Chapter 7

Pharmacogenomics in Drug Development: Conclusions and Perspectives

The investigation described in this thesis focused on assessing the role of genotype differences in explaining inter-individual variability in drug metabolism and the impact of these differences on both the clinical response and the selection of the appropriate dosing scheme.

The impact of genotype resulting from differences in the rate of metabolism between individuals has been found to vary widely between drugs [1]. Primarily this appears to result from factors which include the relative role of the polymorphic enzyme(s) to the clearance of the drug and the type of enzyme or transporter that is involved [2,3]. Furthermore the extent of the influence of these changes is also dependent on where the therapeutic dose is in relation to the exposure response relationships for both efficacy and safety [1]. PK-PD model based approaches to evaluate the impact of these differences including clinical response or surrogate biomarkers, has not been routinely implemented. Model based applications can be used to quantify the differences in drug exposure resulting from genetic differences between individuals whilst also incorporating other factors which may contribute to the inter-individual variably [4-6]. Furthermore the development of a PK-PD model can provide a more comprehensive link between differences in drug exposure and the magnitude of its effect(s) on clinical response.

The focus of this thesis was to apply a PK-PD model based approach in Type 2 Diabetes (T2D), to assess both the short and the long term implications of Pharmacogenomics (PGx) in drug development. The aim was to specifically investigate enzymes that were contributing to the inter-individual variability, to quantify the resulting exposure differences between genotypes, to evaluate the predictability of genotype for exposure and to assess the influence of these differences on the clinical response of efficacy and safety.

Clinical relevance of genetic variants in pharmacokinetic properties

Exploratory preliminary evaluation of genotype during Phase I

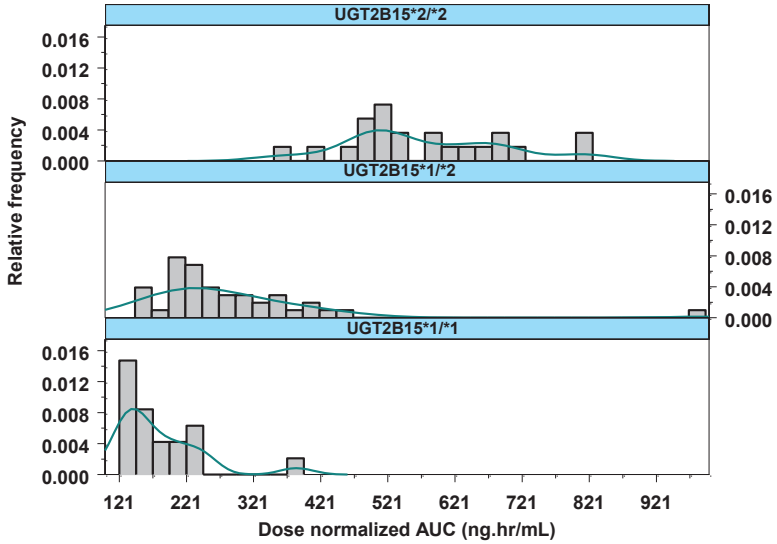
In vitro assessment can be used to determine the involvement of specific isoforms of drug metabolizing enzymes responsible for metabolism of a drug candidate; however since in-vitro

studies are not always quantitatively predictive, confirmation of the relative role of the enzyme in vivo is required [7]. In Chapter 3 the approach to quantify the contribution of the enzymes responsible for the metabolism of the drug sipoglitazar is described. In vitro studies conducted prior to human dosing had predicted a central role for glucuronidation by uridine 5'-diphospate-glucuronosyltransferases (UGTs) in the in vivo biological transformation of sipoglitazar [8,9]. The results of these metabolism studies indicated that multiple UGT isoforms were potentially involved in the metabolism of the drug [8]. Since pharmacogenetic variation has been identified for UGTs [10], the aim of this analysis was to identify which UGTs were potentially correlated with sipoglitazar exposure and then to evaluate the extent of variability explained in part due to genotype.

The results of three preliminary phase I studies of sipoglitazar in healthy volunteers were combined for analysis of the data. There was a total of 82 subjects enrolled for whom both PK and UGT genotype information was available (Chapter 3, Table 1). The dose range included in the studies was 0.2-64mg for sipoglitazar and statistical analysis of area under the plasma concentration–time curve from time 0 to infinity (AUC) revealed dose proportionality across the dose range, with a slope and 95 % confidence interval of 0.99 and 0.92–1.05, respectively (Chapter 3).

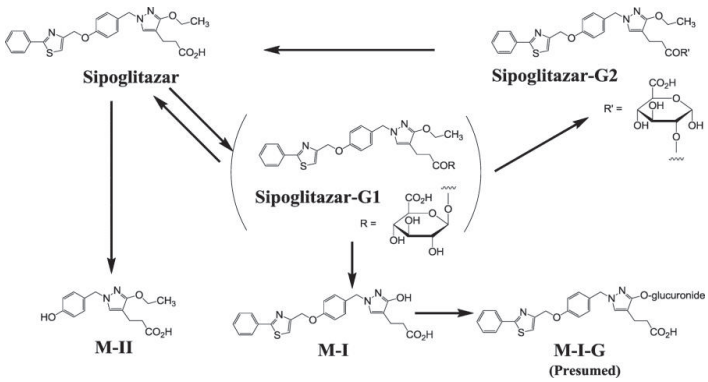
As a first step in the investigation the contribution of each genotype was assessed using Analysis of variance (ANOVA) models on dose normalized AUC. Results of this investigation revealed that variation in UGT2B15 accounted for approximately two-thirds of the variability in sipoglitazar plasma exposure, while no relationship between sipoglitazar plasma exposure and variants of the other UGT enzymes could be identified. This relationship between UGT2B15 genotype and sipoglitazar dose normalized AUC is shown in Figure 1. Considerable overlap was observed between genotype groups, particularly between the UGT2B15*1/*1 and UGT2B15*1/*2 genotypes (Figure 1).

Figure 1. Dose normalized (1mg) histogram for sipoglitazar AUC by UGT2B15 genotype in healthy volunteers (n=82).



