

THE ROLE OF PHARMACOGENETICS IN THE TREATMENT OF DIABETES MELLITUS

ULOGA FARMAKOGENETIKE U LEČENJU DIJABETES MELITUSA

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Summary: Diabetes mellitus is a heterogeneous group of disorders in which particular disease phenotypes can be characterized by a specific etiology and/or pathogenesis of the disease, but in many cases its classification is greatly impeded due to significant phenotype overlapping. Diabetes is a worldwide epidemic with significant health and economic consequences. The frequency of type 2 diabetes (T2D) is much higher than type 1 diabetes (T1D). In adults, around 285 million people suffer from T2DM with a projected rise to 438 million in the next 20 years. A variety of pharmacological treatments exist for patients with T2D, in addition to dietary and physical activity. Pharmacologically, diabetes is treated with nine major classes of approved drugs, including insulin and its analogues, sulfonylureas, biguanides, thiazolidinediones (TZDs), meglitinides, α -glucosidase inhibitors, amylin analogues, incretin hormone mimetics, and dipeptidyl peptidase 4 (DPP4) inhibitors. Treatment strategy for T2D is based mostly on oral hypoglycemic drug (OHD) efficacy assessed usually by HbA1c and/or fasting plasma glucose. The patients are often treated with more than one OHD in combination with the purpose to receive more effective treatment. Characterization of drug response is expected to substantially increase the ability to provide patients with the most effective treatment strategy. If pharmacogenetic testing for diabetes drugs could be used to predict treatment outcome, appropriate measures could be taken to treat T2D more efficiently. To date, major pharmacogenetic studies have focused on response to sulfonylureas, biguanides, and TZDs, the most used OHD. A comprehensive review of the pharmacogenetic studies of specific OHD is presented in this article. Understanding the pharmacogenetics of these drugs

Kratak sadržaj: Dijabetes melitus predstavlja heterogenu grupu poremećaja u kojoj određeni fenotip može karakterisati specifična etiologija i/ili patogeneza bolesti, ali u mnogim slučajevima njegova klasifikacija je vrlo otežana zbog značajnog fenotipskog preklapanja. Dijabetes je globalni epidemiološki problem sa značajnim zdravstvenim i ekonomskim posledicama. Učestalost tipa 2 dijabetesa (T2D) mnogo je veća od tipa 1 dijabetesa. Kod odraslih, oko 285 miliona osoba boluje od T2D, s predviđenim rastom do 438 miliona u sledećih 20 godina. Za pacijente s dijabetesom tipa 2, uz ishranu i fizičku aktivnost, postoji niz farmakoloških lekova. Devet glavnih vrsta lekova odobreno je za lečenje T2D bolesnika: insulin i njegovi analogi, sulfonilureje, bigvanidi, tiazolidindioni, meglitinidi, inhibitori α -glukozi-daze, analogi amilina, mimetici inkretin hormona i inhibitori dipeptidil-peptidaze 4. Strategija lečenja T2D se temelji uglavnom na učinkovitosti oralnih hipoglikemijskih lekova merenjem HbA1c i/ili glukoze natašte. Bolesnici se često leče kombinacijom više oralnih hipoglikemika kako bi se postigla što uspešnija terapija. Karakterizacija odgovora na lek obezbeđuje, kako se očekuje, mogućnost uspešnijeg lečenja, odnosno, da se bolesniku omogući strategija najboljeg lečenja. Ako bi se farmakogenetskim testiranjem dijabetičkih bolesnika mogao predvideti ishod lečenja, odgovarajuće merenje moglo bi se primeniti za mnogo uspešnije lečenje dijabetesa. Do sada, glavne farmakogenetske studije fokusirale su se na istraživanje odgovora na terapiju sulfonilurejama, bigvanidima i tiazolidindionima, najčešće korišćenim oralnim hipoglikemijskim lekovima. Ovaj članak predstavlja sažeti pregled farmakogenetskih studija specifičnih nave-

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Non-standard abbreviations: OHD, Oral hypoglycemic drugs; T2D, type 2 diabetes mellitus; T1D, type 1 diabetes mellitus; SU, sulfonylureas; TZDs, thiazolidinediones; DPP4, dipeptidyl peptidase 4; MODY, the maturity-onset diabetes of the young; SUR, sulfonylurea receptor; HbA1c, hemoglobin A1c; UKPDS, United Kingdom Prospective Diabetes Study; CYP450, cytochrome P 450; CYP2C, cytochrome 2C family; OCT, organic cation transporter; MATE1, multidrug and toxin extrusion 1 protein; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; FFA, free fatty acids.

will provide critical baseline information for the development and implementation of a genetic screening program into therapeutic decision making, enabling a personalized medicine approach for T2D patients.

