STUDIES INVESTIGATING THE IMPACT OF SELECT GENOTYPES ON THE PHARMACOLOGICAL EFFECT OF FENOFIBRATE

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DEDICATION

This dissertation is dedicated to

My mother

Makiah Shehabaldeen

And

My late father

Mustafa Arafah

ABSTRACT

Mixed outcomes from clinical trials that tested the effect of combining statins and fenofibrate, posed a challenge in using this antidyslipidemic medication. One significant challenge of using fenofibrate is the high inter-subject variability in lipid response, which can range from -82 to 132% for triglyceride change from baseline. This magnitude of variability suggests the involvement of non-environmental factors, such as the genetic source of the variability. We identified a genetic variation, UGT2B7 A-327G, harbored in a key metabolizing gene of fenofibrate. It was the source of a 17% differences in the percent-change of triglycerides, post-fenofibrate treatment, between UGT2B7 A-327G genotype groups. We hypothesized that this lipid response variation was due to UGT2B7 A-327G's effect on serum concentration of fenofibrate (exposure). We confirmed this hypothesis by conducting Fenofibrate and the Pharmacogenetic Impact (FPI) study, a pharmacokinetic study aimed at explicitly quantifying the effect of UGT2B7 A-327G on fenofibric acid serum concentration. Furthermore, we discovered that UGT2B7 A-327G modulated the uricosuric effect of fenofibrate in a same manner it modulated the antidyslipidemic response. We considered this as another confirmation of the importance of this genetic variation on fenofibrate's response. We also confirmed that another genetic variation, UGT1A1*28, had an effect on serum concentration of fenofibric. In contrast to UGT2B7 A-327G, UGT1A1*28 did not cause a lipid response variation. However, UGT1A1*28 did

modulated the effect of fenofibrate on bilirubin level. Recognizing and quantifying the effect of these genetic variations will assist in optimizing fenofibrate treatment.

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CHAPTER I

LITERATURE REVIEW

1.1. Introduction:

According to the World Health organization, cardiovascular diseases (CVDs) remain the leading cause of death worldwide representing 30% of all causes of death. This can be explained in part by the rising incidence of obesity, metabolic syndrome and type 2 diabetes (T2DM) worldwide.[1] Dyslipidemia, a main contributor to CVDs, is commonly associated with each of these conditions. The association between dyslipidemia and CVDs had been established since the 1950's and the general belief was that low-density lipoprotein cholesterol (LDL-C) was the main contributor to CVDs. However, the independent contribution of elevated triglyceride (TG) and/or low high-density lipoprotein cholesterol (HDL-C) were not yet fully appreciated, and until today, represented an underappreciated source of risk for CVD.

Fortunately, dyslipidemia is a modifiable risk factor and several approaches have been implemented to control it. Diet and lifestyle-modification were the first to be used; which encouraged the restriction of the daily fat intake and the increase of physical activity. However, dietary lipids represent only a small percent of the lipids found in the body; the majority are manufactured endogenously by the liver. This demonstrated the compelling need for a pharmacological intervention that can better control the endogenous production of lipids. Among the first agents to be used to control dyslipidemia was niacin (vitamin B3). Even though niacin was discovered in 1873, its antidyslipidemic effect was only recognized in 1955.[2] In 1967, clofibrate was

introduced as a prototype of a new category of medication used for the treatment of patients with dyslipidemia, known as the fibrates. However, both niacin and clofibrate had a limited effect on LDL-C, lowering it by 5 to 20%. The search for a more potent antidyslipidemic drugs continued until 1975, when fenofibrate was approved in France as a more potent derivate of clofibrate. Another fibrate, still commonly used, is gemfibrozil, which was introduced in 1982 and later other fibrates followed. Until that date no outcome trial had confirmed the assumption that CVDs incidence can be reduced by controlling lipid levels. It was not until 1978 when the World Health Organization (WHO) trial was published. The WHO trial was a double blinded, multicenter placebo controlled trial. It included over 15,000 middle-aged men who had been followed for an average of 5.3 years. The hypothesis of this trial was that the incidence of ischemic heart disease in middle-aged men can be reduced by lowering high serum cholesterol levels. The WHO trial reported a 21% reduction in the incidence of nonfatal myocardial infarction in patients using clofibrate compared to placebo.[3]

Fibrates were predominantly prescribed for the treatment of dyslipidemia until 1987, when Merck introduced another new class of antidyslipidemic drugs. This new class of agents inhibited the enzyme HMG-CoA reductase. This enzyme is, a rate-limiting step in the endogenous production of cholesterol, a significant source of endogenous cholesterol. This class of medications is commonly referred to as statins.

The first member of the statin class was lovastatin and one year later Merck released a more potent statin, simvastatin (Zocor®). The statins were found to lower LDL-C up to 46% compared to placebo.[4] That was superior to any of the other antidyslipidemic drugs at that time. Even though clinical trials demonstrated that lowering lipid levels can lower the incidence of CVDs, there was still a controversy about its ability to improve survival, particularly in the patients with established CVD. In 1994 the Scandinavian Simvastatin Survival Study (4S) trial was published. The 4S trial hypothesized that lowering lipids with simvastatin would improve survival of patients with established CVD. It included 4444 patients with angina pectoris or previous myocardial infarction. Patients were randomized to a double-blind treatment with simvastatin or placebo and were followed for an average of 5.4 years. The study concluded that simvastatin reduced the overall mortality by 40% compared to placebo.[5] Since then the use of statins has expanded rapidly and includes several agents competing for a market share and are contributing to the evidence of the now widely accepted and substantive morbidity and mortality benefit associated with this class of drugs. This rise in proof of benefit was accompanied by a corresponding increase in use. This was exemplified by the fact that atorvastatin (Lipitor®) has been number one drug in sales in the US market since 2000 until the date of this writing. A 2010 meta-analysis study that included 26 major clinical trials and 170,000 participants who underwent statins treatment for at least 2 years confirmed the safety of statins and its efficacy in reducing CVDs.[6]

Despite the clinical and commercial success of the statins, the interest in fibrates has been renewed following awareness that a substantial risk of CVD events (~60%) remained for patients using statins even after achieving the optimal LDL-C serum levels recommended by the Adult Treatment Panel (ATP) III guidelines.[4,7] This came to be known as "residual cardiovascular risk" or "residual risk". This residual risk is even higher in T2DM patient reaching up to 91%.[8] Furthermore, new evidence has emerged suggesting that TG and HDL-C serum levels are independent CVD risk factors. Statins' effect on TG and HDL-C serum levels is limited, lowering TG by 7-30% and raising HDL-C by 5-15 %.[9] In addition the global rise in obesity, metabolic syndrome and T2DM [10,11], for which high TG and low HDL-C is a common component, emphasize the need for agents like fibrates and niacin. Both fibrates and niacin are very effective in lowering TG and raising HDL-C serum levels. The efficacy of niacin in reducing CVDs has been demonstrated in the Coronary Drug Project (CDP) trial. This was is a placebo-controlled trial which recruited over 8,000 patients with a history of myocardial infarction and followed them for 15 years. The trial concluded that niacin lowered mortality rate by 11% when compared to placebo group.[12] However, the main limitation to niacin use is the flushing of the face and upper body which can last for hours. Flushing occurs in ~80% of patients which can lead up to 6% of the patients to discontinue niacin therapy. Flushing can be managed by dose titration and generally subside after few weeks of continued therapy.[13,14] However, considering that patients

with metabolic syndrome and T2DM represent important candidates for the addition of niacin or a fibrate to their statin therapy, the choice of which class remains a debate. Niacin had been shown to adversely affect the glycemic control in T2DM.[15] Also, niacin can increase the uric acid levels [15,16] which is a common finding in metabolic syndrome patients.[17] In contrast, fibrates have not been shown to adversely affect the glycemic control and significantly lower uric acid levels.[15,16] Furthermore, fibrates, especially fenofibrate, have been shown to lower the incidence of microvascular diseases (vision loss, renal failure and amputation of extremities) in T2DM.[18-21] The two fibrates available in the USA are fenofibrate and gemfibrozil. They are very comparable in their ability to lower lipids levels except that gemfibrozil has almost no capacity to lower LDL-C[22] in contrast to fenofibrate. The advantage of fenofibrate over gemfibrozil is its relative lack of drug interactions. Fenofibrate's relatively clean metabolic profile, compared to gemfibrozil, appears to result in a lower probability of rhabdomyolysis. Although rare, this potential life threatening adverse drug reaction, has been observed with gemfibrozil-statins combination.[23] In a retrospective cohort-study which included over 44,000 patients using a statin and fenofibrate or gemfibrozil from the period of 2004 to 2007 had compared the incidence rates of rhabdomyolysis using claims data from a large United States health insurer. The risk of rhabdomyolysis increased 3.75 fold in patients using fenofibrate-statin combination and 5.11 fold in patients using gemfibrozil-statin compared to patients using statin only.[24] This higher incidence of myopathy observed with gemfibrozil might be due to its ability to inhibit

of CYP2C8 which can affect the metabolism of certain member of the statins group, in contrast fenofibrate dose no affect this enzyme.[23] As a class, fibrates represent a considerable share, ~11%, of the 18 billion dollar in the USA market for dyslipidemia medications [25], with worldwide sales of fenofibrate estimated at over 2 billion US dollars a year. All previously mentioned aspects regarding fenofibrate highlight its role in the upcoming fight against the growing danger of obesity, metabolic syndrome, and T2DM. Both niacin and fenofibrate can be used in the metabolic syndrome and type 2 patients to control lipid levels in attempt to control the CVD residual risk. However, fenofibrate might be a better choice since it eliminates the need for a close monitoring of glycemic index and the uric acid levels. Further, niacin does not provide the microvascular protection that fenofibrate offers.

The main challenge in using fenofibrate is the high inter-subject variability in triglyceride-lowering response. The current lack of understanding of determinants resulting in this high inter-subject variability has limited our ability to identify which patients are more likely to benefit from drug therapy compared to others. This uncertainty in identifying ideal candidates to receive the drug can lead to poor choices resulting in treatment ineffectiveness and overall lack of lipid goal attainment. In order to improve the predictability of optimal response, we must understand the sources for response-variation. Among known sources of response-variability, one important source yet to be fully understood is genetics. Although a systematic exploration of the

relationship between the candidate genes and their effect on pharmacodynamics of fenofibrate has begun a more comprehensive focus on the role of drug metabolism on the pharmacokinetics and hence complementary contribution to response has not been studied. In the upcoming chapters of this thesis, the effect of selected genetic variation, single nucleotide polymorphism (SNP), in genes which are believed to be involved in the metabolism, transportation, elimination, or considered as targets for fenofibrate will be explored. The remainder of this chapter will provide a general background on fenofibrate's mechanism of action, pharmacokinetics, pharmacodynamics, and the pharmacogenetics of fenofibrate. This will shed a light on some of the obstacles in using fenofibrate and can clarify the reasons for the selecting the genes we chose to pursue.

1.2. Mechanism of Action

The following explains how fenofibrate exerts its lipids-altering effect; the most prominent basis for its clinical use. Fenofibrate's effect according to Stael et al. [26] and others [27] occurs in the hepatic cell's nucleus, where it affects the transcription of a number of genes that control lipids catabolism, transportation, storage and metabolism. The net clinical effect in a patient with dyslipidemia is a decrease in serum levels of TG and LDL-C and an increase in HDL-C serum levels. Fenofibrate's mechanism involves the engagement of four main elements; **1**) target genes which possess a specific DNA

sequence know as hormone response elements (**HRE**) located in the promoter area of the gene, **2**) a heterodimer complex which consists of two proteins of the nuclear hormone receptors includes peroxisome proliferator-activated receptor alpha (PPARα) and retinoid X receptor (RXR) component (**PPARα+RXR**), **3**) a **corepressor** protein and finally **4**) a **ligand** which is a signal-triggering molecule.

The heterodimer complex **PPARα+RXR** works as an intermolecular on-off switch for the transcription of a number of genes by attaching to the **HRE.** However, this mechanism in the absence of a **ligand**, endogenous or exogenous, will be stopped when a **corepressor** protein heterodimerizes with **PPARα+RXR**. The exogenous ligand fenofibric acid works by binding to the PPARα of this heterodimer complex dissociating the **corepressor** protein and thus allowing the gene to be transcribed. Those genes which have been indentified to contain the **HRE** and proven to be affected by fenofibrate include lipoprotein lipase (LPL), apolipoprotein C-III (Apo C-III), fatty acid transporter protein (FATP), acyl-CoA synthetase (ACS), apolipoprotein E (ApoE) [28], apolipoprotein A (ApoA-I), ApoA-II, and ApoA-V.[26]

These target genes are what fenofibrate exerts its direct effect on, to modulate its pharmacological effect. Exploring the genetic variations in these genes has the potential to determine the sources and the magnitude of each genetic variation on the response variability to fenofibrate.

1.3. Pharmacokinetics

Pharmacokinetic parameters are used to describe the effect of the body on the drug fenofibrate. It includes the effect on fenofibrate's absorption, distribution, metabolism and elimination. Genetic variations in genes involved in any of the previously mentioned pharmacokinetics processes might alter the response to fenofibrate.

1.3.1 Absorption

Fenofibrate's historically available standard formulations suffered from a low bioavailability of approximately 30%.[29] It is partly due to the high water insolubility of the drug which makes it absorption extremely erratic. To improve fenofibrate's absorption, pharmaceutical companies have worked on improving the particle size and dissolution rate, which are crucial determinants of the bioavailability. Reducing the particle size would increase the overall surface area, which would increase the dissolution rate and subsequently improve the bioavailability.[30] In 1998, a new formulation with a reduced particle of fenofibrate (microparticles) was approved by the USA FDA improving the bioavailability to 65%.[31] However, the bioavailability was dependant on the food intake and it was found that patients consuming high fat had

higher bioavailability.[32] In 2004, a new formulation with nanoparticles of fenofibrate was approved. For this formulation, bioavailability was independent of fed condition.[33] Until 2008, only fenofibrate, which is a prodrug of fenofibric acid, was available. However, in 2008 Trilipix®, which is formulated as choline salt of fenofibric acid, was approved. It achieved a higher absolute bioavailability compared to the nanoparticle formulation, 81% and 69% respectively.[34] Standard formulations required the use a dose of up to 300mg/day. However, improvement in bioavailability lead to a decrease in the amount of fenofibrate dose required in order to realize an equivalent antidyslipidemic effect. This raised the issue of the bioequivalence and therapeutic interchangeability. There has been extensive research regarding fenofibrate bioavailability following its introduction in 1975; every year several new studies are published that assess the effectiveness of the new formulation in improving bioavailability. Some patents held by pharmaceutical companies are for fenofibrate dosage form itself which offers a higher bioavailability. However, a literature search appears to show that the newer formulations have done little more than extending the patent of these new formulations. Furthermore, there is no direct proof that these improvements in the formulations have resulted in an improved outcome or less toxicity.

When comparing studies using fenofibrate to compare the response, consideration of fenofibrate manufacturer, dose, and formulation used can be vital.

This is because the absorption and bioavailability vary considerably the formulations. Furthermore, formulations with different dose or dosage form are not considered as therapeutically interchangeable. For example: a company like Abbott can claim that their older formulation of 160mg Tricor® is equivalent, in lipid-response, to the newer formulation 145mg Tricor®. In contrast, a company like Teva cannot claim that their 160mg Lofibra® is equivalent to Abbott's 145mg Tricor®, thereby they are not therapeutically interchangeable.

It is still unclear if the absorption of fenofibrate from the gastrointestinal tract into blood circulation is achieved by passive diffusion, or through transporters, or both. A future identification of a transporter and a variation in the gene coding it might further explain the variation in absorption. For the meantime, because the antidyslipidemic effect of fenofibrate is correlated to its serum concentration, it is important to control for the erratic absorption of fenofibrate, and consider it as covariate in order to eliminate its effect on fenofibrate response variability.

1.3.2 Distribution

Up to 99.8% of the fenofibric acid in the serum is bound to plasma proteins.[29,35] In a ^{14}carbon labeled (^{14}C) study, the tissue distribution in rats after 8 hours of administering the dose was as follows: liver 29%, kidney 20%, gut 12.5%, blood 10.2%

and the rest in other various tissues.[36] In spite of this extent of serum protein binding, there have been no obvious demonstrations of its clinical relevance. The volume of distribution is ~30L. Maximum serum concentration time (T_{max}) is reached after 2 to 4 hours of oral dose.[33,34]

1.3.3 Metabolism

After the absorption of fenofibrate from gastrointestinal tract it is hydrolysed by the esterase enzymes to form the active moiety, fenofibric acid Figure 1. The T_{max} of the fenofibric acid is ~2 hours, indicating a rapid activation by the serum esterase enzymes. Other secondary metabolites are formed via reduction and decarboxylation of fenofibric acid. The antidyslipidemic activity of those secondary metabolites is unknown. However, arguments based on structure activity relationships suggest that losing the carboxylic group or the chlorine atom diminishes its antidyslipidemic activity[37] Figure 3.

The cytochrome P450 family of enzymes, (phase I enzymes), do not metabolize fenofibrate to a significant extent. An *in vitro* study using human hepatic microsomes containing CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 failed to find any significant metabolism of fenofibrate.[38] UDP-glucuronosyltransferase (UGTs) (phase II enzymes), also metabolize endogenous substrates and xenobiotics to facilitate their elimination.

This process, known as glucuronidation attaches a glucuronic acid molecule to the substrate such as fenofibric acid Figure1. The addition of glucuronic acid renders fenofibric acid up to 4 times [39] more water soluble, and hence more rapidly renally eliminated.[40] Up to 60% of the drug that reached the blood was eliminated as fenofibric acid-glucuronide and 5.7% as reduced-fenofibric acid-glucuronide Figure 2.[39] There are 16 *UGT* gene products in human. These proteins are subdivided into two main families: *UGT1* (chromosome 2q37) and *UGT2* (chromosome 4q13), and they are highly expressed in the liver and kidneys.[41] The prominent UGT enzymes that participate in the glucuronidation of fenofibric acid are UGT1A8, UGT1A9 and UGT2B7 and, to a lesser extent, UGT1A1 and UGT1A3 UGT2B4.[42,43] This means UGTs genes play a vital role in metabolizing fenofibric acid, which can affect the amount of the active form that exerts the antidyslipidemic effect. Identification of genetic variations in these genes can explain some of the variability in response to fenofibrate.

1.3.4 Elimination

From a single dose pharmacokinetic study done in healthy subjects using ^{14}carbon labeled fenofibric acid, they concluded that ~59.3% of the dose was eliminated renally and 24.8% in feces. Furthermore, of the amount of ^{14}C recovered from urine, fenofibrate was eliminated as following: 60% as fenofibric acid-glucuronide, 14% as

fenofibric acid, 6.7% as reduced-fenofibric acid and 5.7% as reduced-fenofibric aicd-glucuronide [39] Figure 2.

With a half life of about 20 (SD, ±7.7) hours [34,44], the pharmacokinetic steady state can be expected to be reached after five days of daily administration. Since fenofibric acid is mainly eliminated renally, patients with end stage renal failure will have prolonged elimination half life of up to 12 times. Hemodialysis is generally able to remove fenofibric acid from the blood.[35]

1.4. Pharmacodynamics

The following section will discuss several of the most common of fenofibrate's desired and adverse pharmacological effects.

1.4.1 The effect on morbidity and mortality

Two clinical trials were specifically designed in order to explore whether the addition of drugs like fibrates or niacin, that can lower TG and raise HDL-C, to a statin can eliminate or lower the residual risk and improve the control of CVDs.

Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial was the first major long term outcomes trial that explored the effect of fenofibrate as

monotherapy compared to placebo. It was a multicenter, double-blinded, and randomized trial that included 9,795 participants aged 50 to 75 years, with T2DM. The FIELD and ACCORD trials both trials investigated in T2DM patients. Both trials failed to show that fenofibrate can reduce the rate of coronary heart disease in DM type 2 patients regardless of their baseline lipid levels. But the question remains; did they use the right lipid levels in their inclusion criteria? According to the APT III guidelines in the management of dyslipidemia the addition of a fibrate or niacin is only required to achieve a secondary goal of a non-HDL-C (your ATP III defined LDL-C goal + 30mg/dL).[4,7] This secondary goal is needed if TG is ≥200mg/dL and the LDL -C goal level had been met using a statin. In the FIELD trial, only 25.7% [45] of the patient included in the study had a TG ≥200mg/dL while in the ACCORD trial it was 33%. [21] Therefore, the majority of the patients in both trials didn't require the addition of a fibrate according to the ATP III guidelines. This raises methodological questions regarding the appropriateness of expecting to demonstrate a drug's effectiveness within a population where almost two-thirds or more of the patients would not qualify to receive the drug under currently accepted guidelines. In fact, positive outcomes had been observed in a subgroup of patients who might meet the ATP III guidelines for the use of fibrates. In the FIELD trial, the analysis that had been done in a prespecified subgroup of patients that consisted of patients with TG of ≥204mg/dL and HDL of ≤40mg/dL for male and ≤50mg/dL for female. In this group, which represented only 20.6% of the recruited patients, the effect of fenofibrate on the time to the first CVD

event was significantly superior compared to the placebo group: 13.5% vs. 17.8% respectively, HR= 0.73 (p=0.0003).[45] In the ACCORD trial, the subgroup consisted of patients with TG of ≥204mg/dL and HDL of ≤34mg/dL, it represented 17.1% of the trial patients. The odds ratio of CVD events was reduced by 35% (p=0.056) in the fenofibrate group compared to the placebo.[21] Similar effects had been seen with other clinical trials with different fibrates.[46] These examples show the importance of recruiting patients who actually, according to the ATP III guidelines, require the addition of a fibrate or niacin to their treatment regimen. Another example of a flaw in the study design is in the AIM-HIGH trial, which the National Institutes of Health (NIH) stopped 18 months earlier than planned. The combination of niacin and statin, compared to statin only, did not reduce the risk of CVD events. According to AIM-HIGH trial inclusion criteria, among other criteria, a patient can be recruited if his TG was at least 150mg/dL off-statins or 100mg/dL on-statins.[47] In fact the median for the TG baseline level was 162mg/dL for all the patients who were recruited to the study.[48] So far, no data have been published on a subgroup analysis like the ones in ACCORD and FIELD trials.

The findings of the trials generally demonstrated that only patients with >200mg/dL and HDL-C< 40mg/dL benefited from using these medications. The details of these trials have been will be discussed later.

