CYP2C8 and SLCO1B1 variants and therapeutic response to thiazolidinediones in patients with type 2 diabetes.

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Abstract

Objective: Thiazolidinediones (TZDs) are putatively transported into the liver by OATP1B1 (encoded by *SLCO1B1)* and metabolized by CYP450 2C8 enzyme (encoded by *CYP2C8*). Whilst CYP2C8**3* has been shown to alter TZD pharmacokinetics, it has not been shown to alter efficacy.

Design: We genotyped 833 Scottish Type 2 diabetes patients treated with pioglitazone or rosiglitazone and jointly investigated association of variants in these two genes with therapeutic outcome.

Result: The CYP2C8**3* variant was associated with reduced glycaemic response to rosiglitazone (P = 0.01) and less weight gain (P = 0.02). The *SLCO1B1* 521T>C variant was associated with enhanced glycaemic response to rosiglitazone (P = 0.04). The super responders defined by combined genotypes at CYP2C8 and *SLCO1B1* had a 0.39% (4 mmol/mol) greater HbA1c reduction (P = 0.006) than the poor responders. Neither of the variants had a significant impact on pioglitazone response.

Conclusion: These results show that variants in CYP2C8 and *SLCO1B1* have a large clinical impact on the therapeutic response to rosiglitazone, and highlight the importance of studying transporter and metabolising genes together in pharmacogenetics.

The TZDs, pioglitazone and rosiglitazone, have been widely used in combination with other oral agents for the treatment of type 2 diabetes. They act as peripheral insulin sensitizers by activating the nuclear peroxisome proliferator-activated receptor-γ (PPARG), which regulates the transcription of genes related to glucose metabolism (1). Following a meta-analysis of 42 studies that linked rosiglitazone to an increased risk of cardiovascular adverse effects (2), its marketing authorisation was withdrawn in Europe, and restricted use in the US. However its restriction has been lifted after the RECORD study failed to show cardiac risks associated with rosiglitazone (3). Pioglitazone is still in clinical use in most countries and its use has been suspended in France, and restricted in Germany, due to a small absolute increased risk in bladder cancer. However a recent multi-population analysis showed no association of pioglitazone or rosiglitazone with the risk of bladder cancer (4).

TZDs are effective at lowering HbA1c by about 1~1.25% (11-14mmol/mol) on average (5). Although TZDs show durability in action greater than seen with either metformin or sulphonylureas (6), weight gain induced by TZDs has restrained their clinical utility (7). For every 1% reduction in HbA1c an estimated 2-3% weight gain is documented (1).

The American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) guidelines continue to highlight the need to individualise treatment in diabetes (8), and this applies particularly for the TZDs where substantial inter-individual variation exists in glycaemic response (9). Epidemiological studies have identified age, gender, baseline weight and HbA1c as significant predictors of response, which can account for up to 49% of the variation in HbA1c reduction (10, 11). Genetic factors are expected to explain at

least part of the remaining variation and may be important to better aid targeted treatment in this patient group.

In-silico modelling has shown that both pioglitazone and rosiglitazone are putative substrates of transporter OATP1B1 which is encoded by *SLCO1B1* (12). Both agents are extensively metabolized in the liver, mainly by the cytochrome P450 2C8 enzyme encoded by CYP2C8 (13, 14). The main metabolites of rosiglitazone are N-desmethyl-rosiglitazone and rosiglitazone-para-O-sulfate that are 20-55 fold less potent compared to the parent drug (15). The principal metabolites of pioglitazone are M-III and M–IV; in contrast to the metabolites of rosiglitazone, they are shown to be pharmacologically active (16). Gemfibrozil, which inhibits both CYP2C8 and OATP1B1 has been shown to increase the plasma concentration Area Under the Curve (AUC) of pioglitazone and rosiglitazone between 2.4 and 3-fold in healthy volunteers (17, 18), suggesting a role for both CYP2C8 and OATP1B1 in pharmacokinetics of the agents.