Keywords: pharmacogenetics, type 2 diabetes mellitus, biguanides, sulfonylurea, thiazolidinediones, candidate gene, personalized medicine

Introduction

Pharmacogenetics

Two interwoven processes, human genome sequencing and the development of new technologies using DNA as an analytical sample and as a reagent, have resulted in the genetic revolution in different fields of medicine, such as the field of medical therapy, leading to personalized medicine through a pharmacogenetics approach (1).

The efficacy of any drug is the result of a balance between pharmacodynamics i.e. drug action and pharmacokinetics, i.e. drug clearance, coupled with a minimal adverse profile. In reality, it is very rare that a given drug has 100% efficacy in 100% of treated patients. There is no doubt that the majority of drugs for common diseases significantly minimize disease burden and improve the quality of patient's life, however, a number of patients suffer from drug side effects. The causes of drug side effects in patients are very different, depending on numerous factors that contribute to interindividual differences. It involves the lifestyle of a patient, biological factors like gender, age, liver and kidney function, and genetic factors. In fact, for some diseases, such as type 2 diabetes mellitus (T2D), pharmacologic treatment of at-risk patients even before manifestation of disease symptoms can significantly reduce disease risk (1–4).

Basically, pharmacogenetics attempts to understand the link between genetic variation and individual response to drugs, i.e. it helps to understand why some patients respond to drugs and others do not, why some of them need higher or lower drug doses in order to achieve an optimal therapeutic response. It can also warn about patients who will have no response to the therapy, as well as about those in whom toxic side effects can occur. Polymorphisms of genes responsible for interindividual differences in drugs efficacy and toxicity can be a cause of alterations on the genes participating in the mechanisms of drug action, such as the genes of drug metabolizing enzymes, transporters, receptors and signal molecules of signal transduction cascades. An individual can be an isolated homozygous and heterozygous carrier of polymorphic alterations, only on one gene or it can simultaneously carry alterations of more genes involved in the drug effect. Whether it is about one or more genes, polymorphisms can contribute to

denih oralnih hipoglikemijskih lekova. Razumevanjem farmakogenetike ovih lekova dobiće se temeljne informacije za razvoj i primenu programa genetskog pretraživanja pri odluci o lečenju, koji bi omogućio pristup personalizovane medicine bolesnicima s dijabetesom tipa 2.

Ključne reči: farmakogenetika, tip 2 dijabetes melitusa, bigvanidi, sulfonilureja, tiazolidindioni, geni kandidati, personalizovana medicina

smaller or higher variability expression in pharmacokinetic processes (absorption, distribution, metabolism and elimination) and pharmacodynamic effects (receptors, ion channels) that result in different response to the drug (5).

At the beginning, the pharmacogenetic field was mainly restricted to observations of familial clustering of drug reactions, but the combination with the Human Genome (6) projects has transformed it, including the area of pharmacogenomics and a wider spectrum of genetic characteristics beyond single nucleotide polymorphisms (SNPs) in the genome. New genetic variants associated with a variety of common diseases identified using genome-wide association studies (8) have elucidated new biological mechanisms underlying not just predisposition to disease, but also response to pharmacologic intervention for disease. So, with other advances in biomedical research, pharmacogenetics has moved from pharmacokinetics to pharmacodynamics. These events bring even closer the prospect of identifying genetic variation that may provide information illuminating which drug at which dose may be the most effective for a given individual. This raises the probability of bringing the so-called personalized medicine to fruition to reduce disease morbidity and mortality, and improve the quality of life for individuals with diabetes mellitus (9).

Diabetes Mellitus

Diabetes mellitus is a heterogeneous group of disorders in which particular disease phenotypes can be characterized by a specific etiology and/or pathogenesis of the disease, but in many cases its classification is greatly impeded due to significant phenotype overlapping.

Type 1 diabetes mellitus (T1D), a multifactorial autoimmune disorder, characterized by absolute insulin deficiency, is the most common form of diabetes in children and the young population. It primarily results from pancreatic β -cell lesions. The pathogenesis of type 1 diabetes includes genetic predisposition for the disease and environmental factors able to activate the mechanisms, which lead to a progressive loss of pancreatic β -cells.

Type 2 diabetes mellitus (T2D) is a heterogeneous multifactorial syndrome characterized by abnormality in insulin action (insulin resistance) and irregular insulin secretion (β -cell failure). Genetic defects may underline each of the two pathogenic mechanisms. In addition, environmental factors such as diet and the sedentary lifestyle can aggravate insulin resistance. T2D includes subtypes which are strongly associated with environmental and genetic factors. Etiologically, it is of utmost importance to differentiate the genes that cause T2D from those that contribute (predispose) to the onset of the disease. These two gene categories have different characteristics and require different methodologies for their detection (1, 10).

