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A genome-wide association study of inflammatory biomarker changes in response to fenofibrate treatment in the Genetics of Lipid Lowering Drug and Diet Network (GOLDN)

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Abstract

Objective—Despite evidence in support of anti-inflammatory and triglyceride-lowering effects of fenofibrate, little is known about genetic determinants of the observed heterogeneity in treatment response. This study provides the first genome-wide examination of fenofibrate effects on systemic inflammation.

Methods—Biomarkers of inflammation were measured in participants of the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN, n=1092) before and after a 3-week daily treatment with 160 mg of fenofibrate. Two inflammatory patterns (hsCRP-IL6 and MCP1-TNF- α) were derived using principal component analysis. Associations between single nucleotide polymorphisms on the Affymetrix 6.0 chip and phenotypes were assessed using mixed linear models, adjusted for age, sex, study center, and ancestry as fixed effects and pedigree as a random effect.

Results—Before fenofibrate treatment, the strongest evidence for association was observed for polymorphisms near or within the *IL2RA* gene with the hsCRP-IL6 pattern (rs7911500, $P=5\times 10^{-9}$ and rs12722605, $P=5\times 10^{-8}$). Associations of the MCP1-TNF- α pattern with loci in several biologically plausible genes (*CYP4F8* (rs3764563), *APBB1IP* (rs1775246), *COL13A1* (rs2683572), and *COMMD10* (rs1396485)) approached genome-wide significance ($P=3\times 10^{-7}$, 5×10^{-7} , 6×10^{-7} , and 7×10^{-7} respectively) before fenofibrate treatment. After fenofibrate treatment, the rs12722605 locus in *IL2RA* was also associated with the MCP1-TNF- α pattern ($P=3\times 10^{-7}$). The analyses of individual biomarker response to fenofibrate did not yield genome-wide significant results, but the rs6517147 locus near the immunologically relevant *IFNAR2* gene was suggestively associated with IL6 ($P=7\times 10^{-7}$).

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Conclusions—We have identified several novel biologically relevant loci associated with systemic inflammation before and after fenofibrate treatment.

Keywords

fenofibrate; inflammation; genome-wide association study

Background

Fenofibrate, a peroxisome proliferator-activated receptor- α (PPAR- α) agonist, has been shown to improve the lipid profile and reduce systemic inflammation [1–3]. However, individual response to fenofibrate is highly heterogeneous. While several genes have been implicated in mediating its lipid-lowering effects [4–6], only a limited number of studies to date have investigated the role of genetic variation in the anti-inflammatory effects of PPAR- α agonist treatment. Specifically, a previous analysis of the Genetics of Lipid Lowering and Diet Network (GOLDN) study data showed robust associations of common C-reactive protein (*CRP*) gene variants with baseline CRP levels, as well as with CRP response to fenofibrate among participants with metabolic syndrome [7]. In contrast, GOLDN investigators reported that two transcription factor 7 like-2 (*TCF7L2*) gene polymorphisms that had been implicated in diabetes were not associated with plasma concentrations of inflammatory biomarkers before or after fenofibrate treatment [8].

A genome-wide approach provides a more comprehensive examination of genetic determinants of fenofibrate response, potentially revealing novel markers associated with systemic inflammation. If successful, a genome-wide approach will identify new biological pathways and thus potential drug targets, as well as more personalized clinical approaches. In this study, we performed association tests between single nucleotide polymorphisms (SNPs) and biomarkers of systemic inflammation before and after three weeks of daily treatment with fenofibrate in participants of European ancestry in the GOLDN study.

Methods

Study Population

The National Heart, Lung, and Blood Institute GOLDN study, described in detail in previous publications [9, 10], was designed to identify genetic determinants of lipid response to treatment with 160 mg of micronized fenofibrate once a day for three weeks. Briefly, Caucasian families with at least 2 siblings were recruited from the genetically homogeneous centers of the National Heart, Lung, and Blood Institute Family Heart study in Minneapolis, MN, and Salt Lake City, UT. Participants were asked to discontinue the use of lipid-lowering agents for at least 4 weeks, to fast for at least 8 hours prior to study visits, and to abstain from alcohol for at least 24 hours prior to study visits. The study protocol was approved by Institutional Review Boards at the University of Minnesota, University of Utah, and Tufts University/New England Medical Center.