Genetic variants CYP2C8*3* (linked polymorphisms of Arg139Lys and Lys399Arg), and *SLCO1B1* 521 T>C (Val174Ala) are commonly seen in populations of European ancestry with allele frequencies at around 12% and 16%, respectively (19). Pharmacokinetic studies of healthy volunteers have established that the gain of function CYP2C8*3* variant is associated with modestly enhanced TZD metabolism. Homozygote CYP2C8*3* carriers had 36% lower rosiglitazone plasma concentration and 39% higher weight-adjusted oral clearance rate compared to the wild type carriers, with clear gene dosage effect seen in the heterozygotes (20, 21). A similar trend has been shown with pioglitazone (22). Despite the pharmacokinetic effect of CYP2C8 variant on rosiglitazone, the studies that have assessed its impact on rosiglitazone efficacy have found no associations in small number of healthy non-insulin resistant volunteers

(20, 21). For *SLCO1B1*, despite the in-silico modelling, a pharmacokinetic study of 32 healthy volunteers found no association between the loss of function 521C allele and weight-adjusted plasma drug AUC after single dose rosiglitazone (4mg) or pioglitazone administration (23). The lack of consistency of these pharmacokinetic and dynamic studies is potentially due to the limited statistical power in the small samples to detect the moderate genetic effect, and the fact that the variants have previously been considered in isolation.

As TZDs have to be transported into the liver to be metabolised by CYP2C8, we assessed the glycaemic response and side effect of weight gain induced by variants in *SLCO1B1* and CYP2C8 together in a large population of patients with Type 2 diabetes treated with rosiglitazone or pioglitazone.

Research design and Methods

Sample ascertainment

Patients were ascertained from the Diabetes Audit and Research Tayside Study (DARTS), which has been described in detail previously (24). In brief, all the patients can be linked to the Medicine Monitoring Unit/Health Informatics Centre Database to retrieve validated prescribing, and to the clinical information system SCI-DC to obtain all biochemistry and clinical phenotypic data back to 1992. Prospective longitudinal data were also collected on these patients. Since October 1997, all patients with diabetes have been invited to give written informed consent to DNA and serum collection as part of the Wellcome Trust United Kingdom Type 2 Diabetes case control collection. As of June 2009, more than 9000 patients have participated in this Genetics of DARTS (Go-DARTS) study.

From 1942 incident TZD users in the Go-DARTS cohort, we identified a study sample of 833 patients who had TZD as their second-line (added to metformin or sulfonylurea monotherapy) or third-line (added to metformin and sulfonylurea dual therapy) treatment according to guideline in Scotland. To be included in the study, individuals had to have complete data with respect to age, gender, weight, oral antidiabetic treatment history, TZD treatment dose, adherence and regular HbA1c measurements. They all had a baseline HbA1c higher than 7%. They were on stable treatment for at least 6 months after TZD was initiated (the index date), which meant they did not start or stop another antidiabetic drug within 6 months either side of the TZD index date. They were not treated with insulin before or during the studied period. This will help to ascertain TZDs related efficacy outcomes. A detailed sample ascertainment procedure is outlined in Supplemental Figure 1. The study was approved by the Tayside Regional Ethics Committee and informed consent was obtained from all subjects.

Drug response definitions

Individuals' glycaemic response to TZDs was modelled as the maximum HbA1c reduction recorded within 1 to 18 months of the index date while maintained on stable treatment. Similarly, TZD induced weight gain was measured as the difference between the last measurement within the study period and the baseline weight. The multivariate linear model equation for these two outcomes is:

HbA1c Reduction (Weight Gain) ~ Baseline HbA1c + Baseline Weight + Adherence + Daily Dose + Study Duration + Age + Sex + Genotype

Baseline HbA1c and baseline weight were defined as the nearest measures taken within the 180 days prior to the TZD index date. Adherence was calculated from the population-based

drug dispensing records as the percentage of maximum possible adherence for each participant. Treatment dose was determined as the mean dose of prescriptions encashed during the three months prior to the minimum HbA1c within the 1-18 months of TZD index date. When the minimum HbA1c happened in less than three months, the average dose before the treatment HbA1c was recorded.

Genotyping

CYP2C8**3* (rs10509681) and *SLCO1B1* 521T>C (rs4149056) were genotyped in the entire Go-DARTS cohort with Taqman-based allelic discrimination assays. As the two CYP2C8**3* variants rs10509681 and rs11572080 are in perfect linkage disequilibrium ($r^2 = 1$ in the 1000 genome CEU panel) (25), only rs10509681 was genotyped in the current study. Assays were performed under manufacturer (Applied Biosystems) recommended standard conditions. Assays were performed on 10ng genomic DNA in 384 well plates; cycled using a H2OBIT thermal cycler (Thermo Scientific, Surrey); fluorescence detection and genotype calling were performed on an ABI 7900FastHT sequence detection system (Applied Biosystems).

