Chapter

Pharmacogenetics of Metformin in Type 2 Diabetes: Perspectives for Latin America

Eloy A. Zepeda-Carrillo, Rafael Torres Valadez, Sara A. Campos Huerta and Elisa María Barrón Cabrera

Abstract

Metformin, in the anti-hyperglycemic pharmacological therapy, is consumed by more than 150 million people annually in the world due to its affordable price, safety, and because of considerable pleiotropic effect that has a positive impact on the control of glycemia, insulin resistance, cardiovascular health, and cancer in patients with type 2 diabetes (T2D). Differences in metformin's effect on glycemic control have been associated with diet, abdominal obesity, years of T2D evolutions, and genetic factors. The Population of Latin America presents an important genetic component of Amerindians that could be explained to some extent in the response to metformin in glycemic control. The most recognized effect of metformin is to inhibit gluconeogenesis hepatica. In recent years, it has been observed to reduce the effect on body mass, positive effects on inflammation, and recently on the intestine with changes in the microbiome that favor suppression of postprandial hyperglycemia. Association studies between genetic variants coding for proteins related to metformin pharmacodynamics have shown different effects on glycemic control in several ethnic groups with European and Asian ancestry, but in Latin America they are scarce or none. Nutrients can interact with metformin favoring or decreasing its anti-hyperglycemic effect, so the diet should be considered.

Keywords: metformin, type 2 diabetes, genes, pharmacogenetics, nutrigenetics, Latin America

1. Introduction

Type 2 diabetes (TD2) shows an upward trend in its prevalence globally, and in the developing countries of Latin America, an increase of up to 50% is estimated by 2045 [1, 2]. Drug treatment to control hyperglycemia in these patients is a daily challenge at the first level of medical care. Metformin is a biguanide that is prescribed as the first drug of choice to control blood glucose based on the treatment guidelines issued by the European Association for the Study of Diabetes (EASD) and the American Association for the Study of Diabetes (ADA), due to its efficacy and safety as well as its low cost which makes this drug widely accessible in any socioeconomic stratum [3]. The main

effect shown by this biguanide is the suppression of hepatic glucose production, but the complete mechanism is not fully demonstrated. Despite the anti-hyperglycemic therapeutic effects demonstrated in several trials since the 1950s of the last century, no more than 35% of patients with this pharmacotherapy achieve the expected glycemic control. In addition, the gastrointestinal side effects of this drug are a reason for non-adherence to treatment, which explains why many patients seek alternatives and delay their therapeutic goal. To explain these effects of metformin, studies have been carried out focused on pharmacokinetics and pharmacodynamics that have shown the important role of proteins related to its intestinal absorption as well as its uptake hepatic. The organic cation transporters OCT1, OCT2, and OCT3 have gained relevance to understanding to a large extent the effects described by metformin to date [4].

Studies of gene variants that code for these proteins and their association with the anti-hyperglycemic effect of metformin in subjects with TD2, have shown that the genetic component of the patient is decisive to explain the anti-hyperglycemic effect of this drug, as well as its gastrointestinal side effects. The focus of these gene-drug association studies has been achieved thanks to the development of Pharmacogenetics [5]. The first pharmacogenetic studies in TD2 emerged in Europe and Asia, and later in the United States of America. From genome-wide association studies (GWAS) in some cohorts, genes with the statistical association of response to metformin were found which are not directly related to the action of the drug, so its usefulness was not expected. Thanks to metformin pharmacodynamic studies it was possible to conduct association studies with candidate genes such as those encoding the organic cation transporters OCT1, OCT2, and OCT3, mainly. The solute carrier family 22 (SLC22) genes encode for these transporters of organic cations located in the plasmatic membranes of intestinal, hepatic, and renal cells, where metformin has its absorption, anti-hyperglycemic effect, and excretion, respectively [5]. The SLC22A1/OCT1 gene encodes for the OCT1 transporter and has been the most explored for its close relationship with the effect of metformin on the liver. More than 30 polymorphic variants of this gene have been described and their association with the effect of metformin has been variable in different populations with TD2. Among the most studied SLC22A1/OCT1 gene polymorphisms in recent times is *Met*408*Val* rs628031, whose frequency of the risk allele variant A (408*Val*) reported for America in the 1000 Genome Project Phase 3 is the lowest [6, 7].

Diet is another factor that affects glycemic control and the therapeutic response of metformin in patients with TD2. The diet of Latin American countries has been modified in recent years influenced by the Westernized diet that is high in saturated fats, simple sugars, cholesterol, and low in fiber which has an impact on the elevation of blood glucose that impacts the expected anti-hyperglycemic effect of metformin. Therapeutic dietary approaches to contribute to glycemic control in TD2 have focused on caloric balance and the percentage contribution of macronutrients in the total color intake of the patient's requirement. The studies of the association of genes with specific nutrients in the diet have begun to demonstrate the importance of the genetic component in response to specific macro or micronutrients, which has allowed the development of nutrigenetics.

