Chapter 4

Evaluation of the Impact of UGT Polymorphism on the Pharmacokinetics and Pharmacodynamics of the Novel PPAR Agonist, Sipoglitazar

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Summary

Sipoglitazar, is a peroxisome proliferator–activated receptor α, δ and γ agonist. During phase I, a wide distribution of clearance between individuals was observed. Hypothesized to result from a polymorphism in the uridine 5'-diphospate-glucuronosyltransferase (UGT)2B15 enzyme, pharmacogenetic samples were collected from each individual for genotyping UGT2B15 in a subsequent phase I trial in healthy subjects (n=524) and in two phase II trials in type 2 diabetes subjects (n=627), total genotype frequency was: *1/*1 (22%), *1/*2 (51%) and *2/*2 (27%). The impact of genotype on exposure was assessed using a pharmacokinetic modelling approach, the influence of genotype on efficacy was evaluated using 12-week HbA1c change from baseline. Model analysis demonstrated UGT2B15 genotype accounted significantly for the variability in sipoglitazar clearance; however, a small fraction of subjects had a clearance that could not be explained entirely by genotype. HbA1c-drop increased with daily drug dose. When stratified by both dose and genotype, HbA1c-drop was larger in the UGT2B15*2/*2 compared with UGT2B15*1/*1 and UGT2B15*1/*2 genotypes (P<.05). In summary, UGT2B15 genotype is a strong predictor for sipoglitazar clearance, a greater clinical response observed in the UGT2B15*2/*2 genotype appears to confirm this. However, overlap in individual rates of clearance across genotypes remains after accounting for genotype.

Introduction

Genetic differences that result in patient variability in drug metabolism, disposition, and response, have led a move towards individualized medicine in which doses are set based on genotype [1,2]. However, the relative contribution of genetic differences to inter-individual variability in exposure varies widely between drugs. In some cases there is too much weight placed on the contribution of a single genotype to drug clearance and too little weight on the contribution of other factors affecting both clearance and the clinical response, such as age, body weight, disease status and environment [3-5].

Polymorphic expressed enzymes, such as cytochrome P450 (CYP) 2C9, CYP2C19, and CYP2D6, have been extensively studied as a large number of drugs are catalyzed through these pathways, including warfarin and metoprolol [6,7]. In addition to the polymorphic CYP mediated metabolism, genetic polymorphisms have been identified for glucuronidation by uridine 5'-diphospate-glucuronosyltransferases (UGTs). Accounting for approximately 10% of the major drug elimination pathways,[4] some of these UGTs have been shown to be polymorphic. An example is the UGT2B15 isoform, which is involved in the inactivation of lorazapam and oxazepam [8,9]. Genetic polymorphisms for UGT2B15 have been identified to result from an amino acid change from aspartic acid (D^{85}) to tyrosine (Y^{85}) at position 85 [10]. Those subjects that are homozygous (*2/*2) with reduced glucuronidation are classified as “poor metabolizers” (PM), compared to those with the wild type (*1/*1) “extensive metabolizers” (EM), and those with the heterozygous allele (*1/*2) exhibiting intermediate levels of metabolic activity “intermediate metabolizers” (IM). The genotype frequencies reported in the Caucasian population for UGT2B15 *1/*1, UGT2B15 *1/*2, and UGT2B15 *2/*2 are 22%, 46% and 32%, respectively [11].

Sipoglitazar, a novel orally-available, peroxisome proliferator–activated receptor (PPAR) agonist with activities for PPAR α, δ, and γ, was targeted for type 2 diabetes mellitus (T2DM). The compound undergoes phase II biotransformation by conjugation catalyzed by UGT [12]. During phase I clinical trials, a bi-or multimodal distribution of exposure/clearance appeared to be more likely than a normal distribution; this was later evaluated using in vitro data and was found to be related to a polymorphism of the UGT2B15 enzyme. Based on this result pharmacogenetic samples for UGT2B15 were collected from each individual in a subsequent phase I trial in healthy subjects and in two phase II trials in

type 2 diabetes subjects for genotyping UGT2B15 (*1/*1, *1/*2 and *2/*2) polymorphisms. The translation of the observed variability in clearance to the pharmacodynamics of the compound was explored in context to the expression of the UGT2B15 enzyme and reviewed in relation to a pre-determined exposure margin.

Methods

Subjects and Data Collection

A summary of studies used in the analysis, as well as demographic and genotype frequency data is given in Table I. One phase I trial in healthy subjects (n=524) and two phase II trials in type 2 diabetes subjects (n=627) were included in the analysis. All studies were conducted in accordance with the Declaration of Helsinki (Edinburgh 2000). Written approval was obtained from the relevant local institutional ethics committee before the start of each study and for the amendments made to the protocols.

Table I. Summary of studies and data used in the analysis

Study name (study number)	Study description	Population	Number of subjects	Number of samples per subject		Gender		Age[b]	Weight[b]	UGT2B15 Genotype		
				PK	PD[a]	Female	Male			*1/*1	*1/*2	*2/*2
Phase I study 1 (006)	Evaluation of the effect of genetic polymorphism on the peak and extent of exposure of sipoglitazar after a single oral dose (64 mg) in healthy male and female subjects, including a range of ethnicity	Healthy volunteers	524	10	n/a	304	220	26 (18-55)	71 (48-113)	124	273	127
Phase II study 2 (EC201)	Efficacy, safety and tolerability of four active doses (8, 16, 32, and 64 mg) and placebo	Patients with T2DM with no prior exposure to antidiabetic medication	320	3	8	155	165	56 (34-75)	90 (60-149)	70	160	90
Phase II study 3 (EC202)	Double-blind, randomized study to evaluate total daily doses of 32 and 64 mg sipoglitazar once daily or twice daily and placebo,	Patients with T2DM with no prior exposure to antidiabetic medication	307	3	8	164	143	57 (34-74)	87 (55-149)	56	157	94

a PD samples were collected for glycosylated hemoglobin. b Values represent median (minimum-maximum). PD = pharmacodynamic; PK = pharmacokinetic; T2DM = type 2 diabetes mellitus; UGT2B15 = uridine 5'-diphospate-glucuronosyltransferase 2B15.