Type 2 diabetes mellitus

In adults, around 285 million people suffer from T2D with a projected rise to 438 million in the next 20 years (10). About 25% of individuals have a pre-diabetic condition in which impaired glucose tolerance or an impaired fasting glucose level bring them at high risk for development of T2D (11). T2D significantly influences the patient's quality of life, and public health in general (11). It is the seventh leading cause of death in the United States and also a risk factor for microvascular complications leading to limb amputations, renal failure and blindness, as well as other disorders such as hypertension, cardiovascular disease, dyslipidemia and infections.

T2D is mostly associated with obesity, sedentary lifestyle, older age, family history and ethnicity. Susceptibility to T2D is also modulated by genetic factors, as evidenced by twin studies (12), familial aggregation (13), and increased disease risk in ethnic minority populations (14–16). The prevalence of T2D is also increasing in youths (11). At present, 8–45% of newly diagnosed pediatric patients have T2D (17).

Beside diet and lifestyle modifications in the therapy of T2D, the oral hypoglycemic drugs (OHD) play a key role. Currently, T2D is treated with nine major classes of approved drugs, including insulin and its analogues, sulfonylureas (SU), biguanides, thiazolidinediones (TZDs), meglitinides, α -glucosidase inhibitors, amylin analogues, incretin hormone mimetics. The most frequently used are sulfonylureas, biguanides, thiazolidinediones, and meglitinides. As mentioned before, there are big interindividual differences in the efficacy of OHD as well as in their side effects, hypoglycemia for example, which are conditioned by genetic polymorphisms. The most oral hypoglycemics are metabolized by the genetically very polymorphic enzyme CYP2C9 (18).

In many T2D patients, treatment with OHD is initially successful, but over time addition of a second antidiabetic agent or transition to insulin becomes necessary to restore acceptable glycemic control. Although

glycemic control has improved over the past decade, still about 40% of patients do not reach the desired hemoglobin A1c (HbA1c) target of <7% (19). So far, there is no single agent that yields optimal glucose-lowering effects in all treated patients (20).

In the study of T2D long-term control, a cumulative incidence of monotherapy failure at 5 years of 15% was found with rosiglitazone (a TZD), 21% with metformin (a biguanide), and 34% with glyburide (a sulfonylurea) (21). These data with respect to monotherapy resulted in a combination therapy being implemented to treat T2D. The general strategy in such combination of therapy is to simultaneously treat multiple components of T2D pathogenesis to control blood glucose levels, including those which contribute to interindividual differences in drug response (22).

A rare autosomal dominant monogenic form of T2D is the maturity-onset diabetes of the young (MODY). MODY exists in six forms due to modifications in six different MODY genes. From them, HNF4A, TCF1 (or HNF1A) and GCK genes which encode two transcriptional factors and glucokinase in the β -cells, respectively, were reliably proved to be involved in T2D. Various phenotypes in MODY patients suggest the disorder is genetically heterogeneous (23, 24).

The pharmacogenetic research assessing the role of genetic determinants of drug responses promises to yield information that may lead to personalized treatment strategies to ensure optimal glucose control in all diabetic patients, improve treatment efficacy, and reduce the risk of adverse drug events in susceptible individuals. In this review of pharmacogenetic investigations, three major classes of oral anti-diabetes drugs: sulfonylureas, biguanides and TZDs, will be discussed.

Sulfonylureas

Sulfonylureas are one of the most widely used classes of oral hypoglycemic agents. The most common sulfonylureas are tolbutamide, gliclazide, glibenclamide and glimepiride. Although most patients respond well to these drugs, 10–20% of treated individuals do not achieve adequate glycemic control using even the highest recommended dose. Five to ten percent of patients who initially respond to sulfonylurea subsequently lose the ability to maintain near-normal glycemic levels (25). Although failure to respond to sulfonylurea therapy may result from a variety of factors, the strongest predictor of failure is deterioration of β -cell function (26).

In a series of studies, Pearson et al. (27) identified rare heterozygous mutations in the potassium inwardly-rectifying channel, sub-family J, member 11 (KCNJ11), more commonly known as the ATP-dependent K^+ channel, representing 30–58% diabetes

diagnosed in patients <6 months of age or in neonatal diabetes. These mutations resulted in continuous activation of the ATP-dependent K⁺ channel, preventing insulin secretion by pancreatic β -cells, and lead to misdiagnosis of type 1 diabetes. This resulted in inadequately treated patients using conventional insulin therapy. Pearson et al. demonstrated that patients with these mutations in *KCNJ11* could be successfully treated with sulfonylureas. Additional studies identified mutations in the ATP-binding cassette, subfamily C (CFTR/MRP), member 8 gene (*ABCC8*), commonly known as the sulfonylurea receptor (SUR), which also result in forms of neonatal diabetes (28). However, only some patients could be successfully treated with sulfonylureas, with carriers of the F132V mutation having to be maintained on insulin therapy.