The principle metabolite of sipoglitazar is the dealkylated derivative M-I. The metabolite is formed in vitro predominantly by the action of cytochrome P450 (CYP) 2C8 on glucuronide intermediates [8]. Based on in-vitro results the metabolic pathway from sipoglitazar to M-I is one in which sipoglitazar is initially metabolized to sipoglitazar-G1 by UDP-glucuronosyltransferase and then sipoglitazar-G1 is metabolized to M-I by O-dealkylation by CYP2C8 and deconjugation [9]. The proposed metabolic pathway is shown in Figure 2.

Figure 2. Postulated metabolic pathways of sipoglitazar. M-I-G, glucuronide of M-I (Reproduced with permission from ref. [**9**]).

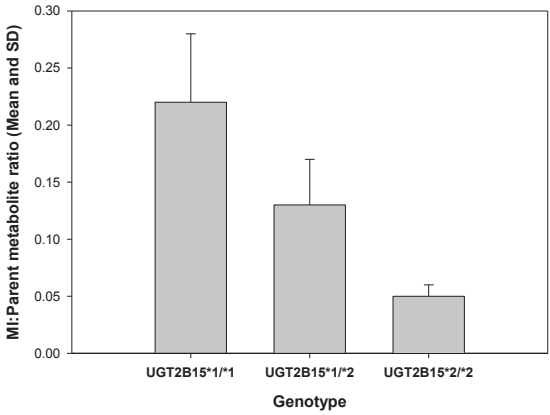


The M-I metabolite also undergoes subsequent conjugation to M-I-G and since a high concentration of M-I-G was present in the urine in monkey studies, it is presumed that the glucuronidation of M-I would also occur in humans [11]. Due to its unique metabolic formation, the metabolite M-I was considered to be a potential marker for the level of metabolic activity of UGT.

Furthermore since sipoglitazar-G1 is deethylated by CYP-2C8 to form M-I, CYP2C8 genotype samples were also collected in one phase I study (n=24) to exclude any influence of CYP-2C8 variants on exposure to sipoglitazar. Following graphical analysis, no relationship was evident between sipoglitazar exposure and CYP2C8 genotypes *1/*1, *1/*3, or *3/*3 (Chapter 3).

Parent to metabolite ratios for AUC were calculated to evaluate if there was a change in the metabolic activity relative to the UGT2B15 genotype. As shown in Figure 3, a reduction could be observed in the metabolite ratio across UGT2B15 genotypes, with the lowest value observed for UGT2B15*2/*2. Consistent with the observed increase in exposure for the UGT2B15*2/*2 genotype, these reductions in metabolite ratios indicate that reduced metabolic activity is associated with UGT2B15*2/*2, supporting the in vitro findings.

Figure 3. Parent to metabolite ratio for area under the plasma concentration–time curve from time 0 to infinity for sipoglitazar by UGT2B15 genotype in healthy volunteers



During the analysis of the early phase I studies two subjects, who were genotyped as UGT2B15*1/*1 and UGT2B15*1/*2 had considerably higher exposure than expected based on their genotype (approximately 2.5-4 fold higher than the mean AUC for the genotype). The metabolic ratios for these two subjects were then compared to the average ratio for the genotype. One of the subjects identified as an outlier had a metabolite ratio consistent with their genotype, indicating that other variables contribute to the disconnect between genotype and exposure. Since rates of glucuronidation are also affected by other factors such as age, diet or disease [10], further extension of this preliminary evaluation was performed through the development of a population PK model to characterize the pharmacokinetic profile and explore other potential sources of variability between individuals.

Initial population PK model development

A population PK model was developed based on the early phase I studies that are described in Chapter 3. The aim was to quantitatively evaluate the differences in clearance (CL) between UGT2B15 genotype and to evaluate other potential covariates that may contribute to explaining the inter subject variability for sipoglitazar.

The data were described using a 2-compartment model with a combined zero and first order uptake process. UGT2B15 genotype was included as a categorical covariate on CL. Figure 4 shows the visual predictive check (VPC) for the observed and predicted data and key parameter estimates are shown in Table 1.

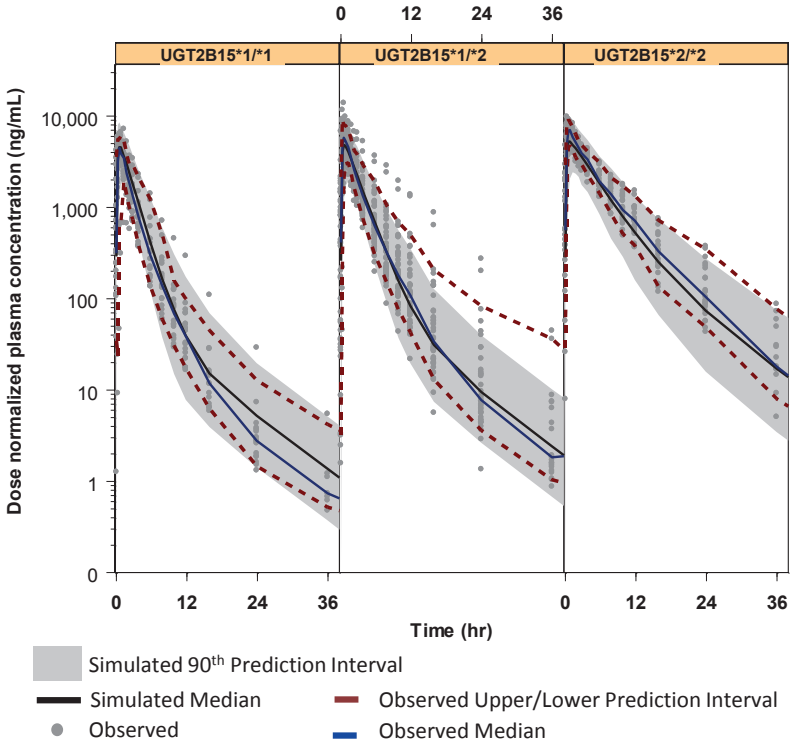
All parameters could be estimated with good precision and the VPC shows that the median trend and variability can be well described in all three genotype groups. ETA shrinkage for clearance and V2 was estimated at 1.6 and 15%, respectively.