Pharmacogenetic Analysis

A blood sample was collected during each study for genotyping. The blood sample was collected into an EDTA tube and stored at -20°C until shipment, then transported frozen to DxS, Manchester, United Kingdom. DNA was prepared from whole blood samples by the AGOWA/Hamilton automated extraction system (Bonaduz, Switzerland). Real-time polymerase chain reaction methods using the Stratagene Mx4000 (La Jolla, CA, USA) and appropriately designed primers allele-specific at the 3′ nucleotide end (Amplification Refractory Mutation System strategy [13]) were used to determine UGT2B15*2 (D85Y) and were analyzed according to their relative capillary electrophoretic mobility using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Warrington, United Kingdom).

Bioanalysis

At each specified time point, plasma samples were collected into sodium heparin–containing tubes. The tubes were inverted gently in order to dissolve the heparin and they were placed on ice until processing. The plasma was separated in a refrigerated centrifuge, within 60 minutes of collection, at approximately 1500 g for 10 minutes. Plasma concentrations for sipoglitazar were quantified in human plasma using a method previously validated by Covance Laboratories Ltd (Harrogate, United Kingdom). This method uses liquid chromatography with tandem mass spectrometric detection, with a validated calibration range from 0.1 to 250 ng/mL for sipoglitazar in human plasma.

Population Data Analysis

Exploratory graphical analysis on the phase I pharmacokinetics of sipoglitazar indicated bi-phasic elimination. The phase I and II datasets were combined and a two-compartmental model with parallel first- and zero-order absorption into the central compartment and first-order elimination was selected as the initial structural model for nonlinear mixed effect model development.

Inter-individual variability (IIV) was explored assuming a log normal distribution of the individual parameter estimates. The IIV (η) for the ith pharmacokinetic parameter, where θi

is the individual pharmacokinetic parameter and θ (mean) is the population predicted mean as shown in the following equation:

θ (i) = θ (mean) * exp(η) EQ(1)

The data included in this analysis are taken from a single dose healthy subject study with dense sampling (0 hours and 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours post dose) and two dose ranging patient studies with 3 trough samples per patient (collected at weeks 4, 6, and 8 of the 12-week phase II trials). The residual error was not expected to be uniform between the two populations. As such different residual errors were applied between the healthy subjects and patient studies (supplemental methods). The IIV for the absorption phase and volume of distribution (V) were only estimated in the healthy subject study. Residual variability was modeled using a proportional model for the subject data *EQ(2)* and a proportional model including IIV for the patient data *EQ(3)* as follows:

Cij = PRED * (1+ERRij) EQ(2)

Cij = PRED * (1+ERRij) * exp(η) EQ(3)

Where *Cij* is the observed concentration, *PRED* is the predicted concentration, and *ERR* is the proportional residual error the for jth prediction for the ith individual.
Eta-shrinkage of all random effects on IIV was computed to inform model validation [14].

In addition to genotype, a covariate analysis was conducted to explore the influence of other individual covariates on the pharmacokinetic parameters, with free fat mass (FFM) used to assess the influence of body weight [15].

All covariates were evaluated in the model using a forward inclusion and backward elimination procedure [16]. Covariates were included in the model using the following equation:

P(mean) = θ (i) * (1+θ(f) * (COV-COV(median))) EQ(4)

Where *P(mean)* is the typical value of the population estimate, θ *(i)* is the individual parameter estimate, COV is the value of the covariate and associated median value and θ*(f)* represents magnitude of the covariate effect.

For safety reasons, an upper limit of chronic exposure was previously determined (area under the curve (AUC) > 73 mg·hr/L) for sipoglitazar. In order to assess the balance between safety and efficacious response, the data are reviewed in context to this level.

Efficacy Data

Analysis of the phase II data was performed in patients with T2DM following 12 weeks of treatment with sipoglitazar. The primary endpoint was the absolute drop in glycosylated hemoglobin (HbA1c) in percentage points observed between day 0 and the last day of dosing, which was stratified by dose and genotype. The HbA1c data were analyzed using analysis of variance and the experiment-wise type 1 error controlled by a combination of Bonferroni correction and Tukey multiple comparison tests. A $P < .05$ was considered statistically significant.

Data Analysis

All population analyses were performed using nonlinear mixed effects modeling on pharmacokinetic and demographic data in the NONMEM software package (version VII, release 1; Icon Development Solutions, Ellicott City, MD, USA) and analyzed using the statistical software package S-Plus® for Windows (version 6.2 Professional, Insightful Corp., Seattle, WA, USA). The first order conditional estimation method was used for estimation.

Visual Predictive Check

Model performance was evaluated using the visual predictive check (VPC), evaluating the ability of the model to predict both the central tendency and the variability of the exposure [17]. The distribution of simulated concentrations for 1000 subjects (median and 90th prediction interval) and the actual individuals, including the median and percentiles was compared graphically. Results

Base Model

A population pharmacokinetic model was developed, with the pharmacokinetics of sipoglitazar being well described using a two-compartmental model with linear kinetics and no observed dose or time dependency. The absorption phase was adequately described using

a combined parallel zero- and first-order uptake process. The IIV was described by an exponential variance model for clearance (CL), volume of distribution (V) and for the duration parameter (D1), which describes the duration of the zero-order process. The residual variability was described using a proportional error model with separate residual variability for phase I and phase II. The addition of IIV on the residual error for the phase II population resulted in a large decrease in the MVOF of -508 points; in addition, the residual error decreased from 131% to 24%.

The median value for CL for the total population was initially estimated at 2.8 L/h.

