Fibrate pharmacogenomics: expanding past the genome

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Fibrates are a medication class prescribed for decades as ‘broad-spectrum’ lipid-modifying agents used to lower blood triglyceride levels and raise high-density lipoprotein cholesterol levels. Such lipid changes are associated with a decrease in cardiovascular disease, and fibrates are commonly used to reduce risk of dangerous cardiovascular outcomes. As with most drugs, it is well established that response to fibrate treatment is variable, and this variation is heritable. This has motivated the investigation of pharmacogenomic determinants of response, and multiple studies have discovered a number of genes associated with fibrate response. Similar to other complex traits, the interrogation of single nucleotide polymorphisms using candidate gene or genome-wide approaches has not revealed a substantial portion of response variation. However, recent innovations in technological platforms and advances in statistical methodologies are revolutionizing the use and integration of other ‘omes’ in pharmacogenomics studies. Here, we detail successes, challenges, and recent advances in fibrate pharmacogenomics.

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Hypertriglyceridemia (triglycerides [TG] > = 150 mg/dl) is a common health problem in the USA, affecting nearly a third of the population over age 20 [1]. Prospective studies have shown that hypertriglyceridemia is a major risk factor for coronary heart disease (CHD) [2], but there is little consensus on the role of TG in the etiology of CHD. The high intercorrelations between lipids, especially TG and low high-density lipoprotein cholesterol (HDL-C), have historically made it difficult to understand the biological mechanisms related to CHD outcomes [3]. Recently, high-impact genetic studies have shed light on this problem by relating variants in lipid metabolism genes with TG levels and CHD risk [4,5]. These studies provide evidence for a causal relationship between TG levels and CHD, which has prompted renewed interest in TG-lowering treatments [6,7]. The genetics of TG in healthy individuals has been extensively studied, with over 120 studies listed in the genome-wide association study (GWAS) catalog for the ‘triglyceride measurement’ trait (accessed December 2019) [8].

Fibrate medications are a class of drugs commonly used to treat hypertriglyceridemia [9]. Globally available fibrates include gemfibrozil, fenofibrate, bezafibrate, etiofibrate and ciprofibrate. Fibrates work to lower blood triglycerides by reducing the liver’s production of very-low-density lipoprotein (VLDL), the triglyceride-carrying particle that circulates in the blood, and by speeding up the removal of triglycerides from the blood. They have also demonstrated a modest increase in HDL-C level but are not generally effective in lowering low-density lipoprotein cholesterol (LDL-C) [10,11].

While these drugs were in use long before their mechanism of action was understood, it is now relatively well comprehended [12]. Fibrates modify TG levels through activation of the nuclear transcription factor PPAR α , which is highly expressed in tissues that metabolize fatty acids such as liver, as well as in heart, muscle and kidney tissues, and in endothelial and smooth muscle cells [13–15]. In a random cross-sectional survey of Lithuanians, men with PPAR α CC allele were less likely to have elevated serum triglycerides [16] and in obese mice, PPAR α downregulation in results in hypertriglyceridemia [17].

Endogenous PPAR α agonists include derivatives of fatty acid catabolism/metabolism, eicosanoids and other downstream moieties from arachidonic acid [18]. Once activated, PPAR α binds to form a dimer with the retinoid

Figure 1. Fibrate mode of action.

LDL-C: Low-density lipoprotein cholesterol; LPL: Lipoprotein lipase; TG-LP: Triglyceride-lipoprotein (complexes).

X receptor, which anneals to specific PPAR α response elements in the genome (Figure 1) via zinc finger motifs and results in upregulated plasma lipoprotein lipase, increased levels of apolipoprotein A-I, apolipoprotein A-II, apolipoprotein A-V and reduced levels of apolipoprotein CIII, promoting increased clearance of circulating TG-rich lipoproteins [19–22]. Combined, these actions function to increase levels of circulating HDL. For more detail in the role of PPAR α in HDL biogenesis, in LDL and VLDL metabolism and in vascular inflammation, Yu *et al.* offer a detailed review of PPAR α [23].

Fibrates have also been shown to shift the density of LDL-C toward larger, more buoyant particles, resulting in LDL-C particles that are less susceptible to oxidation and have an increased affinity for the LDL receptor (Figure 1) [24,25]. Within the fibrate class, fenofibrate has demonstrated TG-lowering and LDL-C-lowering effects in a majority of patients and is the predominant fibrate used in USA although the magnitude of response is highly variable [26].

While the mechanism of lipid lowering is well-understood, the results of studies that evaluate fibrate treatment in terms of cardiovascular disease (CVD) protection have been both positive and mixed [27–29]. For example, results from the Veterans Affairs High-Density Lipoprotein Intervention Trial indicate beneficial effects, with the reduction of major cardiovascular events, especially for patients with Type 2 diabetes [30]. In contrast, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) fibrate trial failed to show a significant improvement in cardiovascular events [31]. A relatively recent meta-analysis summarized the overall results from all trials published between 1950 and 2010 [32]. Across 18 trials with data for over 45,000 patients, fibrate therapy produced statistically significant results, pointing toward a general positive benefit. The meta-analysis revealed an overall 10% relative risk reduction for major cardiovascular events and an overall 13% relative risk reduction for coronary events but found no benefit for stroke [32].