Statistical analysis

One-way ANOVA was used to test for differences in the baseline characteristics by genotype. Allele frequencies difference between subgroups and the full sample was compared in a 2d.f. Chi-Squared test. The exact test of Hardy-Weinberg Equilibrium was carried out with PLINK (26). Multivariate linear regression analyses of HbA1c reduction and weight gain were performed with PLINK under additive genetic model and all the covariates included.

Results

In the 833 patients studied, the allele frequencies of CYP2C8*3 and *SLCO1B1* 521 C were 14.5% and 16%, respectively. The overall genotyping call rate was 94% and both SNPs were in Hardy-Weinberg Equilibrium in the sample ($P > 0.05$). In addition we compared the Taqman genotypes to the existing genotypes from exomechip and the concordance rate for rs10509681 and rs4149056 were 99.8% and 99.7%, respectively. There was no baseline clinical characteristic difference according to CYP2C8 or *SLCO1B1* variant genotypes (Supplemental Table S1).

The number of patients treated with pioglitazone and rosiglitazone were 273 and 519 respectively, with the other 41 patients switched between the two agents. In the combined analysis higher baseline HbA1c, higher baseline weight, older age, being female, higher adherence and longer treatment duration were independently associated with better glycaemic response. Greater weight gain was associated with higher baseline HbA1c, higher baseline weight, higher daily dose, being female and being treated by pioglitazone. No significant association with HbA1c reduction was observed when the CYP2C8*3 and *SLCO1B1* 521C variants were included into the clinical model (Supplemental Table S2). However compared to the wild type, carriers of the *3 allele had less weight gain (β= -0.91, P = 0.006).

Compared to parent drugs, metabolites of rosiglitazone and pioglitazone exert different degrees of glycaemic efficacy (16). In addition, differences in baseline characteristics of pioglitazone and rosiglitazone treated individuals, as shown in Supplemental Table S3 have been observed. Therefore we performed multiple linear regression analysis in the two subgroups separately. The same set of clinical covariates were included in the modelling of weight gain and HbA1c reduction. Table 1 shows the full clinical models in rosiglitazone treated group. A higher baseline HbA1c, higher baseline weight, older age, being female and longer treatment were all

independently associated with better glycaemic response. A higher daily dose was the only strong predictor of weight gain with patients on 8mg/day gaining 2kg more weight than those on 4kg/day (although dose was not associated with glycaemic response to rosiglitazone). For pioglitazone treated patients, a similar pattern of clinical predictors were observed but with less statistical significance due to the smaller number of patients (Supplemental Table S4). In contrast to rosiglitazone, there was no significant effect of pioglitazone dose on weight gain.

When genetic variants were added to the clinical models, patients carrying the CYP2C8**3* variant achieved less HbA1c reduction (allelic beta = -0.21%, $P = 0.01$) and experienced less weight gain (allelic beta = -0.93kg, $P = 0.02$) with rosiglitazone treatment. The *SLCO1B1* 521C variant was associated with greater HbA1c reduction (allelic beta= 0.18%, $P = 0.04$), but not weight gain after rosiglitazone treatment. Neither of the two variants was significantly associated with response to pioglitazone (see Table 2). This could be due to lack of enough statistical power from smaller number of patients treated with pioglitazone. Assuming the **3* variant has the same allelic effect size of 0.21% HbA1c reduction on both rosiglitazone and pioglitazone, the current sample size of 273 pioglitazone users will provide only 37% statistical power to detect the association at an alpha level of 0.05 (27). More than 800 samples are required to provide sufficient (80%) statistical power to detect such an effect size.

To better assess the impact of these variants in rosiglitazone response, we considered a composite model consisting a group of super responders (reduced transport at OATP1B1 (*SLCO1B1* 521 C) and 'normal' metabolisers at CYP2C8 (wild type)), intermediate responders (wild type at CYP2C8 and SLCO1B1) and poor responders ('normal' transport of rosiglitazone into the liver across OATP1B1 (*SLCO1B1* 521 T) and increased metabolism by CYP2C8 (CYP2C8**3*)). When the two variants were considered together, as shown in Figure 1, the super

responders had a 0.39% (4 mmol/mol) (P = 0.006) greater HbA1c reduction than the poor responders. A similar, but non-significant effect was seen on weight gain.