Covariate Analysis

To further investigate the influence of UGT polymorphism, a value for median clearance was optimized separately to each individual according to UGT2B15 genotype (Table II). This resulted in median clearance for genotype groups UGT2B15 *1/*1 and *1/*2, which were respectively, 66% and 53% lower than that of the genotype UGT2B15 *2/*2. Before accounting for any covariates (including genotype) IIV on clearance was 60%, however after including genotype as a covariate, IIV of clearance was reduced to 40%. No differences in degree of variability were observed between UGT2B15 genotype *1/*1, *1/*2 and *2/*2.

Using this pharmacokinetic model, all other candidate covariates were subsequently tested for significance (age, sex, weight, and FFM), separately on V and CL. During forward inclusion only sex, weight or FFM on V resulted in a significant decrease in the MVOF (> 6.63). As the greatest change in the MVOF was observed with FFM, as this was the only covariate retained in the final model after backward deletion, the addition of which resulted in a decrease in IIV of 2%.

Final Model

The parameter estimates for the final model are shown in Table II. The distribution for the post-hoc CL values obtained from the final model are shown in supplemental Figure S1 without stratification and in Figure 1 including stratification by genotype, demonstrating an increasing tendency in CL from UGT2B15 genotype groups *2/*2> *1/*2>*1/*1.

The relationship between dose and AUC (AUC=dose/CL) over the dose interval at steady state was explored and stratified by dose and genotype (Figure 2). As indicated from the median CL values optimized per genotype a higher AUC value was observed in subjects in the UGT2B15 *2/*2 group compared with the other two genotype groups. Although this trend is generally observed, several outlier subjects (>1.5*inter quartile range) were observed in the UGT2B15 *1/*1 and UGT2B15 *1/*2 genotype groups. Subjects from both these genotype groups have overlapping AUC ranges to those values observed for the UGT2B15 *2/*2 genotype.

Table II. Summary of parameter estimates for the final model including covariates

Parameter name	Parameter	Value (CV%)	IIV (%, CV%)
Clearance population 1[a]	CL (*1/*1) (L/h)	4.46 (2.5)	40.25 (7.72)
Clearance population 2[a]	CL (*1/*2) (L/h)	3.25 (2.2)	
Clearance population 3[a]	CL (*2/*2) (L/h)	1.53 (2.2)	
Central volume of distribution[a]	V (L)	9.03 (2.4)	34.21 (13.0)
Peripheral volume of distribution[a,b]	V2 (L)	0.189 (4.9)	--
Intercompartmental clearance	Q (L/h)	0.313 (6.6)	--
Absorption rate constant	ka (1/h)	2.07 (4.8)	--
Duration of zero order process	D1 (h)	0.568 (6.81)	78.29 (14.8)
FFM on central volume of distribution	(L/kg)	0.00349 (27.2)	
Residual variability Phase I (proportional)	σ^2	0.0552 (8.8)	--
Residual variability Phase II (proportional)	σ^2	0.167 (10.2)	76.88 (14.9)

a Bioavailability for sipoglitazar is currently unknown, as such clearance and volume were modeled as CL/F and V/F, respectively.

b The peripheral volume of distribution was implemented as a fraction of the central compartment.

CV% = percent coefficient of variation.

Figure 1. Histogram plot for post-hoc CL values for all subjects included in the analysis stratified by genotype.

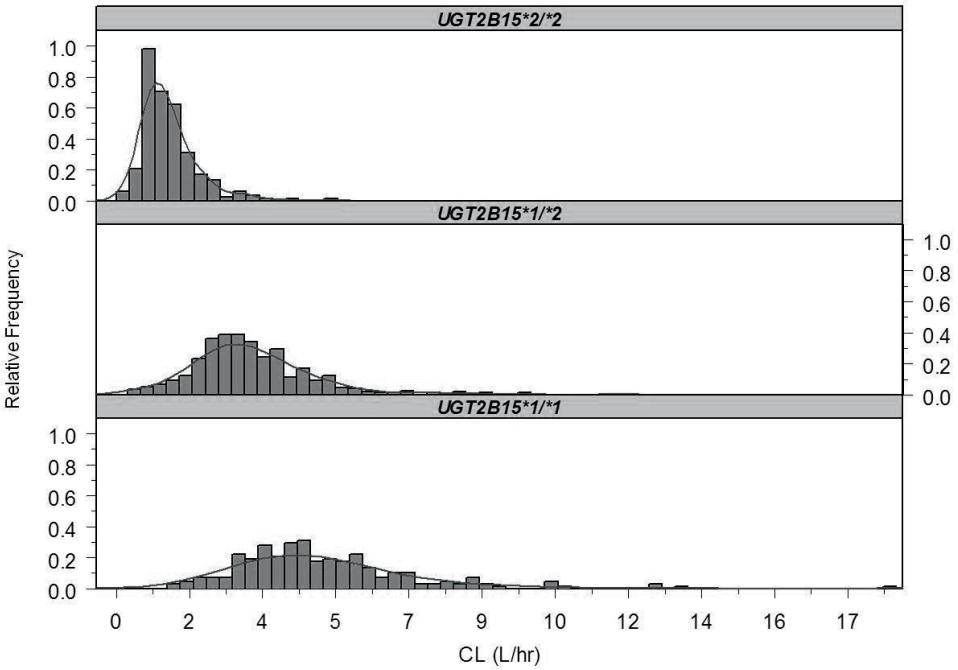
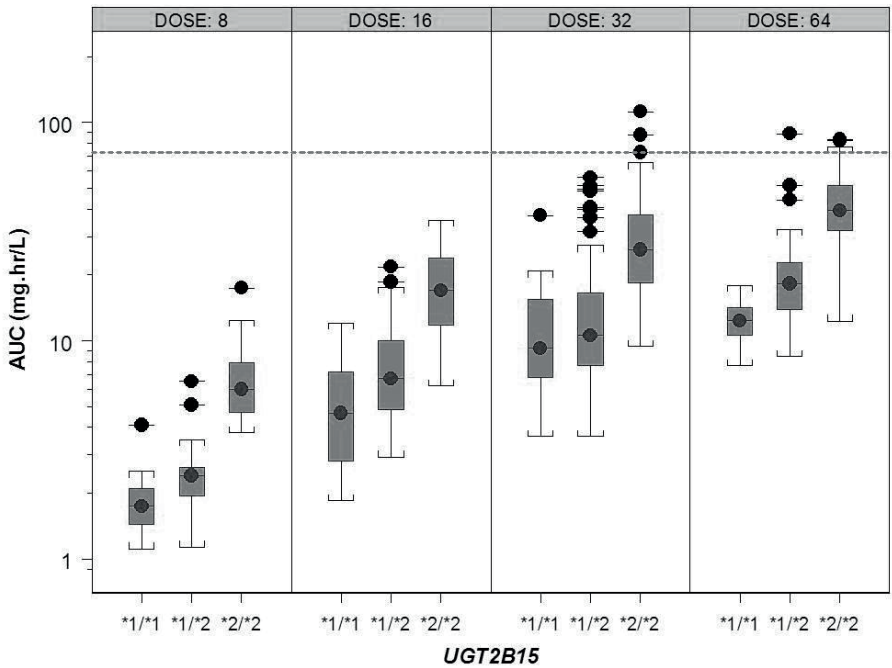