The results of these studies were among the first demonstrating that the genetic etiology of hyperglycemia may modulate response to hypoglycemia agents. Such results yielded strong implications for patient management and paved the way toward elucidating additional genetic factors that might influence drug response in the treatment of T2D.

Sulfonylureas stimulate insulin release from pancreatic β -cells by first binding to the high-affinity plasma membrane receptor (SUR1) coupled to an ATP-dependent K⁺ channel (KATP). This interaction closes the K⁺ channel, which inhibits potassium efflux and depolarizes the plasma membrane, leading to an opening of voltage-gated calcium channels. Calcium influx, and a corresponding increase in intracellular calcium levels, causes release of insulin from the β -cells.

This hetero-octameric protein complex contains four high-affinity sulfonylurea receptor (SUR1) subunits. The genes encoding these proteins are the ATP-binding cassette transporter subfamily C member 8 (*ABCC8*) and potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) genes, respectively.

Rare monogenic mutations in *ABCC8* cause neonatal diabetes (29) and may increase susceptibility to T2D (30–32). Although *ABCC8* encodes the SUR1 receptor, and as such, represents a logical biological candidate for sulfonylurea response, only a few studies have investigated this gene in relation to drug treatment failure (33, 34). In T2D patients (N=228) on SU therapy, carriers with the wild-type CC genotype (exon 16-3>T) had significantly lower HbA1c compared with the TT genotype. On the contrary, wild-type patients with SNP (Glu1273Arg) rs1799859 had significantly higher HbA1c levels compared with the AA genotype (33, 34).

In a study on Chinese T2D patients (N=115) treated with gliclazide and genotyped for marker rs757110, which is located in exon 33 and causes a

ser 1369 ala substitution (35), the G allele carriers were more sensitive to gliclazide and achieved greater decrease in HbA1c compared with individuals carrying the TT genotype (1.60% \pm 1.39 vs. 0.76% \pm 1.70, respectively; P = 0.044). This marker was also examined in two independent cohorts of Chinese T2D patients (N=1,268) treated for 8 weeks with gliclazide. Results revealed that individuals carrying the G allele had greater decreases in glucose levels compared with individuals carrying the wild-type genotype (36). The authors also found a trend toward greater HbA1c reduction in patients with the GG genotype compared with homozygous carriers of the wild-type genotype, although this association did not quite reach statistical significance (P = 0.06) (36). In these individuals, mean gliclazide dosage requirements were ~78% in individuals carrying the G allele compared to ~84% in TT homozygous patients (37). Taken together, these findings provide a rationale for investigating this variant in additional populations and using other sulfonylurea agents.

The *KCNJ11* gene has also been extensively investigated. In humans, *KCNJ11* mutations underlie familial persistent hyperinsulinemic hypoglycemia of infancy (38, 39) and permanent neonatal diabetes (40), and are associated with common forms of T2D (41–48).

Of the known *KCNJ11* variants, the most widely studied is marker E23K (rs5219), which encodes a glu23lys substitution; the variant K allele is associated with increased risk of T2D. Initial studies of this variant did not provide evidence for association with sulfonylurea failure in 364 newly diagnosed patients with T2D from the United Kingdom Prospective Diabetes Study (UKPDS) (49), but a subsequent study (50) in 525 Caucasian T2D patients found a higher frequency of the K allele in patients who failed sulfonylurea therapy compared to those who did not (66.8% vs. 58.0%, respectively). The glibenclamide-stimulated insulin secretion also tended to be lower in patients carrying the K allele, compared to individuals with the homozygous E/E genotype, although this difference was not statistically significant (50). However, differences between genotypes became statistically significant when islets were preexposed to high glucose, suggesting that impairment of insulin secretion in response to sulfonylureas in the presence of the E allele is exacerbated by a hyperglycemic milieu. Holstein et al. (51) in their study in patients with severe sulfonylurea-induced hypoglycemia found the K allele to be associated with higher HbA1c levels compared with the E allele (P = 0.04), which is consistent with previous findings (48).

