Pharmacogenomics in Drug Development:

Implementation and Application of PKPD Model Based Approaches

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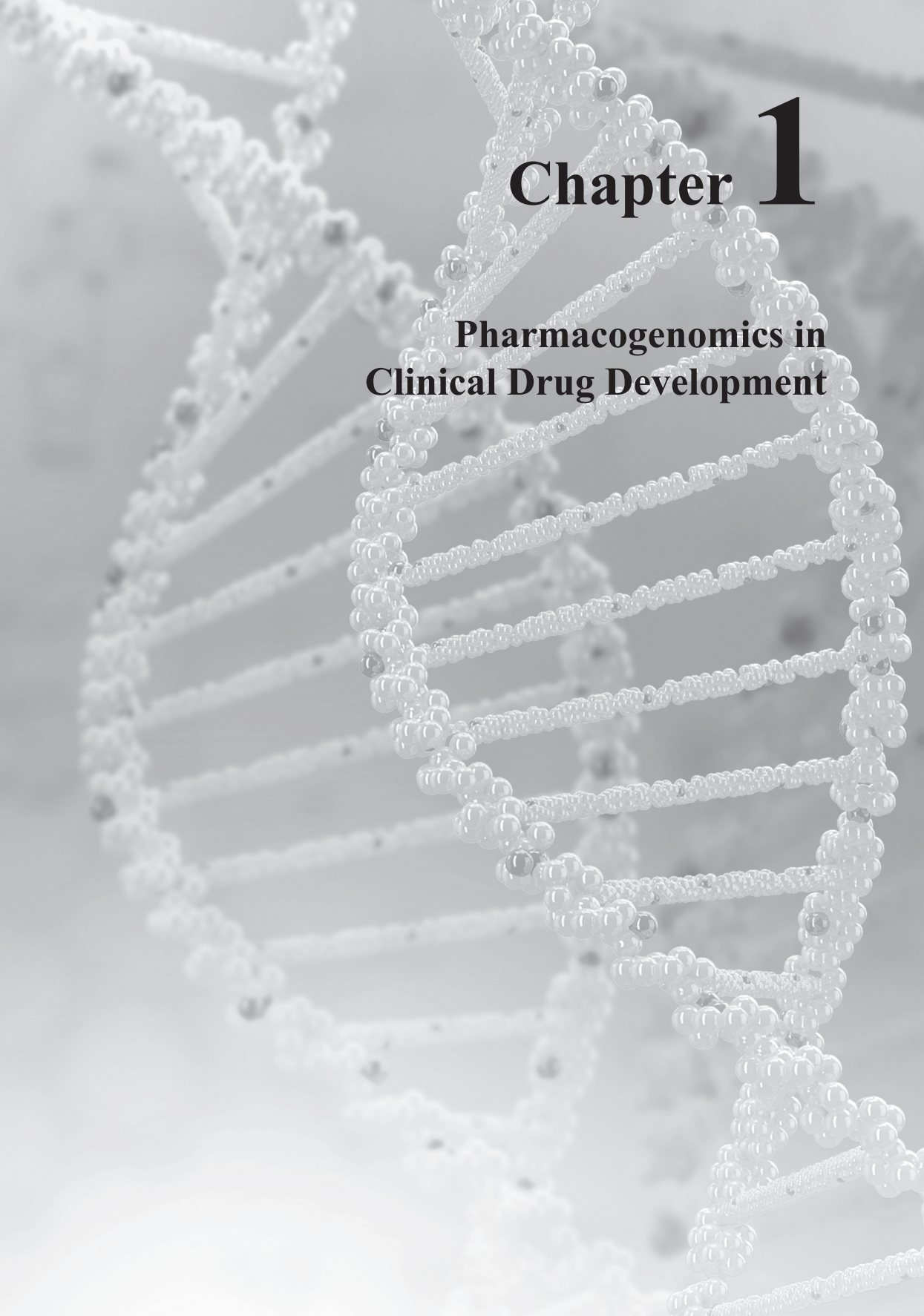

Chapter 1

Pharmacogenomics in Clinical Drug Development

Introduction

Pharmacogenomics (PGx) is defined as 'the study of variations of DNA and RNA characteristics as related to drug response'[1]. To date there are several key areas within clinical development to which PGx has contributed with the greatest impact. These primarily focus on the relationship between the pharmacokinetics/pharmacodynamics in genes encoding the drug metabolizing enzymes. However recent advances in technologies and decreasing costs have broadened the scope of PGx across drug development. Regulatory guidance has recently categorized the scope of impact into four main areas: 1) genes relevant to changes in the pharmacokinetics (PK) 2) genes that code for intended or unintended drug targets and other pathways related to the drug's pharmacologic effect; 3) genes not directly related to a drug's pharmacology that can predispose to toxicities such as immune reactions; and 4) genes that influence disease susceptibility or progression [2]. The goal for the implementation of pharmacogenomics across drug development is to elucidate the genetic basis for inter individual differences in drug response and ultimately utilize this genetic information to predict the safety, toxicity, and efficacy of drugs in specific individuals or subgroups of patients and as the scientific basis for individualized dosing [3].

PGx exploratory study for target selection and toxicity

The genome wide association study (GWAS) has emerged as a powerful tool for identifying disease-related genes for many common human disorders [4]. These studies also have the potential to identify novel drug targets or pathways directly related to the disease [5]. GWAS evaluates DNA sequence variations from across the human genome to identify potential genetic risk factors for diseases that are common in the population [6]. Through the application of GWAS, Complement Factor H gene was identified as a major risk factor for age-related macular degeneration [7]. Furthermore the finding that CYP-2C8 polymorphism is a predictor in multiple myeloma patients to develop bisphosphonate-related oxteonecrosis of the jaw, would not have emerged without GWAS [8]. This was an interesting finding as CYP-2C8, which is expressed in a range of tissues other than the liver, may have a role in the metabolism of inflammatory mediators [9]. In addition to identifying novel associations, GWAS have also been used to evaluate susceptibility to disease across a range of therapeutic areas [10]. GWAS have identified four susceptibility loci for epithelial ovarian cancer and

recently eleven new susceptibility loci for late-onset Alzheimer's disease were identified in a population of approximately 74,000 subjects [11,12]. In other disease areas 50 novel loci are now known to modify individual risk of type 2 diabetes and cardiovascular disease [13].

The rapid increase in the number of GWAS has created an unprecedented opportunity to elucidate the role of common genetic variants in the cause of cancer and other diseases. Statistical designs and methodologies have become increasingly uniform, resulting in more meaningful meta-analysis [10,14]. However there are challenges as GWAS moves into the next phase. The clinical translation of these results also requires substantial efforts in biochemistry and cell biology to confirm the relevance of and elucidate the mechanisms of these findings [10]. The clinical implications of the results also require more efficient genetic testing and improvement in the prediction models [15].

PGx in Early Clinical Development

Early phase implementation of PGx is critical to future clinical study design and development planning since it represents the first exposure of the drug to humans [16]. The potential for pharmacogenetic variation can be predicted from in-vitro data prior to the first in human (FIH) studies [17]. For a drug primarily metabolized by CYP, the isoforms responsible for the metabolism can be identified from in-vitro studies such as recombinant CYP isoforms and correlation analysis [18]. The same principles can also be applied for drugs in which glucuronidation by uridine 5'-diphospate-glucuronosyltransferases (UGTs) are central to the biotransformation. In such circumstances in vitro–in vivo (IVIVE) extrapolation can be implemented to evaluate the impact of any potential polymorphism at the earliest stage. Such an approach can characterize and enhance the understanding of the biological processes directly influencing the PK [19]. The development of a physiologically based PK model to describe these processes can be further utilized in the development paradigm to clarify any requirement for dose reductions by genotype including those in special populations such as hepatic impairment or drug-drug interactions [20,21]. The early identification of the relationship between genetic polymorphism and PK/PD response can also help to guide the future direction of development considering how differences in drug exposure between individuals relates to the safety/efficacy margin.

PGx has been routinely been used in the identification and quantification of inter-individual variability in drug response resulting from differences in the metabolic transformation of a drug. The regulatory authorities have advocated the routine collection of PGx samples in all clinical studies to enable prospective and efficient retrospective evaluation of relationships between genetic polymorphisms and PK/PD response [2,21]. As a result of the increasing evaluation of the relationships between polymorphisms and drug response, the FDA maintains a list of FDA-approved drugs with pharmacogenomic information in their labeling [22].

Clinical relevance of genetic variants in pharmacokinetic properties

There has been extensive evaluation of polymorphic expressed enzymes such as cytochrome P450 (CYP), since more than 80% of drugs in use today are metabolized through this pathway [23]. In addition to the polymorphic CYP mediated metabolism, genetic polymorphisms have been identified for glucuronidation by UGTs [24]. These account for approximately 10% of the major drug elimination pathways. With an increasing number of transporters being identified in drug uptake and disposition, studies have also evaluated relationships between genetic polymorphisms and transporters such as organic anion-transporting polypeptide (OATP) [25].

CYP P450

The most widely described polymorphisms in the P450 (CYP) subfamily have been identified for CYP-2C9, CYP-2C19, and CYP-2D6. A summary of some of the successful examples for the application of pharmacogenomics for CYP-2C9, CYP-2C19 and CYP-2D6 are shown in Table 1.

CYP-2C9 constitutes approximately 20% of the human hepatic P450. Approximately 15% of all clinically used drugs are metabolized by CYP-2C9 including tolbutamide, losartan, diclofenac, celecoxib and several drugs with a narrow therapeutic index, warfarin and phenytoin [26]. There have been several important SNPs identified for CYP-2C9. The genotype, CYP-2C9*3, and to a lesser extent CYP-2C9*2, have shown the most clinical relevance [27].

There are currently three drugs listed in the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling which include specific information on 2C9 genotype, Celecoxib, Flurbiprofen

and Warfarin [22]. For the nonsteroidal anti-inflammatory drug celecoxib, there is a specific dose adjustment included in the drug label for poor metabolizers (i.e. CYP2C9*3/*3) [28]. Flurbiprofen is also a nonsteroidal anti-inflammatory that is indicated for rheumatology. Patients who are known poor metabolizers of CYP-2C9 should be administered Flurbiprofen with caution due to increased plasma levels [29]. Warfarin has two different polymorphic subgroups identified; CYP-2C9 which influences the PK and the PD related genomic variant vitamin K 143 epoxide reductase (VKORC1). The drug label describes a specific dose individualization matrix for 6 different CYP-2C9 genotypes (*1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3) and 3 VKORC1 genotypes (GG, AG and AA). The dose range varies from 0.5-7mg dependent on both the subjects VKORC1 and 2C9 genotype [30].

CYP-2C19 is involved in the metabolism of many drugs across therapeutic areas and is estimated to be involved in the metabolic clearance of approximately 15% of all prescription drugs [31]. There have been several polymorphisms of the gene identified that are known to be associated with reduced enzyme activity, CYP-2C19*2, CYP-2C19*3 and CYP-2C19*17 [32].

The frequency of these poor metabolizers also varies with race. Approximately 1–8% of Caucasians and 13–23% of the Asian populations being poor metabolizers with reduced CYP-2C19 function [33]. Therefore the clinical impact of any polymorphism for this enzyme should be evaluated in context to race differences.

The impact of this polymorphism for clopidogrel, a second-generation thienopyridine that inhibits platelet aggregation has been widely described [34]. Clopidogrel is a pro-drug that requires biotransformation to the active metabolite by CYP-2C19 in order to inhibit platelet aggregation. It was therefore hypothesized that subjects with reduced enzyme function would also be at risk of higher ischemic events due to the lower plasma levels of this active metabolite [34]. The clinical relevance of this was confirmed between carriers of a reduced-function CYP-2C19 allele and a higher rate of major adverse cardiovascular events in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel– Thrombolysis in Myocardial Infarction (TRITON–TIMI) 38. As a result of accumulating evidence the drug label was updated in 2010 to include a “boxed warning” for diminished effectiveness in poor metabolizers of CYP-2C19 [35].

CYP-2C19 is the major metabolic pathway involved in the biotransformation of the proton pump inhibitors (PPI) omeprazole, pantoprazole, lansoprazole, esomeprazole, and rabeprazole [36]. However differences have been identified in the proportional role of CYP-2C19 for each PPI [37]. A study evaluating the relationship between genotype and AUC for omeprazole, lansoprazole, pantoprazole and esomeprazole, identified a 3-10 fold higher exposure range in the poor metabolizers of these drugs (CYP-2C19*3) as compared to subjects in the extensive metabolizer group [38]. Furthermore a relationship was identified between plasma AUC and the observed degree of inhibition of acid secretion for omeprazole [39]. As a result of this the exposure of the drug could be directly related to clinical efficacy [40]. In the drug label for omeprazole, esomeprazole and pantoprazole despite the increase in AUC observed in the poor metabolizers there are no specific dose adjustment requirements based on genotype. However several meta-analysis investigating the relationship between genotype and clinical outcome show potential for improved outcomes with the use of genotyped-based dosing in PPIs [39,41,42].

There are many cardiovascular and central nervous system (CNS) drugs for which CYP-2D6 is central to the biotransformation [43]. It is estimated that CYP-2D6 is involved in the metabolism of approximately 25% of all clinically used medications [44]. CYP-2D6 was first reported to display large inter individual variability following analysis of data from the antihypertensive agent debrisoquine in the mid-1970s [45]. There are currently 4 major subpopulations identified for CYP-2D6, ultrarapid metabolizers (UM), extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM) [46]. The frequency for these allele was also found to vary across different ethnicities. PMs are found in 5-10% of Caucasians however they are rarely found in Asian or African-Americans [47,48].

Metoprolol, used in the treatment of heart failure (HF) and hypertension undergoes O-demethylation catalyzed by CYP-2D6 [49]. Clinical studies have shown that PM subjects have 4- to 6-fold higher plasma concentrations after administration of metoprolol than EM [50]. However in HF patients an evaluation of dose–response association of CYP-2D6 genotype with steady-state metoprolol pharmacokinetics, pharmacodynamics, therapeutic efficacy, and clinical outcome confirmed the association of genotype to PK/PD but found no modulation of treatment efficacy by genotype [51]. An individualized dosing approach is

applied for metoprolol and upward titration that is based on clinical response is recommended for all patients, regardless of CYP-2D6 genotype. There are currently no requirements for dose adjustment based on genotype in the FDA drug label [52].

The opiate Codeine is primarily a pro-drug and its activity is dependent on its conversion to morphine by CYP-2D6. Between PM and UM subjects more than a 30-fold difference in morphine AUCs was found and between EM and UM genotypes a 1.5 fold difference in AUCs was observed [53]. These differences in exposure due to genotype may result in toxic systemic concentrations of morphine even at low codeine doses [54]. The clinical impact of these genotype differences has resulted in a black box warning for CYP-2D6 Ultra-rapid metabolizers for use of the drug for anesthesia in children [55].The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP-2D6, provides an outline of a genotyped-based dosing approach for morphine and recommends alternative analgesics to codeine in patients who are CYP-2D6 poor or ultrarapid metabolizers [54].

Table 1 Examples of the application of pharmacogenomics for CYP-2C9, CYP-2C19 and CYP-2D6

Drug	Therapeutic area	Year of approval	Year PGx information first included in label	Bio-marker	Genotype recommendation (FDA drug label)	Approx. difference in exposure between PM and EM
Celecoxib	Rheumatology	1998	2008	CYP-2C9	50% dose reduction in CYP2C9 poor metabolizers	7 fold [56]
Flurbiprofen	Rheumatology	1988	2010	CYP-2C9	In poor CYP2C9 metabolizers the drug should be administered with caution	3 fold [57]
Warfarin	Cardiology or Hematology	1954	2007	CYP-2C9/ VKORC1	Matrix for dose adjustment by genotype	3 fold [58]
Clopidogrel	Cardiology	1997	2010	CYP-2C19	Boxed warning for diminished effectiveness in poor metabolizers	3 fold [59]
Omeprazole	Gastroenterology	2008	-	CYP-2C19	-	7.5 fold [38]
Pantoprazole	Gastroenterology	2000	2009	CYP-2C19	For known pediatric poor metabolizers, a dose reduction should be considered.	10 fold [38]
Lansoprazole	Gastroenterology	1995	-	CYP-2C19	-	4.5 fold [38]
Esomeprazole	Gastroenterology	2008	-	CYP-2C19	-	3-4 fold [38]
Rabeprazole	Gastroenterology	1999	-	CYP-2C19	-	3 fold [60]
Metoprolol	Cardiology	1992	-	CYP-2D6	-	4-6 fold [50]
Codeine	Anesthesiology	1984	2013	CYP-2D6	Boxed warning for death related to ultra-rapid metabolism of codeine to morphine	30 fold (UM and PM subjects) [53]

Phase II enzymes

Many drugs are subject to phase II biotransformation processes, by which the parent compound or its intermediate metabolites are conjugated and subsequently excreted from the body as water soluble products such as glucuronides [24]. In the United States, glucuronidation is a clearance mechanism that is listed for 1 in 10 of the top 200 prescribed drugs [61]. Pharmacogenetic variation has been identified for UGTs, specifically for the isoforms UGT1A1, UGT1A7, UGT1A9, UGT2B7, and UGT2B15 [24]. However, clinical relevance for polymorphism in UGTs has currently only been identified for a few drugs, primarily catalyzed by UGT1A1 [62]. Examples for the application of PGx for UGT-1A1 are

shown in Table 2. The anti-cancer drug irinotecan, was one of the first drugs to receive pharmacogenomically guided label requirements in 2005 [63,64]. Nilotinib, a tyrosine kinase inhibitor includes information in the label relating to the increased risk of hyperbilirubinemia for subjects genotyped as UGT1A1*28 [65]. UGT1A1 is known to catalyze glucuronidation of hepatic bilirubin in humans [65]. Nilotinib was found to be a potent inhibitor of UGT1A1 in-vitro at clinically relevant concentrations and nilotinib induced-hyperbilirubinemia has been hypothesized to occur as a result of this UGT1A1 inhibition [66].

In addition to UGTs, polymorphism has also been described for other Phase II enzymes such as N-acetyltransferase-2 (NAT2) [21]. Hydralazine is a direct acting arterial vasodilator that is used in the treatment of resistant hypertension. The drug is metabolized by an acetylation reaction mediated by NAT-2 and its activity has been shown to be dependent on NAT2 polymorphism [67]. The FDA drug label for Isosorbide and Hydralazine includes information on the frequency of fast acetylators (approximately 50% of patients are fast acetylators and have lower exposure) but no specific recommendations on dose adjustment are described [68]. Isoniazid is a drug prescribed for the treatment of tuberculosis. It is metabolized primarily in the liver by N-acetyltransferase [69]. Studies evaluating the influence of genotype on efficacy have shown in general that slow and rapid acetylators respond equally well to treatment, however it has been well established that slow acetylators are more likely to develop polyneuropathy during isoniazid therapy [70]. The FDA drug label for Isoniazid includes references to both the efficacy and the safety in slow acetylators but no dose adjustments or genotype based approaches are recommended [71].

Table 2 Examples of the application of pharmacogenomics for Phase II enzymes

Drug	Therapeutic area	Year of approval	Year PGx information first included in label	Biomarker	Genotype recommendation (FDA drug label)	Approx. difference in exposure between PM and EM
Irinotecan	Oncology	1996	2005	UGT1A1	Reduction in the starting dose for UGT1A1*28 allele	2-4 fold [64]
Nilotinib	Oncology	2007	-	UGT1A1	-	inhibitor of UGT1A1 in-vitro [72]
Hydralazine	Cardiology	2005	-	NAT1-2	-	2 fold [68]
Isoniazid	Infections diseases	1994		NAT1-2		4-6 fold [69]

Transporters

In addition to drug metabolizing enzymes, transporters are also major determinants of drug absorption, distribution and elimination with important implications for both safety and toxicity. Based on current knowledge there are genetic polymorphisms identified for 14 transporters important for drug disposition [73]. These include both the influx (e.g. multidrug toxin extrusion proteins (MATEs)) and efflux transporters (e.g. OATP and organic cation transporters (OCTs)) [74]. However the clinical relevance of polymorphism for the different transporters has been found to vary widely between drugs.

The organic anion–transporting polypeptide 1B1 (OATP1B1) transporter facilitates the hepatic uptake of statins. SLCO1B1 is a gene that encodes the protein OATP1B1, expressed on the basolateral membrane of human hepatocytes [75]. Many statins are known to be substrates of OATP1B1 and the effects of polymorphism in SLCO1B1 differ depending on the specific statin that is used [76, 77]. Studies have been conducted evaluating the relationship between the variants of the gene SLCO1B1 and the pharmacokinetics of different statins on the market [78]. The largest influence of this polymorphism was found for simvastatin with exposure of simvastatin acid 120 and 221% higher in participants with the SLCO1B1 c.521CC genotype than in those with the c.521TC and c.521TT genotypes, respectively [79]. An increase in the plasma exposure of simvastatin acid was also found to contribute to an increased risk of myopathy [80]. Further evaluation of this relationship was

performed using GWAS. The study found that common variants of SLCO1B1 were strongly associated with an increased risk of statin-induced myopathy [81]. The FDA label was updated in 2011, limiting the top dose of 80mg to patients only if they have been taking this dose for 12 or more months without evidence of muscle toxicity [82]. There is currently no specific recommendation in the label for genotyped-based dosing as shown in Table 3. However, the Clinical Pharmacogenetics Implementation Consortium Guideline for simvastatin released in 2014, does make recommendations for genotyped-based dosing for subjects with variants of the SLCO1B1 gene [83].

Table 3 Example of the application of pharmacogenomics for OATP1B1

Drug	Therapeutic area	Year of approval	Year PGx information first included in label	Biomarker	Genotype recommendation (FDA drug label)	Range in exposure between genotypes
Simvastatin	Antihyperlipidemic	1991	-	OATP1B1	-	2-3 fold [79]

Clinical relevance of genetic variants in drug targets

Genetic variation in drug targets can have a profound effect on the efficacy of a drug [84]. There have been over 25 examples identified in which genetic polymorphisms in drug target genes can influence drug response [84]. The clinical application for these genetic differences in biomarkers seems to have had the most profound effect in the area of oncology and a summary of successful examples is provided in Table 4. In oncology, 20 PGx markers have been included into the package inserts of 30 FDA-approved anticancer agents to date [5], albeit that the direct clinical application of these PGx markers varies widely across the approved drugs. Specific PGx biomarkers that are known to be present in tumors could potentially be used by the physician to pre-select and tailor a patient's treatment. Such a target approach has the potential to be more selective for cancer cells than normal cells, which may result in improved prognosis and could potentially decrease the toxic effect of anticancer drugs on normal cells [3].

The monoclonal antibodies, cetuximab and panitumumab are designed to inhibit the growth and survival of tumor cells with overexpressed epidermal growth factor receptor (EGFR) and are approved for metastatic colorectal cancer (CRC) [85,86]. Following the approval of these drugs, several research teams identified an association between the resistance for both these drugs and K-ras mutations. Studies found that approximately 40% of these cancer patients contain these mutations [87]. As a result of this, the patients with these mutations are now contraindicated for anti-EGFR therapy and testing for K-ras mutations has been recommended by the FDA before prescribing cetuximab or panitumumab [88].

The leading example of the implementation of PGx in oncology is for the breast cancer drug Herceptin (trastuzumab). The drug is only prescribed for patients, whose tumors overexpress the human epidermal growth factor receptor (HER2) protein, making up approximately 20-30% of breast cancer patients [89]. Early research studies from UCLA had identified relationships between HER2 and the aggressive cancer found in 25% of breast cancer patients. Based on this research Genentech developed Herceptin by humanizing the 4D5 mouse antibody directed at HER2 and subsequently started clinical development of the drug. The Phase III clinical trials were then performed only in subjects who overexpressed HER2 as such the indication for Heceptin in breast cancer was specifically limited for those patients who overexpressed HER2 [90]. The subsequent approval of Herceptin by the FDA was completed simultaneously with Herceptest®, a commercially available test to identify patients who overexpress the HER2 gene [91,92].

Table 4 Examples of the application of pharmacogenomics in oncology

Drug	Therapeutic area	Year of approval	Year PGx information first included in label	Biomarker	Genotype recommendation (FDA drug label)
Cetuximab	Oncology	2004	2012	K-Ras/EGFR	Determine K-Ras mutation and EGFR-expression status prior to initiating treatment
Panitumumab	Oncology	2006	2009	K-Ras/EGFR	Determine K-Ras mutation and EGFR-expression status prior to initiating treatment
Trastuzumab (Herceptin)	Oncology	1998	1998	HER2	Detection of HER2 protein overexpression is required prior to initiating treatment

PGx for Adverse Drug Reactions

Adverse drug reactions (ADR) that occur during clinical development or post approval are an important factor in drug attrition [9]. This remains a major concern for the pharmaceutical industry, between the years 1990 and 2012 there were 43 drugs withdrawn from the market due to ADR [93]. Identifying the genetic contributions to ADR risk may lead to a better understanding of the underlying mechanisms and identification of patients at risk which could ultimately lead to a decrease in the ADR incidence [94]. Studies have evaluated if genetic factors can be used to determine a subject's susceptibility to an ADR and successful examples are presented in Table 5. This has generally involved the use of a case control approach which compares the frequency of the putative PGx predictor in patients with and without the adverse reaction [2].

Drug hypersensitivity reactions (DHRs) are ADR for drugs that occur at a dose tolerated by typical subjects and clinically resemble allergy [95]. The Human leukocyte antigen (HLA) was to found to have a strong association with an increase of drug-induced hypersensitivity [96]. There are two primary examples evaluating the relationship between HLA and ADR, both of which have resulted in the clinical application of genotype-based dosing recommendations. For the drug abacavir, hyper sensitivity was found to be associated with

the HLA-B*5701 allele [97]. As a result, screening for the HLA-B*5701 allele should be performed to assist clinicians in identifying patients who are at risk of developing a hypersensitivity reaction to abacavir [98].

Carbamazepine is an anticonvulsant primarily used in the treatment of epilepsy; however the drug may cause life-threatening allergic reactions such as Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis. Recently the HLA-B*1502 allele was shown to be strongly correlated with carbamazepine-induced SJS and toxic epidermal necrolysis in the Han Chinese and other Asian populations [99]. In 2007, the FDA formally recommended testing for the HLA-B*1502 allele in patients of Asian ancestry, including South Asian Indians, before starting carbamazepine therapy [100].

Drug induced liver injury (DILI) is the most common cause of clinical trial termination of new drugs (approximately 33%) [101]. There have been several drugs withdrawn from the market due to DILI, including Troglitazone, Ximelagatran and Lumiracoxib. Although the pathogenesis of most DILI is unclear, genetic association of individual susceptibility to DILI have been evaluated in several studies [101]. The drug Lumiracoxib, was a selective cyclooxygenase-2 (COX-2) inhibitor, that was withdrawn from the market in 2005 due to concerns over hepatotoxicity [102]. A retrospective GWAS analysis found a strong association between HLA-DQ allelic variants and lumiracoxib-related liver injury. However despite this finding and the potential for pre-selection of subjects based on genotype the drug remains withdrawn from the market and the clinical application of genotyping these subjects was never implemented as an approach to improve patient safety.

Many of the studies evaluating ADR with the use of a PGx approach have used data in patients post approval. At this stage not only has the drug has been exposed to a large number of patients but it also represents the highest risk for the drug company since the maximum investment in any drug has already occurred. The key challenge for PGx is the implementation during the development stage where this would have the greatest potential to improve attrition rates. However there are currently several limitations to this early implementation including the sensitivity and specificity of genetic biomarker tests and the predictive value of these tests as screening tools to predict drug efficacy and prevent ADRs [93].

Table 5 Examples of the application of pharmacogenomics for ADR

Drug	Therapeutic area	Year of approval	Year PGx information first included in label	Biomarker	Genotype recommendation (FDA drug label)
Carbamazepine	Neurology	1968	2007	HLA-B	Screening is required for the presence of HLA-B*1502 prior to initiating treatment
Abacavir	Infectious Diseases	1998	2008	HLA-B	Prior to initiating therapy, screening for the HLA-B*5701 allele is recommended

Conclusions

The applications of PGx throughout the drug development paradigm have increased over the last few years, as the technology improves and becomes cheaper to implement. For many drugs however, despite the explanation of the large inter-individual variability in the PK through the use of genotyping, the clinical application and information in the label directly related to dosing remains limited. Critically most of the information that is currently described in the drug label is based on research conducted after the drug has been approved [103]. Of those approved drug labels in 2012 there are only 14 cases in which labels direct clinicians to utilize PGx testing prior to prescribing, clearly falling short of the intended impact of PGx in the clinic [104].

There appears to be a range of factors that have contributed to this, for drugs in which the polymorphism was reported post-approval many of the studies involve low subject numbers or have inferred the clinical relevance based on the magnitude of change in drug exposure. Some clinical studies address the relationship of genotype to phenotype but primarily focus on the changes observed in mean drug exposure. These studies often do not address the remaining variability of exposure within each genotype or the overlap in exposure between the different genotype groups. Overlap in exposure between genotype is an important consideration if genotype based dosing was to be evaluated. For example, a subject could be classified as a particular genotype but could still receive an inappropriate dose because the predictability of the genotype-phenotype relationship was not fully understood.

Genotype should be considered as an additional covariate for drug exposure with other factors also included during the analysis stage such as age, gender and race. The advantages of such an integrated approach have been clearly demonstrated for warfarin, where both age and CYP-2C9/VKORC1 genotypes were included as covariates for clinical response [58]. A key consideration in such analysis is not only the relationship between genotype and PK, but also including clinical response. The implementation of a population PK-PD model based approach to evaluate the influence of genotype can provide a more comprehensive link between the observed changes in the pharmacokinetics and its influence on the magnitude of response. Thus enabling a comparison of the differences observed between the magnitude of change in the PK and the magnitude of this change on clinical response. For example does a 3-fold increase in plasma exposure by genotype result in a proportional change to the clinical endpoint. To fully evaluate the impact of genotype on clinical response, a comprehensive analysis should be conducted using this approach.

Dose individualization is routinely applied in a range of disease areas from diabetes to CNS, where the patient's phenotype is not determined directly from the exposure of the drug but indirectly measured using a clinical endpoint, i.e. change in HbA1c. In such case, the clinical utility of genotyped-based dosing should be assessed in comparison to the currently applied titration approaches or included as an additional covariate to dose selection. Genotype information could be used at the start of treatment if time to maximal response was important and if the therapeutic window is narrow. However for more chronic dosing approaches where titration is routinely applied, genotype may not offer additional clinical benefit if the variability in the response is high or if clinical biomarkers can be directly related to changes in dose adjustment. This is particularly the case if the biomarker is well established, is low cost and is easily measured.

For new drugs currently in development there remains the possibility to prospectively plan and analyze the data as it emerges from clinical studies. Early implementation allows discussion with the regulators with a focus on the relevance of genotype and the planning of future study design to appropriately characterize the response by genotype. Early consideration of this approach also provides the possibility to develop diagnostic tools that can be used in a clinical setting. As both the drug regulators and industry routinely adopt

these approaches the possibly to enhance patient care and individual patients outcome should only improve.

