofibric acid concentrations in mediating this relationship is unclear. We provide only a preliminary indication that differences in LDL-C reduction in our study, associated with rs135550 may operate through changes in systemic exposure (AUC0–∞) to fenofibrate acid in the serum. This relationship and others related to exposure are more likely to be elucidated with further consideration of genetic variations in the metabolic pathways of fenofibric acid’s elimination.

Our analyses benefited from a strong a priori hypothesis, a good sample size for a pharmacogenetic study, and a healthy population, making generalizations to other samples less problematic than with clinical samples. However, there are some limitations: first, these associations should be tested in more racially diverse populations, and with sample sizes that allow for the stratification by gender, which was only statistically controlled for in this study. Second, it is not clear whether these SNP-phenotype associations would generalize to hyperlipidemic patients, where fenofibrate treatment is targeted. Third, many of our conclusions regarding associations between a variant and both serum fenofibric acid concentrations and responses to fenofibrate did not survive the FDR correction for multiple testing, so should be considered preliminary. Third, it is not clear why some variants in high linkage disequilibrium with each other did not show the same genotype-phenotype associations. This lack of replication within our study suggests that we may have suffered from a power issue with the correction for multiple testing, and replication with a lower number of SNPs, and/or alternative phenotypes would be an important avenue of research. Finally, the finding of an increase in inflammatory markers after fenofibrate treatment was unexpected. Our data suggest that some variants are associated with the expected decrease, while others are associated with an increase in markers of inflammation. As this is the first such finding, we again heavily encourage replication in independent cohorts.

Nonetheless, the SNP-phenotype associations identified in these analyses may have important implications for clinical care. Our data support results showing fenofibrate to be an effective reducer of TG, LDL-C and a modulator of inflammatory markers, and we further highlight the role of genetic variants on the PPARα gene in modulating these responses. Treating CVD, and reducing markers of CVD risk, requires complex lifestyle and pharmacologic intervention paradigms. Newer research is aimed at integrating complex genetic information into these multi-modal networks of treatments to increase their efficacy.41 The data here, which show variants in the PPARα gene modulate the response in LDL-C and IL-6 in response to fenofibrate, may be an important contributor to future paradigms that use pharmacogenetic information.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the staff of the GOLDN study for the assistance in data collection and management. This study was funded by National Heart, Lung and Blood Institute grant number U01HL072724.