Biochemical Measurements

All samples were centrifuged at $2000 \times g$ for 15 minutes at 4 degrees C within 20 minutes of collection, stored frozen at -70 degrees C, and analyzed at the same time for each participant to eliminate inter-assay variability. High-sensitivity C-reactive protein (hsCRP) was measured on the Hitachi 911 using a latex particle enhanced immunoturbidimetric assay (Kamiya Biomedical Company, Seattle, WA). Interleukin-6 (IL6), interleukin-2 soluble receptor (IL2sR)- α , tumor necrosis factor (TNF)- α and monocyte chemoattractant protein-1 (MCP1) were measured using quantitative sandwich enzyme immunoassay techniques

(ELISA kit assays, R&D Systems Inc., Minneapolis, MN) as described in previous publications [12, 13]. The reliability coefficients ranged from 0.76 for TNF- α to 0.99 for hsCRP [13].

Genotyping

DNA extraction and purification in the GOLDN study has been described in detail in Irvin et al [10]. A total of 906,600 SNPs were genotyped using the Affymetrix Genome-Wide Human 6.0 array and the Birdseed calling algorithm [14]. The samples were processed in two different batches by two different technicians. SNPs that were monomorphic (55,530) or had a call rate <96% (82,462) were removed from the analysis. Additionally, SNPs were excluded from the analysis based on the number of families with Mendelian errors as follows: for minor allele frequency (MAF) $\geq 20\%$, removed if errors were present in >3 families (1,486 SNPs); for $20\% > \text{MAF} \geq 10\%$, removed if errors were present in >2 families (1,338 SNPs); for $10\% > \text{MAF} \geq 5\%$, removed if errors were present in >1 family (1,767 SNPs); for $\text{MAF} < 5\%$, removed if any errors were present (9,592 SNPs). In families with remaining errors, SNPs that exhibited Mendelian error were set to missing (31,595 SNPs). Furthermore, 16 participants with call rates <96% were also removed from any subsequent analyses. Subsequently, 748 SNPs failing the Hardy-Weinberg equilibrium (HWE) test at P-value < 10^{-6} were excluded from association analyses. Finally, after excluding markers with $\text{MAF} < 1\%$, HWE P-value < 10^{-6} , missing strand information, or discrepancies with the *mlinfo* file, we used MACH software (Version 1.0.16) to impute untyped SNPs using Human Genome Build 36 as the reference. After the imputation we created a hybrid dataset that included 2,543,887 SNPs, of which 584,029 were initially genotyped in the GOLDN population. Missing typed data were kept as missing in the final genotype data set.

Statistical Methods

Participants were excluded from the analysis if they reported being sick with an infection or fever (n=29), or if they were missing outcome or covariate data, yielding n=1092 for the pre-fenofibrate and n=836 for the post-fenofibrate inflammatory pattern analyses, n=826 for IL2sR- α , IL6, and hsCRP, and n=825 for TNF- α and MCP1. Of those, participants that were missing genotype information at specific loci were excluded from the respective analyses.

Outcomes were defined using the individual inflammatory measures as ratios of pre- to post-fenofibrate treatment plasma concentrations of the biomarkers (hsCRP, IL2sR- α , IL6, MCP1, TNF- α), as well as inflammatory patterns pre- and post-fenofibrate treatment. Log- or square root- transformations were carried out for non-normally distributed ratios of individual inflammatory biomarkers. For the inflammatory biomarker with the strongest evidence of association (IL2sR- α), a sensitivity analysis was conducted with the outcome defined as the difference in post- and pre-fenofibrate treatment concentrations with the model adjusted for baseline concentrations. Patterns were derived as proposed by Kabagambe *et al.* [13] using principal component analysis (PROC FACTOR in SAS); all five inflammatory biomarkers, measured before fenofibrate treatment, were entered into the model, producing two inflammatory patterns based on evaluation of eigenvalues and the Scree plot. The patterns were rotated using the VARIMAX option to improve interpretability [15]. To derive post-fenofibrate inflammatory patterns, the procedure was repeated with the same markers measured in fasting serum collected at the end of the treatment period. Because none of the pre- or post-fenofibrate inflammatory principal components were normally distributed, PROC RANK was used to compute ranks of their values, which were subsequently used as the primary outcomes.