Table 1. Key pharmacokinetic parameter estimates from small phase I study (n=82) in healthy volunteers

Parameter	**Parameter (CV%)**	**IIV (%, CV%)**
Clearance population, UGT2B15*1/*1 (L/hr)	4.9 (9.8)	30.2 (26.4)
Clearance population, UGT2B15*1/*2 (L/hr)	3.98 (4.2)	
Clearance population, UGT2B15*2/*2 (L/hr)	2.2 (5.1)	
Volume of central distribution (V2) (L)	10.5 (3.6)	15.7 (24.7)
Peripheral volume of distribution (L)	1.2 (5.4)	
Residual error (proportional)	0.08 (17.8)	

Based on this preliminary evaluation there was approximately a 2.3 fold decrease in CL between the UGT2B15*1/*1 and UGT2B15*2/*2 genotype groups. Before accounting for UGT2B15 as a covariate on CL, inter individual variability (IIV) was estimated at 49%. After accounting for UGT2B15 genotype as a covariate, IIV on CL was reduced from 49 to 30%.

Figure 4. Visual predictive check for dose normalized (64mg) plasma concentration data in 82 subjects from phase I data in healthy volunteers by genotype group. UGT2B15*1/*1 (n=19), UGT2B15*1/*2 (n=41), UGT2B15*2/*2 (n=22)



Potential effects of the demographic covariates age, weight and gender were evaluated in the model using a forward inclusion procedure [12]. Based on the data in this healthy volunteer population none of the tested covariates at this stage were found to be significant. However as the distribution volume for body weight would be expected to be higher in diabetes patients and 96% of subjects enrolled in this study were Caucasian, a further covariate analysis was undertaken during the Phase II population PK analysis described in Chapter 4.

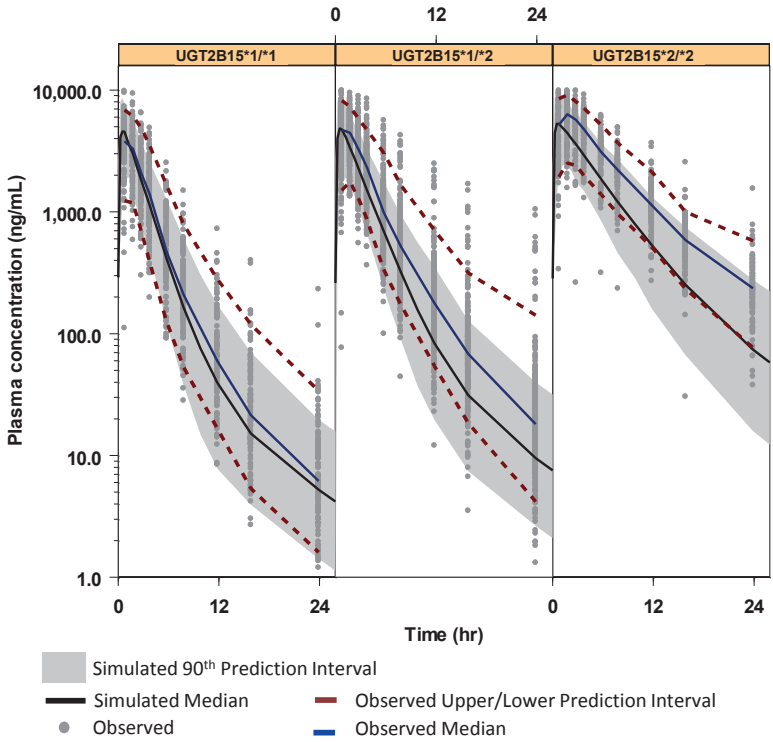
Pharmacokinetic studies in healthy volunteers

During the early development phase, an important consideration is the characterization of the relationship between genotype and drug exposure. Pharmacogenomic guidelines from both the EMA and FDA highlight that conventional pharmacokinetic approaches (frequent blood sample collections), should be performed to evaluate the role of genotype on the disposition and recommend that these studies follow a similar approach to the evaluation in organ impairment where subjects are matched between groups for intrinsic factors such as age or body weight which may influence the PK of the drug [7, 13]. Evaluating genotype during phase I should be used in an exploratory context and for generating hypotheses that can be tested during the later development phase [13].

To this end in addition to the preliminary phase I studies described in Chapter 3, an additional large phase I study for sipoglitazar was conducted in healthy volunteers (study overview presented in Chapter 4). The aim of this study was to the further investigate the correlation between UGT2B15 genotype and sipoglitazar metabolic phenotype in the context of all other potential sources of variation, in a diverse study population of approximately 500 healthy male and female subjects. As such five hundred and twenty-four subjects (mean age of 29.8 years), including 220 male, 304 female, 108 Black or African American, and 104 Hispanic subjects were enrolled into the study.

It was evaluated if the PK model that had been developed only on the preliminary phase I studies could then predict the mean and variability in such a large, diverse population. This was performed using an external VPC, where the median and variability simulated from the small population PK model are overlaid with the individual, median and observed variability from this large phase I trial. The results of this external VPC are shown in Figure 5.

Figure 5. External visual predictive check (observed data from large phase I study in healthy volunteers n=524) and simulated median and prediction interval using the model developed on preliminary phase I data in healthy volunteers (n=82).



Although the median and the extent of the variability can be well described for the UGT2B15*1/*1 genotype, there appears to be a modest under prediction of the extent of absorption and of the elimination phase for the typical subject in the UGT2B15*2/*2 group. There may be a number of explanations for this difference based on differences in the population characteristics of the subjects enrolled. To evaluate these covariate differences further, a visual inspection of the demographic data and the CL from the small phase I study

and CL from the model developed on the large phase I dataset (Chapter 4) was performed. The results of this are shown in Figures 6 and 7.