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Chapter 2

PKPD Modeling of Pharmacogenomics in Early Drug Development. Scope and Motivation of the Investigations

Introduction

Phase I studies conducted early in the clinical development program provide the first opportunity to assess not only the pharmacokinetic (PK) profile of a drug but also to evaluate the extent of differences between individuals. Evaluating the basis for differences between individuals considers a range of factors including, routes and rates of elimination, permeability/solubility data or covariates such as age or gender. Identification of the enzymes catalyzing the in-vitro-metabolism is a key component in determining the source and possible clinical relevance of this variation. For drugs in which in-vitro studies show that a polymorphic gene is central to the metabolism of the drug (in vitro data predict >50% of the drug be cleared by a single polymorphic enzyme) [1], it is important to consider the role of this enzyme to the PK variability. The implementation of genetic factors into the development program has three key aims 1) to understand how genetic factors contribute to inter-individual variability in PK and PD 2) to inform the optimal design of phase 2/3 studies 3) to provide evidence/information to support the labeling [2].

Application of model based approaches in PK

The role of polymorphic changes in variability of drug exposure should be considered relative to the other intrinsic or extrinsic factors. The understanding of specific covariates (e.g., age and race) and gene-covariate interactions on the variability in drug response is useful in understanding the relative impact of genetics, versus other nongenetic factors on the both the PK and safety/efficacy of the drug [3]. This is important to consider when evaluating if dose individualization and/or therapeutic drug monitoring (TDM) could actually be used to improve patient outcomes. Population PK models are a powerful tool to quantify and identify sources of variability both within and between subjects [4]. By implementing a model based approach, differentiation between variability both within and between subjects can enhance the statistical power to identify the different factors influencing the pharmacokinetic profile [5]. The value of a applying a model based approach was demonstrated for tacrolimus where studies investigating the effect of CYP3A4*22 genotype on tacrolimus PK had been limited in their approach by only considering the trough concentrations and not fully evaluating the use of co-medication [5]. Moes et al quantified the effect of CYP3A4*22 genotype for cyclosporine, everolimus, and tacrolimus clearance and found that the effect was a reduction

in clearance of less than 20%. They therefore concluded that dose adjustments based on CYP-3A4*22 were not required. The study further confirmed the role of CYP-3A5*3 genotype and found this was a suitable predictive marker for tacrolimus clearance, but close TDM remains essential due to the remaining variability between patients within the same genotype group.

Other sources of variability were also incorporated into the population PK analysis of the HIV-1 protease inhibitor, Atazanavir [6]. The drug exhibits high inter- and intrapatient variability and sources of variation between individuals were attributed to a number of sources not only related to pharmacogenetic (CYP3A5) factors but also including the effect of food on the bioavailability and adherence to therapy. An integrated population PK analysis revealed that a 28% increase in clearance was observed in subjects with at least one CYP3A5*1 allele, however the between subject variability decreased by an additional 40% when adherence was also considered in the model. This approach was able to delineate the effects resulting from genotype whilst considering the other components contributing to the variability in exposure. Such a comprehensive model based approach can then be used to further evaluate the necessity of individualized dosing.

Application of model based approaches in PK-PD

Whilst population PK models can be used to characterize the PK properties of a drug, they can also be linked either directly or indirectly with pharmacodynamic (PD) response. Through the use of a PK-PD model based approach to evaluate the influence of genotype a more comprehensive link between changes in the PK and its influence on the magnitude of response can be established. This is an important consideration to assess the clinical relevance of these changes in exposure as a direct result of genotype since overall variability in the PD, which appears random until relevant covariates have been identified, can be much greater than that observed in the PK [7]. Currently, the application of a model based approach to evaluate the influence of different genotypes, by linking pharmacokinetic changes with response seems limited to only a few drugs. Many studies have evaluated the effect of pharmacogenetics for warfarin and several models have been published describing the relationship between PK and the PD marker, International Normalized Ratio (INR) [8]. The application of a PK-PD model based approach for warfarin has not only been limited to adults where both age and CYP-2C9/VKORC1 genotypes were included as covariates for

clinical response (INR), but this was recently extended to evaluate if the same relationships could be identified in children [9]. Hamberg et al applied a PK-PD model based approach to a data set on 163 children. They evaluated if another genetic covariate (CYP-4F2 genotype), which had recently been shown to influence warfarin dose requirement should be included in the current pharmacogenetic dosing algorithms. The PK-PD analysis showed that variability in children was found to be comparable with that reported in adults and that CYP-2C9 genotype could explain up to a four-fold difference in dose with VKORC1 genotype explaining up to a two-fold difference in dose. The analysis also found that bodyweight, age, baseline and target INR, time since initiation of therapy, but not CYP-4F2 genotype, were found to influence significantly typical warfarin dose requirements in children. This model based approach can now be further utilized to improve the improving efficacy and safety of warfarin therapy in children.

Furthermore physiologically based pharmacokinetic (PBPK) models have been linked to PD response integrated to estimate the drug concentration at the site of action. This may offer a better understanding of true PD variability vs. variability resulting from drug disposition at the site of action [10]. Through the development of a PBPK-PD model, Rose et al demonstrated that by using the local concentration at the effect site to drive the PD response they were able to explain why there was a disconnect observed between the effect of (organic anion-transporting polypeptide) OATP1B1 polymorphism on rosuvastatin plasma concentration and the lack of impact observed on the PD response. They show that plasma concentration is different between individuals due to genotype, but a significant proportional reduction in the PD marker (mevalonic acid) does not occur as the concentration at the effect site has already reached Emax. Such an approach is able to describe the physiological implications behind these differences and enhance the understanding of the eventual impact of genotype on clinical response.

Genotype influences on model based approaches in disease progression analysis

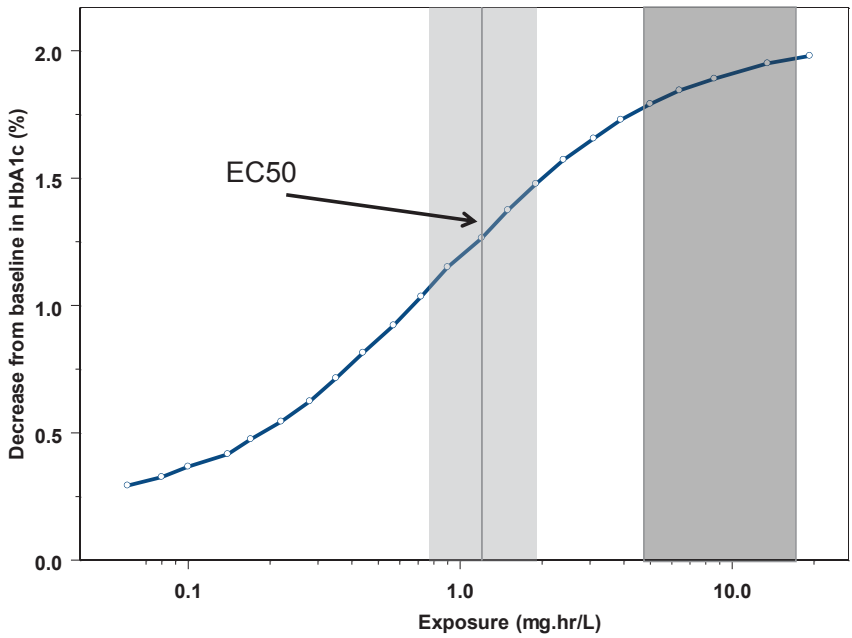
Disease progression analysis is a model based approach applied to describe and explain changes in disease status as a function of time and drug therapy [11]. The advantage of implementing a model based approach being the key characterization of the relationship between treatment and the physiology of the disease over time. Many of the published

models evaluating disease progression have focused in the areas of Type 2 Diabetes (T2D), Parkinson's and Alzheimer's disease [12, 13]. Recently the application of the genome wide association study (GWAS) has emerged as a powerful tool for identifying disease-related genes for many common human disorders. The application of these studies have identified eleven new susceptibility loci for late-onset Alzheimer's disease, in T2D significant associations were identified for more than 35 independent loci and in Parkinson's disease many GWAS are currently on-going to evaluate susceptibility to the disease [14, 15, 16]. Data from these GWAS could also be an important covariate as an extension of the current model based approaches applied in these disease areas. Such an approach would enable treatment specific effects to be evaluated on the time course of the disease profile and enable evaluation of disease modifying drug effects in the different genetic populations. This comprehensive approach could then be used to evaluate if specific genetic sub-populations respond differently to drug treatment.

Understanding the dose response relationship relative to genotype

Genotype difference should be considered relative to the dose response relationship [3]. For example, for a drug which has a steep dose response curve, small changes in exposure resulting from genotype differences will have a greater impact depending on where the therapeutic dose sits on the curve. Whilst for a drug at which the therapeutic dose is given close to the Emax these differences resulting from genotype will be less apparent. Figure 1 shows how these changes in exposure can be related to the drug response and how the therapeutic dose should be evaluated relative to the exposure response relationship. The magnitude of the influence of exposure changes on response (i.e. decrease from baseline in HbA1c) is different depending on the drug exposure.

Figure 1 Exposure response relationship for the decrease from baseline in HbA1c (%) at 3 months, light grade shade shows exposure range between EM and PM subjects at 64mg, dark grey shade shows exposure range for EM and PM at 400mg



When the drug exposure is close to the EC50, greater changes in HbA1c between different genotypes would be observed. The influence of this is illustrated in Figure 2a and Figure 2b.

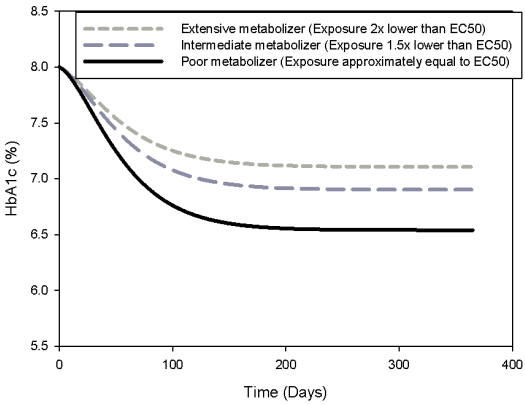


Figure 2a. HbA1c simulation over time for a dose of 64mg

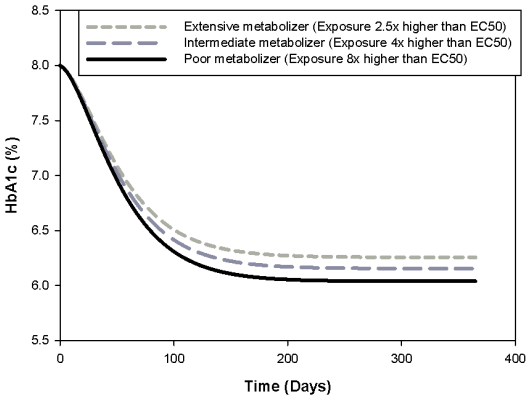


Figure 2b. HbA1c simulation over time for a dose of 400mg

When the genotype influence is simulated for a dose close to the EC50 (light graded shaded area on Figure 1), the difference in the change from baseline in HbA1c between extensive (EM) and poor metabolizers (PM) is -0.6%. However, when a higher dose is given (dark gray shaded area on Figure 1), a large change in exposure results in a less than proportional change in the clinical response. The difference in the change from baseline in HbA1c between EM and PM subjects is now only -0.2%. Therefore this genotype difference would not be clinically relevant when a higher dose was given. Whilst increasing the exposure can negate the influence of genotype, this should routinely be considered on balance to the safety/efficacy margin. A comprehensive model based approach would incorporate not only PD response related to efficacy but also including safety. Often in clinical development the relationship between drug exposures and efficacy can be well defined, but the relationship between drug exposure and safety may only be defined as linear as dose escalation would be limited by exposure margins or would be stopped before the maximum effects are reached. This could have an influence for subjects who are poor metabolizers as these subjects would be expected to have higher exposure than the average population. Additional studies at higher doses than previously studied in Phase I may be needed to cover the exposure range for the

subjects who are poor metabolizers. This may be the case if the frequency distribution/ratio of genotypes for EM/PM is highly unbalanced and no poor metabolizers had been enrolled at the top dose level in phase I or II.

Evaluating the clinical consequence of genetic differences

Dosing recommendations should ensure that a patient receives a drug that is both safe and effective [1]. Genotype based dosing recommendations are expected to follow the same expected level of evidence as other adjustments made for subpopulations where dose adjustment are made based on renal function or weight. Different routes for dose recommendations may include dose titration, optional gene based dosing or dosing based on genotype [1]. Since dose titration approaches are widely applied in many therapeutic areas, for existing therapies dose titration approaches should be compared with genotype based dosing to evaluate the additional utility of implementing a genetic dosing algorithm. As such the application of a model based approach can be used to simulate different scenarios and address clinical questions such as the time to reach maximum effect or the % of subjects achieving a certain target for both genotyped-based dosing vs. titration-based approaches. Clinical trial simulation can also be used to look at clinical outcomes in each of the genotype sub-populations for upcoming pivotal studies.

Conclusions

The application of model based approaches to evaluate the influence of genotype, have primarily focused on the use of genotype as a covariate on drug exposure. These models should preferably also be extended during the drug development program to include clinical response, evaluating safety or efficacy markers to design the appropriate genetic based dosing algorithms or compare different study designs i.e. genotype-based dosing vs. a single dose level for all subjects. Further extension has focused on the use of PB-PK models which can be developed during the non-clinical stage and combined with PD models for safety or efficacy. Ultimately these model based approaches can be used to determine if covariate-based dose individualization would be required to normalize exposure and minimize variability in clinical outcomes across population subgroups and inform label recommendations that can improve individual patient outcomes [17].

Outline of the investigations in this thesis

This thesis starts with an overview of the current applications of Pharmacogenomics (PGx) across drug development with an emphasis on the implications of polymorphism in drug metabolizing enzymes and transporters. The second section (Chapter 2) focuses on the application of model based approaches to evaluate differences in drug exposure and response as a result of these genetic differences between individuals.

In Chapters 3, 4 and 5 the focus for this thesis is on a clinical example for the T2D drug, sipoglitazar. Sipoglitazar undergoes phase II biotransformation by conjugation catalyzed by UDP-glucuronosyltransferase (UGT) [18]. Clinical data from four phase I studies in healthy volunteers and two phase II trials in T2D subjects were utilized in the analysis. PGx samples for determination of UGT genotype were collected for all subjects enrolled in the trials. The objectives of the investigation in Chapters 3-5 was to evaluate the role of UGT genotype differences in explaining inter-individual variability for sipoglitazar and to then investigate the impact of these differences on both the clinical response and the selection of the appropriate dosing scheme for future trials.

In Chapter 3, an investigation was conducted to evaluate the enzymes that were contributing to the inter-individual variability of sipoglitazar and to then quantify the resulting exposure differences between genotype. Here the importance of considering genotype relative to other intrinsic and extrinsic factors is investigated and discussed. The analysis in Chapter 3 was conducted using data from a trio of phase I clinical pharmacology studies in healthy volunteers. The studies included a single ascending dose (n=39), multiple ascending dose (n=19) and a single dose age/gender study (n=30). The dose range for sipoglitazar was 0.2-64mg.

The investigation and analysis conducted in Chapter 4 was then focused on evaluating genotype influences in the target population, T2D patients. In this chapter data from two phase II randomized, double-blind studies (sipoglitazar once daily: 8, 16, 32, or 64 mg; sipoglitazar twice daily: 16 or 32 mg; rosiglitazone 8 mg once daily and placebo for 13 weeks; n = 780) were included in the analysis. For evaluation of the exposure data the phase II trials were combined with a large phase I single dose (64mg) study in a diverse ethnic study population of 524 healthy male and female subjects. The magnitude of exposure

differences resulting from polymorphic differences in the UGT2B15 enzyme were quantified and considered relative to other covariates in the target population using pharmacokinetic modelling. The predictability of the genotype-exposure relationship was investigated and an approach to evaluate the predictability of this relationship is then described. The consequences of disconnect between genotype and exposure are discussed. In addition, a preliminary analysis was conducted to evaluate the relationship between genotype and efficacy, using change from baseline in HbA1c at 3 months by dose and genotype as a pharmacodynamic endpoint. Exposure data were then reviewed relative to the safety margin and discussed in context to the therapeutic dose.

This analysis was extended in Chapter 5 and a population PK-PD model was implemented to evaluate the relationship between exposure differences resulting from genotype and its magnitude of impact on the clinical response (FPG and HbA1c). This analysis showed how the development of PK-PD model can be used to test different dosing scenarios to appropriately plan future clinical studies and to evaluate the impact of genotype on dosing relative to current dosing practices in T2D.

The next section (Chapter 6) focusses on PD model based approaches in T2D over a much longer time period (>2.5 years). Since T2D is a slowly progressing disease, the importance of considering both the drug and disease effects on the time course of the relevant biomarkers is investigated. A phase IV study that was conducted in Japanese T2D subjects was used for the analysis. In this study (n=587) subjects received pioglitazone in combination with other oral glucose-lowering drugs or oral glucose-lowering drugs excluding thiazolidinedione (control group). Treatment was adjusted to achieve HbA1c<6.9% following the standard treatment guidelines for T2D in Japan. In the control group, either the dosage of the current therapy was increased, or a concomitant oral glucose-lowering drug was added. In the pioglitazone group, the preferred adjustment was to increase the dose of pioglitazone. The study was conducted over a period of 2.5-4 years and all subjects included in the trial were treatment experienced. Throughout the study biomarker samples for FPG and HbA1c were collected. A population PD model simultaneously incorporating FPG and HbA1c was developed to describe the time course of the drug and disease effects in both treatment groups. The aim of this analysis was to further enhance the understanding of the treatment and time course effects on FPG and

HbA1c and the development of the PD model enabled simulations to be performed to compare the longer term glycemic durability between treatment groups.

In Chapter 7 the investigations are reviewed and discussed with a focus on the application of model based approaches across clinical drug development to evaluate and understand genotype differences in enzymes and transporters. Furthermore the applications of PGx in treatment approaches to T2D over the long term are hypothesized. The future perspectives on the applications of model based approaches to evaluate the impact of genotype in clinical development are presented. The focus for discussion is on the utilization of PBPK models throughout clinical development in understanding the role of genotype relative to the other intrinsic and extrinsic factors and for considerations in study design.

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Chapter 3

The Effect of Genetic Polymorphisms in UGT2B15 on the Pharmacokinetic Profile of Sipoglitazar, a Novel Anti-diabetic Agent

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Summary

Purpose

Sipoglitazar was a novel, azolealkanoic acid derivative that possesses selective activity for the peroxisome proliferator-activated receptors (PPAR) PPARγ, PPARα, and PPARδ. The compound undergoes phase II biotransformation by conjugation catalyzed by UDP-glucuronosyltransferase (UGT); the aim of this analysis was to explore the influence of genetic polymorphism in UGT on the pharmacokinetics of sipoglitazar.

Methods

Three preliminary phase I clinical pharmacology studies were conducted in tandem in healthy human subjects. Genotyping was undertaken in a total of 82 subjects in the phase I program for the purpose of genotyping UGT polymorphisms. Plasma samples were collected for up to 48 hours post dose to characterize the pharmacokinetic profile following a single oral dose of the drug.

Results

Plasma concentrations of sipoglitazar and the distribution of dose-normalized individual values for area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) before any stratification were considerably skewed with a multi-modal distribution. The proportion of variability in $AUC_{0-\infty}$ explained by UGT2B15 was 66.7% ($P<0.0001$); the addition of other genetic or demographic factors was not statistically significant. Subjects homozygous for the UGT2B15 D85Y variant (UGT2B15 *2/*2) were exposed to greater plasma concentrations of sipoglitazar compared with subjects homozygous for the wild-type allele UGT2B15 *1/*1 (3.26 times higher) or heterozygous allele UGT2B15 *1/*2 (2.16 times higher).

Conclusions

These results indicate that sipoglitazar clearance is substantially modified by UGT2B15 enzyme variants, with higher exposure observed in the UGT2B15 *2/*2 genotype group.

Introduction

Sipoglitazar, a novel, orally-available, azolealkanoic acid derivative, has selective peroxisome proliferator-activated receptor (PPAR) agonist activities for PPARγ, PPARα, and PPARδ. As such, sipoglitazar was developed to improve peripheral insulin sensitivity, normalize circulating lipid profiles, and reduce body weight in patients with metabolic syndrome and type 2 diabetes mellitus (T2DM). A preliminary phase I program of clinical pharmacology studies was conducted in healthy human subjects to examine the pharmacokinetics, safety, and tolerability of sipoglitazar as single and multiple doses.

Many drugs are subject to phase II biotransformation processes, by which the parent compound or its intermediate metabolites are conjugated and subsequently excreted from the body as water soluble products such as glucuronides [1]. Pharmacogenetic variation has been identified for glucuronidation by uridine 5'-diphospate-glucuronosyltransferases (UGTs), specifically for the isoforms, UGT1A1, UGT1A7, UGT1A9, UGT2B7, and UGT2B15 [1]. However, clinical relevance for polymorphism in UGTs has currently only been identified for a few drugs, primarily catalyzed by UGT1A1. The anticancer drug, irinotecan, includes a label recommendation to lower the starting dose for subjects with the homozygous allele UGT1A1*28/*28 and nilotinib, carrying a label warning of increased risk of hyperbilirubinemia for subjects genotyped as UGT1A1*28 [2].

Preclinical studies of sipoglitazar metabolism conducted *in vitro* using human and animal liver microsome preparations suggest that enzymatic glucuronidation is central to its biotransformation [3]. Sipoglitazar is relatively stable in the absence of the UGT co-substrate, uridine diphosphoglucuronic acid (UDP), whereas the parent compound is susceptible to conjugation by the active enzymes UGT1A1, UGT1A3, UGT1A6, and UGT2B15. The principal metabolite of sipoglitazar is the the dealkylated derivative M-I. The potency of metabolite M-I relative to that of the parent sipoglitazar was 33%, 37%, and 17% for PPARγ, PPARα, and PPARδ, respectively. The metabolite is generated *in vitro* by the action predominantly of cytochrome P450 (CYP) 2C8 on the glucuronide intermediates. It is therefore hypothesized that initially sipoglitazar is metabolized to the glucuronide conjugate, sipoglitazar-Glu, by UDP glucuronyl transferase and secondly sipoglitazar-Glu is metabolized to M-I by dealkylation by CYP2C8 and deconjugation. Therefore, due to its

unique metabolic formation, the metabolite M-I was considered to be a marker for the level of metabolic activity of UGT.

On the basis of the *in vitro* findings, UGTs are hypothesized to play an important role in the disposition of sipoglitazar, and it is therefore postulated that abnormalities in the gene encoding UDP-glucuronosyl transferase may alter the rate of clearance of sipoglitazar from the body. Therefore, during the first human studies of sipoglitazar, pharmacogenetic investigation of relevant drug metabolizing enzymes was focused on UGT genetic polymorphisms.

Methods

Subjects

Three phase I studies, referred to as Studies EC001, EC002, and EC003, were undertaken, all in the United Kingdom. A summary of the demographic and genotype information across the studies is described in Table 1.

Table 1 Demographic and genotype information for subjects with known UGT2B15 genotype included in the phase I trials (n=82)

Genotype	UGT2B15*1/*1	UGT2B15*1/*2	UGT2B15*2/*2
Number of subjects	19	41	22
Proportion	23%	50%	27%
Age (years) mean ± SD[a]	34.1 ± 12.9	31.6 ± 9.7	32.6 ± 11.0
Age elderly cohort (years) mean ± SD	72 ± 5.7[b]	71.7 ± 3.8[c]	77[d]
Height (cm) mean ± SD	173.7 ± 7.3	171.9 ± 11.3	172.8 ± 8.4
Weight (kg) mean ± SD	71.4 ± 6.5	70.0 ± 10.1	72.4 ± 12.3
BMI (kg/m^2) mean ± SD	23.7 ± 2.3	23.7 ± 3.0	25.0 ± 2.8
Sex (male/female)	15/4	25/16	15/7
Race (Caucasian/Asian/Mixed race)	17/1/1	40/1/0	22/0//0

a Excluding elderly cohort. b n=2. c n=9. d n=1.
BMI=body mass index.

Table 2 shows the genotype information by dose group for all studies.

Table 2 Genotype information for UGT2B15 by dose group for all studies

Dose (mg)	0.2 (n=5)	0.4 (n=3)	1 (n=5)	2[a] (n=3)	4[a] (n=3)	8[a] (n=4)	16[a] (n=16)	32[a] (n=8)	64[a] (n=35)	Total (n=82)
Genotype										
UGT2B15*1/*1	1	1	2	0	1	2	5	0	7	19
UGT2B15*1/*2	3	2	2	2	0	0	5	5	22	41
UGT2B15*2/*2	1	0	1	1	2	2	6	3	6	22

[a] In EC001 the same subjects received two different doses of sipoglitazar, the number included in each dose represents the first dose received for all subjects

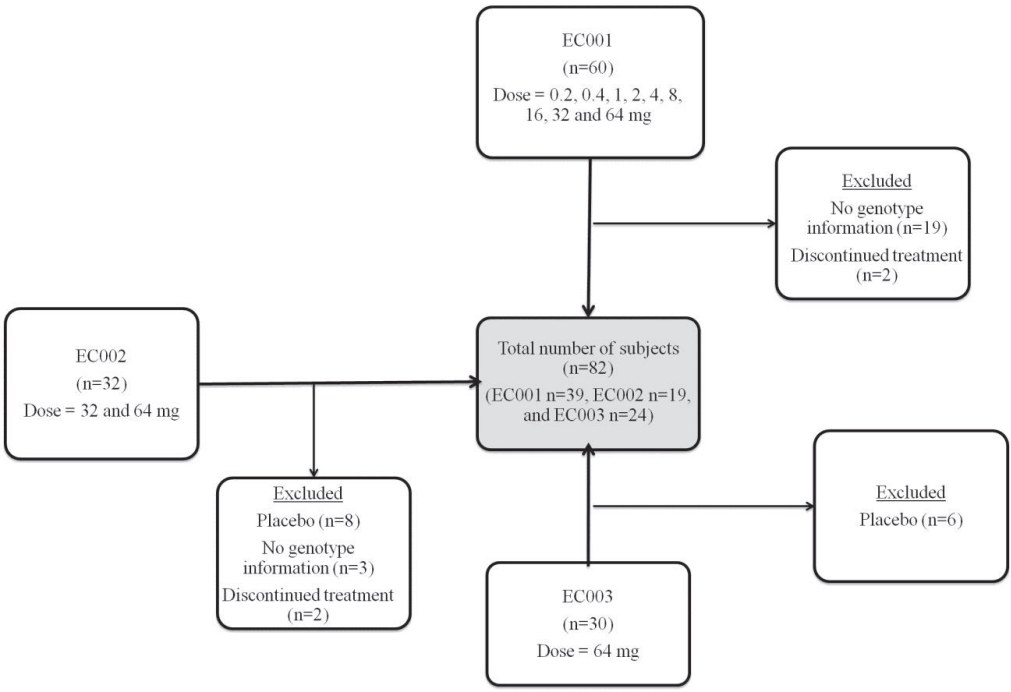
Study EC001 was a double-blind, placebo-controlled, cross-over study. A total of 60 healthy male and female subjects aged 18 to 55 years took part, of whom, 58 completed the investigation. In the ascending dose part of the study, 48 subjects received one dose of placebo and two single doses of sipoglitazar

(3-(3-ethoxy-1-{4-[(2-phenyl-1,3-thiazol-4-yl)methoxy]benzyl}-1*H*-pyrazol-4-yl)propanoic acid, also known as TAK-654), Takeda Pharmaceutical Company Limited, Osaka, Japan, at doses of 0.2, 0.4, 1, 2, 4, 8, 16, 32 and 64 mg. Study EC002 was a double-blind, placebo-controlled, parallel groups study. A total of 32 healthy male and female subjects aged 18 to 55 years took part, of whom, 30 completed the investigation. An equal number of male and female subjects who had been allocated to the active treatment received either a single dose 32 or 64 mg of sipoglitazar. Study EC003 was a double-blind, placebo-controlled, parallel groups study. A total of 30 healthy male and female subjects took part; 15 subjects aged 18 to 45 years (young cohort) and 15 subjects aged ≥65 years (elderly cohort), received a single 64mg dose of sipoglitazar.

Samples were collected for PK at the following time points in all studies: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h postdose.

Figure 1 shows the disposition of subjects who were included in the analysis. The total number of subjects who received an active dose of sipoglitazar and for whom genotype information was collected, was 82 (EC001 n=39, EC002 n=19, and EC003 n=24).

Figure 1 Flow chart of subjects included in the analysis, EC001, EC002, EC003 Phase 1 double-blind, placebo controlled studies (see Subjects section for details)



Ethical Considerations

All three studies were conducted in accordance with the Declaration of Helsinki (Edinburgh 2000). Written approval was obtained from the relevant local independent ethics committee before the start of each study and for the amendments made to the protocol.

In the case of Study EC001, 39 subjects were traced retrospectively and provided consent so that samples might be taken for genotyping. In Study EC002, consent was prospectively obtained for limited CYP genotyping only: 23 subjects (19 who received sipoglitazar and 4 who received placebo) were later traced and provided consent to allow UGT genotyping also. In study EC003, *CYP2C8* and *UGT2B15* genotyping were incorporated into the original protocol, and consent for all study procedures and analyses was obtained from every subject prospectively.

Analytical methods

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.

Sipoglitazar was analyzed in plasma by a validated liquid chromatography method with tandem mass spectrometry (HPLC/MS/MS) in selected reaction monitoring mode using turbo ionspray. The method was validated by Covance Laboratories Ltd (Harrogate, United Kingdom).

Briefly, plasma (0.25 mL), was mixed with internal standard (25 µL, 1 µg/ml sipoglitazar-d5, 5 µg/mL M-I-d5), 0.05 M phosphate buffer (0.75 mL, pH 3) and diethyl ether (3 mL). The organic layer was evaporated under nitrogen at 40°C and reconstituted in methanol:water:acetic acid (50:50:0.4 v/v/v). Following mixing and centrifugation, the supernatant fraction (5 µ L) was injected onto a Xetarra RP18, 5 µm, 150 x 2.1 mm (i.d.) HPLC column (Waters, Milford, MA) at 40°C. The mobile phase was water:acetic acid (100:0.2 v/v) and methanol:acetic acid (100:0.2 v/v) at a gradient of 40/60 from 0 to 3 min after injection, 20:80 from 3 to 4.5 min and 40:60 for 0.1 min. The mass spectrometer was operated under the following conditions: ionspray voltage; 5200V, heated capillary temperature; 425°C, auxillary gas flow; 8000 cc/min and nebuliser pressure; 15 psi.

The validated calibration range for sipoglitazar in human plasma was from 1 to 2500 ng/mL. Quality control samples were prepared in control human plasma with concentrations of sipoglitazar as follows: 3 (low), 50 (mid), and 1750 (high) ng/mL. For samples with concentrations higher than the assay range, a validated dilution (with human plasma) procedure was adopted. In study EC001, EC002 and EC003 assay precision for sipoglitazar was ≤9.1%, ≤9.4%, ≤7.1% and accuracy was 94.0-110.0%, 93.6-106.0% and 96.4-105.9%, respectively.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined study-by-study using noncompartmental analysis at Covance Clinical Research Unit Limited, Harrogate, United Kingdom, using WinNonlin Version 3.2 (Study EC001), at Medeval Limited, Manchester, United Kingdom,

using WinNonlin Version 4.0 (Study EC002), and at Data Magik Limited, Salisbury, United Kingdom, using procedures implemented in SAS Version 8.2 (Study EC003).

Genotyping

Venous blood for genotyping was taken into an EDTA tube and the whole blood sample (4 ml) stored at -20°C pending shipping in the frozen state (-20°C) to the site of analysis.