REFERENCES

1 Krauss RM. Triglycerides and atherogenic lipoproteins: rationale for lipid management. Am J Med 1998; 105: 585–625.

2 Wilson PW, Garrison RJ, Castelli WP, Feinleib M, McNamara PM, Kannel WB. Prevalence of coronary heart disease in the Framingham Offspring Study: role of lipoprotein cholesterol. Am J Cardiol 1980; 46: 649–654.
23 Liu Y, Ordovas JM, Gao G, Province M, Straka RJ, Tsai MY et al. The SCARB1 gene is associated with lipid response to dietary and pharmacological interventions. J Hum Genet 2008; 53: 709–717.
24 Straka RJ, Burkhardt RT, Fisher JE. Determination of fenofibric acid concentrations by HPLC after anion exchange solid-phase extraction from human serum. Ther Drug Monit 2007; 29: 197–202.
25 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 1995; 57: 289–300.
26 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263–265.
27 Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol 2004; 26: 61–69.
28 Caslake MJ, Packard CJ, Gaw A, Murray E, Griffin BA, Vanceao BD et al. Fenofibrate and LDL metabolic heterogeneity in hypercholesterolemia. Arterioscler Thromb 1993; 13: 702–711.
29 Kraja AT, Province MA, Straka RJ, Ordovas JM, Borecki IB, Arnett DK. Fenofibrate and metabolic syndrome. Endocr Metab Immune Disord Drug Targets 2010; 10: 138–148.
30 Reinhard W, Stark K, Sedlacek K, Fischer M, Baessler A, Neureuther K et al. Association between PPARalpha gene polymorphisms and myocardial infarction. Clin Sci 2008; 115: 301–308.
31 Brisson D, Ledoux K, Bosse Y, St-Pierre J, Julien P, Perron P et al. Effect of apolipoprotein E, peroxisome proliferator-activated receptor alpha and lipoprotein lipase gene mutations on the ability of fenofibrate to improve lipid profiles and reach clinical guideline targets among hypertriglyceridemic patients. Pharmacogenomics 2002; 12: 313–320.
32 Foucher C, Rattier S, Flavell DM, Talmaud PJ, Humphries SE, Kastelein JJ et al. Response to micronized fenofibrate treatment is associated with the peroxisome proliferator-activated receptors alpha/gC intron7 polymorphism in subjects with type 2 diabetes. Pharmacogenetics 2004; 14: 823–829.
33 Jun M, Foote C, Lv J, Neal B, Patel A, Nicholls SJ et al. Effects of fibrates on cardiovascular outcomes: a systematic review and meta-analysis. Lancet 2010; 375: 1875–1884.
34 Enquobahrie DA, Smith NL, Bin JC, Cartl CY, Rice KM, Lumbey T et al. Cholesterol ester transfer protein, interleukin-8, peroxisome proliferator activator receptor alpha, and Toll-like receptor 4 genetic variations and risk of incident nonfatal myocardial infarction and ischemic stroke. Am J Cardiol 2008; 101: 1683–1688.
35 Shin T, Kuboki S, Huber N, Eismann T, Galloway E, Schuster R et al. Analysis of C-reactive protein genotypes for measurement of cardiovascular risk by HPLC after anion exchange solid-phase extraction from human serum. J R Stat Soc Ser B 2002; 64: 241–265.
36 Ballantyne CM, Nambi V. Markers of inflammation and their clinical significance. Atheroscler Suppl 2005; 6: 21–29.
37 Cesari M, Penninx BW, Newman AB, Newman AB, Kritchevsky SB, Nicklas BJ et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation 2003; 108: 2317–2322.
38 Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 2000; 86: 943–949.
39 Hausenloy DJ, Yellon DM. Targeting residual cardiovascular risk: raising high-density lipoprotein cholesterol levels. Postgrad Med J 2008; 84: 590–598.
40 Knopp RH, Walden CE, Warnock GR, Albers JJ, Ginsberg J, McGinnis BM. Effect of fenofibrate treatment on plasma lipoprotein lipids, high-density lipoprotein cholesterol subfractions, and apolipoproteins B, A1, AI, and E. Am J Med 1987; 83: 75–84.
41 Sebestjen M, Keber I, Zegura B, Simic S, Bozic M, Fressart MM et al. Statin and fibrate treatment of combined hyperlipidemia: the effects on some novel risk factors. Thromb Haemost 2004; 92: 1129–1135.
42 Rosenson RS. Effect of fenofibrate on adiponectin and inflammatory biomarkers in metabolic syndrome patients. Obesity 2009; 17: 504–509.
43 Rosenson RS, Huskin AL, Wolff DA, Helenowski IB, Rademaker AW. Fenofibrate reduces fasting and postprandial inflammatory responses among hypertglycemic patients with the metabolic syndrome. Atherosclerosis 2008; 198: 381–388.
44 Jonkers U, Mohrschlad MF, Westendorp RG, van der Laarse A, Smelt AH. Severe hypertriglyceridemia with insulin resistance is associated with systemic inflammation: reversal with bezafibrate therapy in a randomized controlled trial. Am J Med 2002; 112: 275–280.
45 Okopien B, Krysik R, Kowalski J, Madej A, Belowski D, Zielinski M et al. The effect of statins and fibrates on interferon-gamma and interleukin-2 release in patients with primary type II dyslipidemia. Atherosclerosis 2004; 176: 327–335.
46 Keating GM, Ommrod D. Miconised fenofibrate: an updated review of its clinical efficacy in the management of dyslipidaemia. Drugs 2002; 62: 1909–1944.
47 Ordovas JM. Pharmacogenetics of lipid diseases. Hum Genomics 2004; 1: 111–125.
48 Smith JA, Arnett DK, Kelly RJ, Ordovas JM, Sun YV, Hopkins PN et al. The genetic architecture of fasting plasma triglyceride response to fenofibrate treatment. Eur J Hum Genet 2008; 16: 603–613.
49 Liu Y, Ordovas JM, Gao G, Province M, Straka RJ, Tsai MY et al. Pharmacogenetic association of the APOA1/C3/A4/A5 gene cluster and lipid responses to fenofibrate: the genetics of lipid-lowering drugs and diet network study. Pharmacogenet Genomics 2009; 19: 161–169.
50 Shen J, Arnett DK, Parnell LD, Peacock JM, Lai CQ, Hixson JE et al. Association of common C-reactive protein (CRP) gene polymorphisms with baseline plasma CRP levels and fenofibrate response: the GOLDN study. Diabetes Care 2008; 31: 910–915.
51 Mandard S, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. Cell Mol Life Sci 2004; 61: 393–416.
52 Spinelli SL, O'Brien JJ, Bancos S, Lehmann GM, Springer DL, Blumberg N et al. The PPAR-platelet connection: modulators of inflammation and potential cardiovascular effects. PPAR Res 2008; 2008: 328172.
53 Rizzo G, Fiorucci S. PPARs and other nuclear receptors in inflammation. Curr Opin Pharmacol 2006; 6: 421–427.
54 Pankow JS, Province MA, Hunt SC, Arnett DK. Regarding “Testing for population subdivision and association in four case-control studies”. Am J Hum Genet 2002; 71: 1478–1480.
55 Patsch JR, Miesenbock G, Hopfenviesser T, Muhlberger V, Knapp E, Dunn JK et al. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb Vasc Biol Am Heart Assoc 1992; 12: 1336–1345.