Since dosing is a strong predictor of rosiglitazone induced weight gain, we performed a stratified genetic analysis of the rosiglitazone treated patients by daily dose. As shown in Supplemental Table S5, the CYP2C8*3 variant had a similar impact on weight gain and HbA1c reduction in those treated with 4mg/day and 8mg/day. The *SLCO1B1* variant had a stronger impact on glycaemic response in those treated with 8mg/day than those treated with 4mg/day. Due to the limited sample size, this observed pharmacogenetic difference is not statistically significant in a formal gene by dose interaction test (P = 0.73).

Conclusion

In this large population pharmacogenetic study of patients with type 2 diabetes, we have jointly investigated whether variants in the putative drug transporter gene *SLCO1B1* and the metabolizing enzyme gene CYP2C8 contribute to variation in glycaemic response and weight gain in response to treatment with TZDs. We confirm previous reports that TZDs work better in women, and with increasing obesity (28, 29). The combined genotypes at CYP2C8 and *SLCO1B1* can be used to define a super response and a poor response groups to rosiglitazone, who differ in HbA1c reduction by approximately 0.39% (4 mmol/mol). This effect size is about one-third of the average HbA1c reduction achieved by 8mg daily rosiglitazone (5) or about half of the HbA1c reduction related to DPP-4 inhibitors monotherapy (30). Therefore, the effect size observed in this study could be clinically relevant in stratified medicine. On the other hand these variants do not alter pioglitazone response.

We showed rosiglitazone treated individuals carrying the CYP2C8*3 variant had poorer glycaemic response but less weight gain in a gene-dosage dependent manner compared to the wild type carriers. These results are consistent with previous pharmacokinetic studies which showed that the CYP2C8*3 variant was associated with higher rosiglitazone oral clearance, and lower plasma concentration AUC (20, 21). Other previous investigations into the pharmacodynamic impact of CYP2C8 variations on rosiglitazone response have found no evidence in small samples of normal insulin sensitivity subjects (20, 21). However association of the CYP2C8*3 variant with impaired HbA1c lowering has been reported in type 2 diabetes individuals (31). The current study has demonstrated that the mild pharmacokinetic difference between CYP2C8*3 genotype can be translated into pharmacodynamic difference in rosiglitazone treated type 2 diabetes individuals, with the lower drug exposure among the CYP2C8*3 variant carriers resulting in less HbA1c reduction and weight gain.

In this study we showed association of CYP2C8*3 with response to rosiglitazone but not pioglitazone despite an established role of CYP2C8 in pioglitazone pharmacokinetics. This is entirely consistent with the contrast between the pharmacological properties of the two agents (Figure 2). As the main rosiglitazone metabolites are less potent, pharmacokinetic difference of the parent drug were translated into efficacy difference. For pioglitazone, the principal biotransformation products, M-III and M-IV, are reported to exert sustained hypoglycaemic action, therefore ameliorate the pharmacokinetic difference in parent drug on overall efficacy (32).

In this study, we have for the first time showed that the *SLCO1B1* 521C allele is associated with better glycaemic response in patients treated with rosiglitazone. Our results also indicated that the pharmacogenetic effect of *SLCO1B1* 521 T>C variant on rosiglitazone response was

more pronounced in the 8mg/day group than in the 4mg/day group. This might explain why previous rosiglitazone pharmacokinetic studies reported no significant association between *SLCO1B1* 521 T>C genotypes and drug exposure after 4mg/day treatment and suggests the variant becomes rate limiting only at high doses (19, 20).