2. Epidemiology impact of T2D and their comorbidities in Latin America

The prevalence of diabetes in Latin America in adults in 2019 ranged from less than 6% in Ecuador and Argentina to 17% in Belize. On average, the prevalence for Latin

America was 9.7%, with an increase of 7.4% compared to 2010. The largest increase was 10 percentage points recorded in Belize, while the largest decrease was 6 percentage points recorded in Uruguay and Venezuela between 2010 and 2019 [1]. Other countries where it decreased were Peru, Panama, and Ecuador but with less than 5 percentage points in the same period. Two Latin American countries that are Brazil and Mexico in the global Top Ten of T2D with sixth and seventh place, respectively, contributing almost 30 million people with diabetes in general globally [8]. When considering the Americas region, the World Health Organization WHO includes Canada and the USA. In the Region of America, about 62 million people have T2D, contributing 15% to the global prevalence of 422 million individuals diagnosed with T2D that was reported in 2014, and with a trend towards an increase in cases. In the Americas region, prevalence has tripled since 1980 and is estimated to reach 109 million by 2040 [2]. In 2019, diabetes was the sixth leading cause of death, with an estimated 244,084 deaths directly caused by T2D. In addition, it is the second leading cause of disability-adjusted life years (DALY's), which reflects the limiting complications for their daily activity that people with T2D suffer throughout their lives. In North America and the Caribbean alone, 1 in 7 adults lives with T2D (51 million) and is expected to reach 57 million adults by 2030 and 63 million by 2045. In this region, it caused 931,000 deaths and 415 million USD was spent due to this disease in the year 2021 alone [9, 10].

The International Diabetes Federation (IDF) divides the Americas region into North America and the Caribbean (NAC), and South and Central America (SACA). The NAC region has the second highest prevalence of diabetes among IDF regions at 14% and the number of people with diabetes for this region is projected to increase by 24% to 63 million by 2045. Of this region, Mexico is the second with the largest population with diabetes (14.1 million), below the USA. In this NAC region, mortality from diabetes is the second highest with 931,000 and the second highest percentage with 18.4%, in people of working age. In the SACA region, it is estimated that 1 day in 11 adults has diabetes (about 33 million), and it is estimated that it will increase by 50%, that is, to 49 million in 2045. Of this region (SACA), Brazil is in first place with the highest number of people with diabetes (15.7 million), followed by Colombia (3.4 million), Venezuela (2.3 million), Argentina (8 million), and Chile (1.7 million [9]. In the NACA region, 415 billion USD was spent due to this disease, while the SACA region 65 billion USD was spent [9]. In addition, Brazil of the SACA region and Mexico of the NAC, are two Latin American countries that are in third and eighth place with the highest total expenditure due to diabetes with 42.9 and 19.9 USD billion, respectively, [9]. Of these amounts reported by the IFD in its Diabetes Atlas, about 90% of the total of these amounts correspond to TD2.

Projections on the prevalence of TD2 estimate that developing countries will be among those with the greatest increase due to the social determinants of health such as diet, physical activity, access to health systems for timely diagnosis, and the budget available for TD2 care. The increase in population in urban areas and the consequent "Westernized" type diet in combination with a decrease in physical activity favor overweight and obesity which are risk factors for the development of TD2, which allow us to assume an unfavorable future for the control of TD2 and its comorbidities in this geographical region.

2.1 Pharmacotherapy with metformin in TD2

In medieval times, the plant *Galega officinalis* (Galega or French lilac) was used in Europe as a popular remedy to treat diabetes. Phytochemical studies in the late 1800s

revealed that the plant was rich in guanidine. In 1918, animal studies showed that guanidine had hypoglycemic effects, but with toxic effects for clinical use. In 1929 the biguanide "metformin" was synthesized, which demonstrated the hypoglycemic effects observed by biguanide, but without the toxic effects [4]. The first clinical trial using metformin to treat T2D was reported in 1957. In the mid-1950s of the last century, metformin was approved in Europe as a treatment for T2D and in the United States of America (USA) since1995 [4]. After more than 60 years of clinical use for T2D, metformin has demonstrated safety and efficacy, making it the most commonly prescribed oral drug globally to control T2D [4]. Metformin is prescribed as the first choice of anti-hyperglycemic treatment worldwide, based on international guidelines for the management of T2D issued by the European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) [3]. Metformin is taken by about 150 million people each year for its safety, efficacy, and low cost, in addition to the pleiotropic effects that have a positive impact on glycemic control, insulin resistance, cardiovascular health, and cancer in patients with T2D [11]. In addition, metformin improves the inflammatory effects associated with obesity and reduces body mass, which is why it is widely prescribed in Mexico and the USA, where the prevalence of overweight and obesity is high [12, 13]. Despite the beneficial effects described, only about 30% achieve the goal of glycemic control (HbA1c <7%), so the pharmacokinetics and pharmacodynamics of this drug have been studied to explain these results. Studies in this regard have shown that in the intestine, the Organic Cation Transporter (OCT1 and OCT3), a protein with transmembrane channel functions in "enterocyte" epithelial cell, is responsible for the absorption of metformin, while in the liver this same protein is responsible for internalizing it to exert one of the main anti-hyperglycemic effects, suppression of hepatic glucose production "hepatic gluconeogenesis," and in the kidney OCT1 and OCT2 are the route of excretion of metformin (**Figure 1**). This knowledge has allowed the search for genetic variants in the SLC22A1/OCT1 gene which codes for the OCT1 protein, associated with response to metformin with a disciplined approach known as Pharmacogenetics.