While more investigation is required to understand these mixed results, the results as a whole demonstrate large interindividual variation in response to fibrate treatment [33]. Family-based studies have established that variation in lipid fibrate response is a heritable trait, with heritability values at the same order of magnitude as other complex traits [33]. The establishment of heritability is a necessary but not sufficient test to motivate further gene mapping with candidate gene or genome-wide approaches under the common disease–common variant hypothesis. Gene mapping has produced results that mimic what has been found for other complex traits, namely a number of successes but few that can be reliably replicated. A ‘missing heritability’ problem is evident from these studies, and the field has responded by interrogating additional genetic models, including rare-variant testing, searching

for gene–gene interactions and epigenomic mapping [34]. Additionally, recent studies have taken an integrative genomics approach to discover exciting new associations across ‘-omes’. Enclosed is a review of important findings in fibrate pharmacogenomics, highlighting recent studies that provide new insights using novel genomic tools and cutting-edge modeling approaches.

Evidence of genetic & epigenetic response variation to fibrate

Candidate gene studies

Initial pharmacogenomics studies focused on candidate gene approaches to identify genetic variants associated with fibrate response. With such a well understood mechanism of action, studies have focused on genes involved in the pharmacokinetics and pharmacodynamics of these drugs. As mentioned earlier, fibrates activate $PPAR\alpha$, which modulate transcription of key drug target genes. These genes include *APOA5*, *LPL* and *APOC3*, which are involved in the metabolism of TG-rich lipoproteins as their promoters contain a *PPAR* response element. Fibrates also increase *ABCA1* transcription, resulting in increased synthesis of HDL in the liver in a *PPAR*-dependent manner [35]. There have been a number of associations in these genes of polymorphisms with fibrate response. Some of the earliest pharmacogenomics work in fibrates evaluated variants in *PPAR\alpha*, with the first studies in one of the older fibrate medications – gemfibrozil [36] and fenofibric acid [37]. Hu and Tomlinson [38] provide an excellent table of such associations in their 2013 review, and we include these findings in Table 1.

Additionally, it is well-known that a major predictor of fibrate response is baseline TG level [70]. Polymorphisms in the *APOA1/C3/A4/A5* cluster have been reliably associated with baseline TG levels in a number of studies [71]. As such, this cluster has also been interrogated for its role in drug response, with several associations found, including a common variant (rs662799) in the promoter region in *APOA5*. The -1131T>C variant in *APOA5* has been associated with higher baseline and postprandial TG levels and enhanced TG response to fenofibrate in patients with dyslipidemia or metabolic syndrome [37]. Additionally, variants in the encoding *LFABP* have been shown to mediate lipid level response to fenofibrate [55].

While these results are certainly not exclusive to one cohort, the majority of candidate gene associations have been found in one cohort from the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study (ClinicalTrials.gov Identifier: NCT00083369). The GOLDN study had two main goals, namely to identify the genes that determine the response of lipids to two interventions: one to raise lipids (a postprandial lipemia challenge through the ingestion of high-fat meal) and another to lower lipids through a 3-week regimen of fenofibrate. The study population was Caucasian families with at least two siblings from the National Heart, Lung, and Blood Institute Family Heart Study at the Minneapolis, MN and Salt Lake City, UT centers. Participants could not have taken lipid-lowering agents for at least 4 weeks prior to their initial visit. A total of 861 participants in the open-label trial received once-daily, 160-mg fenofibrate for 3 weeks and were followed for treatment response. Details of the GOLDN cohort can be found in Lai *et al.* [40].

Because results from the GOLDN dominate the literature for candidate gene associations, findings in this study and their contextualization reflect much of the current state of candidate gene studies with short-term lipid response. Smith *et al.* provide an excellent summary of the effects of 91 variants in 25 candidate genes examined in the GOLDN cohort. Using a systematic approach, the authors summarize the role of genes, demographic and clinical covariates, and their interactions [72]. They find that the most influential factor by far for fibrate response is a patient's baseline TG level. Baseline TG explained 48.1 and 46.7% of the variance in triglycerides and VLDL cholesterol responses to fenofibrate, respectively. Further, the four top-ranked single nucleotide polymorphisms (SNPs) in *APOA4*, *APOC3*, *ABCA1* and *LIPC* explained 11.9% more variation in the change in triglycerides, and four SNPs in *ABCG8*, *LIPC* and *FABP1* explained 7.8% more variation in the change in VLDL cholesterol than baseline triglycerides alone, suggesting an independent effect of genetic variants on lipid response to fenofibrate. Attempts at replicating these top findings have produced mixed results. The association of *ABCA1* variants with fibrate response in GOLDN was replicated in the Bosse study mentioned above (the small clinical trial of gemfibrozil conducted in obese men) [36] but was not supported in the Veterans Affairs HDL Intervention Trial [47].