Several possible factors may explain the discrepancies between these studies. First, the different definitions of secondary failure: in the UKPDS, failure was defined as patients who needed additional therapy, regardless of the type and control of hyper-

glycemia, while the second study defined failure solely in terms of progression to insulin therapy. Second, duration of therapy with OHD before failure differed between the two studies (1 yr after randomization in UKPDS vs. 12 yrs in the second study); the shorter duration of therapy in the UKPDS does not allow the possibility that some individuals carrying the K allele may be destined to experience secondary failure, but had not yet done so. Third, the class and type of the sulfonylurea drug differed between the studies (chlorpropamide vs. glibenclamide), which may have influenced the response based upon the genotype at this marker. Finally, the clinical characteristics of patients differed between the studies; the UKPDS recruited newly diagnosed patients, while the second study recruited patients with known diabetes. Patients with a new diagnosis would be expected to have better β -cell function compared to patients with a longer duration of T2D, which again could confound the basis for secondary failure independent of genotype at this locus.

CYP2C9 and CYP2C19

Most OHD are metabolized by class 2C genetically polymorphic CYP450 enzymes. Whereas sulfonylureas are mostly CYP2C9 substrates, CYP2C8 is the main enzyme responsible for the biotransformation of thiazolidinediones (rosiglitazone and pioglitazone) and repaglinide. (52). Many CYP2C9 have been identified, but the most common allele is designated as *1, which is the most frequent across populations and is generally considered the wild-type allele of the gene (52). The most studied allelic variants of this gene are Arg144Cys (i.e. rs1799853 or CYP2C9*2) and Ile359Leu (i.e. rs1057910 or CYP2C9*3), which have respective frequencies of 11% (*2) and 7% (*3) in Caucasians (53, 54). Most studies have found that individuals carrying at least one *2 or *3 allele exhibit reduced CYP2C9 activity, while those with either the *2/*3 or *3/*3 genotype show reduced drug-metabolizing activities, with a lower dose requirement, compared with individuals having the wild-type Arg144/Ile359 (CYP2C9*1) allele (55–57). Even in healthy volunteers receiving glimepiride, the CYP2C9 genotype altered the pharmacokinetic profile of the drug significantly, with a much slower elimination of glimepiride in individuals carrying the *3 allele compared to those with the *1/*1 genotype (58). The decrease in activity is the most profound for the CYP2C9*3 allele: mean clearances in homozygous CYP2C9*3/*3 individuals are 25% of that of wild-type for a number of substrates, while heterozygosity for this variant corresponded to clearance of 29% compared to wild type. For the CYP2C9*2 allele, the V_{max} displays a 50% reduction compared to the CYP2C9*1 allele and residual clearance of tolbutamide for CYP2C9*1/*2 heterozygotes was 70% compared to CYP2C9*1/*1 individuals (59, 60).

For tolbutamide, an oral sulfonylurea hypoglycemic drug used in the treatment of T2D for many years, the contribution of CYP2C9 genetic polymorphisms to pharmacokinetics and blood glucose lowering effects was very well documented. Consequently, a careful monitoring of the hypoglycemic effects upon tolbutamide administration in patients heterozygous and especially those homozygous for CYP2C9*3, which is an allele with decreased enzymatic activity, was recommended. Moreover, dose adjustments for carriers of a CYP2C9*3 polymorphism were suggested i.e. half and 20% of tolbutamide standard dose, respectively, for the heterozygous and homozygous carriers of CYP2C9*3 (53). The impact of CYP2C9 polymorphism on the pharmacokinetics of second generation sulfonylurea drugs like glibenclamide (glyburide), glimepiride and glipizide has also been studied. Similarly, it was shown that the total clearance of these oral antidiabetics in the carriers of CYP2C9*3/*3 genotype was only about 20% of that in wild types (CYP2C9*1/*1), whereas in heterozygotes, this parameter was reduced to 50–80%. Interestingly, the resulting magnitude of differences in drug effects (insulin concentrations) seems to be much less pronounced than for the pharmacokinetic parameters. Nevertheless, it has been considered that respective CYP2C9 genotype-based dose adjustments may reduce the incidence of possible adverse reactions. At the same time, the presence of another common CYP2C9 variant allele i.e. CYP2C9*2 seems to be without clinical relevance for the therapy with sulfonylureas, since it has been considered to reduce the CYP2C9 enzymatic activity to a minor extent only (54).

The second important enzyme of the CYP2C subfamily is CYP2C19, for which the first genetic polymorphism was identified based on aberrant metabolism of the anticonvulsant drug mephenytoin: 3–5% of Caucasians, 12–23% of Asians and 4% of Africans were shown to be CYP2C19 poor metabolizers. The two predominant variant alleles, encoding CYP2C19 protein lacking enzymatic activity, are CYP2C19*2 (681G>A) and CYP2C19*3 (636G>A) (61, 62).