Genotyping was performed by 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 (PCR) methods using the Stratagene Mx4000 (La Jolla, California, USA) and appropriately designed primers allele-specific at the 3′nucleotide end (Amplification Refractory Mutation System strategy [4]) were used to determine UGT1A6*2 (T181A, R184S), UGT1A7*2 (N129K, R131K), UGT1A7*3 (N129K, R131K, W208R), and UGT2B15*2 (D85Y), and the products of UGT1A1*28 (promoter $A(TA)_6TAA$ to $A(TA)_7TAA$) were analyzed according to their relative capillary electrophoretic mobility using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Warrington, United Kingdom). Similar real time PCR methods were used to determine CYP2C8*3 and CYP2C8*4 (A1196G and C792G, respectively).

To confirm the results in the two subjects in whom there appeared to be disconnect between genotype and sipoglitazar pharmacokinetic phenotype, all six exons plus nearby intronic regions of UGT2B15 were amplified and the fragments sequenced in forward and reverse directions using Big-Dye Terminators (Applied Biosystems). The reaction products were purified by gel exclusion chromatography and analyzed using an ABI Prism 3100 Genetic Analyzer.

Statistical methods

A dose proportionality assessment was performed on area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) by combining data from all three studies. The analysis was performed on the log transformed parameter, $AUC_{0-\infty}$ (dose range 0.2 to 64mg). For subjects who received two different doses of sipoglitazar, the $AUC_{0-\infty}$ from both doses was included in the analysis. The power model was used for analysis, $\ln(AUC_{0-\infty}) = a + b*\ln(dose) + error$ where a is the intercept and b is the dose-proportionality coefficient. For dose proportionality the slope of the regression line (b) = 1 and for dose independence b

= 0. The degree of proportionality was assessed using the value of b and the associated 95% confidence interval (CI). If the 95% CI for the slope of the regression line was close to unity, the relationship between dose and the pharmacokinetic parameter was concluded to be dose proportional for the dose range studied.

Analysis of variance (ANOVA) models were used to explore the effects on dose-normalized $AUC_{0-\infty}$ using log transformed data. For each UGT enzyme, a one-way ANOVA model with the genotype enzyme as a fixed effect factor was used to evaluate differences between levels of each UGT enzyme. Then separate ANOVA models, including UGT2B15 as a fixed effect factor and other covariates, were also produced. The coefficient of determination (R-square) from these models was used to estimate the proportion of variance accounted for by each statistical model. SAS version 9.1.3 (SAS Institute Inc. Cary, NC, USA) was used to produce all the analyses.

Results

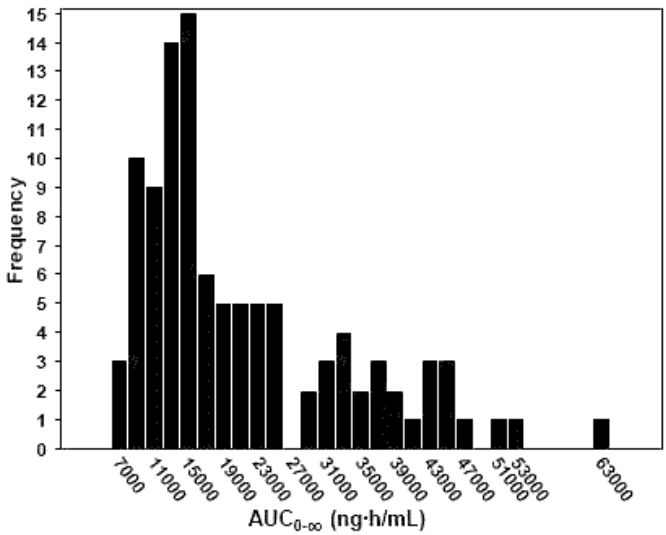
Pharmacokinetic results

A program of three phase I clinical pharmacology studies was undertaken in tandem to investigate the pharmacokinetics of single and repeated oral doses of sipoglitazar administered to male and female healthy subjects. In male subjects, single oral doses of sipoglitazar, 0.2 to 64 mg, were well tolerated, as were single oral doses of 16 to 64 mg in female subjects. Sipoglitazar was rapidly absorbed with a maximum observed plasma concentration occurring 0.6 to 1 h postdose across the dose levels investigated. Plasma concentrations of sipoglitazar declined with bi-phasic kinetics and with a terminal elimination half-life ($T_{1/2}$) of approximately 3 to 5 h. Statistical analysis of $AUC_{0-\infty}$, revealed dose proportionality across the dose range with a slope and 95% CI of 0.99 (0.92 - 1.05). The plasma exposure of the major metabolite M-I was approximately 10% of the parent, with a $T_{1/2}$ of approximately 6 to 7 h. After correcting for body weight, plasma concentrations of sipoglitazar were only slightly greater in female (n=27) subjects than in male subjects (n=55). Exposures to sipoglitazar and M-I metabolite were somewhat increased in elderly subjects (>65 years) compared with younger subjects.

The distribution of sipoglitazar plasma $AUC_{0-\infty}$ values for all subjects who took at least one dose of sipoglitazar (n=82) in the phase I program, normalized to a dose of 64 mg, is shown

in Figure 2. The distribution of $AUC_{0-\infty}$ values was notably skewed with an apparently multi-modal disposition suggesting the existence of a number of potential subpopulations.

Figure 2 Histogram plot of dose-normalized AUC0-∞ values for sipoglitazar in all phase I studies. AUC0-∞ Area under the plasma concentration– time curve from time 0 to infinity



Impact of UGT polymorphisms on pharmacokinetic parameters

In vitro studies conducted prior to human dosing predicted a central role for glucuronidation in the in vivo biological transformation of sipoglitazar. Following the analysis of the results from the first two phase I studies and the potential existence of subpopulations, a total of 62 subjects who took part in these studies (Studies EC001 and EC002) were retrospectively traced for variants of UGT1A1, UGT1A6, UGT1A7, and UGT2B15. Fifty-eight of these subjects had taken active drug. Based on the results, subjects who took part in the third phase I study (Study EC003) gave consent prospectively for genotyping for UGT2B15 and CYP2C8 only.

The majority of the subjects who took part in these studies were classified as Caucasians (Table 1). The proportion of participants shown to have UGT2B15*1/*1, UGT2B15*1/*2,

and UGT2B15*2/*2 genotypes was consistent with the literature for subjects having this ethnic background [5].

ANOVA was used to examine the influence of the four UGT enzymes on sipoglitazar $AUC_{0-\infty}$. Initially, separate one-way ANOVAs were performed for each enzyme in turn for subjects taking part in the first two studies (in whom all four enzymes were genotyped). The proportion of $AUC_{0-\infty}$ variance explained by UGT2B15 was 71% ($P < 0.0001$). Addition of the other genotype enzymes in turn, to this model, achieved no increase in the proportion of $AUC_{0-\infty}$ variance explained in the model (Table 3). The proportion of $AUC_{0-\infty}$ variance explained by UGT2B15 when this was tested across all three studies was 66.7% ($P < 0.0001$). Addition of demographic factors age, gender, and body weight did not explain additional $AUC_{0-\infty}$ variance with statistical significance (Table 3). No relationship between sipoglitazar $AUC_{0-\infty}$ and variants of glucuronosyltransferase genes other than UGT2B15 was evident on inspection.

Table 3 ANOVA results for sipoglitazar to determine the effect of UGT2B15 on dose-normalized $AUC_{0-\infty}$ (log transformed)

Effect of UGT2B15 and other genotypes (Studies EC001 and EC002; n=58)			
Source	**df**	**P value**	**R-square**
Model UGT2B15 alone			
UGT2B15	2	<0.0001	0.709
Model with UGT2B15 plus			
+UGT1A6	3	0.6505	0.718
+UGT1A1	3	0.8458	0.713
+UGT1A7	5	0.5438	0.731
+UGT1A6+UGT1A1+UGT1A7	-	-	0.737
Effect of UGT2B15 and demographic factors (Studies EC001, EC002, EC003; n=82)			
Source	**df**	***P* value**	**R-square**
Model UGT2B15 alone			
UGT2B15	2	<0.0001	0.666
Model with UGT2B15 plus			
+Gender	3	0.0464	0.683
+Age	3	0.0703	0.680
+BMI	5	0.8737	0.666

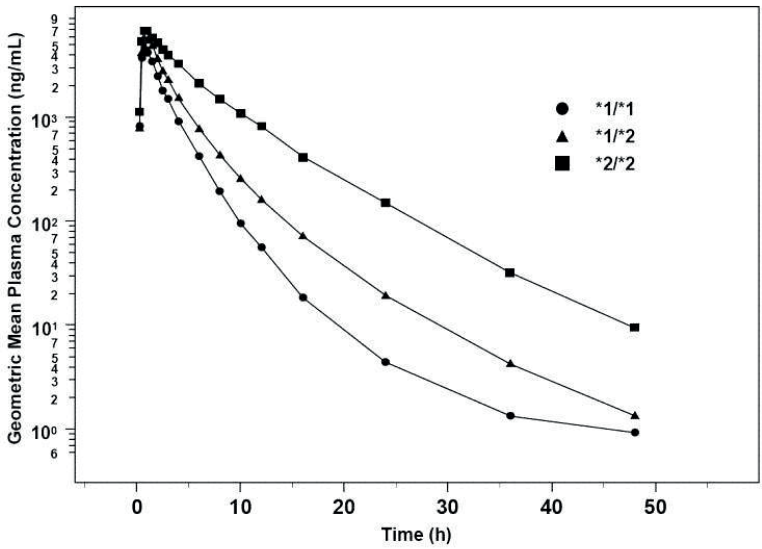
ANOVA, analysis of variance; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; BMI=body mass index.

Impact of UGT2B15 polymorphisms on pharmacokinetic parameters

The summary of pharmacokinetic parameters by UGT2B15 genotype for sipoglitazar and its main metabolite M-I are shown in Table 4. Figure 3 shows the corresponding plasma concentration-time profiles of sipoglitazar for the three UGT2B15 genotypes. Sipoglitazar $AUC_{0-\infty}$ was increased by approximately two- to three-fold in subjects with UGT2B15*2/*2 genotype as compared with subjects with genotype UGT2B15*1/*2 or UGT2B15*1/*1.

Parent to metabolite ratios for AUC (AUC-MR) were calculated with respect to UGT2B15 genotype and were found to vary across the UGT2B15*1/*1 (22%) and UGT2B15*1/*2 (13%) or UGT2B15*2/*2 (5%) genotype groups. The geometric mean AUC for sipoglitazar increased by approximately 51% from UGT2B15*1/*1 to UGT2B15*1/*2 groups.

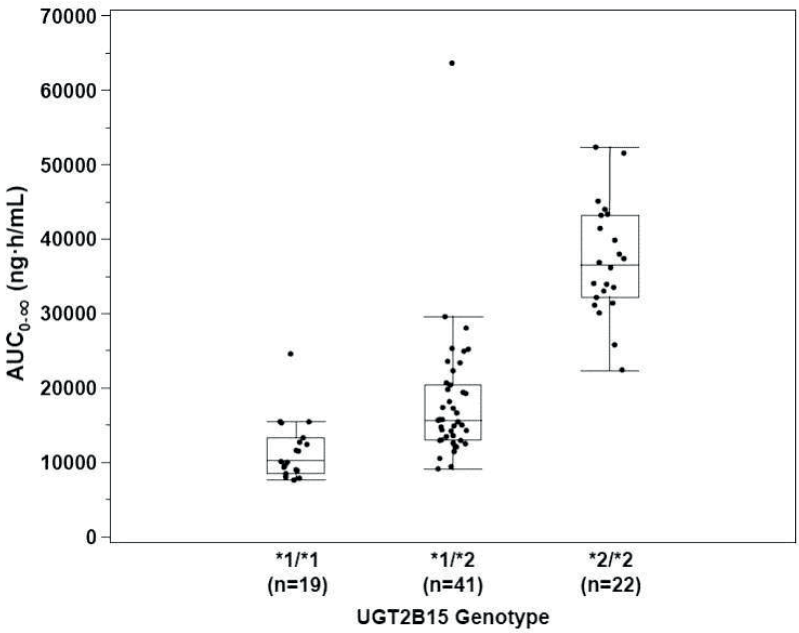
Figure 3 Plasma concentration–time profile dose-normalized (sipoglitazar, single 64 mg dose) by genotype group



$T_{1/2}$ values were comparable between the genotype groups for both sipoglitazar and M-I; however, the concentration 24 h postdose (C24) for sipoglitazar was approximately 52 and 21 times higher in UGT2B15*2/*2 as compared with that of UGT2B15*1/*2 or UGT2B15*1/*1, respectively. The M-I C24 in UGT2B15*2/*2 was approximately double that of UGT2B15*1/*2 or UGT2B15*1/*1.

The 25th and 75th percentile distributions show overlap between the UGT2B15*1/*1 and UGT2B15*1/*2 genotype (Figure 4); however, two outlier subjects (>1.5 times the interquartile range) were observed in the UGT2B15*1/*2 and UGT2B15*1/*1 groups. The UGT2B15*1/*2 and UGT2B15*1/*1 status of the outliers was later confirmed by direct gene sequencing.

Figure 4 Box plot of relationship between genotype and exposure for sipoglitazar. UGT Uridine 5′ -diphosphate-glucuronosyltransferase



Based on the results of the two outlier subjects, it was thought probable, then, that variations in CYP2C8 activity could contribute to the variation in sipoglitazar exposure observed in these subjects. To formally exclude an important influence of CYP2C8 variants on exposure to sipoglitazar, CYP2C8 genotype samples were collected in subjects who received active compound in the third clinical study (EC003). Exposure values for sipoglitazar were clearly correlated with UGT2B15 genotypes when data from all three studies were combined (Figure

4, n=82); however, no relationship was observed between sipoglitazar exposure and CYP2C8 genotypes *1/*1, *1/*3, or *3/*3 when this data was explored in study EC003 (Figure 5, n=24). In addition, the two outlier subjects observed in Figure 4 did not correlate with the outlier subjects observed in Figure 5.

Figure. 5 Box plot of relationship between the cytochrome P450 2C8 (CYP2C8) genotype and exposure for sipoglitazar (study EC003 only)

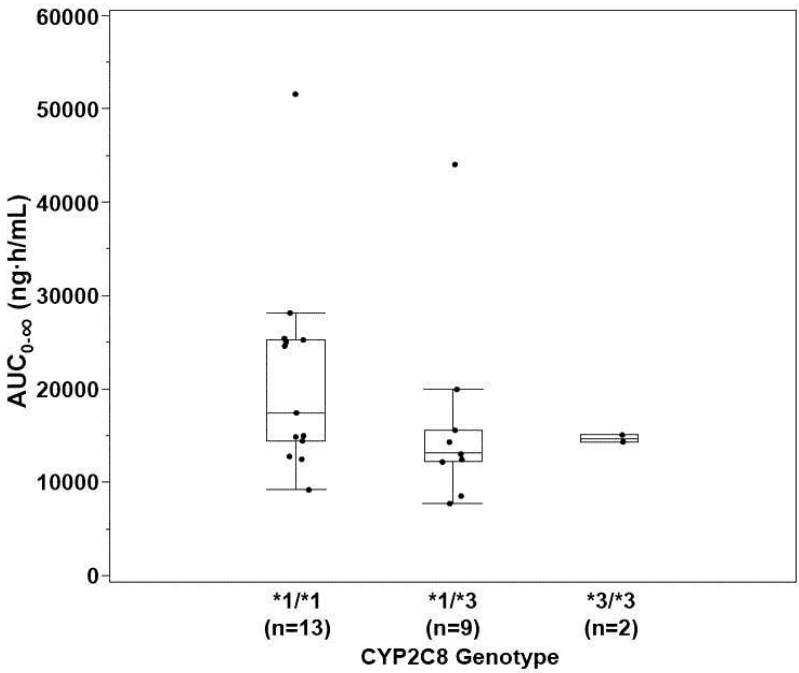


Table 4 Dose-normalized pharmacokinetic parameters (GM (%CV)) of sipoglitazar and metabolite M-I

Genotype	UGT2B15*1/*1 (n=19)	UGT2B15*1/*2 (n=41)	UGT2B15*2/*2 (n=22)	GM ratio 90% CI *2/*2 vs *1/*1	GM ratio 90% CI *2/*2 vs *1/*2
Sipoglitazar					
Cmax (ng/mL)	4,695 (39.5)	6,283 (30.1)	7,169 (24.0)	1.53 (1.30, 1.79)	1.14 (0.99, 1.30)
C24 (ng/mL)[a]	2.52 (0–29.3)	6.37 (0–275)	131.6 (48–354)	-	-
$AUC_{0-\infty}$ (ng·h/mL)	11,194 (30.7)	16,910 (36.9)	36,476 (20.9)	3.26 (2.77, 3.83)	2.16 (1.88, 2.47)
$T_{1/2}$ (h)[b]	3.82 (52.6)	3.29 (36.6)	5.05 (11.0)	1.32 (1.10, 1.59)	1.53 (1.31, 1.79)
$AUC_{0-\infty}$ MR	0.22 (27.5)	0.13 (35.2)	0.05 (19.4)	0.25 (0.21, 0.29)	0.41 (0.36, 0.47)
M-I					
Cmax (ng/mL)	591.4 (29.4)	468.2 (27.1)	233.5 (24.1)	0.39 (0.34, 0.45)	0.50 (0.44, 0.56)
C24 (ng/mL)[a]	8.08 (0–24.5)	7.48 (0–25.6)	15.9 (0–34.72)	-	-
$AUC_{0-\infty}$ (ng·h/mL)	2,484 (24.5)	2,223 (26.6)	1,986 (27.1)	0.80 (0.70, 0.92)	0.89 (0.80, 1.00)
$T_{1/2}$ (h)[b]	7.30 (51.8)	6.42 (36.5)	7.85 (17.7)	1.08 (0.89, 1.29)	1.22 (1.05, 1.43)

$AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; C24, concentration at 24 hours; CI, confidence interval; Cmax, maximum observed plasma concentration; CV, coefficient of variation; GM, geometric mean; $T_{1/2}$, terminal elimination half-life; MR, metabolite ratio.

[a] median and range. [b] not dose normalised.

Discussion

The investigation we report here of UGT genetic polymorphisms in human subjects dosed with the novel, nonthiazolidinedione, insulin-sensitizing agent sipoglitazar was prompted by observing considerable inter-subject variability in drug plasma concentration profiles as data emerged from an on-going trio of clinical pharmacology studies. Further inspection of the skewed distribution of sipoglitazar $AUC_{0-\infty}$ values suggested the presence of subpopulations. The results presented show a strong correlation between the genetic variants of UGT2B15 and the sipoglitazar exposure. Approximately two-thirds of the inter-subject variability in sipoglitazar plasma exposure is explained by UGT2B15 genetic variation and no relationship between sipoglitazar plasma exposure and variants of the other UGT enzymes was found.

UGTs, together with acetyltransferases, glutathione-S-transferases, and sulfotransferases, are responsible for the phase II biotransformation of many drugs. Amongst these enzyme families, UGTs are considered to show the most profound effects on drug elimination [5]. Examples where inter-patient differences in drug elimination may result from differences in glucuronidation rates and underlying UGT allelic variation include lorazepam [6], the toxic irinotecan metabolite SN-38 [7,8], and mycophenolic acid [9]. For S-oxazepam [10] and rofecoxib [11], provocative in vitro data show, respectively, that polymorphisms of UGT2B15 and UGT2B7/UGT2B15 differ in their activity with respect to drug and have not as yet been shown to cause inter-patient differences in drug exposure. Polymorphisms of UGT1A6 appear to exert little effect in practice on the rate of paracetamol elimination [12,13]. However, glururonides have been relatively under studied compared with the CYP mediated metabolism; the literature is divided upon the impact of this variant on enzyme function and further in vivo studies are necessary to evaluate the clinical significance [14].

Although the exposure was approximately two- to three-fold higher in the UGT2B15*2/*2 genotype than either UGT2B15*1/*1 or UGT2B15*1/*2, two outlier subjects genotyped as UGT2B15*1/*1 and UGT2B15*1/*2 were observed. These subjects genotyped as UGT2B15*1/*1 and UGT2B15*1/*2, but had considerably higher exposure than expected based on their genotype. Gene sequencing confirmed that heterozygosity for the D85Y mutation had been correctly identified on initial investigation and revealed no alternative unexpected genetic mutations. Our analysis showed that across the population UGT2B15 genotype could explain 66% of the variability of sipoglitazar exposure as determined by

$AUC_{0-\infty}$. Other factors such as age, body mass index or sex appeared to contribute little to explaining the additional variability or outlying subjects. This experience suggests that analysis of UGT2B15 genotype alone may not invariably predict the extent of individual exposure to sipoglitazar and as yet other unidentified factors may affect the clearance of sipoglitazar in subjects. In addition, the rates of glucuronidation are affected not only by genetically determined variation but also by age, gender, disease, diet, and other environmental influences [5]. The relationship between exposure to sipoglitazar and UGT2B15 genotype in phase I trials significantly accounts for the variability. The extensive sipoglitazar metabolizer phenotype associated with UGT2B15*1/*1 and the relatively poor metabolizer phenotype associated with UGT2B15*2/*2 genotype can be inferred from the results presented. Rates of drug metabolism were not directly measured; however, there was a significant decrease in the AUC metabolite ratios of UGT2B15*1/*1 or UGT2B15*1/*2 as compared with UGT2B15*2/*2, indicating reduced levels of metabolic activity associated with UGT2B15*2/*2. The conclusion that subjects with UGT2B15*2/*2 genotype metabolize sipoglitazar poorly is nonetheless consistent with the results of others who, using S-oxazepam as a substrate, have shown that the UGT2B15 D85Y variant is less active than the wild-type enzyme [10]. Potentially, the UGT2B15 D85Y variant could have a significant impact because of its high population frequency (approximately 50% of all alleles) [15]. Due to this frequency, approximately 22% of the Caucasian population is homozygous for this allele with a potentially significant impact on their ability to metabolize drugs and other chemicals by this pathway [5].

As predicted from the mechanism of action and from its pharmacological profile in animal models, sipoglitazar exerts little effect over blood glucose levels in healthy, nonobese human subjects with normal insulin sensitivity. However, following completion of the phase I clinical pharmacology studies described here, studies of sipoglitazar have been completed in patients with T2DM. All patients taking part in these studies were genotyped for UGT2B15 prospectively to assess the clinical relevance of variants of this enzyme [16].

In summary, it is clear that the activity of UGT2B15 transferase is important for the elimination of sipoglitazar, and that individual exposure to sipoglitazar is dependent on the differential activity of naturally occurring enzyme variants.

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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.

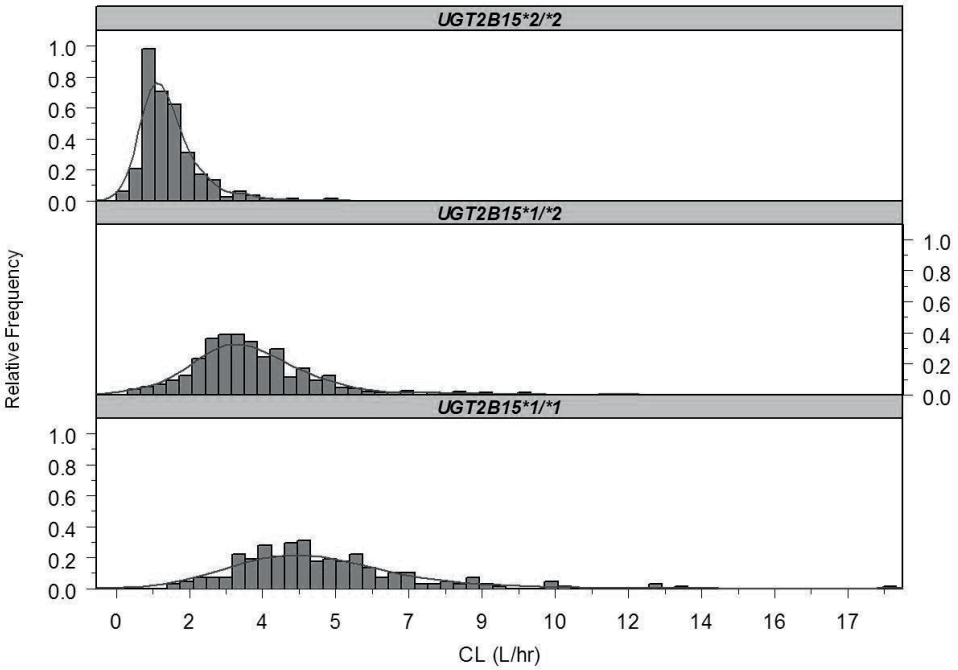
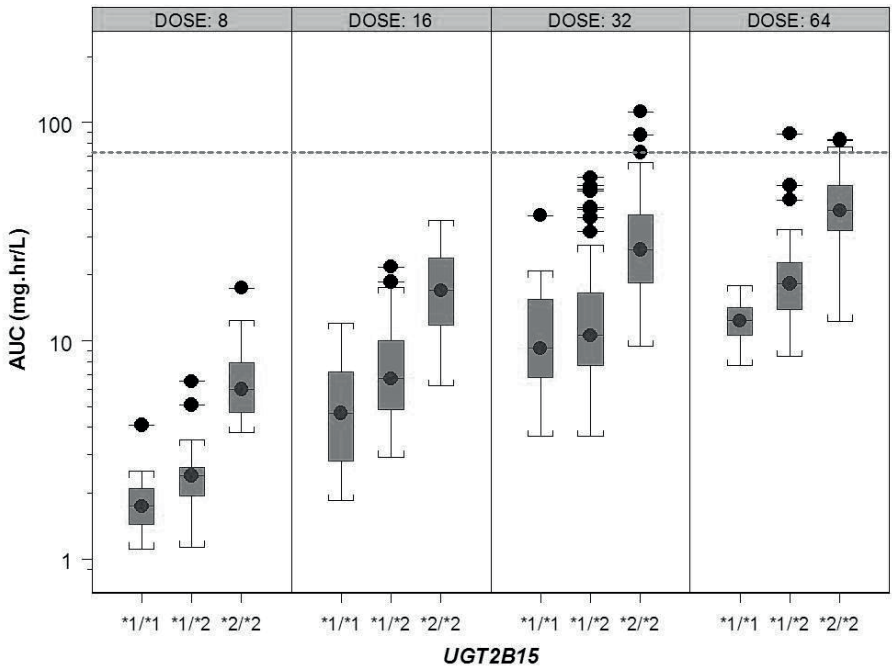


Figure 2. Box plots (median, 25th and 75th percentiles) for the estimated area under the concentration-time curve from 0 to 24 hours (AUC) by genotype and dose. Gray line exposure limit 73 mg·hr/L.



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)).

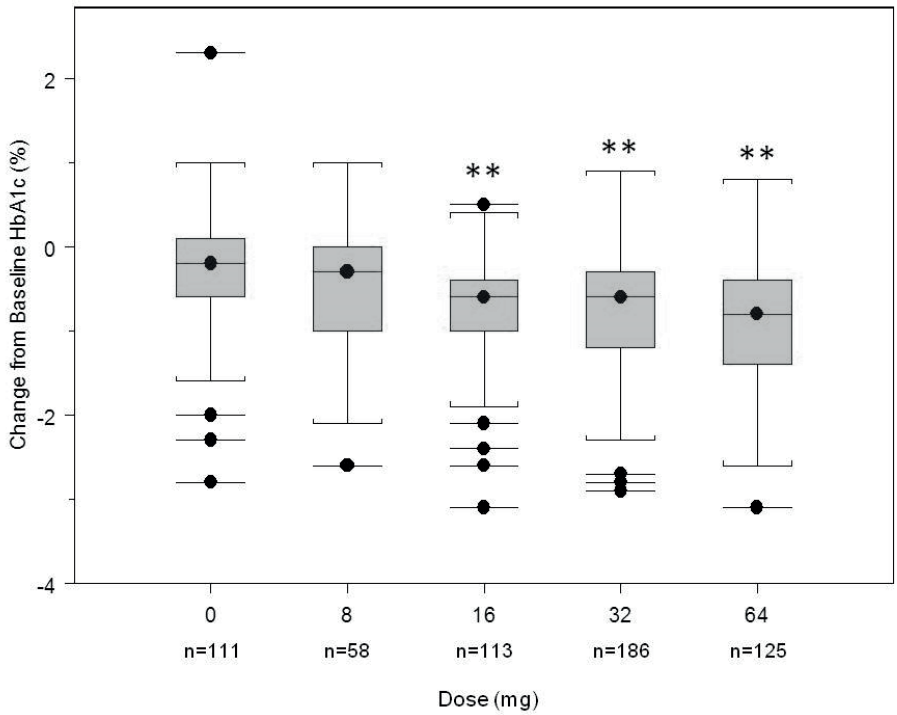
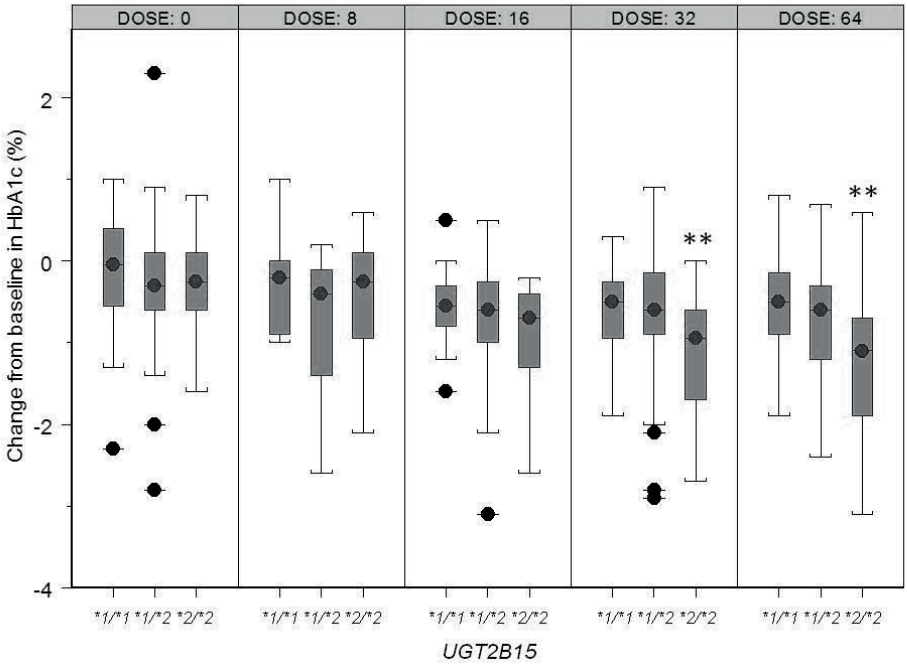


Figure 3.(b) Box plots (median, 25th and 75th percentiles) for the change from baseline in HbA1c by genotype and dose. **=statistically significant at $P < .05$.



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.