An important 2012 candidate gene study using the GOLDN cohort integrated GWAS results from blood lipid traits [49]. In this study, the candidate gene list was generated from 95 loci found to be significant in a meta-analysis of GWAS performed for blood lipid traits (discussed more below). The authors then tested the 95 loci associated with lipid traits for association with fibrate response. They reported a statistically significant association in a SNP near *APOA1* to fenofibrate response for HDL and triglycerides. Importantly, the association was replicated in the Pharmacogenetics of Hypertriglyceridemia in Hispanics study [49]. Suggestive associations with fenofibrate response

Table 1. Fibrate pharmacogenomic associations.

Study type	Gene/region	Subjects	Cohort	Ref.
Candidate gene studies	<i>ABCA1</i>	287	GOLDN	[39]
	<i>APOA5</i>	791	GOLDN	[40]
	<i>APOA5</i>	861	GOLDN	[41]
	<i>APOA5</i>	2228		[37]
	<i>LPL/APOA5</i>	110		[42]
	<i>APOE/APOA4/APOA5</i>	861	GOLDN	[43]
	<i>APOE/LPL/PPARα</i>	292		[44]
	<i>APOE</i>	272		[45]
	<i>CYP7A1</i>	864	GOLDN	[46]
	<i>PPARα</i>	63		[36]
	<i>PPARα</i>	827	VA-HIT	[47]
	<i>LPL</i>	899	VA-HIT	[48]
	<i>APOA1</i>	861/ 267	GOLDN/ HyperTG	[49]
	<i>PPARα</i>	300	GOLDN	[50]
	<i>LPL/APOC-III</i>	2385		[51]
	<i>APOA5</i>	50		[52]
	<i>APOB</i>	958	GOLDN	[53]
	<i>APOE</i>	136		[45]
	<i>GCKR/APOA5</i>	844	GOLDN	[54]
	<i>LFABP</i>	130		[55]
	<i>LPL</i>	145		[56]
Genome-wide studies	<i>SCARB1</i>	861	GOLDN	[57]
	<i>PPARα</i>	155	DAIS	[58]
	<i>C10:37</i>	822	GOLDN	[59]
	<i>CPT1A/RNMT/C6orf42</i>	1105	GOLDN/GAW20	[60]
	<i>SMAD3/IPO11/AKR7A3/HSD17B13</i>	1264	ACCORD	[61]
	<i>PBX4</i>	813 781	GOLDN ACCORD	[26]
	<i>HLA-A*33:01</i>	Various	Various	[62]
	<i>12q24/CDH13/PCK1/ZBP1/TMEM19 SCUBE1</i>	793	GOLDN	[63]
	<i>AHCYL2/CD36</i>	817	GOLDN	[64]
Epigenetics	<i>IL2RA/MCP1-TNA-α/IFNAR2</i>	1092	GOLDN	[65]
	<i>1p35.2/3q28</i>	859	GOLDN	[66]
	<i>CPT1A/ETV6/ABCG1</i>	1105	GOLDN/GAW20	[67]
	<i>CPT1A/SARS/ABCG1</i>	1105	GOLDN/GAW20	[68]
	<i>RPTOR/FAM50B/PRR3/GNL1/hsCRP</i> <i>ILF3/SYNPO2/SMPD3/KIAA122L</i>	750	GOLDN	[69]

were observed for markers in or near *PDE3A*, *MOSC1*, *FLJ36070*, *CETP*, the *APOE/C1/C4/C2* cluster and *CILP2*. While the main result reinforces some of the findings from other candidate gene studies, it also suggests novel associations. Arguably, the most impactful part of the study is the replication of *APOA1* in the Hispanic study. *APOA1* was identified as a candidate gene in the GOLDN cohort, so this replication is an important contribution to the field.

All the studies summarized so far have focused on common variants. Recent candidate gene studies have interrogated rare variants for fibrate response. One of the first studies to evaluate rare variants also used the GOLDN cohort [50]. Patients from the extreme tails of response to fibrate (highest and lowest changes in triglycerides) were selected for *PPAR α* resequencing, with a focus on exonic and regulatory variants. This study identified a number of variants within *PPAR α* associated with triglyceride response. Rare variants were also associated with relative change in total cholesterol but not with changes in LDL or HDL.

Another rare-variant candidate gene study evaluated the impact of variants in genes involved in TG and HDL-C metabolism on lipid response to fenofibric acid therapy [51]. This study evaluated 2385 Caucasian patients with dyslipidemia from three concurrent, prospective, randomized, double-blind, clinical trials that examined the efficacy

of fenofibric acid therapy [73,74]. The researchers sequenced the *APOA1*, *APOC2*, *APOC-III* and *LPL* genes and, using rare-variant collapsing and SKAT methods, identified associations. They found that synonymous rare variants in *LPL* and *APOC-III* were significantly associated with post-treatment changes in HDL-C and TG.