Population substructure was assessed using principal components generated using EIGENSOFT 3.0 and found to be limited in the GOLDN data. The first 10 principal

components were retained and tested for association with dependent variables. If an association was found, a given principal component was included in the model as a covariate to adjust for population stratification. Of the 10 principal components initially retained to characterize population substructure, one (PC3) was found to be associated with the dependent variable in one of the models (IL6); therefore, only PC3 was retained as a covariate in the association model where the outcome was defined as pre- to post-fenofibrate treatment ratio of IL6 concentrations. The other models were not adjusted for ancestry as associations between the outcomes and the principal components were not statistically significant in the study population. Additionally, statistical adjustment for pedigree further removed heterogeneity due to population substructure in our data set. The associations of interest were assessed using linear mixed models, adjusted for sex, age, and center as fixed effects, and phenotypic dependence among family members as a function of their kinship (R software, *kinship* package [16]). The *lme4* function fits a linear mixed effects model, which incorporates relatedness between individuals into the covariance structure of the random effects, while the fixed effects are used to test for associations and adjust for potential confounders. The additive assumption was used to model genotypes. P-values were adjusted for multiple testing using the Bonferroni approach based on 2,543,887 typed and imputed SNPs, yielding an α -level of $0.05/2,543,887 = 1.97 \times 10^{-8}$. Quantile-quantile (Q-Q) plots of p-values were constructed to evaluate deviations from the expected test statistic distribution. Genome-wide Manhattan plots were generated to visualize the results.

Results

General characteristics of the study population are summarized in Table 1. At baseline, the mean age of participants was 48 ± 16 years; about half of the participants were female (51%) and about half were recruited at the Minnesota center (51%). All participants were of self-reported European ancestry. At baseline, 15% of all participants had triglyceride levels exceeding 150 mg/dL, 67% had high-density lipoprotein cholesterol levels lower than 50 mg/dL, 14% had low-density lipoprotein cholesterol levels higher than 150 mg/dL, and 31% had total cholesterol levels exceeding 200 mg/dL, indicating relatively low prevalence of dyslipidemia in the study population. Serum concentrations of all inflammatory biomarkers except hsCRP slightly increased from baseline to post-fenofibrate treatment, while dyslipidemia improved, as evidenced by decreases in triglycerides, low density lipoprotein- and total cholesterol, and a small increase in high density lipoprotein cholesterol. However, among subjects with baseline hypertriglyceridemia, defined as serum triglycerides >200 mg/dL, the levels of all inflammatory biomarkers except for IL2sR- α actually decreased (data not shown). The top two loci associated with the pre- to post-fenofibrate treatment ratio for each inflammatory biomarker are described in Table 2. Additionally, rs11206628, rs17471855, and rs11101115 were among the associated loci but are not included as they were in linkage disequilibrium ($r^2 > 0.8$) with the top hits. None of the markers reached the threshold for genome-wide significance (P -value $< 1.97 \times 10^{-8}$) for any of the individual inflammatory biomarkers. The strongest evidence for association (P -value $= 7 \times 10^{-7}$) was observed between rs6517147 and IL2sR- α concentration. The top finding was validated (P -value $= 2 \times 10^{-7}$) in a sensitivity analysis with the outcome defined as the difference between pre- and post-fenofibrate treatment concentrations, with the model adjusted for baseline IL2sR- α values (data not shown). Additionally, we observed associations between the pre- to post-fenofibrate treatment MCP1 concentration ratio and markers in the *KIF6* gene on chromosome 6 (rs9394587, P -value $= 2 \times 10^{-6}$ and rs721755, P -value $= 5 \times 10^{-5}$). Although these associations did not reach genome-wide statistical significance, they may be biologically relevant as *KIF6* polymorphisms have been implicated in mediating statin response and cardiovascular disease risk [17].