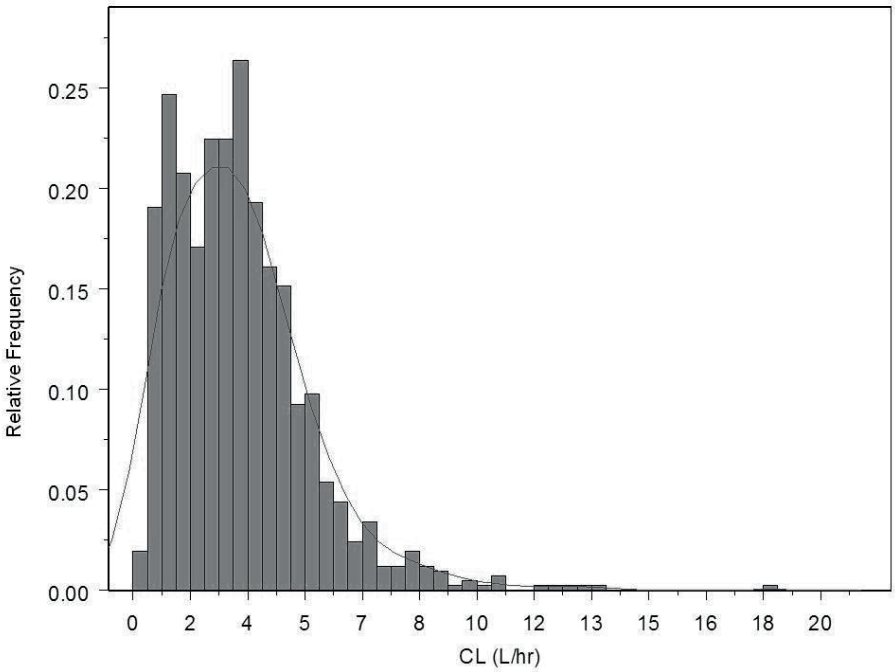


Figure S2.(a) Visual predictive check for observed and predicted single dose (64 mg) data for 006.

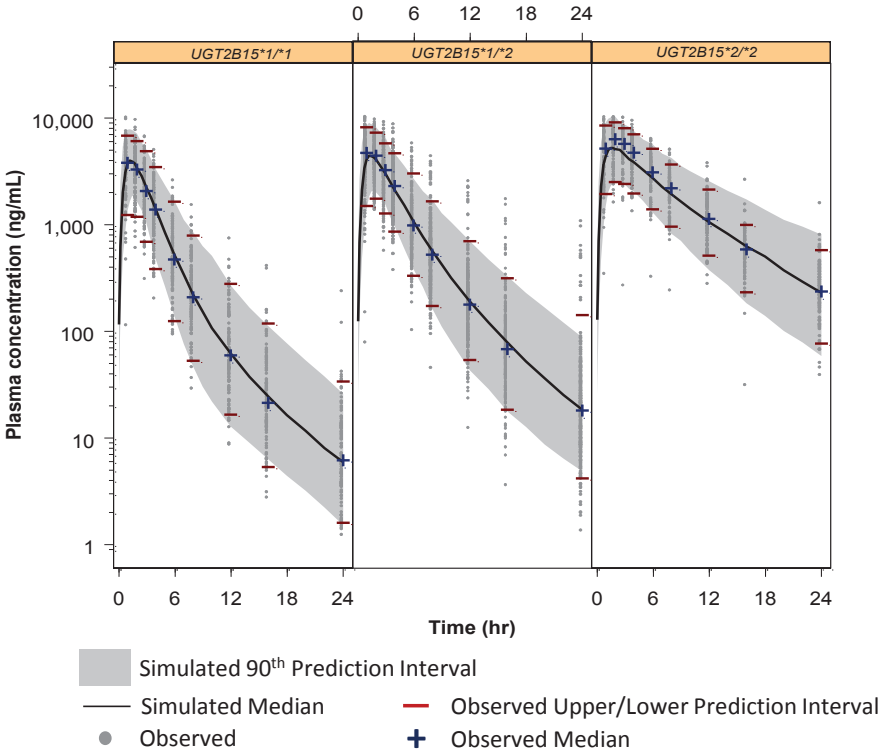
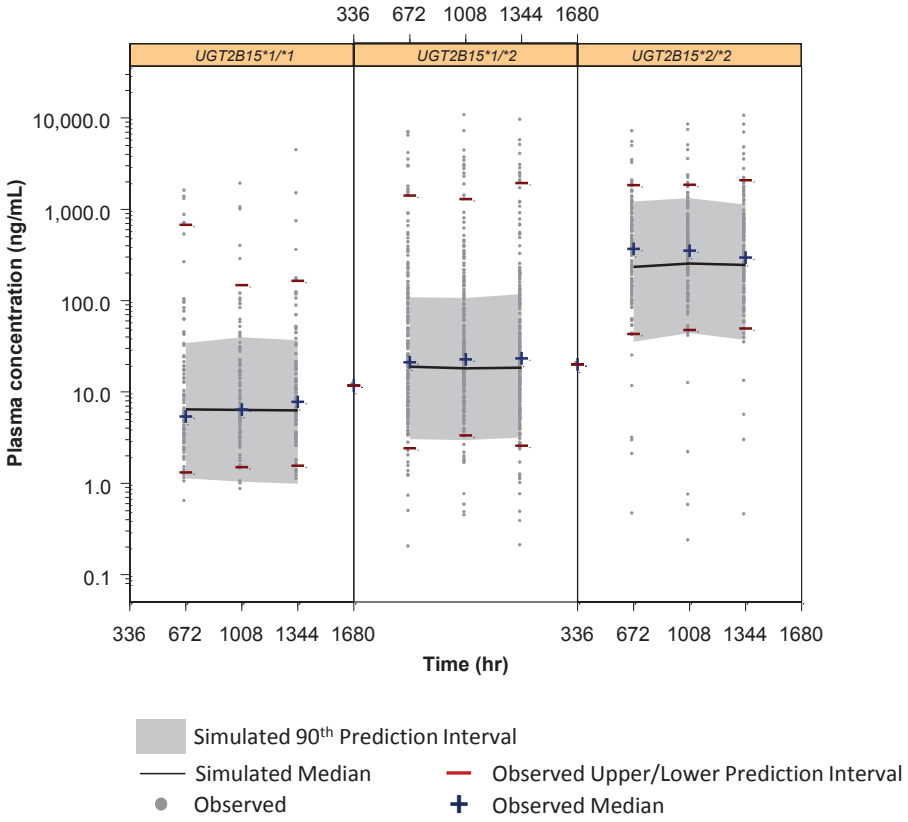


Figure S2.(b) Visual predictive check for dose normalized observed and predicted data from the combined phase II trials (EC201 and EC202)



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

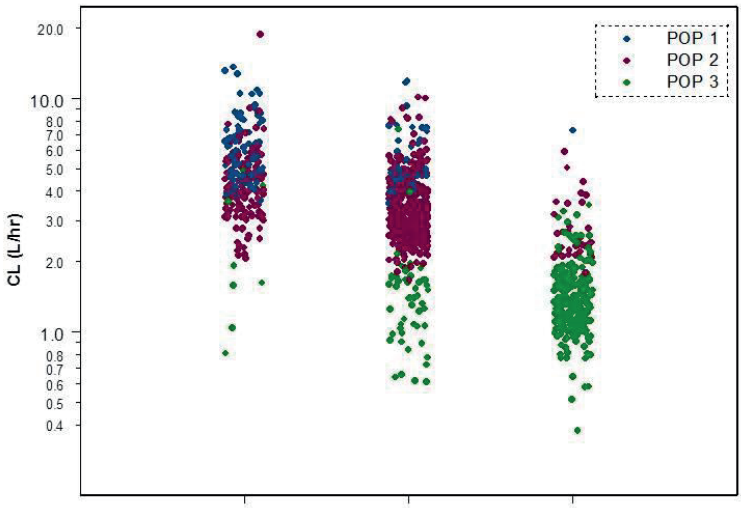
POP3 (CL3 PM) = UGT2B15*2/*2

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.

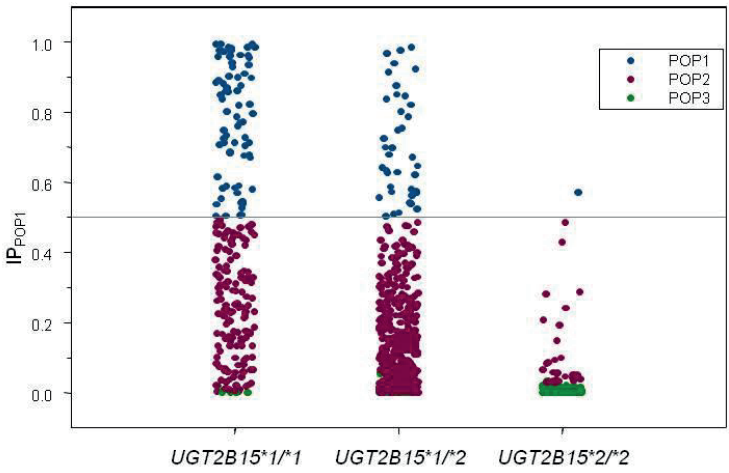


Figure S4(b) The individual probablity (IP) for subjects assigned to mixture 2 by genotype and population.

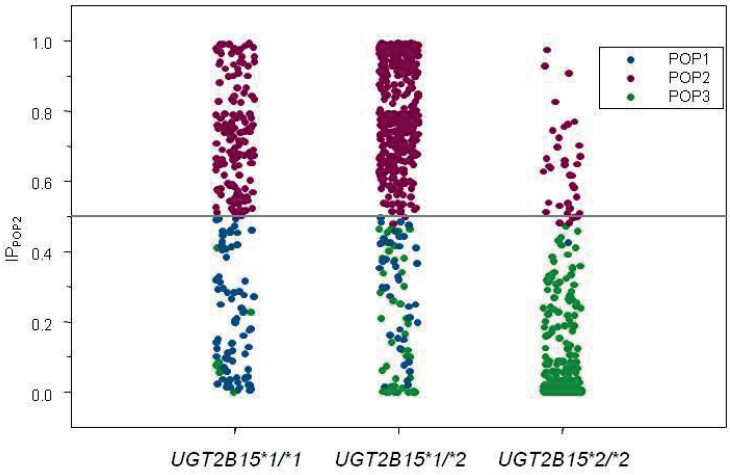


Figure S4(c) The individual probablity (IP) of beloning to mixture 3 by genotype and population.

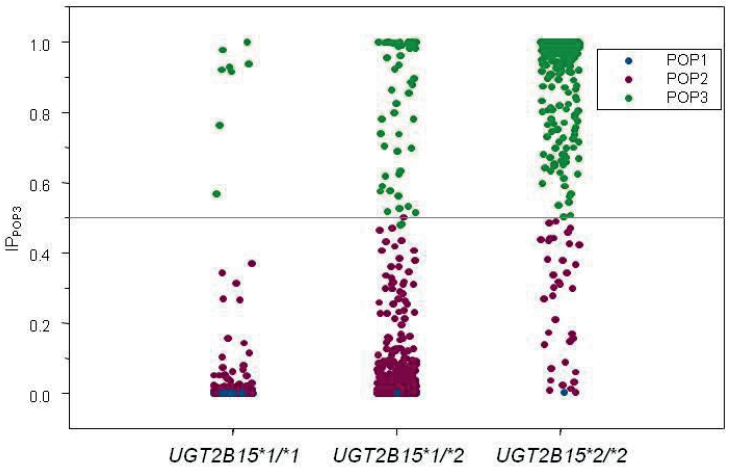


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Table AI. Summary of parameter estimates for the mixture model including covariates

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

Chapter 5

A Model-Based Approach to Analyze the Influence of UGT2B15 Polymorphism Driven Pharmacokinetic Differences on the Pharmacodynamic Response of the PPAR Agonist Sipoglitazar

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Abstract

The pharmacokinetics of sipoglitazar, a peroxisome proliferator activated receptor agonist, are subject to high inter-individual variability resulting from a polymorphism of the UGT2B15 genotype. The aim of the current analysis was to apply a PK-PD model-based approach to evaluate the influence of UGT2B15 driven pharmacokinetic differences on the clinical response. Efficacy and safety of sipoglitazar compared to placebo were assessed in Type 2 Diabetes Mellitus patients in two Phase II randomized, double-blind studies (sipoglitazar QD: 8, 16, 32 or 64 mg; sipoglitazar BID: 16 or 32 mg; rosiglitazone 8mg QD and placebo for 13 weeks) (n=780). Changes in fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) levels over time were described as a function of individual drug exposure using a simultaneous, cascading indirect response model structure. The effects on FPG and HbA1c could successfully be described for placebo, rosiglitazone and sipoglitazar treated groups in all three UGT2B15 genotypes. Differences in drug effects between genotypes were fully explained by differences in drug exposure. The current PK-PD analysis confirms that UGT2B15 genotype is a major determinant for differences in FPG and HbA1c response to sipoglitazar treatment between Type 2 Diabetes mellitus patients, due to related differences in drug exposure.

Introduction

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) as a next generation insulin sensitizer. The compound undergoes Phase II biotransformation by conjugation through UDP-glucuronosyltransferase (UGT) [1]. Following a population pharmacokinetic analysis, UGT2B15 genotype was found to be a covariate for the clearance (CL) of sipoglitazar both in healthy subjects and T2DM patients [2]. Higher plasma exposure of sipoglitazar was observed in the UGT2B15*2/*2 genotype than subjects homozygous for the wild-type allele UGT2B15*1/*1 (3.3-fold higher) or heterozygous allele UGT2B15*1/*2 (2.2-fold higher) [3].

T2DM is a complex multi-factorial disease and current therapies target a range of disease pathways, promoting insulin secretion or improving insulin sensitivity [4]. Many of these drugs involve upward titration to effect, based on glycemic targets and/or the addition of combination therapy [5]. Titration based approaches are also applied to limit adverse events such as hypoglycemia or weight gain [6]. Individualized dosing is widely applied and responder rates to treatment may depend on factors such as the duration of the disease or prior anti-diabetic medication, although response rates can be variable and difficult to predict [7]. Consideration should be given not only to factors directly affecting the pathology of the disease but also to those which influence the plasma exposure of these drugs such as genetic polymorphisms which may be additional covariates for clinical response [8]. Genetic polymorphisms in the enzyme responsible for the metabolism of several glucose lowering drugs including rosiglitazone (CYP-2C8) and glimepiride (CYP-2C9) have been identified [9]. For rosiglitzone, 1.5 fold higher clearance was observed in the CYP2C8*3/*3 subjects compared with wild-type (CYP2C8*1/*1) carriers [10]. Whilst in healthy Korean subjects clearance of glimepiride was 1.6 fold higher in CYP2C9*1/*1 subjects than in CYP2C9*1/*3 subjects [11]. However the clinical relevance of these differences in pharmacokinetics has not resulted in specific genotype based dosing recommendations [12].

The implementation of a PK-PD model-based approach to evaluate the influence of genotype provides a more comprehensive link between changes in the pharmacokinetics and its

influence on the magnitude of response [13,14]. *In vitro–in vivo* extrapolation may also be useful when considering the impact of any potential polymorphism and to help define the exposure response relationships [15]. Currently the application of a model-based approach to evaluate the influence of different genotypes, by linking pharmacokinetic changes with response seems limited to only a few drugs. Warfarin has been extensively studied due to its wide use, narrow therapeutic range and large inter patient variability, all making response unpredictable [16]. However genetic data are now an integral part of the drug development program and a model-based approach can aid in addressing the complex nature of the relationship not only between genotype and PK but also including clinical response.

The present population PK-PD analysis was performed to evaluate the role of UGT (UGT2B15*1/*1, UGT2B15*1/*2, and UGT2B15*2/*2) driven exposure differences on the pharmacodynamic response for sipoglitazar in both fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c).

Methods

Subjects and Data Collection

Data from 2 Phase II trials conducted over 13 weeks in T2DM patients were included in this analysis. The baseline patient characteristics are summarised in Table S1. Patients were treated with sipoglitazar, rosiglitazone or placebo: sipoglitazar 8mg QD, 16mg QD, 16mg BID, 32mg QD, 32mg BID and 64mg QD, placebo or rosiglitazone 8mg QD. Serial blood samples for FPG and HbA1c were collected throughout the study (-1, 0, 2, 4, 6, 8, 10 and 12 weeks). The disposition of subjects was as follows: Sipoglitazar n=572, rosiglitazone n=72 and placebo n=136 (total = 780). All subjects were provided with dietary advice for the entire duration of the trial. The three main inclusion criteria were drug naïve patients with a diagnosis of type 2 diabetes, an HbA1c of >7.0% and <10.0% at Screening and age >35 years and <75 years.

An accredited central laboratory (Medical Research Laboratories International, Brussels, Belgium) was responsible for the analysis of samples for FPG and HbA1c. 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.

Population Data Analysis

Changes in FPG and HbA1c levels over time were described as a function of individual drug exposure using a simultaneous, cascading indirect response model structure. The model was parameterised in terms of a zero order rate constant for the production of FPG (KinG) and a first-order rate constant for the removal of FPG (KoutG). Changes in HbA1c were modelled as secondary changes to FPG, with a first order rate constant (KinH) and a first order HbA1c degradation rate constant, KoutH. As reported by Hamren et al, the relationship between FPG and HbA1c was found to be non-linear, and HbA1c was described as a function of FPG using a power function (FPG^{γ}) [17].

A lower FPG baseline value was observed in the 32mg BID sipoglitazar group compared to all other treatment groups. The addition of a separate FPG baseline for this group was included in the model.

The drug effect (DEF) was incorporated using an Emax model driven by AUC, where Emax is the maximal effect and AUC50 is the AUC0-24h at steady state achieving half the maximal response, implemented with the following equation:

$$DEF = Emax \cdot AUC/AUC50 + AUC \qquad EQ(1)$$

The individual PK parameters derived from a previous analysis were used to calculate individual exposure, AUC (AUC=dose/CL) over the dose interval at steady state [2]. The drug effect was evaluated for FPG on both KinG and KoutG.

In the rosiglitazone group, no plasma concentration data were collected during the treatment period and as such the treatment effect for rosiglitazone (ROTE) was included using a stimulatory step function on KoutG. The FPG data in the placebo group on average showed no change over time. However at the individual level a lifestyle effect in both placebo and actively treated subjects for FPG (LEFPG) was observed. This effect was described by an additive random effect, capturing both the positive effects of intervention due to diet or

exercise and the negative effects in subjects who showed a loss of glycemic control. HbA1c in the placebo group however, showed on average a gradual decease in HbA1c over time. This reduction in HbA1c was found to be independent of the lifestyle effect identified on FPG and a step function directly inhibiting KinH could be identified for this direct lifestyle effect on HbA1c (LEHB). A difference was also found in LEHB between active and placebo arms. For the active groups this was described relative to the placebo group as shown in EQ(2).

$$LEHBactive = LEHBplacebo - LEHBfactor \quad \text{EQ(2)}$$

The overall model structure for FPG and HbA1c is shown in EQ(3) and EQ(4) respectively.

$$\frac{dFPG}{dt} = KinG \cdot (1 \pm LEFPG) - KoutG \cdot (1 + DEF + STEF) \cdot FPG \quad \text{EQ(3)}$$

$$\frac{dHbA1c}{dt} = KinH \cdot (1 - LEHB) \cdot FPG^{\lambda} - KoutH \cdot HbA1c \quad \text{EQ(4)}$$

In EQ(3) LEFPG is included as a additive random effect with the structural parameter fixed at zero.

To explore any potential differences between daily dosing regimens a different AUC50 value was tested between the BID and QD groups.

Intra-individual Variability and Residual Error

Intra-individual variability (IIV) on FPG baseline was explored assuming a log normal distribution of the individual parameter estimates. However IIV on the baseline for HbA1c was evaluated using a Box-Cox transformation model, which was applied to account for the skewness observed in the individual data [18]. Residual variability was included using a proportional model. The correlation between IIV on baselines was included using the OMEGA BLOCK option. Genotype information was not collected in 10% of the population however these subjects were included in the analysis using an average clearance value for the population.

Covariate Analysis

Potential covariates (Age, sex, weight and duration of disease) were evaluated in the model using a forward inclusion and backward elimination procedure [19].

Data Analysis

All population analyses were performed using nonlinear mixed effects modeling on pharmacodynamic data in the NONMEM software package (version 7, release 1; Icon Development Solutions, Ellicott City, Maryland) and analyzed using the statistical software package S-Plus for Windows (version 6.2 Professional, Insightful Corp, Seattle, Washington). The first-order conditional estimation method with interaction was used with ADVAN6 (general nonlinear model). Berkeley Madonna version 8.3.13 (Macey & Oster, University of California, Berkeley) was used to perform simulations of the time profile for FPG and HbA1c.

Model Qualification

The visual predictive check (VPC) was used to evaluate the ability of the model to predict both the central tendency and the variability of FPG and HbA1c (median and 90th prediction interval) [20]. The VPC for the sipoglitazar treatment groups was performed using the overall proportion of subjects in each genotype (UGT2B15*1/*1=21%, UGT2B15*1/*2=51%, and UGT2B15*2/*2=28%). The clearance values used for each genotype are shown in Table 1.

The stability of the model was tested by using 500 bootstrap replicates of the original dataset. To further inform model validation ETA shrinkage was estimated for all the random effects [21].

Model Selection

No further improvement in the model was considered by a change in objective function of less than 10.8 ($P \leq 0.001$) [19]. In addition to this graphical analysis enabled assessment of bias and the biologically plausibility of the parameter estimates was evaluated.

Evaluation of the Influence of Genotype on FPG and HbA1c Treatment Effects

Simulation of FPG and HbA1c Time Course

To evaluate the influence of genotype on the time profiles for both FPG and HbA1c these data were simulated over a one year time period. Using this data the change from baseline in FPG and HbA1c at 6 months was determined since this could be the primary endpoint for a Phase III trial. Rosiglitazone 8mg QD and sipoglitazar 64mg for all three genotypes were simulated.

Simulation of Clinical Response at 6 months

The model was used for clinical trial simulation to evaluate the impact of genotype on glycemic responder rates, an additional endpoint to allow further appreciation of response to treatment. Two approaches were compared using either pre-selection of dose based on genotype or a single dose level for all subjects. For genotype driven dose selection, three dosing approaches were compared. The doses were selected to target comparable drug exposure levels in each genotype group. Dose assignment was based on the population estimated CL values for each genotype. The dose levels and CL values are shown in Table 1. Clinical response was defined as the % of subjects achieving a HbA1c reduction >0.7% at 6 months [22]. 100 subjects were simulated for each genotype and 300 subjects for the single treatment. For reference, rosiglitazone 8mg QD (n=300 subjects) response was simulated.

Evaluation of genotyped-based and titration-based dosing approaches

The potential differences between genotyped-based dosing, in which subjects already begin their treatment at the optimal dose and titration-based dosing, in which subjects start at a fixed dose and are subsequently titrated to effect was evaluated through simulation. For genotyped-based dosing, Design C (UGT2B15*1/*1=96mg, UGT2B15*1/*2=64mg and UGT2B15*2/*2=32mg) was used and subjects would begin treatment at each of the dose levels depending on their UGT2B15 genotype.

For titration based dosing, all subjects would receive the same 32mg dose level at the start of treatment. Subjects could be titrated up to a maximum of 96mg using 32mg and 64mg tablets. The FPG value was assessed every two months. For those subjects in the UGT2B15*2/*2

genotype, 32mg already represents the optimal dose for this group based on the low drug clearance. Therefore simulations using only the UGT2B15*1/*1 and UGT2B15*1/*2 genotype were performed

Results

PK-PD model

The developed indirect response model adequately described the change in FPG and HbA1c over time for all treatment groups (Figure 1a and Figure 1b). The model parameters are shown in Table 2 along with the bootstrap estimate. During conduct of the bootstrap, 92.6 % of runs minimized successfully. The parameter estimates from the model were consistent with those estimated from the bootstrap and all of the parameters from the final model were within the 95% confidence interval of the bootstrap estimate supporting the robustness and stability of the model. No substantial ETA shrinkage was observed (<12%), and all CV% for each parameter was less than 35%.

Figure 1. (a) Visual predictive check for observed and predicted FPG data.

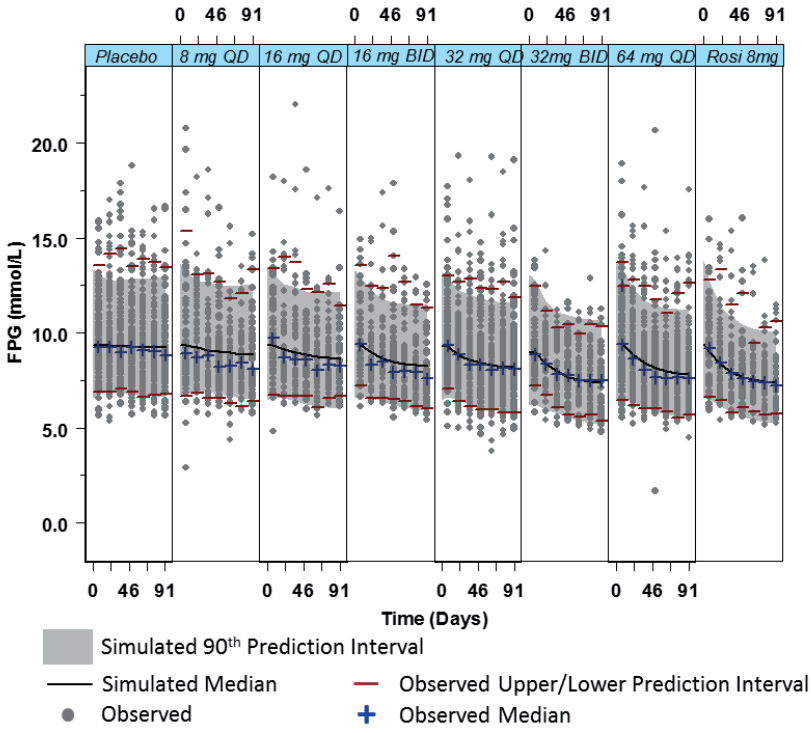
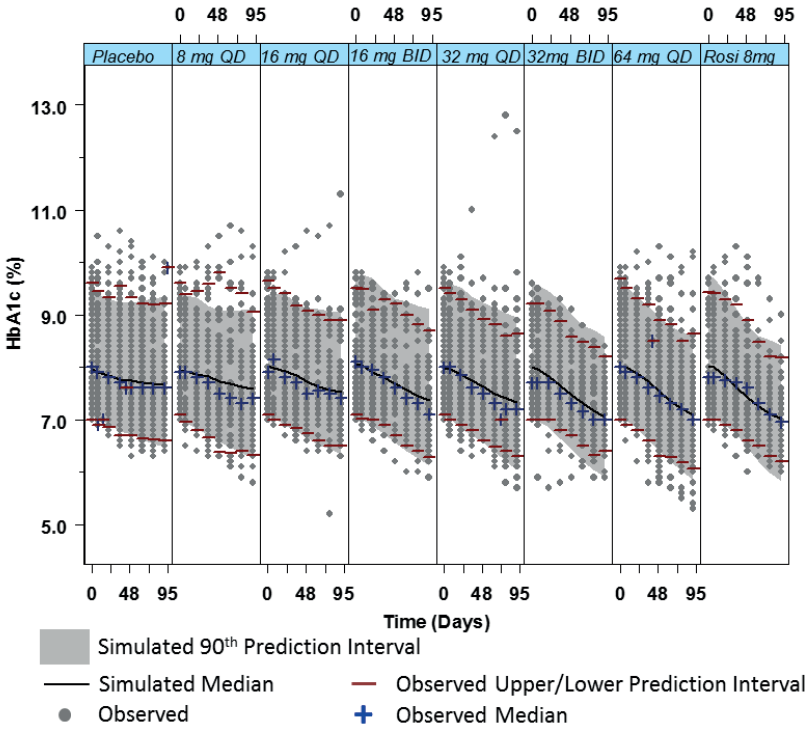


Figure 1. (b) Visual predictive check for observed and predicted HbA1c data.



The inclusion of the Box-Cox transformation on IIV for the baseline on HbA1c (BSLH) resulted in a significant ($P \leq 0.001$) change in the objective function. The inclusion of the Box-Cox transformation on the baseline for FPG (BSLG) did not result in a significant improvement in the model; this is likely a result of the screening criteria (HbA1c>7%) being based solely on HbA1c levels.

Histogram plot for FPG and HbA1c showing the model fitted baseline distributions are presented in supplemental Figures S1 and S2, respectively.

No bias was observed in the diagnostic plots by genotype for HbA1c or FPG. These are shown in supplemental Figures S4-S6 and S7-S9, respectively.

Drug effect model

The effect of sipoglitazar was included as a stimulatory effect acting on KoutG. The Emax of this effect was estimated at 49% and AUC50 was 1.2 mg.day/L. This Emax is similar to that reported from other PPAR agonists, 41% and 43% [17,23]. Treatment effect (ROTE) for rosiglitazone was also included as a stimulatory effect on KoutG with a population mean value of 28% at the studied dose level. The relationship between FPG and HbA1c could be described using a power function with an estimated slope of 0.7. The estimated Kout value for both FPG and HbA1c was 0.027 days-1 and 0.031 days-1 respectively, this is consistent with the values reported by other studies [24].

Using the model, the overall effect of sipoglitazar treatment (Drug + placebo) at 3 months was derived and is shown in comparison to the rosiglitazone group in Figure S3. The median value and associated 25th and 75th percentiles for the 8mg QD rosiglitazone treatment effect relative to the sipoglitazar groups shows that a total daily dose of at least 64mg for sipoglitazar (for all genotypes) would be needed to achieve comparable treatment response. For the different genotype groups the treatment effect was found to increase in the order UGT2B15*2/*2 > UGT2B15*1/*2 > UGT2B15*1/*1.

Lifestyle effect model

The lifestyle effect for FPG (LEFPG) was zero for the mean population but individual effects were observed and implemented in the model using an additive random effect. A lifestyle effect on HbA1c (LEHB) was best described by an effect on KinH during the course of the study. This effect was independent of the drug treatment effects which were carried over from changes in FPG to HbA1c. The lifestyle effect was found to be lower in actively treated groups compared to the placebo group. Specifically, a population mean decrease of 3.7% was identified for the lifestyle effect in the placebo group, whilst in the actively treated groups the reduction was slightly lower, 2%. No significant ‘lifestyle’ effect on HbA1c could be identified on the rosiglitazone group.

Covariate Analysis

Potential covariates on IIV were evaluated, during forward inclusion for BSLG, BSLH, LEFPG and LEHB. However neither sex, weight, duration of disease or age had a significant effect on any of these parameters and no covariates were retained in the final model.

Evaluation of the Influence of Genotype on FPG and HbA1c Treatment Effects

Simulation of FPG and HbA1c Time Course

Based on the simulated time profiles for sipoglitazar by genotype and for rosiglitazone over a one year period, 90% of steady state for FPG and HbA1c is expected to be reached at approximately 2.3 months and 3.7 months, respectively (Figure 2a and 2b).

Figure 2. (a) Simulated FPG over time for sipoglitazar 64 mg by genotype and rosiglitazone (8mg QD).

