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Pharmacogenetics and future strategies in treating hyperglycaemia in diabetes

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1. ABSTRACT

This review focuses on current evidence for pharmacogenetics for the 3 commonly used drug classes in treating diabetes: metformin, sulphonylureas and thiazolidinediones. Currently, metformin pharmacogenetics is focussing on drug transport with the recent finding that variation in OCT transporters might affect metformin response. An aetiological approach has identified monogenic patients with diabetes due to *TCF1* mutations who are particularly sensitive to the hypoglycaemic effects of sulphonylureas, and *KCNJ11* or *ABCC8* mutations in which sulphonylureas can be used in place of insulin treatment. In Type 2 diabetes sulphonylurea response has been shown to be associated with variants *TCF7L2* associated with type 2 diabetes risk. For thiazolidinediones, focus has been on PPARgamma variants although with no consistent result. Genome wide association studies offer great potential to unravel what genetic factors influence response and side effects of diabetes therapies. Large numbers of well phenotyped patients for response and side effect as well as similarly sized similarly phenotyped replication cohorts are required. Establishing such cohorts is a priority in diabetes pharmacogenetics research.

2. INTRODUCTION

The last five years have seen a significant breakthrough in the understanding of the genetic aetiology of both common type 2 diabetes and rare mendelian forms of diabetes. The impact of these genetic discoveries on clinical care to date remains largely limited to these rarer types of diabetes; but our increasing understanding of the molecular mechanisms of diabetes offers hope that we might be able to better target existing therapy and design new therapies. However, except for a few examples the clinical impact of pharmacogenetics is minimal, and no more so than in the management of diabetes. This review will highlight key areas where progress has been made – in Maturity Onset Diabetes of the Young and Neonatal Diabetes – and will outline some recent studies that are starting to unravel genetic determinants of drug response in type 2 diabetes.

The response to a drug is determined by the concentration of active drug available at its site(s) of action (pharmacokinetics), and then the ability of the drug to elicit an effect at its site of action (pharmacodynamics). In simple terms, the pharmacokinetics of a drug are determined by: its absorption, which may be passive or

active; metabolism to an active form if required; distribution to the drugs site of action, which again can be passive or active e.g. into the liver or across the blood brain barrier; the rate of drug clearance which might include metabolism to an inactive form, and excretion into the bile or urine. The pharmacodynamics of a drug can be broadly divided into direct and indirect factors. The direct drug effects will be influenced by its ability to bind to a receptor, the function of that receptor and function of the downstream pathways. The indirect factors are those that are distinct from the effector pathway e.g. response to a drug that increases insulin secretion may well be more effective in a patient that is more insulin sensitive although the drug effect has no effect on insulin action. For a disease like Type 2 diabetes that is highly heterogeneous and where the drugs used target the disease causing defects, both direct and indirect pharmacodynamics of a drug will be influenced by disease aetiology i.e. someone may respond well to sulphonylureas because their diabetes is aetiologically distinct from someone who responds poorly.

Pharmacogenetic studies investigate the role of genetic variation in pharmacokinetics or pharmacodynamics on drug response, where drug response can be positive (therapeutic) or negative (side effect). Until recently pharmacogenetic studies have focussed primarily on genetic variation in drug metabolism enzymes following early discoveries that this trait was inherited. The most extensively studied of these systems is the cytochrome p450 family. Other than metformin, the oral agents used to treat type 2 diabetes are metabolised via these enzymes and examples of this will be discussed. More recently, genetic studies have investigated other aspects of pharmacokinetics, including the role of drug transporters and this can be seen in recent advances in metformin pharmacogenetics. Although quite rare, monogenic diabetes has provided two dramatic examples of aetiological pharmacogenetics - how disease aetiology can impact on treatment response (1, 2). However, the increasing data available from genome wide association studies of type 2 diabetes offer the exciting potential to be able to tease out polygenic variants that impact on response to commonly used therapies.

This review will present an overview of established and potential genetic variants that influence response to the three commonly used classes of oral agents in diabetes – sulphonylureas, biguanides and thiazolidinediones. There is no pharmacogenetic data available for the newer agents such as the GLP-1 analogues and DPP-IV inhibitors. For each therapy, the mechanism of action will be discussed and data on genetic variation of pharmacokinetics and both monogenic and polygenic aetiological pharmacogenetics will be presented.

3. SULPHONYLUREAS

3.1. Mechanism

Sulphonylureas primarily promote pancreatic insulin secretion. They bind to the SUR1 moiety of the pancreatic beta-cell K_{ATP} channel causing the channel to close. The K_{ATP} channel is the key regulator of insulin secretion in the beta-cell, and channel closure causes membrane depolarisation which triggers calcium influx and release of intracellular calcium stores, which in turn stimulates translocation of insulin containing vesicles to the cell membrane, exocytosis and subsequent insulin release. There are a number of different sulphonylureas that vary in their duration of action and pancreatic specificity and these are reviewed in (3). Commonly used sulphonylureas include gliclazide, glibenclamide, glimepiride and glipizide.