1.4.2 Effect on lipid

Fenofibrate is only approved by the FDA for the treatment of the certain types of dyslipidemia, in addition to diet and therapeutic life style changes: **primary hypercholesterolemia, mixed dyslipidemia (Frederickson Type IIa and IIb), and h**ypertriglyceridemia (Frederickson types IV and V). When used as monotherapy it lowers total cholesterol between 14-24%, LDL-C 10-27 %, TG 33-53%, and raise HDL-C between 5-15%.[31,49-51] This contrasts with gemfibrozil, which has virtually no LDL-C lowering effect.[22] The maximum antidyslipidemic effect from fenofibrate was achieved after two weeks of continuous administration.[52,53]

One of the key determinants of the percent change in lipid levels is baseline lipid levels, i.e. higher baseline levels lead to higher percentage change. However, in patients with severe hypertriglyceridemia (type V), TG>500mg/dL and modest level of LDL-C~100 mg/dL, LDL-C level will increase to up to +46% and TG drop by as much as -45%.[49] The antidyslipidemic effect of fenofibrate is significantly increased when used with a statins. Combination trials show a percentage drop in total cholesterol between 19-42%, LDL-C 21-42%, TG 39-49% and increased HDL-C between 14-28%. The effect varies depending on which statins was used, its dose, and lipid baseline levels.[54-57] The combination of fenofibrate and ezetimibe has a complementary, not a

synergistic, effect. Ezetimibe has an additive LDL-C lowering effect, although they are not commonly used together.[58,59]

1.4.3 Effect on uric acid

Elevated levels of uric acid have been linked to CVD as an independent risk factor[60-62], due to uric acid's detrimental effect on endothelial function.[63-66] Fenofibrate lowers uric acid, 21-28%.[67,68] The proposed mechanism of this action suggests that fenofibrate acts as a uricosuric by facilitating the renal elimination of uric acid through inhibiting the urate transporter 1 (*URAT1*). This transporter is found in the renal proximal tubules and is responsible for the reabsorption of uric acid.[68] In a study, a single dose of fenofibrate was administered in healthy volunteers, there was a gradual decrease in serum concentrations of uric acid level by 8.6% after 3 hours and 25.8% at 10 hours after administering the dose. The urinary excretion of uric acid was increased by 76.5% at 3 hours and 123.5% at 10 hours post dose.[68] In patients with gout who were undergoing stable urate lowering agents, allopurinol or benzbromarone, fenofibrate was able to lower uric acid levels by 19% after 3 weeks of treatment [69] and 23% after two months of treatments.[70] These results seem to suggest that fenofibrate has an advantage over niacin, which has been shown to increase uric acid levels between 11-14%.[15,16] *Chapter 4 of the thesis discusses details related to the effect of fenofibrate on uric acid.*

1.4.4 The effect on bilirubin

Unlike uric acid, chronic elevation of bilirubin levels has been shown to be protective against CVD.[71-73] The proposed mechanism of this beneficial association may relate to bilirubin's antioxidant properties.[74-77] The effect of fenofibrate on bilirubin levels is not clear. Some studies report no effect [78] whereas others reported substantial changes.[79] None of the studies were specifically designed to answer this question directly, and most reported bilirubin levels as part of a panel of safety endpoint. Most of these studies were conducted in a relatively small sample size (n=20 or less) and did not consider pharmacogenetics factors which may explain differences in fenofibrate's effect on bilirubin within a given population. *Chapter 3 of the thesis discusses details related to the effect of fenofibrate on fenofibrate on bilirubin.*

1.4.5 The effect on microvascular circulation

1.4.5.1 Renal function

Several clinical trials have shown a renal protective effect in DM type 2 patients using fenofibrate. In the FIELD trial progression to albuminuria was significantly reduced in fenofibrate recipients, 10% compared to 11% in placebo; (p=0.002).[51] In ACCORD trial the incidence of **mic**roalbuminuria was lower in fenofibrate recipients: 38.2%, compared to placebo 41.6%; (p=0.01), also, the incidence of **mac**roalbuminuria

was lower, 10.5% compared to 12.3%; (p=0.04).[21] Creatinine serum concentration was also significantly higher, 17-22%, in fenofibrate recipients than placebo in the previously mentioned trails. Fenofibrate increases serum creatinine, this gave the impression that fenofibrate might be injuring the kidneys. However, based on long term prospective clinical studies, FIELD and ACCORD, fenofibrate in fact had been shown to be renoprotective.[80] Furthermore, this increase in serum creatinine is reversible, levels returns to normal within 50 day from discontinuing fenofibrate.[81]

1.4.5.2 Retinopathy

In the ACCORD trial, patients who received fenofibrate showed a slower progression of diabetic retinopathy in patients with T2DM compared to placebo, 6.5% vs. 10.2% (p=0.006). Regardless, there was no significant difference in the percent of moderate loss of vision between the two groups, fenofibrate (23.7%), and placebo (24.5%) p=0.57.[82] In the FIELD trail, there was a reduction in the need for first laser treatment for retinopathy in patients using fenofibrate (6.9%) compared to placebo (10.9%), HR=0.63, (p=0.0003). This protection can be seen after 8 months of the start of fenofibrate treatment. When the patients are subdivided into patients with and without history of retinopathy, the protective effect of fenofibrate is not seen in patients with history of retinopathy (p=0.1).[18]

1.4.5.3 Amputation

The risk of any amputation in the FIELD study in patients using fenofibrate was lowered, 0.9% vs. 1.4% in placebo, hazard ratio=0.63 (p=0.04).[19] Between 2002 and 2009, there was a 117% increase in the fenofibrate prescriptions in the USA, even after the negative results of the FIELD trial in 2005.[83] Furthermore, Abbott announced that in the first quarter of 2011, sales of Tricor® and Trilipix® increased by more than 20% and this was after the negative results of ACCORD in 2010.[84] There are several plausible reasons for this increase in fenofibrate use: 1) the lag time between the publication of these clinical trials and the change in the treatment guidelines and their utilization by health care providers, 2) increased use in DM type 2 patients due to the microvascular protective effects, 3) the immense direct to consumer and health care providers advertisement. The latter explanation has been extensively discussed by Jackevicius et al.[83]

1.4.6 Effect on macrovascular circulation

The Diabetes Atherosclerosis Intervention Study (DAIS) [50] was the first major study to assess the effect of correcting dyslipidemia using fenofibrate on the atherosclerosis progression in coronary arteries in 418 DM type 2 patients. It was a placebo controlled, double-blinded, randomized, and multicenter study that lasted 3 years. The average baseline level of TG was 230 mg/dL and HDL-C of 39.8mg/dL. The fenofibrate group had a smaller increase in percentage diameter stenosis compared

to placebo group, 2·11% and 3·65% respectively, p=0·02 and a smaller decrease in minimum lumen diameter, 0·06mm and 0·10mm respectively, p=0·029. They concluded that treatment with fenofibrate reduced the angiographic progression of coronary-artery disease in T2DM.

1.4.7 Adverse effects

Two long terms major outcome clinical trials, FIELD and ACCORD, which followed patients for an average of 4.7 to 5 years, have reported that fenofibrate was generally well tolerated and had a good safety profile. However, in the FIELD trial patients on fenofibrate were at higher risk for pancreatitis than those on placebo, 23 out of 40 out of 4,895 *(*0.8%) vs. 4,900 (0.5%); (p=0·031). Also, there was a higher risk of pulmonary embolism in the fenofibrate group compared to placebo, 1.1% vs. 0.7%; (p=0·022). Rhabdomyolysis was reported in 3 fenofibrate recipients (0.06%) and one placebo recipient (0.02%). Creatine phosphokinase (CPK) levels of >10 the upper limit of normal (ULN) was reported in 4 fenofibrate recipients (0.08%) and 3 in placebo recipients (0.06%). In the ACCORD trial, history of pancreatitis became an exclusion criteria due the higher incidence of pancreatitis reported in FIELD trail. This explains the low incidence rate of pancreatitis reported in ACCORD trial; only two cases and both in the placebo group. No incidence of pulmonary embolism was reported in either arm of the study. Myopathy, myositis, or rhabdomyolysis was reported in 4 out of

2,765 fenofibrate recipients (0.1%) and 3 out of 2753 placebo recipient (0.02%). Creatine phosphokinase (CPK) levels of >10 the upper limit of normal (ULN) was reported in 10 fenofibrate recipients (0.4%) and 9 in placebo recipients (0.3%).[21] In the FIELD trial, the number of patients who discontinued fenofibrate treatment compared to placebo was 954 out of 4,895 (20%) and 950 out of 4,900 (19%) respectively.[51] Similarly, in the ACCORD trial, the number of patients who discontinued fenofibrate treatment was comparable to placebo, 61 out of 2765 (2.2%) and 49 out of 2753 (1.78%) respectively.[21] In both trials the discontinuation rate of study medication was not higher in those allocated to fenofibrate. Given the size and nature of the population studied, this suggests fenofibrate to be a reasonably well tolerated agent.

A general concern with fibrates treatment when combined with statins is the increased incidence of rhabdomyolysis, given the large number of combination studies, which continued for several months to several years, of treatment with fenofibrate and different members of the statins group. These studies have shown a good safety and tolerability profile.[21,55,56,85,86] In fact, the incidence of muscle related injuries varies between fibrates, with fenofibrate demonstrating a superior level of tolerance compared to gemfibrozil.[87-89] For example, this superior tolerability could be due to the differences in the enzymes involved in their metabolism, or the difference in their ability to induce or inhibit certain enzymes that can interfere with the pharmacokinetic or pharmacodynamic of statins. Unlike gemfibrozil, fenofibrate does not induce or

inhibit the cytochrome P450 enzymes, which are involved in the metabolism of statins.[90] Furthermore, unlike gemfibrozil, fenofibrate is not metabolized by CYP450, so it will not compete with statins for metabolism. Both fenofibrate and statins are metabolized by the *UGT* enzymes, but pharmacokinetic studies have shown no significant effect on the statins or their metabolites' pharmacokinetic profile, at least not to the extent that can induce toxicities.[91,92]

1.4.7 Others effects

Fenofibrate lowers the level of fibrinogen [93], C-reactive protein, interleukin-6 and tumor necrosis factor-α.[94]

1.5. Pharmacogenetics

The completion of the human genome project has and will continue to reap countless benefits and controversies. We are already harvesting its benefits in the clinical research field. An increasing number of clinical research studies are incorporating genotyping into the design of their studies and taking advantage of the open source of the DNA sequencing data available online. Pharmacogenetics is the science studying the effect of genetic variations on the drug's kinetics and dynamics, which can manifest as inter-subject response variability. Pharmacogenetics potentials had never been better, with the cost of genotyping consistently declining and the rapid

advancement in the genotyping machines increasing their accuracy and efficiency. In the pharmacy field genetic information advances can serve the **Translational clinical research**, **pharmaceutical industry** and **applied medical field**.

1.5.1 Translational Clinical Value of Pharmacogenetic Research Findings. Clinician's Perspective:

Pharmacogenetic based knowledge can and has been used to:

1.5.1.1 Predict treatment outcome:

Patients who can benefit the most from drug treatment and individuals, who might benefit least, may represent a translational impact of pharmacogenomic based research. In the former, it may provide guidance to clinicians to select between treatment options or propose alternative treatments for those projected to represent inferior responders. For example patients using tamoxifen as breast cancer recurrence prevention, and have genotype of CYP2D6 *4, *5, 10* or *41 are not an ideal candidate for this treatment. They had more recurrences of breast cancer, shorter relapse-free periods, and worse event-free survival rates compared with carriers of functional alleles.[95]

1.5.1.2 Predict adverse drug reactions:

Select patients who are more prone to suffer adverse drug reactions when using a specific drug may be identified through pharmacogenomic based research. For

example patients that harbor the SLCO1B1*5 SNP have a higher probability of statin-induced myopathy.[96]

1.5.1.3 Dose individualization:

Pharmacogenomics could prove valuable when determining the optimal dose for a given patient. For example, one could maximize the desired effect and possibly lower side effects for select agents with narrow therapeutic indices. For example: genotyping patients for certain SNPs in the VKORC1, CYP2C9*2, or CYP2C9*3 genes may represent a superior basis to estimate the needed dose of warfarin that can maintain the balance between preventing clots formation and serious hemorrhage.[97]

1.5.1.4 Predict drug-drug interaction:

Understanding the effect of the genetic variation can clarify the mechanism for some of the drug-drug interactions (DDI), especially when some of the drugs mimic the effect of those genetic variations. For example: the DDI between clopidogrel and the drug class of proton pump inhibitors (PPIs). PPIs inhibit the CYP2C19 gene, which is needed for the activation of clopidogrel. Patients who used this combination had higher rates of myocardial infarction.[98] Similarly patients with genotypes that code for lower or diminished activity of CYP2C19 gene and using clopidogrel were more likely to have a cardiovascular ischemic event or death.[99]

1.5.2 Pharmaceutical industry perspective:

1.5.2.1 Current view

The pharmacogenetics information emerging can help the pharmaceutical industry to 1) determine patients who are more likely to suffer an adverse drug reaction, lowering the chance of the withdrawal of their product from the market and possible lawsuits by injured patients, 2) improve their dosing recommendation using the patient genetic variations, 3) shorten the time for a molecule to reach the market by expediting the safety clinical studies.

1.5.2.2 Futuristic view

The advance in the pharmacogenetics field will force the pharmaceutical industry to leave the one-drug or one-dose fits all products to more specific and individualized ones. Also, with a more genetic level understanding of the diseases' etiology, there will be a movement away from the manufacturing of drugs that treat the *symptoms* of a disease, toward drugs that treat the *sources* of a disease.

1.5.3 Applied medical field

The incorporation of the pharmacogenetic findings into the medical field includes the use of new genetics approaches selection of treatment remains at its infancy. This limited use can be ascribed to a number of factors: 1) education: clinicians of all type need to be educated on the advantages of the new genetics approaches and how to interpret and utilize it in treatment, 2) cost: the cost of most of these genetics approaches are not covered yet by insurance companies and hospitals favor less expensive conventional approaches, 3) lack of supporting data: the cost effectiveness of the genetics approach have not yet been established by the pharmacoeconomic studies, and 4) ethical issues: there are a lot of ethical issues that have arisen with the decoding of the human genome. The following are some examples of the ethical issues; a) psychological impact on family members who unwillingly discovered, through the genotyping of another family member, their risks in developing a life threatening disease, b) fear of discrimination by health insurance companies after attaining the patients' genetic data, c) lack of pharmacogenetic-clinical data in racially minor or rare genotypes groups, and d) how to handle genetic information on a genetic variation that was believed to be linked to a drug's metabolism but afterward was associated to a devastating disease.

To increase the utilization of the pharmacogenetic-guided therapy in the clinical field, the emergence of the following must happen: 1) A more readily available and increasingly less expensive genotyping technologies, 2) Studies that prove the

superiority and cost-effectiveness of the pharmacogenetic-guided therapy over the conventional treatment approaches. The successes of the pharmacogenetic-guided therapy can help optimize our treatment methods, which can assist us in saving numerous lives, improve the patient quality of life, and drive the cost of the healthcare down.

1.5.4 Pharmacogenetics of fenofibrate

One important challenge for using fenofibrate is the high inter-subject variability in lipid response, even after controlling for known traditional sources of variability, ranging from -82 to 132% for TG change from baseline.[100] This magnitude of variability suggests the involvement of non-environmental factors, like the genetic source of variability. The Genetics Of Lipid Lowering Drugs and Diet Network (GOLDN) trial was the first to explore and characterize the genetic basis for the variation in TG response in two contexts, one that raises TGs, and one that lowers TGs (the fenofibrate treatment). The NIH funds the study, which is also a part of the PROGENI Network, a group of family intervention studies focusing on gene-environment interactions. Twelve hundred family members were recruited into the study from two geographical locations (Minneapolis, Minnesota and Salt Lake City, Utah). The participants from both locations are considered genetically homogenous, and are considered to be of European American descent (CEPH). After 10 hours of

fasting, lipid levels were measured at baseline, 0hr (pre-fenofibrate), for two consecutive days. Then participants underwent a high fat load challenge, and lipid levels were also measured at 3.5 and 6hr. Participants were then started on oral fenofibrate 160mg (Tricor®) once daily for 21 days. After that, another fasting lipid levels, 0hr (post-fenofibrate) were collected and another fat challenge was performed and lipid levels were also measured at 3.5 and 6hr. Fenofibric acid serum concentration was measured at 0, 3.5 and 6hr post fenofibrate dose. For complete description of the GOLDN trial refer to Liu et al. [101]

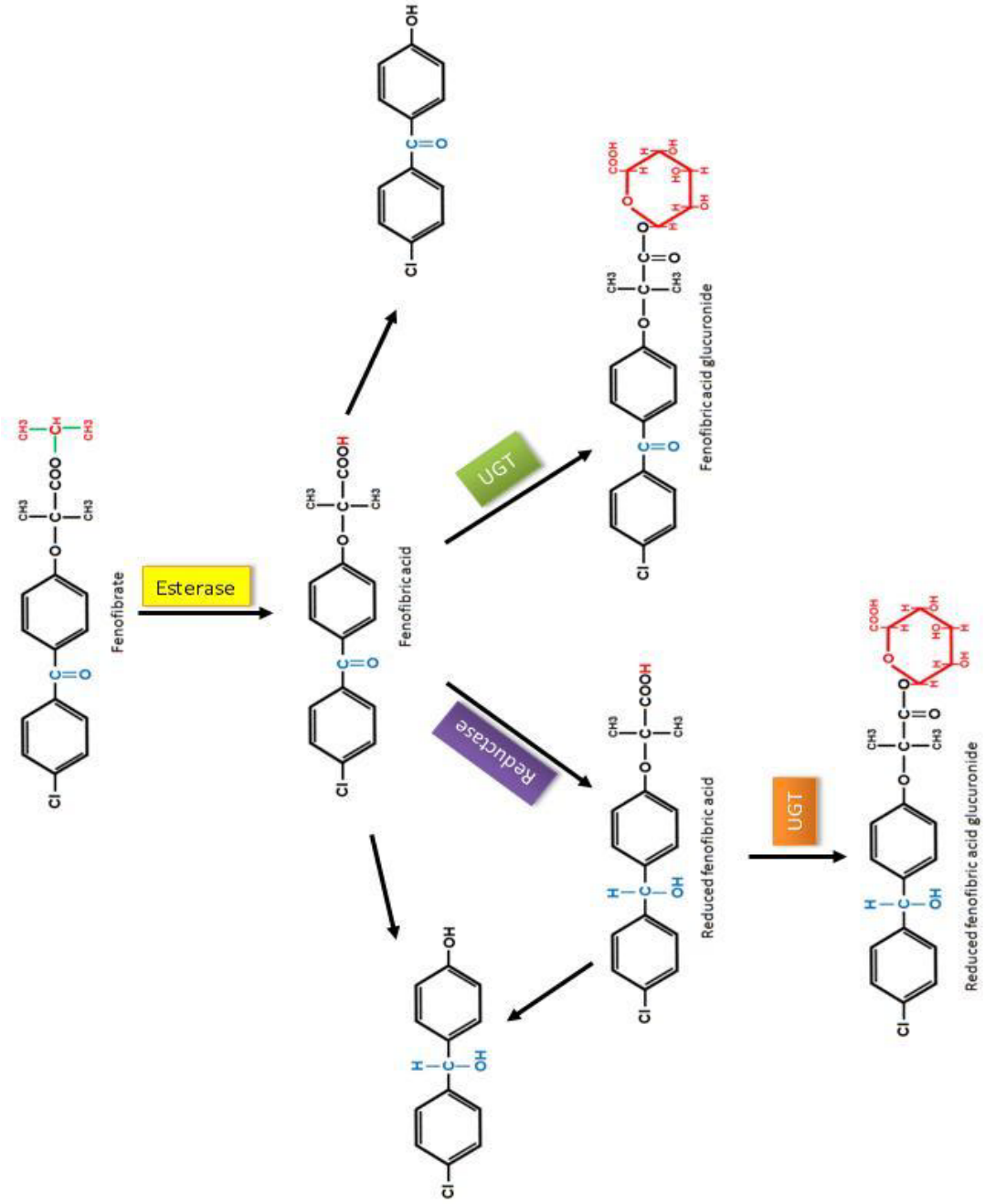
From the GOLDN study, a SNP in a metabolizing gene, *UGT2B7*, had explained some of the variation in fenofibric acid serum concentration (trough) and the high lipid-response variability. The fenofibric acid serum concentration was measured at only 0, 3.5 and 6hr, so a full pharmacokinetic profile and the full potential effect of the SNPs cannot be established. For these reasons, among others, the Fenofibrate and Pharmacogenetics Impact (FPI) study was completed. The FPI study served as a confirmation of the effect of UGT2B7 A-327G (rs7662029) SNP on the pharmacokinetics and pharmacodynamics of fenofibrate. Healthy participants were recruited according to their genotype of the UGT2B7 A-327G SNP. Also, the FPI study gave a full pharmacokinetics profile which might empower or clarify the effect of some of the SNPs that was not found to be linked to fenofibric acid serum concentration-variation because only one time point (trough) was available in GOLDN study. The

FPI was designed to match the GOLDN study design as close as possible. However, there are several differences; 1) the fat challenge was not administered, 2) the time point were more comprehensive (8 time points) giving an AUC of 0-24, and 3) participants took fenofibrate *145mg* (Tricor®) for 28 days instead of 21day as in GOLDN study. *Chapter 2 of the thesis will discuss the FPI study.*

Following a mechanism based approach, the GOLDN trial genotyped the participants for SNPs in genes that might be affecting the response to fenofibrate. The genes were classified into three categories: target (affecting lipid metabolism), metabolizing, and eliminating genes. Also, the effect of genetic variations on other pharmacological effects, other than antidyslipidemic effect, of fenofibrate had been also explored.

Given this background chapter on fenofibrate, the following topics will be discussed in Chapters 2, 3 and 4. Chapter 2 will focus on a major clinical trial conducted on healthy subjects evaluating the effect of a SNP, in the UGT2B7 gene, on pharmacokinetic parameters of fenofibrate. Chapters 3 and 4 of this thesis will explore the pharmacogenetic basis for differential response to fenofibrate on bilirubin and uric acid. Finally a summary chapter will provide a perspective on our findings and conclusions. Collectively the above chapters represent my new knowledge in the area of pharmacogenetics and its consequences on fenofibrate metabolism and potential clinical use.

Figure 1: metabolic pathway of fenofibrate and the formation of its metabolites.



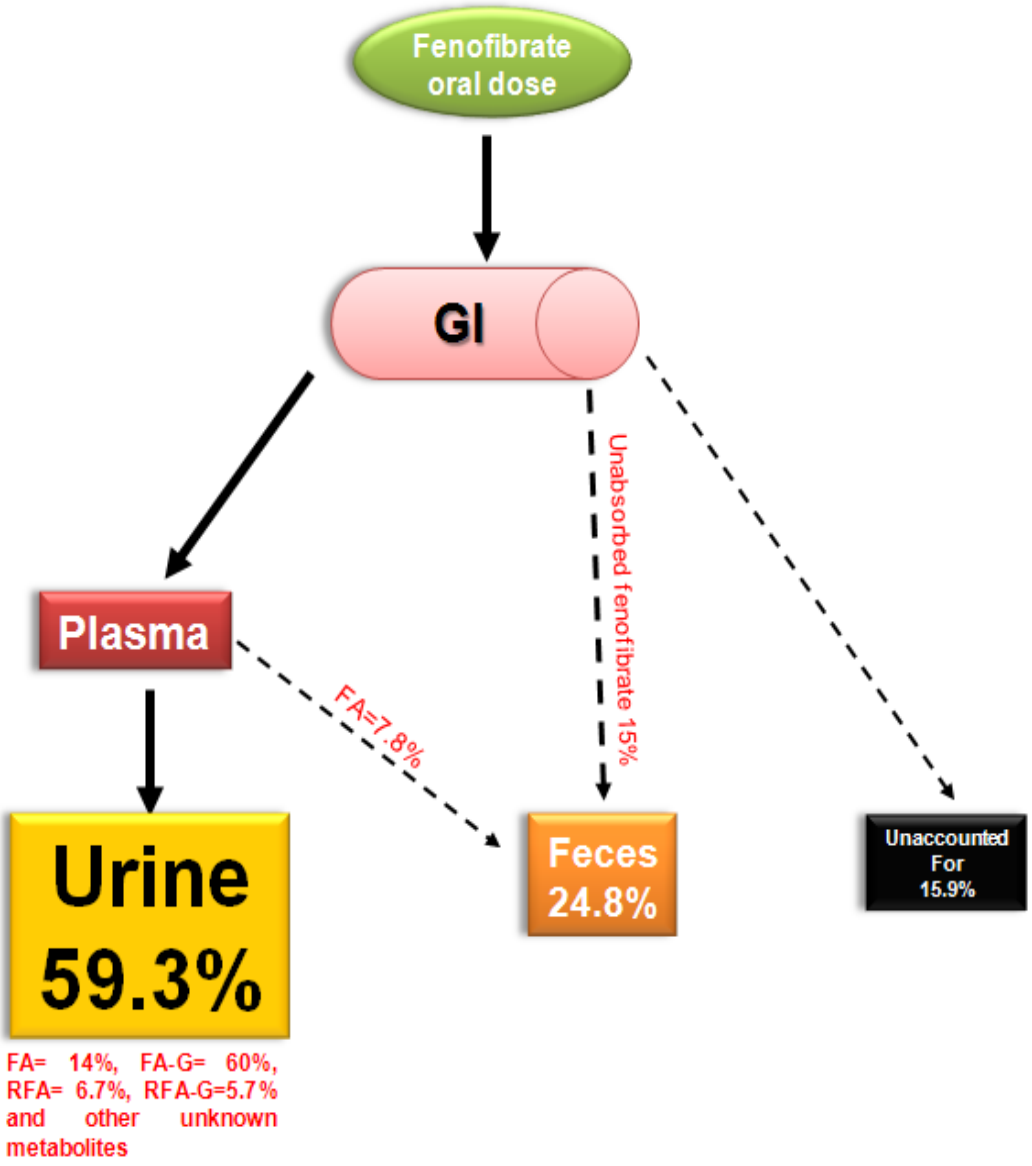


Figure 2: The fate of oral dose fenofibrate according to Weil et al.

FA=fenofibric acid, FAG=fenofibric acid-glucuronide, RFA=reduced-fenofibric acid, RFAG= reduced-fenofibric acid-glucuronide.

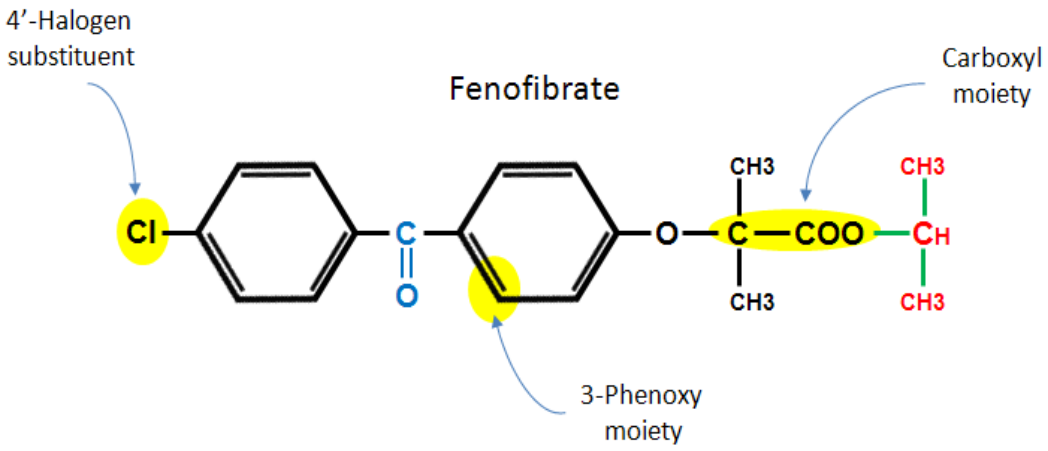


Figure 3: Chemical structure of fenofibrate and structure activity relationship.