2.2 Mechanism of action of metformin and its effect on T2D

Although it is recognized that the main effect of metformin is the suppression of hepatic glucose production, this drug has multiple mechanisms of action and the clear benefits of its use in the treatment of T2D in relation to glucose metabolism are complex and not fully understood [14]. Physiologically, metformin has been shown to have a key role in the liver and intestine. At the molecular level, evidence shows that metformin acts by mechanisms related to AMP-activated protein kinase (AMPK) and by inhibition of mitochondrial respiration, which improves blood glucose by acting on these mechanisms, both dependent and independent mechanisms [15].

Traditionally, metformin acts on the liver through organic cation transporters 1 (OCT1), which absorbs the drug from the enterocytes into the portal vein through the basolateral membrane and enters the hepatocyte, reducing hepatic glucose production after a high-fat diet, which improves blood glucose levels [16]. In this context, metformin reduces hepatic gluconeogenesis by inhibiting respiratory chain complex I, which suppresses ATP production and increases cytoplasmic AMP → ATP and ADP → ATP ratios, changes that activate AMPK mechanisms, which is the main cellular bioenergetic sensor (**Figure 2**). Due to membrane potentials, metformin accumulates up to 1000 times more within the mitochondria than in the cytoplasm of

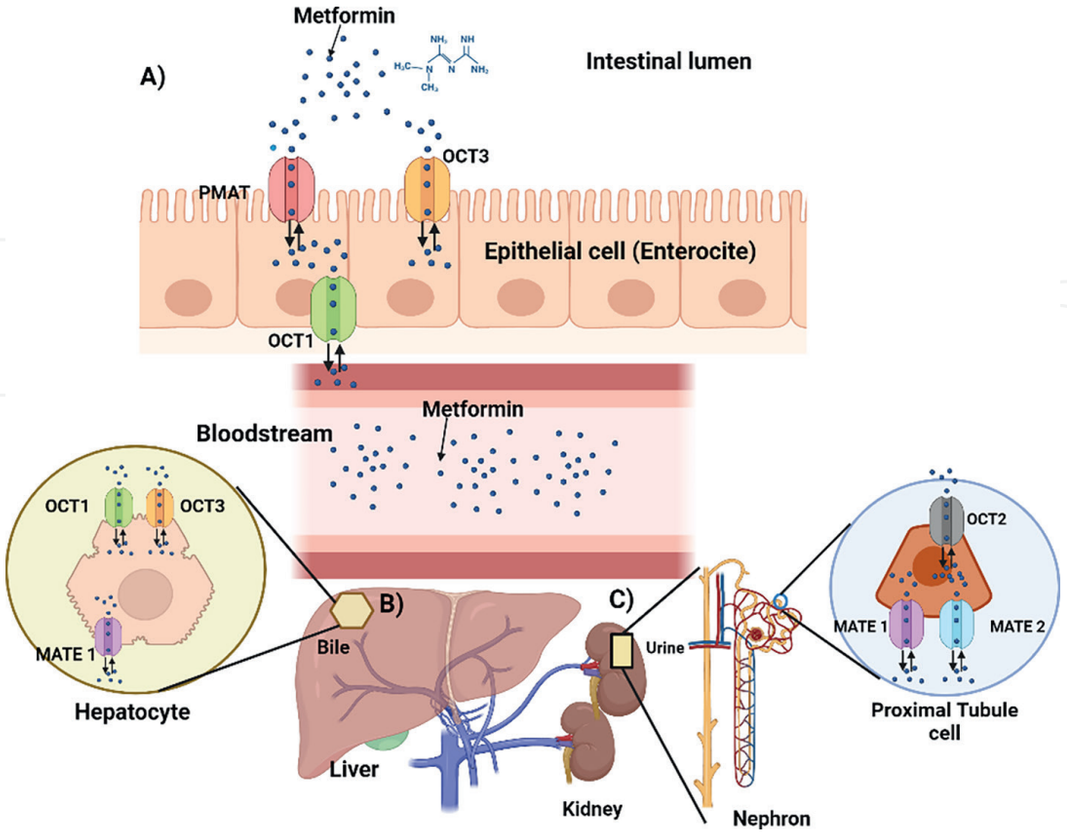

Figure 1.
Metformin pharmacodynamics. Scheme with the transporters responsible for the absorption, distribution, and elimination of metformin. (A) Intestinal absorption through the plasma membrane monoamine transporter (PMAT) and the Organic Cation Transporter 3 (OCT3), once in the "enterocyte" epithelial cell the Organic Cation Transporter 1 (OCT1) which is located on the basolateral surface, transports metformin to the serosal side of the endothelium, allowing its arrival in the bloodstream; (B) the transporters OCT1 and OCT3 are expressed in the sinusoidal membrane of hepatocytes where they capture metformin from the bloodstream. The elimination of metformin from inside the hepatocyte is by the Toxic Compound and Multidrug Extrusion Transporter (MATE1) helping to excrete metformin in bile, although in humans most of the metformin is excreted in the urine through MATE1; (C) the uptake of metformin from the bloodstream to renal epithelial cells is mainly facilitated by the Organic Cation Transporter 2 (OCT2) located on the basolateral membrane of renal proximal tubule cells, in turn, MATE1 and MATE2 expressed in the apical membrane of renal proximal tubule cells contribute to the excretion of metformin in the urine.

the cell, this energy-intensive process does not have the required supply of ATP and the concomitant suppression of this pathway could explain the effects obtained in the T2D treatment [17].