3.2 Efficacy and variation in response.

There is no difference in efficacy of the different sulphonylureas (4-7) with a mean reduction in HbA1c of between 1.5 and 2%, depending on the baseline HbA1c (8). 10 to 20% of patients will have a poor response to sulphonylureas with a decrease in fasting glucose < 1.1mmol/L and these are considered to have primary failure (6). Looking retrospectively within Tayside, Scotland, for patients with a pre-treatment HbA1c between 7 and 9%, the mean HbA1c reduction on Sulphonylurea initiation is 1%; however, 15% of patients have a reduction greater than 2% and 15% have primary failure with deterioration in HbA1c despite treatment (Pearson, unpublished data).

3.3. Pharmacokinetics

In 1979, a ninefold variation in the rate of tolbutamide disappearance from plasma was described with a trimodal distribution suggestive of monogenic inheritance (9). This variation in hydroxylation of tolbutamide was subsequently shown to be due to variation in CYP2C9 (10, 11). CYP2C9 has also been shown to be a rate-limiting enzyme in the metabolism of other sulphonylureas including glibenclamide (12), gliclazide (13), glipizide (14) and glimepiride (15-17). Two variants in CYP2C9 affect the catalytic function of the enzyme: Arg144Cys (2C9*2; allele frequency 11%) and Ile359Leu (2C9*3; allele frequency 7%) (18-20). For glibenclamide the clearance for the *2/*2 individuals was reduced by 25% and for the *3/*3 individuals by 57% compared to wild type (12). Similar figures for tolbutamide are 25% and 84% (21).

3.4. Genetic variation in pharmacokinetics

There have been only a few studies looking at the impact of CYP2C9 variation on sulphonylurea response in patients with type 2 diabetes. In a study of just 20 diabetic patients admitted to the emergency room with severe hypoglycaemia during sulphonylurea treatment compared with 337 patients with type 2 diabetes and no history of severe hypoglycaemia, the *3/*3 and *2/*3 were over-represented in the hypoglycaemia group (10% vs 2.1%). However, this result is based on just 2 patients in the hypoglycaemia group and as such could easily be a false positive. In a recent observational population study of 172 patients prescribed tolbutamide, those individuals carrying the *3 allele (n=20) had a significantly lower dose escalation of tolbutamide compared to wild type, consistent with a greater therapeutic response to the low initiation doses in this group. Numbers carrying the *3 allele were low and a similar result was not seen with glibenclamide (n=34) and glimepiride (n=42) use. However, this is the first study to suggest a sulphonylurea dose effect of

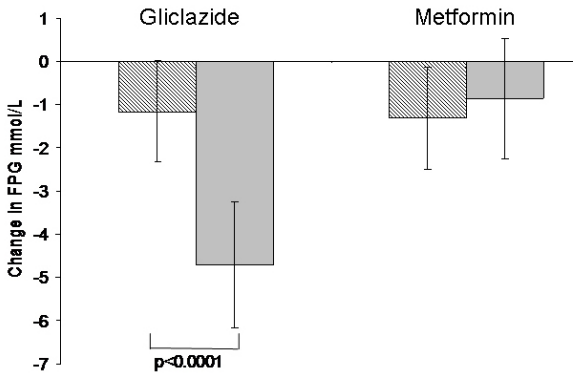

Figure 1. Change in fasting plasma glucose in response to 6 weeks of gliclazide or metformin in patients with *HNF1A* mutations or Type 2 diabetes. Bars represent mean, and error bars the 95% confidence limits. Type 2 diabetes represented by diagonal striped shading, HNF1A by grey shading. Reproduced with permission from (1).

CYP2C9. Only minimal data were available on glycaemic response and no data were available on hypoglycaemia. There is clearly a requirement for a large-scale study on CYP2C9 variation on glycaemic response and hypoglycaemia in sulphonylurea users, however, despite the considerable effects of CYP2C9 variants on pharmacokinetics of sulphonylureas, currently genotyping is not considered to be of value prior to sulphonylurea administration.

3.5. Aetiological pharmacogenetics – Monogenic Diabetes

In the last 5 years there have been two examples where diabetes aetiology determines treatment response in diabetes. These are sulphonylurea sensitivity seen in monogenic diabetes (Maturity onset diabetes of the young or MODY) due to heterozygous mutations in *TCF1* (encoding HNF-1alpha) (1), and the finding that two novel types of neonatal diabetes due to mutations in *KCNJ11* (encoding Kir6.2) and *ABCC8* (encoding SUR1) can be treated with sulphonylureas rather than lifelong insulin (2, 22). Although these types of diabetes are rare, they are often mistaken for other forms of diabetes and they present a good paradigm for aetiological pharmacogenetics that should be translatable to polygenic disease.