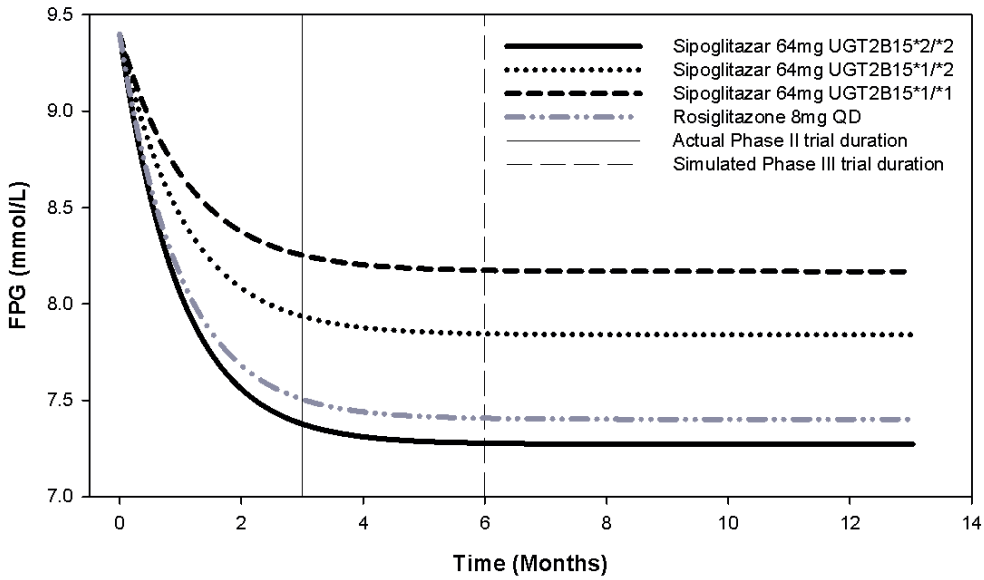
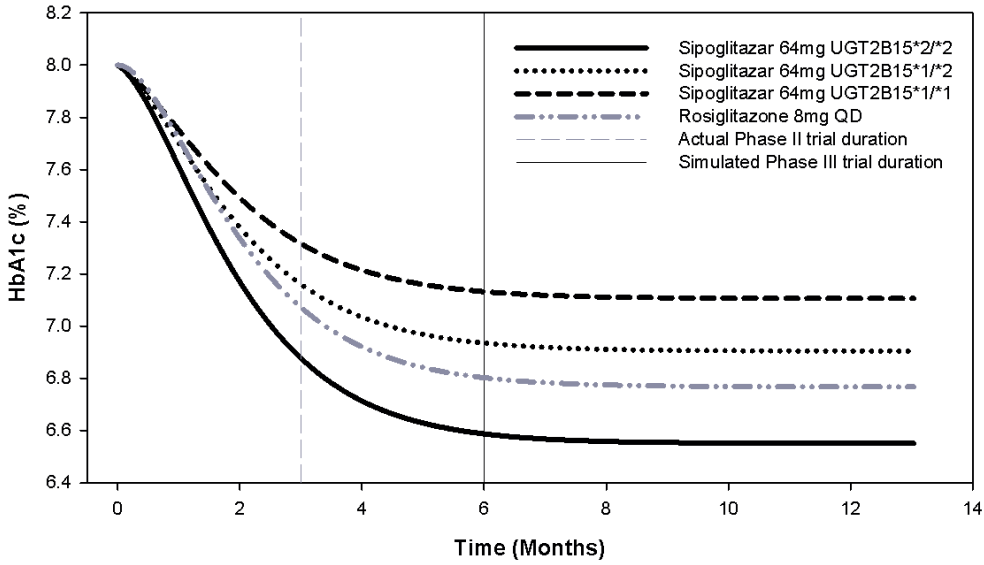


Figure 2. (b) Simulated HbA1c over time for sipoglitazar at 64 mg by genotype and rosiglitazone (8mg QD)

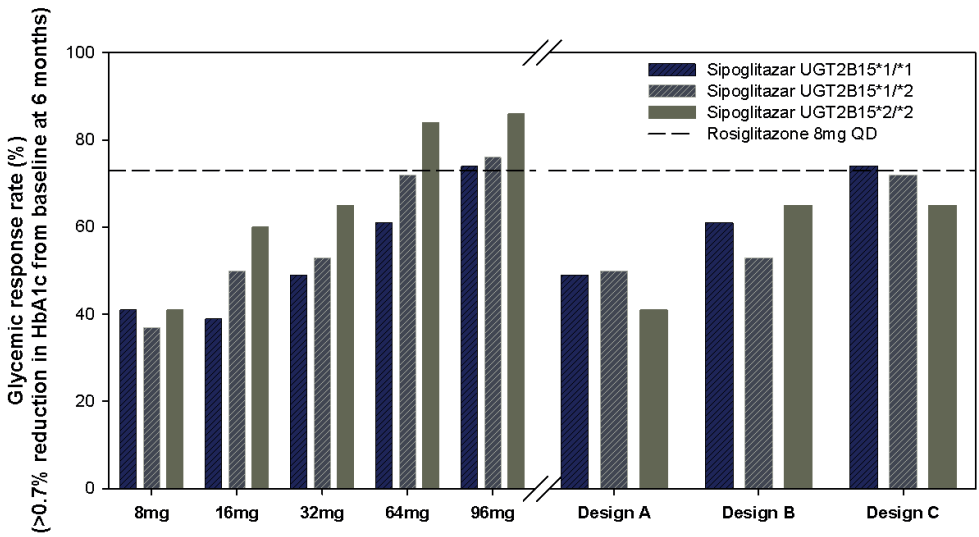


The simulated median change from baseline in FPG for a total daily dose of 64mg sipoglitazar at 6 months was -1.2 mmol/L, -1.6 mmol/L and -2.1 mmol/L for UGT2B15*1/*1, UGT2B15*1/*2 and UGT2B15*2/*2 genotypes respectively. The same trend is observed for HbA1c, the median change from baseline by genotype was -0.9%, -1.1% and -1.4% for the UGT2B15*1/*1, UGT2B15*1/*2 and UGT2B15*2/*2 genotypes, respectively. For the reference treatment rosiglitazone 8mg QD, predicted changes in FPG and HbA1c at 6 months were -2.0 mmol/L and -1.2%, respectively.

Simulation of Clinical Response at 6 Months

The percentage of patients achieving a reduction in HbA1c > 0.7% at 6 months for doses of 8, 16, 32, 64 and 96 mg by genotype were simulated and are shown in Figure 3.

Figure 3. Bar graph for the % of responders (>0.7% reduction in HbA1c at 6 months) by dose and genotype. Design A (UGT2B15*1/*1=32mg, UGT2B15*1/*2=16mg and UGT2B15*2/*2=8mg) Design B (UGT2B15*1/*1=64mg, UGT2B15*1/*2=32mg and UGT2B15*2/*2=16mg) Design C (UGT2B15*1/*1=96mg, UGT2B15*1/*2=64mg and UGT2B15*2/*2=32mg)



A comparable result to the 8mg rosiglitazone treatment was obtained for all genotypes at a dose level of 96 mg sipoglitazar (single dose approach). For genotyped-based dose assignment this was achieved in all three genotypes using Design C (UGT2B15*1/*1=96mg, UGT2B15*1/*2=64mg and UGT2B15*2/*2=32mg) with lower doses being administered to the UGT2B15*1/*2 and UGT2B15*2/*2 groups than with the single dose approach. Within Design C the percentage of subjects achieving a target reduction in HbA1c were 74, 72 and 65% for the UGT2B15*1/*1, UGT2B15*1/*2 and UGT2B15*2/*2 groups respectively, as compared to 73% for rosiglitazone.

Evaluation of genotyped-based dosing and titration-based dosing approaches

Figures 4a and 4b show the response for FPG and HbA1c over a 12 month period for genotyped and titration-based dosing approaches for the UGT2B15*1/*1 and

UGT2B15*1/*2 genotypes respectively. For the UGT2B15*1/*1 genotype, FPG at 2 months was 8.8 mmol/L and 8.1 mmol/L for the titration and genotyped based dosing respectively. At 2 months in the titration-based approach the dose was increased from 32mg to 64mg. At 4 months the median value for FPG was 8.3 mmol/L in the titration-based group and a further titration step up to 96mg was included.

Figure 4. (a) Simulated FPG and HbA1c profiles for genotyped and titration-based dosing approaches for UGT2B15*1/*1.

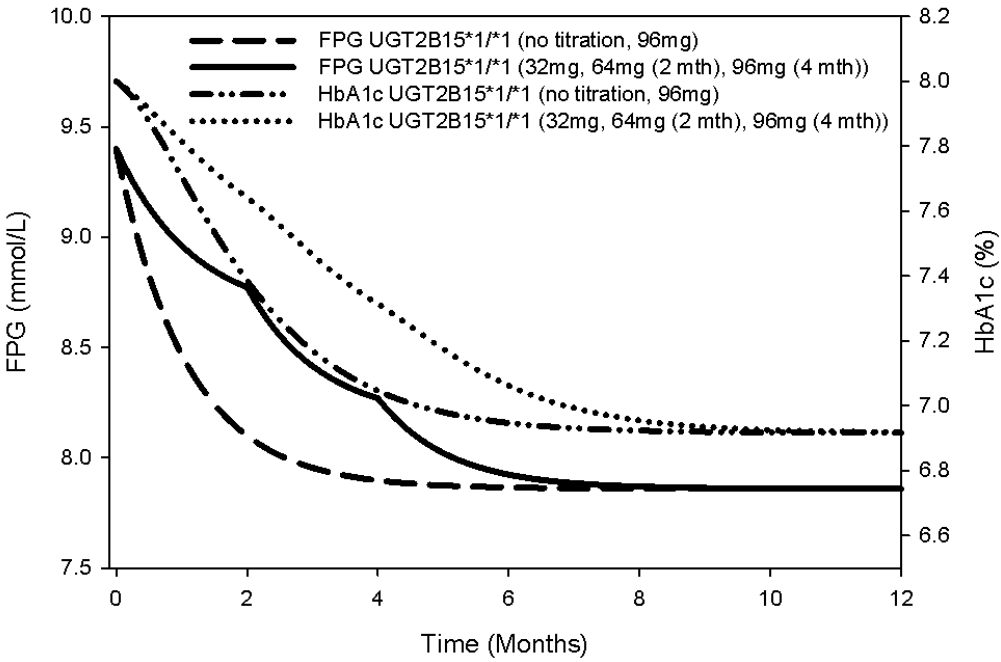
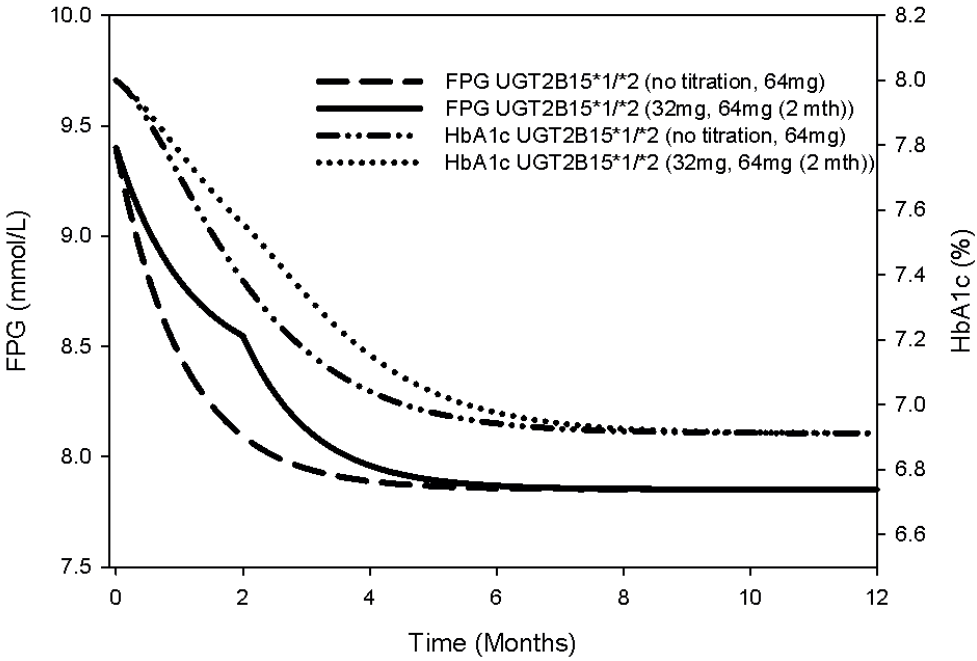


Figure 4. (b) Simulated FPG and HbA1c profiles for genotyped and titration-based dosing approaches for UGT2B15*1/*2.



For genotyped based dosing, where this subject receives 96mg from the start a stronger reduction in FPG was observed over the first 6 months of treatment. The difference in FPG between the two approaches at 2 months and 4 months was -0.7 mmol/L and -0.4 mmol/L. The time to achieve 90% of FPG steady state was approximately 2.4 months and 4.6 months for genotype and titration-based dosing respectively

For the UGT2B15*1/*2 genotype, only one titration step was included at 2 months from 32mg to 64mg. The value for FPG at 2 months was 8.6 mmol/L and 8.1 mmol/L for the

titration and genotype designs respectively. For FPG, 90% of steady state for the two approaches was reached at approximately 1.7 and 2.7 months.

Discussion

A PK-PD model-based approach was applied to simultaneously evaluate changes in FPG and HbA1c in type 2 diabetic patients with no prior exposure to anti-diabetic medication. The aim of our analysis was to characterize the relationship between differences in exposure due to genotype and the clinical response using FPG and HbA1c data as biomarkers. After accounting for genotype-related differences in exposure, a single unique PK-PD relationship was found to apply to the entire patient population. We found that genotype driven differences in exposure resulted in differences in clinical response for both FPG and HbA1c. The application of genotype-based dosing was found to normalize the differences in HbA1c response whilst minimizing the potential for over exposure of the drug in the UGT2B15*1/*2 and UGT2B15*2/*2 genotypes.

The mean influence of genotype on FPG and HbA1c response was simulated over a one year time period. Based on these simulations, greater reductions in both FPG and HbA1c were observed in the UGT2B15*2/*2 genotype as compared to the other groups. At a dose of 64mg for sipoglitazar, the values of the HbA1c decrease at 6 months were -0.9% versus -1.4% for the UGT2B15*1/*1 and UGT2B15*2/*2 genotype respectively. This may translate into clinically meaningful differences, since reducing hyperglycemia improves morbidity and mortality in T2DM patients [25]. Long term studies (10 years in duration) showed that a 1% reduction in HbA1c was associated with a reduction in risk of 21% for any end point related to diabetes, of 21% for deaths related to diabetes, of 14% for myocardial infarction, and of 37% for microvascular complications [26]. Furthermore a 0.5% decrease in HbA1c could avert 10% of cardiovascular complications over the course of 5 years [27]. Subjects with the UGT2B15*1/*1 genotype, would have the potential to gain clinical benefit from individualized dosing in order to achieve further reductions in HbA1c.

A simulation study was performed to evaluate the utility of genotype based dosing to account for the differences in exposure due to genotype. The number of subjects achieving a HbA1c

reduction >0.7% at 6 months was used for evaluation. Based on these results to achieve equivalence to the reference PPAR agonist rosiglitazone, for all subjects irrespective of genotype, a dose of 96mg of sipoglitazar would be required. However using genotype based dosing uniform response rates could be achieved with lower doses for the UGT2B15*2/*2 and UGT2B15*1/*2 groups. The responder rates of genotype design C (UGT2B15*1/*1=96mg, UGT2B15*1/*2=64mg and UGT2B15*2/*2=32mg) were comparable to that of 8mg rosiglitazone. As previously described some outlying subjects had considerably higher exposure than expected based on their genotype [3]. For these outlying subjects for whom there is disconnect between genotype and exposure a therapeutic drug monitoring approach or further safety evaluation at higher exposures would probably still be needed even though genotype based dose selection is a valid approach to improve HbA1c reduction.

Through simulation, genotype and titration based-dosing approaches were compared. As shown in Figures 4a and 4b the advantage for genotype dosing is that glycemic control can be achieved in a shorter time duration. The difference in the time to 90% of steady state between genotyped and titration-based dosing was approximately 1 and 2 months for the UGT2B15*1/*2 and UGT2B15*1/*1 genotypes. However, ultimately the magnitude of FPG reduction achieved for the two approaches would be expected to be the same. The biggest impact of genotyped-based dosing seems to be observed for those subjects with UGT2B15*1/*1. In the Caucasian population the frequency for UGT2B15*1/*1 is reported between 19-22% which is consistent with our own studies [28]. However in Asian American and Japanese American subjects the frequency was reported as 47% and 100% respectively [28]. Therefore in the Asian population the impact of this polymorphism would be greater on a larger percentage of the population and may have the potential to impact on any future clinical trial results if genotype frequency information was not collected or considered. Those subjects with UGT2B15*1/*1 would have a lower clinical response if only a fixed dose approach was used or a longer time to maximum effect if titration based dosing was applied. Newly approved therapies such as dipeptidyl peptidase 4 inhibitors have no requirement for dose titration to effect included in the label, which is in contrast to older drugs such as

rosiglitazone [29]. Therefore the impact of genotyping and dose titration for this drug would have to be considered in relation to new and upcoming therapies that do not require titration to effect.

There are several limitations of our model. During the trial no PK data were collected for rosiglitazone. In addition the 6 month simulation is based only on trial data up to 3 months both of which may increase the uncertainly in our extrapolations. However, simulated changes in FPG and HbA1c at 6 months for rosiglitazone 8mg total daily dose, based on the currently developed model, were fairly comparable to those previously reported in T2DM patients over the same time period. The mean change from baseline for rosiglitazone 4mg twice daily for FPG and HbA1c was reported as -3.0 mmol/L and -1.5%, respectively [30]. Simulated responder rates were slightly higher than those reported using the same criteria, 54% (actual) vs. 73% (simulated) [31]. However a direct comparison may be confounded by differences in the patient baseline characteristics and enrolment criteria.

A non-linear relationship between FPG and HbA1c was observed. This is consistent with other reports, and was best described with the use of a power function with a value of 0.7. This value is comparable to a previous PK-PD analysis with tesaglitazar, where this value was reported as 0.7 [17]. In addition a reduction in HbA1c that was visibly observed in the placebo group could also be identified in active treatment. This effect was found to be independent of changes in FPG. It is hypothesized that this disconnect between FPG and HbA1c may result from the contribution of post-prandial glucose (PPG) since the value of HbA1c is the result of both fasting and postprandial hyperglycemia [32]. Ozmen et al also showed that mean plasma glucose (the arithmetic mean of FPG and PPG) may better correlate with HbA1c [33]. This relationship between average glucose and HbA1c was explored using a semi-mechanistic model-based approach by Garcia et al. However, a model-based approach incorporating both post-prandial and fasting plasma glucose simultaneously with HbA1c may offer further insight into the complex relationship between HbA1c and glycemia.

In conclusion, we show how the genotype effect on the PK does translate to differences in FPG and HbA1c response and this could be addressed with a genotype-based dosing

approach. A model-based analysis should be performed to elucidate these genotype differences while considering the other components affecting clinical response.

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Supplemental Appendix

Table S1. Baseline characteristics

Characteristics	Median and range (or count)
Age (years)	56 (34 – 75)
Sex (male:female)	388:392
Body weight (kg)	88.8 (55 – 160)
Duration of disease (years)	1.0 (0 – 30.9)
FPG baseline (mmol/L)	9.3 (2.9 – 20.8)
HbA1c baseline (%)	7.9 (6.9 – 9.9)
UGT2B15 genotype* (*1/*1:*1/*2:*2/*2)	149:357:194

*genotype information not collected in 80 subjects

FPG, fasting plasma glucose; HbA1c, glycosylated haemoglobin

Figure S1. Histogram plot for baseline FPG for all subjects included in the analysis

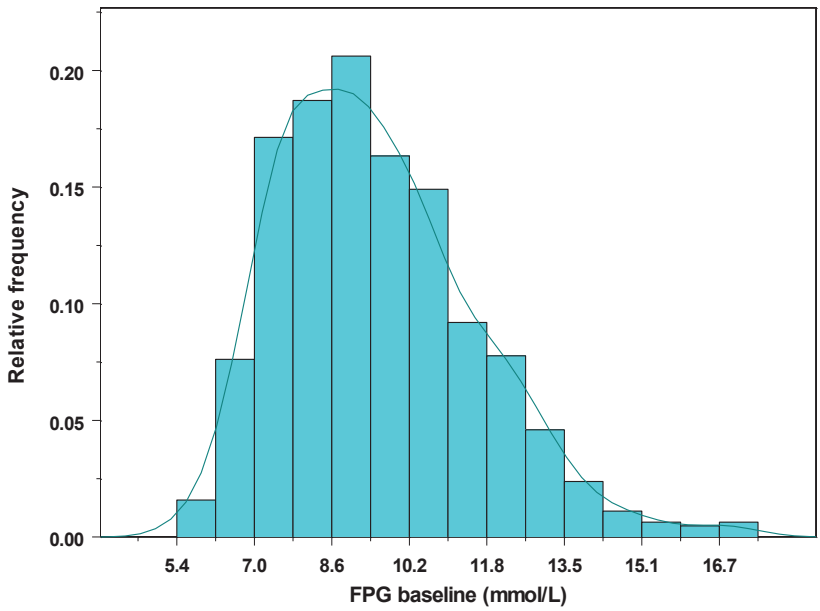


Figure S2. Histogram plot for baseline HbA1c for all subjects included in the analysis

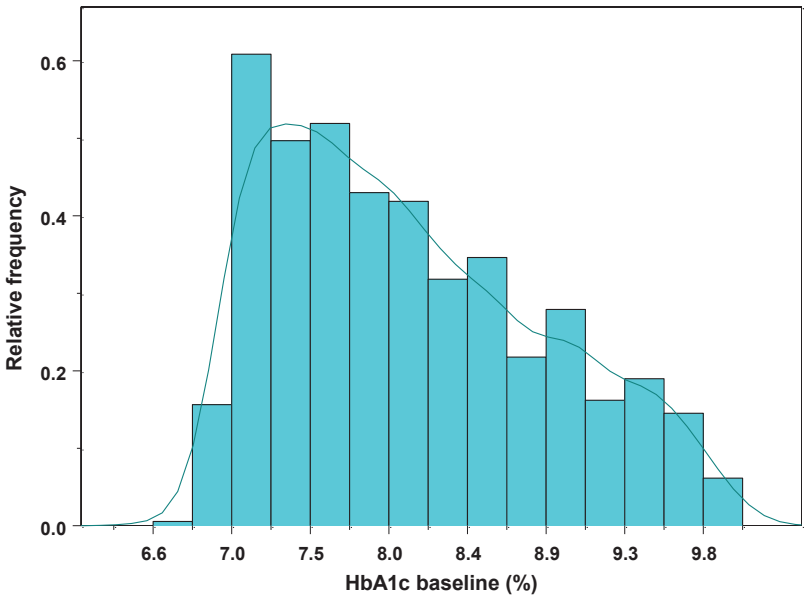


Figure S3. Box plots (median, 25th, and 75th percentiles) for the effect (Drug + placebo) by genotype and treatment group for placebo and sipoglitazar. (solid grey line = median rosiglitazone, dashed greyline=rosiglitazone 25th, and 75th percentiles). (1= UGT2B15*1/*1, 2= UGT2B15*1/*2, 3=UGT2B15*2/*2)

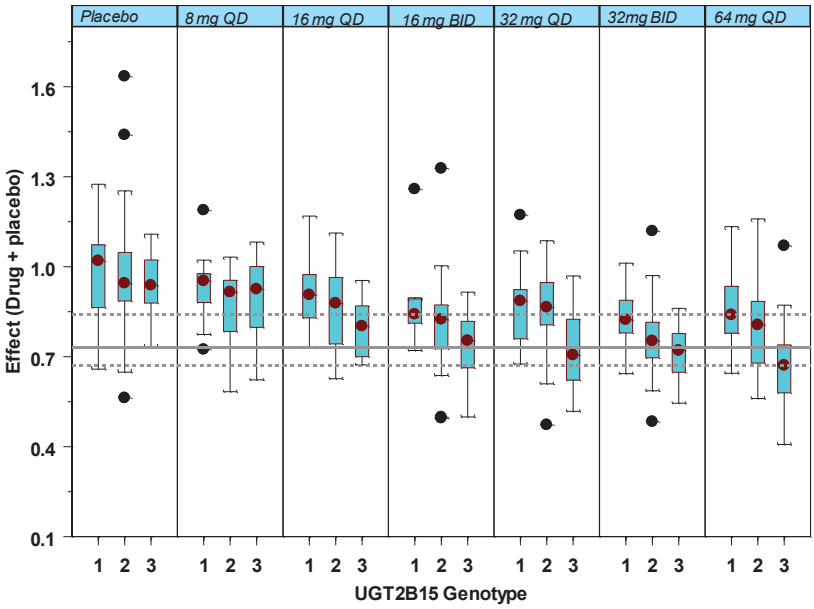
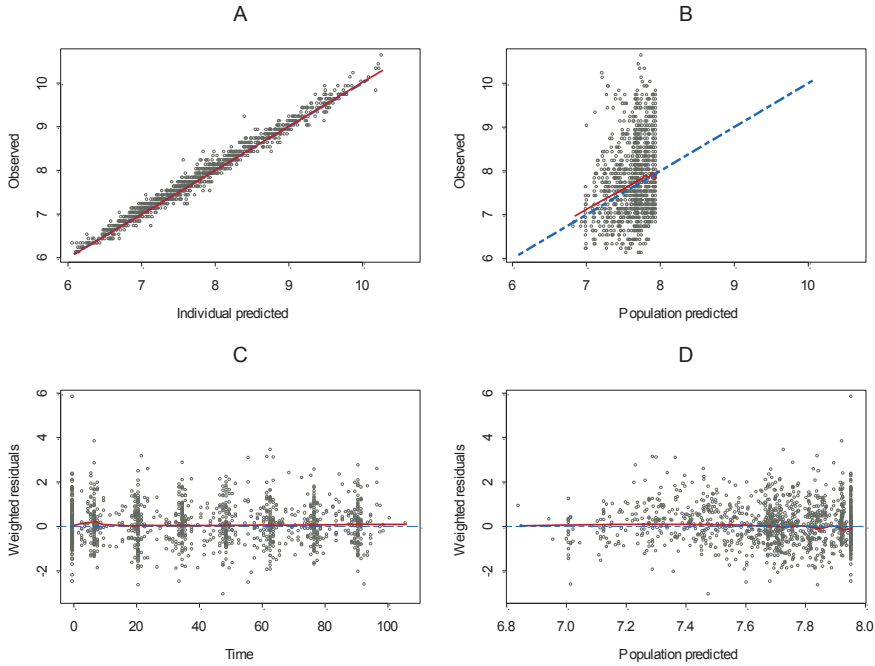


Figure S4. Diagnostic plots of HbA1c for all treatment groups of sipoglitazar UGT2B15*1/*1



A: Observations vs. individual fitted values

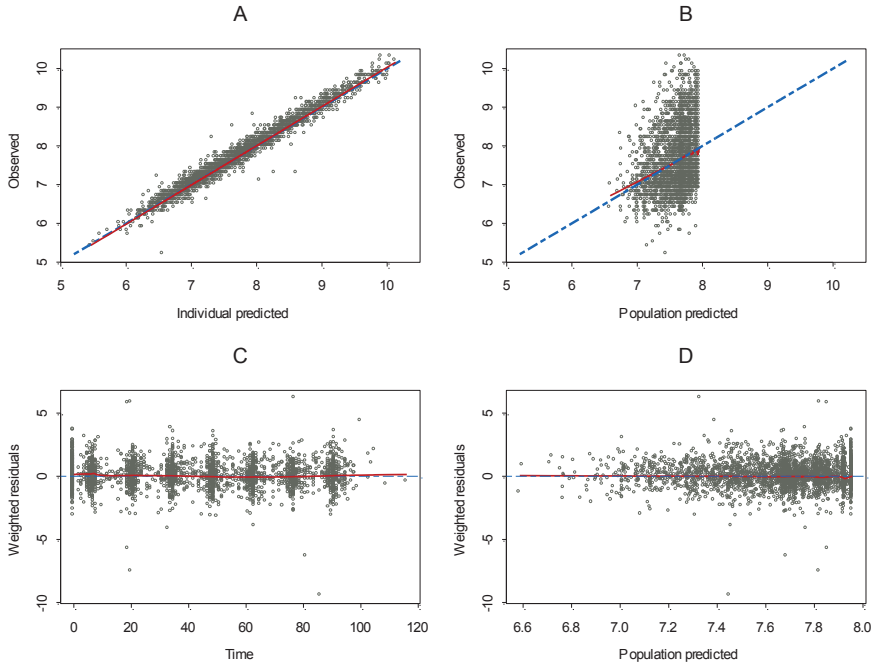
B: Observations vs. population fitted values

C: Conditional weighted vs. time

D: Conditional weighted vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S5. Diagnostic plots of HbA1c for all treatment groups of sipoglitazar UGT2B15*1/*2



A: Observations vs. individual fitted values

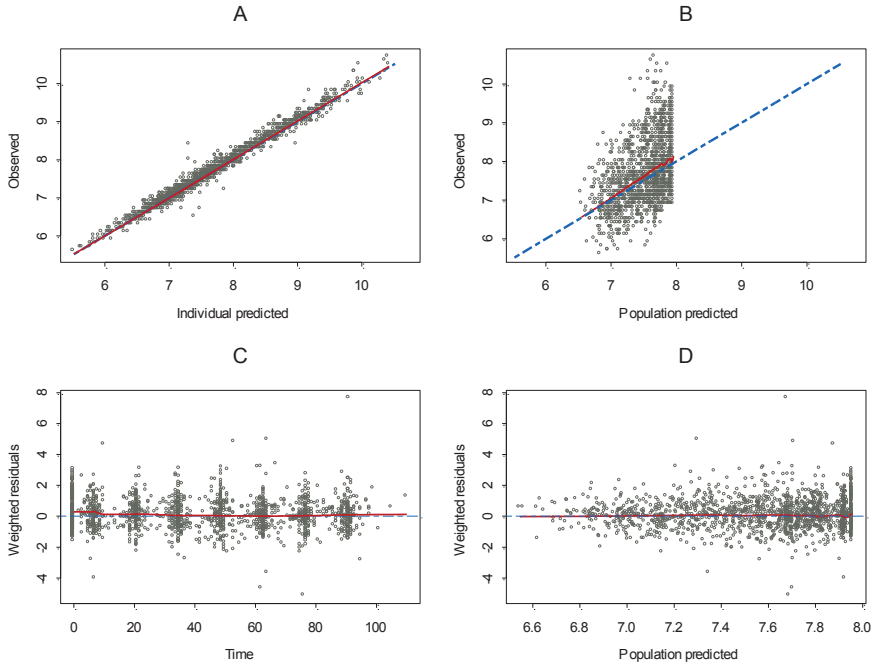
B: Observations vs. population fitted values

C: Conditional weighted vs. time

D: Conditional weighted vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S6. Diagnostic plots of HbA1c for all treatment groups of sipoglitazar UGT2B15*2/*2