The effect of metformin on hepatic gluconeogenesis goes beyond mitochondrial control. Increases in the AMP → ATP ratio directly affect the activity of fructose-1,6-bisphosphatase, one of the key enzymes in this metabolic pathway, resulting in its acute inhibition. Due to the cellular energy imbalance, AMPK seeks to restore energy homeostasis by activating the catabolic pathways that generate ATP while deactivating the cellular processes that consume ATP. Activated AMPK phosphorylates the ACC1 and ACC2 isoforms of acetyl-CoA carboxylase (ACC), which inhibits fat synthesis and promotes fat oxidation, reducing hepatic lipid stores and improving hepatic insulin sensitivity in T2D patients [18, 19].

Although AMPK activation has not yet been determined exactly how it occurs, in this sense the effect of metformin is extremely complex and occurs at multiple levels by phosphorylating binding proteins, disassembling transcriptional coactivation complexes, and even deacetylating proteins that limit the rate of energy

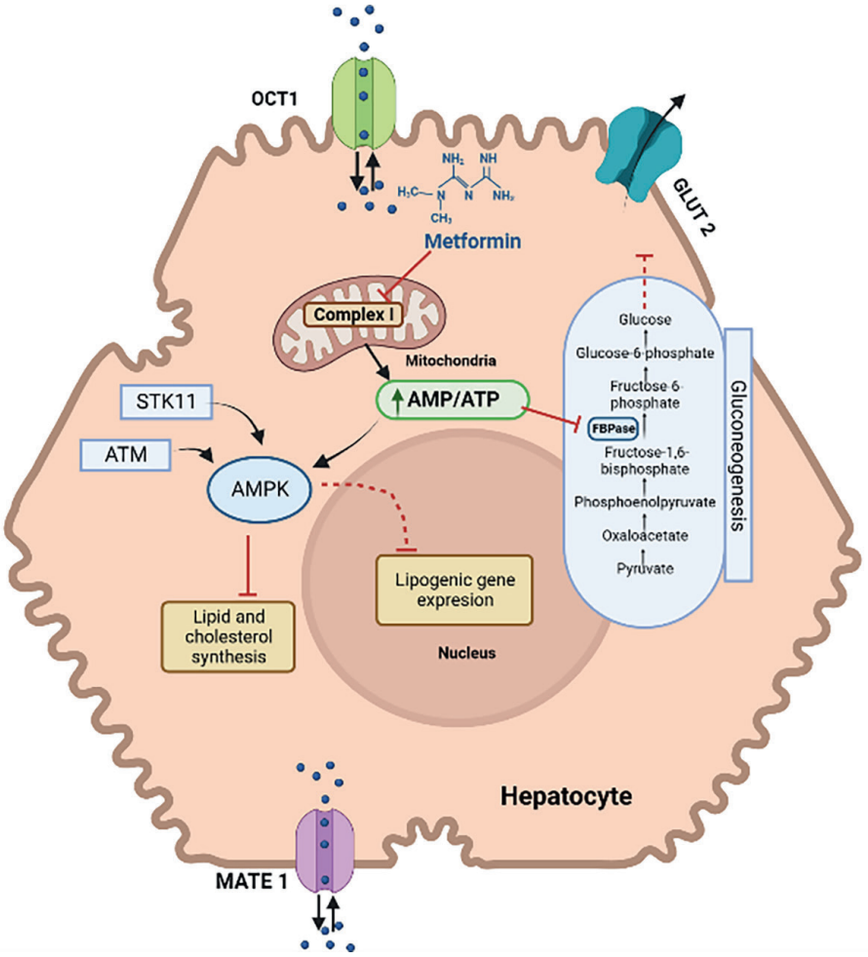

Figure 2.
Mechanism of action of metformin in the liver. Metformin reduces hepatic gluconeogenesis by inhibiting respiratory chain complex I, which suppresses ATP production and increases cytoplasmic AMP/ATP. Increases in the AMP/ATP ratio directly affect the activity of fructose-1,6-bisphosphatase, one of the key enzymes in gluconeogenesis, resulting in its acute inhibition. In addition, those changes activate AMP-activated protein kinase (AMPK), which is the main cellular bioenergetic sensor. Activation of AMPK results in the inhibition of lipogenic gene expression and cholesterol synthesis. AMPK activity may also be modulated by metformin through kinases such as the serine/threonine kinase encoded by ataxia telangiectasia mutated gene (ATM) or serine/threonine kinase 11 (STK11). FBPase, fructose-1,6-biphosphatase; GLUT 2, glucose transporter; MATE, Toxic Compound and Multidrug Extrusion Transporter.

biosynthesis [20]. Even if controversies persist about the effects of metformin on AMPK, the reduction in the expression of mRNAs encoding key enzymes of hepatic gluconeogenesis, and the improvement in hepatic insulin sensitivity, are proven long-term clinically relevant effects of metformin that are mediated by AMPK [21].