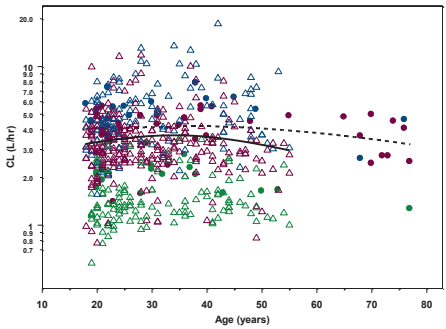


Figure 6. Plots for age vs CL. Individual (triangle – large phase I, circles – small phase I) and smoothing spline (solid line – large phase I, dashed line – small phase I). Color by genotype UGT2B15*1/*1 (blue), UGT2B15*1/*2 (red), UGT2B15*2/*2 (green)

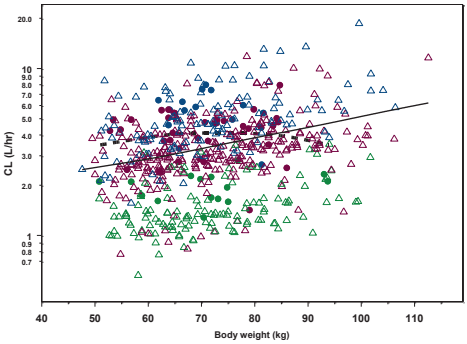


Figure 7. Plots for body weight vs CL. Individual (triangle – large phase I, circles – small phase I) and smoothing spline (solid line – large phase I, dashed line – small phase I). Color by genotype UGT2B15*1/*1 (blue), UGT2B15*1/*2 (red), UGT2B15*2/*2 (green)

Based on this graphical analysis, the relationship between age and CL appears to be comparable between the two datasets but some differences appear to be present in the relationship between body weight and CL. This is likely resulting from the larger body weight range in the large phase I trial that creates a higher sensitivity for the existence of an inter-relationship. One of the major differences in the large phase I trial is the enrollment of a diverse ethnic population. A summary of the data for CL by race is shown in Figure 8 for the large phase I study. Of the 122 subjects that were enrolled in the UGT2B15*2/*2 genotype group, 70% were non-hispanic white, 18% were Hispanic and 12% were Black or African American. In the UGT2B15*2/*2 genotype in the small phase I trial only Caucasian subjects were enrolled in this genotype group. Furthermore in the UGT2B15*2/*2 genotype group in

the small phase I studies, 73% of subjects were male as compared to only 39% in the large phase I trial (Figure 9).

Figure 8. Box plot for CL by ethnicity and UGT2B15 genotype for large phase 1 study in healthy volunteers. 1=American Indian or Alaskan native, 2=Asian, 3=Black or African American, 4=Native Hawaiian or Other Pacific Islander 5= non-hispanic white, 6= Multiracial

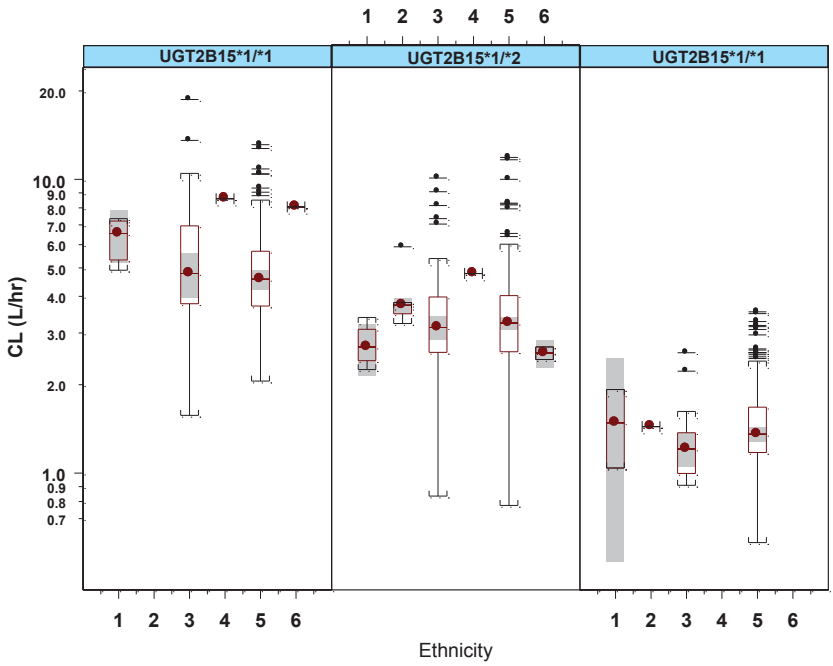
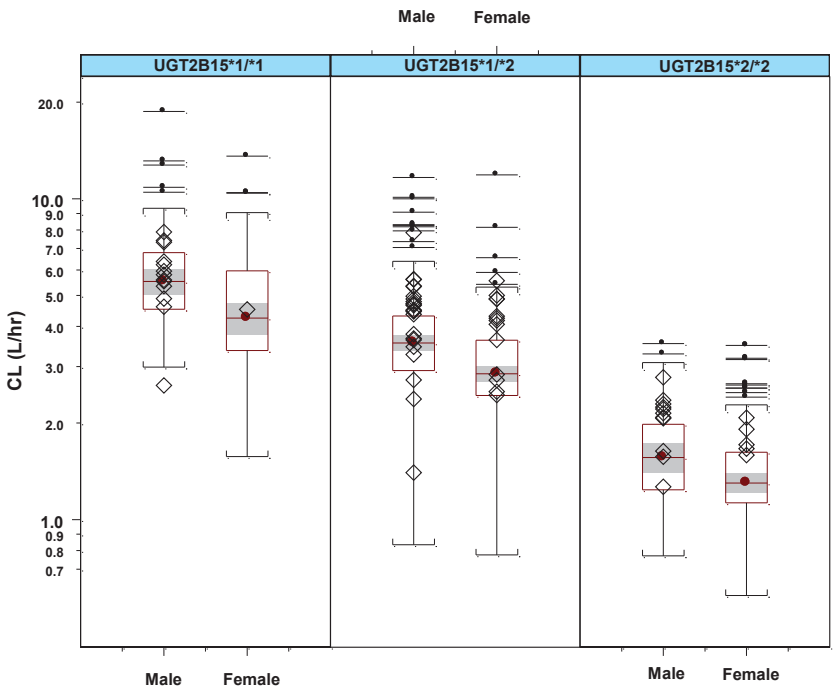


Figure 9. Box plot for CL and gender for large phase I study in healthy volunteers. Scatter plot of CL (triangles) for small phase I in healthy volunteers by gender.



The differences in CL by genotype between the studies are summarized in Table 2. Approximately a 1.4-fold difference is observed between the CL estimates for the UGT2B15*2/*2 genotype between the studies.