CYP2C19 may also play a role in sulfonylurea metabolism. Two common markers in this gene, *2 (rs4244285) and *3 (rs4986893), produce a non-functional enzyme, and individuals with either allele are referred to as poor metabolizers (62). Because the *3 allele is more frequent in the Asian population, it is not surprising that the poor metabolizer phenotype is more common in Asians compared to Caucasians, 2–6% vs. 10–25%, respectively (63, 64). In healthy Chinese males, the AUC of gliclazide was increased 3.4-fold in poor metabolizers compared to the wild-type genotype carriers (61). In poor metabolizers the half-life of gliclazide was also prolonged from 15.1 to 44.5 h (61).

Other genes

A few additional genes have also been investigated as modulators of sulfonylurea response in T2D. Three genes encoding the insulin receptor substrate-1 (*IRS1*), the transcription factor 7-like 2, T-cell specific, HMG-box (*TCF7L2*) and nitric oxide synthase 1 adaptor protein (*NOS1AP*) have been found to have an association with sulfonylurea response (65–67). The studies performed in association with these genes in T2D patients are presented in *Table 1*.

Biguanides (Metformin)

Metformin belongs to oral antidiabetics widely used in overweight patients with type 2 diabetes. It is often the first drug used to treat newly diagnosed T2D. It ameliorates hyperglycemia by decreasing hepatic glucose output and gastrointestinal glucose absorption and improving insulin sensitivity. However, only about 60–65% of patients achieve acceptable control of fasting glucose levels. Metformin is not metabolized, but undergoes rapid renal elimination. The genetic component contributing to variation in the renal clearance of metformin is >0.9, suggesting that genetic factors underline variability in the elimination of this drug (68, 69). The molecular mecha-

nisms of metformin are initiated by its activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), resulting with suppression of glucose production via gluconeogenesis and increased peripheral glucose uptake (70). Inhibition of hepatic gluconeogenesis by metformin occurs through AMPK-dependent regulation of the orphan nuclear receptor small heterodimer partner, SHP (71), and a protein-threonine kinase (LKB1), which phosphorylates and activates AMPK, is critical for the glucose-lowering effects of metformin in the liver (72).

Metformin may also exert a direct effect on pancreatic β -cells increasing insulin release in response to glucose (73) and may help to preserve β -cell function (10). However, the molecular mechanisms are so far unknown.

Organic cation transporters and related proteins

Metformin serves as a substrate for organic cation transporters (OCTs), including OCT1 and OCT2, expressed in the liver and in the kidney, respectively (74, 75). Organic cation transporter 1 (OCT1) is mainly responsible for metformin entry into enterocytes and hepatocytes. Several genetic polymor-

Table 1 Some other genes involved in the pharmacogenetics of sulfonylurea.

SNP	Study population	Associated response phenotype	References
<i>TCF7L2</i>			
rs12255372 rs7903146	4469 participants from the Genetics of Diabetes Audit and Research Tayside (GoDARTs)	May affect susceptibility to T2D, and modulate response to sulfonylurea therapy; in both cases, the pathophysiology likely stems from impaired insulin secretion due to deteriorating β -cell function.	Pearson et al. 2007 (65)
rs12255372 rs7903146		Insulin secretion is reduced in individuals with the risk alleles at rs12255372. Individuals with the TT genotype were less likely to respond to sulfonylurea treatment with a target HbA1c < 7% compared to carriers of the GG genotype (57% vs. 40%). Individuals with the TT genotype were much less likely to achieve a target HbA1c of 7% within one year of initiating sulfonylurea treatment compared with carriers of the GG genotype. rs7903146 carriers may respond suboptimally to sulfonylurea therapy due to decreased β -cell function.	
<i>IRS1</i>			
Gly972Arg	477 Caucasians with T2D who were treated with sulfonylurea agents	Genotype frequency of the variant allele (Arg972) was almost twice as high in patients who experienced secondary sulfonylurea failure compared to individuals with controlled glycemia.	Sesti et al. 2004 (66)
<i>NOS1AP</i>			
rs10494366	Patients on glibenclamide (N=250)	Prescribed doses of glibenclamide were higher in individuals carrying the TG genotype compared with those with the TT genotype.	Becker et al. 2008 (67)

phisms in OCT1, some of them leading to reduced transporter activity, have been identified. Results of one clinical study stated that carriers of at least one OCT1 variant allele reduced function of the transporter, showed higher glucose levels following administration of metformin (59).