It has recently been postulated that the liver may not be the main target organ for metformin action in T2D patients as might be assumed. In this sense, in the gut, significant effects of metformin on anaerobic glucose metabolism have been observed. The reduction in net glucose uptake and the increase in lactate have made the intestines an important therapeutic target in the treatment of T2D with metformin, highlighting three main lines:

- Extended-release metformin is primarily retained in the gut, with minimal systemic absorption, and is as effective in lowering blood glucose as the standard formulation commonly implemented in T2D patients [22].

- The reduction of endogenous glucose production in the liver can only be partially explained by the effect of metformin, indicating the existence of other extrahepatic glucose-lowering mechanisms [23].

- Human studies have established that loss-of-function variants of the *SLC22A1* gene, encoding the OCT1 protein, reduce hepatic uptake of metformin but do not affect its efficacy in lowering HbA1c in T2D patients [24].

Metformin increases intestinal uptake of fluorodeoxyglucose in the colon, an effect that is accompanied by an increase in AMPK phosphorylation in colonic enterocytes, which affects energy homeostasis and glucose metabolism. In addition, at the intestinal level, metformin also affects glucose metabolism by increasing the secretion of glucagon-like peptide 1 (GLP-1), a mechanism of action that has been described for both immediate-release and prolonged-release metformin [25]. However, the potential mechanism of action of metformin mediated by the intestine that has recently generated the most interest has to do with the alteration of the intestinal microbiome and its inflammation.

In type 2 diabetes, metformin has been shown to have positive effects on intestinal inflammation, by inhibiting NF-κB signaling and differentiation of monocytes into macrophages, as well as suppression of proinflammatory cytokines from these macrophages [26]. In humans, metformin-dependent effects on the increase in *Escherichia spp.* and decrease in *Intestinibacter spp.* in the intestinal microbiome, have been linked to a decrease in adipose tissue inflammation and significant suppression of postprandial hyperglycemia [27]. This emphasizes that the changes in the microbiome in T2D are predominantly associated with the mechanism of action of metformin and not with the disease itself, although its role as a cause or consequence of the therapeutic effect still requires further investigation.

2.3 Pharmacogenetics of metformin in Latin America

From the development of technology with which it was possible to sequence the human genome and the software designs for the analysis of complete genomes that have emerged in the last decade, which are more accessible to the international scientific community, GWAS whole genome association studies have been generated to expand the knowledge of genes associated with susceptibility to develop T2D. Thus, since the first study of GWAS in T2D more than 100 loci of susceptibility have been described in different regions of the world. With the development of drugs for the treatment of hyperglycemia in TD2 with different effects and target tissues, studies have been carried out to advance the understanding of the variability in the response to these drugs based on the genetic component of the subjects studied. With this approach to determine the interindividual genetic variability of response to specific drugs arises pharmacogenetics, which focuses on the search for specific variants of genes whose expression products "proteins" are associated with lower pharmacological response (efficacy) or side effects to prescribed drugs.

The mechanisms of action of metformin have not been fully elucidated. Pharmacokinetic studies of metformin have shown that this biguanide is not metabolized in the body and is excreted without changes in its chemical composition through active tubular secretion in the urine by the kidney. With advances in the pharmacodynamics and pharmacokinetics of metformin, it has been possible to conduct studies in TD2 pharmacogenetics in several population groups in recent years. Until

the end of 2020, about 30 genes have been found with specific variants associated with therapeutic response to metformin or side effects that limit their therapeutic use, so they are considered candidate genes. There are three large-scale genome-wide association studies (GWAS) conducted in different cohorts. In 2011, the first in a European cohort "Genetics of Diabetes Audit and Research Tayside" (GoDART) was completed and included 1024 patients affected by TD2 [28]. The main results of this study showed 14 single nucleotide polymorphisms (SNPs) mapped on chromosome 11 locus 11q22, which were successfully associated with treatment for achieving HbA1c < 7% goal in the 18 months after starting metformin treatment. Subsequently, the rs11212617 was studied in 2 independent cohorts, GoDARTS with 1783 patients treated with metformin and the UK Prospective Diabetes Cohort (UKPDS) with 1113 patients, resulting in a significant association with response to metformin in both studies [29]. This genetic variant rs11212617, is located in a large block of linkage disequilibrium, which includes a set of genes among which is the ATM gene, which had been one of the candidate genes in greater perspective since that study, by the replication of result emerged in other independent cohorts such as China Han and Western Saudi Arabia. However, no significant difference was found between the response to metformin and rs11212617 in patients with TD2 in the Iranian and Indian populations [5, 30]. The ATM gene encodes for a protein belonging to the PI3/PI4 kinase family whose function is an important cell cycle checkpoint, which is not directly related to pharmacodynamics, pharmacokinetics or described mechanisms of intracellular action of metformin [31].