3.5.1. Maturity Onset Diabetes of the Young due to HNF-1alpha mutations

Maturity onset diabetes of the young (MODY) is defined by the presence of a pedigree with at least a 2 generation family history of non-insulin requiring diabetes that presents before the age of 25 in one family member. It is heterogeneous with mutations in at least 6 genes identified to date (reviewed in (23)). In the UK, and probably internationally, the most common form of MODY is due to heterozygous mutations in the transcription factor HNF-1alpha, encoded by *TCF1*. These account for 1% of all diabetes (24). HNF-1alpha MODY usually presents in teenage or early adult years, and after initial management with diet, requires oral treatment before progressing to insulin. It is associated with microvascular complications of diabetes, and increased macrovascular disease. Other features of diabetes due to HNF-1alpha are a decreased renal threshold for glucose, and a marked sensitivity to sulphonylurea medication.

In a randomised trial of sulphonylureas and metformin in patients with diabetes due to HNF1A mutations and Type 2 diabetes, the fall in fasting plasma glucose to gliclazide was 3.9-fold greater in patients with *HNF1A* mutations than their response to metformin (p=0.002); as expected, no difference in response to gliclazide or metformin was apparent in those with type 2 diabetes (1) (Figure 1). The mechanism probably results from the fact that the major β-cell defects due to reduced HNF-1alpha function are in glucose metabolism, and are therefore bypassed by sulphonylureas which act on the K_{ATP} channel to stimulate insulin release (1). This study highlighted, for the first time, the importance of genetic aetiology in determining response to treatment in diabetes and has led to change in clinical management of patients with *HNF1A* mutations. Sulphonylureas are now recommended as the first line anti-diabetic therapy for these patients. Excitingly patients who have been assumed to have type 1 diabetes and treated with insulin, who are subsequently found to have an HNF1A mutation have been able to transfer off insulin onto sulphonylurea therapy (25).

3.5.2. Infancy Onset Diabetes

Diabetes diagnosed before the age of 6 months is only rarely due to autoimmune type 1 diabetes (26, 27) yet until recently only a few isolated causes had been identified (reviewed in (28)). In 2004, a third of cases of diabetes diagnosed before 6 months of age were found to be due to activating mutations in the *KCNJ11* gene encoding the Kir6.2 subunit of the beta-cell K_{ATP} channel (29). Subsequently in 2006, mutations in the *ABCC8* gene encoding the other subunit of the K_{ATP} channel, SUR1 were also found to cause neonatal diabetes, although less commonly (22, 30).

In normal insulin secretion, the pancreatic K_{ATP} channel is a key regulator, converting glucose metabolism within the cell into changes in membrane potential. As intracellular ATP rises following glucose metabolism, the K_{ATP} channels close causing membrane depolarisation and subsequently insulin secretion. In neonatal diabetes due to Kir6.2 or SUR1 mutations, the KATP channel is insensitive to changes in intracellular ATP, so whatever the prevailing glucose the beta-cell will not produce insulin (29).

It was hypothesised that because these mutations were in the K_{ATP} channels where sulphonylureas bind, oral sulphonylureas might work to produce insulin secretion in these patients who have been 'insulin dependent' from soon after birth. Following promising physiological studies (29), sulphonylureas were used successfully in enabling patients to transfer off insulin (31). In a large series, over 90% of patients were able to transfer off insulin, with an age ranging from 3 months to 36 years (2). A large number of these patients had normoglycaemia with sulphonylurea treatment without significant hypoglycaemia suggesting

that the sulphonylureas were enabling meal regulated insulin secretion. This is because the predominant effect of the sulphonylureas in these patients is not to directly stimulate insulin secretion but to promote action of non-glucose secretagogues such as the incretins (2). There can be no doubt that for these rare patients, finding a genetic cause for their diabetes has had a huge impact on their quality of life and long term health outcomes.

3.6. Aetiological Pharmacogenetics – Type 2 diabetes

Given that sulphonylureas bind to the beta-cell K_{ATP} channel, candidate aetiological variants that might influence response to sulphonylureas will encode the K_{ATP} channel itself (*KCNJ11* and *ABCC8*), upstream pathways including genes encoding enzymes of glycolysis and mitochondrial metabolism and transcription factors involved in these pathways (e.g. *TCF1* and *HNF4A*), and downstream pathways including the voltage gated calcium channel, the insulin gene and the molecular machinery involved in insulin exocytosis. Indirect aetiological variants will be those that otherwise affect beta-cell function, possibly by decreasing beta-cell mass, or alter insulin sensitivity. Recently a number of established variants have been identified as associated with type 2 diabetes risk that impact primarily on beta cell insulin secretion, and these include variants in *TCF7L2* (32), *KCNJ11*, *CDKAL1,CDKN2A-2B, WFS1, HHEX-IDE, SLC30A8* (reviewed in (33)). To date the only aetiological candidate genes published investigating association with sulphonylurea response are in *KCNJ11, ABCC8 and TCF7L2*. These studies are generally small, and none have been independently replicated and the results should not be over interpreted.