Figure 2. Box plots (median, 25th and 75th percentiles) for the estimated area under the concentration-time curve from 0 to 24 hours (AUC) by genotype and dose. Gray line exposure limit 73 mg·hr/L.



Model Validation

The observed and predicted plasma concentration-time profile following a single 64 mg dose in healthy subjects are shown using the VPC (supplemental Figure S2a). The VPC for the dose normalized phase II data is shown in supplemental Figure S2b. The model-predicted median and 90th prediction interval closely resemble those for the actual data, demonstrating the ability of the model to describe the data well. No substantial eta-shrinkage was observed for CL (2.3%), V (4.1%) or IIV for omega on sigma (-1.7%); however, for D1, eta-shrinkage was fairly high (28.1%), but was considered acceptable for the aims of this analysis.

Influence of Genotype and Dose on Efficacy

Figure 3(a) shows a clear pattern for the dose response relationships based on the median 12-week change from baseline of HbA1c with total daily dose. For the entire study population, doses equal to or greater than 16 mg showed a significant ($P < .05$) change from baseline in HbA1c compared with placebo. However, when stratified by UGT2B15 genotype and dose (Figure 3(b)), subjects with the UGT2B15*2/*2 genotype showed a significantly larger reduction ($P < .05$) in HbA1c compared with the UGT2B15*1/*1 and UGT2B15*1/*2 genotypes at 32 mg and 64 mg. At 32 mg, the median change from baseline in HbA1c for the UGT2B15*2/*2 genotype was -0.95% (n=36) compared with -0.6% (n=100) and -0.5% (n=50) in the UGT2B15*1/*2 and UGT2B15*1/*1 groups, respectively.

Figure 3.(a) Box plots (median, 25th and 75th percentiles) for the change from baseline in HbA1c by dose (placebo (n=111), 8 mg (n=58), 16 mg (n=113), 32 mg (n=186), 64 mg (n=125)).

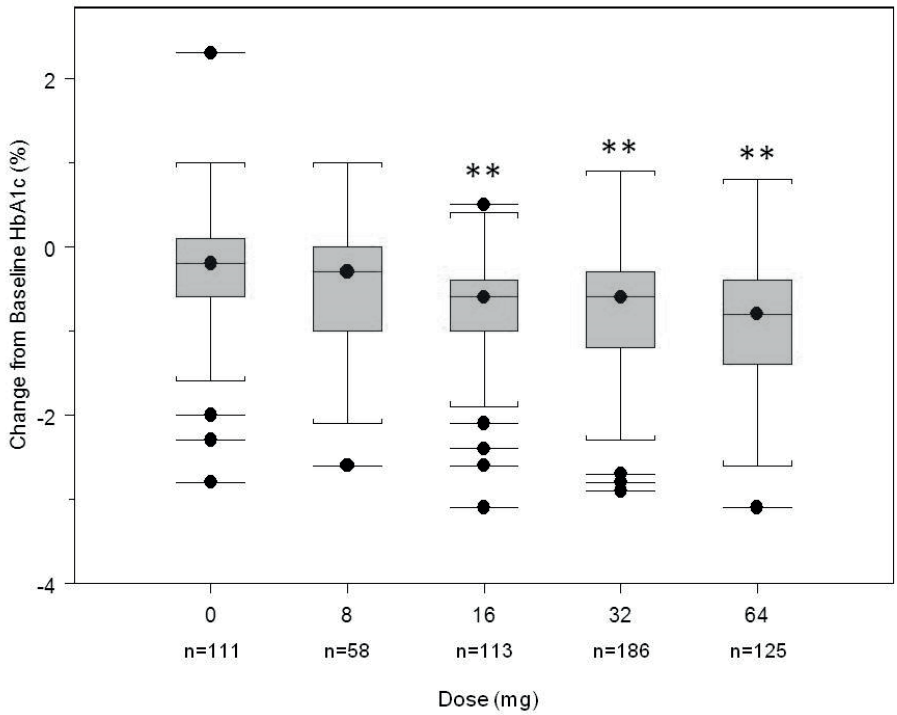
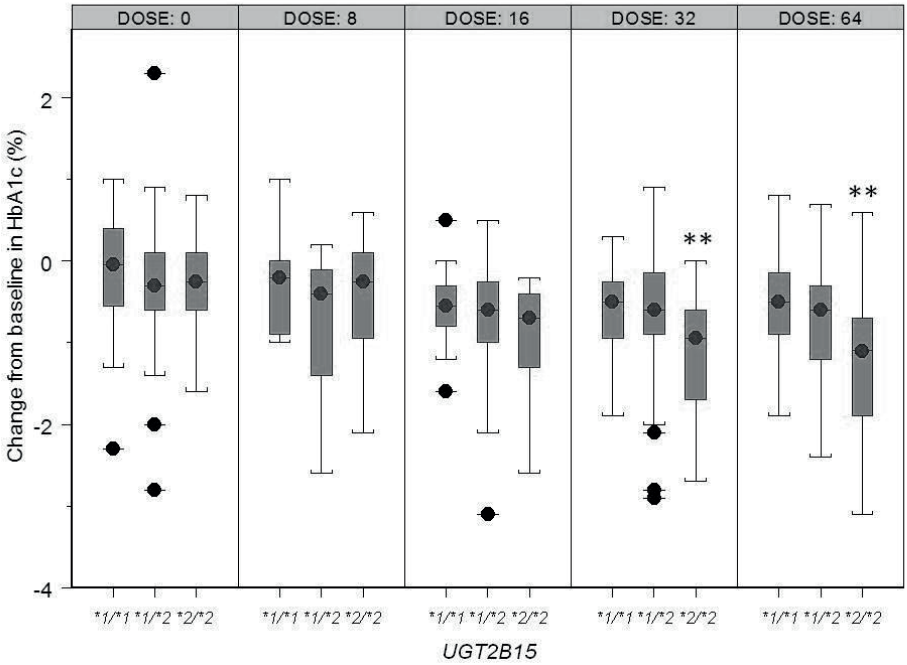