In 2016, the Metformin Genetics (MetGen) Consortium conducted a three-stage GWAS that included nearly 13,500 participants from different ancestry. The SLC2A2 gene encoding the glucose transporter GLUT2, an integral membrane glycoprotein expressed in the intestine, liver, islet beta cells, and kidney, presented a C-allele variant rs8192675 with 0.17% ($p = 6.6 \times 10^{-14}$) greater metformin-induced HbA1c in 10,577 participants with European ancestry [32]. The association of this SNP rs8192675 with metformin response was replicated in the Germany Diabetes Study. In a meta-analysis with 13,123 participants of any ancestry (European, Latino, African American) the frequency of the C-allele associated with response to metformin varied widely, but there was no genetic heterogeneity between the different ethnic groups [32, 33].

The other GWAS-focused study that sought the association of genetic variants with HbA1c change in response to metformin pharmacotherapy in a TD2 cohort was the one applied in the cohort of Action to Control Cardiovascular Risk in Diabetes (ACCORD), which includes subjects from the USA. The rs254271 variant in PRPF31 gene and the variant rs2162145 in CYPA6 gene was found to be associated with worse and better responses to metformin, respectively ($p = 3.79 \times 10^{-6}$, beta = 0.16; $p = 4.04 \times 10^{-6}$, beta = −0.197), these results were similar in a meta-analysis of independent cohorts with response to metformin [34]. Previous studies that had identified the rs11212617 variant in the ATM gene and the rs8192675 variant in the SLC2A2 gene were not replicated in this study.

GWAS studies are very useful to identify genomic variants statistically associated with a given phenotype such as a disease. These early genetic association studies focused on the analysis of family-based linkage as well as candidate genes in small groups of TD2 patients. It has been observed that this approach to studies is usually useful only for the identification of genetic variants with large effects. Thus, the selection of candidate genes for the study of metformin response in patients with TD2 was then focused on genes coding for proteins related to the pharmacodynamics of

this biguanide. Therefore, genes whose expression products such as organic cation transporters OCT are determinants for intestinal absorption, uptake liver, and renal excretion have been the most explored.

2.4 Pharmacogenetics of response to metformin in TD2 based on its pharmacodynamics

Organic cation transporters (OCT1, OCT2, and OCT3) are directly related to metformin pharmacodynamics as shown in **Figure 1**. The SLC22A1, SLC22A2, and SLC22A3 genes code for these proteins OCT1, OCT2, and OCT3. The OCT2 and OCT3 proteins are associated with intestinal absorption and renal clearance of metformin, so the genes that code for them have been studied more with side effects than with anti-hyperglycemic effects mainly. The OCT1 transporter is expressed in the intestine allowing its absorption, in the liver, it is the one that allows the entry to the hepatocyte to exert the anti-hyperglycemic effect by reducing hepatic gluconeogenesis and in the kidney for its elimination, which has become relevant for its close relationship with the efficacy of metformin [35, 36]. In humans, the SLC22A1/OCT1 gene (ID6580), located on the chromosome 6 locus (6q25.3), is among the most studied regarding the pharmacological response to metformin in glycemic control of patients with T2D. More than 30 polymorphic variants of this gene associated with different effects on metformin response have been identified in various ethnic groups, mostly from European and Asian regions, suggesting a population-specific response. At the end of the last decade, a systematic review highlighted and summarized the overall effects of polymorphisms in the SLC22A1/OCT1 gene in response to metformin and evaluated the role of these in interethnic differences. In total, 34 polymorphisms were found in 10 different ethnic groups. The response to metformin from these studies was measured with different variables such as %HbA1c, fasting plasma glucose (FPG), and postprandial plasma glucose (PPG). The *Met*408*Val* polymorphism of the SLC22A1/OCT1 gene (rs628031) has been the most widely studied with response to metformin and its genetic effect resulting in differences in glycemic control and side effects in the groups studied. This allows us to suggest the variable response of metformin based on the genetic variants according to the population studied [29]. These gene-metformin association studies have arisen in subjects with TD2 but with European and Asian ancestry mainly, which forces studies to be carried out in populations of Latin America, which presents a heterogeneity of ethnic groups with varied ancestry.

2.5 Global distribution of most studied SLC22A1/OCT1 gene variants with metformin response

The *Met*408*Val* polymorphism of the SLC22A1/OCT1 rs628031 gene has three A/C/G alleles, the ancestral allele G (Met 408) and the minor allele A (408Val), the latter associated with lower response to metformin. The global frequency reported in the 1000 Genomes Project Phase 3 for this SNP is 69% for the G allele and 31% for the A allele. The 1000 Genomes Project has the global regions defined in a particular way in Africa, America, East Asia, Europe, and Southeast Asia. For the Region of America, the frequency reported for the G allele is 78% and for the A allele 22%, whose frequency of the risk A allele is the lowest. While in Europe the frequency of allele G is 59% and for allele A is 41%, region that has the highest frequency of allele A. In the Southeast Asia region, the frequency is 61% for the G allele and 39% for the risk A allele, while in the African region the frequency of the G allele is 73% and 27% for the risk A allele [7]. With the frequency of the risk A allele in America at a low

percentage compared to the other regions, a higher proportion of subjects with T2D who present a response to metformin for glycemic control would be expected. If we consider that Mexico and Brazil are two countries with a considerable prevalence of overweight and obesity in addition to TD2, which condition the metabolic control of these patients, metformin should be a promising drug to contribute to metabolic control in these subjects, but studies are required to determine the response to metformin considering in addition to these variant other variables of importance in TD2 such as age, years with TD2, physical activity, diet, among others. With these pharmacogenetic studies that are increasingly accessible, it is possible to demonstrate the association and magnitude of the effect of metformin in glycemic control that allows us to know who are the patients with TD2 responders of the non-responders to metformin, which will be a significant advance for the health of patients with TD2.