The loss of any the highlighted functional group will lead to complete loss of the antidyslipidemic activity of fenofibrate.

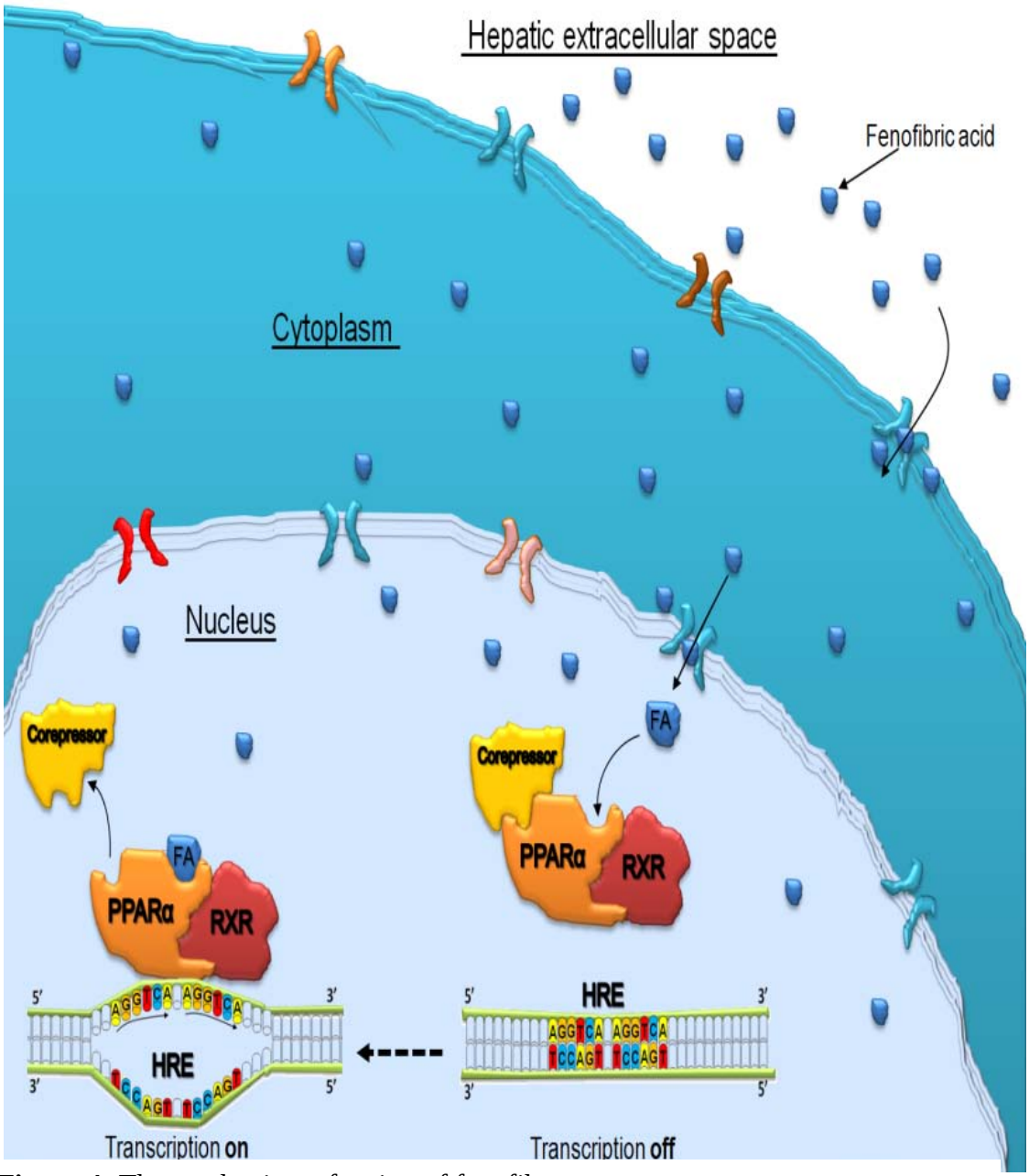


Figure 4: The mechanism of action of fenofibrate
FA: fenofibric acid, HRE: hormone response elements, PPARα: peroxisome proliferator activated receptor alpha, RXR: retinoid X receptor.

CHAPTER II

The *in vivo* role of UGT2B7 A-327G as a determinant of serum fenofibric acid levels in healthy subjects from the Fenofibrate and Pharmacogenetic Impact (FPI) Study

2.1. BACKGROUND

For patients who have already achieved optimal LDL-C targets, fibrates represent one of only two classes of agents recommended to manage a patient's "residual risk"[1] for cardiovascular events.[2] However, the high inter-subject variability of fenofibrate's lipid-lowering response and mixed results from recent outcome-based clinical trials of fenofibrate - such as the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD)[3] and Action to Control Cardiovascular Risk in Diabetes (ACCORD)[4] studies - have called to question our ability to identify which patients represent optimal candidates to receive these agents. We posit that one important source of variability in a patient's lipid-lowering response to fenofibrate can be traced to fenofibrate's metabolic fate. Glucuronidation is a well-recognized metabolic pathway influencing the elimination of various therapeutic agents.[5] *In vitro* evidence of *UGT2B7*'s role in eliminating fenofibric acid, the active form of the lipid-lowering drug fenofibrate, has been demonstrated.[6] From the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study[7], we have specifically reported that *UGT2B7* is an important determinant of lipid lowering response.[8] However, evidence supporting the pharmacokinetic basis of this pharmacogenetic association is still lacking. Consequently, in the absence of any other *in vivo* evidence elucidating the significance of genetic variants of drug metabolizing genes on serum pharmacokinetics of fenofibric acid, this study sought to test that question using UGT2B7 A-327G, a single nucleotide polymorphism (SNP), as a basis for its role in modulating fenofibrate's lipid-altering

response. An enhanced understanding of genetic sources of lipid-altering response may eventually provide a basis for genetic-guided selection of optimal candidates to receive fenofibrate drug therapy while avoiding those least likely to respond or possibly display toxicities. By providing direct evidence of altered disposition based on a specific UGT variant – which has been linked to *in vivo* lipid response to fenofibric acid – significantly adds to the accumulating data supporting the importance of pharmacogenetic-based sources of drug response variability.

2.2. METHODS

2.2.1. Study design

This prospective pharmacokinetic clinical study recruited male and female subjects >18yrs of age who were not on lipid-altering medications including nutraceuticals and OTC agents. Exclusion criteria included: current use of insulin or warfarin, pregnancy or any women of childbearing potential not using an acceptable form of contraception, history of an allergy or hypersensitivity to fenofibrate, investigational drug use within 30 days of the study and history, or presence of liver, kidney, pancreas, gall bladder disease or malabsorption (e.g. Crohn's disease). There were no specific criteria regarding lipid baseline values, and this study was approved by University of Minnesota Committee on the Use of Human Subjects in Research (IRB

Code Number: 0708M15441) and registered with ClinicalTrials.org (Protocol ID: NCT00613613).

Subjects responding to an ad were first interviewed by phone and given a brief explanation of the study to determine their general eligibly for the study. They were instructed to fast for≥10 hours and consume no alcohol for 24 hours prior to their scree ning visit (V0). Subjects who declared using any antihyperlipidemic drug were required to discontinue taking the drug (after gaining their physician's approval) for at least four weeks before conducting the screening visit (V0)

Upon receiving a thorough explanation of the study and signing the approved consent form, we collected demographic, anthropometric, medical history, current medical conditions, and a blood sample for the purpose of obtaining genomic DNA, serum biochemical and lipid screening tests. Blood pressure was also taken by trained study personnel at this visit.

Our criteria for including subjects in the study primarily included meeting our genomic based criteria of being either AA or GG for UGT2B7A-327G and having a minimum baseline fasting triglyceride level ≥ 90 mg/dL. Their genotype for UGT2B7 A-327G (rs7662029) SNP was not shared with study subjects or with personnel directly involved with the conduct of this study.

During the second phase, all subjects were provided fenofibrate 145mg (TriCor)® and advised to take each capsule once daily in the morning for 28 days. The phase consisted of four separate visits (V1- 4) where V1 and V2 were held one day apart before fenofibrate administration and V3 and V4 were also held one day apart after fenofibrate administration. All lipid fraction levels taken pre- and post-fenofibrate represent the average of the V1 and V2 measures or V3 and V4 measures respectively, this repeated measurements reduce the high variability in triglyceride serum level. Preceding each of the four visits subjects fasted ≥10hours and consumed no alcohol 24 hours before the visits. For safety and the compliance reasons subjects were contacted by phone after 10 and 24 days after starting fenofibrate. The serum sampling scheme beginning at V3 included a 0, 2, 3.5, 6, 8, 10, 12 and 24 hour time point following the 28th dose and was used for the purposes of calculating fenofibric acid's AUC 0-24. For V3, all subjects were served a breakfast meal which consists of 30% fat. Urine was also collected at intervals of 0-6hr, 6-12hr and 12-24hr following V3.

2.2.2. Processing plasma and serum samples

Plasma sample: 8mL of blood were placed in a lavender top tube and inverted 8 times. Next tube is placed in an ice bath, then centrifuged for 10 min at 32000 rpm *x* g at 4-10°C. After that transfer equal volume of plasma into 2-mL microvial and freez in -80°C freezer. *Serum sample*: 8mL of blood were placed in a red top tube and inverted 8

times and left in room tempreture for 30-45min Next tube is placed in an ice bath, then centrifuged for 10 min at 32000 rpm *x* g at 4-10°C. After that transfer equal volume of serum into 2-mL microvial and freez in -80°C freezer.

2.2.3. DNA extraction procedure

DNA is extracted from peripheral white blood cells. Blood is drawn into yellow top tubes containing ACD solution A. 15mL of whole blood is mixed with 35mL of 1x RBC Lysis Buffer and incubated for 15 minutes at 37ºC in 50 mL conical tubes. Tubes are then centrifuged to pellet the white blood cells. To the pellet, 3mL of WBC Lysis Buffer containing sodium dodecyl sulfate is added ands the cells are incubated at 37ºC overnight. After incubation, solutions of RNAse A and Proteinase K are added and the solution is incubated at 37ºC for at least 60 minutes. For DNA precipitation, 1.5mL of 7.5M ammonium acetate is added and the solution gently mixed. The extract is transferred into a 15mL conical tube and centrifuged at 3000 rpm for 20 minutes to remove protein. The supernatant is then transferred to a second 15mL conical tube leaving the debris pellet behind. To this, 10mL of absolute ethanol is added, the tube is gently inverted to mix the two solutions and the DNA is allowed to precipitate. The DNA is removed with a glass pipette, washed with 70% ethanol and the DNA resuspended in 3mL of TE buffer and incubated at 37ºC. When the DNA is dissolved,

the solution is divided equally among three labeled storage tubes and store at 4ºC. DNA yields are typically 200 ng/µl or more with 260:280 ratios of 1.8.

2.2.4. Genotyping

UGT2B7 A-327G: genotyping for the genetic marker UGT2B7 A-327G, was performed at Laval University by re-sequencing. In brief, 30ng of genomic DNA was combined with 250 nM of each of the amplification primers (CAAAAATATGTGGACCA TGTTTAGTCAAA TAAGTTAGAGCTTCATG TTACTGATTG), 200 nM of dNTP, 3mM MgCl2, 1X Taq buffer (50mM KCl, 10mM Tris, 0,01% gelatin, pH8,3), and 1 unit of Taq DNA polymerase in a final volume of 50ul. Each reaction was incubated at 94°C for 30s followed by 35 cycles at 94°C for 30s, 56°C (annealing temperature) for 30s, and 72°C for 30 min, with a final step at 72°C for 5 min. Amplification products were purified onto silica matrix, and were sequenced using the Big Dye terminator v3.1 cycle sequencing kit and were resolved onto an ABI PRISM 3730XL DNA analyzer (ABI, Foster City, CA, USA). All sequences were analyzed with the Staden package (Open Source Technology Group, http://staden.sourceforge.net/). Note that rs7662029 variant also refers to position -268 relative to the hepatic start site in the *UGT2B7* promoter.[9,10]

UGT1A1*28: genotyping the UGT1A1*28 SNP of interest was conducted at the University of Minnesota BioMedical Genomics Center from 20ng of genomic DNA, a

98 bp segment including the TA repeat region was amplified using the forward primer 5' GTCACGTGACACAGTCAAAC 3' and reverse primer 5' TTTGCTCCTGCCAGAGGTT 3' with 6-FAM tag attached to the 5' end of the reverse primer.[26,27] These primers anchor the TA locus in the promoter region of the UGT1A1 gene. They amplify a 98 bp fragment when a $(TA)_6$ allele is present and 100 bp fragment when a $(TA)_7$ allele is present. The DNA was amplified for 35 cycles and the PCR fragments and was run on gel electrophoresis to be analyzed. Control DNAs from individuals known to have a $TA_{6/6}$, $TA_{6/7}$ and $TA_{7/7}$ genotype were included in the PCR analysis and electrophoresis. Amplified regions were then analyzed on a ABI 3130XL capillary size fragmenter to determine the number of the TA repeats.[28] TA counts were called visually utilizing Peak Scanner® version 1.0

2.2.5. Lipid and biochemistry analysis

Analyses of serum biochemistries as well as serum lipid levels; high density lipoprotein-cholesterol (HDL-C), triglyceride (TG), and total cholesterol; were conducted by the Department of Laboratory Medicine and Pathology, School of Medicine, University of Minnesota. Specifically, total cholesterol was measured using a cholesterol oxidase method on a Roche Modular P Chemistry analyzer (Roche Diagnostics Corporation, Indianapolis, IN). The same reaction was also used to measure HDL-C after precipitation of non-HDL-C with magnesium/dextran. Friedewald equation was used to calculate low density lipoprotein-cholesterol (LDL-C) levels (LDL-C = Total cholersterol – HDL-C - Triglicerides/5). Non-high density lipoprotein-cholesterol (non-HDL-C) was calculated as non-HDL-C= T.choleterol – HDL-C. Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation, Indianapolis, Ind) on a Roche

Modular P Chemistry analyzer (Roche Diagnostics Corporation). Glucose, high-sensitivity C-reactive protein, and were also determined using the Roche Modular P Chemistry analyzer. Insulin determination was done using the Roche Elecsys Analyzer with the sandwich immunoassay method, while HOMA-IR and HOMA-β were calculated using the following equation: (Glucose (mg/dL) X insulin (mU/L)) / 405, ((360 X Insulin (mU/L)) / (Glucose (mg/dL)-63)%) respectively.[11] The inter-laboratory coefficients of variation in a pooled plasma control were 4.0% for triglycerides, 1.6% for cholesterol and 2.9% for HDL-C. Total bilirubin (direct + indirect) was measured in serum using a diazonium salt/ion colorimetric assay (Roche Diagnostics, Indianapolis, IN) and read on the Roche Modular P Chemistry Analyzer (Roche Diagnostics). Total bilirubin (conjugated + free) was measured in serum using a diazonium salt/ion colorimetric assay and read on the Roche Modular P Chemistry Analyzer (Roche Diagnostics). The reference range is 0.2 – 1.3 mg/dL with an inter-assay CV of 3.1%. Conjugated bilirubin is measured in serum on the Roche Modular P Chemistry Analyzer using a colorimetric assay based on the diazo Jendrassik-Grof procedure. The reference range is 0 – 0.2 mg/dL with an inter-assay CV of 4.7%.

2.2.6. Fenofibric acid serum and urine determination

HPLC MS/MS:

Serum and the urine samples were processed similarly for measurements of fenofibric acid. Briefly, the samples were thawed at room temperature, and 25ul of serum or urine was transferred to a tube. Five milliliters of Methanol:Water 80:20 (containing fenofibric acid d6) was added and vortexed for 30 seconds. After that, the samples

were centrifuged at 2500 X g. One hundred microliters were transferred to HPLC vial insert prior to injection. The system was controlled through Analyst Software, version 1.5 from Applied Biosystems. The chromatographic system consisted of an HPLC Prominence (Shimadzu Scientific instruments, Inc, Columbia, MD, USA). The chromatographic separation was achieved with a Synergie RP Hydro 4 um packing material, 100 X 4.6 mm (Phenomenex,Torrance, USA). The mobile phases were (solvent A) water with 0.02% formic acid (v/v) and (solvent B) methanol with 0.02% formic acid (v/v) at a flow rate of 0.9 ml/min. The analytes were eluted using isocratic conditions: 90% B. The LC-MS/MS system consisted of a mass spectrometer (model API 4000, Concord, Canada). It was operated in multiple reactions monitoring mode (MRM) and equipped with a turbo ion-spray source. Electrospray ionization was performed in negative ion mode with an ionization voltage of -4500 V, a declusting potential voltage of -40V, a collision energy voltage of -16V and a heater probe temperature of 500°C. The analytes were detected using the following mass transition: 316.9 → 230.9 (fenofibric acid) and 322.9 →230.9 (fenofibric acid d6). The resolution used in this method for Q1 and Q3 was Unit/Unit. A six-point calibration curve (from 500 to 50,000 ng/ml) was prepared by spiking blank plasma with appropriate amounts of fenofibric acid. The linear regression of fenofibric acid peak area ratio was weighted by 1/x.

2.2.7. Pharmacokinetics analysis

Pharmacokinetic parameters including, maximum serum drug concentration (Cmax), time at maximum serum drug concentration (Tmax), apparent oral clearance (total clearance) (Cloral/F), elimination rate constant (Ke), apparent volume of distribution (Vd/F), and half-life(T1/2) were calculated using the WinNonlin® (v6.2) software. The primary endpoint of area under the curve (AUC0-24) of fenofibric acid serum concentration was analyzed by the linear trapezoidal rule, as calculated by a noncompartmental analysis using WinNonlin®.

2.2.8. Statistical analysis

Since the impact of UGT polymorphism on the pharmacokinetics of fenofibric acid were emerging, we projected the ratio of exposures for the high and low UGT activity groups to have a 2-fold difference in AUC (AUC0-24h, low activity/AUC0-24h, high activity). This was based on the significance of the glucuronidation pathway (>60%) in the overall elimination of fenofibric acid.[12] We further projected the standard deviation of AUC0-24 to be 50% of the AUC value based upon results from the GOLDN study.[7] Based on these projections, we predicted the need to recruit 22 subjects per group using a two-sample t-test power calculation (two-sided) with an α of 0.05 and a β of 0.2 (power of 80%). To account for drop outs, 50 subjects were recruited (25 subjects per group).

All statistical analyses were conducted using Stata (version 12.1, Stata Corp, College Station, TX). Baseline characteristics were compared between the two genotype groups (AA vs. GG) using unpaired t-test for continuous variables and Fisher's exact test for categorical variables. Multiple linear regressions were performed to test for association between fenofibric acid AUC_{0-24} and UGT2B7 A-327G (AA vs. GG). Several covariates were considered: age, sex, free bilirubin level at visit 3, body mass index, creatinine clearance, use of oral contraceptive or hormone replacement therapy; but only free bilirubin level at visit 3 and body mass index were used in the final model. Serum lipid changes from baseline were calculated as (post-fenofibrate lipid level - pre-fenofibrate lipid level)/Pre-fenofibrate lipid level. Associations between lipid changes and fenofibric acid AUC_{0-24} and UGT2B7 A-327G were tested using multiple linear regression, adjusting for the pre-fenofibrate lipid level. When triglyceride was examined, indirect bilirubin level was also included as a covariate.

2.3. RESULTS

Two hundred and eighty subjects consented to be screened for their fasting lipid levels and were genotyped for UGT2B7 variant. From this pool, 56 subjects who were either AA or GG for UGT2B7 A-327G enrolled in the treatment phase of the study. The drug was generally well-tolerated; however, 7 individuals did not complete the entire study. The reasons for non-completion included 1 individual who decided to withdraw due to a skin rash and 6 others who failed to return for followed up visits for

unspecified reasons. Other minor adverse effects reported by those completing the entire study included one with GI upset, one with self-resolving rash and one reporting dizziness. Ultimately, 49 subjects (30 [61%] are females) with an overall mean (±sd) age of 33 (±11.8) years completed the pharmacokinetic study and constitute our study population. None of these subjects reported having any preexisting diagnosis of dyslipidemia, diabetes or cardiovascular disease (CVD). Consistent with our inclusion/exclusion criteria (Table 5), none of the subjects who were enrolled reported using any lipid-altering medication or nutraceuticals. The ethnicity of the participants were as follows; 73.5% (36/49) Caucasian, 8.2% (4/49) Hispanic, 6.1% (3/49) African American, 2.0% (1/49) Asian/Pacific Islander, and 10.2% (5/49) specified "Other". The observed allelic frequency of the polymorphism, UGT2B7 A-327G (rs7662029), amongst all of those screened (n=280) was comparable to HapMap data (build 37.1), the minor allele frequency is 49.6% in the CEPH population.

Using UGT2B7 A-327G SNP, the 49 participants formed the two genotype groups: 26 in AA and 23 in GG. There were no significant differences in age, gender, body mass index (BMI), lipid profiles, insulin, HOMA-IR, HOMA-B, hs-CRP, vitals such as blood pressure and prevalence of metabolic syndrome between the two genotype groups at baseline (Table 1).

The overall pharmacokinetic parameters for the combined population and those for the AA vs. GG individuals are summarized in Table 2 while overall renal clearance and fractional excretions are represented in Table 3. There was no statistically significant difference between the two genotypes in percent of fenofibric acid collected from urine. Our main outcome of fenofibric acid AUC_{0-24} was significantly different between the two genotype groups, with the GG group exhibiting a 27% higher AUC_{0-24} compared to the AA group. The multiple regression analysis was performed to test the effect of UGT2B7 A-327G on fenofibric acid AUC_{0-24}, and post-fenofibrate free-bilirubin level and BMI were the only significant covariates that remained in the model.. Specifically the mean (±sd) AUC_{0-24} was 300 (±131)μg*hr/mL for GG and 218.4 (±98)μg*hr/mL (p=0.023) for AA individuals respectively (Figure 1 and Table 2). Higher free bilirubin level were associated with higher AUC_{0-24}. In contrast higher BMI were associated with lower AUC_{0-24}. We also note a significant difference in the C_{max} of fenofibric acid in the serum, with GG achieving higher C_{max} values relative to individuals who were AA for UGT2B7. No significant difference in the time at which C_{max} was reached (T_{max}) was observed. No statistically significant differences were seen in renal clearance (Cl_{renal}) or the percent of fenofibric acid recovered from urine in any of the time points collected (0-6, 6-12, and 12-24hr), between genotype groups.

Overall, there was an expected significant change (p<0.001) in the mean (±sd) serum lipid values from baseline for total cholesterol, LDL-C, TG, nonHDL-C but not HDL-C, -16.2% (±11.1%), -18.6% (±14.6), -29.9 (±19.3), -20.6% (±13.3) and 0.64% (±14.9)

respectively. There was, however no distinction observed between each of the UGT2B7 A-327G genotype groups in terms of the magnitude of lipid-lowering effect for each of these lipid fractions (p>0.05) (Table 4).

From Pearson's correlation coefficient test there was, an association between higher fenofibric acid AUC_{0-24} (exposure) and percent change in serum lipid fraction from baseline was confirmed for TG (r^2=0.26, p= 0.0002), total cholesterol (r^2=0.12, p=0.014), LDL-C (r^2=0.17, p= 0.0032), and non-HDL-C (r^2=0.11, p=0.019) but not HDL-C (p=0.56), stressing the importance of exposure to lipid response.

2.4. DISCUSSION

UGT genes have been recognized as a key regulatory pathway for the metabolism and elimination of fenofibric acid on the basis of *in vitro*[13] and *in vivo*[12] evidence. This is the first study to quantify the *in vivo* impact that a key genetic polymorphism within the *UGT* family has on the disposition of fenofibric acid. Specifically, this study confirms UGT2B7 A-327G, or a variation closely linked to this SNP, is a significant determinant of systemic drug exposure, accounting for a 27% (p=0.026) difference in AUC_{0-24} between UGT2B7 AA versus GG individuals. Our final multiple regression analysis identified two significant covariates. Those with a higher BMI and lower free bilirubin level at visit 3 were associated with higher apparent oral clearance of fenofibric acid. Although we cannot be certain as to why higher BMI

may be associated with higher apparent oral clearance of such compounds, the higher clearance associated with lower bilirubin levels may represent competition for glucuronidation pathways. Our subjects were genotyped for UGT1A1*28 which is a known contributor to serum bilirubin levels.[14] However, the distribution and frequency within UGT2B7 AA or GG individuals in our limited sample precluded our ability to ascribe this as a definitive source of observed differences in oral clearance. Rather, those with higher free bilirubin levels may simply represent greater competition for those with compromised capacity to glucuronidate fenofibric acid (GG individuals) relative to those with greater capacity (AA individuals).