Joint investigation of variants in genes encoding for proteins involved in pharmacokinetics and pharmacodynamics of a given drug is believed to give better understanding of the role of genetics in drug response than individual variants per se. For example, studies investigating joint effect of variants in metformin transporters has been published elsewhere (33-35). With this in mind, we have investigated joint effect of variants in genes encoding TZD transporter (*SLCO1B1*) and metabolizer (CYP2C8). In a composite model that consists of super responders and poor responders, the glycaemic effect of the *SLCO1B1* variant is much greater when considered on a CYP2C8 wild type background (allelic effect 0.22) compared to on a CYP2C8 variant background (allelic effect 0.1). This finding highlights the importance, when considering drug transporters and drug metabolizing enzymes, to assess variants that alter drug availability for metabolism and variants that alter rate of metabolism together, otherwise clinically important variants may be overlooked. Moreover, other functional variants such as those regulatory variants in these two genes could also affect the pharmacokinetics of TZDs, therefore contribute to the variation in treatment outcome. Locus-wise genetic screening would be useful to identify other functional variants in these two genes. In addition, further functional studies investigating the joint role of these variants on HbA1c reduction and weight gain are also warranted.

There were some limitations of our study. The main limitation is the observational nature of our dataset which may introduce bias. Response modelling has shown baseline HbA1c and

weight, the dose given, treatment duration, age and sex all added variation to TZD response among the patients. Despite adjusting for these clinical characteristics in the model, the association between genetic variants and drug response could still be confounded. However, there was no phenotypic difference by genotype in our study sample as shown in Supplemental Table S1, and the clinicians and participants were clearly blind to genotype, so these extrinsic factors will not introduce bias to the pharmacogenetic effect. A further limitation is our measure of weight gain. It is not possible to differentiate if measured weight gain reflects fluid retention or increase in fat mass or both. Finally, our sample size, despite being much larger than any published study, is still small. This in particular limits the phenotypes we are able to study. For example, it is not possible to assess the impact of these variants on other side effects such as incident heart failure due to a major lack of power.

Finally we acknowledge that we have undertaken a number of statistical tests in this study. We performed a total number of eight independent genetic association tests (two variants against two outcomes in two treatment groups) which carry a threshold of $p=0.006$ (0.05/8) for any individual signal to be study-wide significant under a stringent Bonferroni correction. As shown in table 2, three independent signals did reach the conventional threshold of $p<0.05$ with the current sample size. In addition when the genotypes of the two variants were combined together based on known biological mechanism, a study-wide significant ($p=0.006$) result was observed between super responders and poor responders to rosiglitazone.

This study established that glycaemic response and weight gain in rosiglitazone treated type 2 diabetes individuals were associated with genetic variants in the drug transporter gene *SLCO1B1* and the metabolizing enzyme gene CYP2C8, and highlighted the importance of studying pharmacokinetic genes together. The genetically defined super responders had an

extra 0.39% (4 mmol/mol) HbA1c reduction than those non-responders. Whilst our results establish key pharmacogenetic variants that alter response to rosiglitazone, there could be factors that hinder its direct clinical applicability. The variants that increase glycaemic efficacy to rosiglitazone also increase weight gain i.e. the 'benefit' and 'harm' are both increased. With the increasing awareness of risk associated with TZDs there is a need to optimize the benefit and reduce the risk for an individual. We believe that this is a key opportunity for pharmacogenetics to potentially identify individuals who can benefit from the considerable therapeutic advantages of TZDs, who are least at risk of the side effects. Rather than letting TZDs slide into disuse, efforts should concentrate on identifying predictors of response or harm to TZDs.

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Conflict of interest

All authors declare no conflict of interest pertaining to this manuscript.

Author contributions

A.D, wrote manuscript, performed research, analysed data. K.Z. wrote the manuscript, designed research, performed research, and analyzed data. L.D. analyzed data. R.T. analyzed data. F.C. analyzed data. G.L. performed research. C.N.A.P. wrote the manuscript, designed research, performed research. E.R. P. wrote the manuscript, designed research, performed research, and analyzed data. K.Z. and A.Y. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Figure Legends

Figure 1. Rosiglitazone response by *SLCO1B1* and CYP2C8 genotypes. Super responders (wild type at CYP2C8 and one or more variant C allele at SLCO1B1), Intermediate responders (wild type at both CYP2C8 and SLCO1B1), Poor responders (one or more **3* allele at CYP2C8 and wild type at SLCO1B1). The error bars represented the standard error of the mean. ***P* < 0.01.