Genome-wide studies & meta-GWAS

While the candidate gene studies discussed above have yielded important insights about fibrate pharmacogenomics, less than 10% of variation in fibrate responses is explained by candidate genes [33], which has motivated genome-wide interrogations aimed at uncovering novel associations across the genome. Both genome-wide association and genome-wide linkage approaches have been used, with a number of successes. The majority of GWAS results indicate new associations that weren't included in candidate gene studies, and while candidate genes had nominal significance in many studies, they did not emerge as top hits.

The first GWAS of fibrate response was a meta-analysis of common variants from both the GOLDN study (described above) and the ACCORD study [26]. Briefly, the ACCORD study was a two-by-two factorial trial designed to determine the effects of intensive treatment of blood glucose versus standard treatment and either blood pressure or plasma lipids on CVD outcomes in high-risk patients with Type 2 diabetes (ClinicalTrials.gov Identifier: NCT00000620) [75]. The ACCORD trial tested whether the addition of fenofibrate to background statin treatment further reduced CVD risk compared with statin treatment alone by decreasing TG and increasing HDL-C. In total, 2229 participants with available genotype data were randomized to fenofibrate, and 781 Caucasian patients met additional inclusion criteria for GWAS analysis of the ACCORD data. Fibrate response was measured as the change in lipid levels after 30 days of fibrate exposure in patients naive to fibrate therapy. Meta-analysis of the results for the Caucasian samples from the two cohorts revealed a significant genome-wide association with the *PBX4* gene on chromosome 19 and LDL-C response to fenofibrate. *PBX4* is a known lipid-level gene but was not included in the earlier candidate gene study [49]. Importantly, this locus was replicated at a nominally significant level using data from the Hispanic samples in the ACCORD trial.

The second GWAS study of fenofibrate response was within the ACCORD clinical trial, in which all racial groups were included in stratified analyses [61]. This study was also the first to evaluate the role of genome-wide rare variants in fibrate pharmacogenomics, using both Affymetrix and Illumina exome chip data. Common variant analysis for African-American subjects revealed marginally associated variants in the *SMAD3* and *IPO11* genes. Gene-based rare-variant analysis found novel associations with variants in *AKR7A3* and *HSD17B13* in Caucasian patients. Unfortunately, no replication cohorts were available for African-American samples or for rare variants in Caucasians. These novel findings were followed up with gene expression experiments in mice, which demonstrated reduced expression of *HSD17B13* in mice fed fenofibrate, providing important functional support for this rare-variant finding [61].

The next study to evaluate rare variants at the genome-wide level was performed with the GOLDN cohort [76]. Exome-wide sequencing was used to evaluate associations with fibrate response and interactions with high-fat diets. The results revealed that rare coding variants in *ITGA7*, *SIPA1L2* and *CEP72* were respectively associated with several measures of fibrate response, namely fasting LDL-C change, triglyceride postprandial area under the increase (AUI), and triglyceride postprandial AUI response. Replication efforts using the Heredity and Phenotype Intervention Heart Study [77] were unsuccessful, but functional validation efforts were successful. Gene transcript levels of *SIPA1L2* were found to be associated with triglyceride postprandial AUI. Again, this study highlights the need for continued replication efforts for rare-variant results.

The family-based data within the GOLDN cohort has also been used for genome-wide linkage analysis. Linkage analysis using microsatellite markers identified regions on 1p35.2 and 3q28 weakly linked ($\text{LOD} > 2$) to changes in plasma adiponectin concentrations before and after fibrate therapy [66]. The peak on chromosome 1 included two possible candidate genes – *IL22RA1* and *IL28RA* – and the peak on chromosome 3 is near the location of the adiponectin gene [66]. Other regions suggested modest linkage with logarithm of the odds (LOD) between 1.5 and 2.0 [66]. In a later study using SNP data, Hildalgo *et al.* identified significant genomic regions relevant to fibrate response [78]. They performed a multipoint linkage scan and then selected families demonstrating evidence of linkage. The results showed a highly significant linkage peak on chromosome 7 with a change in LDL, even after adjusting for baseline values. While the peak was broad, this suggests that the *ABCB4* and *CD36* genes within this peak may be biologically relevant. With linkage analysis to identify both rare and common variant signals, these candidates could be investigated as candidate genes in studies such as the ACCORD trial.