The SNP in gene SLC22A1/OCT1 rs594709 is another polymorphism that presents two variants, the ancestral allele A and the minor allele G, the latter associated with a lower response to metformin in glycemic control. The global frequency reported in the 1000 Genomes Project Phase 3 for this SNP is 68% for the G allele and 32% for the A allele. For the region of America, the frequency reported for allele A is 78% and 22% and for the risk allele G, the lowest frequency reported in all regions. While in Europe the frequency of allele A is 59% and 41% for allele G, a region that has the highest frequency of the risk allele G. The Southeast Asia region is the most frequent follower of the G risk allele with 39% and 61% for the A allele. In the African region, it has a frequency of 29% for the risk allele G and 71% for the allele A [37].

These two SNPs rs628031 and rs594709 show that their frequency in Latin America is the lowest compared to the other regions, as well as other SNPs studied in Europe and Asia. A la fecha se han encontrado cerca de 15 variantes geneticas asociadas con respuesta a metformina **Table 1**. In Latin America, the association studies of these SNPs should be an important issue to determine their association and magnitude with respect to the effect on glycemic control in different populations to approach the personalized medicine required by the patient with TD2. This personalized medicine would allow us to approach finding responders versus non-responders to establish metformin treatments with more patient achieving the therapeutic goal (%HbA1c <7).

2.6 Nutrition and dietary interactions between macro and micronutrients with metformin in T2D

Lifestyle modifications involve the incorporation of regular physical exercise, as well as complete, varied, sufficient, and balanced nutrition to ensure a correct supply of carbohydrates, protein, fiber, and polyunsaturated fats in patients with TD2, who present impaired metabolism of carbohydrates, lipids, and proteins. The typical nutritional approach includes a diet with caloric reduction to achieve weight loss and control the percentage of body fat. The different editions of the international guidelines of the American Diabetes Association (ADA) highlight the importance of macronutrient consumption and control, however, specific recommendations for micronutrients in diabetes control are limited in the scientific literature [3]. In addition, nutrition is directly related to the success of the anti-hyperglycemic drug therapy of metformin because the absorption of nutrients can influence the pharmacokinetics of the drug and consequently its therapeutic effect [13].

Some studies have explored the relationship between the consumption of macro and micronutrients with the effect of metformin on T2D. Diets high in saturated fat were found to have a negative impact on the effect of metformin on glycemic control

Gene symbol ID	Region	dbSNP ID	SNP	Alleles	Effect	Risk allele				
						AFR	AMR	EAS	EUR	SAS
SLC22A1 6580	6q25.3	rs628031	Missense Met408Val	A/G	↓SE	A 27%	A 22%	A 26%	A 41%	A 39%
		rs12208357	Missense Arg61Cys	C/T	↓	T 0%	T 2%	T 0%	T 6%	T 2%
		rs34130495	Missense Gly401Ser	A/G	↓	A 0%	A 1%	A 0%	A 2%	A 0%
		rs622342	Intronic	C/T	↓	C 18%	C 40%	C 15%	C 38%	C 25%
		rs683369	Missense Leu160Phe	G/C	↓	G 1%	G 11%	G 15%	G 21%	G 17%
		rs36056065	Indel GTAAGTTG	−/GTAAGTTG	SE	ND	ND	ND	ND	ND
		rs594709	Intronic	A/G	↑	G 29%	G 22%	G 26%	G 41%	G 39%
		rs2282143	Missense Pro341Leu	C/T	↑	T 8%	T 2%	T 13%	T 1%	T 8%
		rs72552763	IndelGAT	−/GAT	↓	AT 5%	AT 29%	AT 0%	AT 18%	AT 15%
		rs316019	Missense Ala270Ser	G/T	↓	A 19%	A 9%	A 14%	A 11%	A 13%
		rs145450955	Missense Thr201Met	G/A	↓	A 0%	A 0%	A 1%	A 0%	A 0%
		rs201919874	Missense Thr199Ile	C/T	↓	A 0%	A 0%	A 0%	A 0%	A 0%

ID: Gene ID. dbSNP ID: a unique identifier assigned to a single nucleotide polymorphism (SNP) when it is submitted to the SNP database. Met, Methionine; Val, Valine; Arg, Arginine; Cys, Cysteine; Gly, Glycine; Ser, Serine; Leu, Leucine; Phe, Phenylalanine; Pro, Proline; Ala, Alanine; Thr, Threonine; Ile, Isoleucine. A, Adenine; G, Guanine; C, Cytosine; T, Thymine. ↑, increased response to therapy (in relation to the minor allele); ↓, reduced response to therapy (in relation to the minor allele); SE, side effect. AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, South Asian.