Consistent with findings by Kabagambe *et al.* [13], two inflammatory patterns were identified each before and after fenofibrate treatment: hsCRP-IL6 and MCP1-TNF- α . Scree plots characterizing each pattern are presented in Supplemental Digital Content 1 and 2 respectively. The hsCRP-IL6 pattern was dominant pre-, while the MCP1-TNF- α pattern was dominant post-fenofibrate treatment (factor loadings not shown). Table 3 presents genetic markers that were most significantly associated with the inflammatory patterns at both time points of the study. The top two loci for the hsCRP-IL6 pattern before fenofibrate, both of which reached the genome-wide significance level, were in or within 20 kb of the *IL2RA* (interleukin 2 receptor- α) gene. One of the two *IL2RA* SNPs (rs12722605) was also the locus that associated most significantly with the MCP1-TNF- α pattern after fenofibrate. Additionally, a number of biologically relevant yet borderline statistically significant markers in *CYP4F8*, *AOBB1IP*, *COL13A1*, and *COMMD10* genes (P-values ranging from 3×10^{-7} to 7×10^{-7}) were associated with the MCP1-TNF- α pattern. Similarly to the analysis of individual biomarkers, SNPs were excluded if they were in linkage disequilibrium ($r^2 > 0.8$) with the other top hits, leaving out rs10488183, rs4666349, and rs786870. The quantile-quantile (Q-Q) plots and Manhattan plots summarizing the results of the genome-wide analyses for each outcome are shown in Supplemental Digital Content 3–11 and Figure 1, respectively. The following lambda values were estimated for each model: 1.00 (hsCRP), 1.04 (IL2sR- α), 1.00 (IL6), 1.08 (MCP1), 1.02 (TNF- α), 1.12 (hsCRP-IL6 pattern at baseline), 1.11 (hsCRP-IL6 pattern after fenofibrate), 1.15 (MCP1-TNF- α pattern at baseline), 1.08 (MCP1-TNF- α after fenofibrate).

Discussion

To our knowledge, we have conducted the first genome-wide study on the effect of fenofibrate on systemic inflammation and found several robust associations with loci in biologically plausible regions of the genome. Among individual inflammatory biomarkers' responses to fenofibrate, the strongest association was observed between rs6517147 and the IL2sR- α concentration ratio. The rs6517147 marker is located on chromosome 21 within 100 kb of the *IFNAR2* (interferon receptor 2) and within 150 kb of the *IL10RB* (interleukin 10 receptor β) genes; it has a minor allele frequency of 0.20 in the HapMap European (CEU) population. Despite not reaching genome-wide statistical significance, this finding is of biological relevance, as evidence from animal and human studies supports the role of both genes in the systemic inflammatory response. Specifically, *IFNAR*-knockout mice were shown to exhibit decreased resistance to inflammation-induced apoptotic stressors and impaired replenishment by precursors following infection with *Pneumocystis* [18]. Additionally, a family study in humans showed that mutations in the *IL10RB* gene impact secretion of pro-inflammatory cytokines from peripheral blood mononuclear cells in the setting of inflammatory bowel disease [19].

In the analysis of both inflammatory patterns, the top statistically significant markers varied from pre- to post-fenofibrate treatment, suggesting involvement of different biologic pathways. At baseline, the strongest and the only genome-wide statistically significant signal for the hsCRP-IL6 pattern was found with rs7911500, an intergenic marker between the *IL15RA* and the *IL2RA* genes, and with rs12722605, located in the 3' region of the *IL2RA* gene. The association with rs12722605 also approached statistical significance post-fenofibrate treatment for the MCP1-TNF- α pattern. These results are consistent with previously published reports that have linked this region of chromosome 10 to a variety of immunologically relevant phenotypes including response to seasonal influenza vaccine [20], Graves' disease [21], and type 1 diabetes [22]. Additionally, rs12722605 was found to be borderline associated with susceptibility to multiple sclerosis [23]. The IL2R- α /CD25 subunit encoded by the *IL2RA* gene is a component of the interleukin 2 receptor involved in control of T-cell response and autoimmunity [21, 24]. Studies have shown that regulatory T-

cells play a role in atherogenesis [25], suggesting that variation in the *IL2RA* gene is a possible mediator of the relationship between fenofibrate treatment, systemic inflammation, and cardiovascular health.