Table 2. Model estimated CL values by genotype for small (n=82) and large phase I (n=524) models in healthy volunteers

Genotype	Small phase I model estimated population CL (CV%)	Large phase I model estimated population CL (CV%)
UGT2B15*1/*1 (L/hr)	4.9 (9.8)	4.82 (2.5)
UGT2B15*2/*1 (L/hr)	3.98 (4.2)	3.29 (9.2)
UGT2B15*2/*2 (L/hr)	2.2 (5.1)	1.55 (2.0)

A combination of the differences in body weight, gender or ethnicity may contribute to explaining the difference that is observed between studies in the UGT2B15*2/*2 genotype group. Some limitations may have been observed in the predictability of these small phase I studies to a more diverse population, but the value of this early preliminary work is shown as the general trends in the genotype-exposure relationship can already be identified and this information can then be used to inform the design of future trials to appropriately characterize these relationships in the target population.

Evaluating the clinical relevance of genotype differences in exposure

As a result of the preliminary evaluation described in Chapter 3, genotype analysis was carried out for the UGT2B15 polymorphism in all subjects enrolled in the subsequent phase II trials (n=627). The aim of the work described in Chapters 4 and 5 was to develop a population PK-PD model to describe the relationship between changes in exposure and clinical response and to evaluate the necessity of genotype-based dosing in relation to current dosing practice in T2D.

Development of a population PK model for sipoglitazar in T2D patients

Phase II clinical studies provide the opportunity to assess the exposure of a drug in the target patient population and to evaluate the effect of genotype relative to other intrinsic or extrinsic factors. Diabetes may have the potential to alter the PK of a drug due to its effects on protein levels, lipids and carbohydrate metabolism [14]. These factors may result in changes in absorption due to decreased gastric emptying, distribution changes related to non-enzymatic glycation of albumin and biotransformation or excretion changes due to regulation of enzymes or nephropathy [14].

In Chapter 4 a population PK analysis was conducted with the aim to quantify the differences in exposure in the target population between UGT2B15 genotype, to evaluate other potential sources of variability and to derive exposure values by dose for comparison to the safety margin. The model estimated median clearance values for UGT2B15*2/*2 genotype were found to be approximately 2-fold and 3-fold higher than those subjects with the UGT2B15*2/*1 or UGT2B15*1/*1 genotypes, respectively.

Before accounting for any covariates (including genotype), IIV on clearance was 60%; however, after including genotype as a covariate, the IIV of clearance was reduced to 40%. Only one other covariate (Free fat mass) was found to be significant during the covariate analysis and accounted for an additional 2% of the IIV. This analysis confirmed the earlier findings of the relationship of UGT2B15 genotype to sipoglitazar exposure in the target population. Although, during the analysis of the small phase I studies a somewhat lower (2.3-fold) difference in CL was observed between the UGT2B15*1/*1 and UGT2B15*2/*2 genotypes.

Post-hoc CL values were then used to determine individual exposure over the dose interval at steady state (AUC24). These exposure values were then compared to the safety margin for the therapeutic dose and were used as the input into the PK-PD model to evaluate the exposure response relationship.

Predictability of the genotype-phenotype relationship

Once a relationship has been established between genotype and exposure, a key question is the determination of not only the magnitude of the variability between genotypes but also how predictable the genotype-phenotype relationship is. This becomes important if dosing based on genotype were to be considered. If subjects have a higher exposure than predicted based on their genotype, a genotype-based dosing approach may unintentionally result in several fold higher exposure than expected and could exceed safety margins depending on the therapeutic window of the drug.

An approach to evaluate the predictability of the genotype-phenotype relationship is described in Chapter 4. The predictive strength of genotype for apparent drug clearance was

investigated by analyzing the data without *a priori* consideration of UGT2B15 genotype in the model. Without this knowledge, the individual value of CL was assigned over one of three distributions using a probability model (NONMEM $MIX) to assign subjects to one of three subpopulations having either a low, intermediate or high CL, based on the joint model-optimization of probability and population parameters [15]. These three populations (POP1, POP2, and POP3) were generated for post-hoc evaluation against the actual UGT2B15 genotype *1/*1, *1/*2 and *2/*2 and the difference in subject assignment between categories was then compared (appendix Chapter 4).

From the results of the comparison between actual assignment of genotype and assignment to a population based on the model parameters, in total, 27% (278/1023) of all subjects had been assigned to a different population category than expected based on their genotype. The highest number of subjects misclassified was for the UGT2B15*1/*1 genotype. This is likely due to the large overlap in CL distribution between UGT2B15*1/*1 and UGT2B15*1/*2 subjects; 62% of the UGT2B15*1/*1 subjects had been assigned to the POP2 (intermediate CL) category. However the consequence of this depends on the specific type of genotype-based dosing approach that would be applied clinically. For example, the biggest impact of a misspecification of CL class based on genotype would occur if a subject who was genotyped as an extensive metabolizer actually appeared to have a clearance within the range associated with that in the poor metabolism group. That subject would then receive a dose that could result in the exposure for that subject being several fold greater than expected. For a drug with a wide therapeutic index this may not be of clinical relevance but for a drug of which the top dose is close to the exposure margin, the risks of overdosing subjects should be considered on balance to the risk/benefit profile.

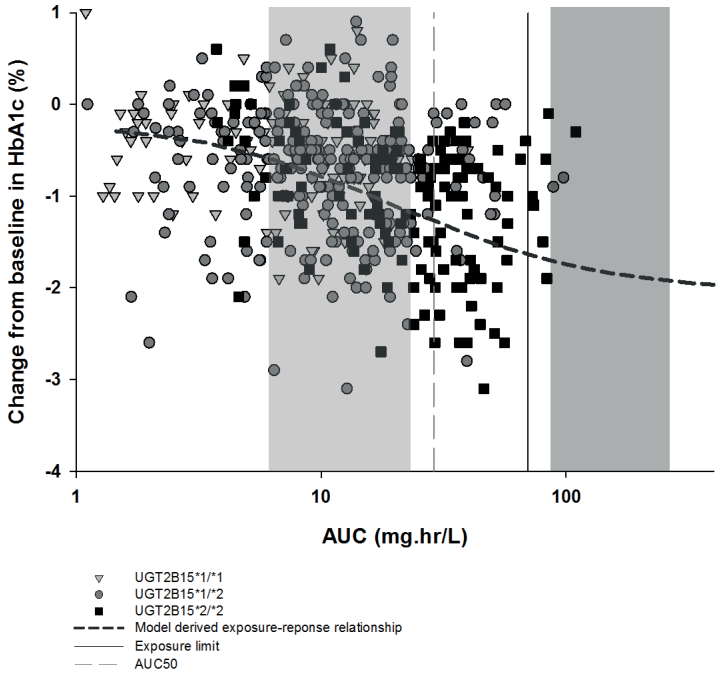
Evaluating the influence of genotype on clinical response

A quantitative and descriptive analysis of the influence of genotype on the pharmacokinetic properties of sipoglitazar was described in Chapters 3 and 4. The question for the clinical development program now focuses on evaluation of the relationship between changes in the exposure due to genotype and its magnitude of effect on the clinical response. In Chapter 5, it was addressed if the relationship between changes in the PK due to genotype would result in

clinically relevant change in response using fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) as surrogate biomarkers for clinical response.