The E23K variant of KCNJ11 was robustly associated with type 2 diabetes in a large meta-analysis (34). The insulin secretion has been reported to be reduced (35), or normal (36) in carriers of the K allele. In a study of human donor islets, glibenclamide induced insulin secretion was impaired in the KK islets (37). The association of response to sulphonylureas and the E23K variant was studied in the UKPDS cohort, where in a study of 360 type 2 diabetic patients there was no effect of the genotype on the change in fasting plasma glucose in the first year of treatment (38). In a subsequent study of 525 patients with type 2 diabetes, sulphonylurea failure was confusingly defined as failure of combination sulphonylurea and metformin therapy rather than sulphonylurea alone. In this study carriers of the K allele had a relative risk for failure of this combination of 1.45 (95%CI 1.01 – 2.09, p=0.04). However, it is unclear whether this reflects sulphonylurea failure, metformin failure or simple differential rates in diabetes progression by genotype. Using this same approach, the authors have also described an association between the G972R IRS-1 variant and 'sulphonylurea' failure (OR 2.1) (39).

Although variants in ABCC8 (encoding SUR1) have not been robustly associated with type 2 diabetes, the A1369S (40) and the silent AGG1273AGA (41) variants have been associated with progression to diabetes from impaired glucose tolerance. No studies have looked at ABCC8 variants on glycaemic response to sulphonylureas, however, one study has demonstrated decreased tolbutamide-stimulated insulin secretion in 10 subjects carrying a combined genotype associated with diabetes risk.

In 2006 the DeCODE group published an association between *TCF7L2* variants and type 2 diabetes risk, such that the 10% of the population homozygous for the risk variant were twice as likely to develop diabetes as the wild type population (32). This has been widely replicated and remains the strongest genetic association for type 2 diabetes described to date. The mechanism for how *TCF7L2* variants cause diabetes remains unclear, although a number of studies point to this being due to decreased beta cell function (42, 43), possibly mediated by an impaired incretin response (43). Given the potential role of *TCF7L2* in insulin secretion, and its large effect (by type 2 diabetes standards) it is a good candidate gene for assessing impact on sulphonylurea response. In a large study from Tayside, Scotland, of 901 incident users of sulphonylureas, patients with type 2 diabetes who were homozygous for the diabetes risk allele (G) at SNP rs12255372 were twice as likely not to be treated to below a target HbA1c of 7% in the first 3 -12 months of treatment compared to patients homozygous for the T allele (OR 1.95, p=0.005) (44). Importantly there was no effect of this variant on metformin response (n=945) (44) showing that the association is with sulphonylurea response rather than diabetes severity or progression. Figure 2 shows the Kaplan Meier plot for time to failure for both sulphonylurea and metformin use by *TCF7L2* genotype (44).

4. METFORMIN

4.1. Mechanism

Despite metformin's first description over 50 years ago, its mechanism of action, both at a physiological and a molecular level, remains hotly debated. Metformin, the only licensed biguanide, is widely accepted to be an 'insulin sensitizer'. In a systematic review(45), metformin's primary effect was to reduce hepatic glucose output by increasing insulin suppression of gluconeogenesis. Despite some studies showing an effect of increasing insulin mediated glucose disposal into muscle, this review did not conclude this to be an important effect. Interestingly a recent very carefully controlled clamp study comparing metformin and pioglitazone treatment showed no effect of metformin on insulin action at the liver (46). An important and often overlooked mechanism of metformin is a reduction in non-insulin mediated glucose clearance (45), which explains as much of the effect of metformin on glucose lowering as its role on hepatic glucose production, and a reduction in glucose absorption from the gut.

At the molecular level, the effects of metformin are mediated via AMP activated protein kinase (AMPK)(47), an effect that requires phosphorylation of AMPK by LKB1 (48), but metformin does not directly activate AMPK or LKB1 and the mechanism where by metformin activates