Additionally, our findings for the MCP1-TNF- α pattern at baseline highlight several variants that warrant further examination. We have observed associations approaching genome-wide significance with markers in the following biologically pertinent genes: *CYP4F8* (rs3764563), *APBB1IP* (rs1775246), *COL13A1* (rs2683572), and *COMMD10* (rs1396485). The *CYP4F8* gene encodes a member of the arachidonic acid pathway that affects inflammation by oxygenating and hydroxylating COX-derived products to prostaglandin E2 [26]. Laboratory studies have shown that the protein encoded by *APBB1IP* interacts with Rap1, which in turn is an important modulator of T-cell responses and thus plays a crucial role in the context of inflammation [27, 28]. Collagen type XIII- α 1, encoded by the *COL13A1* gene, has been previously linked to inflammation in the liver [29] and intestine [30]. Finally, the protein encoded by *COMMD10* associates with and inhibits NF- κ B, a transcription factor involved in regulating innate and adaptive immunity, and has been implicated in autoimmune conditions such as multiple sclerosis [31, 32].

The results of this study must be interpreted in light of several limitations. First, as this is the first genome-wide study of inflammatory response to PPAR- α agonist treatment, the findings need to be replicated in independent cohorts to provide evidence of validity. However, due to the uniqueness of the GOLDN intervention and the limited availability of information on inflammatory phenotypes in other clinical trials, replication presents a serious challenge common to pharmacogenomics. Second, because the magnitude of effect of each marker on the complex phenotype of inflammatory response is likely to be small, our sample size may not have been sufficient to identify all relevant loci. Third, the use of principal component analyses to identify patterns from individual inflammatory biomarkers may not fully capture the state of systemic inflammation [13] and does not provide an intuitive interpretation of regression coefficients from the final models. However, prior work from our group showed that individuals with higher scores on either pattern also had higher concentrations of the biomarkers with high factor loadings (i.e. hsCRP and IL6 or MCP1 and TNF- α) [13], suggesting that the pattern scores have a distinct biological meaning. Fourth, as our study population is a priori at lower risk for cardiovascular disease than those with a true indication for fenofibrate, the generalizability of our findings may be limited. Finally, our statistical models do not take into account potential epistatic interactions that may impact the final estimates of association for each individual locus.

In summary, we have identified a number of biologically plausible loci for systemic inflammation both before and after fenofibrate treatment. The results of this genome-wide study can inform future investigations of interactions between genetic factors and fenofibrate in the treatment of cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Manhattan plots of genome-wide results of testing for association between SNPs and inflammatory response to fenofibrate treatment (A–E) or with pre- (F,G) and post-fenofibrate (H,I) inflammatory patterns. The X-axes display the chromosome on which the SNP is located, the Y-axes display $-\log_{10}(\text{P-value})$.

- A) High-sensitivity C-reactive protein
- B) Interleukin-6
- C) Interleukin-2 soluble receptor- α
- D) Tumor necrosis factor- α
- E) Monocyte chemoattractant protein-1
- F) The hsCRP-IL6 pattern pre-fenofibrate treatment
- G) The MCP1-TNF- α pattern pre-fenofibrate treatment
- H) The hsCRP-IL6 pattern post-fenofibrate treatment
- I) The MCP1-TNF- α pattern post-fenofibrate treatment

Table 1

Characteristics of the GOLDN study participants (n= 1092 at baseline).