Table 1.
Genetic variants ton gene SLC22A1 influence metformin therapy outcomes and frequencies by region.

in animal models [38]. Also, it has been reported that the consumption of leucine at doses of 24 g / kg combined with the use of metformin in mice with obesity induced by a high-fat diet, significantly reduced the degree of adiposity, liver weight control, liver, and plasma lipids, as well as inflammatory markers in mice. One of the characteristics of patients with advanced T2D is the accumulation of liver fat and increased dyslipidemias. Therefore, focusing nutritional therapy with combined leucine–metformin intake, even at low doses, appears to have a significant effect on adiposity and completely reverses hepatic steatosis in diet-induced obese mice [39]. The amino acid leucine participates in the activation of the AMPK/Sirt1 signaling pathway, thus modulating energy, lipid, and glycolytic metabolism. Leucine has the ability to activate Sirt1, in part, by reducing energy in NAD+ activation, and as a consequence other sirtuin activators [40]. Also, the combined effect of leucine with resveratrol (nutrient–nutrient interaction) has been evaluated, and increased insulin sensitivity, muscle glucose utilization, and lipid oxidation were found. In this sense, the authors of this study propose the consumption of leucine as an adjuvant amino acid in the efficiency of metformin, since, as this is a drug that also converges in the AMPK/Sirt pathway, the combination of both could be a first-line proposal in patients with T2D, especially those who are overweight or obese [41, 42].

There is increasing evidence that metformin is associated with physiological processes in the gastrointestinal tract, since a pronounced effect can be observed when metformin is administered orally, in that sense, evaluating its interaction with the absorption of specific nutrients from the diet becomes highly relevant within the area of pharmacokinetics [43, 44]. Under this hypothesis, a study in animal models was conducted to evaluate microbiota changes in mice that were fed a high-fat diet and, in addition, received metformin treatment. Stool samples were collected before and after drug treatment and found 100 different species related to metformin. Functional analysis targeting carbohydrate, lipid, and amino acid metabolism pathways revealed 14 modified hierarchies. Sex-specific differences in response to metformin treatment were also observed. Males experienced greater changes in metabolic markers, while females showed important changes in the gut microbiome [45].

Micronutrients such as calcium, magnesium, zinc, potassium, and sodium (Ca, Mg, Zn, K, and Na) are cations that are absorbed in the intestine through the transmembrane channel "transporter of organic cations" (OCT1) mainly. This is the same protein related to the absorption of metformin, so the study of the interactions between the dietary intake of micronutrients with specific variants of the *OCT1* gene in subjects receiving metformin, may explain to some extent the differences in terms of the effect on glycemic control observed in subjects with this pharmacotherapy. A recent study reported that there is a significant interaction between the consumption of specific micronutrients such as calcium and potassium with the risk variant 408*Val* rs628031 of the *SLC22A1/OCT1* gene and glycemic control in T2D patients receiving metformin [13].

With the above, the importance of carrying out Nutrigenetics and Pharmacogenetics studies in patients with T2D is highlighted that allows the timely detection of subjects who do achieve glycemic control based on personalized diet and metformin, while in non-responders seek the particular treatment that guarantees the goal in glycemic control that is HbA1c <7%.

3. Conclusions

The most recognized effect of metformin is the suppression of hepatic glucose production, which favors glycemic control in subjects with TD2. The response to

metformin in glycemic control is variable in each population studied, which forces us to study genetic factors related to the pharmacokinetics and pharmacodynamics of this drug to find associated genetic variants that explain these differences to some extent. Metformin is eliminated without structural modification by urine mainly, so genes closely related to its pharmacodynamics such as solute carriers (SLC) are of greatest interest in future studies in Latin America. With the Pharmacogenetics approach, it will be possible to predict the genetic component that differentiated responders from non-responders to drug treatment with metformin for glycemic control in patients with TD2 in Latin America.

The mechanisms of action of metformin are not fully understood, there is evidence of some physiological effects in the intestine that suggest a mechanism in glucose homeostasis and the intestinal microbiome that favors glycemic control in patients with TD2, which makes a promising future of this drug by expanding the target organ.

Interaction studies between metformin and macro or micronutrients with genes encoding organic cation transporters (SLC22), may help improve glycemic control in patients with TD2 from specific populations in Latin America.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Eloy A. Zepeda-Carrillo[1*], Rafael Torres Valadez[1,2], Sara A. Campos Huerta[1] and Elisa María Barrón Cabrera[3]

1 Specialized Unit in Research, Development and Innovation in Genomic Medicine, Nayarit Center for Innovation and Technology Transfer, Autonomous University of Nayarit, Tepic, Mexico

2 Integral Health Academic Unit, Autonomous University of Nayarit, Tepic, Mexico

3 Faculty of Nutrition and Gastronomy Sciences, Autonomous University of Sinaloa, Culiacan, Mexico

*Address all correspondence to: eloyz@uan.edu.mx

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