Figure 3.(b) Box plots (median, 25th and 75th percentiles) for the change from baseline in HbA1c by genotype and dose. **=statistically significant at $P < .05$.



Discussion

During this analysis the pharmacokinetic and pharmacodynamic data for sipoglitazar in healthy subjects and T2DM patients were analyzed in relation to the polymorphic expression of the UGT2B15 enzyme. Based on in-vitro metabolism studies, UGT2B15 was expected to contribute to the inter-subject variability. Firstly, a population pharmacokinetic model was developed for sipoglitazar, evaluating the individual relationship of UGT2B15 genotype to clearance. This analysis revealed that genotype significantly accounted for the variability in clearance of sipoglitazar. Secondly, the marker for efficacy, HbA1c change from baseline, when stratified by dose and genotype revealed that a greater clinical response was observed in patients in the UGT2B15*2/*2 group compared with patients in the UGT2B15*1/*1 and UGT2B15*1/*2 genotype groups. Thus the UGT2B15 enzyme was found to play an important role in the disposition of sipoglitazar, the results of which impacted on the clinical efficacy.

Using the pharmacokinetic model, the influence of genotype on the IIV on clearance was explored. By accounting for genotype as a covariate on clearance the IIV was reduced from 60% to 40%. Additional covariates were tested on both clearance and volume; however, only FFM on volume was found to be significant, reducing the IIV on distribution volume by 2%. The results of this work showed genotype can indeed explain the variability in clearance however only to a certain degree, with 40% IIV on clearance remaining. Thus genotype alone cannot explain entirely the observed degree of variation in exposure and various other factors are apparently contributing to the variability. Results from the current analysis showed that a small fraction of the population of either UGT2B15*1/*1 or *1/*2 groups have widely overlapping ranges in individual clearance between genotype groups. To evaluate this further, a mixture model was developed in parallel by optimization of individual probabilities to estimate the category of metabolism on the basis of apparent clearance, without taking the information on the genotype into account (supplementary material). This analysis estimated the percentage of subjects in the UGT2B15*1/*1 and *1/*2 groups in whom the phenotype was not corresponding with the genotype as 8% (61/744). In other words, in these subjects, genotype was not predictive of the actual observed clearance (supplementary Figure S3).

These subjects had an apparent clearance value that falls into the range observed for the UGT2B15*2/*2 group, resulting in potentially 2-3 times lower clearance than the median value for these groups based solely on genotype.

Phase II results showed a clear dose-dependent reduction in the pharmacodynamic marker, HbA1c, with sipoglitazar treatment. When stratified by genotype, this effect was lower in the UGT2B15*1/*1 and *1/*2 groups compared with the UGT2B15*2/*2 genotype group, confirming the clinical relevance of genotype-based differences in exposure of this drug. For various drugs, studies are reported that address the relationship of genotype to phenotype, with a primary focus on the mean change in pharmacokinetic parameters when stratified by genotype [18-20]. However, these studies often do not address the remaining variability of exposure within each genotype at the individual level or the overlap in exposure between different genotype groups. Other studies, focus directly on stratification by genotype to clinical outcome [21]. Individual differences in pharmacokinetics caused in part by polymorphism are not necessarily of clinical relevance [22]. This is generally due to a number of factors such as a very large range of overlap in exposure between genotypic groups [23], and/or wide safety to efficacy margins that allow a single treatment to be both efficacious and safe for all patients irrespective of genotype. Under certain conditions, pre-selection of doses based on one of several genotypes could potentially lead to efficacy or safety concerns if the phenotype overlap between genotype groups is not adequately understood. For example, a subject could be classified as a particular genotype but could still receive an inappropriate dose because other structural and/or random factors also contribute to the individual exposure. Although there is now a wide interest in the use of genotype-based dosing to account for differences in efficacy due to the polymorphic driven changes in pharmacokinetics, currently, very few drugs on the market have a specific dose adjustment recommendation included in the label [24]. In addition, for some cases the study population was too small to confirm the clinical relevance of such polymorphisms [25,26].

Based on the current results for sipoglitazar, the use of a genotype approach in which doses are set for individuals based on a genetic sample was considered as a potential method of individualized dose selection. From the results of this analysis, a dose of 32 mg appears to

achieve an optimal reduction in HbA1c in the UGT2B15*2/*2 group with comparability to other diabetic agents that achieve reductions in HbA1c of around 0.7%-1% in short term trials [27]. Thus, genotype-based dosing would target comparable AUC values to be achieved for all UGT2B15 genotype groups. However under such circumstances at this exposure level those subjects with disconnect between genotype and clearance may exceed the exposure margins, especially in the UGT2B15*1/*1 and UGT2B15*1/*2 genotype groups. Given the potential disconnect between individual clearance and genotype and the potential in these subjects to exceed exposure limits a more balanced approach may combine therapuetic drug monitoring in addition to the pre-selection of doses based on genotype.