Other fibrate response measure studies

While the above-mentioned studies are focused on changes in lipid levels as a direct measure of drug response, recent studies have expanded genome-wide approaches to evaluate other effects of drug treatment, including adverse events. The GOLDN cohort was again used to perform GWAS analysis on variability in levels of adiponectin after fenofibrate treatment [63]. Adiponectin is an adipose-secreted protein that plays a role in insulin sensitivity, HDL-C levels and inflammatory patterns. Fenofibrate treatment has been shown to raise adiponectin, with broad variability associated with this change across patients. The analysis revealed two significant results and several novel suggestive associations. The study replicated a previously reported association with the *CDH13* gene and found a significant association between rs2384207 in 12q24 and fenofibrate-induced change in circulating adiponectin. This region has previously been linked to several metabolic traits [63]. The suggestive results include associations with SNPs near the *PCK1*, *ZBP1*, *TMEM18* and *SCUBE1* genes. The findings from single-marker tests were corroborated in gene-based analyses.

Another recent study evaluated associations of drug exposure with drug-induced liver injury (DILI), which has an estimated annual incidence between 10 and 15 per 10,000 to 100,000 persons exposed to prescription medications [79]. DILI accounts for approximately 10% of all cases of acute hepatitis and is the most common cause of acute liver failure in USA [80]. Using a diverse, international case-control cohort of over 11,000 patients, genome-wide association analysis identified SNPs associated with DILI as an adverse outcome to a range of drugs and drug classes, including fibrates. A known association with rs114577328 (a proxy for *A*33:01*, an HLA class I allele) was reproduced, and a new association with rs72631567 on chromosome 2 was also identified [81].

Complex etiology

While there have been a number of provocative findings using single-nucleotide variants, fibrate response is a complex trait. As with almost all complex traits in humans, it is clear that only a small portion of variability in the trait is explained by single-nucleotide variants. Drug response is highly heritable, but studies so far have suffered from the grand ‘missing heritability’, now ‘hidden heritability’, challenge that is so common in human genetics [34,82]. Because of this, pharmacogenomic studies have expanded to other ‘-omes’ and to analyses that evaluate more complex models such as gene–gene interactions.

Epigenetic evidence of interindividual responses to fibrate

For all complex traits, epigenetic programs may account for a significant fraction of ‘missing heritability’ [83]. Epigenetic modifications such as DNA methylation and chromatin assembly states reflect the high plasticity of the genome and contribute to altered gene expression in a stable manner without modifying genomic DNA sequences. An epigenetic etiology of fibrate response is especially promising due to biological links between the PPAR α pathway, homocysteine and S-adenosylmethionine, a source of methyl groups for the DNA methylation reaction [84]. Technological advances have made the epigenome more readily accessible, and a growing literature shows consistent components of complex traits found to respond to environmental conditions such as drug exposure to also be epigenetically inherited. It is also well-established that a number of associations with differentially methylated regions (DMRs) and lipid traits are the target traits of fibrate therapy, making investigation of interactions between DMRs and drug treatment an intuitive next step [85]. Recently, Nustad *et al.* demonstrated significant genome-wide DNA methylation heritability changes (21%) in lipid response pre- and post-fibrate treatment [86].

The first studies on differential methylation with fibrate treatment were completed using the GOLDN cohort. A 2015 study used the Illumina Infinium HumanMethylation450 array to assess methylation both before and after a 3-week treatment regimen with fibrate [87]. An epigenome-wide study was conducted assess associations with TG, HDL and LDL. The results were generally negative at either the genome-wide or nested-candidate gene level. As the GOLDN trial only evaluated changes with 3 weeks of exposure, it is unclear if long-term exposure would result in more significant changes. Thus, evaluating changes after long-term exposure is an important goal for the field. This study adopted the relatively straightforward approach of testing each DMR for lipid-level changes before and after treatment, and a mixed-effects model was fit for each DMR [87].

Subsequent analysis of methylation data from the GOLDN cohort using a different approach found DMRs to be associated with lipid-level changes even at 3 weeks of exposure. Namely, Almeida *et al.*’s approach based on analysis of genetic variance components returned a few promising results [88]. As shown by other studies, highly heritable lipid traits before treatment need to be considered in analysis [85,89,90]. Principal components analysis was used to decorrelate the methylation data before and after fibrate treatment, allowing comparison of the two distributions.

The data for DMRs located near genes were filtered and a random-effects association test was implemented to screen for genes whose methylation patterns explain substantial portions of HDL heritability. This analysis revealed a novel association with the *TMEM52* gene (now *CEMIP2*), which is a cell-surface hyaluronidase transmembrane protein [88]. Further follow-up is needed to replicate and/or functionally validate this association, but this represents an important new finding.

Another re-analysis, again based on the GOLDN data, also found significant differences in methylation sites in response to fenofibrate [91]. Again, the study involved a strikingly different indirect approach to analysis based on the reasoning that CpG sites that exhibit a genetic response to fibrate would exhibit highly familial and variable methylation levels post-treatment but not pretreatment. Using post-treatment sibling correlations and standard deviations, and comparing values before and after treatment, the researchers identified significant association within the *KIAA1804* and *ANAPC2* genes. These genes also had highly significant methylation quantitative trait loci (meQTL). As with the previous analysis, replication and functional studies are needed for follow-up.