Variable	Mean or Median ^I ± SD or %
Age, years	48 ± 16
Sex, % female	51
Field center, % from Minnesota	51
High-sensitivity C-reactive protein, mg/dL	
Baseline	0.12 ± 0.35
After fenofibrate treatment	0.12 ± 0.47
Interleukin-2 soluble receptor-α, pg/mL	
Baseline	943 ± 361
After fenofibrate treatment	1039 ± 530
Interleukin-6, pg/mL	
Baseline	1.40 ± 3.13
After fenofibrate treatment	1.47 ± 3.32
Tumor necrosis factor-α, pg/mL	
Baseline	2.88 ± 5.21
After fenofibrate treatment	3.13 ± 4.06
Monocyte chemoattractant protein-1, pg/mL	
Baseline	200 ± 16
After fenofibrate treatment	209 ± 75
Triglycerides, mg/dL	
Baseline	141.65 ± 119.47
After fenofibrate treatment	90.78 ± 55.22
High density lipoprotein cholesterol, mg/dL	
Baseline	47.20 ± 13.15
After fenofibrate treatment	49.47 ± 13.41
Low density lipoprotein cholesterol, mg/dL	
Baseline	121.63 ± 31.46
After fenofibrate treatment	104.30 ± 31.30
Total cholesterol, mg/dL	
Baseline	191.20 ± 38.97
After fenofibrate treatment	166.83 ± 34.37

^I Medians were used only for the inflammatory biomarkers due to skewness in the data.

Table 2
Top two loci associated with inflammatory response to fenofibrate treatment in GOLDN study participants.

Marker	Chromosomal Position	Major/Minor Alleles	Minor Allele Frequency	Gene	P-value	n
C-reactive protein						
rs13122273	4p15.33	G/T	0.23	--	9×10^{-7}	787
rs17460823	14q13.1	A/G	0.05	<i>NPAS3</i>	2×10^{-6}	787
Interleukin-6						
rs10888935	1p32.3	A/T	0.38	--	7×10^{-7}	787
rs4513299	2q14.1	C/G	0.42	--	4×10^{-6}	775
Interleukin-2 soluble receptor-α						
rs6517147	21q22.11	A/G	0.23	<i>IFNAR21</i>	7×10^{-7}	788
rs11661856	18q23	A/G	0.03	--	1×10^{-6}	788
Tumor necrosis factor-α						
rs17556665	11p15.2	G/T	0.11	<i>SPON1</i>	1×10^{-6}	786
rs11979476	7q32.1	G/T	0.26	<i>AHCYL2</i>	2×10^{-6}	772
Monocyte chemoattractant protein-1						
rs12220898	10q11.23	C/T	0.11	<i>DRGX</i>	1×10^{-6}	786
rs4909764	8q24.23	C/T	0.04	<i>FAM135B</i>	2×10^{-6}	786

¹ Within a 100 kb window

Table 3
Top loci associated with inflammatory patterns pre- and post-fenofibrate treatment in GOLDN study participants.

Marker	Chromosomal Position	Major/Minor Alleles	Minor Allele Frequency	Gene	P-value	n
The hsCRP-IL6 pattern pre-fenofibrate						
rs7911500	10p15.1	C/T	0.07	<i>IL2RA</i>	5×10^{-9}	796
rs12532960	7p14.1	G/T	0.12	--	3×10^{-7}	796
The hsCRP-IL6 pattern post-fenofibrate						
rs6728440	2p42.1	A/G	0.15	--	2×10^{-7}	788
The MCP1-TNF-α pattern pre-fenofibrate						
rs3764563	19p13.12	C/T	0.08	<i>CYP4F8</i>	3×10^{-7}	785
rs786870	10p12.1	A/G	0.28	<i>APBB1IP</i>	5×10^{-7}	789
rs17564315	2p24.1	A/G	0.17	--	7×10^{-7}	789
rs1396485	5q23.1	A/G	0.17	<i>COMMD10</i>	7×10^{-7}	787
The MCP1-TNF-α pattern post-fenofibrate						
rs12722605	10p15.1	A/T	0.18	<i>IL2RA</i>	3×10^{-7}	789
rs391317	11p12	A/T	0.23	--	5×10^{-7}	789