Alternative dosing approaches based on monitoring of individual efficacy directly after the start of dosage could also be considered The current anti-diabetic agents requiring dose titration can reach the highest dose in 2 to 3 titration steps and usually only requiring 2 visits. For sipoglitazar and other PPAR agonists, the longer time to effect equilibration likely indicates that monitoring would be required over a longer period than for metformin [28], at similar time frames as for rosiglitazone (8 to 12 weeks). This would likely characterize those subjects in the UGT2B15*2/*2 group since the higher exposure seems to result in a stronger effect; however, longer titration steps and a wider range from the initial starting dose to the maximum dose would likely be required in the UGT2B15*1/*1 or UGT2B15*1/*2 genotype group.

In summary, it can be concluded that genotype explains a large part of the observed variability in exposure to sipoglitazar, but other factors which remain largely unexplained at the moment may cause a level of exposure that is either too low to achieve the desired effect or so high that exposure limits will be exceeded. A genotype-based dosing approach alone would thus not be a viable strategy for sipoglitazar, however, a combination of therapeutic drug monitoring combined with an efficacy-based approach may offer an alternative to mitigate the risks in subjects who have disconnect between genotype and drug exposure.

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Supplemental Appendix

Figure S1. Histogram plot for post-hoc clearance (CL) values for all subjects included in the analysis.

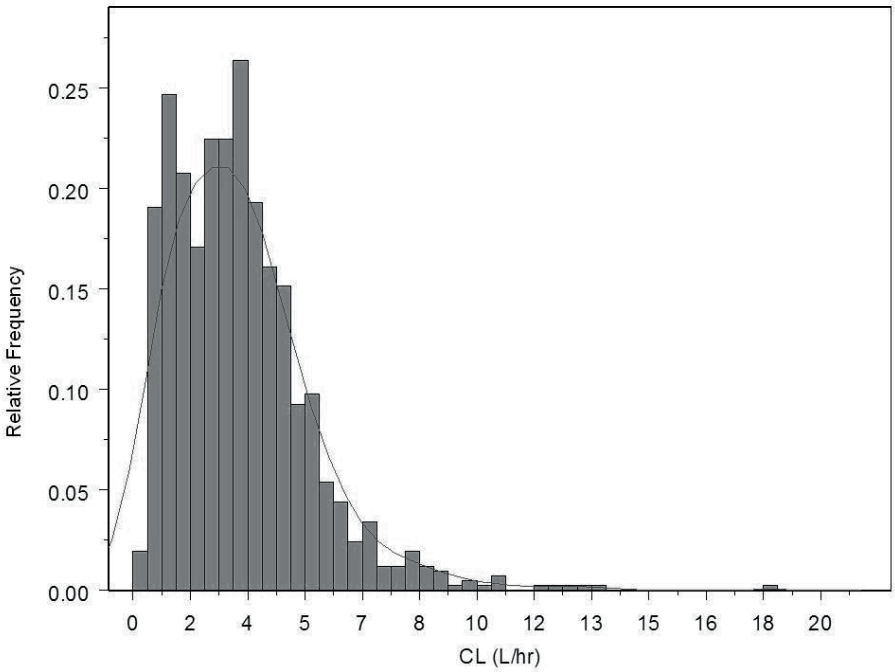


Figure S2.(a) Visual predictive check for observed and predicted single dose (64 mg) data for 006.

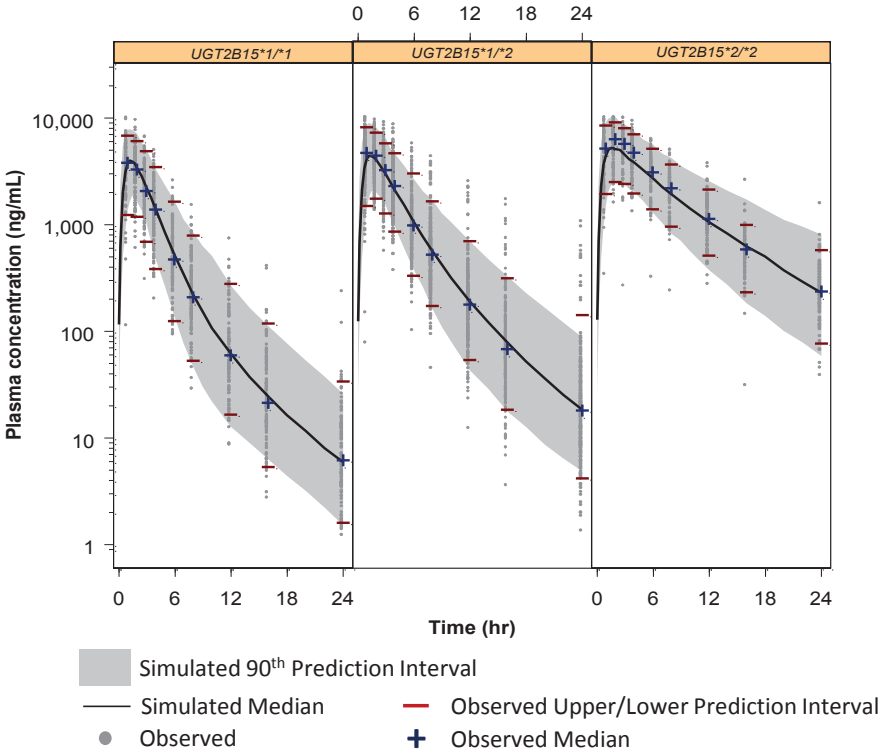
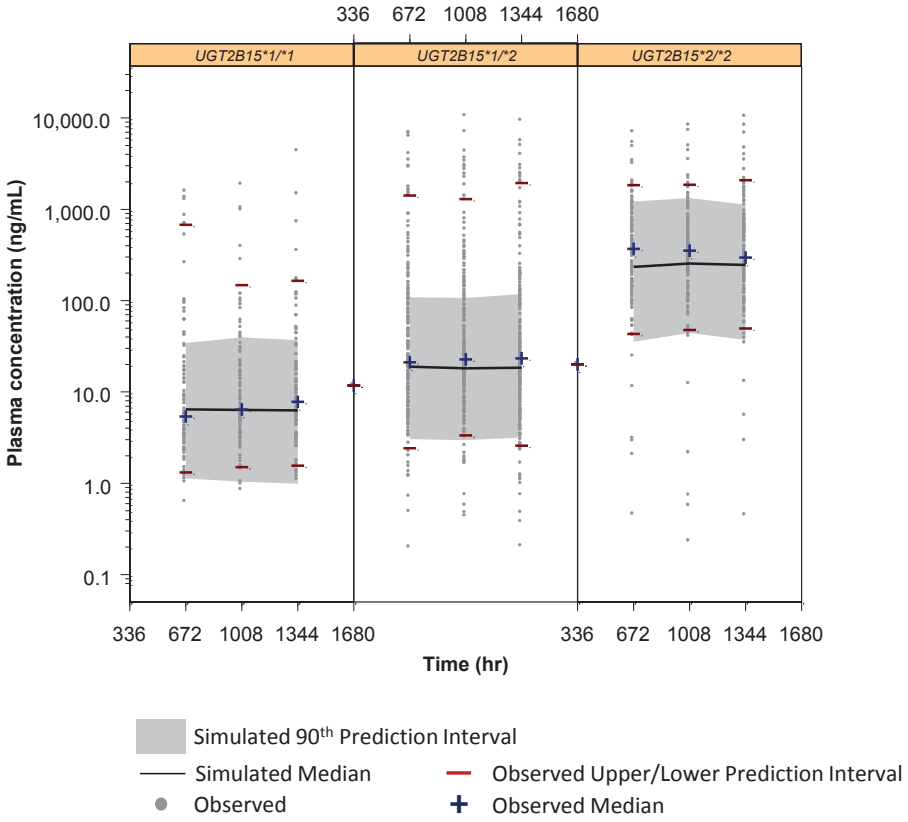