Typically administered as a prodrug, fenofibrate is rapidly converted to its active moiety, fenofibric acid, by esterases within the gastrointestinal tract. By one account, [12] 59% of a single dose was determined to be eliminated renally while 25% eliminated via the fecal route, predominantly as fenofibric acid-glucuronide. Our motivation to document the magnitude of impact this *UGT2B7* variation has on the pharmacokinetics of fenofibrate follows a logical path of exploration from earlier evidence of the clinical relevance of this specific SNP, compared to other *UGTs.*[8] From a comprehensive analysis of several candidate SNPs within several *UGT* super families (*1A1*, *1A6*, *1A9*, *2B4* and *2B7*), our GOLDN study of 861 individuals identified the UGT2B7 A-327G SNP to have the greatest and most consistent association with fenofibrate's lipid-altering response. *In vitro* analysis of the role of select *UGT*'s corroborated the

significance of relevant *UGT*s; however, we lacked ideal pharmacokinetic proof of their role *in vivo*. Therefore, the present study's verification of the pharmacokinetic significance of this SNP would be expected to advance our pursuit of genetic and non-genetic determinants of lipid-altering response to fenofibrate, which may ultimately lead to potential guidance when selecting this drug for individuals of a known genotype and/or phenotype.

We decided to utilize the promoter UGT2B7 A-327G (rs7662029) SNP and not the non-synonymous H268Y (rs7439366) SNP in spite of being in linkage disequilibrium (0.99). This decision was based on our own data and that of others, who have studied compounds following analogous pathways of elimination. Specifically, an *in vitro* analysis demonstrated UGT2B7 H268Y to have either limited[15] or no[16] effect on mycophenolate's metabolic fate. Similarly, our own data [6] from *in vitro* work failed to detect a statistically significant difference for the H268Y SNP. In contrast, an *in vivo* study examining the H268Y SNP effect on mycophenolate metabolite demonstrated a 22% ($p \leq 0.05$) difference in mycophenolate serum metabolite $AUC_{0\text{-}12}$[17], which is entirely consistent with the findings from this study and GOLDN *in vivo* studies.[8] This incongruity between *in vitro* and *in vivo* studies may be explained by mechanisms by which a promoter SNP affects the overall expression of *UGT2B7* gene and, perhaps, in a tissue-dependent manner. Although this nonsynonymous change (UGT2B7 Y268H) did not have an effect on fenofibric acid or mycophenolate, we cannot rule out the

possibility that it may have an effect on the glucuronidation of other drugs. Future studies quantifying mRNA of *UGT2B7* in relation to promoter SNPs, including the -327 variant, may lend greater clarity to the mechanism behind this observation.

Since there is an association between higher fenofibric acid concentrations and greater lipid-altering effects [18], we hypothesized the observed difference in this response may be modulated, at least in part, by this SNP's effect on the fenofibric acid's AUC. Consequently, subjects who are GG for rs7662029 would be expected to have a higher AUC due lower glucuronidation activity compared to AA subjects. Their lower apparent oral clearance may thus result in a greater potential exposure to the site of action and thus lipid-altering response compared to the AA genotype. Since 59% of the dose is renally eliminated, we would also anticipate these variations to be reflected in estimates of renal clearance of fenofibric acid. However, AA individuals did not exhibit higher fenofibric acid concentrations in urine compared to GG subjects. This observation was again consistent with the pattern of serum and urine data collected from studies of mycophenolic acid.[17] Although the design of this study precludes the specific identification of a basis for this observation, we hypothesize that the alternative routes of elimination, such as the fecal route which represents ~25% of fenofibric acid's elimination, may be playing a larger role for AA vs. GG individuals.[12]

The present study complements the GOLDN study by substantiating this SNP's impact on the pharmacokinetics of fenofibric acid in a prospective manner. As such, it was designed with the intent to quantify the apparent oral and renal clearance of fenofibric acid using an adequate sampling strategy. Given its primary purpose, it contrasts with the GOLDN study whose design, power and sample size was chosen primarily for the discovery of genetic determinants (drug target and drug disposition sources) of lipid response. Although the GOLDN study initially identified UGT2B7A-327G to be an important determinant of serum trough concentration as well as lipid-altering response, it lacked a sampling strategy to more fully characterize fenofibric acid's disposition. On the other hand, given that baseline lipid values are an important determinant of fenofibrate's lipid lowering effect[19] and our present study included subjects with minimal restrictions on baseline fasting lipid values (Table 1), our ability to distinguish lipid-altering effect between targeted genotype groups was naturally limited. Indeed, in order to replicate the magnitude of lipid changes observed in the GOLDN study, we would estimate the need for at least ~40 subjects (vs. ~25) for each genotype group. Although the subjects recruited for this study would not represent clinical candidates for this drug, overall the expected changes for all lipid fractions were observed across both groups (Table 4). In summary, data generated from both studies provide the pharmacokinetic evidence supporting the observed lipid changes based on this SNP's impact on drug disposition and therefore supports the genetic basis for observed response differences between the genotypes studied.

Ultimately, forecasting drug responsiveness for those drugs whose concentrations relate to response will require approaches which combine important genetic determinants of drug metabolizing and drug target genotypes. One such example is represented by warfarin dosing algorithms, which have had some success to date.[20] Although dosage selection would not be the primary objective of such an approach for fenofibrate, the value of understanding genetic determinants of fenofibrate's response would be linked to its ability to determine a candidate's eligibility to receive the drug on the basis of its predictive power to improve lipid profiles or outcomes. Fibrates, unlike many therapeutic agents, are administered as a fixed dose for all but a select few adults with renal impairment. As such it is unique compared to most other therapeutic agents in that it is not titrated to response. This practice underscores the importance of considering the impact of genetic variants as determinants of fenofibric acid's disposition as a determinant of potential response to its lipid-altering effects. Whereas a clinician may consider adjusting the dose to facilitate achievement of a particular magnitude of response with other drugs, say a statin, the same is not true for fibrates. Consequently, achieving a desired response is simply left to simple trial-and-error for a given individual based on the recommended single dose suggested for all individuals. In so far as an individual's genotype provides some predictability of their likelihood of being either an optimal or sub-optimal responder, such knowledge would provide valuable guidance to clinicians selecting drug therapies

for prospective candidates. Before such an approach becomes clinically feasible, studies the predictive performance of these genetic markers of responder phenotype, especially when combined with additional genetic and non-genetic predictors of response, must be completed along with feasibility studies.

The importance of UGT2B7 A-327G genotype can best be ascertained in the context of a comprehensive model considering other genetic and non-genetic (e.g. renal function) factors. Our attempts to understand sources of variability will hopefully lead to an improved understanding as to why some patients respond well to this drug while others do not - both from an efficacy and toxicity perspective. Understanding drug response variability in patients may provide insight towards understanding the mixed outcomes in major clinical trials such as the ACCORD[4] and FIELD [3] studies, which sought to assess the role of fenofibrate in patients with type 2 diabetes to lower the risk of CVD and other endpoints. Evidence of fenofibrate's benefit either independent of or in addition to statin therapy was not readily apparent from the primary analyses of either the FIELD or ACCORD studies. Notwithstanding sub-analyses which identified select subgroups who derived benefit from fenofibrate drug therapy[21], debate continues as to the basis for the overall observation of a lack of overall outcome benefit from fenofibrate. At present, it appears obvious that at the very least, the identification of optimal candidates for fenofibrate has not yet occurred. Ultimately, analysis of the lipid and clinical outcomes from the FIELD and ACCORD study on the basis of genetic

determinants of variability in lipid altering response may provide insight as to the true utility of genotype-guided selection of optimal candidates for receiving fenofibric acid therapy.

In conclusion, data indicate that UGT2B7 A-327G (rs7662029) represents a significant determinant of serum drug concentrations of fenofibric acid, which appear to result in a substantive 27% difference in overall exposure between the two genotypes studied. Given the association between drug concentration and lipid-lowering response, this observation lends credibility to the contention that UGT2B7 A-327G is a potentially significant predictor of lipid response phenotype for fenofibrate. The eventual combined results of this SNP in the context of other relevant drug target SNPs are expected to further differentiate responder phenotypes which can support clinical guidance for the selection of superior vs. inferior candidates to receive fenofibrate.

2.5. ACKNOWLEDGEMENTS

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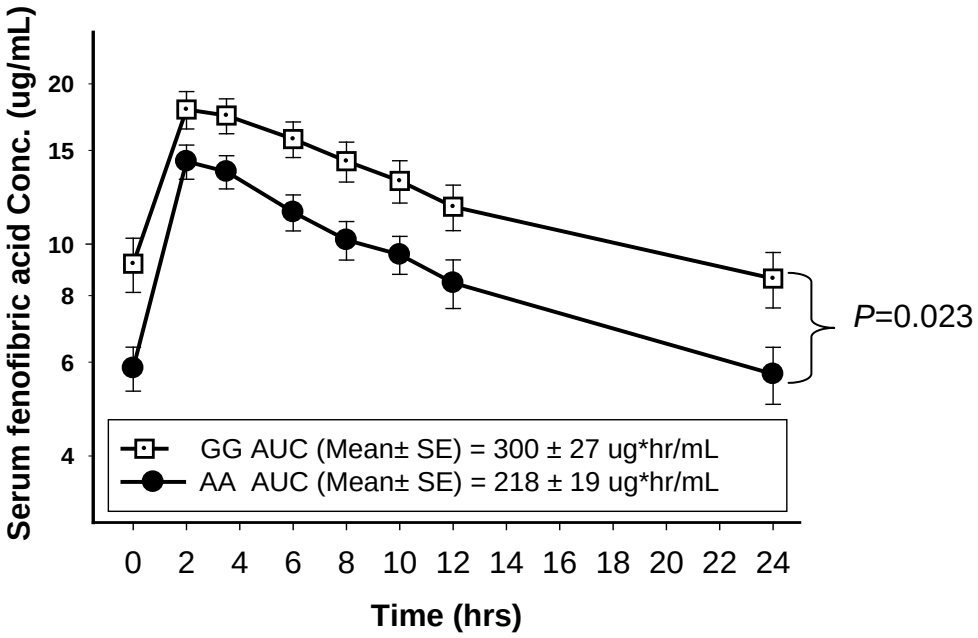


Figure 1: Mean and standard error (SE) of the serum concentrations of fenofibric acid on a log scale from the 8 blood samples collected in the 24 hour period. The open squares are the GG (n=23) genotype and solid circles are the AA (n=26) genotype for UGT2B7 A-327G. $AUC_{0\text{-}24}$ =

Table1: Baseline value of subjects n=49.

	UGT2B7 A-327G (n=49)				
	AA (n=26)		GG (n=23)		
Variable	**Mean (±sd)**	**Median**	**Mean (±sd)**	**Median**	***P*-value**
Gender (male/female)	10/16		9/14		0.59
Age (years)	34.4 (12.9)	30.5	30.7 (10.6)	28	0.28
Body mass index (kg/m2)	29.8 (6.1)	29	28.4 (5.7)	28	0.41
Creatinine clearance (mL/min)	101 (22)	96	112 (25)	109	0.11
Total cholesterol (mg/dL)	181 (35)	184	192 (33)	191	0.26
HDL-C (mg/dL)	44 (11)	42	47 (12)	47	0.42
LDL-C (mg/dL)	108 (30)	105	119 (28)	112	0.20
non-HDL-C (mg/dL)	136 (36)	133	143 (34)	144	0.52
Triglyceride (mg/dL)	141 (81)	115	129 (57)	116	0.53
Glucose (mg/dL)	95 (23)	90	90 (10)	89	0.28
Insulin (μIU/mL)	10.2 (7.5)	9.5	9.2 (5.3)	7.9	0.59
Direct bilirubin (mg/dL)	0.14 (0.06)	0.13	0.14 (0.04)	0.13	0.93
Total bilirubin (mg/dL)	0.48 (0.24)	0.4	0.53 (0.26)	0.5	0.53
HOMA-B	130 (112)	96	131 (69)	113	0.98
HOMA-IR	2.5 (2.2)	2.1	2.1 (1.3)	1.7	0.39
hs-CRP (mg/L)	3.8 (6.1)	1.6	4.2 (5.7)	1.9	0.49
No. subjects on OC or HRT	3		7		0.10
No. subjects with metabolic syndrome	7		3		0.19
Blood pressure (mmHg)	120/77 (11.1/9.1)	118/ 76	122/76 (14.7/11.8)	120/76	0.62/0.82

The *p*-values are a comparison of AA vs. GG genotype groups. Continuous data were determined using T-Test two sample with unequal variance, while a Chi Squared analysis or Fisher's exact test were used for categorical data (where appropriate).

HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, non-HDL-C=non-high density lipoprotein cholesterol, HOMA-IR=homeostasis model assessment–insulin resistance, HOMA-B=homeostasis model assessment–Beta, OC= oral contraceptive, HRT= hormone replacement therapy, hs-CRP= high sensitivity C-reactive protein

Table 2: Effect of UGT2B7 A-327G on the pharmacokinetic of fenofibrate in serum.

	UGT2B7 A-327G SNP (n=49)			
	AA (n=26)	GG (n=23)		All (AA+GG) (n=49)
Variable	**Mean (±sd)**	**Mean (±sd)**	***P*-value**	**Mean (±sd)**
AUC_{0-24} (µg*hr/ml)	218 (96)	300 (130)	0.026§	256 (120)
C_{max} (µg/mL)	15 (4.9)	18.9 (6.5)	0.027†	16.9 (6.2)
T_{max} (hr)	2.4 (0.69)	2.6 (1.01)	0.85†	2.59 (0.98)
Cl_{oral}/F (L/hr)	0.74 (0.3)	0.59 (0.28)	0.035†	0.69 (0.32)
K_e (hr^{-1})	0.044 (0.018)	0.036 (0.013)	0.12†	0.039 (0.016)
V_d/F (L)	18.4 (5.7)	16.6 (5.7)	0.28†	17.6 (5.7)
$T_{1/2}$ (hr)	19 (9.9)	22 (9.1)	0.34†	20 (9.6)

§ The *p*-value as determined by multiple regression analysis model, which included the following covariates: free bilirubin level at visit 3 and body mass index.

† *P*-values for continuous data were determined using T-Test two sample with unequal variance.

AUC_{0-24} = Area under the curve of the serum concentration of fenofibric acid for 24 hours period. C_{max}= maximum serum drug concentration, T_{max}= time at maximum serum drug concentration (C_{max}). Cl_{oral}/F=apparent oral clearance (total clearance), K_e= elimination rate constant, V_d/F=apparent volume of distribution, $T_{1/2}$= half-life.

Table 3: Effect of UGT2B7 A-327G on the pharmacokinetic of fenofibrate in urine.

	UGT2B7 A-327G SNP (n=49)			
	AA (n=26)	GG (n=23)		All (n=49)
Variable	Mean (±sd)	Mean (±sd)	*P*-value	Mean (±sd)
Amount of fenofibric acid 0-6h (µg)	8.97 (4.2)	9.04 (3.7)	0.91	9.00 (3.9)
Amount of fenofibric acid 6-12h (µg)	8.41 (5.4)	7.79 (4.3)	0.79	8.11 (4.8)
Amount of fenofibric acid 12-24h (µg)	4.96 (2.4)	5.17 (2.1)	0.95	5.06 (2.2)
% of dose as fenofibric acid in urine	14.8 (6.2)	15.2 (4.6)	0.89	14.9 (5.5)
Cl_{renal} (L/hr)	0.108 (0.05)	0.089 (0.05)	0.16	0.09 (0.05)

P-values for continuous data were determined using T-Test two sample with unequal variance.

Table 4: Change of lipids from baseline (%).

	UGT2B7 A-327G SNP (n=49)		
	AA (n=26)	**GG (n=23)**	
Lipid	**Mean (±sd)**	**Mean (±sd)**	***P*-value**
Total cholesterol	-15.6 (8.6)	-16.9 (13.6)	0.081
HDL-C	2.1 (12.3)	0.96 (17.5)	0.78
LDL-C	-17.9 (12.4)	-19.2 (17.1)	0.079
non-HDL-C	-20.8 (11.3)	-20.5 (15.6)	0.065
Triglyceride	-28.4 (19.5)	-31.7 (19.4)	0.719

The *p*-value as determined by multiple regression analysis model, which included the following covariates: fenofibrate serum concentration (AUC_{0-24}) and the baseline level of corresponding lipid.

% change from baseline= (Post-fenofibrate lipid level - Pre-fenofibrate lipid level)/Pre-fenofibrate lipid level*100, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, non-HDL-C=non-high density lipoprotein cholesterol. *P*-values are based on a multiple regression analysis; including AUC_{0-24} of fenofibric acid, baseline (pre-fenofibric acid) lipid level

Table 5: Inclusion and exclusion criteria of the study.

Inclusion criteria

1. >18year old
2. Be willing to participate in the study and attend the scheduled clinic exams.
3. Did not use lipid lowering therapy for the past 4 weeks.

Exclusion criteria

1. <18 years old.
2. History of liver, kidney, pancreas, gall bladder disease or malabsorption (e.g. Crohn's disease).
3. Use of insulin or currently taking warfarin.
4. Pregnant women or women of childbearing potential not using an acceptable form of contraception.
5. History of an allergy or hypersensitivity to fenofibrate.
6. Investigational drug use within 30 days of the study.

CHAPTER III

Comparing The Effect Of Fenofibrate On Serum Bilirubin Levels Between UGT1A1*28 Genotypes.

3.1. BACKGROUND

Epidemiological evidence has provided a basis for the association between serum bilirubin level and cardiovascular diseases (CVD) such that low bilirubin levels have been associated with a higher risk of coronary artery disease [1] and stroke [2], while chronic elevations of bilirubin levels have been shown to be protective against CVD.[3-5] The proposed mechanism of this beneficial association may relate to bilirubin's antioxidant properties which it is discussed in detail by others.[6-9]

Bilirubin is produced by the degradation of the heme found in the red blood cells. Alterations in serum bilirubin levels can occur as a result of either a decrease in elimination, an increase in production or some variation in both. Both genetic factors and pharmacological agents can influence bilirubin levels and therefore should be simultaneously considered while evaluating their potential impact on cardiovascular event rates.

The genetically based condition known as Gilbert syndrome is characterized by moderately elevated levels of total bilirubin. Gilbert syndrome is typically associated with a genetic variant (rs8175347) found on chromosome 2 within the uridine diphosphate glucuronyltransferase (UGT) 1 family, polypeptide A1 (UGT1A1 gene). This variation is a short tandem repeat (microsatellite) variation of TA repeats found in the TATA-box promoter area of the *UGT1A1* gene. Whereas most commonly

occurring count of TA repeats is 6 (designated as $TA_{6/6}$ or UGT1A1*1/*1), patients with Gilbert syndrome have 7 TA repeats ($TA_{7/7}$ or UGT1A1*28/*28).[10] Individuals with Gilbert's syndrome are reported to have as much as 52% lower glucuronidation activity compared to UGT1A1*1/*1 individuals, leading to a lower levels of conjugated (direct) bilirubin and consequently higher levels of free (uncongugated or indirect) bilirubin.[10,11] The allele frequency of UGT1A1*28 is not insignificant (~29-34%) within Caucasians and other populations.[12-14]

Serum bilirubin levels may also be affected by pharmacological agents. For example, the antidyslipidemic drug clofibrate has been demonstrated to lower the levels of bilirubin [15] by inducing the *UGT1A1* gene [16] resulting in the increased creation of mono-, di- or even tri-conjugated-bilirubin. The conjugate, known as glucuronide, renders the complex more hydrophilic facilitating its elimination by renal or intestinal routes. Fenofibrate is a commonly used antidyslipidemic agent of the same general class as clofibrate which is known to be extensively metabolized by UGT enzymes. *In vitro* [17] and *in vivo* [18] studies have shown that both genes families *UGT1A* and *UGT2B* are involved in the glucuronidation (conjugation) of fenofibrate in addition to bilirubin. However, in contrast to clofibrate, studies reporting fenofibrate's overall effect on bilirubin levels have been inconsistent with some studies reporting no change [19], while other reporting either decreases [20] or even increases in serum bilirubin levels.[21]

We posit that a careful analysis of the impact of fenofibrate on bilirubin levels while considering genetic and non-genetic factors may provide insight as to the basis for these apparent discrepancies. Given the association between serum bilirubin levels and cardiovascular disease, clarity with respect to the role of *UGT's* on the relationship between serum bilirubin levels and fenofibrate use, may provide insight as to what, if any role *UGT's* may play in modulating fenofibrate's effect on CVD. Understanding the genetic determinants of how fenofibrate may affect serum bilirubin levels may provide guidance for interpreting the mixed results from recently completed outcome-based clinical trials of fenofibrate.[22,23]

As we are not aware of any previous investigation evaluating the effect of fenofibrate on serum bilirubin level on the basis of the three different genotypes of UGT1A1*28, we sought to do so utilizing data generated from the Genetics of Lipid lowering Drug Network (GOLDN) trial. Consequently, the objective of our analysis was to assess the effect of 160mg of once daily fenofibrate on serum bilirubin levels in 861 subjects participating in the GOLDN study. Furthermore, we sought to compare the effect of fenofibrate on serum bilirubin levels between UGT1A1*28 genotypes ($TA_{6/6}$, $TA_{6/7}$ and $TA_{7/7}$), and examine the effect of UGT1A1*28 on area under the serum concentration time curve (AUC_{0-6}) of fenofibric acid. Finally we assessed the possible impact of UGT1A1*28 on the variability in the antidyslipidemic response to fenofibrate.

3.2. METHODS

3.2.1. Study Population:

Subjects participating in the Genetics of Lipid lowering Drug Network (GOLDN) trial formed the basis for the study population used for this analysis. The GOLDN study which is part of the PROGENI (PROgram for GENetic Interactions) Network was sponsored by the NIH through the University of Minnesota in collaboration with the University of Utah, Washington University, Tufts University, University of Texas, University of Michigan, University of Alabama and Fairview-University Medical Center.

The GOLDN study was an open-labeled study of male and female subjects taking once daily fenofibrate (160 mg TriCor®) for 21 days. It included two protocols: a full or an abbreviated protocol. The full protocol consisted of an initial screening visit, followed by four study visits. All participants were instructed to fast for ≥12 hours and abstain from using alcohol for ≥24 hours before all clinic visits. The first two study visits were separated by 24 hours as were the third and fourth visit. Visits 2 and 3 were separated by approximately 21 days of fenofibrate administration. The abbreviated fenofibrate protocol was offered only to those individuals who initially refused the full fenofibrate protocol and was identical to the full protocol except for

having two less fasting lipid profiles compared to those participating in the full fenofibrate protocol. Specifically, the abbreviated protocol forewent visit 2 and visit 4 thereby having only one pre-fenofibrate and one post-fenofibrate fasting lipid panel. This study also included two assessments of the effect of a dietary fat challenge on post-prandial lipemia. One assessment was conducted while participants were taking no lipid-lowering medications, and once following the 3 week administration of study medication. Subjects were provided a high fat load on visit 2 and immediately after fenofibrate administration on visit 4. Serum samples were taken at 0, 3.5 and 6 hour post administration of this high fat load. Fasting lipid panels were taken at all 4 visits.

The primary efficacy endpoint (for GOLDN study) was the change in triglycerides relative to baseline. Secondary efficacy endpoints are the changes from baseline for low-density lipoprotein (LDL-C), total cholesterol, high-density lipoprotein (HDL-C), and non-HDL cholesterol. The lipid levels were calculated by averaging the levels of visits 1 and 2 representing the baseline levels (pre-exposure to fenofibrate) and visits 3 and 4 levels for post-exposure levels. Written informed consent was obtained from all GOLDN subjects at the screening visit. The protocol of the study was approved by the investigational review board (IRB) of the University of Minnesota, University of Utah, and Tufts University. The detailed design and methodology of the GOLDN study has been previously described.[24,25] The GOLDN study was registered with Clinicaltrial.gov (NCT00083369).

3.2.2. SNP Selection and genotyping:

Genomic DNA was isolated from peripheral blood leukocytes using Puregene DNA reagents following the vendor's protocol. Genotyping the UGT1A1*28 SNP of interest was conducted at the University of Minnesota BioMedical Genomics Center from 20ng of genomic DNA, a 98 bp segment including the TA repeat region was amplified using the forward primer 5' GTCACGTGACACAGTCAAAC 3' and reverse primer 5' TTTGCTCCTGCCAGAGGTT 3' with 6-FAM tag attached to the 5' end of the reverse primer.[26,27] These primers anchor the TA locus in the promoter region of the UGT1A1 gene. They amplify a 98 bp fragment when a $(TA)_6$ allele is present and 100 bp fragment when a $(TA)_7$ allele is present. The DNA was amplified for 35 cycles and the PCR fragments and was run on gel electrophoresis to be analyzed. Control DNAs from individuals known to have a $TA_{6/6}$, $TA_{6/7}$ and $TA_{7/7}$ genotype were included in the PCR analysis and electrophoresis. Amplified regions were then analyzed on a ABI 3130XL capillary size fragmenter to determine the number of the TA repeats.[28] TA counts were called visually utilizing Peak Scanner® version 1.0

3.2.3. Statistical analysis:

Demographic and laboratory characteristics are summarized for all participants taking fenofibrate. Categorical variables are summarized by frequencies while continuous variables are reported by mean± standard deviation (SD) for normally distributed variables or as median and inter-quartile range with minimum and maximum values for those values not normally distributed. For the analysis of the percentage change in lipid levels, the logarithm of the ratio from the mean of two post exposure levels to the mean of two pre-exposure levels (baseline) was used as a dependant variable. Protocol violators or participants less than 75% compliant with fenofibrate were excluded from the overall analysis. All tests were performed at an $\alpha = 0.05$, two-tailed. Statistical tests with *P* value of 0.05 or less were considered statistically significant. Continuous variables not normally distributed were log (natural) transformed prior to calculating inferential statistics. Allele frequencies were estimated for TA stretch and a chi squared test used to examine for deviations of genotypes from Hardy-Weinberg equilibrium (HWE).