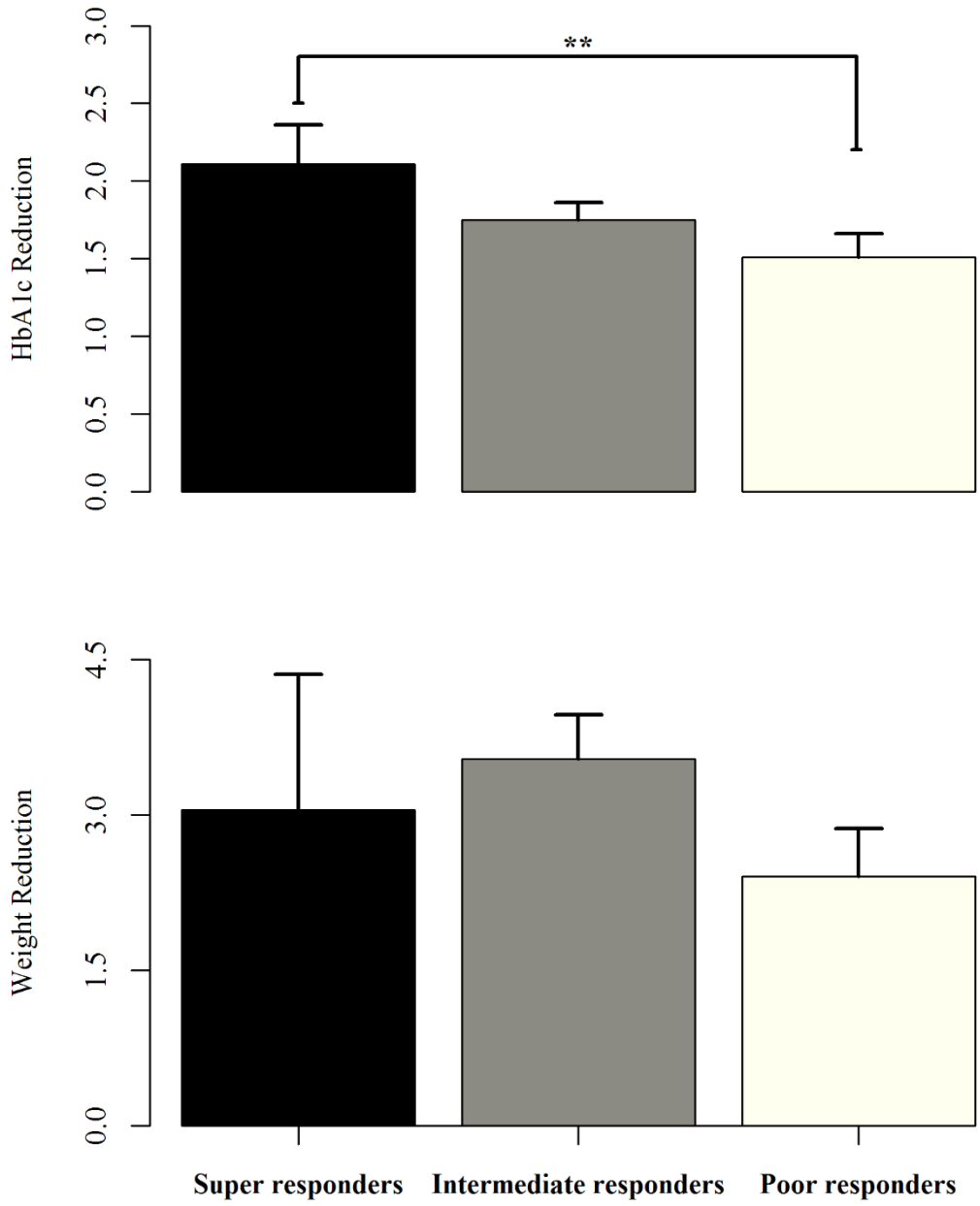

Figure 2. Pharmacogenetic effect of CYP2C8 and *SLCO1B1* on TZDs pharmacokinetics and pharmacodynamics. Pharmacogenetic influence by CYP2C8 and *SLCO1B1* variants is expected to affect rosiglitazone pharmacodynamics because both its main metabolites (N-desmethyl-rosiglitazone and rosiglitazone-para-O-sulfate) are less potent than its parent drug and pharmacokinetic differences will alter the drug exposure of active components (the parent drug, rosiglitazone) and therefore therapeutic response. Patients carrying the wild type *SLCO1B1* allele and gain of function CYP2C8 variants are expected to eliminate rosiglitazone much faster (poor responders) than carriers of the loss of function *SLCO1B1* variants on a wild type CYP2C8 background (super responders). In comparison, no pharmacogenetic effect is expected on pioglitazone response as its main metabolites (M-II, M-III and M-IV) remain active and the exposure of total active drug components is not altered by pharmacokinetic difference.