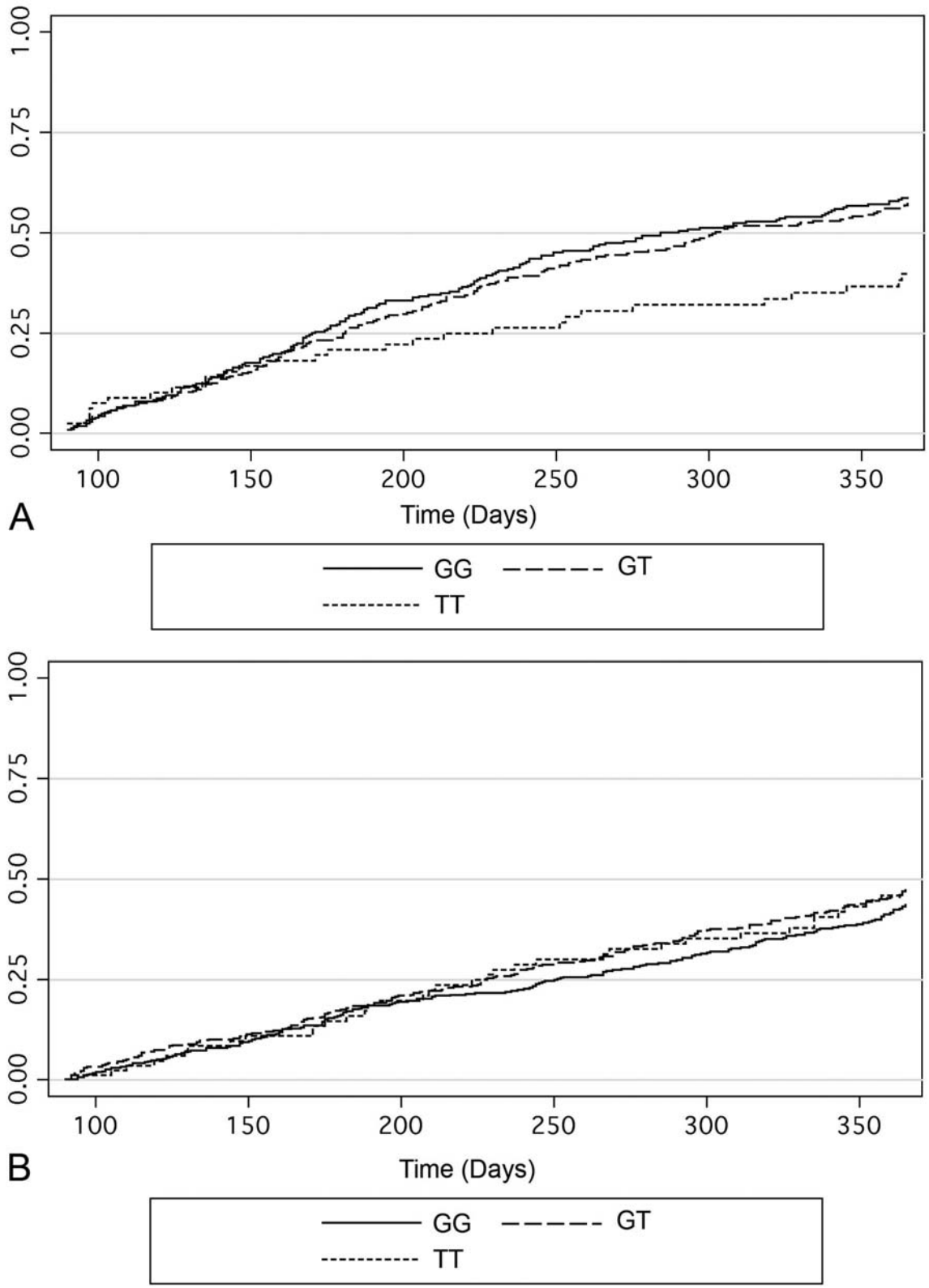

Figure 2. Sulphonylurea response in patients with Type 2 diabetes according to TCF7L2 genotype. Kaplan-Meier Plots showing the proportion of patients, by genotype at rs1225372, who achieve a target HbA1c <7% after being initiated on treatment with a sulfonylurea (panel A) or metformin (panel B). Reproduced with permission from (44).

AMPK remains to be determined (49). One possible mechanism could be that metformin acts to inhibit the mitochondrial respiratory chain (50) and thus indirectly activates AMPK by altering cellular ATP and AMP.

4.2. Efficacy and variation in response

Metformin is recommended as first line oral agent in overweight individuals in the joint ADA/EASD guidelines(51). With sulphonylureas, it is one of two oral hypoglycaemic agents proven to decrease microvascular complications of diabetes (52). Furthermore in the UKPDS, metformin was the only treatment to reduce all-cause mortality (52). Metformin is equivalent in efficacy to sulphonylureas (53, 54), and depending on the baseline HbA1c will decrease HbA1c by approximately 1.5% (55). Up to 25% of patients develop intestinal side effects with metformin which are intolerable in between 5-10% of patients. The mechanism for this GI intolerance and what factors determine who is affected remain uncertain. In Tayside, Scotland, we show a similar degree of variability in glycaemic response to metformin as seen with Sulphonylureas i.e. about 15 % do not respond, and 15% have a striking response (Pearson, unpublished).

4.3. Pharmacokinetics.

The oral bioavailability of metformin ranges between 40% and 60%. It is not metabolised and is primarily excreted unchanged in the urine (56, 57). Approximately 20-30% of the dose is recovered in the faeces unchanged (56, 58). There is decreased bioavailability at higher doses suggesting active saturable intestinal absorption (56, 57). As the rate of absorption of metformin is slower than its elimination, intestinal absorption is the rate limiting step of metformin disposition. A recent study using intestinal caca cells showed that metformin is actively transported at the apical and paracellular surface, and this paracellular transport accounts for 90% of metformin absorption (59). *In vitro* studies have suggested the role of a recently described cation transporter PMAT (plasma membrane monoamine transporter) (SLC29A4) in gut absorption (60). This has a high affinity for metformin, is expressed on the tips of the small intestinal mucosal epithelial layer and its uptake is increased by the more acidic environment of the upper small intestine. Clearly a further understanding of the active and passive processes involved in metformin uptake is required to understand variability in efficacy and gastrointestinal side effects of the drug.