The primary objective of the analysis is to characterize the bilirubin response profile in relation to factors affecting bilirubin response to fenofibrate Table 3. Since participants were recruited from families, a linear mixed model was used implemented in R (or SAS version 9.1 SAS Institute, Cary, NC, USA). Within the model, genotypes of UGT1A1*28 were treated as fixed effects and dependencies among members within each family were treated as random effects.[29] The following covariates were included

in the model for testing factors that influence **baseline bilirubin** (Table 3): UGT1A1*28 genotype groups, sex, body mass index and smoking status. The following covariates were included in the model for testing the effect of fenofibrate on **percent change in bilirubin** from baseline (Table 3): UGT1A1*28 genotype groups, fenofibrate serum concentration (AUC_{0-6}), and body mass index**.** Pearson's correlation coefficient test was used to the relation between fenofibric acid serum concentration and percent change in bilirubin from baseline.

3.2.4. Measuring bilirubin levels:

Total bilirubin (conjugated + free) is measured in serum using a diazonium salt/ion colorimetric assay (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Modular P Chemistry Analyzer (Roche Diagnostics). The reference range is 0.2 – 1.3 mg/dL with an inter-assay CV of 3.1%. Conjugated bilirubin is measured in serum on the Roche Modular P Chemistry Analyzer using a colorimetric assay based on the diazo Jendrassik-Grof procedure (Roche Diagnostics). The reference range is 0 – 0.2 mg/dL with an inter-assay CV of 4.7%.

3.2.5. Measuring fenofibric acid serum concentration:

Serum fenofibric acid concentrations were quantified using HPLC in samples drawn at 0, 3.5, and 6hour after administering the last dose of fenofibrate at visit 4.

Briefly, samples were extracted using an anion-exchange solid-phase extraction procedure. Chromatographic separation was performed using isocratic conditions, with an ultraviolet detection at 285 nm. A complete procedure has been previously published.[30]

3.2.6. Pharmacokinetics analysis

A partial (0-6 hour) area under the serum concentration-time curve (AUC_{0-6}) of fenofibric acid was calculated by the linear trapezoidal rule, using a noncompartmental analysis on WinNonlin®(v6.2), from 0, 3.5, and 6hour time points.

3.2.7. Measuring lipid levels:

Triglycerides were measured by glycerol-blanked enzymatic method on the Roche COBAS FARA centrifugal analyzer (Roche Diagnostics Corporation). The GOLDN study measured NMR LDL-C and HDL-C particle size in addition to triglyceride -rich lipoproteins and remnant particles. This method uses signal amplitudes of the lipoprotein subclasses of difference sizes as its basis of quantification. Comparison of NMR and ultracentrifugation separation in this study population showed a high degree of correlation, suggesting that NMR is a valid alternative method for measuring triglyceride -rich lipoproteins. Blind duplicate samples from 5% of participants were sent to the laboratory to assess repeatability. For all lipid

subfractions, the repeatability was above 90%. All blood samples from each individual were stored until the completion of their participation and then analyzed together.

3.3. RESULTS

Subject characteristics and UGT1A1*28 genotype, baseline characteristics of the patient population studied are summarized in Table 1. Both genders were equally represented with an overall mean (range) age of 48.4 (18-83) years. Twenty six perecent had the diagnosis of hypertension, 44% had metabolic syndrome and 8% with type 2 diabetes mellitus. The majority of the participants had a normal renal function with a mean (range) creatinine clearance of 97 (30-263) ml/min.

Eight hundred and thirty five subjects were genotyped for UGT1A1*28 variants. From this pool, 384(45.9%) were $TA_{6/6}$, 374(44.7%) were $TA_{6/7}$, 77(9.2%) were $TA_{7/7}$, 1(0.1%) were $TA_{5/7}$ and 1(0.1%) were $TA_{6/8}$ Table 2. The observed allelic frequency of the polymorphism, UGT1A1*28, was comparable to HapMap data (build 37.3). There were no significant differences among the three genotypes groups in age, BMI, creatinine clearance and the percent of the following: alcohol drinker, current smokers, oral contraceptive and hormone replacement therapy use, number of participants recruited from Utah center compared to Minnesota, participants diagnosed with hypertension, diabetes and metabolic syndrome. The only exception is sex, where the males represented only 34% of the individuals with the $TA_{7/7}$ genotype (P = 0.0088) Table 2.

Bilirubin levels

Baseline analysis of bilirubin levels: the overall baseline mean (±sd) of total bilirubin was 0.54mg/dL (±0.26), conjugated bilirubin 0.15mg/dL (±0.06) and free-bilirubin 0.39mg/dL (±0.22). Considerable differences were observed according to UGT1A1*28 genotype. For example, the baseline mean (±sd) of total bilirubin in the 7/7 group was 0.85mg/dL (±0.41) which was significantly higher than both the 6/6 and 6/7 individuals at 0.48mg/dL (±0.2) and 0.55mg/dL (±0.22) respectively (p<0.0001 for both). A similar pattern emerged for the conjugated bilirubin where the mean (±sd) value for 7/7 individuals was 0.21mg/dL (±0.07) which was again significantly higher than both the 6/6 and 6/7 individuals at 0.14mg/dL (±0.05) and 0.16mg/dL(±0.06) respectively (p<0.0001 for both). Finally, the pattern was again followed for the free-bilirubin values Table 3.

Fenofibrate's effects on bilirubin levels: The overall change from baseline post-fenofibrate exposure mean (±sd) of total bilirubin -5.65% (±32.5), conjugated bilirubin 5.14% (±33.9) and free-bilirubin -6.44% (±43.3) Figure 1. The post-fenofibrate change from baseline varied considerably according to the UGT1A1*28 genotype. For example, the mean (±sd) change from baseline of total bilirubin in the $TA_{7/7}$ group was -24.7% (±27.6) which was significantly different from both the $TA_{6/6}$ and $TA_{6/7}$ groups whose changes from baseline were -1.77% (±30.4) and -6.57% (±33.5) respectively

(p<0.0001 for both). An identical pattern existed for the conjugated bilirubin and free-bilirubin Figure 2(a) and Table 3. Impact of smoking: In a sub-analysis of the effect of smoking on baseline bilirubin levels, smokers had a significantly lower total- and free-bilirubin baseline levels compared to non-smokers. Specifically mean (±sd) total bilirubin baseline levels were 0.45 (±0.21) vs. 0.55 (±0.26) mg/dL (p<0.001) for smokers vs. non-smokers, while mean (±sd) free bilirubin baseline levels were 0.31 (±0.17) vs. 0.4 (±0.22) mg/dL (p<0.001) again for smokers vs. non-smokers. However, the differences in conjugated bilirubin baseline levels, for smokers 0.14 (±0.05)mg/dL vs. non-smokers 0.16 (±0.06) did not achieve statistical significance(p<0.071). When further sub-grouped according to UGT1A1*28 genotype, the only significant difference observed between smokers and non-smokers was that within the $TA_{7/7}$ genotype group Table 4.

Analysis within smokers alone, indicate the effects of fenofibrate on percent change in bilirubin levels from baseline (V1) through post-fenofibrate exposure (V3) are null, regardless of UGT1A1*28 genotype Table 4 and Figure 2(b). This contrasts with the clear pattern of statically significant differences for the effects of fenofibrate on percent change in bilirubin levels from baseline (V1) through post-fenofibrate exposure (V3) within the non-smoking group with only one exception – that within the $TA_{6/7}$ group for conjugated bilirubin Table 4 and Figure 2(c).

Fenofibric acid concentrations: The overall mean (±sd) of serum AUC_{0-6} of fenofibric acid for all participants was 69.7 (±32.3) μg*hr/mL. According to UGT1A1*28 variant the mean (±sd) serum fenofibric acid (AUC_{0-6}) for the $TA_{6/6}$ and $TA_{6/7}$ genotype groups was some 13.6% (p=0.03) and 11.9% (p=0.025) lower than that for the $TA_{7/7}$ group. There were no significant differences between genotype groups when individual time points (0, 3.5 and 6hour) were considered Figure 3 and Table 5.

Lipids levels: An association between higher fenofibric acid AUC_{0-6} (exposure) and higher percent change in serum lipid fraction from baseline, were confirmed for triglyceride p<0.0001, HDL-C p=0.0054, total cholesterol p<0.0001, LDL-C p<0.0001, non-HDL-C p<0.0001. No effect of the UGT1A1*28 genotype was noted for any baseline value for serum lipids with the exception of a lower mean (±sd) LDL-C level for the $TA_{7/7}$ individuals compared to those who were $TA_{6/7}$ (124 (±32) vs. 117mg/dL (±30) mg/dL (p=0.044) respectively). No significant differences in the serum lipid change from baseline between the three genotypes groups were noted Table 6.

3.4. DISCUSSION:

Although, fenofibrate is primarily prescribed for the treatment of dyslipidemia, its ancillary pharmacological effects include its ability to alter levels of bilirubin. Since elevated bilirubin levels have been associated with cardioprotection [3-5], the clinical significance of this pleotropic effect warrants further study in order to interpret its potential relevance to fenofibrate's cardiovascular outcomes from clinical trials.[22,23]

Previous studies examining fenofibrate's bilirubin-altering effect typically report no clear pattern of response.[19-21,31] However given their modest sample sizes ranging from 17-40, these studies were either inadequately powered or lacked an appropriate design to examine this outcome. In contrast, the present study represents the largest (n=835) conducted with the capacity to provide clarity and insight as to the specific direction and magnitude of fenofibrate's bilirubin-altering effect based on our capacity to examine this outcome from the perspective of known UGT1A1*28 genotype. Consequently, the present study provides the first ever comprehensive report of the effect of fenofibrate on bilirubin by UGT1A1*28 genotype. In doing so, it serves to explain why other analyses produced mixed results simply due to the heterogeneity of outcome for the group as a whole and simultaneously provides a basis to examine this relationship as a factor when selecting optimal candidates to receive fenofibrate as a therapeutic entity.

Overall, our study demonstrated that fenofibrate lowers total-bilirubin by 5.7%. This was accompanied by a reduction in free-bilirubin by 6.4% and an increase in conjugated-bilirubin by 5.1%, from baseline levels. Although the specific mechanism by which fenofibrate alters bilirubin levels cannot be determined from this study, these observations are consistent with the hypothesis that fenofibrate may up-regulate *UGT* activity. This up-regulation in turn, leads to the observed increase in the conjugation of

the free-bilirubin an observation consistent with fenofibrate's effect on bile acid elimination also mediated by *UGTs*.[32]

Examination of these overall effects on bilirubin levels do not provide a complete story without consideration of two critical factors affecting bilirubin levels. The present study establishes, for the first time, UGT1A1*28 to be a critical determinant of fenofibrate's bilirubin-altering response and provides novel insight as to the differential impact of smoking within UGT1A1*28 genotype.

Specifically, subjects who are $TA_{7/7}$ exhibit a significant reduction in total bilirubin levels while subjects who are $TA_{6/6}$ exhibit the least predictable reduction in total bilirubin levels in response to fenofibrate. Individuals who are $TA_{6/7}$ are intermediate in their magnitude of fenofibrate's tendency to decrease in bilirubin levels. These differences are detailed in Table 3 and Figure 2(a).

Furthermore, fenofibrate's effect within UGT1A1*28 genotypes vary-in part based on the differences between UGT1A1*28 genotypes' baseline (or pre-fenofibrate) bilirubin values. For example, there was a profound difference for change in total bilirubin levels in response to fenofibrate between $TA_{7/7}$ (-24.7%) and either of the $TA_{6/7}$ (-6.7%) or $TA_{6/6}$ (-1.8%) group Table 3. Notably a 2-fold higher total bilirubin baseline level was observed within the $TA_{7/7}$ genotype group and a 1.5-fold higher free- and

conjugated-bilirubin baseline level compared to either the $TA_{6/7}$ or $TA_{6/6}$ genotype groups. Consequently, the decrease in conjugated-bilirubin level after the treatment with fenofibrate in the $TA_{7/7}$ genotype, which contrasts with to the increases observed for the $TA_{6/7}$ and $TA_{6/6}$ genotype groups, is likely due to the overall decrease in the free-bilirubin level. The differences noted in the baseline bilirubin levels between $TA_{6/7}$ and $TA_{6/6}$ individuals relative to $TA_{7/7}$ individuals for UGT1A1*28 follow a pattern which would be expected from a loss of function for those with higher repeat numbers.[11] Peters et al.[11] have suggested that the *UGT1A1* gene for those who are $TA_{7/7}$, may be functioning at a level of less than 50% of that of the $TA_{6/7}$ or $TA_{6/6}$ individuals. Consequently, we would expect $TA_{7/7}$ individuals to have a higher baseline bilirubin level and, once induced by the exposure to fenofibrate, display greater reductions of bilirubin levels to nearly normal levels. Indeed, those observations were evident within our subjects Table 3.

As mentioned, our ability to identify a large number (n=77) of individuals with the $TA_{7/7}$ genotype represents a strength of our study. This 9.2%, prevalence within of our 837 subjects was consistent with its prevalence observed within other Caucasian populations.[12-14] Logically studies with a smaller sample sizes[19-21,31] of less than 50 would not be able to distinguish extreme responders, high total bilirubin reduction, within a genotype from potential outliers and thus would lack the ability detect these genotype-based observations.

We confirm smoking status to play its expected role in contributing to lower baseline bilirubin-levels relative to non-smokers.[33,34] Others [35,36] have shown the proposed mechanism for this observation appears to be mediated by the induction of *UGT* genes. In the present study, cigarette smoking was significantly associated with a 22.2% lower total bilirubin level compared to non-smokers. What has not yet been reported however is the relative effect of smoking within the $TA_{7/7}$ genotype group compared to other genotype groups. For example, individuals who were $TA_{7/7}$ current smokers had a statistically significant (p=0.002), 51% lower level of total bilirubin compared to $TA_{7/7}$ nonsmokers. This differential effect for those who are reported to be cigarette smokers could explain the partial disconnect between genotype and phenotype within the $TA_{7/7}$ genotype group; where a smoker's genotype would suggest they would have a high bilirubin level yet phenotypically, they resembled those with a bilirubin level more typical of either a $TA_{6/7}$ or $TA_{6/6}$ individual. Furthermore, smokers unlike non-smokers, displayed no significant differences in baseline bilirubin level across any of the UGT1A1*28 genotypes. We conclude from the previous observations that UGT1A1*28 genotype will not be a predictor of bilirubin level change in response to fenofibrate treatment in smokers.

Glucuronidation is essential for the effective elimination of endogenous compounds and xenobiotics. Fenofibrate is rapidly hydrolyzed to its active form, fenofibric acid, and

thus further metabolized almost exclusively by UGT's enzymes.[37] From analysis of our serum concentration data, it appears as if the $TA_{7/7}$ individuals had a decreased ability to glucuronate and thus eliminate fenofibric acid. This was evident by the ~15% higher serum concentration of fenofibric acid (AUC_{0-6}) within the $TA_{7/7}$ compare to $TA_{6/6}$ and $TA_{6/7}$ genotype groups, $p<0.05$. In spite of previously demonstrated associations of between higher concentration of fenofibric acid and greater lipid-altering response, the difference in AUC_{0-6} observed between the UGT1A1*28 genotype groups did not translate into detectable differences in lipid-altering response. From previous *in vitro* work, fenofibric acid is predominantly metabolized by *UGT1A9* and *UGT2B7* and to lesser extent *UGT1A1* [17,38], so this genetic variation in *UGT1A1* might not have a sufficient impact on fenofibric acid's pharmacokinetics parameters such that it would affect its lipid lowering response. Furthermore, there are most certainly other genetic and non-genetic factors contributing to lipid altering response which were not considered in this analysis.

Advances understanding to the effect of fenofibrate on bilirubin levels may provide insight for interpreting the mixed outcomes associated with fenofibrate use within recently conducted clinical trials.[22,23] From these trials, fenofibrate's clinical benefits were limited to clinical outcomes related to microvascular disease and/or limited to those patients with atherogenic dyslipidemia. Based on the findings from the present study, we would posit UGT1A1*28 genotype and smoking status to be potential

covariates worthy of analysis within these outcome-based studies. Thus, we would advocate for such analysis of outcome-based studies to determine if subjects with $TA_{7/7}$ genotype are inferior candidates to receive fenofibrate. Even though $TA_{7/7}$ individuals had higher concentrations of fenofibric acid within our study, these elevated concentrations did not translated into superior antidyslipidemic response. This may be due to the relatively modest metric of exposure (limited to a "partial" AUC_{0-6} from only 3 time points and/or possibly the nature of the subjects of our study representing relatively few who displayed atherogenic dyslipidemia. Specifically, the majority of participants of this study were had average triglyceride and HDL-C levels of 139 and 46.7mg/dL respectively and thus would not represent ideal candidates for the use of fenofibrate. Nonetheless, the enhanced lowering of cardioprotective levels of bilirubin normally associated with individuals who were $TA_{7/7}$ for UGT1A1*28 within outcome-based studies may represent a population within which fenofibrate's effect of lowering their bilirubin levels actually deprive such individuals from their otherwise advantageous cardioprotective levels of bilirubin.

In conclusion we confirmed that both UGT1A1*28 and smoking status may be important determinants of fenofibrate's bilirubin-altering effect. The apparent differential induction of *UGT*'s by fenofibrate within UGT1A1*28 genotypes along with cigarette smoking status, may represent important covariates to consider when selecting optimal candidates to receive fenofibrate.

Table 1: baseline value between three genotype groups of the UGT1A1*28

UGT1A1*28 genotype	All (n=835)		$TA_{6/6}$ (n=384)		$TA_{6/7}$ (n=374)		$TA_{7/7}$ (n=77)		*P*-value
	$\bar{X}$	Range	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	
Age, years	48.4	18-83	48.2	16.4	48.6	15.6	48.3	15.4	0.9395
Sex (male) %	50	-	53	-	50	-	34	-	**0.0088**
BMI, kg/m²	28.4	16.6-52.7	28.4	5.4	28.4	5.5	29.0	6.3	0.6019
Drinkers, %	49	-	46	-	52	-	53	-	0.1539
Smokers %	7.5	-	7	-	7	-	10	-	0.3494
OC or HR %	16	-	15	-	15	-	19	-	0.6457
Hypertension %	26	-	25	-	26	-	31	-	0.5571
Creatinine clearance ml/min	97	30-263	97.5	32.1	97.3	29.8	93.1	26.5	0.5054
Utah center %	49.9	-	52.6	-	47.3	-	49.4	-	0.3446
Diabetes type 2, %	8	-	7	-	8	-	10	-	0.5116
Metabolic syndrome %	44	-	45	-	44	-	43	-	0.9144

P-values for continuous data were determined using analysis of variance (ANOVA) while a Chi Squared analysis or Fisher's exact test, were used for categorical data (where appropriate). $\bar{X}$ = mean, SD=standard deviation OC= oral contraceptive, HR= Hormone Replacement therapy. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene.

Table 2: TAn/n genotypes frequency (n=837)		
Genotype	**Frequency**	**Percent**
$TA_{6/6}$	384	45.9
$TA_{6/7}$	374	44.7
$TA_{7/7}$	77	9.2
$TA_{5/7}$*	1	1
$TA_{6/8}$*	1	1
*excluded from the analysis		

Table 3: Bilirubin baseline level and percent change post-fenofibrate treatment according to UGT1A1*28 genotypes

UGT1A1*28 genotype	All		$TA_{6/6}$		$TA_{6/7}$		$TA_{7/7}$		***P*-value¥**		
	n=835		n=384		n=374		n=77				
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$TA_{6/6}$ vs. $TA_{7/7}$	$TA_{6/7}$ vs. $TA_{7/7}$	$TA_{6/6}$ vs. $TA_{6/7}$
Pre-fenofibrate (V1)											
Total bilirubin (mg/dL)	0.54	0.26	0.48	0.20	0.55	0.22	**0.85**	0.41	**<0.0001**	**<0.0001**	**<0.0001**
Conjugated bilirubin (mg/dL)	0.15	0.06	0.14	0.05	0.16	0.06	**0.21**	0.07	**<0.0001**	**<0.0001**	**<0.0001**
Free-bilirubin(mg/dL)	0.39	0.22	0.34	0.16	0.39	0.18	**0.64**	0.36	**<0.0001**	**<0.0001**	**0.0001**
Change											
Total bilirubin %	-5.65	32.5	-1.77	30.4	-6.57	33.5	**-24.7**	27.6	**<0.0001**	**<0.0001**	**0.0263**
p-value£	**<0.0001**		**0.000537**		**<0.0001**		**<0.0001**				
Conjugated bilirubin %	5.14	33.9	8.66	31.1	3.94	36.2	**-8.13**	31.4	**<0.0001**	**0.003**	**0.0314**
p-value£	0.75		**0.000878**		0.385		**0.0022**				
Free-bilirubin%	-6.44	43.3	-2.46	43.6	-7.74	42.1	**-28.6**	32.3	**<0.0001**	**<0.0001**	0.0567
p-value£	**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**				

¥ The *p*-value as determined by multiple regression analysis model, which included the following covariates: sex, body mass index and smoking status for the portion of the table corresponding to the **pre-fenofibrate** and fenofibrate serum concentration (AUC_{0-6}) and body mass index for the **change** portion of the table, the 7/7 is the reference group.

£ The *p*-value to test the significance of change from baseline (pre-fenofibrate), as determined by t-test: two-sample assuming equal or unequal variances (where appropriate).

$\bar{X}$ = mean, SD=standard deviation. Pre-fenofibrate corresponds to baseline bilirubin values whereas change in bilirubin fractions were calculated by (post-pre)/pre, expressed as a %, where post corresponds to bilirubin fractions measured after administration of fenofibrate for 21 days. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene.

Table 4: Bilirubin change according to UGT1A1*28 genotypes and smoking status.

		All (n=769)						UGT1A1*28											
								$TA_{6/6}$ (n=355)						$TA_{6/7}$ (n=343)					
		V1		V3		P¥	N	V1		V3		P¥	N	V1		V3		P¥	N
		$\bar{X}$	SD	$\bar{X}$	SD			$\bar{X}$	SD	$\bar{X}$	SD			$\bar{X}$	SD	$\bar{X}$	SD		
Total Bilirubin	Non-smokers	0.55	0.26	0.48	0.20	**<0.001**	**714**	0.48	0.20	0.45	0.18	**<0.001**	**330**	0.56	0.22	0.49	0.19	**<0.001**	**321**
	Smokers	0.45	0.21	0.42	0.17	**0.13**	**56**	0.43	0.22	0.40	0.18	0.4	**25**	0.47	0.20	0.43	0.17	0.41	**23**
	P-value£	**<0.001**						0.21						0.053					
Conjugated Bilirubin	Non-smokers	0.16	0.06	0.15	0.06	0.66		0.14	0.05	0.15	0.06	**0.018**		0.16	0.06	0.16	0.05	0.12	
	Smokers	0.14	0.05	0.14	0.06	0.8		0.13	0.05	0.14	0.08	0.8		0.14	0.06	0.14	0.05	0.79	
	P-value£	**0.071**						0.48						0.12					
Free Bilirubin	Non-smokers	0.40	0.22	0.33	0.15	**<0.001**		0.34	0.16	0.31	0.14	**<0.001**		0.40	0.18	0.33	0.15	**<0.001**	
	Smokers	0.31	0.17	0.28	0.12	0.26		0.30	0.18	0.26	0.12	0.27		0.32	0.15	0.30	0.13	0.35	
	P-value£	**<0.001**						0.19						0.064					

		$TA_{7/7}$ (n=71)					
		V1		V3		P¥	N
		$\bar{X}$	SD	$\bar{X}$	SD		
Total Bilirubin	Non-smokers	0.90	0.40	0.61	0.25	**<0.001**	**63**
	Smokers	0.44	0.21	0.39	0.15	0.38	**8**
	P-value£	**0.002**					
Conjugated Bilirubin	Non-smokers	0.21	0.06	0.19	0.06	**0.003**	
	Smokers	0.15	0.05	0.13	0.04	0.21	
	P-value£	**0.01**					
Free Bilirubin	Non-smokers	0.69	0.35	0.42	0.20	**<0.001**	
	Smokers	0.29	0.16	0.26	0.11	0.55	
	P-value£	**0.002**					

¥ The *p*-value as determined by paired t-test or Wilcoxon signed-rank test (where appropriate).
£The *p*-value to test the effect of smoking on baseline bilirubin (V1) as determined by t-test: two-sample with equal or unequal variances (where appropriate).