Figure S2.(b) Visual predictive check for dose normalized observed and predicted data from the combined phase II trials (EC201 and EC202)



Population Data Analysis

Methods

Inter-individual variability on the residual error for the patient trial was included using NONMEM's omega-sigma interaction option, since all samples were intended to be collected at trough, the actual time after the administration of the dose was not recorded in either of the phase II trials. Therefore this helps to account for high fluctuation in trough samples for some subjects due to sampling error or apparent non-compliance [1], recognizing that CL estimates could potentially be confounded by adherence. However, in this analysis, we assume complete compliance.

Mixture Model Analysis

Methods

The relationship between drug clearance and genotype was additionally determined without the use of the pertinent information on the UGT2B15 genotype in the model. Without this knowledge, the inter-individual variation was described using a probability model (NONMEM $MIX) to assign subjects to one of the three populations based on the model-estimated parameters [2-4]. The individual probability of belonging to a subpopulation was estimated and compared to the actual genotype catagory.2 Subjects assigned to a different population than expected based on their genotype may have been misclassified if indicated by an individual value of belonging to that population (IPk) close to 0.5.

Subjects were assigned to one of the three populations (POP1, POP2, and POP3); these were expected to approximate the *UGT2B15* genotype *1/*1, *1/*2 and *2/*2. This subpopulation assignment was then compared to actual genotype categorization and corresponded as follows:

POP1 (CL1 EM) = UGT2B15*1/*1

POP2 (CL2 IM) = UGT2B15*1/*2

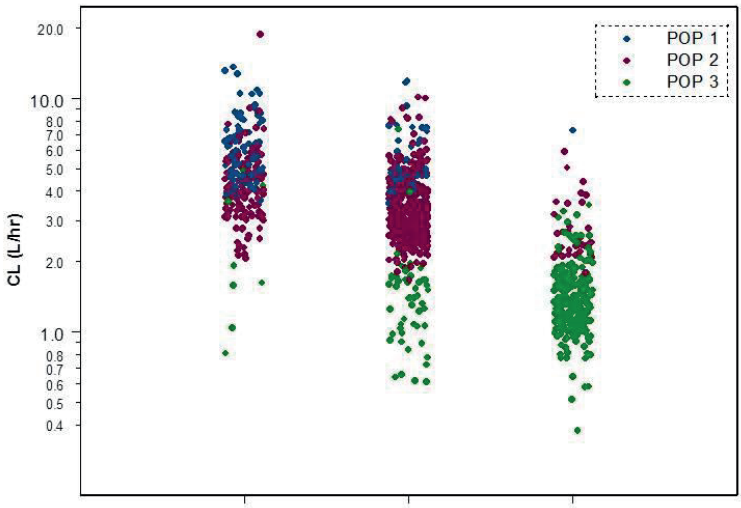
POP3 (CL3 PM) = UGT2B15*2/*2

Subjects who were classified as UGT2B15 *1/*1 or *1/*2 based on genotype, but were assigned to the PM category (POP 3) by the model are expressed as a percentage of the total UGT2B15 *1/*1 and *1/*2 genotype groups.

Results

The pharmacokinetic parameter estimates for the mixture model are shown in Table AI. The IIV on CL was estimated as 38%. As shown in Figure S3, a total of 61 (8%) subjects with genotype UGT2B15 *1/*1 or UGT2B15 *1/*2 were assigned to the PM category (POP3) by the mixture model.

Figure S3. Clearance (CL) estimates grouped by genotype and assigned population. The number of subjects assigned to a population (nPOP) and actual genotype group is shown below the graph. UGT2B15 = uridine 5'-diphospate-glucuronosyltransferase 2B15.



Assigned Population	*UGT2B15*1/*1* (n=227)	*UGT2B15*1/*2* (n=517)	*UGT2B15*2/*2* (n=279)
nPOP1 (n=119)	78	40	1
nPOP2 (n=600)	141	424	35
nPOP3 (n=304)	8	53	243

The individual probability of each subject of belonging to the EM (POP1), IM (POP2), or PM (POP3) subpopulation was calculated (Figures S4(a), S4(b) and S4(c)). A wide range of individual probability values between 0 and 1 was observed for the EM and IM populations for genotypes UGT2B15*1/*1 and UGT2B15*1/*2, this range of probabilities indicate that assignment to the EM or IM population is associated with uncertainty for these genotypes.