The approach was taken to develop a population PK-PD model to describe the changes in FPG and HbA1c as a function of individual exposure, whilst PD response data from rosiglitazone at a therapeutic dose of 8mg QD were incorporated into the analysis as reference data. The model could describe the individual and median profiles for all dose levels (8-64 mg total daily dose of sipoglitazar) and no differences in the shape of the exposure response relationship were found between genotypes. The model derived median exposure response relationship for the typical patient between AUC and change from baseline in HbA1c is shown in Figure 10 in relation to the actual observed data from the Phase II trials. As outlined in Chapter 2, the therapeutic dose should be considered relative to the exposure response relationship and evaluated in context to the safety margin. For sipoglitazar, AUC at steady state achieving half the maximal response (AUC50) and the established exposure limit are shown in Figure 10. The median exposure range between UGT2B15*1/*1 and UGT2B15*2/*2 genotypes for a dose of 32mg are shown on Figure 10. At this dose level the response in HbA1c is different by genotype as the exposure range between genotypes sits in the middle of the dose response curve (Table 3). If the dose was closer to the Emax for glycemic control, i.e. exposure for all genotypes was above the exposure limit of 73 mg.hr/L (corresponding to a dose of approximately 400mg for all subjects), changes in exposure caused by genotype would have less of an impact on the predicted/expected change in HbA1c (Figure 10, Table 3). However if higher exposure levels were to be achieved for all subjects, the exposure would then exceed the safety margin for a substantial fraction of the population. The current exposure limit is based on mean data from non-clinical studies; however this margin also includes a degree of uncertainty on clinical relevance and as well as on variability within the patient population. Significantly exceeding this would require additional insight in clinical safety and tolerability.

Figure 10. Observed change from baseline in HbA1c (%), observed AUC for all sipoglitazar dose levels in the Phase II trials (3 months) and simulated median exposure response relationship between HbA1c and AUC. (median exposure range between UGT2B15*1/*1 and UGT2B15*2/*2 genotypes for a dose of 32 mg, median exposure range between UGT2B15*1/*1 and UGT2B15*2/*2 genotypes for a dose of 400 mg).



The developed PK-PD model was used to simulate the expected FPG and HbA1c change from baseline at 6 months (duration of a Phase III trial). The simulation showed that for

sipoglitazar, a dose of 32 mg in the UGT2B15*2/*2 genotype would be expected to provide an equivalent result to the reference treatment rosiglitazone (Table 3). The results of the simulation also show that for a dose of 32 mg, a less than proportional change in HbA1c was observed relative to the changes in drug exposure across genotypes. In the phase II population, approximately a 3.3-fold difference in CL is observed between UGT2B15*1/*1 and UGT2B15*2/*2 genotypes, however this results in only a 1.8-fold difference in HbA1c drop relative to the baseline. Although a dose of 32 mg in the UGT2B15*2/*2 subjects can achieve reductions in HbA1c equivalent to rosiglitazone, a clinically significant difference (0.5% change from baseline in HbA1c [16]) is observed between the UGT2B15*2/*2 and UGT2B15*1/*1 genotypes as a result of the differences in drug exposure. It was therefore postulated that genotyped based dosing could contribute to the normalization of response across individuals by achieving comparable exposure levels across genotype groups.

Table 3. Simulated median change from baseline in HbA1c at 6 months by genotype for sipoglitazar at a dose of 32 and 400 mg and difference in CL between UGT2B15 genotypes (T2D subjects)

Genotype/Treatment	CL (L/hr)	Change from baseline HbA1c at 6 months for 32 mg (%) (Exposure range 6-21 mg.hr/L)	Change from baseline HbA1c at 6 months for 400 mg (%) (Exposure range 79-261 mg.hr/L)
UGT2B15*1/*1	5.04	-0.6	-1.7
UGT2B15*1/*2	3.35	-0.8	-1.8
UGT2B15*2/*2	1.53	-1.1	-1.9
Rosiglitazone 8mg		-1.2	

Evaluating genotyped-based dosing approaches

When genetically determined differences in exposure have been observed, there are specific approaches recommended by the regulatory authorities to determine the appropriate dosing adjustment [7]. These include dose titration, optional gene base dosing or dosing based on genotype. The PK-PD model developed in Chapter 5 was then used to simulate these various scenarios and evaluate the most efficient dosing strategy to achieve optimal therapeutic response for all genetic subgroups for sipoglitazar.

Simulations were performed evaluating three different approaches, (1) a single dose level for all subjects, (2) genotype-based dose adjustment (where genotype is used to estimate the starting dose) or (3), titration based on therapeutic response. Based on the simulation at 6 months, using a single dose level for all subjects, a dose of 96mg would be expected to provide a comparable result to the rosiglitazone treatment arm in all genotype groups. However a dose of 96mg would be expected to exceed the currently defined safety margin, particularly for subjects in the UGT2B15*2/*2 genotype and would be a dose higher than had previously been administered during either Phase I or Phase II. If such an approach was to be taken, additional safety evaluation and/or TDM of plasma levels in an early stage of the study would be needed at these higher exposures. The use of TDM may be an alternative approach to prevent over exposure of subjects [17,18]. This could be of particular value if there was a disconnect between the genotype-exposure relationship. TDM has been routinely used as tool to individualize drug dosage in many therapeutic areas and further discussion of this approach is out of scope of this thesis [18,19,20].