The GOLDN study has also been used for epigenomic mapping of inflammatory response to short-term fibrate treatment [69]. In this study, the researchers sorted blood cells to reduce cell-type heterogeneity and performed epigenotyping on CD4+ T cells using the Illumina Infinium HumanMethylation450 array. They conducted association analysis at the genome-wide level with a change in concentration of a number of inflammatory cytokines before and after treatment. The most significant results were for changes in hsCRP, IL-2sR, and IL-6. Highly significant associations were found in intergenic regions and in the following genes: *KIAA1324L*, *SMPD3*, *SYNPO2*, *ILF3*, *PRR3*, *GNL1*, *FAM50B*, *RPTOR*. Additionally, they evaluated epigenetic associations with overall immune response profiles quantified using principal components analysis and, simultaneously using this collapsed information from all cytokines, identified additional associations.

Epistasis modeling

In attempts to continue to explore more complex genetic models, studies have expanded from the relatively standard approach of testing for single SNP and single DMR associations, and the search for ‘hidden heritability’ has been extended by examining other known sources of variation. The importance of epistasis (gene–gene and gene–environment interactions) in pharmacogenomics traits in general is well-established, and studies have begun to explore such interactions with fibrate [92,93]. Integrative genomic studies have the aim of enabling a better understanding of the complex biology of drug response, with promising initial successes.

A candidate gene study within the GOLDN cohort was one of the first to investigate gene–gene interactions [49]. Genes reliably associated with blood lipid traits were used as candidate genes to test for association with the lipid-lowering effects of fenofibrate. The researchers identified several genes associated with changes in HDL-C, LDL-C, total cholesterol and triglyceride concentrations and followed up by conducting tests for gene–gene interactions among the significant SNPs. Within the GOLDN cohort, linear modeling revealed a highly significant epistatic association between rs10401969 near *CILP2* and rs4420638 in the *APOE/C1/C4/C2* cluster with total cholesterol response to fenofibrate. The researchers sought to replicate this epistasis model and successfully replicated this interaction in the HyperTG cohort. Generally, replication studies and successes for gene–gene interactions are relatively limited in pharmacogenomics, so this result is exciting.

Several studies have looked for interactions with environmental factors. While all pharmacogenomics studies can be considered gene–environment interaction studies, with drug exposure the environmental exposure, some studies have demonstrated that other environmental factors such as diet mediate both baseline lipid values and fenofibrate response. Irvin *et al.* examined the impact of a high-fat diet on drug response in a candidate gene study but did not detect a direct gene–environment–environment interaction [94]. A previous study established that high-fat meals modify fibrate response [76,94], and genetic data from the GOLDN cohort was used to determine the impact of fibrate treatment on the association of *APOE* genotype differences and association with baseline lipids. The researchers found that while *APOE* was not directly associated with drug response (change in lipid levels), the association of overall lipid levels with *APOE* were conserved even after fibrate treatment, with slight mediation of effect sizes.

Complex modeling through the genetic analysis workshop

With the goals of further interrogating complex models of fibrate response while also evaluating and comparing different analysis tools, the GOLDN data were the focus of the 20th Genetic Analysis Workshop (GAW; www.gaworkshop.org). GAWs are a major collaborative effort among genetic epidemiologists to evaluate and compare

statistical genetic methods for relevant current analytical challenges in the field. While normally concentrated on disease or other complex trait questions, the 20th GAW was focused on fenofibrate pharmacogenomics [95]. As the focus of the workshop, simulated data based on the GOLDN cohort was used to rigorously evaluate the statistical methods used, and the real data were used to deliver new insights into fibrate response [95]. While a review of all the statistical methods used in the workshop is beyond the scope of this paper, Cherlin *et al.* [96] and Darst *et al.* [97] provide excellent perspectives on the diversity of methods employed. The following outlines some of the major findings of the 20th GAW.

The GOLDN data present unique challenges and opportunities from a methods perspective. While the vast majority of pharmacogenomics studies are performed using unrelated individuals [98], GOLDN is a repeated-measures family-based cohort. Analysts that participated in the workshop were challenged to address issues related to epigenetics, genetics of treatment response, analysis of repeated measures, GWAS, genotype-by-smoking and genotype-by-age interactions and joint analyses of multiple phenotypes, among others. Many of the methods used rely on careful consideration of the family structure, including estimates of heritability. Several gene-based analyses were proposed, many of which incorporated SNP and methylation data. Generally, these gene-based methods have superior performance compared with variant-by-variant approaches. Genomic prediction and genetic risk score methods were used and generated promising results. Integrative genomics approaches were also used. A special issue of BMC Genetics published many papers with results from the workshop [95]. A few highlights are mentioned here.