Metformin primarily exists in a protonated cation form at physiological pH. Therefore in addition to PMAT, there has been a lot of interest in other organic cation transporters (OCT) that may have a role in absorption, distribution and clearance of metformin. Metformin is a good substrate for human OCT1 (SLC22A1) and OCT2 (SLC22A2) (61, 62). hOCT1 is primarily expressed in the liver, whereas hOCT2 is expressed in the kidneys. Therefore variants in hOCT1 might be expected to alter metformin availability at the liver, one of its key sites of action, whereas variants in hOCT2 might alter serum metformin levels and metformin efficacy. A further organic cation transporter, hMATE1 (human multidrug and toxin extrusion 1) has been shown to mediate the final excretion step for organic cations both into the bile canaliculi in the liver, or the renal tubules (63). Metformin is a substrate for hMATE1 and hMATE2 (64).

4.4. Genetic variation in Pharmacokinetics

A number of groups have identified functional polymorphisms in *SLC22A1* (encoding OCT1) in Caucasians(65-67) which are distinct from polymorphisms in Japanese populations (68, 69) consistent with ethnic differences in metformin pharmacokinetics. In Caucasians, for the Arg61Cys polymorphism (Minor allele frequency 11%), transport of MPP^+ was reduced by 70% (65); and for Gly401Ser (MAF 3.3%) transport of MPP^+ was reduced by more than 98% (65); . A common polymorphism, 420del, present with a MAF of 16% in Caucasians did not show reduced MPP^+ transport (65) but did show reduced transport of metformin (67).

There has been some recent exciting work on the role of *SLC22A1* variation on metformin response. In a transgenic mouse model, knockout of liver *Slc22a1* virtually abolished hepatic lactate production supporting a key role of Oct1 in transporting metformin into the hepatocytes (70). An elegant study by Shu *et al.* took this concept further and showed that OCT1 plays an important role in determining metformin response in humans (67). They showed that deletion of *Slc22a1* in mouse liver reduced metformin effects on AMPK phosphorylation and gluconeogenesis and as a consequence the glucose lowering effect of metformin was abolished. In addition, they described four loss of function polymorphisms in *SLC22A1* that, in a study of 20 normal glucose tolerant individuals, reduced the effect of metformin on response to oral glucose(67). Interestingly in a subsequent paper they demonstrate higher serum metformin concentrations in those carrying the reduced function *OCT1* polymorphisms suggesting that this is due to reduced hepatic uptake of the drug (71). In contrast to the findings by Shu *et al.*, a study of 24 'responders' and 9 'non-responders' to metformin showed no difference in the prevalence of OCT1 or OCT2 variants between these two groups. A large study of *SLC22A1* variation on glycaemic response and side effects to metformin is required to determine the clinical impact of variation in this drug transporter in the diabetic population.

Similar to *SLC22A1*, a number of reduced function polymorphisms in *SLC22A2* (encoding OCT2) have been described (72-74). In a comprehensive study across 5 ethnic groups, Leabman *et al.* described 28 polymorphisms of which 4 were common (MAF >1%), but only one (Ala270Ser; MAF 16% Caucasians) was found in all ethnic groups and this had no effect on metformin or MPP^+ transport (72). However, in a recent study in a Korean population, 3 non-synonymous SNPs were identified in *SLC22A2*, including the G808T (Ala270Ser) mutation common to other populations (75) and in this study MPP^+ transport by the Ala270ser mutant was reduced. In a subsequent study, plasma metformin concentration after 500mg oral metformin was increased with reduced renal

clearance in humans heterozygous or homozygous for the mutant allele 808T(76) with a gene dose effect seen. At present there is no data on the clinical impact of this polymorphism on metformin response in normoglycemic controls or patients with type 2 diabetes.

4.5. Aetiological Pharmacogenetics

Unlike sulphonylureas, the mechanism of metformin remains unclear. Also, the majority of type 2 diabetes genes discovered to date affect beta-cell insulin secretion and are thus good candidates for affecting sulphonylurea action. It is difficult to know what similar candidates would be for metformin action. Given its action, in the broad sense, as an insulin sensitizer then one might hypothesise gene variants involved in insulin sensitivity would affect metformin response. At present these are limited to *PPARgamma* and *FTO*, although we have shown that BMI does not strongly correlate with metformin response (77). No studies have looked at these variants. Interestingly, in the Diabetes Prevention Program the E23K *KCNJ11* variant interacted with metformin treatment on diabetes progression, such that those carrying the K allele had less protective effect of metformin than the wild type EE (40). This unexpected result has not been replicated.

A few studies have investigated the association between variants in genes involved in the metformin pathway and type 2 diabetes. A Japanese study of 192 cases and 272 controls identified a haplotype across *PRKAA2* (encoding the AMPK-alpha2 subunit) that was associated with type 2 diabetes, was replicated in two independent cohorts, and associated with insulin resistance assessed by HOMA (78). However a haplotype analysis in 4206 Scandinavian and Canadian individuals showed no association between *PRKAA2, PRKAB1* (encoding the AMPK beta1-subunit) and *PRKAB2* (endoding beta2 subunit) and type 2 diabetes risk or insulin sensitivity. Furthermore, a study of 1787 unrelated Japanese subjects found no association between *PRKAA2*, *STK11* (LKB1), *CRTC2* (TORC2) variants and type 2 diabetes that withstood correction for multiple testing. So there is no convincing evidence for an association between the pathways of metformin action and diabetes risk, however this does not preclude an effect on metformin response and the haplotypes identified would be good candidates to assess metformin response. No studies have looked at this to date.