As shown from the simulation of a genotype based dosing approach in Chapter 5, a result equivalent to the rosiglitazone reference dose could (also) be achieved for all genotypes by administering lower doses to the UGT2B15*2/*2 and UGT2B15*1/*2 genotype groups. The optimal genotype-based approach would have the following fixed dosing scheme: UGT2B15*1/*1=96 mg, UGT2B15*1/*2=64 mg, and UGT2B15*2/*2=32 mg. The design of the Phase III study would then include pre-selection of dose based on genotype for all subjects enrolled in the trial. Such an approach would also require the development of an assay for UGT2B15 genotype for the relevant genetic testing to be performed in the clinic if genotyped-based dosing was then included in the label [21].

Although a genotype-based dosing approach could be used to normalize response between the genetic subgroups, in T2D a titration approach based on efficacy/safety is routinely applied. A comparison was therefore simulated between genotyped-based dosing and titration based approaches, with all subjects in the titration group starting at 32mg. Subjects in the UGT2B15*2/*2 group would not need to undergo dose titration as 32mg appears to be the optimal dose for this genotype group. The results of this simulation highlight two key points.

The magnitude of reduction in FPG or HbA1c between the genotype and titration approaches would be expected to be the same but the time taken to eventually achieve that maximum response would be shorter when pre-selection of dose was based on genotype. The differences between genotyped and titration approaches in the time to maximum effect was estimated at 2 and 3 months for the UGT2B15*1/*2 and UGT2B15*1/*1 genotypes respectively (Chapter 5, Figure 4a and 4b). Since there is a causal link established between hyperglycemia and diabetic complications, earlier reduction in glycemic markers through the use of genotyped-based dosing may offer additional clinical benefit in specific cases or patient populations [22].

The frequency of the UGT2B15*2/*2 genotype is approximately 22% in the Caucasian population, but in Japanese American subjects, in a sample size of 77, there were no subjects reported as UGT2B15*2/*2 genotype [10]. This is an import consideration for comparing genotype-based dosing and titration approaches since the benefit of genotype-based dosing would affect a lower number of subjects if there was a higher proportion of UGT2B15*2/*2 genotype subjects as they would already start treatment at the most efficacious dose without the need for genotyping. If the frequency of the UGT2B15*1/*1 and UGT2B15*1/*2 genotypes was higher, genotyped-based dosing may be advantageous as these subjects would start at the correct dose and would not require additional titration steps. Therefore, the frequency of the genotype in different ethnic populations should also be considered in evaluating the most appropriate dosing scheme.

Genotype influences on model based approaches in disease progression analysis

Analysis conducted using Genome Wide Association Studies (GWAS) in T2D have identified significant associations for more than 35 independent loci [23]. These studies are conducted not only to identify new disease genes but also to evaluate the mechanisms behind the disease, with initial studies identifying loci that impact directly on beta cell function [24]. In type 1 diabetes (T1D) the concept that candidate genes may affect disease progression by modulating survival and function of the β-cells has already been evaluated for the gene cathepsin H [27]. Results in children with T1D showed that carriers of the T allele required a significantly higher insulin dose to maintain glycemic control and carriers of this genotype

had faster disease progression, leading to a more prominent β-cell dysfunction [27]. The application of disease progression models incorporating drug and genetic information may offer further insight into these interesting findings on disease differences by genotype.

The aim of the work described in Chapter 6 was to evaluate the drug and disease effects on FPG and HbA1c over a long term period in treatment experienced Japanese T2D subjects receiving the current standard of care. Using the model developed on this long term data (>2years) it can be hypothesized how PGx in T2D may influence drug response through both symptomatic and disease modifying effects.

In Chapter 5 it was demonstrated that pre-selection of an optimal dose based on genotype would result in a decrease in the time to reach maximum effect as compared to using titration based on efficacy. However T2D is a slowly progressing disease and the symptomatic benefits of this early optimization of dose should also be evaluated considering the influence of disease progression [25]. It could be postulated that through genotyping a subject a more efficacious starting dose could be selected that would reduce the time taken for titration. A simulation was therefore performed to evaluate how reducing the time taken to reach the maximal dose during titration would influence the FPG profile over a period of 5 years.

Simulation for a range of ET50 values was performed (0-150 days); where ET50 represents the time taken to achieve half the maximal dosage for a subject undergoing titration. Results in Figure 11 show that decreasing titration time has several consequences on the long term. As the time to maximal effect is reduced greater symptomatic benefit of FPG reduction can be obtained, however as there is no change in the underlying disease rate symptomatic benefits observed early in the treatment period have almost disappeared after 5 years. This is consistent with the profile for a disease independent symptomatic effect [26].

In Figure 12, the effects of differences in the disease progression rate for the FPG profile are shown. This may be as a result of a treatment that directly targets a novel disease pathway identified from GWAS or that a subject's disease progression rate, as observed in T1D, can be different depending on the genotype. Interestingly, this simulation shows that changes in disease progression rate would only appear to have a substantial influence on FPG levels in

this treatment experienced patient population approximately 1.5 years from the start of treatment.

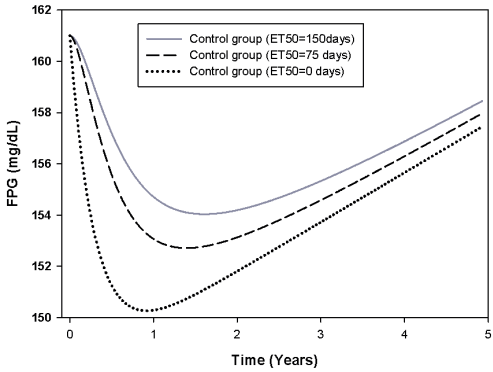


Figure 11. Simulation of median FPG change over time for a range of ET50 values (all simulations performed using a DPRC value in the model of 0.013 year^{-1}).

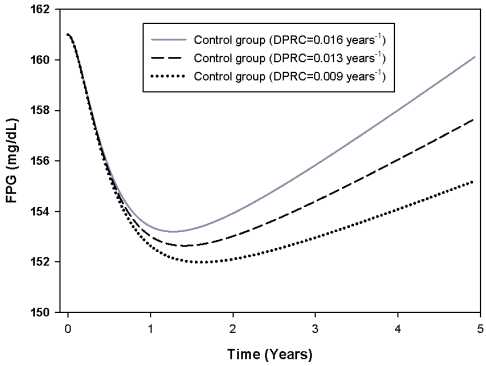


Figure 12. Simulation of median FPG change over time for a range of disease progression rate (DPRC) values (all simulations performed using an ET50 value in the model of 75 days).