However, the individual probability of belonging to the PM population appears to be associated with less uncertainty, since the majority of probabilities by UGT2B15 genotype are closer to 0 or 1.

Figure S4(a) The individual probablity (IP) of beloning to mixture 1 by genotype and population.

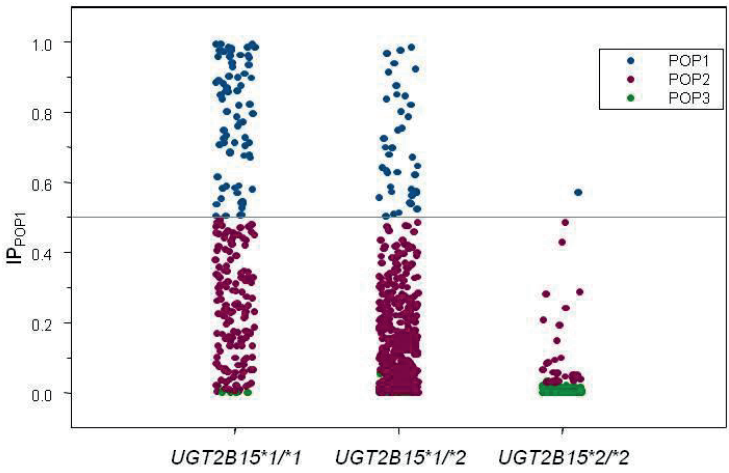


Figure S4(b) The individual probablity (IP) for subjects assigned to mixture 2 by genotype and population.

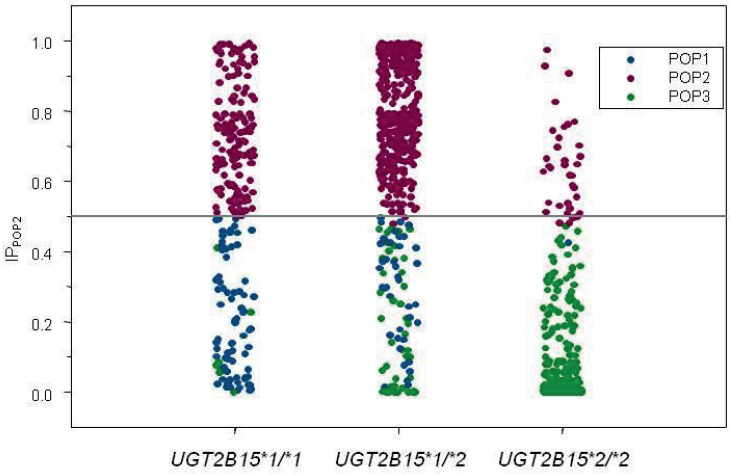
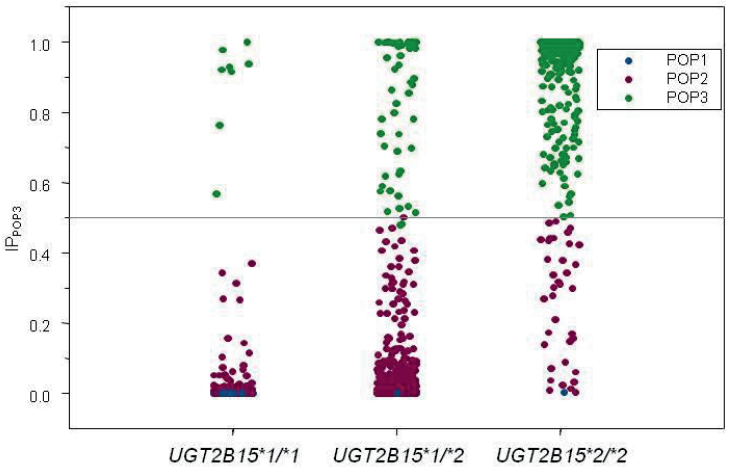


Figure S4(c) The individual probablity (IP) of beloning to mixture 3 by genotype and population.



References:

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Table AI. Summary of parameter estimates for the mixture model including covariates

Parameter name	Parameter	Value (CV%)	IIV (%, CV%)
Clearance population 1[a]	CL POP1 (EM) (L/h)	5.04 (3.85)	38.21 (10.7)
Clearance population 2[a]	CL POP2 (IM) (L/h)	3.35 (2.38)	
Clearance population 3[a]	CL POP3 (PM) (L/h)	1.53 (2.64)	
Central volume of distribution[a]	V (L)	9.06 (2.41)	34.50 (13.4)
Peripheral volume of distribution[a,b]	V2 (L)	0.188 (4.93)	--
Intercompartmental clearance	Q (L/h)	0.311 (6.72)	--
Absorption rate constant	ka (1/h)	2.15 (6.19)	--
Duration of zero order process	D1 (h)	0.637 (3.69)	77.20 (16.9)
FFM on central volume of distribution	(L/kg)	0.00556 (16.5)	
Probability fraction[c]	PROB	0.367 (3.69)	
Probability of belonging to POP 1[c]	POP 1	0.18 (18.6)	
Probability of belonging to POP 2[c]	POP 2	0.522	--
Probability of belonging to POP 3[c]	POP 3	0.30	
Residual variability Phase I (proportional)	σ^2	0.05487 (9.23)	--
Residual variability Phase II (proportional)	σ^2	0.167 (10.2)	72.18 (15.6)

[a] Bioavailability for sipoglitazar is currently unknown, as such clearance and volume were modeled as CL/F and V/F, respectively.

[b] The peripheral volume of distribution was implemented as a fraction of the central compartment

[c] The probability of belonging to the populations 2 and 3 was estimated as:

POP2 = (1-POP1)*PROB

POP3= (1-POP1)*(1-PROB)

CV% = percent coefficient of variation