The multidrug and toxin extrusion 1 protein (MATE1) facilitates metformin excretion from these cells into bile and urine, respectively. Drug transporter gene polymorphisms may underlie variation in drug response (68), and a number of studies have focused on the genes encoding the OCTs as mediators of variability in glycemic response or renal elimination of metformin. OCT1 and OCT2 belong to the SLC22A family of solute carriers and are encoded by the *SLC22A1* and *SLC22A2* genes, respectively. MATE1 is encoded by the *SLC47A1* gene.

SLC22A1

A critical step for achieving the metformin hypoglycemic effects is uptake of metformin into hepatocytes by OCT1, so it may be expected that variants in *SLC22A1* contribute to different glycemic response to the drug. The first who addressed this possibility by investigating four nonsynonymous *SLC22A1* variants (i.e. R61C, G410S, 420del, and G465R) were Shu et al. (76, 77).

In 21 volunteers given metformin, no association between *SLC22A1* genotype and plasma glucose concentration or AUC after OGTT was observed, however, following metformin dosing, volunteers carrying risk alleles had significantly higher plasma glucose concentrations and greater AUC for most of the sampling time compared to those with wild-type alleles (76). Shu (77) also showed in the group of volunteers with known *SLC22A1* (N=21) that plasma metformin concentration tended to be higher in individuals carrying *SLC22A1* risk alleles vs. wild-type allele carriers. These individuals also had a significantly higher maximal plasma concentration of metformin and lower oral volume of distribution (77).

OCT2 is expressed in the basolateral membrane of the renal epithelium and transport of metformin through the membrane may be the first step for its tubular secretion. Several studies confirmed that a variant T allele at marker 808G > T in *SLC22A2* was associated with reduced renal clearance of metformin and lower renal tubular clearance (78–81). However, an investigation by Tzvetkov et al. (82) in 103 healthy participants did not find significant evidence for an association between 14 *SLC22A2* markers, including 808G > T, and renal metformin clearance. It is possible that *SLC22A2* markers are largely important for the renal elimination of metformin in individuals of Asian origin, which may explain the discrepancies between the first two reports and this study. Despite these differences, findings reported for *SLC22A2*

polymorphisms may have clinical relevance and should be studied further.

The *SLC47A1* gene encodes the MATE1 protein, which is located in the bile canicular membrane in the hepatocyte and the brush border of the renal epithelium. Its function is to excrete metformin through the bile and urine. It is colocalized with OCT1 and OCT2 in the hepatocyte and renal epithelium respectively (83) and may contribute to the variability in response to the drug. Little is known of the effect of genetic variants in *SLC47A1* and metformin response. To date, only one study has investigated this gene, in which an association was observed with only one marker, rs2289669, and metformin response, as defined by a decrease in HbA1c levels (84). For each minor A allele in this study, the decrease in HbA1c level was 0.3%. The clinical impact of both rs2289669 and *SLC47A1* needs to be evaluated further and confirmed in other populations.

Thiazolidinediones

Thiazolidinediones (TZD) act by activating their molecular target, peroxisome proliferator-activated receptors (PPARs). The exact mechanism by which TZDs act has not been clearly known; however, data indicate that TZDs increase insulin sensitivity with direct and indirect effects on adipose tissue and muscle (85).

So far, three known forms of the nuclear receptor PPAR exist: PPAR- α , PPAR- γ , and PPAR- δ , which are encoded by distinct genes and have different tissue expression (86). TZDs are selective agonists for PPAR γ , which is predominantly expressed in adipose tissue, and appear to have minimal activity on PPAR α or PPAR δ (87). TZD stimulation of PPAR γ results in increased adipocyte differentiation (87) and has been shown to reduce hyperglycemia in patients with T2D (88, 89).

TZDs appear to be metabolized through the family of cytochrome P450 enzymes. Troglitazone is metabolized into sulphate and glucuronide conjugates and a quinone-type metabolite (90, 91), and its metabolism appears to inhibit activities of other cytochrome P450 enzymes, suggesting it may interact with other medications. In contrast, pioglitazone is metabolized into five metabolites, mainly by CYP3A4, CYP2C8 and CYP2C9, and three of these metabolites appear to be active (92). Unlike troglitazone, pioglitazone does not appear to inhibit the activity of other cytochrome P450 enzymes and therefore is expected to have few drug interactions (93, 94).

Nonresponse rates in TZD therapy appear to be similar across diverse populations, suggesting little to no contribution from environmental exposures to differences in response. Furthermore, issues related to ethnic/racial differences or compliance are not likely

to significantly contribute to response given the observed similarity across very diverse studies. These observations led to the hypothesis that genetic variation may be an important and significant contributor to the TZD response mechanism. However, given the observation that individuals may have differential response to different TZDs, it is possible that gene variants that underlie response to one TZD may not contribute to response to another.