$\bar{X}$ = mean, SD=standard deviation. V1= visit 1 as it corresponds to bilirubin fraction measured before administration of fenofibrate (Pre-fenofibrate) values, V3= visit 3 as corresponds to bilirubin fractions measured after administration of fenofibrate (Pre-fenofibrate) for 21 days. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene.

Table 5: Serum concentrations of fenofibrate (160mg) between the three genotype groups of the UGT1A1*28 at three time points

UGT1A1*28 genotype	All n=835		$TA_{6/6}$ n=384		$TA_{6/7}$ n=374		$TA_{7/7}$ n=77		*P*-value¥	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$TA_{6/6}$ vs. $TA_{7/7}$	$TA_{6/7}$ vs. $TA_{7/7}$
0hr conc. (µg/mL)	8.03	4.99	7.99	5.23	7.9	4.71	8.92	5.09	0.236	0.153
3.5 hr conc. (µg/mL)	12.6	5.99	12.31	5.93	12.5	5.80	14.3	7.00	0.056	0.055
6 hr conc. (µg/mL)	14.3	6.4	13.79	6.12	14.4	6.37	15.93	7.38	0.101	0.330
AUC_{0-6} (µg*hr/mL)	69.7	32.3	68.11	32.9	69.4	31.5	78.86	37.4	**0.030**	**0.025**

¥ The *p*-value as determined by multiple regression analysis model, which included the following covariates: age, age, creatinine clearance and smoking status. $\bar{X}$ = mean, SD=standard deviation. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene.

Table 6: Lipid baseline level and percent change from baseline after treatment with fenofibrate (160mg) between three genotype groups of the UGT1A1*28

UGT1A1*28 genotype	All		$TA_{6/6}$		$TA_{6/7}$		$TA_{7/7}$		*P*-value¥	
	n=835		n=384		n=374		n=77			
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	6/6 vs. 7/7	6/7 vs. 7/7
Pre-fenofibrate										
Triglyceride (mg/dL)	139	96	138	107	141	89	139	76	0.32	0.45
HDL-C (mg/dL)	46.7	13	47	14	46	13	48	13	0.95	0.97
LDL-C (mg/dL)	123	31	124	31	124	32	117	30	0.067	**0.044**
Total Cholesterol (mg/dL)	192	39	192	40	193	40	188	38	0.25	0.26
non-HDL-C (mg/dL)	145	39	145	40	147	40	140	39	0.24	0.25
Change										
Triglyceride %	-30.2	21.8	-30.0	22.8	-30.6	20.5	-33.9	22.6	0.84	0.79
HDL-C %	6.8	11.2	6.2	10.4	7.1	11.5	7.4	13.0	0.55	0.78
LDL-C %	-14.8	17.7	-14.4	18.2	-15.5	17.3	-15.2	17.0	0.47	0.27
Total Cholesterol %	-12.7	9.99	-12.6	10.0	-12.9	9.9	-12.9	10.2	0.33	0.31
non-HDL-C %	-19.1	13.3	-18.9	13.2	-19.6	13.3	-19.7	13.8	0.21	0.27

¥ The *p*-value as determined by multiple regression analysis model, which included the following covariates: age, sex, body mass index and diagnosis of diabetes for the portion of the table corresponding to the **pre-fenofibrate** and age, sex, body mass index, baseline of each respective lipid level, creatinine clearance and fenofibrate serum concentration (AUC_{0-6}) for the **change** portion of the table.

$\bar{X}$ = mean, SD=standard deviation. Pre-fenofibrate corresponds to baseline lipid values whereas change in lipid fractions were calculated by (post-pre)/pre, expressed as a %, where post corresponds to lipid fractions measured after administration of fenofibrate for 21 days. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene.

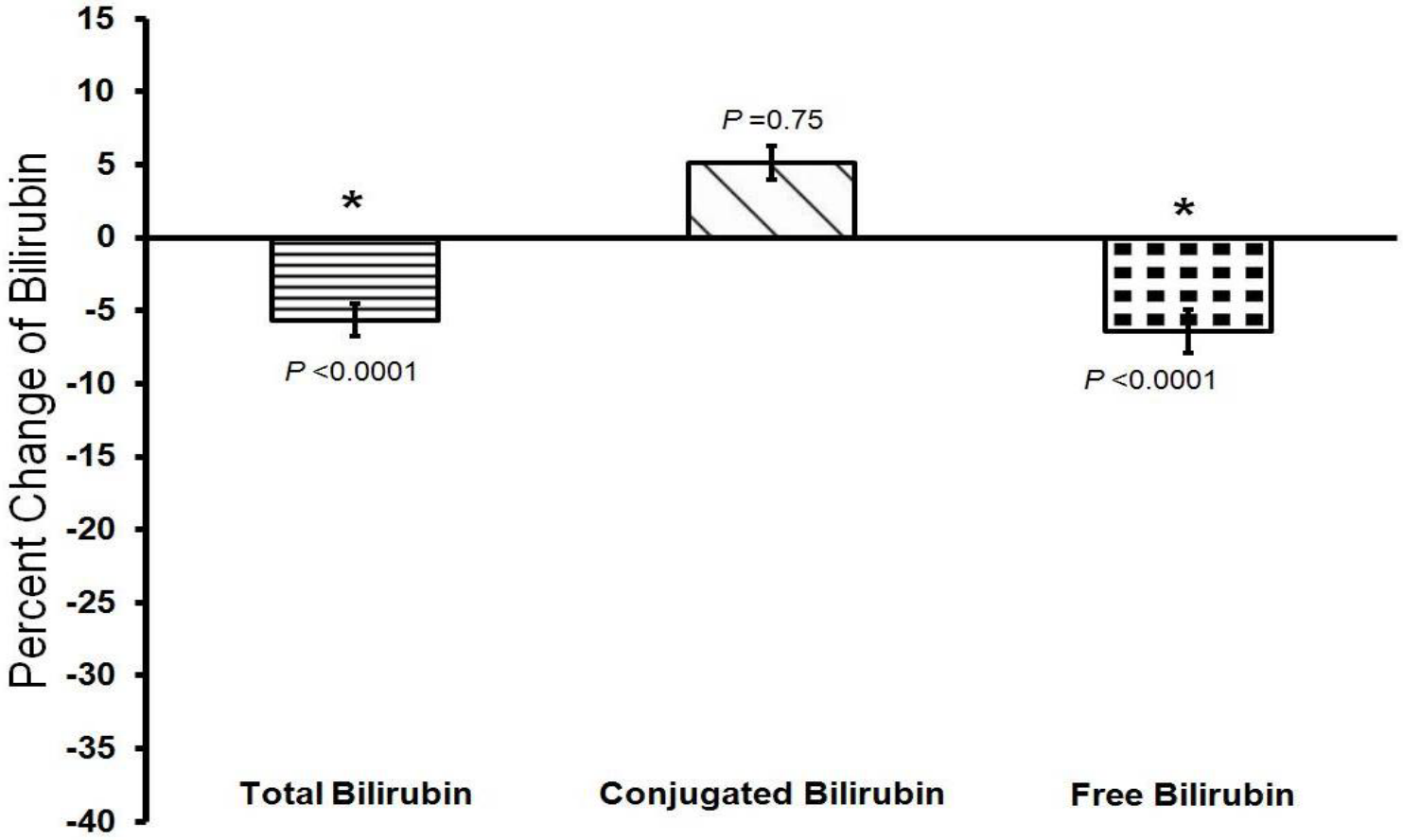


Figure 1: Percent change (mean and standard error) of bilirubin. Percent change was determined by (post - pre fenofibrate)/pre-fenofibrate. The asterisk (*) indicate a significant change from baseline.

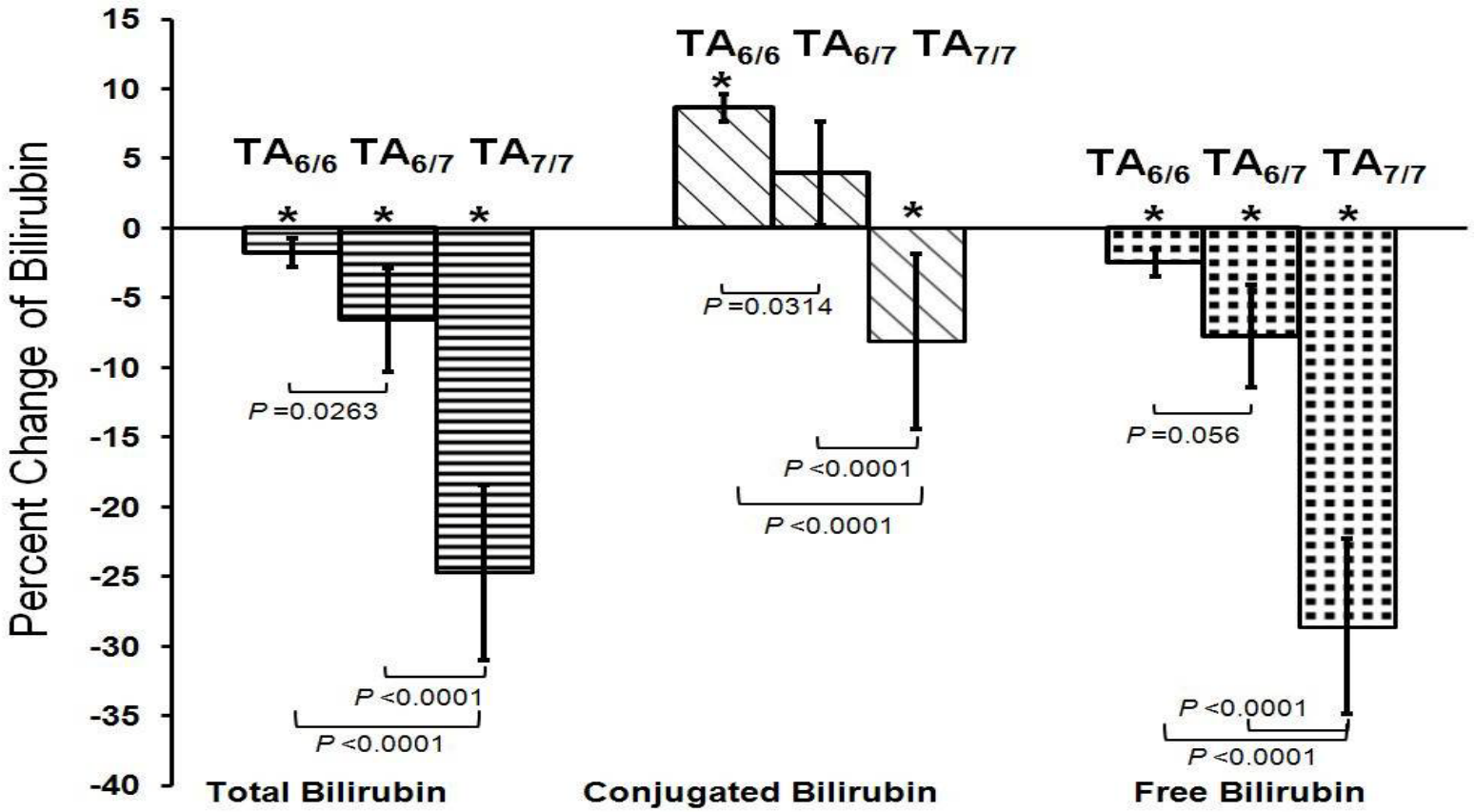
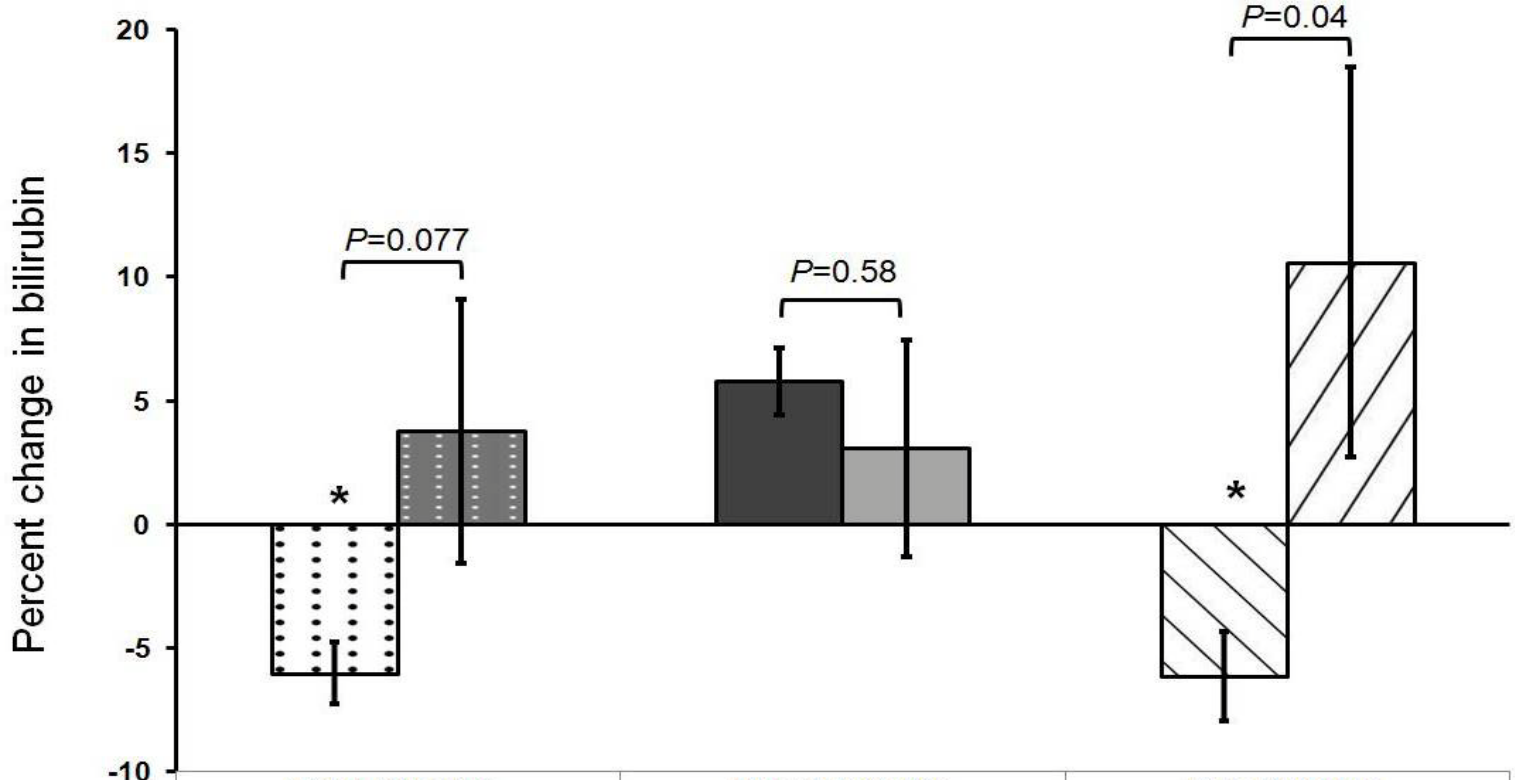
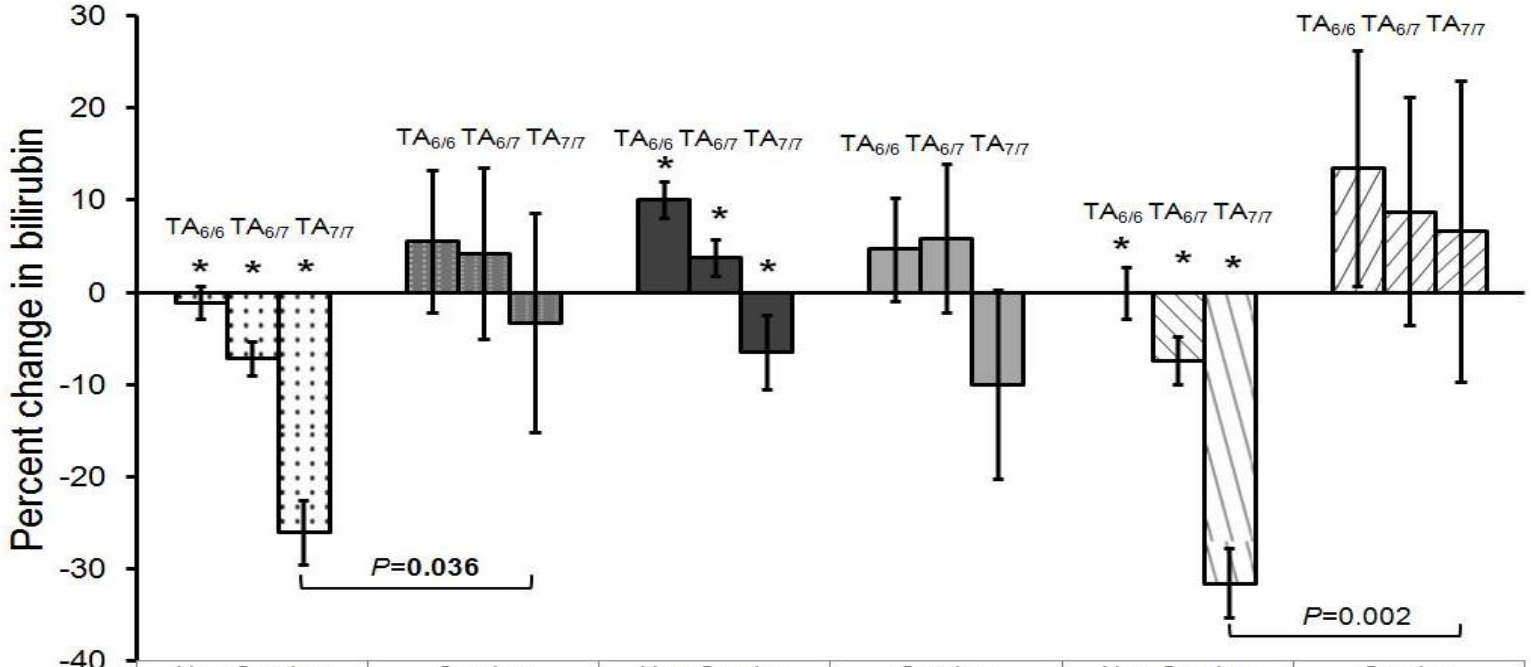


Figure 2(a): Percent change (mean and standard error) of bilirubin according to **UGT1A1*28 genotypes**. Percent change was determined by (post - pre fenofibrate)/pre-fenofibrate. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene. Percent change was determined by (post-pre fenofibrate)/pre-fenofibrate. The asterisk (*) indicate a significant change from baseline.



	Total Bilirubin	Direct Bilirubin	Free Bilirubin
Non-Smoker	-6.01	5.80	-6.14
Smoker	3.77	3.07	10.60

Figure 2(b): Percent change (mean and standard error) of bilirubin according to **smoking status**. Percent change was determined by (post - pre fenofibrate)/pre-fenofibrate. The asterisk (*) indicate a significant change from baseline.



	Non-Smoker	Smoker	Non-Smoker	Smoker	Non-Smoker	Smoker
	Total Bilirubin		Conjugated Bilirubin		Free Bilirubin	
6/6	-1.05	5.57	10.10	4.68	-0.10	13.50
6/7	-7.18	4.26	3.79	5.87	-7.37	8.82
7/7	-26.04	-3.27	-6.51	-10.00	-31.56	6.65

Figure 2(c): Percent Change (mean and standard error) of bilirubin according to **UGT1A1*28 genotype** and **smoking status**. $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene. Percent change was determined by (post-pre fenofibrate)/pre-fenofibrate. The asterisk (*) indicate a significant change from baseline.

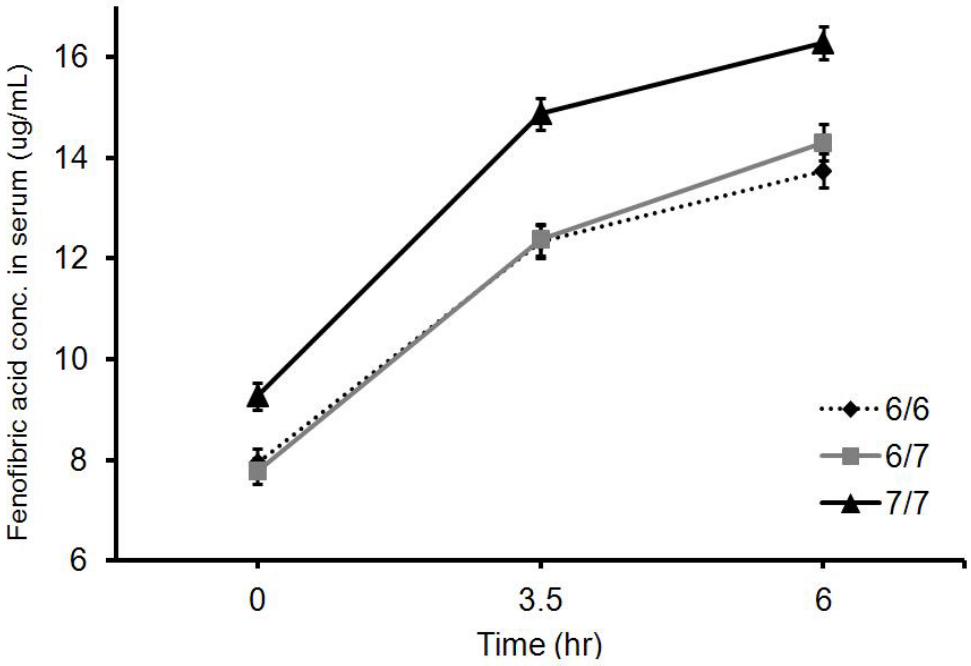


Figure 3: Partial AUC_{0-6} of fenofibric acid (mean and standard error) according to UGT1A1*28 genotypes. Diamonds are the $TA_{6/6}$= 6 TA repeats in the TATA-box in both copies of the *UGT1A1* gene, squares are the $TA_{6/7}$= six TA repeats in the TATA-box in one copy of the *UGT1A1* gene and seven TA repeats on the other gene copy, triangles are the $TA_{7/7}$= 7 TA repeats in the TATA-box in both copies of the *UGT1A1* gene.

CHAPTER IV

Modulation of the Uricosuric Effect of Fenofibrate by UGT2B7 A-327G.

4.1. Background

Epidemiological studies have established an association between elevated levels of uric acid and all-cause mortality [1,2] in addition to several clinically important conditions including hypertension [3,4], metabolic syndrome [5], diabetes [6] and chronic kidney disease.[7,8] Furthermore, evidence is emerging from studies investigating the mechanism of uric acid's detrimental effect on endothelial function [9-12] and the beneficial effects of uric acid-lowering agents preserving renal function [13-16] and mitigating retinopathy.[17] In spite of uric acid's association with disease and the beneficial effects of agents which lower uric acid on outcomes, our knowledge of factors, governing an agent's uricosuric effect for a given patient is lacking. The purpose of this study was to explore this gap in knowledge as it relates to a specific therapeutic agent in order to provide a basis that may ultimately serve clinicians seeking to consider such therapy for patients with hyperuricemia.

The antidyslipidemic agent known as fenofibrate has been shown to lower uric acid levels by ~24% (range 15 to 34%) Table 1. The mechanism of fenofibrate's ability to lower serum uric acid is unknown. As is common to many pharmacodynamic responses, the magnitude of a response is often associated with exposure yet, somewhat uniquely and with rare exceptions, fenofibrate is most typically prescribed at one dosage rate and not titrated to response. Explorations of fenofibrate's variability in its lipid-lowering response, has identified concentration to be a key factor.[18] Specific

detailed examination of likely drug disposition-based contributors to serum fenofibric acid concentrations have identified Uridine 5'-diphospho-glucuronosyltransferase (UGT) gene family and *UGT2B7* specifically to have played the most prominent role.[19] For example, we have previously have shown that a genetic variation, UGT2B7 A-327G (rs7662029), caused a significant difference in the steady state area under the serum concentration-time curve (AUC_{0-24}) of fenofibric acid.[20] Given that this difference in serum concentration of fenofibric acid has been linked to a significant difference in fenofibrate's lipid-lowering effect, we conducted this analysis to ascertain whether UGT2B7 A-327G (rs7662029) may affect uric acid-lowering response to fenofibrate treatment. To accomplish this objective, we examined the impact of UGT2B7 A-327G genotype on the uricosuric response to fenofibrate on within 861 participants of the Genetics of Lipid Lowering Drug Network (GOLDN) trial.[21]

4.2. Method

4.2.1. Study Population

Male and female subjects participating in the GOLDN trial formed the basis for the study population used for this analysis. The GOLDN study, which is part of the PROGENI (PROgram for GENetic Interactions) Network, was as multi-institutional study sponsored by the NIH.