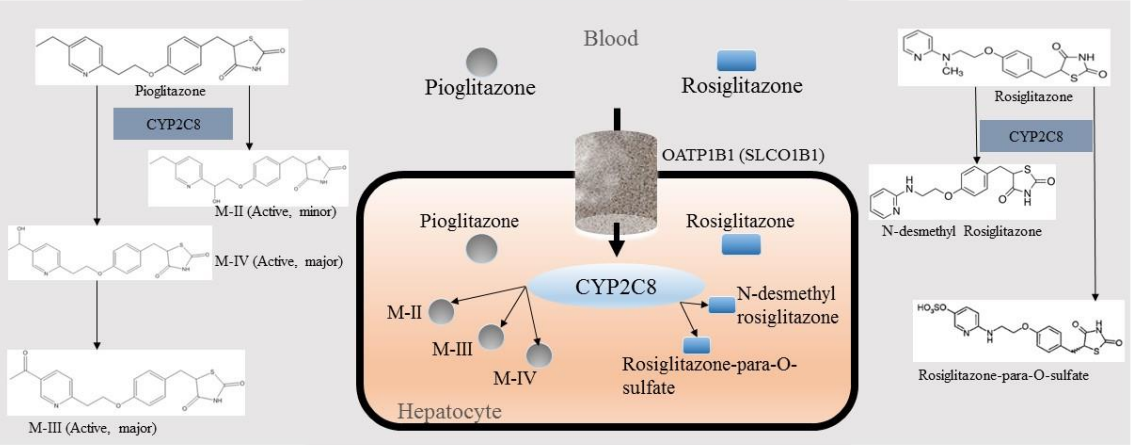

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Table 1. Multiple linear models for HbA1c reduction and weight gain in rosiglitazone.

	Weight Gain			HbA1C Reduction		
	Beta	95% CI	*P*-value	Beta	95% CI	*P*-value
Baseline HbA1c	0.33	[0.15,0.65]	0.04	0.65	[0.59,0.72]	< 0.001
Baseline Weight	0.23	[-0.01,0.47]	0.06	0.07	[0.02,0.13]	0.004
Age	0.19	[-0.19,0.58]	0.33	0.23	[0.15,0.31]	< 0.001
Sex	0.82	[-0.12,1.66]	0.06	0.28	[0.09,0.46]	0.003
Dose	0.41	[0.25,0.59]	< 0.001	0.03	[-0.01,0.06]	0.19
Adherence	0.23	[-0.06,0.51]	0.11	0.05	[-0.01,0.11]	0.09
Study Duration	-0.08	[-0.20,0.04]	0.18	0.06	[0.03,0.08]	< 0.001

Sex was coded 1 and 2 for male and female respectively; Age was coded in the unit of 10 years; Baseline HbA1C was measured as percentage; Dose was measured as 10% of the recommended maximum daily dose; Adherence was measured in 10%; Baseline weight was measured in 10kg; and the study duration was measured in month as the time from TZD index date to the treatment outcome measurement date.

Table 2. Genetic effect of CYP2C8 and *SLCO1B1* variants on HbA1c reduction and weight gain (additive genetic model).

Treatment	Gene	Weight Gain			HbA1C Reduction		
		Beta	95% CI	*P*-value	Beta	95% CI	*P*-value
Rosiglitazone (n=444)	CYP2C8**3*	-0.93	[-1.73,-0.13]	**0.02**	-0.21	[-0.38,-0.04]	**0.01**
	SLCO1B1	-0.13	[-0.92,0.67]	0.75	0.18	[0.01,0.34]	**0.04**
Pioglitazone (n=239)	CYP2C8**3*	-0.46	[-1.45,0.51]	0.34	0.14	[-0.10,0.38]	0.26
	SLCO1B1	-0.02	[-0.92,0.87]	0.96	-0.10	[-0.32,0.12]	0.37