PPARG

As mentioned before, the TZD are a natural target of PPARG. Although initially a specific common variant in *PPARG* (rs1801282; Pro12Ala) was shown to be associated with T2D and insulin sensitivity (95, 96), it was demonstrated in the TRIPOD study that rs1801282 was not associated with a troglitazone-induced improvement in insulin sensitivity assessed by the intravenous glucose tolerance test (97). No association was found between this variant and response to troglitazone therapy assessed as a change in HOMA-IR, an indirect measure of insulin sensitivity, reported by the Diabetes Prevention Program (DPP) (98, 99). These results suggested that pioglitazone therapy is not associated with the *PPARG* variant rs1801282 and improvement in fasting glycemia or HbA1c (100).

The lack of association of this single *PPARG* variant with T2D did not exclude the possibility that variation elsewhere in *PPARG* could contribute to TZD response. Upon sequencing the coding region of *PPARG* and tested variants for association with TZD response in the TRIPOD study, among the 133 identified SNPs, eight showed evidence for association with response to troglitazone monotherapy, which was defined as an improvement in insulin sensitivity measured using the intravenous glucose tolerance test with minimal model analysis. The odds ratios for these associations ranged from 2.04 to 2.36 (101). These observed odds ratios for troglitazone response are in stark contrast to the relatively small odds ratios observed for disease susceptibility (102), but are consistent with other pharmacogenetic studies in which relatively large effect sizes are observed (103). An important observation was also that SNPs showing association with TZD response, defined by a change in insulin sensitivity, did not show evidence for association with fasting glucose. Because the glucose regulatory system is designed to tightly regulate glycemia, this metric should be sensitive enough to detect relatively large changes in TZD response. This is consistent with the observation that in the progression to T2D, large changes in glycemia are only observed when β -cell failure ensues (104).

Regarding the discrepancies between the results in TRIPOD and DPP (105), which raise important questions in both the conduct of and the comparison

between pharmacogenetics studies, one should consider the differences between the two studies, such as duration of treatment, mean age, ethnic/racial composition of the study cohort, and T2D risk (gestational diabetes vs. impaired glucose tolerance), as potential explanations for the divergent association results. However, they do not negate the fact that both studies showed a significant effect of troglitazone to reduce risk for T2D, and both studies observed responders and nonresponders (106, 107).

Adipokines

It is known also that TZDs significantly reduce triglyceride content in adipose tissue, skeletal muscle and liver, and increase leptin concentrations (108–110). Together, these changes lead to a decrease in circulating free fatty acids (FFA), which reduces FFA-induced insulin resistance in skeletal muscles. It has been shown as well that TZD therapy alters concentrations of other adipokines, such as leptin, adiponectin and TNF- α (111–114). Data also suggests that troglitazone-induced changes in insulin sensitivity are not associated with changes in total adiponectin concentration, but with changes in the high molecular weight subfraction (113). Responders to troglitazone showed a significant increase in the high molecular weight subfraction, while nonresponders showed no change (114). These observations make adiponectin (*ADIPOQ*) an attractive target for further genetic analysis.

Study of the association between two variants in *ADIPOQ* rs1501288 and rs2241766 and response to rosiglitazone assessed by changes in fasting glucose and HbA1c in Korean patients with T2D (114) demonstrated that these two variants in *ADIPOQ* are associated with reduced changes in both fasting glucose and HbA1c in response to 12 weeks of rosiglitazone therapy.

Additional studies have shown varying levels of evidence for an association between response to TZDs and leptin (115), TNF- α (115) and resistin (116). Although these results are relatively underpowered, they point to the adipokine signaling system and a potential neuroregulatory mechanism underlying TZD response. Independent replication of these findings in larger sample sizes will be required before they can be accepted as valid associations.

Conclusions

Pharmacogenetic research provides a means to better understand and improve pharmacotherapy. However, pharmacogenetics provides only information on associations regarding specific genetic markers that can be predictive of drug efficacy. So far, association studies have not formally assessed the

specificity or sensitivity of genetic markers in T2D, although T2D has been studied extensively at the clinical and epidemiologic levels. Among them, several studies have identified variants that have the potential to become genetic markers if investigations in larger, well-designed cohorts confirm their potential roles in optimal drug selection and individualized pharmacotherapy in patients with T2D. At this time, larger, well-powered studies with clearly defined outcomes and utilizing a global approach are needed, as they will not only be more informative than extant candidate gene investigations, but will also be neces-

sary to define the array of genetic variants that may underlie drug response. Such results will probably enable achievement of optimal glucose control, improvement of therapeutic efficacy, and reduction in risk of adverse drug reaction in at-risk patients, which together will lead to personalized treatment strategies for all individuals with T2D.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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