Although described in greater detail elsewhere[22], the GOLDN study was an open-labeled study with one daily tablet of fenofibrate (160 mg TriCor®) taken for 21 days. The protocol consisted of an initial screening visit, followed by four study visits. All participants were instructed to fast for≥12 hours and abstain from using alcohol for ≥24 hours before all clinic visits. Visit 1 (V1) and visit (V2) (pre-fenofibrate), were separated by 24 hours as were the third (V3) and fourth (V4) visits (post-fenofibrate). Visits 1 and 3 were separated by approximately 21 days of fenofibrate administration.

The primary efficacy endpoint (for the GOLDN study) was the change in triglycerides pre- and post-fenofibrate administration. Secondary efficacy endpoints are the changes of low-density lipoprotein (LDL-C), total cholesterol, high-density lipoprotein (HDL-C), and non-HDL cholesterol pre- and post-fenofibrate administration. Written informed consent was obtained from all GOLDN subjects at the screening visit. The protocol of the study was approved by the investigational review board (IRB) of the University of Minnesota, University of Utah, and Tufts University. The GOLDN study was registered with Clinicaltrial.gov (NCT00083369).

4.2.2. Measuring fenofibric acid serum concentration:

Serum fenofibric acid concentrations were quantified using HPLC in samples drawn at 0, 3.5, and 6 hour after administering the last dose of fenofibrate on day 21.

Briefly, samples were extracted using an anion-exchange, solid-phase extraction procedure. Chromatographic separation was performed using isocratic conditions with an ultraviolet detection at 285 nm. A complete procedure has been previously published.[23]

4.2.3. Pharmacokinetics analysis

A partial (0-6 hour) area under the serum concentration-time curve (AUC_{0-6}) of fenofibric acid was calculated by the linear trapezoidal rule applied to the three measures at 0, 3.5 and 6 hours using a non-compartmental approach implemented on WinNonlin®(v6.2).

4.2.4. Uric acid measurement

Uric acid was measured in serum on visit 1 (V1 or pre-fenofibrate) and then at visit 3 (V3 or post-fenofibrate), using an enzymatic colorimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Modular P Chemistry analyzer (Roche Diagnostics). The reference range was 3.4 – 7.0 mg/dL. The inter-assay CV range for the laboratory was 1.9%. Uric acid levels were measured pre-fenofibrate treatment at visit 1 and post-fenofibrate treatment at visit 3 of the study.

4.2.5. DNA extraction procedure

Genomic DNA was isolated from peripheral blood leukocytes using Puregene DNA reagents following the vendor's protocol.

4.2.6. Genotyping

4.2.6.1. UGT2B7 A-327G (rs7662029)

Genotyping was performed using iPLEX Gold method using Bruker Autoflex II MALDI/TOF Mass Spectrometer. iPLEX reagents and protocols for multiplex PCR, single base primer extension (SBE), and generation of mass spectra. Multiplexed assays typically contain 10–36 SNPs. Multiplexed PCR was performed in 5-µl reactions on 384-well plates containing 10 ng of genomic DNA. Reactions contained 0.5 U HotStar Taq polymerase (QIAGEN), 100 nM primers, 1.25X HotStar Taq buffer, 1.625 mM MgCl2, and 500 µM dNTPs. Following enzyme activation at 94 °C for 15 min, DNA was amplified with 45 cycles of 94 °C x 20 sec, 56 °C x 30 sec, 72 °C x 1 min, followed by a 3-min extension at 72 °C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom). Single-base extension was carried out by adding SBE primers at concentrations from 0.625 µM (low MW primers) to 1.25 µM (high MW primers) using iPLEX enzyme and buffers (Sequenom, San Diego) in 9-µl reactions. Reactions were desalted and SBE products were measured

using the MassARRAY system, and mass spectra was analyzed using TYPER software (Sequenom, San Diego) in order to generate genotype calls and allele frequencies.

4.2.6.2. SLC2A9 (rs734553)

Genotypes were determined using the TaqMan genotyping assay (Applied Biosystems, Inc. Foster City, CA) with primers designed by Applied Biosystem e2s Primer-by-Design service. Genotypes were visualized using a PRISM 7500 and data was analyzed using the ABI Sequence Detection Software. The reverse primers were (AAGGCGGGCTGACTGATTAGATCCC[G/T]GAAAGCACAATAATCA GATCATGGG)

4.2.7. Statistical analysis

Demographic and laboratory characteristics were summarized for all subjects taking fenofibrate. Categorical variables were summarized by frequencies while continuous variables were reported by mean ± standard error (±se) for normally distributed variables or as median and inter-quartile range with minimum and maximum values for those values not normally distributed. Protocol violators, or participants less than 75% compliant with fenofibrate, were excluded from the overall analysis. All tests were performed at an $\alpha = 0.05$, two-tailed. Statistical tests with *P*-value of 0.05 or less were considered statistically significant. Continuous variables not normally distributed were log (natural) transformed prior to calculating inferential statistics. Allele frequencies were estimated for UGT2B7 A-327G and SLC2A9 (rs734553) SNPs and a chi-squared test used to examine for deviations of genotypes from Hardy-Weinberg equilibrium (HWE).

The primary objective of the analysis was to characterize the uric acid response profile in relation to factors, UGT2B7 A-327G, and SLC2A9 rs734553, affecting uric acid response to fenofibrate. Since participants were recruited from families, a linear mixed model was used and implemented in R. Within the model, genotypes were treated as fixed effects, and dependencies among members within each family were treated as random effects.[24] The following covariates were included in the model for testing the effect of UGT2B7 A-327G or SLC2A9 SNPs on baseline uric acid and percent change in uric acid from baseline: age, age^2, sex, baseline creatinine clearance, baseline body mass index, and baseline serum creatinine**.** Pearson's correlation coefficient test was used to the relation between fenofibric acid serum concentration and percent change in uric acid from baseline.

4.3. Results

Baseline characteristics and levels:

Baseline characteristics of the patient population studied are summarized in Table 2. Both genders were equally represented with an overall mean (range) age of 48.6 (18 – 83) years. The majority of the participants had a normal renal function with a mean (±sd) creatinine clearance of 97ml/min (30). Eight percent were current smokers, and 49% were current alcohol drinkers. Twenty-six percent were diagnosed as hypertensive patients, 45% had metabolic syndrome, and 7% were type II diabetics (T2DM). Also, there was no was no significant difference in baseline characteristics and levels between the UGT2B7 A-327G genotypes Table 2.

Genotypes frequency:

Of the 861 subjects participating in this analysis, 816 (94.8%) were successfully genotyped for SLC2A9 rs734553. T/T genotype represented 56% (459), T/G 38% (313), and G/G 6% (44). Of the 861, 812 or (94.3%) were successfully genotyped for UGT2B7 rs7662029. A/A genotype represented 27% (217), A/G 50%, and G/G 23% (186) Table 3.

Uric acid levels:

The average (±se) baseline uric acid (pre-fenofibrate) was 5.48 (±0.051) mg/dL, ranging from 1.8–9.8 mg/dL. After treatment with fenofibrate (post-fenofibrate), the average (±se) uric acid (post-fenofibrate) was 4.3 (±0.041) mg/dL, a 20.8% (±0.46) reduction from the baseline ($p < 0.0001$) Figure 1.

Fenofibric acid levels:

The overall mean (±se) of serum AUC_{0-6} of fenofibric acid for all participants was 69.7 (±1.17)μg*hr/ml. The mean (±se) within genotypes of UGT2B7 A-327G was as follows: A/A 62.2 (±2.25)μg*hr/ml, A/G 69.9 (±1.66)μg*hr/ml, and G/G 75.5 (±2.54)μg*hr/ml, which were significantly different between each of the genotypes ($p<0.0001$). There was a significant ($p<0.0001$) correlation between the serum concentration of fenofibric acid (exposure) and the percent change of serum uric acid, in which higher concentrations of fenofibric acid led to further reduction in uric acid Figure 4.

SLC2A9 rs734553 and serum uric acid levels:

There was a 12.4% difference in mean uric acid levels at baseline between individuals who were T/T vs. G/G for SLC2A9 SNP (p<0.0001), but there was no significant difference between T/T and T/G, $p > 0.05$. The difference in uric acid levels between T/T and G/G post-fenofibrate treatment was still significant ($p = 0.0051$), but it dropped to 8.1% Table 4 and Figure 2(a). However, there was no significant difference, mean (±se), in the percent change of uric acid between any of the three genotypes Table 4 and 2(b).

UGT2B7 A-327G (rs7662029) and uric acid levels:

There was no significant difference between the three genotypes in pre-fenofibrate uric acid levels. The mean (±se) A/A were 5.51 (±0.1) mg/dL, A/G 5.49 (±0.07) mg/dL, and G/G 5.42(±0.11) mg/dL (p>0.05)). In the uric acid level post-fenofibrate, the only significant difference was between A/A and G/G, 4.43 (±0.08) mg/dL and 4.19 (±0.08) mg/dL respectively ($p = 0.001$), but not between A/G 4.29 (±0.06) mg/dL and A/A or G/G (Table 5 and Figure 3(a)). However, there was a significant difference between A/A and A/G, -19% (±0.91) and -21% (±0.67) respectively ($p = 0.009$). Also there was a significant difference between A/A and G/G, -19% (±0.91) and -22% (±0.90) respectively ($p = 0.003$), but not between A/G and G/G, -21% (±0.67) and -22% (±0.90) respectively ($p = 0.38$). For this reason we combined A/G and G/G genotypes into one group (G/X) where the x means A or G alleles (Table 5 and Figure 3(b)). All the p-values were adjusted for the following covariates: age + age^2 + sex + baseline creatinine clearance + baseline body mass index + baseline serum creatinine + the effect of family relations among participants. However, SLC2A9 rs734553 did not have a significant

effect on the percent change of uric acid between the UGT2B7 rs7662029 genotypes, and it was not included in the final multi-regression analysis model.

4.4. Discussion

To our knowledge, this study represents the first report of a key genetic polymorphism found to be a determinant of fenofibrate's uric acid-lowering response. Specifically our data confirm that either UGT2B7 A-327G, or a SNP which is linked to it, represents a significant determinant of fenofibrate's uricosuric response event while controlling for the SLC2A9 SNP (rs734553).

Fenofibrate's uricosuric effect is an important pleiotropic property, especially given evidence linking uric acid levels with various cardiovascular outcomes and co-morbidities. Fenofibrate's utility in patients with atherogenic dyslipidemia [25] and the common occurrence of both hyperuricemia and atherogenic dyslipidemia in patients with the metabolic syndrome [26] or T2DM, [27] underscores the preferential utility of fenofibrate for such patients relative to other anti-dyslipidemic medications without this property.

Contemporary guidelines [25] for the management of patients with atherogenic dyslipidemia (a phenotype with elevated triglyceride and low high density lipoprotein) at target LDL-C, identify fibrates and niacin as optimal choices. Compared to other fibrates, fenofibrate represents the only one with notable uricosuric properties.[28-33]

Niacin on the other hand, has been shown to increase uric acid levels as much as 11-14%.[34-37] Consequently, fenofibrate's potential advantage over other therapeutic choices for patients with atherogenic dyslipidemia and hyperuricemia are obvious.

The importance of selecting optimal candidates to receive fenofibrate is underscored by the findings of recent studies evaluating its clinical benefits in patients at risk for cardiovascular events. Two large clinical studies exemplify this point. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD)[14] and Action to Control Cardiovascular Risk in Diabetes (ACCORD)[13] studies evaluated fenofibrate's utility to reduce cardiovascular endpoints when compared to placebo in patients with T2DM. Both studies consistently observed a reduced incidence of microvascular disease [13-16] while at the same time, apparently failing to positively affect the incidence of macrovascular events for those taking fenofibrate relative to placebo. For example, fenofibrate was more effective than placebo in preserving renal function and/or mitigating the development of retinopathy [17] while overall outcomes from macrovascular event rate reductions associated with fenofibrate were mixed. Overall, the mixed results from these outcome-based clinical trials of fenofibrate have called to question our ability to identify which patients represent optimal candidates to receive these agents. From the ACCORD trial [13] the distinction between superior and inferior candidate to receive fenofibrate, in part, was defined by their baseline levels of triglycerides and HDL-C.[13,38,39] We posit that another important source of variability in

a patient's lipid-lowering response and *now*, uric acid-lowering response to fenofibrate, can be traced to fenofibrate's metabolic fate.

Glucuronidation is a well-recognized metabolic pathway influencing the elimination of various therapeutic agents.[40] *In vitro* evidence of *UGT2B7*'s role in eliminating fenofibric acid, the active form of the lipid-lowering drug fenofibrate, has been confirmed.[19] From the GOLDN Study[21], we have reported that *UGT2B7* is an important determinant of lipid lowering response[18] and the likely mechanism for this observation relates to its role in modulating the disposition of fenofibric acid.[20] Specifically, on the basis of our analysis conducted within participates in the GOLDN study population, we found those with the G/G or G/X genotype for UGT2B7A-327G would represent optimal candidates for this drug in terms of its uric acid-lowering and lipid-lowering properties. Although we can only speculate, it is entirely possible that the microvascular event rate lowering experienced participants in either the FIELD or ACCORD trials may differ on the basis of their UGT2B7A-327G genotype. If found to be true, then the translational value of proposing to prospectively genotype individuals to qualify their candidacy to receive fenofibrate may well be justified.

The SLC2A9 SNP (rs734553) appears to be an important determinant of baseline uric acid levels as well as a marker for risk to develop hypertension [41] and gout.[42] This SNP's association with baseline uric acid was confirmed within our study

whereby T/T and T/G individuals had a ~12 % higher uric acid level compared to G/G individuals. This represents an absolute difference of ~0.6mg/dL between these genotype groups. To provide some context, Kodama et al[6] reports that for every 1 mg/dL increase in serum uric acid, the relative risk of (T2DM) increases by 1.17-fold. In contrast to the SLC2A9 SNP (rs734553), UGT2B7 A-327G appears to modulate the response to fenofibrate such that there was a 13.6% relative difference (p=0.0019) in percent change in uric acid between A/A vs. G/X individuals. UGT2B7 A-327G did not affect baseline uric acid.

There are several limitations to our study worthy of some discussion. The present study was conducted in subjects who were, for the most part, not hyperuricemic. Consequently our findings of the influence of UGT2B7 A-327G on fenofibrate's uricosuric effect may not be representative of those who begin with elevated uric acid levels. Furthermore, given that our study was relatively short in duration (~21 days of fenofibrate), the confidence of our estimation of magnitude of effect and/or persistence of difference between genotypes, may be called to question. Although Takahashi et al [33] conducted a 6 month study of fenofibrate use within subjects with gout and did see a persistent lowering of fenofibrate, no genetic analysis was offered.

Confirmation of the pharmacodynamic significance of UGT2B7 A-327G advances the possibility of considering additional genetic and non-genetic determinants

of the uricosuric response to fenofibrate such that we may enhance our prediction of superior candidates to receive fenofibrate. This potential to individualize therapeutic choices holds the promise of optimizing outcomes while minimizing un-necessary risks where alternative therapies may prove superior.

In conclusion fenofibrate's uricosuric response, and hence potential clinical benefit, appears to be modulated through UGT2B7 A-327G.

Table 1: Studies that reported the effect of fenofibrate on uric acid

Author	Duration	Dose	Sample size	Type of participants	Baseline serum uric acid (mg/dL)	% change uric acid	Other medication affect UA	Country	Placebo controlled
Bastow et al. 1988 [28]	6 weeks	100mg TID	10	Hyperuricemic	7.7	- 20%	Compared to bezfibrate	UK	Yes crossover
Jen et al. 1997 [32]	3 months	100mg TID	12 (1 female)	Hyperlipidemic	6.3	- 24.4%	Compared to gemfibrozil	China	No crossover
Elisaf et al. 1999 [43]	8 weeks	200mg QD	25	Hypertensive, hyperuricemic	4 weeks FA only 7.6 4 weeks FA + losartan 5.6	4 weeks FA only - 26% 4 weeks FA+ losartan additional - 12.5%	Losartan was added	Greece	no
Idzior-Walus et al. 2000 [44]	12 weeks	200mg QD	37 (All males)	Hyperlipidemic	7.2	- 20%	-	France	no
Insua et al. 2002 [31]	6 weeks	200mg QD	21	Hyperlipidemic	5.1	- 15.6%	Compared to gemfibrozil	Argentina	Yes crossover
Kiortsis et al. 2001 [45]	12 weeks	200mg QD	75	Hyperlipidemic	5.3	Compliers - 28% Poor compliers - 8.7%	-	Greece	no
Feher et al. 2003 [46]	3 weeks	200mg QD	10	Gout	6.3	- 19%	Added to allopurinol	UK	
Kazumi et al. 2003 [47]	8 weeks	300mg QD	43	Hyperlipidemic and / or hyperuricemic	7.8	- 24%	-	Japan	yes
Milionis 2003 [48]	12 weeks	200mg QD	22	Hyperlipidemic	5.6	- 16%	Compared to Atorvastatin	Greece	
Melenovsky et al. 2003 [49]	9 weeks	200mg QD	37	Hyperlipidemic	6.8	- 28%	-	Czech republic	no
Noguchi et al. 2004 [50]	12 weeks	300mg QD	40	Hyperlipidemic	7	- 25%	-	Japan	no
Lee et al. 2006 [51]	2 months	160mg QD	14	Gout	6.9	- 23%	Added to allopurinol or benbromaron	Korea	no
Takahashi et al. 2007 [33]	6 months	300mg QD	26	Gout	5.9	- 10.5%	Added allopurinol or benbromaron and compared to Bezafibrate	Japan	no
Wu et al. 2007 [52]	12 weeks	200mgQD	39	DM II	6.4	-23%	-	Taiwan	no
Patterson et al. 2009 [53]	2 weeks	200mg QD	10	Healthy participants	4.75	- 34%	-	Czech republic	no
Uetake et al. 2009 [54]	Single dose	300mg	9 (All males)	Healthy participants	5.8	- 25.8% in 10 hrs	-	Japan	no

Table 2: Baseline characteristics of three genotype groups of the UGT2B7 A-327G

	All participants		UGT2B7 A-327G genotype						
	n=812		A/A (n=217)		A/G (n=409)		G/G (n=186)		*P*-value
	$\bar{X}$	Range	$\bar{X}$	se	$\bar{X}$	se	$\bar{X}$	se	
Age (years)	48.6	18 - 83	48.4	1.07	47.7	0.77	50.6	1.22	0.13
Sex (male) (%)	50		50		51		49		0.93
BMI (kg/m^2)	28.4	16 - 52	28.7	0.38	28.2	0.27	28.7	0.43	0.51
Total cholesterol (mg/dL)	192	97 - 332	191	2.65	191	1.93	196	2.79	0.41
Drinkers (%)	49		50		47		52		0.50
Smokers, (%)	8		10		6		8		0.25
OC or HR (%)	15		16		14		17		0.77
Hypertension (%)	26		29		48		23		0.62
Creatinine clearance (ml/min)	97	30 - 263	97		99		94		0.21
Utah center (%)	50		49		52		50		0.80
Diabetes type II (%)	7		8		8		4		0.20
Metabolic syndrome (%)	45		48		43		43		0.43

P-values for continuous data were determined using an analysis of variance (ANOVA) while a chi-squared analysis, or Fisher's exact test, were used for categorical data (where appropriate). BMI= body mass index, OC= oral contraceptive, HR= Hormone replacement therapy. Drinkers=alcohol drinkers. $\bar{X}$= mean, se=standard error, n=sample size.

Table 3: Frequencies for SLC2A9 SNP (rs734553) and UGT2B7 A-327G (rs7662029) genotypes

SNP	SLC2A9 (n=816)			UGT2B7 A-327G (n=812)		
	G/G	G/T	T/T	G/G	A/G	A/A
number/genotype	44	313	459	186	409	217
prevalence	6%	38%	56%	23%	50%	27%

Table 4: SLC2A9 SNP (rs734553) effect on uric acid baseline and percent change

Uric acid (mg/dL)	SLC2A9 SNP (rs734553)						
	T/T (n=430)		T/G (286)		G/G (n=40)		*p*-value
	$\bar{X}$	se	$\bar{X}$	se	$\bar{X}$	se	
Pre-fenofibrate	5.57	0.07	5.41	0.09	4.88	0.24	<0.0001*§
Post-treatment	4.33	0.05	4.28	0.07	3.98	0.20	0.0051*
Uric acid % Δ	-21.50	0.60	-20.07	0.77	-17.88	1.40	0.7255¥

*The *p*-value as determined by the multiple regression analysis model, which included the following covariates: age, age^2, sex, baseline creatinine clearance, body mass index, baseline serum creatinine, and family effect for the portion of the table corresponding to the pre-fenofibrate and post-treatment.

¥ The *p*-value as determined by the multiple regression analysis model, which included the following covariates: age, age^2, sex, baseline creatinine clearance, body mass index, baseline serum creatinine, baseline uric acid, and family effect for the portion of the table corresponding to the Uric acid % Δ.

§ Significant difference between T/T or T/G vs. G/G, but no significant difference between T/T and T/G.

Pre-fenofibrate corresponds to baseline uric acid values whereas change in uric acid fractions were calculated by (post-pre-)/ pre-, expressed as a percent, where post- corresponds to uric acid fractions measured after administration of fenofibrate for 21 days. $\bar{X}$= mean, se=standard error, n=sample size. Δ=change.

Table 5: Uric acid baseline level and % change in men according to UGT2B7 A-327G genotypes

	UGT1A2B7 A-327G genotype													
	A/A (n=205)		A/G (n=374)		G/G (n=176)		*p*-value			A/A (n=205)		G/X (n=550)		*p*-value
Uric acid (mg/dL)	$\bar{X}$	se	$\bar{X}$	se	$\bar{X}$	se	A/A vs. A/G	A/A vs. G/G	A/G vs. G/G	$\bar{X}$	se	$\bar{X}$	se	
Baseline	5.51	0.10	5.49	0.07	5.42	0.11	0.99	0.219	0.16	5.51	0.10	5.47	0.06	0.6265*
Post-treatment	4.43	0.08	4.29	0.06	4.19	0.08	0.07	0.001	0.051	4.43	0.08	4.25	0.05	0.0087*
Uric acid % Δ	-19	0.91	-21	0.67	-22	0.90	0.01	0.003	0.38	-19	0.91	-22	0.51	0.0019¥

*The *p*-value as determined by the multiple regression analysis model, which included the following covariates: age, age^2, sex, baseline creatinine clearance, body mass index, baseline serum creatinine, SLC2A9 SNP rs734553, and family effect for the portion of the table corresponding to the pre-fenofibrate and post-treatment.

¥ The *p*-value as determined by multiple regression analysis model, which included the following covariates: age, age^2, sex, baseline creatinine clearance, body mass index, baseline serum creatinine, baseline uric acid, SLC2A9 SNP rs734553, and family effect for the portion of the table corresponding to the Uric acid % Δ.

Pre-fenofibrate corresponds to baseline uric acid values whereas change in uric acid fractions were calculated by (post-pre)/pre, expressed as a %, where post corresponds to uric acid fractions measured after administration of fenofibrate for 21 days. $\bar{X}$= mean, se=standard error, n=sample size. Δ=change. G/X= A/G+G/G genotype groups.