A: Observations vs. individual fitted values

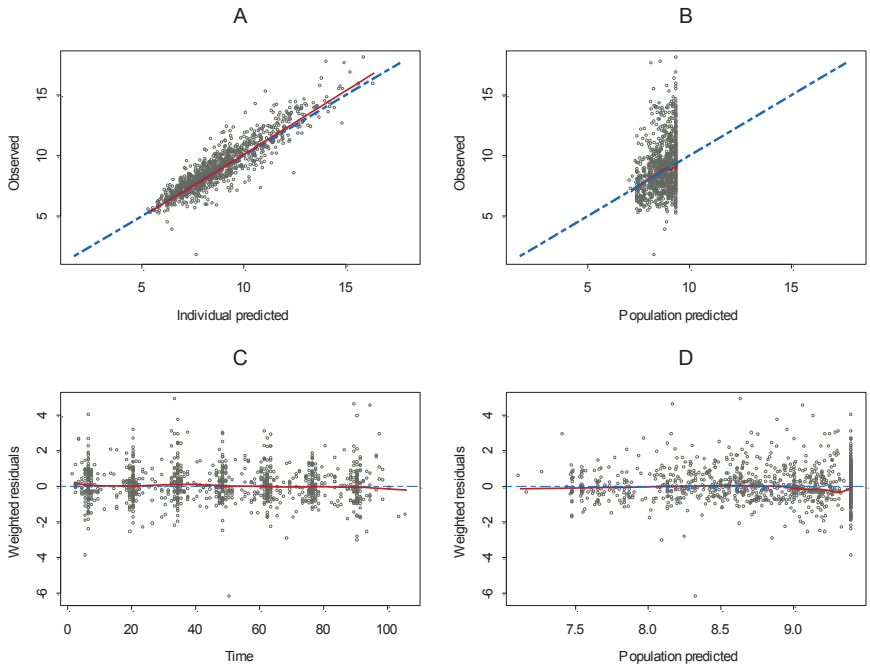
B: Observations vs. population fitted values

C: Conditional weighted vs. time

D: Conditional weighted vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S7. Diagnostic plots of FPG for all treatment groups of sipoglitazar UGT2B15*1/*1



A: Observations vs. individual fitted values

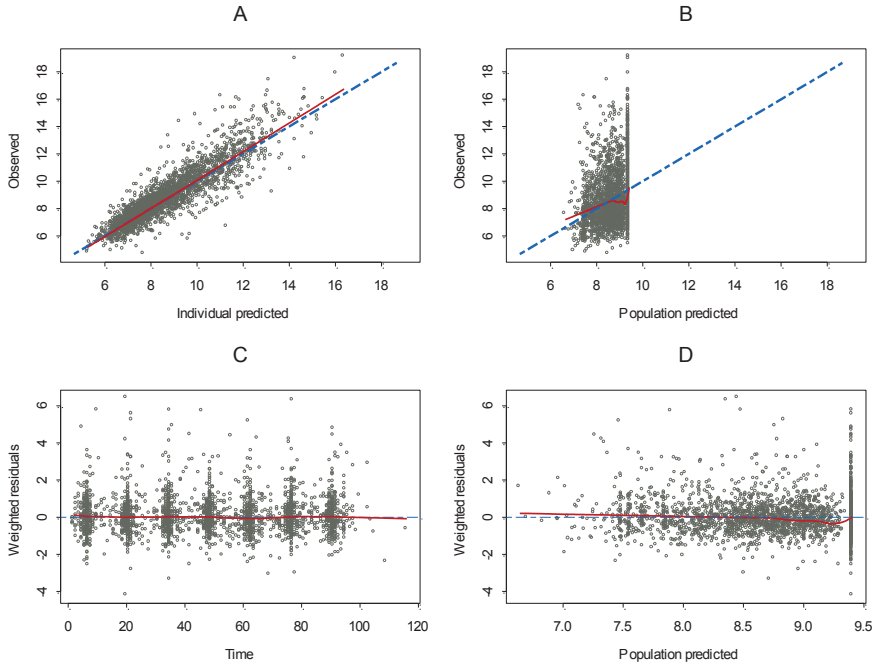
B: Observations vs. population fitted values

C: Conditional weighted vs. time

D: Conditional weighted vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S8. Diagnostic plots of FPG for all treatment groups of sipoglitazar UGT2B15*1/*2



A: Observations vs. individual fitted values

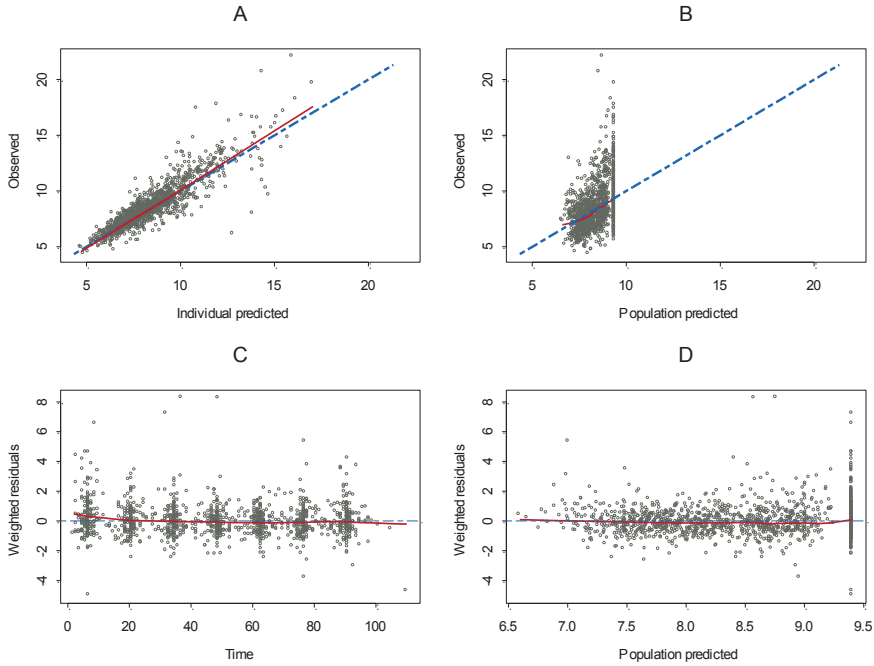
B: Observations vs. population fitted values

C: Conditional weighted vs. time

D: Conditional weighted vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S9. Diagnostic plots of FPG for all treatment groups of sipoglitazar UGT2B15*2/*2



A: Observations vs. individual fitted values

B: Observations vs. population fitted values

C: Conditional weighted residuals vs. time

D: Conditional weighted vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Chapter 6

Evaluation of the Long-term Durability and Glycemic Control of FPG and HbA1c for Pioglitazone in Japanese Patients with Type 2 Diabetes

Frances Stringer, Joost DeJongh, Kazuaki Enya, Emiko Koumura, Meindert Danhof and Kohei Kaku

Diabetes Technology and Therapeutics, In press

Abstract

Aim: Application of a model-based approach to evaluate long term durability and glycemic control of pioglitazone in comparison to other oral glucose-lowering drugs in Japanese type 2 diabetes mellitus (T2DM) patients.

Methods: Japanese T2DM patients were enrolled in a prospective, randomized, open-label, blinded-endpoint study and received pioglitazone ± other oral glucose-lowering drugs (excluding another thiazolidinedione (TZD)) (n=293) or oral glucose-lowering drugs excluding TZD (n=294). Treatment was adjusted to achieve HbA1c<6.9% and samples for FPG and HbA1c were collected over 2.5-4 years. A simultaneous cascading indirect response model structure was applied to describe the time course of FPG and HbA1c. HbA1c levels were described using both an FPG-dependent and an FPG-independent function. To account for titration, drug effects for both treatment groups were implemented using a time dependent Emax model.

Results: Pioglitazone was superior in both time to maximum effect and the magnitude of reduction achieved in FPG and HbA1c. Greater reduction (2-fold) in FPG was observed with pioglitazone compared to the control group. Maximum drug effect for FPG was predicted to occur earlier (11 months) for pioglitazone than the control group (14 months). The simulated additional reduction in FPG and HbA1c achieved with pioglitazone was predicted to be maintained beyond the currently observed study duration.

Conclusion: Pioglitazone was found to result in improved glycemic control and durability compared to control treatment. This model-based approach enabled the quantification of differences in FPG and HbA1c for both treatment groups and simulation to evaluate longer term durability on FPG and HbA1c.

Introduction

The prevalence of diabetes in Japan has been increasing over the past two decades, primarily driven by lifestyle changes [1,2]. There will be an estimated number of diabetes cases in Japan of 8.9 million by the year 2030, following the same trend as other Asian countries [3]. Epidemiological studies have established that hyperglycemia is a significant risk factor for the development of cardiovascular disease (CVD) [4,5]. Japanese type 2 diabetes mellitus (T2DM) subjects have been shown to have a three-fold higher risk for CVD than non-diabetic subjects and the Ministry of Health, Labour and Welfare in Japan has now identified diabetes as a healthcare priority [1].

Current guidelines in Japan recommend achieving a target HbA1c <7.0% to inhibit the progress of and prevent the onset of macrovascular disease [6]. There are currently seven groups of oral agents currently used in Japan: Sulfonylurea drugs, fast-acting insulin secretion stimulators (glinides), biguanides, thiazolidines (TZD), alpha-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors, and sodium glucose cotransporter-2 inhibitors [7]. However there are differences in the usage patterns compared to North America and Europe [1]. Furthermore the underling pathology of T2DM was found to be different between Japanese and Caucasian subjects [8,9]. In comparison to Caucasians, Japanese are unable to compensate insulin resistance with increased insulin secretion to the same extent. A recent study identified body composition as the major determinant for these pathophysiological differences between Japanese and Caucasian T2DM subjects [9]. As a result of differences in glycemic targets and in the pathophysiological features of diabetes, treatment guidelines in Japan differ from those in Western countries [7].

T2DM is a slowly progressing disease and glycemic deterioration is predominantly due to insulin resistance and beta-cell failure [10]. Currently there are no available therapies that can completely stop the progressive loss of glycemic control, although different therapies can delay the extent of this loss by different degrees [11]. To evaluate this further, determination of a coefficient of failure (which is the slope obtained by performing regression analysis) was proposed as an approach to assess beta-cell failure from any index of glycaemia [12]. Further extension of this approach can be undertaken using pharmacokinetic-pharmacodynamic

models developed to characterize the time course of drug effects. The advantage of implementing a model based approach being the key characterization of the relationship between treatment and the physiology of the disease over time [13]. Traditional approaches such as last observation carried forward result in a collapse in the time dimension of the data and therefore disregard the actual trajectory of change in disease status over time [14]. As a result of this, crucial information on disease progression over time is ignored and short-term hypoglycemic effects of a treatment are combined with its longer term effects on the disease.

In contrast however, a model based approach is applied to describe and explain changes in disease status as a function of time and drug therapy. In Caucasian T2DM patients these approaches have been widely applied to discriminate between standard of care and new therapies, assessing alternative treatment strategies and by using meta-analysis to evaluate the current competitive landscape for anti-diabetic therapy [15-18]. Furthermore the regulatory authorities advocate the application of these model based approaches with a particular focus on understanding exposure response relationships [19,20].

Up until now, no model based approach in Japanese T2DM patients has been undertaken on mid to long-term data. Therefore this analysis represents to our knowledge, the first model based approach to evaluate the drug specific effects in Japanese T2DM patients for pioglitazone in combination with other oral glucose lowering drugs as compared to oral glucose-lowering drugs alone simultaneously on FPG and HbA1c. The aim of this analysis is to further enhance our understanding of the treatment and time course effects on FPG and HbA1c whilst the development of a model will enable the simulation for both groups to compare the longer term glycemic durability.

Methods

Subjects and Data Collection

The data used in this analysis are from a multicenter, prospective, randomized, open-label, blinded-endpoint (PROBE) study that was designed to assess the glycemic effects of pioglitazone and their impact on cardiovascular outcomes in Japanese patients with type 2 diabetes over a period of 2.5–4 years. Patients received pioglitazone and other oral

glucose-lowering drugs (excluding another TZD) (n=293) or oral glucose-lowering drugs excluding TZD (n=294). Treatment was adjusted to achieve HbA1c<6.9%. The primary results of this study have already been described in detail elsewhere [21,22].

Population Data Analysis

During the study HbA1c was collected every 12 weeks and FPG was collected every 24 weeks. The baseline characteristics are described in Table 1. The changes in FPG and HbA1c levels over time were described using a simultaneous, cascading indirect response model structure, similar to the approach previously described in Caucasian patients [14,23]. HbA1c (%) data were collected using the Japanese Diabetes Society values and then converted to the National Glycohemoglobin Standardization Program (NGSP) values [24].

Disease progression submodel for FPG and HbA1c

The model was parameterized in terms of a zero order production rate for FPG (KinG) and a first-order rate constant for the removal of FPG (KoutG). Changes in HbA1c were initially modelled as secondary changes dependent on FPG, with a first order rate constant (KinH) for production and a first order HbA1c degradation rate constant, KoutH for disappearance. The description of HbA1c production also included the use of a power function on FPG^{γ} [15,25]. Disease progression for FPG was implemented as a proportional increase in the FPG level with a slope (FPGDP), relative to the baseline at study start. A number of different models for disease progression were explored including exponential and log-linear, however these were not found to be superior.

The overall model structure is described below in equations 1-3.

$$KinGDP = (BSLG * KinG) * (1 + FPGDP * TIME) \quad \text{EQ(1)}$$

$$\frac{dFPG}{dt} = KinGDP - KoutG \cdot (1 + DEF) \cdot FPG \quad \text{EQ(2)}$$

$$\frac{dHbA1c}{dt} = FPGind + KinH \cdot FPG^{\lambda} - KoutH \cdot HbA1c \quad \text{EQ(3)}$$

During model development, it was noted that the changes in HbA1c over time could not be fully described by the changes observed in FPG alone. Furthermore, there were differences observed in the rate of change over time between FPG and HbA1c following graphical inspection of the data. This is consistent with previous reports and is likely to result from the co-contribution and input of non-fasting glucose since HbA1c is a measure of average glucose comprising of both fasting and postprandial hyperglycemia [26,27]. A separate FPG-independent effect to describe the source of changes in HbA1c was therefore included which resulted in significantly improved model diagnostics and fit. This FPG independent input was described using a zero order rate constant (KinZ) and a linear time dependent parameter (DPind) included in the following equation:

$$FPGind = KinZ * (1 + DPind * TIME) \qquad \text{EQ(4)}$$

Drug effect Model

The drug effect (DEF) on FPG was incorporated using an Emax model driven by TIME, where Emax is the maximal effect of overall exposure to FPG-lowering drugs. ET50 is the time required for titration to half of the maximal exposure. For both treatment groups DEF was implemented as a stimulatory effect on KoutG. The Emax model approach was applied to account for the titration of anti-diabetic medication in the early phase of the study. This information could not be directly included in the analysis at the individual patient level due to the way in which time was recorded in the case report form for the titration schemes. To account for titration-related dose changes in both patient populations the drug effect was described with the following approach:

$$DEF = Emax \cdot TIME/ET50 + TIME \qquad EQ(5)$$

ET50 in the pioglitazone group was estimated with a value close to 0 and with low precision. This is likely to result from the maximum effect of titration being achieved for drugs in the pioglitazone group by the time of first FPG sample collection at 3 months. As a result, this parameter was fixed to zero for all remaining model development without any loss in goodness-of-fit.

Subjects who entered the trial were already receiving anti-diabetic medication, the details of which are shown in Table 1. However the assumption was used that these subjects were indeed at steady state on their baseline medication when they entered the trial. Further refinements to the model to account for this additional background therapy at the individual patient level did not result in any improvements in model diagnostics.

Table 1. Baseline characteristics

Characteristics	Pioglitazone Group (n=293)	Control Group (n=294)
Age (years)[a]	58.0 (35.0 – 74.0)	58.0 (37.0 – 74.0)
Sex (male:female)	184:109	181:113
Body weight (kg)[a]	69.0 (45.0 – 107.0)	68.0 (44.0 – 116.0)
BMI (kg/m^2)[a]	26.5 (18.5 – 37.3)	26.2 (19.0 – 42.6)
FPG baseline (mg/dL)[a]	153.0 (77.0 – 304.0)	157.0 (81.0 – 371.0)
HbA1c baseline (%)[a]	7.9 (6.9 – 11.4)	7.6 (6.9 – 11.8)
Number of non-TZD mediations at baseline[a]	1 (0 – 4)	2 (0 – 4)
Non-TZD diabetic medication at baseline by type		
Sulphonylureas (%)	73.0	81.6
alpha-Glucosidase inhibitors (%)	35.8	55.8
Biganides (%)	42.6	67.7
Rapid-acting insulin secretagogue drugs (%)	6.5	12.9
Number of non-TZD diabetic medications at baseline		
0 (%)	10.6	1.4
1(%)	42.0	37.1
2 (%)	39.2	46.9
>=3 (%)	8.2	14.6

[a]Median and range

Data Analysis

All analyses were performed using the nonlinear mixed effects modeling approach in NONMEM (version 7, release 1; Icon Development Solutions, Ellicott City, Maryland). The first-order conditional estimation method with interaction (ADVAN6, TOL=5) was used. Statistical analysis was performed in S-Plus (version 8.1 Professional, TIBCO Software Inc.).

Simulations of FPG and HbA1c were performed in Berkeley Madonna version 8.3.13 (Macey & Oster, University of California, Berkeley).

Inter-individual Variability and Residual Error

Inter-individual variability (IIV) on FPG baseline and EMAX was explored assuming a log normal distribution of the individual parameter estimates. However IIV on the baseline for HbA1c was evaluated using a Box-Cox transformation model to account for skewness observed in the individual data, likely resulting from inclusion criteria based on HbA1c>6.9% [25,28]. IIV on FPGDP was described by an additive random effect. Residual variability was included using a proportional model and the correlation between IIV on baselines was included using the OMEGA BLOCK option.

Covariate Analysis

Potential covariates at baseline included age, sex, weight, BMI, number of non-TZD medications, type of non-TZD medication and baseline FPG and HbA1c. These were evaluated in the model using a forward inclusion and backward elimination procedure [29]. Before performing the covariate analysis the most appropriate distribution of the covariates was evaluated.

Model Qualification

The visual predictive check (VPC) was used to evaluate the ability of the final model to predict both the central tendency and the variability of FPG and HbA1c (median and 90th prediction interval) [30]. The stability of the model was tested by using 100 bootstrap replicates of the original dataset. To further inform model validation ETA shrinkage for the random effects was estimated [31].

Model Selection

No further improvement in the model was considered by a change in objective function of less than 10.8 ($P \leq 0.001$) for each additional degree of freedom (=extra parameter). In addition graphical analysis enabled assessment of bias and the biologically plausibility of the parameter estimates was evaluated.

Evaluation of the treatment effects on glycemic markers

Based on observed data the % of subjects achieving a HbA1c<7.0% and the mean HbA1c values at 2.5 years (the minimum study duration for all subjects) were estimated and compared for both treatment groups.

Observed change from baseline in HOMA-IR was calculated and a two-sample t-test was used to compare the mean values of change from baseline in HOMA-IR at each visit between treatment groups. To further explore the predictability of the model, change from baseline in HOMA-IR was recalculated using the model predicted FPG values.

To explore the influence of drug effects on the time course for pioglitazone and the control group the median FPG and HbA1c time profiles were simulated.

Results

The results presented here were based on the simultaneous analysis of FPG and HbA1c data in 587 T2DM subjects with median treatment duration of 3.14 years (maximum 3.9 years). Results of the VPC are shown in Figure 1a and 1b and indicate adequate precision and accuracy of the model. The results for the model parameters are specified in Table 2 along with their bootstrap estimate. All model parameters could be obtained with adequate precision, and all parameters fall within the 95% CI's. Estimated shrinkage for all random effect parameters was low (< 21%). Additional diagnostics are provided in Supplemental Figures S1-S4.

Figure 1. (a) Visual predictive check for observed and predicted FPG data.

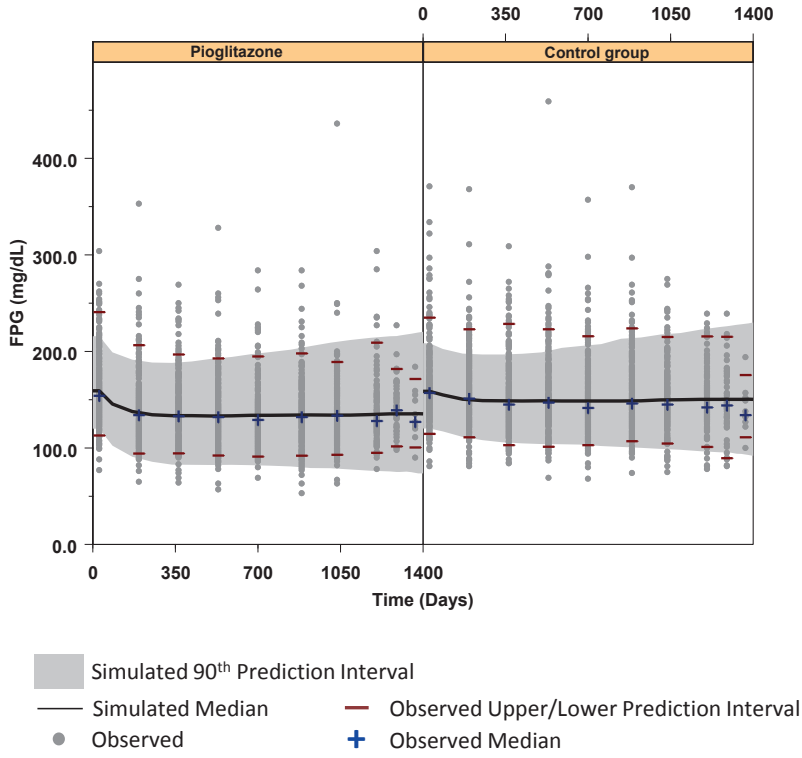


Figure 1. (b) Visual predictive check for observed and predicted HbA1c data.

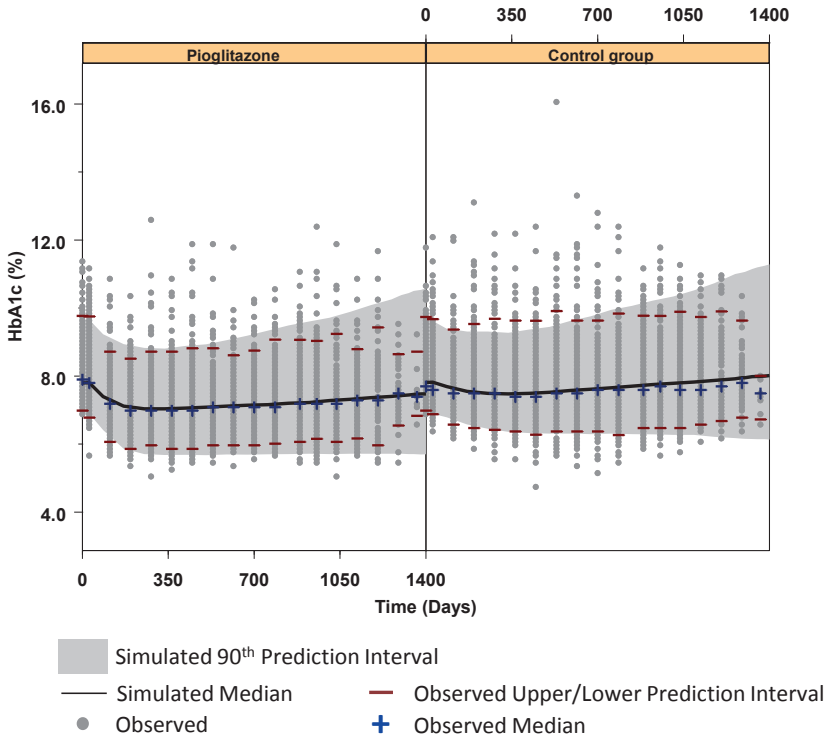


Table 2. Summary of parameter estimates for the final model including bootstrap estimates

Parameter	Model Estimate (CV%)	Mean Bootstrap Estimate (95% CI)[a]
	Fixed effects	
BSL FPG (Females) (mg/dL)	156.0 (1.1)	155.7 (152.5-158.9)
KoutG ($days^{-1}$)	0.0089 (11.4)	0.0089 (0.0073-0.011)
BSL HbA1c (%)	7.83 (0.5)	7.83 (7.75-7.91)
Box Cox	3.28 (14.8)	3.28 (2.27-4.29)
KoutH ($days^{-1}$)	0.072 (13.1)	0.071 (0.051-0.116)
Emax Piogliazone (%)	17.3 (8.3)	17.5 (14.4-20.5)
Emax Control (%)	8.4 (14.8)	8.5 (5.7-11.2)
ET50 Pioglitazone (days)	0 FIX	0 FIX
ET50 Control (days)	49.2 (46.3)	49.0 (-0.9-100.8)
KinZT ($days^{-1}$)	0.28 (16.9)	0.29 (0.17-0.41)
FPGDP ($years^{-1}$)	0.017 (28.3)	0.016 (0.006-0.027)
DPind ($years^{-1}$)	0.03 (14.8)	0.03 (0.006-0.06)
Gamma	1.91 (21.8)	1.84 (1.0-2.7)
Gender on FPG BSL	0.05 (22.9)	0.05 (0.03-0.08)
	Random effects: inter-individual variability (IIV)	
ω^2 BSL FPG	0.03 (7.2)	0.03 (0.023-0.033)
ω^2 BSL HbA1c	0.01 (8.5)	0.01 (0.008-0.011)
ω^2 FPGDP	0.004 (19.7)	0.003 (0.002-0.005)
ω^2 Emax	0.75 (11.8)	0.74 (0.57-0.90)
Correlation (ω^2 BSL HbA1c, ω^2 BSL FPG)	0.01 (10.0)	0.01 (0.009-0.013)
	Random effects: residual error	
Residual error FPG (%)	14.4 (4.6)	14.4 (13.7-15.1)
Residual error HbA1c (%)	5.8 (5.1)	5.8 (5.4-6.1)

[a] During conduct of the bootstrap, 97.0 % of runs minimized successfully.

CV, coefficient of variation; CI, confidence interval; BSL FPG, baseline for fasting plasma glucose; KoutG, first-order rate for fasting plasma glucose; BSL HbA1c, baseline for glycosylated hemoglobin; KoutH, first-order rate constant for glycosylated hemoglobin; Emax, is the maximal effect of overall exposure to FPG-lowering drugs; FPGDP, disease progression rate for FPG; DPind disease progression rate for FPG-independent input; KinZT, zero order rate constant for FPG-independent input; ET50, the time required for titration to half of the maximal exposure; ω^2, inter individual variability.

During the study period (2.5-4 years), 90 subjects (9% Pioglitazone, 6% control group) discontinued before 2.5 years. This was based on a number of criteria including: adverse

event, voluntary withdrawal or major protocol deviation. Patient discontinuation may possibly influence model parameters when caused by selective drop-out of certain subpopulations of patients. This may be due to lack of efficacy, occurrence of side effects, and/or compliance. To exclude this, models were run using all the data and excluding the drop-out subjects. All model parameters were compared by including and excluding these discontinued subjects. Only a slight difference was observed in the FPG disease progression rate parameter (FPGDP) 0.017 $year^{-1}$ (with all subjects) and 0.016 $year^{-1}$ (excluding discontinued subjects). This result showed only a very minor influence of these subjects was observed on the FPG profile and due to the low observed drop out in the study further model development including drop-out was not performed.

Drug effect model

Differences in the effect due to maximum drug exposure (EMAX) on FPG were observed between the two treatment groups. The model derived Emax values for pioglitazone and the control group were 17% and 8%, respectively. Resulting in approximately 2-fold greater reduction in FPG for pioglitazone as compared to the control treatment (median maximum simulated change from baseline in FPG was -21 mg/dL compared to -9 mg/dL for pioglitazone and the control group, respectively). An ET50 value of 49 days for the control group indicated that half the maximum exposure level of drugs affecting FPG was achieved in approximately 2 months. However for the pioglitazone group, ET50 was fixed at 0 indicating that apparent steady state for the titration of treatment for FPG occurs earlier than the control group. Based on simulation, the resulting maximum drug effect for FPG was achieved at approximately 14 and 11 months for the control and pioglitazone groups, respectively.

Disease progression was parameterized as a proportional increase over time relative to the FPG baseline. The model predicted increases over time were estimated at approximately 2 mg/dL (95% CI 0.9 – 3.5)/per year for FPG and 0.2 % (95% CI 0.15 – 0.25)/per year for HbA1c (Figure 2a and 2b). A second contribution to HbA1c production was described using an additional FPG- independent input (DPind); this FPG-independent input was estimated to contribute an additional 0.03% to the increase in HbA1c per year. Simulated FPG and HbA1c

median values over time for pioglitazone are shown simultaneously in Figure 2c. As shown in the simulation the additional FPG-independent input to HbA1c results in differences in the rate of change over time between FPG and HbA1c.

Figure 2. (a) Simulated FPG time profiles for pioglitazone and control group over 5 years

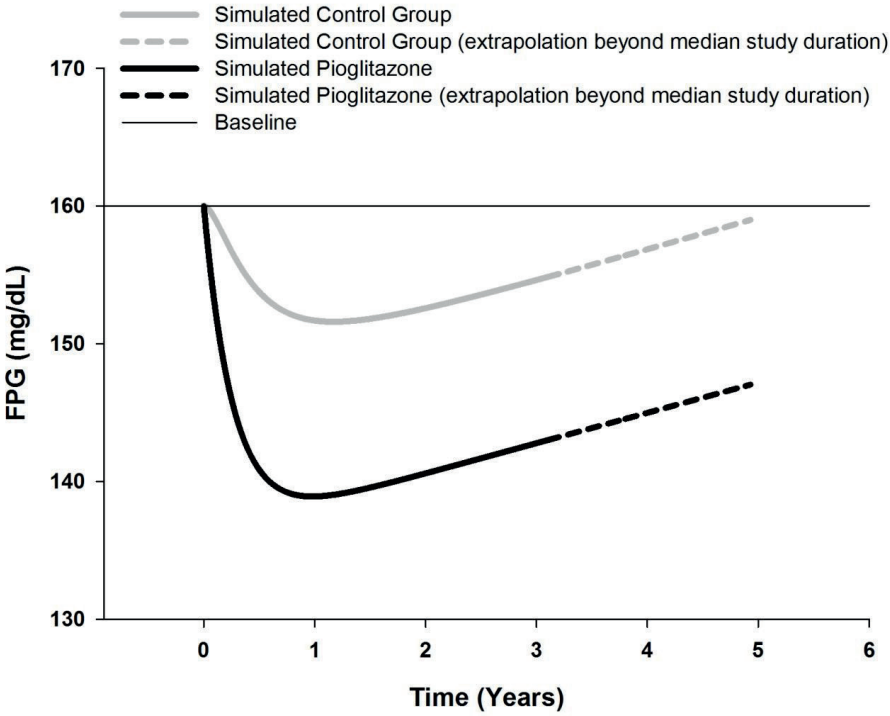


Figure 2. (b) Simulated HbA1c time profiles for pioglitazone and control group over 5 years

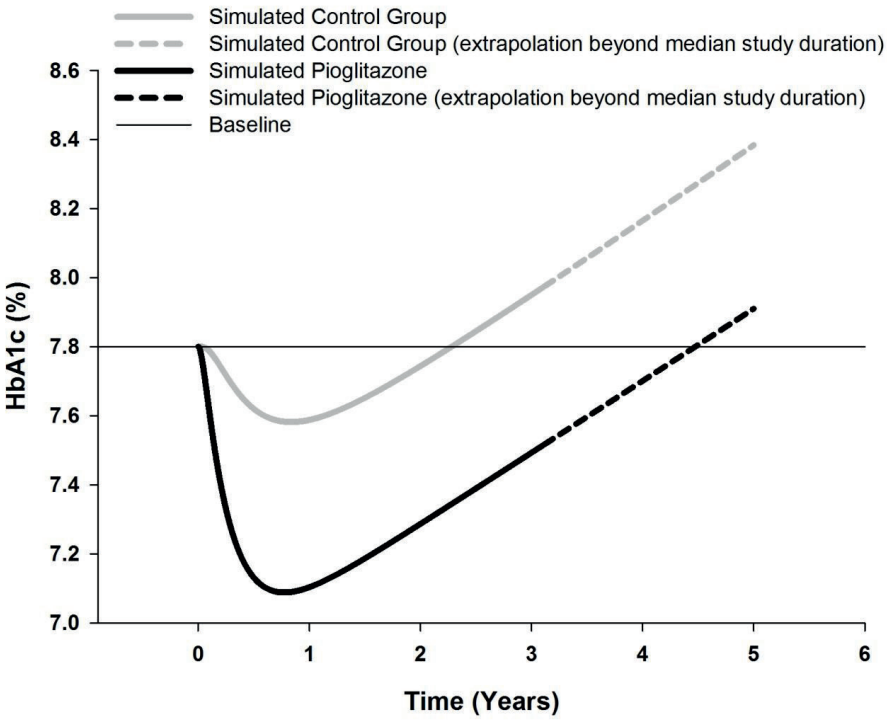
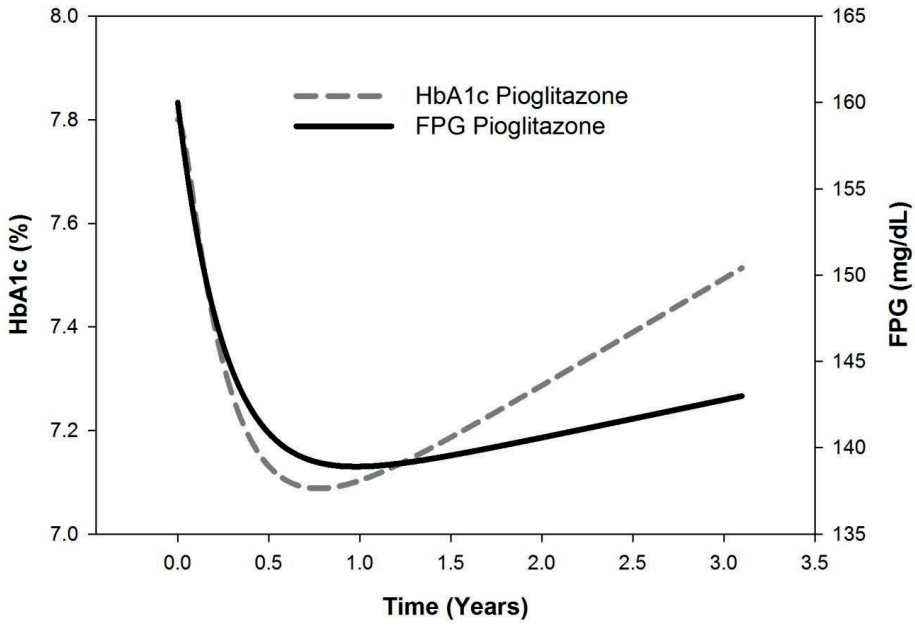


Figure 2. (c) Simulated comparison of FPG and HbA1c time profiles for pioglitazone



No differences in the apparent disease progression rates (FPGDP or DPind) between treatments could be identified from the model predicted post-hoc parameters.