Pathway and network approaches resulted in a number of compelling findings and expanded on modeling the complex etiology. Lim *et al.* performed network analysis to understand the interactions between fibrate treatment, methylation and lipid levels [99]. They identified a candidate probe-set list by testing for nominal association with pretreatment triglyceride levels. This candidate set was used as input for weighted gene correlation network analysis (WGCNA) to construct pre- and post-treatment methylation networks of these probes. The researchers then identified modules/clusters that were topologically different between the pre- and post-treatment measurements. They then tested the modules for overrepresentation of biological pathways and found significant enrichment in the sphingolipid signaling pathway, proteoglycans in cancer and several metabolic pathways. Nustad *et al.* also successfully applied network and pathway approaches to identify modules of CpGs that changed topology before and after treatment [100]. Further, Wei and Wu used gene set enrichment analysis and found overrepresentation of the cancer- and metabolism-related pathway [68].

Other important contributions were the evaluation of novel linkage approaches [59], interrogation of the parent of origin effects [101] and genomic prediction using genomic-best linear unbiased prediction methods to demonstrate the potential for drug response prediction [102]. Additionally, causal inference and Mendelian randomization approaches were used to move beyond association analysis [60]. Mendelian randomization analysis can also be used for causal inference. Such analysis can test whether an observational association between variants and drug response reflects a causal relationship correcting for the confounding, reverse causation and various biases that are challenges in observational studies. While such approaches have been used since the early 1990s, their application in pharmacogenomics is a new area of research [103].

Discussion

The enclosed review aims to summarize the current state of the field in fibrate pharmacogenomics. There have been a number of recent promising findings regarding both common and rare genetic variants. Further, associations have been identified with both candidate gene and genome-wide approaches, and replication of several of the genes identified has begun. PharmGKB (www.pharmgkb.org) is an excellent resource for the evolving list of reported SNP associations and provides detailed information on the direction of effect of the variants reported. (www.pharmgkb.org/chemical/PA449594/variantAnnotation). Consistent findings regarding *PPARA*, *APOE E2*, *APOA1*, *APOB*, *APOC3*, *APOA5*, *IL6* and *LPL* are promising but have not yet risen to the level of actionable findings. Extensive work toward validation and replication are needed. Despite the broad use of fibrates in the population, there have been a limited number of cohorts that have been amenable to pharmacogenomic interrogations. New cohorts need to be evaluated. Future studies should focus in particular on increasing the diversity of patients included. The GOLDN cohort is entirely Caucasian, and while there is some diversity in other cohorts, the vast majority of patients studied are Caucasian and greatly limits the understanding of drug response across the entire population.

Overall, this review reveals that like many drug response traits, fibrate response is complex. This complexity has resulted in challenges, which have been heavily reviewed in the literature [34]. Two broad approaches have been used

to address these challenges. First, technologies have advanced, and the definition of pharmaco-‘genomics’ within the field has grown. Further, gene mapping efforts have been expanded from the ‘genome’ to the ‘epigenome’, with promising results that support further interrogation of the epigenetic mechanisms of response. A major limitation of the epigenetic work so far is that it has all been conducted within a single cohort – GOLDN. To truly move forward with this line of investigation, the work must be expanded to additional cohorts, either through replication efforts for the initial associations or further work on epigenome-wide studies. To the best of the authors’ knowledge, there is no other cohort with available genome-wide epigenetic data. However, this could be an important direction for cohorts, including ACCORD, the Fenofibrate Intervention and Event Lowering in Diabetes study, HyperTG, and potential resources related to major biobanking and electronic health records studies, that would enable them to expand to the epigenome.

Second, complex analysis strategies that embrace rather than ignore the apparent complex etiology have been used. The application of methods to identify gene–gene interactions, model changing pathways and networks and integrate different types of data has expanded the understanding of fibrate response genetics. The use of the GOLDN data in a GAW competition was an exciting opportunity that both motivated and evaluated new statistical approaches for fibrate pharmacogenomics. This exercise not only found new associations with fibrate response, but also resulted in novel statistical approaches that will benefit pharmacogenomics in general.

A number of directions are likely for fibrate pharmacogenomics in the near future. Continued efforts regarding replication and validation should be a priority. Again, the limited availability of cohorts with fibrate use is a challenge, and it is likely that electronic health record data will be needed for such efforts. Functional follow-up in cell lines and model organisms will play a crucial role given the limited opportunities for replication. Naturally, in addition to expanding epigenome studies, it is anticipated that the expansion to other ‘-omes’ will continue, and studies have already focused on metabolomics [104] and the microbiome [105]. Another challenge in fibrate pharmacogenomics is that fibrates are rarely used alone at the population level. For example, fibrates are commonly used along with statins, and the complexity of response to combination therapies add additional layers of complexity for gene mapping. For pharmacogenomics findings to translate into clinical applications, such context will be key.