5. THIAZOLIDINEDIONES

5.1. Mechanism

Thiazolidinediones are PPARgamma ligands that induce binding of PPARgamma with one or more coactivator proteins to a DNA PPAR response element, promoting transactivation of a large number of target genes. PPARgamma is primarily expressed in adipose tissue, but is also present in pancreatic beta-cells, vascular endothelium and macrophages. PPARgamma promotes adipocyte differentiation and increases fatty acid uptake and storage in adipocytes. Additional effects include increased glut4 expression in skeletal muscle, increased adiponectin concentrations and increased intravascular lipolysis by lipoprotein lipase. Physiologically, thiazolidinediones increase insulin stimulated glucose uptake into muscle, insulin suppression of hepatic glucose output and insulin stimulated lipolysis (79). One possible mechanism is termed the 'fatty acid steal' hypothesis – that PPARgamma increases the number of small adipocytes in the subcutaneous tissue and increases fatty acid uptake, thus promoting sequestration of fatty acids away from sites of potential harm (liver and beta-cell). Additionally, or alternatively, the insulin sensitising effects of thiazolidinediones may be mediated by their effect on adipocyte related cytokines as they have been shown to markedly increase the insulin sensitizing adipokine Adiponectin, and to alter expression of the insulin resistance associated adipokines TNF-alpha, resistin and 11-betahydroxysteroid 1 (79). Interestingly, recent studies have suggested a non-PPAR effect of the thiazolidinediones being mediated via AMPK in rat muscle (80) and in aortic endothelial cells (81).

5.2. Efficacy and variation in response

The thiazolidinediones are of similar efficacy to sulphonylureas producing, on average, a 1.5% reduction with a baseline HbA1c of approximately 9% (82, 83). The glycaemic benefit of thiazolidinediones seems to be sustained. In a recent trial of monotherapy, there was only a 15% failure of monotherapy in patients using rosiglitazone, compared with 21% of metformin users and 34% of those on glibenclamide (84). Anecdotally there is considerable variation in response to thiazolidinediones, and this is best highlighted by the TRIPOD study, where insulin sensitivity was assessed before and after troglitazone therapy. One third of the subjects had no improvement in insulin sensitivity (or indeed a deterioration in insulin sensitivity) (85).

5.3. Pharmacokinetics

The oral bioavailability of rosiglitazone is 99% with a time to peak concentration of 0.5 to 1 and an elimination half life of 3-7 hours. However, its major metabolites persist for longer with an elimination half life of 5 days (86). After being metabolised the metabolites are primarily excreted in the urine (approx 65% of the dose). The major pathway for Rosiglitazone metabolism is via CYP 2C8, with a minor role for CYP2C9 (87).

Pioglitazone also has high oral bioavailability (>80%) with a time to peak concentration of 1.5 hours and an elimination half life of approximately 9 hours, although the major active metabolites have a much longer half life of approximately 26-28 hours (88). Pioglitazone is extensively metabolized in the liver, with the majority excreted as inactive metabolites in the faeces (88). Like Rosiglitazone, Pioglitazone is primarily metabolised by CYP 2C8, although CYP3A4 plays a lesser role (89).

5.4. Genetic variation in pharmacokinetics

Although CYP2C8 is polymorphic the impact of CYP2C8 variation on thiazolidinedione pharmacokinetics is minor and inconclusive. In response to rosiglitazone, carriers of the 2C8*3 polymorphism (Arg139Lys & Lys399Arg substitutions) had lower elimination half lives than wildtype but showed no difference in glucose lowering (90). For pioglitazone, 2C9*3 polymorphisms reduced the area under the plasma concentration-time curve (91) but no studies have looked at the effect of this genotype on pharmacodynamic response.

5.5. Aetiological Pharmacogenetics

Variation at PPARgamma was one of the first loci to be robustly associated with type 2 diabetes, with the finding that carriers of the Ala variant at codon 12 were protected against diabetes with a per-allele RR of 1.25 compared to the Pro/Pro individuals (92). As the Pro12Ala variant influences transcriptional activity of PPARgamma and is located in the ligand binding domain, this variant is a good candidate to affect thiazolidinedione response (93). A number of groups have studied this and found variable results probably reflecting the small sample sizes in each group. The first study, 131 patients were treated with pioglitazone for ≥ 26 weeks. In a multivariate analysis the Pro12Ala genotype had no significant effect on glycaemic or lipid response (94). In the TRIPOD study improvement in insulin sensitivity was used as an outcome measure. In this study, 93 hispanic women with previous gestational diabetes had an assessment of insulin sensitivity by intravenous glucose tolerance test carried out before and after 3 months of treatment with troglitazone. One third of patients had no beneficial effect of troglitazone on insulin sensitivity, yet the Pro12Ala genotype was no different between the different response groups (85). However, in a haplotype analysis of this same TRIPOD cohort a weak association with response was found with certain haplotypes although the small numbers here makes this haplotype analysis underpowered (95). Finally, in a study of 198 korean patients with type 2 diabetes given 4mg rosiglitazone for 3 months those carrying the Ala allele had a greater response to rosiglitazone than the pro/pro homozygotes however the allele frequency in the ala group was very low (3%) and this result is only based on 11 pro/ala and no ala/ala individuals (96).