Evaluation of the treatment effects on glycemic markers

Model-based simulation results

Glycemic durability was evaluated using the model optimized parameters to simulate the median FPG and HbA1c time profiles over a 5 year period (Figure 2a and 2b). Median FPG in the control group was predicted to almost return to baseline levels (160 mg/dL) 5 years after starting treatment, however at 5 years in the pioglitazone group predicted FPG levels were still considerably lower (147 mg/dL) (Figure 2a). The duration of time required for median HbA1c levels to return to baseline (HbA1c=7.8%) was approximately 2.1 years in the control group and approximately 4.5 years for the pioglitazone group, for the typical patient

in this population (Figure 2b). The differences between pioglitazone and the control group in simulated FPG and HbA1c median values was approximately 13 mg/dL and 0.5%, respectively at 5 years.

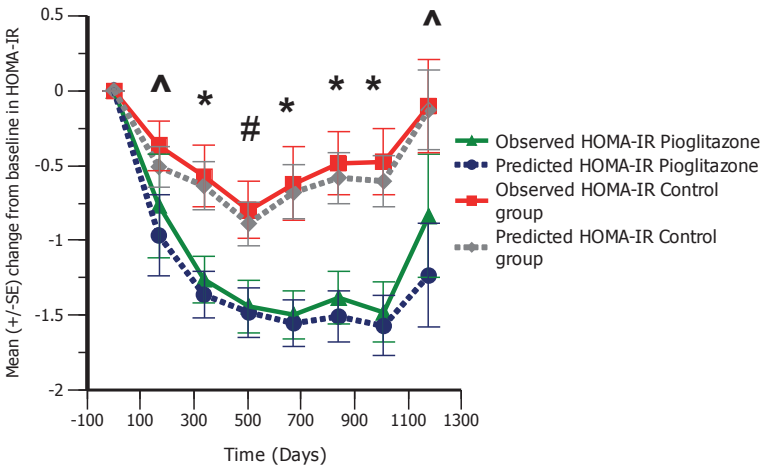
Observed data analysis

At 2.5 years (the minimum study duration for all subjects), 34% of the patients in the pioglitazone group had an observed HbA1c level <7.0%, as compared to only 18% in the control group. Mean observed HbA1c values at 2.5 years were 7.3% and 7.8% ($p<0.001$) for the pioglitazone and control groups, respectively.

This is comparable to data observed in Caucasians when pioglitazone was added to metformin, at 2 years 31% of patients had an HbA1c<7.0% [32].

Figure 3a shows the observed and predicted change from baseline in HOMA-IR by treatment group. A statistically significant difference ($p<0.05$) from the control group for pioglitazone in observed HOMA-IR was maintained from 168 days until the last visit. The observed and predicted change from baseline HOMA-IR values are compared graphically (Figure 3a). Observed and predicted values are in close agreement, confirming the good predictability of the model.

Figure 3. (a) Observed and predicted change from baseline in HOMA-IR over the study duration. (not significant = ^) (# = p<0.05) (*= p<0.01).



Covariate Analysis

Following the covariate analysis, only one relationship was identified as significant. Male subjects were found to have a slightly (5%) higher BSLG than female subjects. These differences in FPG levels by gender have been previously reported in Japanese subjects [33].

Discussion

Here we present the first application of a model based approach to evaluate drug and disease effects in Japanese T2DM patients over a 2.5-4 year treatment period. Our analysis enabled the determination of disease progression rates in Japanese treatment experienced T2DM patients for both FPG and HbA1c and a comparison of the drug effects between treatment groups. Stronger drug effects (2-fold greater) could be identified for pioglitazone as compared to the control group. Furthermore these effects could be maintained over a longer period, indicating that pioglitazone in combination with other oral glucose lowering drugs in Japanese T2DM patients can result in improved glycemic durability.

Greater reductions in both FPG and HbA1c data were observed in the pioglitazone group. Data in Caucasian subjects evaluating the treatment effects of pioglitazone in combination with other glucose lowering drugs have also shown favorable glycemic results in both short and long term studies [34]. When comparing gliclazide or metformin alone with pioglitazone given as add-on therapy improved and sustained glycemic control was maintained over a 2 year study period [32]. In a longer term study, glycemic durability over 3.5 years in Caucasian subjects for Pioglitazone in combination with metformin revealed significant benefits in glycemic control compared with glibenclamide [35]. Furthermore reductions in HOMA-IR were also maintained out to 3.5 years in the same study. Indicating that pioglitazone through lowering the burden of insulin resistance could lead to increased protection of the beta-cells [35].

A difference in the rate of change over time between FPG and HbA1c was identified. These differences were accounted for in the model using a separate FPG-independent and time-dependent effect on HbA1c. Studies have shown that mean plasma glucose (the arithmetic mean of FPG and PPG) correlates better with HbA1c than FPG alone [26,16]. It is therefore hypothesized that the different rates of change observed between FPG and HbA1c are due primarily to the input related to PPG. To demonstrate the magnitude of this FPG-independent contribution, the HbA1c time profile is simulated with and without the hypothesized contribution of PPG (Figure S5). An absolute difference of approximately 1% in HbA1c is observed between the HbA1c simulation dependent only on FPG and the

simulation dependent on FPG and PPG. Recent publications have reported that PPG has an absolute contribution of approximately 1.3-1.6 % to overall HbA1c levels [36]. Treatments which specifically target PPG have also shown reductions in HbA1c in the region of 1-1.5% [36]. Based on these studies the estimated absolute contribution of PPG to HbA1c is between 1%-1.6%. This is consistent with our findings and therefore supports the hypothesis that PPG is the main driver for the differences we observed between the rate of change over time in FPG and HbA1c.

The model enabled the quantification and evaluation of apparent disease progression rates for FPG and HbA1c. Although no differences could be identified in the underlying disease progression rate that is estimated here in either FPG or HbA1c between groups, pioglitazone is shown to maintain glycemic control over a longer duration whilst reducing insulin resistance. As predicted form its mechanism of action, pioglitazone maintains a statistically significant decrease in HOMA-IR until the last visit compared to the control group (Figure 3a). A clinically meaningful difference in HbA1c [37], between the two groups at 5 years was also predicted in the current study. The difference between pioglitazone and the control group in the simulated FPG and HbA1c median values was predicted as approximately 13 mg/dL and 0.5%, respectively at 5 years. Apparent disease progression rates in this trial are lower than those reported in the UKPDS study in Caucasian subjects determined using the coefficient of failure [12], however comparison to other studies maybe confounded by both the baseline characteristics, the combination of different glycemic treatments and titration schemes and ethnic background. Therefore, a model based approach that combines glycemic data from Caucasian and Japanese T2DM patients simultaneously, should be performed to elucidate any differences in disease progression rates while considering the other covariates affecting clinical response.

There are several limitations of our current model analysis. Due to study limitations no individual dose titration data could be included in the analysis and no pharmacokinetic data was collected for any of the treatments, in addition any long term simulation is based only on trial data with a median duration of 3.1 years. Each of these factors contribute to a certain degree of uncertainly in our extrapolations beyond the actual study duration.

In summary, the application of a model based approach quantified differences in FPG and HbA1c for both treatment groups and enabled simulation to evaluate the longer term durability on FPG and HbA1c data for both pioglitazone and the control group. Based on this result pioglitazone when given in combination with other oral glucose lowering drugs in Japanese T2DM patients was found to result in improved glycemic control and durability as compared to oral glucose lowering treatment alone.

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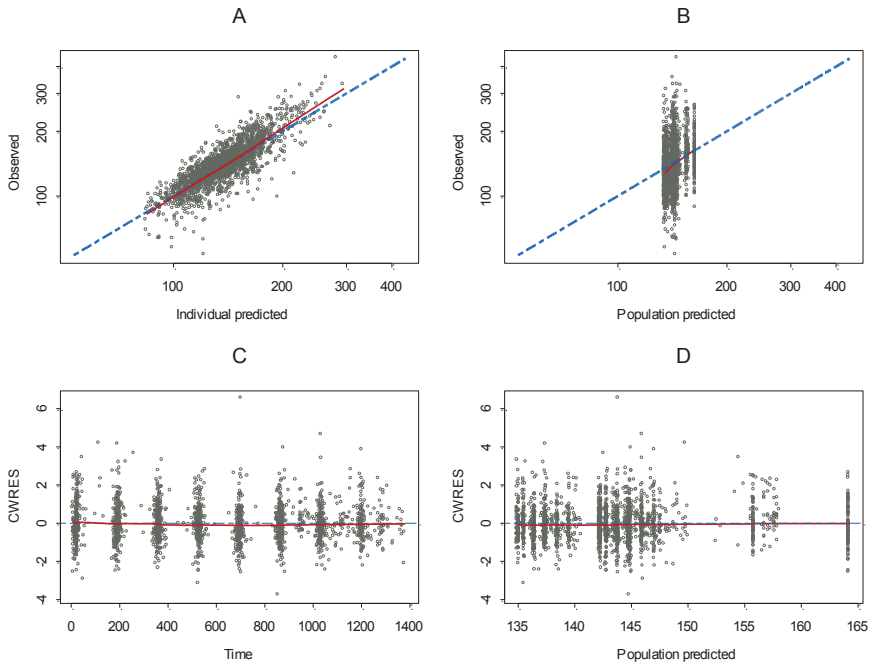
Supplemental Appendix

Figure S1. Diagnostic plots of FPG for pioglitazone



A: Observations vs. individual fitted values

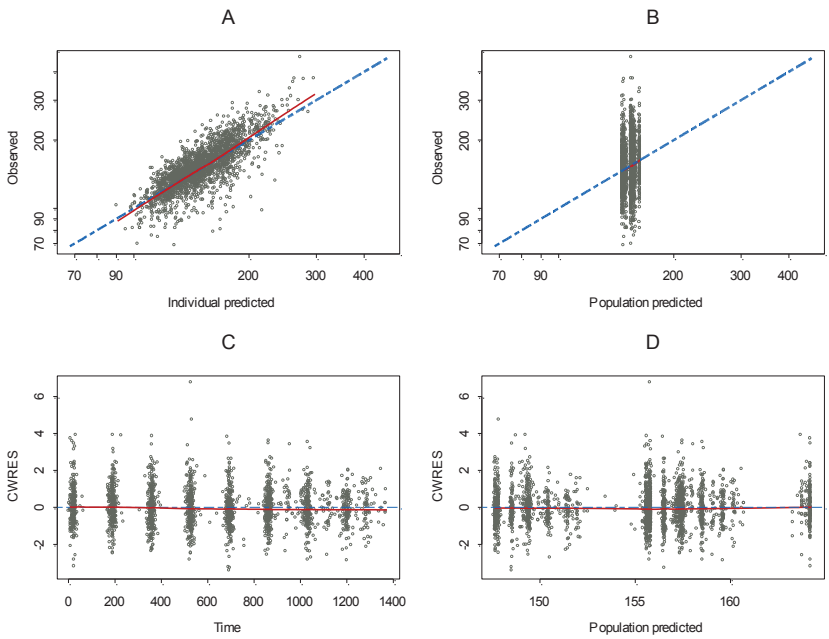
B: Observations vs. population fitted values

C: Conditional weighted residuals vs. time

D: Conditional weighted residuals vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S2. Diagnostic plots of FPG for control group



A: Observations vs. individual fitted values

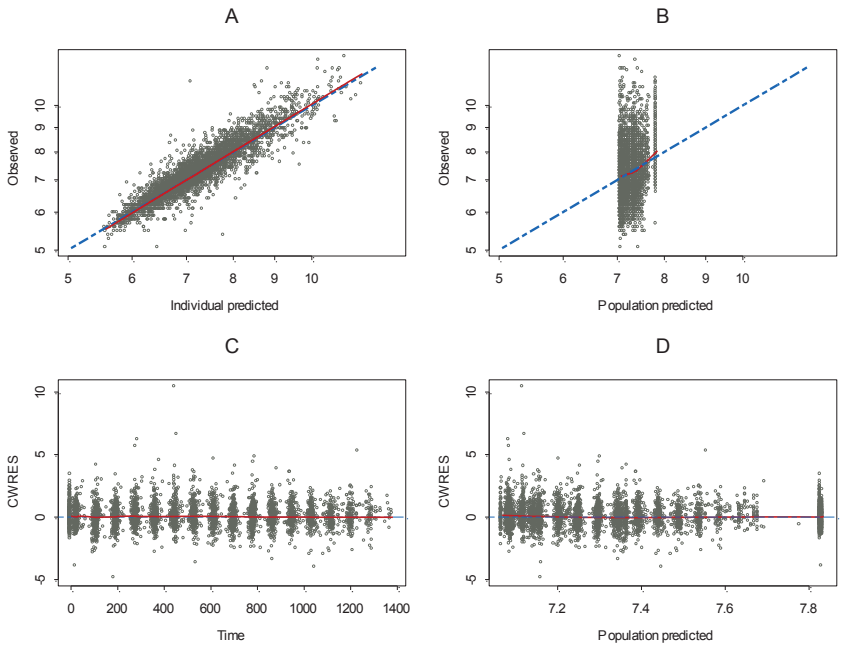
B: Observations vs. population fitted values

C: Conditional weighted residuals vs. time

D: Conditional weighted residuals vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S3. Diagnostic plots of HbA1c for pioglitazone



A: Observations vs. individual fitted values

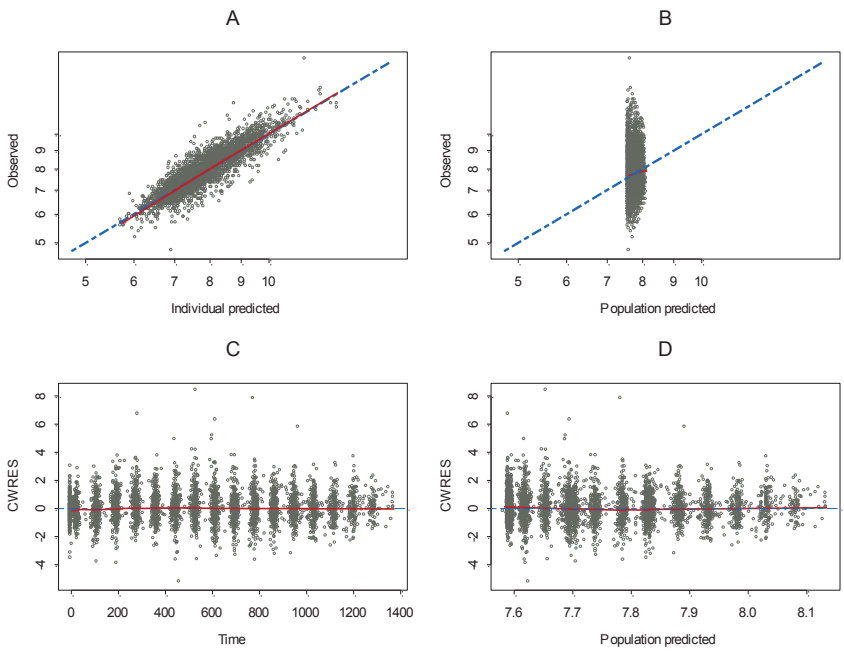
B: Observations vs. population fitted values

C: Conditional weighted residuals vs. time

D: Conditional weighted residuals vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S4. Diagnostic plots of HbA1c for control group



A: Observations vs. individual fitted values

B: Observations vs. population fitted values

C: Conditional weighted residuals vs. time

D: Conditional weighted residuals vs. population fitted values

Dashed line: line of identity (A and B) or line indicating 0 (C and D)

Figure S5. Observed (dot), individual predicted (IPRED) and the population prediction (PRED) for the FPG time profile of typical representative subjects

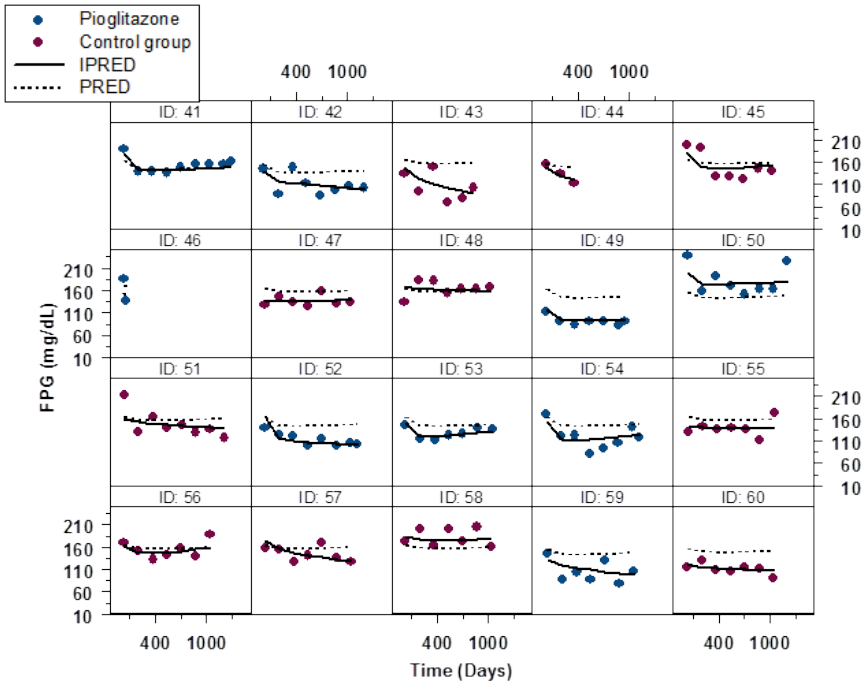


Figure S6. Observed (dot), individual predicted (IPRED) and the population prediction (PRED) for the HbA1c time profile of typical representative subjects

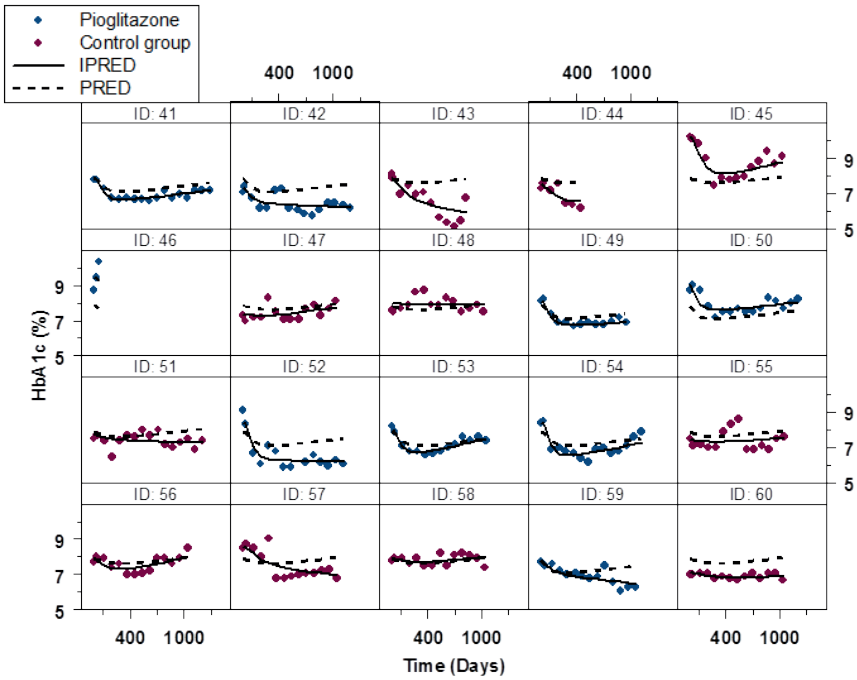
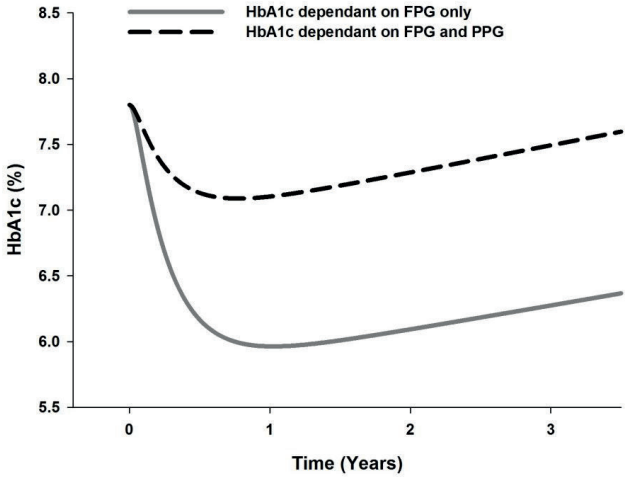


Figure S7. Simulated Pioglitazone HbA1c time profile for HbA1c dependent on FPG only and HbA1c dependent on both FPG and PPG



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

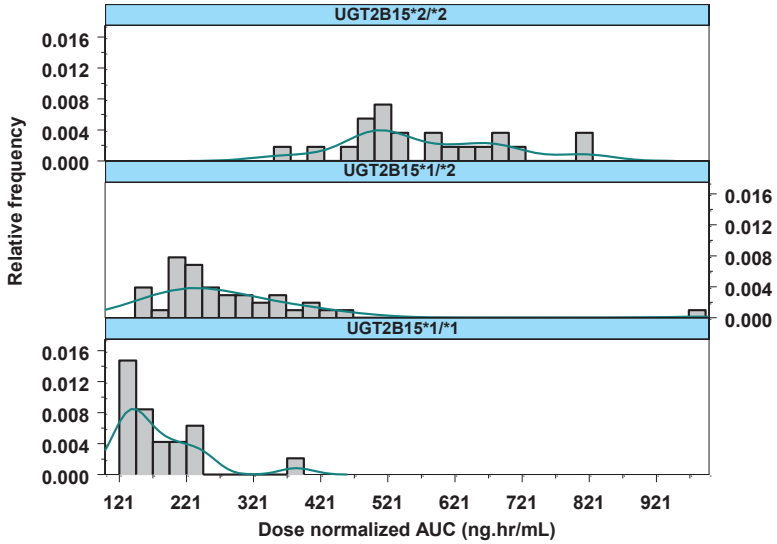
In vitro assessment can be used to determine the involvement of specific isoforms of drug metabolizing enzymes responsible for metabolism of a drug candidate; however since in-vitro

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).

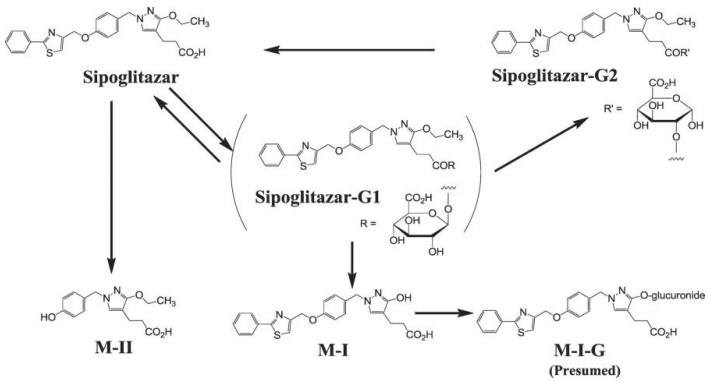
As a first step in the investigation the contribution of each genotype was assessed using Analysis of variance (ANOVA) models on dose normalized AUC. Results of this investigation revealed that variation in UGT2B15 accounted for approximately two-thirds of the variability in sipoglitazar plasma exposure, 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**]).

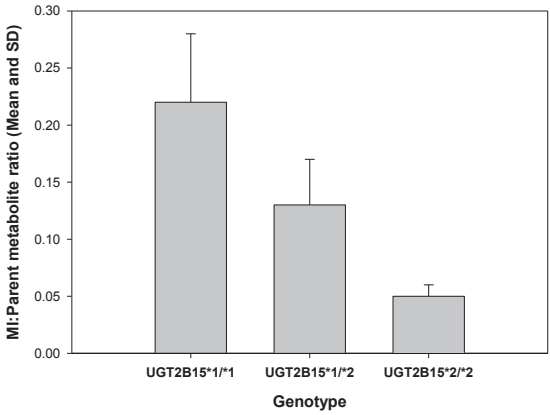


The M-I metabolite also undergoes subsequent conjugation to M-I-G and since a high concentration of M-I-G was present in the urine in monkey studies, it is presumed that the glucuronidation of M-I would also occur in humans [11]. Due to its unique metabolic formation, the metabolite M-I was considered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

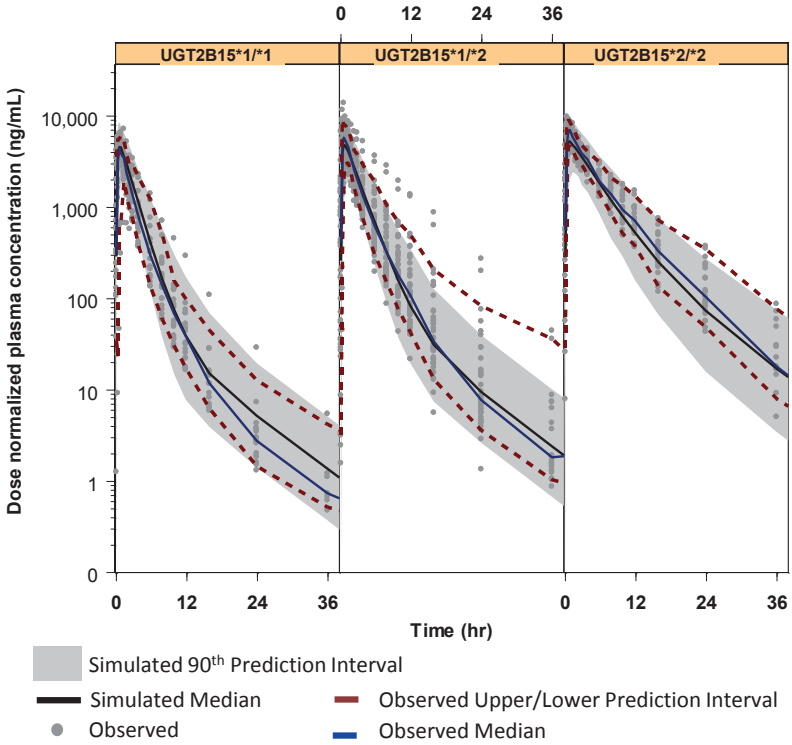
All parameters could be estimated with good precision and the VPC shows that the median trend and variability can be well described in all three genotype groups. ETA shrinkage for clearance and V2 was estimated at 1.6 and 15%, respectively.

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.

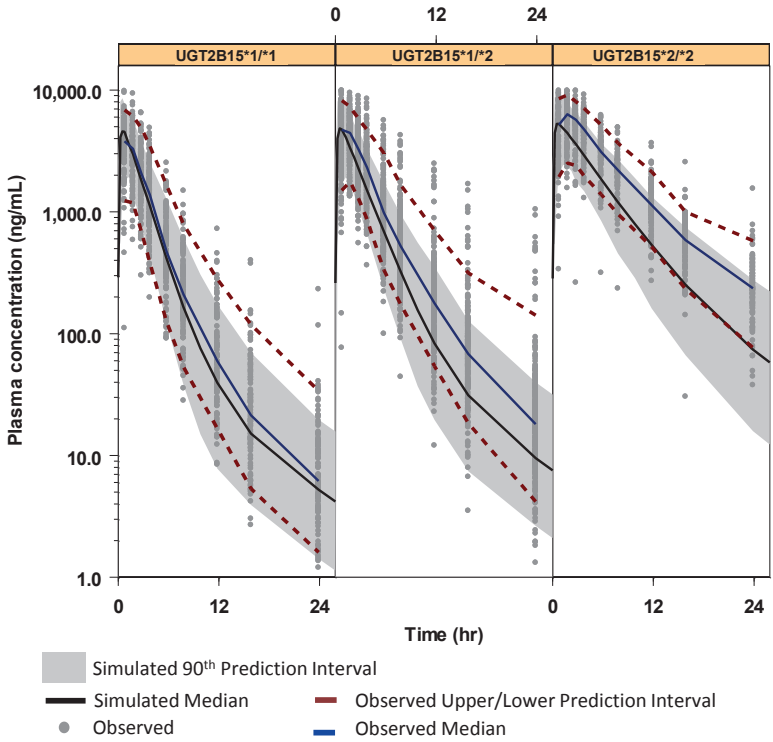
Pharmacokinetic studies in healthy volunteers

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.