As the field worked toward an actionable understanding the pharmacogenetics, it will be important to keep in mind the changing use of fibrates in clinic. Fibrates are prescribed as ‘broad-spectrum’ lipid modifying agents and in the 1990s their role in preventing cardiovascular outcomes seemed clear. The Helsinki Heart Study [27] and Veterans Affairs High-Density Cholesterol Intervention Trial [106] initially demonstrated significant benefit, especially in patients with atherogenic dyslipidemia. However, this clarity disintegrated following the negative outcomes reported by the Bezafibrate Infarction Prevention, Fenofibrate Intervention and Event Lowering in Diabetes and ACCORD randomized controlled trials. While there is a clear impact of lipid levels with fibrate treatment, as summarized by an important meta-analysis in 2010, the overall efficacy of fibrate monotherapy in preventing cardiovascular events is modest at best [32].

The strongest reduction in cardiovascular events appears to be in patients with high triglyceride and low HDL cholesterol levels, but whether the addition of fibrates to statin therapy will further reduce CVD is uncertain [107]. There is also emerging evidence that fibrates may have an important clinical role in reducing microvascular complications in diabetic patients, including progression of retinopathy, progression of microalbuminuria and nephropathy, development of sensory neuropathy and leg amputation [108]. It is certainly clear with such modest effects, the number to treat is high and identifying individuals that would respond would improve overall care. The median absolute risk reduction for 5-year cardiac outcomes is estimated at about 2.15% for patients with dyslipidemia, corresponding with a number needed to treat of 47, and 0.22% in patients without dyslipidemia (number needed to treat 455) [109]. The ability to predict those that would respond, and reduce treatment of likely nonresponders would dramatically improve these statistics while saving patients and healthcare systems unnecessary costs [110]. Further, as alternative treatment options emerge, finding the optimal drug with pharmacogenomics is a critical future direction for the field [111]. With over 10 millions fibrate prescriptions in the USA a year, there is an enormous opportunity for impact if biomarkers can be found [112].

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Executive summary

- In USA, hypertriglyceridemia (excessive triglyceride [TG] levels above 150 mg/dl) affects nearly a third of the adult population.
- Coronary heart disease (CHD) is a complex trait and recent studies have provided genetic evidence for a relationship between TG levels and CHD.
- Fibrates are a class of drugs effective in lowering TG levels with a modest increase in CHD-protective high-density lipoprotein cholesterol (HDL-C) levels.
- Fibrates primarily function to effect levels of TG and HDL-C by activating the nuclear transcription factor peroxisome proliferator-activated receptor- α (PPAR α) resulting in nuclear localization and altered transcriptional regulation of multiple genes involved in lipogenesis and metabolism and transport ultimately resulting in:
 - Increased TG clearance.
 - Increased lipoprotein lipase levels.
 - Decreased low-density lipoprotein cholesterol expression, density and oxidation.

Genetic evidence modulating fibrate response – candidate gene studies

- Polymorphisms in the *APOA1/C3/A4/A5* cluster associated with fibrate TG responses.
- Variants in this cluster in addition to variants in *LFABP*, *LIPC*, *ABCG8* and *FABP1* explain almost 20% of the variation in lipid responses to fibrates.
- Rare variant analyses have identified additional variants in *PPAR α* , *LPL* and *APOC-III* associated with TG and HDL-C fibrate treatment responses.

Genetic evidence modulating fibrate response – genome-wide association & meta-genome-wide association studies

- Initial genome-wide association study in the GOLDN and ACCORD trials combined identified variants in *PBX4*, *SMAD4*, *IPO11*.
- Rare variant analyses in ACCORD discovered variants in *AKR7A3* and *HSD17B13* associated with fibrate response, the latter of which was followed up and replicated in mouse studies.
- Rare variant analyses in GOLDN discovered additional hits in *ITGA7* *SIPA1L2* and *CEP72* associated with dramatic responses to fibrates.
- Genetic linkage analyses in GOLDN identified regions on 1p35.2 and 3q28 linked to changes in plasma adiponectin concentrations before and after fibrate therapy.

Other fibrate response measure studies

- Associations between adiponectin response to fibrates and rs2384207 in *12q24*, with suggestive associations in or near *PCK1*, *ZBP1*, *TMEM18* and *SCUBE1* genes.
- Adverse drug induced liver injury response to fibrate associated with *A*33:01*, an HLA class I allele.

Epigenetic evidence modulating fibrate response

- Differential methylation of the *KIAA1804* and *ANAPC2* genes was found to be associated with fenofibrate responses.
- In GOLDN, inter-individual variation in inflammatory responses in fenofibrate were found to be highly significantly associated *hsCRP*, *IL-2* and *IL-6*, as well as significant findings in *KIAA1324L*, *SMPD3*, *SYNPO2*, *ILF3*, *PRR3*, *GNL1*, *FAM50B*, *RPTOR*.

Epistasis modeling of hidden heritability

- Modeling of gene–gene and gene–environment interactions has identified epistatic associations with sentinel single nucleotide polymorphisms near *CILP2* and the *APOE/C1/C4/C2* cluster with cholesterol responses to fibrates. More importantly, these findings were replicated.

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