In keeping with some of the pharmacological effects of the thiazolidinediones, glycaemic response to other genes has been studied in downstream candidate pathways, but again here the studies are small and underpowered. Two studies on *ADIPOQ* (encoding adiponectin) showed differing effects: In one study of 166 Korean patients the T45G polymorphism reduced the effect of rosiglitazone on adiponectin whereas a G276T polymorphism increased the effect on blood glucose response (97); in a study of 42 patients no effect was seen on glucose reduction with rosiglitazone by the T45G variant, but a -11377C/G SNP did affect adiponectin response (98). One interesting candidate gene arose from mouse work where beta-cell knockout of *Abca1* (the rate limiting step in HDL biogenesis) led to impairment in beta-cell insulin secretion due to cholesterol toxicity. *Abca1* is activated by rosiglitazone which decreases this lipotoxicity and improves beta-cell insulin secretion. In mice lacking beta-cell *Abca1,* rosiglitazone had no effect on glucose tolerance and beta-cell insulin secretion (99). Subsequently, response to rosiglitazone was assessed in 88 diabetic subjects at 3 SNPs in *ABCA1*. One of these SNPS, R219K, showed nominal association with response, however there were only 17 KK patients, and no adjustment for multiple testing was made (100). The biological rationale and previous mouse data makes this an interesting candidate worthy of further large-scale pharmacogenetic studies.

Thiazolidinediones are associated with significant morbidity due to peripheral oedema and a 2-fold increased risk of heart failure (101) due to fluid retention. This has been attributed to PPARgamma regulation of a renal collecting duct sodium transporter (ENAC) (102, 103), and polymorphisms in the gene encoding the ENAC beta-subunit have been associated significantly with oedema in a study of 207 patients receiving Farglitazar in phase 3 clinical trials (104). In a study of another glitazar (dual acting PPARgamma/alpha agonists), ragaglitazar, oedema was less in those carrying the protective ala allele at Pro12Ala PPARgamma than the wildtype patients, and was not influenced by the Leu162Val SNP in PPARalpha ï□.

6. FUTURE THERAPEUTIC AND PHARMACOGENOMIC STRATEGIES

This review has focussed on genetic determinants of response to commonly used established therapies in type 2 diabetes. Despite, in some cases, strong evidence for variation in pharmacokinetics of a drug or in the drug pathway there has been only a few examples where genotype convincingly associates with response. This in part reflects the multiple studies of small sample size, but may reflect our poor understanding of disease aetiology and drug mechanism (for example, of metformin). The recent advances in genome wide technology that now allow relatively cheap genome wide association studies (GWAS) offers a new approach to study pharmacogenomics (33). In the first instance, the increasing number of genes associated with diabetes in GWAS of Type 2 diabetes case/control studies will reveal new aetiological pathways for diabetes, and hence new potential mechanisms for aetiological pharmacogenetics of existing therapies, and the development of new therapies. However, what is potentially more exciting is the use of GWAS where drug response is the main outcome. GWAS is hypothesis generating and does not rely on prior knowledge (or lack of knowledge) about drug

mechanism or disease aetiology. So, for instance, if this approach is applied to a large study of metformin response it could help unravel new mechanisms in metformin action or could provide an alternative approach to reveal new aetiological variants. The challenge is to collect suitable cohorts of suitable size (at least >1000) where metformin response can be defined, and similar sized or larger cohorts where any hits from the GWAS can be independently replicated.

7. CONCLUSIONS.

There have been recent advances in pharmacogenetics of diabetes therapies. In particular there has been an exponential increase understanding of the role of variation in drug transporters, which has led to the finding that *SLC22A1* variants might affect metformin response. There has also been an increase in our understanding of how certain types of monogenic diabetes can be treated differently and this has had dramatic clinical benefit for the small percentage of patients with this type of diabetes. There has been a little progress in what genetic factors determine response in polygenic type 2 diabetes, although here it should be emphasised the clinical impact is currently small (or absent). For example, despite the odds ratio of 2 in the study on sulphonylurea response by *TCF7L2* genotype, the absolute difference in treatment HbA1c between the homozygous groups was only 0.3%. This highlights the relatively small effects that are likely to be found with pharmacogenetics of common disease and hence studies of large cohorts will be required. Given these small effects is there likely to be any clinical impact of these polygenic variants on drug response? Hope can be drawn from studies in diabetes risk genetics, where the small effects of each gene variant are log additive, such that those with a number of risk alleles are at greatly increased risk compared with those who only have low risk alleles (106). However, before risk alleles can be combined robust associations with response need to be identified. Hopefully this first step is something that can be achieved within the next few years.

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