As shown in Figures 11 and 12, the advantages of symptomatic and disease modifying benefits occur on different timescales. Early symptomatic improvements would generate a short-term improvement that decreases over time. In contrast, the disease modifying effects on FPG propagate over time. An optimized treatment approach in T2D would therefore not only have symptomatic improvement but could also interact with the disease progression rate. It may therefore be that the most optimal PGx driven treatment approaches come from a range of different studies involving genes that target different pathways.

Perspectives on approaches to evaluate the impact of genotype in clinical development

During the non-clinical stage if a polymorphic gene is identified to play a central role in the metabolism of the drug, consideration should be given to this during the design of the first in human trial (FIH) [28]. A key component of this is the prediction of the influence of genotype

differences from non-clinical data. As proposed by Zhou et al, a predict, learn and confirm approach towards clinical development should be implemented [29]. Physiologically-based pharmacokinetic (PBPK) models are built mainly from drug-independent "system" information and incorporate both intrinsic and extrinsic factors [30]. These models can be used to assess the influence of genotype on human drug exposure before the conduct of the FIH trial by utilizing the non-clinical animal and in vitro data. This is an important consideration for making predictions on genotype effects on exposure as PBPK models can incorporate metabolic intrinsic clearance values for multiple CYP enzymes and information on the frequency and activity of different allelic forms [31,32]. The influence of genotype can then be assessed relative to the contribution of enzymes and transporters on intestinal and hepatic availability, with the aim to fully understand the impact of these variables on the bioavailability in vivo [33].

If the frequency of the genotype for the enzyme is already known, subjects can be enrolled into the phase I trials to evaluate the differences in exposure between these genetic subgroups using a stratified approach. These early phase I studies can then be used to further validate or challenge the PBPK model assumptions. Such an approach would also enable simulations to be performed to evaluate potential differences by genotype in drug-interaction and organ impairment studies [33]. Lower doses in subjects with organ impairment maybe required for subjects who are poor metabolizers of a drug and the application of PBPK simulations could be used to assess the exposure changes by genotype relative to the changes in hepatic function or protein binding. This information can be used to appropriately plan and prioritize studies in special populations in the clinical development program and inform patient inclusion/exclusion criteria in phase II.

Population PK analysis of clinical data, including maximum likelihood or Bayesian methodology can be used in combination with bottom-up PBPK approaches [31]. PBPK models can be combined with population PK approaches to evaluate PK sample collection and optimal design for the phase II or III trials [31,34,35]. Virtual populations can be simulated using the PBPK models and these simulations can be evaluated by population PK methods. This combined approach has already been demonstrated to assess co-medication as

a covariate, and further extension of this approach would include genotype as an additional covariate in the analysis [34,35]. It would also be of value to use PBPK models for trial simulation if only limited subjects from a particular genotype subgroup have been enrolled in the phase I studies or if the phase II studies are expanded into subjects of a different ethnic background as such information can be incorporated into the simulation.

Following the phase II studies, a population PK approach would be applied to evaluate the influence of genotype as a covariate on exposure data relative to the other intrinsic and extrinsic factors which may also contribute to the variability both within and between subjects in the target patient population. The data from this population PK approach can then be compared to the earlier derived PBPK model forecast to validate the model assumptions in special populations.

Understanding the dose-exposure-response relationship is a key component in evaluating how genotype differences in exposure may result in a different clinical response. This should be evaluated relative to any appropriate safety margins, whilst the magnitude of influence of genotype should be considered relative to the other covariates identified. The development of a PK-PD or PBPK-PD model incorporating safety and/or efficacy can be used to understand the shape of this dose response relationship. One of the advantages of implementing PBPK models is the ability to predict the impact of specific mechanistic processes and determinants on the tissue dose [36]. Further extension of these models by linking PBPK to PD response can be considered in the simulation, and the relationships between drug exposure and efficacy or toxicity can be evaluated [37]. An additional advantage of linking PBPK models to PD response is that the local concentration at the effect site can be determined and used as the input for the PD response, rather than plasma concentration. This is particularly important when transporters are involved in drug disposition at the effect site, as there may be disconnect between the plasma concentration and the concentration at the site of action [38]. The development of models linking exposure with clinical response would then be used for clinical trial simulation evaluating different dosing scenarios such as genotype-based dosing or TDM approaches to appropriately design further studies. As clinical trials expand into other regions, the frequency of the genotype should then be considered relative to ethnicity

and regional lifestyle differences. The necessity of genotype-based dosing approaches may also depend on the frequency of the genetic subgroups enrolled.

Less progress has been made in understanding the role of PGx differences directly influencing PD response. In oncology there are several examples where drugs are administered only in certain genetic subpopulations, for example genetic testing for K-Ras mutation and EGFR-expression are required prior to initiating treatment for cetuximab and panitumumab [39]. As shown for warfarin, a genotype-based dosing approach is not only limited to genetic differences which influence the PK, but also including genetic differences that directly affect the PD response. Further expansion of the current model based approaches for warfarin would link PBPK models with PD response and incorporate the differences due to VKORC1 genotypes [40]. When evaluating the variability between individuals in PD response consideration should not be limited to the multiple CYP enzymes or transporters that are involved in the metabolism or uptake of the drug, but also to the possibility that genetic subgroups in the PD may also contribute to the variability observed in the response.

Conclusions

The applications of PGx across the clinical development paradigm are starting to change the approach to evaluating clinical response between individuals. As PGx sample collection becomes routine in clinical studies, the possibility to integrate this into our understanding of drug effects should only increase. Model based approaches integrating physiological based parameters or linking exposure with response are powerful tools to quantify and evaluate the impact of genetic differences resulting from either change in drug exposure or directly related to clinical response. Evaluating this impact early in the development phase is important to appropriately design future clinical studies and to ensure that the exposure response relationship can be appropriately determined for all genetic subgroups. Such a comprehensive approach should only improve study design and patient outcomes and ultimately help to reduce drug attrition across the pharmaceutical industry.

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