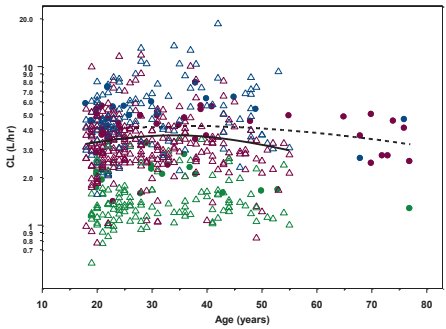


Figure 6. Plots for age vs CL. Individual (triangle – large phase I, circles – small phase I) and smoothing spline (solid line – large phase I, dashed line – small phase I). Color by genotype UGT2B15*1/*1 (blue), UGT2B15*1/*2 (red), UGT2B15*2/*2 (green)

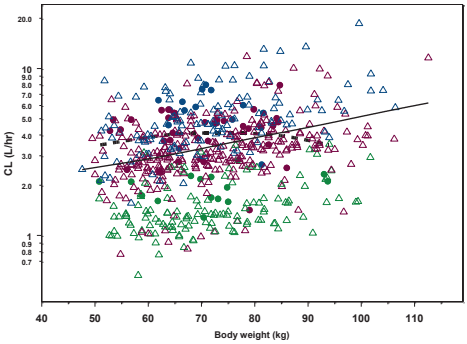


Figure 7. Plots for body weight vs CL. Individual (triangle – large phase I, circles – small phase I) and smoothing spline (solid line – large phase I, dashed line – small phase I). Color by genotype UGT2B15*1/*1 (blue), UGT2B15*1/*2 (red), UGT2B15*2/*2 (green)

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

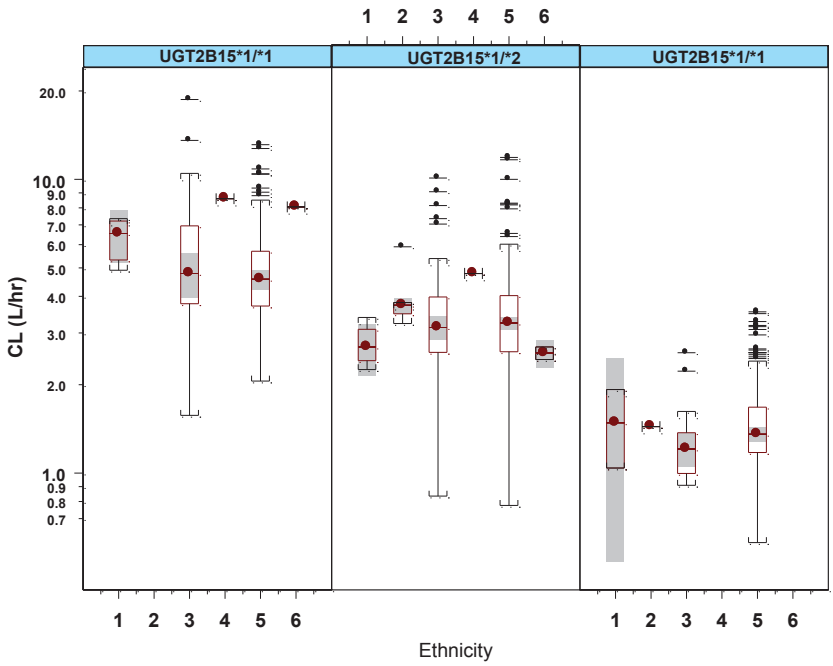
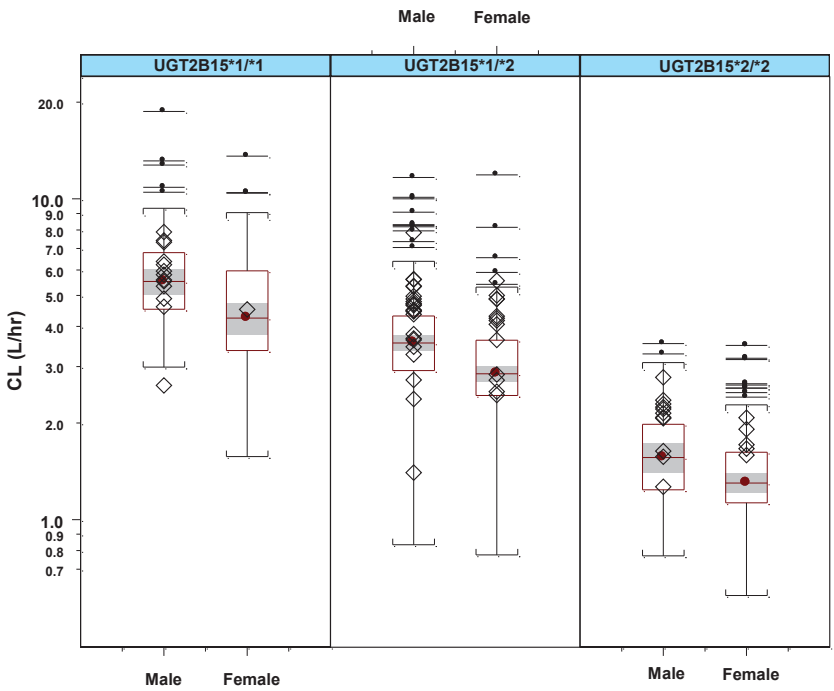


Figure 9. Box plot for CL and gender for large phase I study in healthy volunteers. Scatter plot of CL (triangles) for small phase I in healthy volunteers by gender.



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.

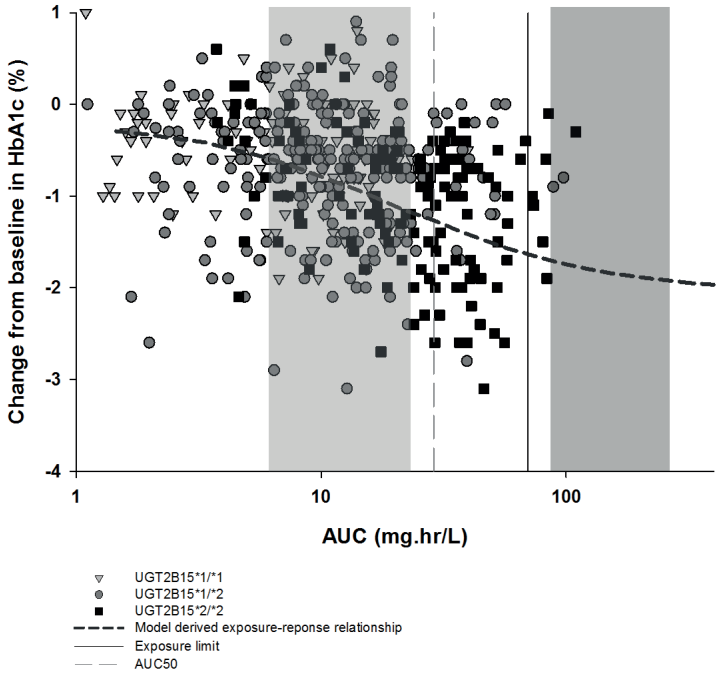
Evaluating the influence of genotype on clinical response

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.

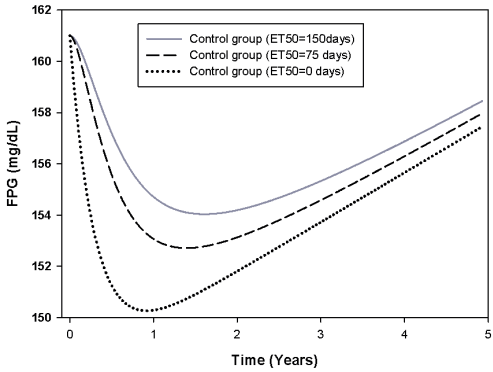


Figure 11. Simulation of median FPG change over time for a range of ET50 values (all simulations performed using a DPRC value in the model of 0.013 year^{-1}).

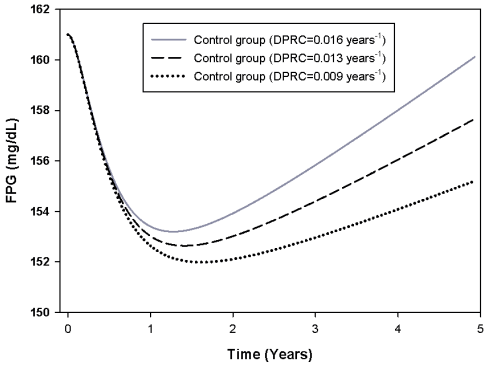


Figure 12. Simulation of median FPG change over time for a range of disease progression rate (DPRC) values (all simulations performed using an ET50 value in the model of 75 days).

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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Summary in English

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. In Chapter 1 this thesis starts with an overview of the current applications of Pharmacogenomics (PGx) across drug development with an emphasis on the implications of polymorphisms in drug metabolizing enzymes and transporters. The second section (Chapter 2) focuses on the application of model based approaches to evaluate differences in drug exposure and response as a result of these genetic differences between individuals. In Chapters 3, 4 and 5 the focus for this thesis is on a clinical example for the oral glucose lowering drug, sipoglitazar which undergoes phase II biotransformation by conjugation catalyzed by UDP-glucuronosyltransferase (UGT). Clinical data from four phase I studies in healthy volunteers and from two phase II trials in subjects with type 2 diabetes mellitus (T2D patients) were utilized in the analysis. PGx samples for determination of UGT genotype were collected for all subjects enrolled in the trials

Clinical relevance of genetic variants in pharmacokinetic properties

Exploratory preliminary evaluation of genotype during Phase I clinical trials

In Chapter 3, an investigation was conducted to evaluate the enzymes that were contributing to the inter-individual variability of sipoglitazar and to then quantify the resulting differences in exposure between genotypes. The analysis in Chapter 3 was conducted using data from a trio of phase I clinical pharmacology studies in healthy volunteers (n=82). The dose range for sipoglitazar was 0.2-64mg. Statistical analysis of area under the plasma concentration–time curve from time 0 to infinity (AUC) revealed dose proportionality across the dose range (slope = 0.99; 95% confidence interval 0.92-1.05), (Chapter 3). As a first step in the PGx investigation, the contribution of each genotype to the variation in dose normalized AUC was assessed using Analysis of variance (ANOVA). 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. Considerable exposure overlap was observed between genotype groups, particularly between the UGT2B15*1/*1 and UGT2B15*1/*2 genotypes and the exposure was found to be approximately two- to three-fold higher in the UGT2B15*2/*2 genotype than either UGT2B15*1/*1 or UGT2B15*1/*2. Two outlier subjects were identified. These subjects, which were genotyped as UGT2B15*1/*1 and UGT2B15*1/*2, had considerably higher exposure than expected based on their genotype. This analysis showed that across the population UGT2B15, genotype could explain 66% of the variability of sipoglitazar exposure as determined by dose-normalized AUC. Other factors such as age, body mass index or sex appeared to contribute little to explaining the additional variability or outlying subjects in this healthy volunteer population.

Development of a population PK model for sipoglitazar in T2D patients

The investigation and analysis conducted in Chapter 4 was then focused on evaluating genotype influences in the target population, T2D patients. In this chapter data from two phase II randomized, double-blind studies (sipoglitazar once daily: 8, 16, 32, or 64 mg; sipoglitazar twice daily: 16 or 32 mg; rosiglitazone 8 mg once daily and placebo for 13 weeks; n = 780) were included in the analysis 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. 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*1/*2 or UGT2B15*1/*1 genotypes, respectively. Before accounting for any covariates (including genotype), inter-individual variability (IIV) on clearance was 60%; however, after including genotype as a covariate, the IIV of clearance was reduced to 40%. Only one other covariate (fat free 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. Post-hoc CL values

were then used to determine individual exposure over the dose interval at steady state (AUC24). These exposure values were then were used as the input into the PK-PD model to evaluate the exposure response relationship described in Chapter 5.

Evaluating the clinical relevance of genotype differences in exposure

Evaluating the influence of genotype on clinical response through disease progression analysis

In Chapter 5, 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 a reference group The developed PK-PD 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 PK-PD model was used to simulate the expected FPG and HbA1c change from baseline at 6 months (duration of a Phase III trial) by UGT2B15 genotype. 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. The results of the simulation also show that for a dose of 32 mg, the change in HbA1c was less than proportional 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, the reduction in HbA1c was significantly less in the UGT2B15*1/*1 genotype as compared to the UGT2B15*2/*2 genotype.

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. 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. The percentage of subjects achieving HbA1c reduction >0.7% at 6 months was used for evaluation. Based on these results to achieve equivalence to rosiglitazone (73%), for all subjects irrespective of genotype, a dose of 96mg of sipoglitazar would be required (a single dose level for all subjects). However using genotype based dosing uniform response rates could be achieved with lower doses for the UGT2B15*2/*2 and UGT2B15*1/*2 groups (UGT2B15*1/*1=96mg, UGT2B15*1/*2=64mg and UGT2B15*2/*2=32mg).

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 on therapeutic response, with all subjects in the titration group starting at 32mg. 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 difference in the time to 90% of steady state between genotyped and titration-based dosing was approximately 1 and 2 months for the UGT2B15*1/*2 and UGT2B15*1/*1 genotypes.

Application of a PD model based approach in Japanese T2D subjects to describe the drug and disease effects on FPG and HbA1c for pioglitazone over 2.5-4 years

The next section (Chapter 6) focusses on PD model based approaches in T2D over a much longer time period (>2.5 years). Since T2D is a slowly progressing disease, the importance of considering both the drug and disease effects on the time course of the relevant biomarkers is investigated. A phase IV study that was conducted in Japanese T2D subjects was used for the analysis. In this study (n=587) subjects received either pioglitazone (+/-oral glucose-lowering drugs) or oral glucose-lowering drugs alone (control group). Treatment was adjusted to achieve HbA1c<6.9% and all subjects included in the trial were treatment experienced. A simultaneous cascading indirect response model structure was applied to describe the time

course of FPG and HbA1c. HbA1c levels were described using both an FPG-dependent and an FPG-independent function. To account for titration, drug effects for both treatment groups were implemented using a time dependent Emax model.

Differences in the effect due to maximum drug exposure on FPG were observed between the two treatment groups. The model derived Emax values for pioglitazone and the control group were 17% and 8%, respectively and resulted in approximately 2-fold greater reduction in FPG for pioglitazone as compared to the control treatment. Disease progression was parameterized as a proportional increase over time relative to the FPG baseline. The model predicted increases resulting from disease progression were estimated at approximately 2 mg/ml/per year for FPG and 0.2%/per year for HbA1c. Simulations of FPG and HbA1c over 5 years were performed. The maximum drug effect for FPG was forecasted to occur earlier (11 months) for pioglitazone than the control group (14 months). The simulated additional reduction in FPG and HbA1c achieved with pioglitazone was predicted to be maintained beyond the currently observed study duration. Through the development of a model on this long term data (>2years) simulation can be used to hypothesize how PGx in T2D may be used to influence drug response through both symptomatic and disease modifying effects.

Conclusions

The application of model based approaches to evaluate the influence of genotype, have primarily focused on the use of genotype as a covariate on drug exposure. These models should preferably also be extended during the drug development program to include clinical response, evaluating safety or efficacy markers to design the appropriate genetic based dosing algorithms or compare different study designs i.e. genotype-based dosing vs. a single dose level for all subjects. The implementation of a population PK-PD model based approach to evaluate the influence of genotype provides a more comprehensive link between the observed changes in the pharmacokinetics and its influence on the magnitude of response. Thus enabling a comparison of the differences observed between the magnitude of change in the PK due to genotype and the magnitude of this change on clinical response. As PGx sample collection becomes routine in clinical studies, the possibility to integrate this into our understanding of drug effects should only increase. 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.

Samenvatting in het Nederlands (Summary in Dutch)

Het onderzoek zoals beschreven in dit proefschrift richt zich op het vaststellen van de rol van genotypische verschillen om individuele variabiliteit in geneesmiddelenmetabolisme te verklaren en de invloed van deze verschillen op zowel het klinische effect als de keuze van het passende doseringsschema vast te stellen.

In Hoofdstuk 1 geeft het proefschrift een overzicht van de huidige toepassingen van Farmacogenetica (PGx) bij ontwikkeling van geneesmiddelen met de nadruk op de gevolgen van polymorfismen voor de activiteit van geneesmiddel-metaboliserende enzymen en transporters. Het tweede deel (Hoofdstuk 2) concentreert zich op de toepassing van de wiskundige modellen waarmee verschillen in blootstelling aan, en de werking van, geneesmiddelen als gevolg van bovengenoemde genetische verschillen tussen personen kunnen worden vastgesteld. De Hoofdstukken 3, 4 en 5 richten zich op een voorbeeld uit de kliniek. Het betreft het oraal toegediende, glucose verlagende middel sipoglitazar dat fase II biotransformatie ondergaat door koppeling aan glucuronzuur, gekatalyseerd door het enzym UDP-glucoronosyltransferase (UGT). Klinische data uit vier fase I studies in gezonde vrijwilligers en uit twee fase II onderzoeken in proefpersonen met type 2 diabetes mellitus (T2D patiënten) zijn gebruikt voor de analyse. PGx monsters ter bepaling van UGT genotype werden verzameld bij alle proefpersonen die deelnamen aan het onderzoek.

Klinische relevantie van genetische varianten? in farmacokinetische eigenschappen

Voorlopige evaluatie van de invloed van genotype tijdens fase I klinisch onderzoek

In Hoofdstuk 3 werd een onderzoek uitgevoerd om variatie in de activiteit van de enzymen die bijdragen aan de individuele spreiding in de farmacokinetiek van sipoglitazar te evalueren om zo de hieruit voortkomende verschillen in blootstelling tussen genotypes te kwantificeren. De analyse in Hoofdstuk 3 werd uitgevoerd met data van een drietal fase I klinisch farmacologische onderzoeken in gezonde vrijwilligers (n=82). De dosering van sipoglitazar varieerde van 0.2-64 mg. Statistische analyse van het oppervlak onder de plasma concentratie

tijdscurve vanaf punt 0 tot oneindig (AUC), toonde een evenredig verband aan over het gehele dosisbereik (hellingsgraad=0.99; 95% betrouwbaarheidsinterval 0.92-1.05), (Hoofdstuk 3). Als een eerste stap in het PGx onderzoek, werd de bijdrage van elk genotype aan de variatie in de waarde van de dosis genormaliseerde AUC, bepaald met behulp van variantieanalyse (ANOVA). De resultaten van dit onderzoek lieten zien dat variatie in UGT2B15*1/1 ongeveer twee derde van de variabiliteit in plasma concentraties van sipoglitazar verklaart, terwijl er geen relatie tussen de blootstelling en varianten van de andere UGT enzymen kon worden vastgesteld. Er werd een aanzienlijke overlap in blootstelling waargenomen tussen de genotype groepen, vooral tussen UGT2B15*1/*1 en UGT2B15*1/*2. Na toediening van dezelfde dosis is de blootstelling ongeveer twee- tot driemaal hoger in het UGT2B15*2/*2 genotype dan in ofwel het UGT2B15*1/*1 of het UGT2B15*1/*2 genotype. Twee afwijkende individuen zijn geïdentificeerd. Deze personen met genotypen UGT2B15*1/*1 en UGT2B15*1/*2 vertoonden een beduidend hogere blootstelling dan verwacht op basis van hun genotype. Deze analyse bewijst dat gemeten over de gehele UGT2B15 populatie, genotype 66% van de spreiding in sipoglitazar blootstelling, uitgedrukt op basis van de dosis genormaliseerde AUC, kan verklaren. Andere factoren als leeftijd, body mass index of geslacht lijken weinig bij te dragen aan een verklaring van de resterende variabiliteit in deze populatie van gezonde vrijwilligers.

Ontwikkeling van een populatie PK model voor sipoglitazar in Type-2 diabetes mellitus (T2D) patienten

Het onderzoek en de analyse uitgevoerd in Hoofdstuk 4 richt zich vervolgens op het evalueren van de invloed van het genotype in de doelgroep, T2D patiënten. In dit hoofdstuk zijn data uit twee fase II gerandomiseerde , dubbelblind onderzoeken (sipoglitazar eenmaal daags: 8, 16, 32 of 64 mg; sipoglitazar tweemaal daags: 16 of 32 mg; rosiglitazone 8 mg eenmaal daags of een maal daags placebo, gedurende 13 weken; n=780) opgenomen in de analyse. PK analyse is uitgevoerd om samenhang tussen het UGT B15 genotype en de

verschillen in blootstelling te kwantificeren, om mogelijke andere bronnen van spreiding te ontdekken en te kunnen beschrijven en om de blootstelling per dosis te bepalen. De door het model geschatte gemiddelde klaringswaarde voor het UGT2B15*2/*2 genotype bleek ongeveer tweemaal tot driemaal hoger te zijn dan in de proefpersonen met respectievelijk de UGT2B15*1/*2 of UGT2B15*1/*1 genotypen. Wanneer er geen rekening werd gehouden met invloeden van covariaten (inclusief genotype), bleek de inter-individuele variabiliteit (IIV) in de klaring 60% te zijn; echter, na het toevoegen van het genotype als covariaat, was de IIV voor de klaring verminderd tot 40%. In de covariatenanalyse werd nog één andere covariaat (vetvrije massa) gevonden met een effect op de klaring. Deze covariaat was verantwoordelijk voor 2% van de IIV. Deze analyse bevestigde de eerdere bevindingen over de relatie tussen enerzijds het UGT2B15 genotype en anderzijds de sipoglitazar blootstelling in de doelgroep. De post-hoc klaringswaarden die in deze studie werden gevonden, werden gebruikt om individuele blootstelling te bepalen tijdens het doseringsinterval in steady-state na herhaalde toediening (AUC24). Deze blootstellingswaarden werden ingevoerd in PK-PD model om de relatie tussen de blootstelling en het effect zoals beschreven in Hoofdstuk 5 te bepalen.

De beoordeling van klinische relevantie van genotype verschillen in blootstelling

Het beoordelen van de invloed van genotype op klinische respons door middel van ziekteprogressie-analyse

Hoofdstuk 5 beschrijft de ontwikkeling van een populatie PK-PK model om de veranderingen in de waarden van de biomarkers FPG en HbA1c te beschrijven als een functie van individuele blootstelling. In deze analyse werden data van de effecten van rosiglitazone, na toediening van de therapeutische dosis van 8 mg 4 maal daags, als referentiegroep meegenomen. Het resulterende PK-PD model gaf een goede beschrijving van de individuele en de gemiddelde profielen van de beide biomarkers voor alle doseringen (8-64 mg, totale

dagelijkse dosis sipoglitazar). Er werden geen verschillen gevonden tussen de genotypen voor wat betreft de relatieve respons op blootstelling. Het PK-PD model werd gebruikt om de verwachte verandering in FPG end HbA1c, in vergelijking met de basislijn bij 6 maanden (Fase III onderzoeksperiode), voor de verschillende UGT2B15 genotype na te bootsen. De resultaten hiervan lieten zien dat een sipoglitazar dosis van 32 mg in het UGT2B15*2/*2 genotype een zelfde verandering in FPG en Hb1Ac oplevert als de referentiebehandeling met rosiglitazone. De resultaten van de simulatie lieten ook zien dat voor een dosis van 32 mg, de verschillen in mate van daling van HbA1c tussen alle genotypen relatief klein zijn in vergelijking met de verschillen in blootstelling. In de fase II populatie kan ongeveer een 3.3-voudig verschil in blootstelling aan sipoglitazar worden waargenomen tussen de UGT2B15*1/*1 en UGT2B15*2/*2 genotypen, maar dit leidt tot een slechts 1.8-voudig verschil in HbA1c-daling ten opzichte van de basislijn. Hoewel een dosis van 32 mg in de UGT2B15*2/*2 proefpersonen een HbA1c-daling kan bewerkstelligen gelijk aan die van rosiglitazone, was deze daling beduidend minder in het UGT2B15*1/*1 genotype dan in het UGT2B15*2/*2 genotype.

Op grond van de hiervoor beschreven veranderingen werd er verondersteld dat genotype-gestuurde dosering, zou kunnen bijdragen aan normering van de individuele respons door het bereiken van vergelijkbare blootstellingsniveaus in alle genotype groepen. Om dit verder te onderbouwen werden simulaties uitgevoerd, waarin drie verschillende scenario's met elkaar werden vergeleken, (1) toediening van dezelfde dosis voor alle proefpersonen, (2) een op genotype-gebaseerde aangepaste dosis (waar genotype wordt gebruikt om de startdosis in te schatten) of (3) titratie van de dosis gebaseerd op de therapeutische respons. Het percentage proefpersonen dat een HbA1c vermindering >0.7% behaalde na 6 maanden werd gebruikt als eindpunt voor de evaluatie. Deze resultaten laten zien dat om een vergelijkbaar effect als voor de referentiebehandeling met rosiglitazone (73%) te behalen, voor alle proefpersonen, ongeacht het genotype, een dosis van 96 mg sipoglitazar nodig is (een gelijke dosis voor alle proefpersonen). Echter gebruikmakend van dosering op basis van genotype, kon een uniforme responsgraad worden behaald met lagere

doseringen voor de UGT2B15*2/*2 en UGT2B15*1/*2 groepen (UGT2B15*1/*1=96 mg, UGT2B15*1/*2=64 mg en UGT2B15*2/*2=32mg).

Hoewel een op genotype gebaseerde methode kan worden gebruikt om de respons tussen de genetische subgroepen te normeren, wordt in T2D routinematig een titratiemethode toegepast gebaseerd op doelmatigheid/veiligheid. Daarom werd een simulatie uitgevoerd om de een genotype gebaseerde dosering met een titratie gebaseerd op therapeutische respons te vergelijken, waarbij alle proefpersonen in de titratie groep startten op een dosis van 32 mg. Twee belangrijke punten vallen op bij het resultaat van deze simulatie. In lijn met de verwachting was de daling in de waarde van FPG of HbA1c dezelfde voor enerzijds de op genotype-gebaseerde en anderzijds de op titratie gebaseerde methodes, maar het tijdsduur binnen welke de maximale respons werd behaald was korter indien de voorselectie van dosering gebaseerd werd op genotype. Het verschil in tijdsduur benodigd voor het bereiken van 90% van de uiteindelijke steady-state waarde van de biomarkers tussen op genotype en op titratie gebaseerde doseringsmethoden was ongeveer 1 en 2 maanden voor de UGT2B15*1/*2 en UGT2B15*1/*1 genotypen.

Toepassing van een PD model om het effect van pioglitazone op de veranderingen in de waarden van FPG en HbA1c in Japanse TdD patiënten over een periode van 2.5-4 jaar te beschrijven

Het onderzoek dat wordt beschreven in hoofdstuk 6 heeft betrekking op het beschrijven van de invloed van pioglitazone op de veranderingen in de waarden van FPG en Hb1Ac over een langere periode (> 2.5 jaar) in Japanse T2D patiënten. Dit is belangrijk omdat T2D een langzaam voortschrijdende ziekte is en er daardoor een behoefte is aan geneesmiddelen waarmee de mate van deze ziekteprogressie kan worden afgeremd.

Bij deze studie werden data uit een fase IV onderzoek in Japanse T2D patiënten gebruikt. In dit onderzoek (n=587) ontvingen proefpersonen ofwel pioglitazone in combinatie met een of

meerdere oraal toegediende, glucose verlagende geneesmiddelen of uitsluitend orale, glucose verlagende geneesmiddelen (controle groep). Het behandelingsdoel was om een HbA1c waarde <6.9% te bereiken. Een getrapte indirect respons modelstructuur werd toegepast om het profiel van de concentraties van FPG en HbA1c gedurende de studieduur te beschrijven. Voor HbA1c werd het verloop beschreven met een combinatie van zowel een FPG afhankelijke als een FPG onafhankelijke functie. Om rekening te houden met het effect van eventuele titratiestappen, werd de aanpassing van de geneesmiddeldosering voor beide groepen beschreven met een tijdsafhankelijke, niet-lineaire functie.

Er kon een verschil in effect op FPG bij maximale blootstelling worden vastgesteld tussen de twee groepen. De model-afgeleide Emax waarden voor pioglitazone en de controle groep waren respectievelijk 17% en 8%. Dit resulteerde in een circa twee maal sterkere verlaging van FPG waarden in de pioglitazone groep vergeleken met de controle behandeling. Ziekteprogressie werd uitgedrukt als een tijdsafhankelijke functie van FPG ten opzichte van de (FPG) basislijn. De door het model voorspelde toename kon worden geschat op ongeveer 2mg/ml per jaar voor FPG en 0.2% per jaar voor HbA1c. Vervolgens werden simulaties van de veranderingen in FPG en HbA1c over en periode van 5 jaar uitgevoerd. Op basis van deze simulaties werd er voorspeld dat het maximale effect van het geneesmiddel voor FPG eerder optreedt voor pioglitazone dan voor de controlegroep (11 vs. 14 maanden). De voorspelde initiële daling van FPG en HbA1c behaald met pioglitazone zou volgens deze voorspelling langer aanhouden dan de huidige studieduur . Door de ontwikkeling van het huidige model, gebaseerd op lange termijn data(>2 jaar), is het mogelijk geworden via simulaties hypothesen op te stellen over de rol van PGx op het verloop van T2D en de behandeling hiervan, zowel van de symptomatische als van de ziekte remmende effecten.

Conclusies

De toepassing van op wiskundige modellen gebaseerde methoden om de invloed van

genotype op de werking van geneesmiddelen te bepalen, heeft zich primair geconcentreerd op het gebruik van genotype als een covariaat voor de blootstelling. De toepassing van deze modellen zou bij voorkeur moeten worden uitgebreid naar latere fasen geneesmiddelenontwikkeling waar klinische uitkomsten worden vastgesteld en de veiligheid wordt geëvalueerd. Dat maakt het mogelijk doseringsalgoritmes te optimaliseren voor de verschillende genotypen. Bovendien wordt het zo mogelijk om verschillende onderzoeksvragen te vergelijken; zoals bijvoorbeeld de vergelijking tussen de op genotype gebaseerde dosering tegenover de toediening van een eenheidsdosis voor alle proefpersonen. Toepassing van populatie PK-PD modellen om de invloed van genotype op de blootstelling te bepalen, levert een belangrijke schakel in het onderzoek naar de relaties tussen de veranderingen in de farmacokinetiek en de daaruit voortvloeiende verandering in effecten.

Door de routinematige bepaling van PGx eigenschappen in klinische studies zal het begrip hierover op mogelijke effecten van geneesmiddelen verder kunnen toenemen. Integratie van deze informatie verkregen uit de vroege fasen van geneesmiddelenontwikkeling is essentieel voor de juiste opzet van toekomstige klinische studies naar de relatie(s) tussen blootstelling en effecten in alle genetische subgroepen. Uiteindelijk zal een samenhangende benadering hiervan (dienen te) leiden tot een zo efficiënt mogelijke studie-opzet als tot het meest kansrijke klinische resultaat voor individuele patiënten.

Curriculum Vitae

Frances Stringer was born on August 11th 1979 in Durban, South Africa. In 1997 she completed her high school education at Hanson Upper and in the same year started her undergraduate degree at Nottingham University in England. She completed her undergraduate degree in Environmental Chemistry in 2000 and from 2000-2001 studied for a masters degree in Medicinal Chemistry and Drug Metabolism at Loughborough University.

In 2001 she started working in the pharmaceutical industry, with her first position as a Pharmacokineticist at Elan Drug Delivery in Athlone, Ireland. In 2002 she moved back to the UK to work for UCB-Celltech within the clinical assays department. From 2006-2014 she worked as a Clinical Pharmacologist for Takeda Pharmaceutical Company. Working firstly in the European headquarters in London before moving to Japan from 2010-2014. In Summer 2014, Frances joined Model Answers in Brisbane, Australia as a Pharmacometrician.

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