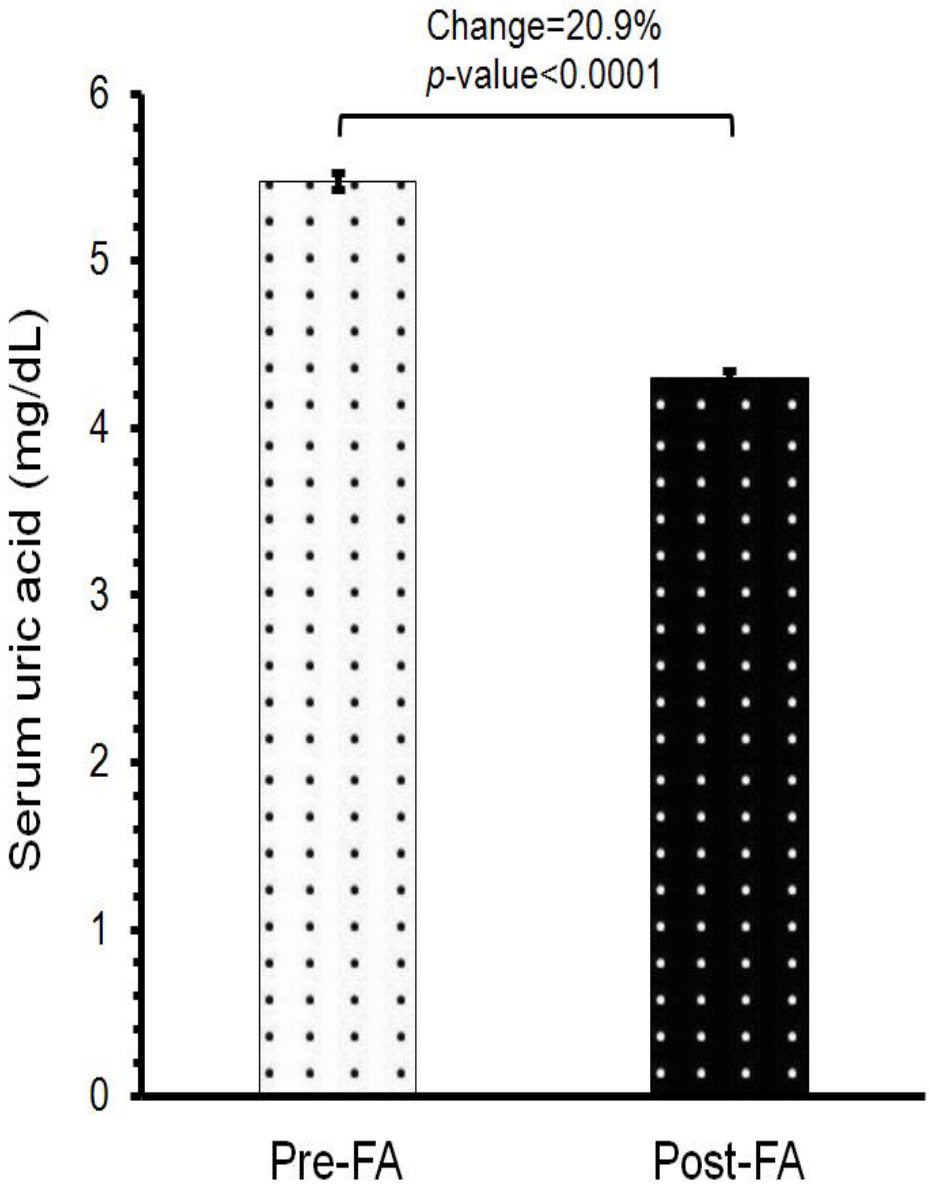


Figure 1: Average change (mean and standard error) in uric acid after 21 days of fenofibrate treatment. Pre-FA= before fenofibric acid treatment, Post-FA= after fenofibric acid

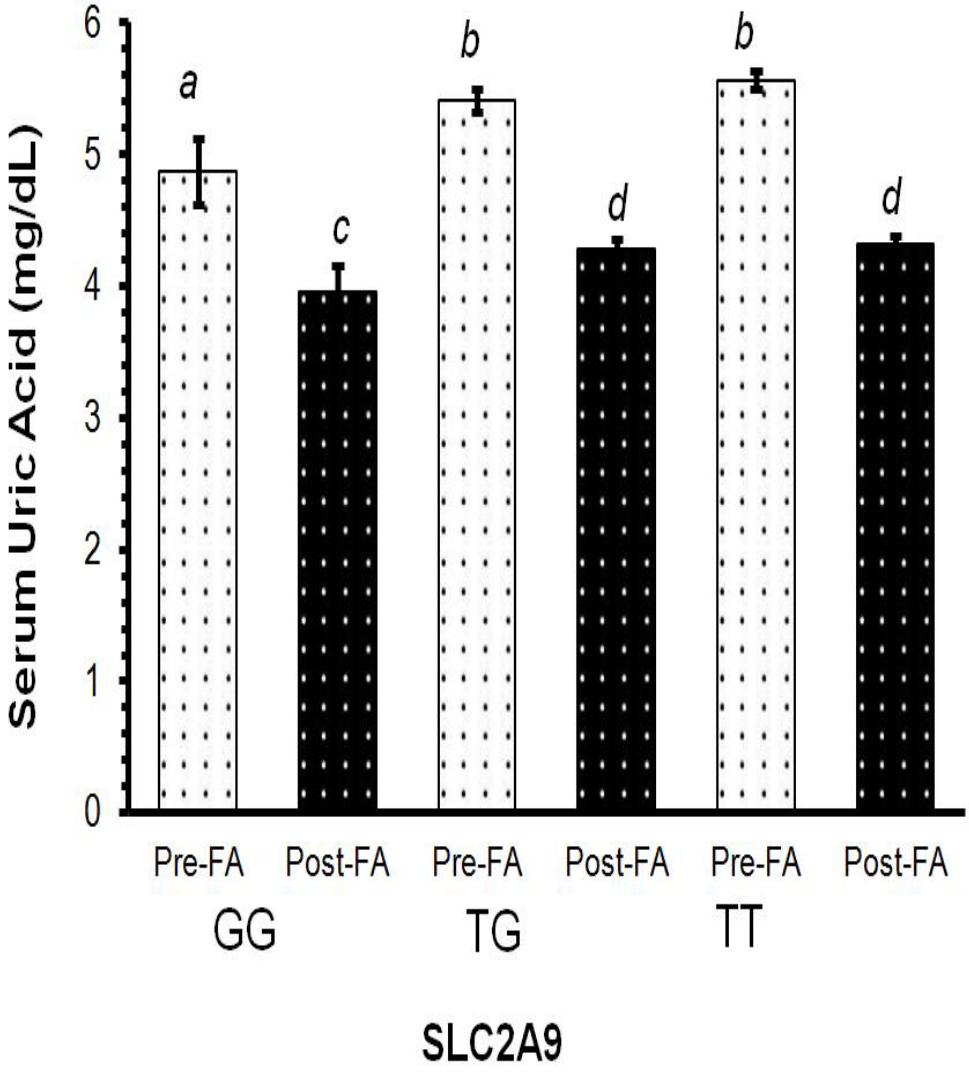


Figure 2(a): Change in uric acid according to SLC2A9 SNP (rs734553). Pre-FA= before fenofibric acid treatment, Post-FA= after fenofibric acid treatment. Letters a, b, c, and d are indications of *p*-value, were different letter indication a significant difference ($P<0.01$).

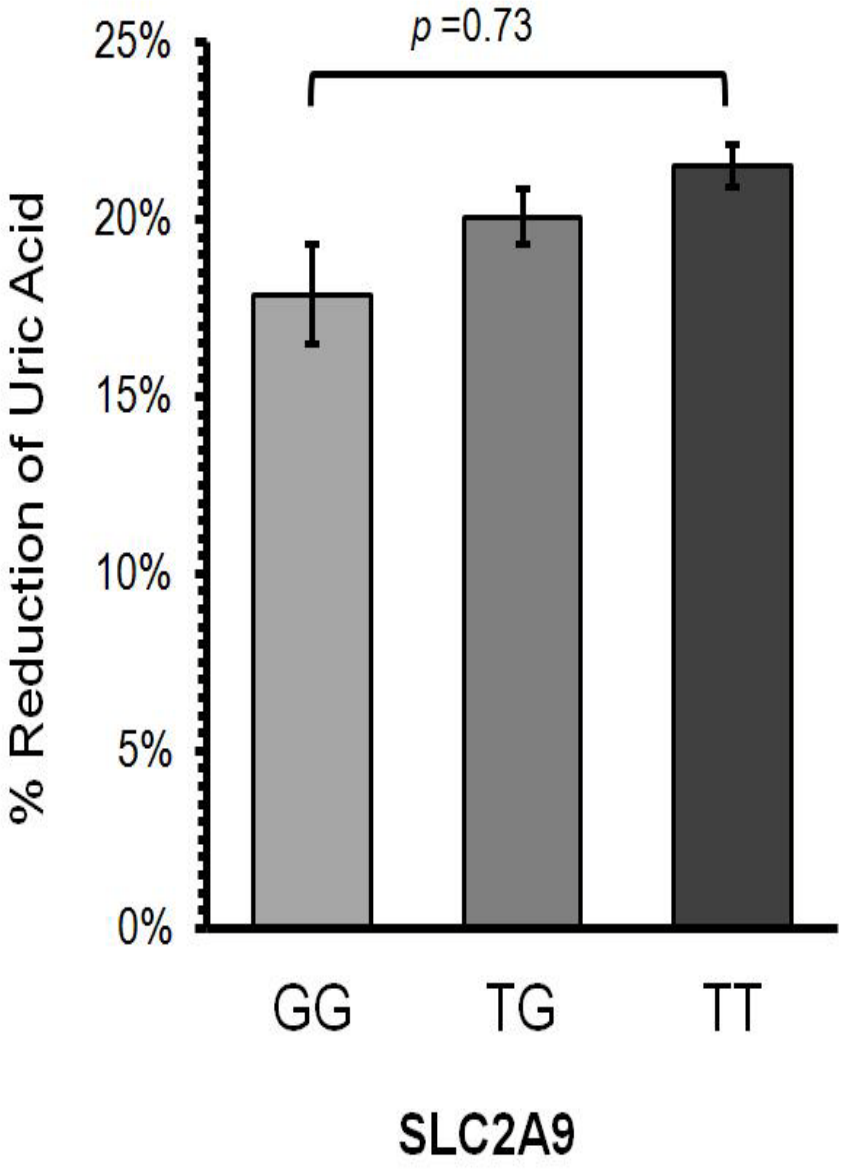


Figure 2(b): Percent change in uric acid according to SLC2A9 SNP (rs734553).

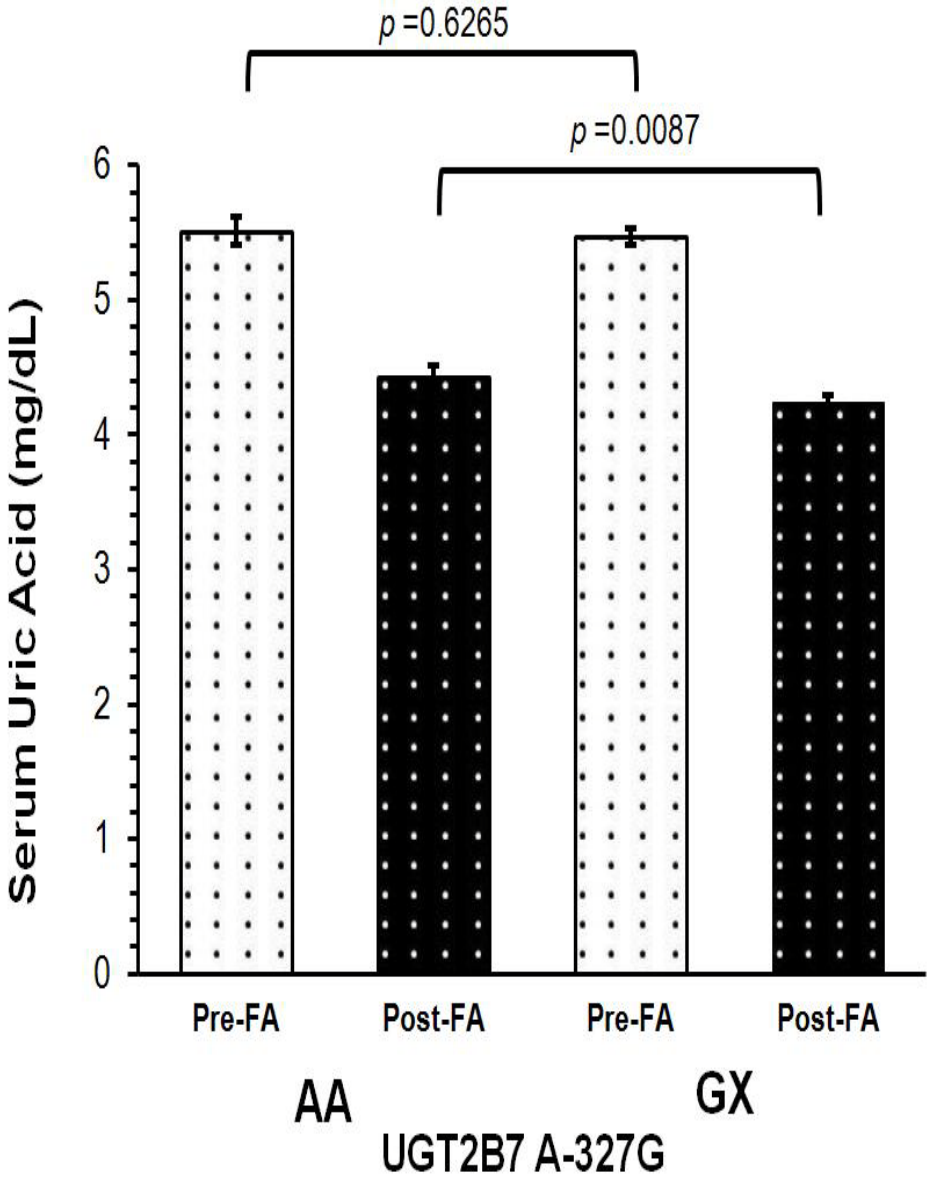


Figure 3(a): Change in uric acid according to UGT2B7 A-327G. A/A= A/A genotype, G/X = GA or GG genotype. Pre-FA= before fenofibric acid treatment, Post-FA= after fenofibric acid treatment.

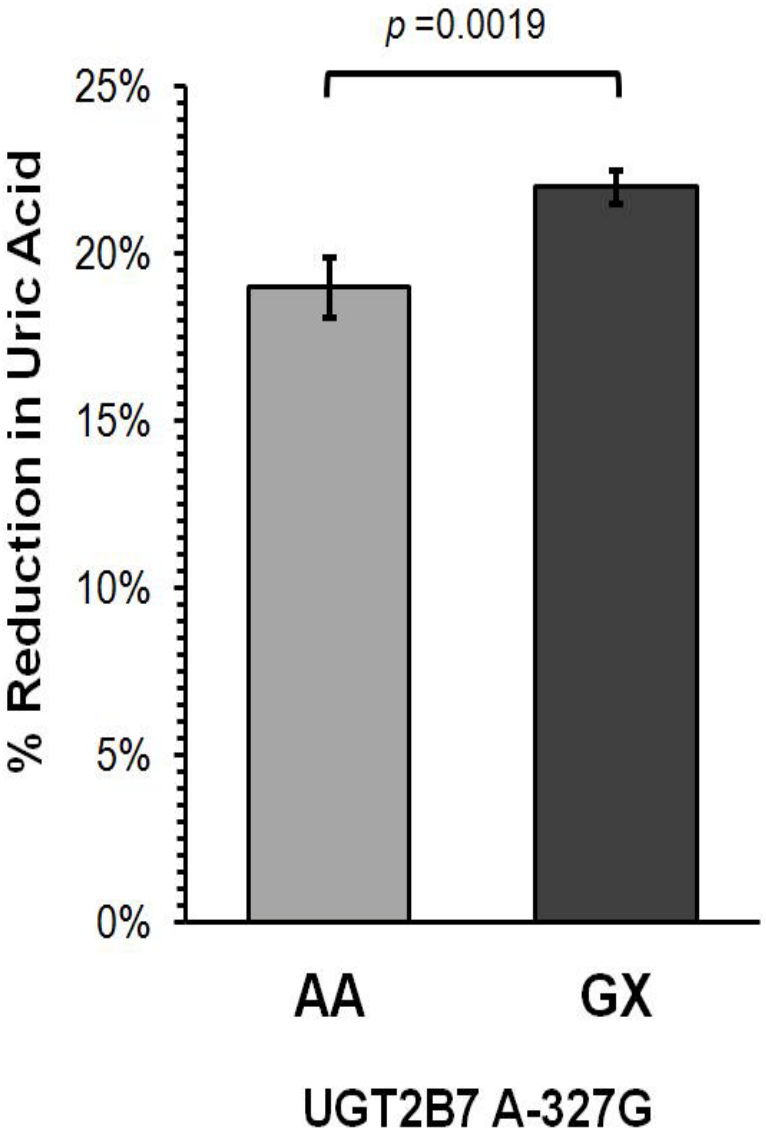


Figure 3(b): Percent change of uric acid according to UGT2B7 A-327G. AA= AA genotype, GX= GA or GG genotype.

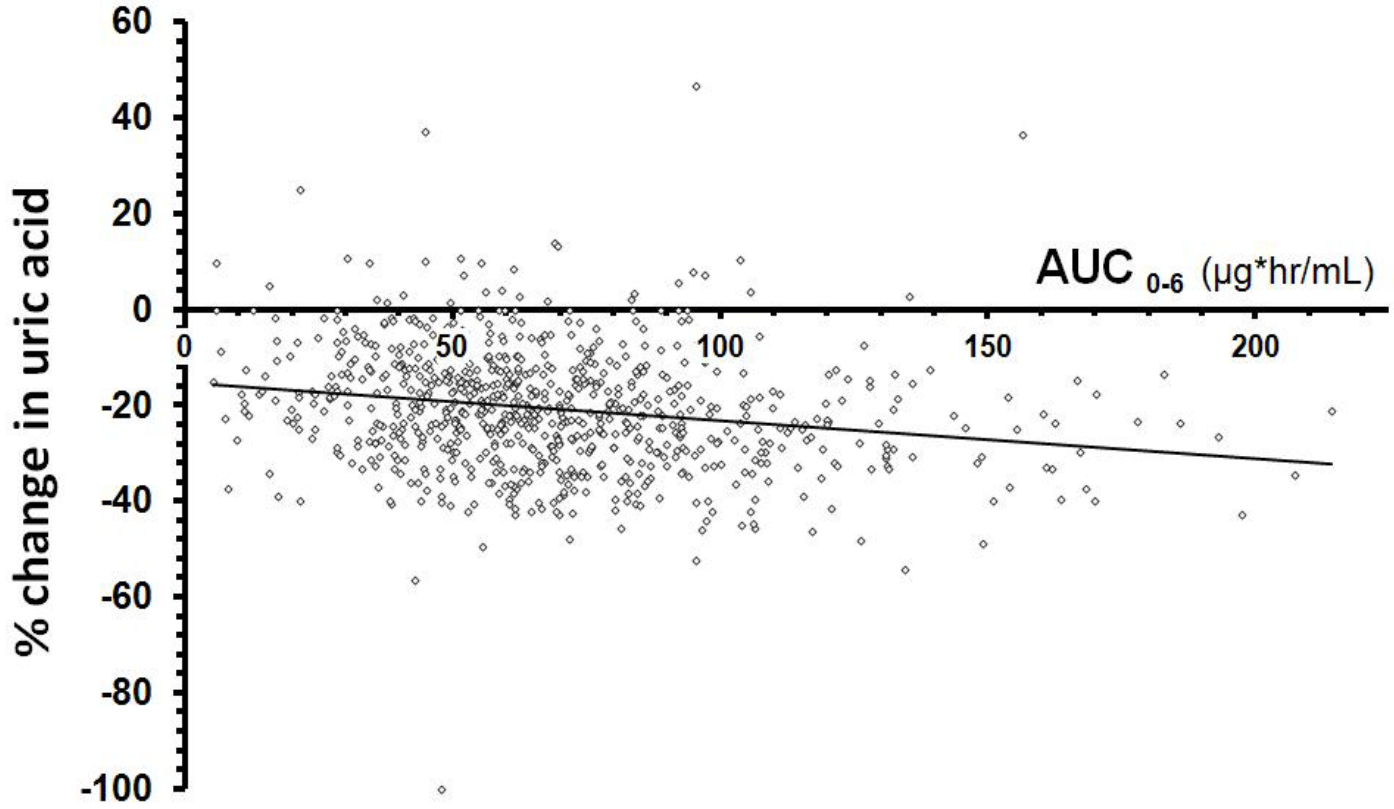


Figure 4: The correlation between percent change of uric acid from baseline and the area under the curve (AUC_{0-6}) of the serum concentration of fenofibric acid. AUC_{0-6} consists of three time points at 0, 3.5 and 6 hours.

CHAPTER V

Summary and Conclusions

The recognition of the residual cardiovascular risk, a substantial risk of a cardiovascular disease event (~60%), still exists in patients using statins who have attained their optimal low density lipoprotein cholesterol (LDL-C) levels recommended by the Adult Treatment Panel III guidelines.[1,2] An important contributor to the residual cardiovascular risk is the inability of statins to treat atherogenic dyslipidemia, a phenotype characterized by a reduced high density lipoprotein cholesterol and elevated triglyceride levels. This inability of statins renewed the interest in medications that can treat atherogenic dyslipidemia like fibrates and niacin. However, the mixed outcomes from clinical trials that tested the effect of combining statins and fenofibrate [3,4] or niacin [5] posed a challenge in using these two medication groups. One significant challenge of using fenofibrate is the high inter-subject variability in lipid response, which can range from -82 to 132% for TG change from baseline.[6] This magnitude of variability suggests the involvement of non-environmental factors, such as the genetic source of the variability. The Genetics Of Lipid Lowering Drugs and Diet Network (GOLDN) trial was the first to explore and characterize the genetic basis for the variation in lipid response.[7] One important genetic variation discovered in the GOLDN trial was a single nucleotide polymorphism (SNP), UGT2B7 A-327G, which was the cause of a 17% difference in the percent change of triglycerides, post-fenofibrate treatment, between its genotype groups.[6] The UGT2B7 A-327G is harbored in a key gene for the metabolism of fenofibric acid.[8,9] We hypothesized that the UGT2B7 A-327G caused this response variation in triglycerides due to its effect on serum concentration of fenofibric acid

(exposure). Furthermore, there is a positive correlation between serum concentration of fenofibric acid and percent change in lipid levels.[10] However, the GOLDN trial had only one reliable serum concentration for fenofibric acid, which was the reason for the conduction of Fenofibrate and the Pharmacogenetics Impact (FPI).

In the FPI study we sought to explicitly confirm the impact of UGT2B7 A-327G on serum fenofibric acid concentrations in humans, and therefore a potential marker of therapeutic response to fenofibrate. We pre-screened subjects for A/A or G/G genotype for UGT2B7 A-327G, and they received one daily dose of fenofibrate (145mg) for 28 days. On day 28, we measured the area under the serum fenofibric acid concentration (AUC_{0-24}). We concluded for the first time that UGT2B7 A-327G was a significant determinant of serum fenofibric acid concentrations causing a 27% difference in the AUC_{0-24} between A/A and G/G genotypes, and thus provides evidence for a key source of fenofibrate's high inter-subject variability in lipid response.

The antidyslipidemic effect is not the only pharmacological effect for fenofibrate; other effects had also been reported, such as its effect on serum bilirubin, fibrinogen [11], *C-reactive protein, interleukin-6, and tumor necrosis factor-α*[12], and uric acid level. The goals of the next two chapters are to quantify the effect of fenofibrate on both serum uric acid and bilirubin while controlling for SNPs that might affect baseline levels and percent change post-fenofibrate treatment of uric acid and bilirubin.

Elevated bilirubin levels had been linked to a lower incidence of CVD, while lower bilirubin levels were linked to a higher incidence of CVD.[13-15] The effect of fenofibrate treatment on bilirubin level was not clear; therefore, in chapter three we explored the effect of fenofibrate on bilirubin level in general, as well as the role that the UGT1A1*28 genetic variation plays in this effect. This effect has been explored in GOLDN trial subjects. Furthermore, since *UGT* genes are almost exclusively metabolize fenofibric acid [8,9], we explored the possible effect of UGT1A1*28 on fenofibric acid serum concentration, which like the UGT2B7 A-327G can influence the percent change in lipid levels. We found that fenofibrate lowered total-bilirubin levels 6.5% by increasing the conjugation of the free-bilirubin, which was revealed by an increase in the conjugated-bilirubin level. However, this effect was most evident in the UGT1A1*28/*28 genotype since they inherently have higher baseline bilirubin levels. However, in smokers the effect of fenofibrate on bilirubin levels was not significant even in the UGT1A1*28/*28 genotype, which could be because smokers had significantly lower baseline bilirubin levels compared to non-smokers. According to Lin et al.[14], UGT1A1*28 allele carriers who have higher bilirubin levels display a strong association with a lower risk of CVD, so we can conclude that the UGT1A1*28/*28 genotype might not be an optimal candidate to receive fenofibrate treatment due to its bilirubin lowering effect, therefore, depriving subjects with UGT1A1*28/*28 genotype of the advantage of CVD protection.

In chapter four of this thesis, we explored the uricosuric effect of fenofibrate. In this study we sought to quantify the effect of fenofibrate, SNPs, and environmental factors on the uricosuric effect within the GOLDN trial subjects. This is important specifically because studies link elevated serum uric acid levels with all-cause mortality [16,17], hypertension [18,19], metabolic syndrome [20], diabetes [21] and chronic kidney disease [22,23]. Previously we determined UGT2B7 A-327G to affect fenofibrate's disposition and lipid response; thus we sought to examine its impact on fenofibrate's uricosuric effect. We found that on average fenofibrate lowers serum uric acid; mean (±se) 20.8% (±0.46). Also, higher serum concentrations of fenofibric acid were associated with greater percent change in uric acid. We also identified a significant effect of UGT2B7 A-327G on percent change in uric acid for GG and GA relative to AA but not between G/G and GA, therefore a collapsed model combining AG and GG as GX was used. There was a significant difference, mean (±se) between AA and GX, -19% (±0.91), and -22% (±0.51) respectively. We concluded that fenofibrate's uricosuric response variability and potential clinical benefits appear to be modulated through UGT2B7 A-327G in a manner consistent with that predicted by serum fenofibric acid levels.

Additional clinical studies are required to test and apply our findings in which we used a pharmacogenetic-guided therapy. If these findings were confirmed, it could help in determining optimal patients who would benefit greatly from fenofibrate treatment,

which means patients would receive the utmost desired outcome and the least risk of adverse drug reaction. The successes of this pharmacogenetic-guided therapy could help in saving numerous lives, improve the patients' quality of life, and drive down the cost of healthcare.

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