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FACULTAD DE CIENCIAS QUÍMICO BIOLÓGICAS
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Efecto de las variantes comunes en los genes *SLC22A1* y *SLC22A2* sobre el control glicémico de pacientes con diabetes tipo 2 tratados con metformina

T E S I S

QUE PARA OBTENER EL TITULO DE

MAESTRO EN CIENCIAS BIOMEDICAS

PRESENTA:

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APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 18 días del mes de junio de dos mil dieciocho se reunieron los miembros del Comité Tutoral designado por la Academia de Posgrado de la Maestría en Ciencias Biomédicas, para examinar la tesis titulada **"Efecto de las variantes comunes en los genes SLC22A1 y SLC22A2 sobre el control glicémico de pacientes con diabetes tipo 2 tratados con metformina"**, presentada por el alumno Carlos Alberto Reséndiz Abarca, para obtener el Grado de Maestría en Ciencias Biomédicas. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

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29-Dec-2018

Dear Dr. Flores-Alfaro:

Your manuscript entitled "Altered glycemic control associated with polymorphisms in the SLC22A1 (OCT1) gene in a Mexican population with type-2 diabetes treated with metformin: a cohort study" has been successfully submitted to The Journal of Clinical Pharmacology and is presently being given full consideration for publication. Your assigned manuscript ID is JCP-18-Dec-443.

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Este trabajo se realizó en el Laboratorio de Investigación en Epidemiología Clínica y Molecular de la Facultad de Ciencias Químico-Biológicas de la Universidad Autónoma de Guerrero en Chilpancingo, Guerrero, México, así como en la Unidad de Investigación en Bioquímica del Hospital de Especialidades Siglo XXI, CDMX, México.

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Durante el período en que cursó la Maestría en Ciencias Biomédicas, la QBP. Carlos Alberto Resendiz Abarca recibió beca para estudios de maestría del CONACYT con número 777313.

Index

Table index

Altered glycemic control associated with polymorphisms in the *SLC22A1* (OCT1) gene in a Mexican population with type-2 diabetes treated with metformin: a cohort study

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Abstract

The organic cation transporters (OCT1 and OCT2) and the multidrug and toxin extrusion transporter 1 (MATE1), encoded by the *SLC22A1*, *SLC22A2*, and *SLC47A1* genes, respectively, are responsible for the absorption of the metformin in enterocytes, hepatocytes, and kidney cells. The aim of the study was to evaluate whether a genetic variation in the *SLC22A1*, *SLC22A2*, and *SLC47A1* genes could be associated with an altered response to metformin in patients with type-2 diabetes (T2D). A cohort study was conducted in 308 individuals with a diagnosis of T2D of less than 3 years and who had metformin monotherapy. Three measurements of blood glycosylated hemoglobin (HbA1c) were obtained at the beginning of the study, and at 6 and 12 months. Five polymorphisms were analyzed in the *SLC22A1* (rs622342, rs628031, rs594709), *SLC22A2* (rs316019), and *SLC47A1* (rs2289669) genes by real-time PCR. The results showed a significant association among genotypes CC-rs622342 (β=1.36; p <0.001), AA-rs628031 (β=0.98; p = 0.032), and GG-rs594709 (β=1.21; p = 0.016) in the *SLC22A*1 gene with the average increase in HbA1c levels during the follow-up period. Additionally, a significant association was found in the CGA and CAG haplotypes with the average increase in HbA1c levels compared to the highest frequency haplotype (AGA). In conclusion, the genetic variation in the *SLC22A1* gene is significantly related to the variation of the HbA1c levels, an important indicator of glycemic control in diabetic patients. This information may contribute to identify patients with an altered response to metformin prior to starting their therapy.

Keywords

Type-2 diabetes, Metformin, *SLC22A1*, *SLC22A2*, *SLC47A1*

Introduction

Diabetes is a chronic disease that occurs when the pancreas does not produce or secrete sufficient insulin or when the body is not able to use this hormone effectively; thus, glucose does not appropriately become incorporated into the cells and a hyperglycemic state is generated. Initial treatment in patients with diabetes is a combination of changes in lifestyle, mainly healthy eating and exercise; in addition, the use of drugs can be recommended. Metformin is the drug-of-choice to treat diabetic patients, due to their low cost and euglycemic effectiveness. Additionally, in contrast to other medications, metformin does not cause hypoglycemia or hyperinsulinemia, common side effects associated with other antidiabetic drugs.[1] Metformin is not metabolized, and is excreted unchanged in urine, with a half-life of ~5 h. The classic functional mechanism of metformin is decreasing the state of hepatic energy, decreasing hepatic glucose production and fatty acids synthesis, as well as reducing gastrointestinal glucose absorption and improving peripheral sensitivity to insulin.[2]

Metformin is widely distributed to several tissues and enters the cells by the action of membrane transporters such as the organic cation transporters (OCT1, OCT2, and OCT3), the multidrug and toxin extrusion transporters (MATE1 and MATE2), and the plasma membrane monoamine transporter (PMAT).[2] In the cell, metformin inhibits the complex I of the mitochondrial electron transport chain, which results in a decrease of ATP and an increase in ADP and AMP concentrations, causing a low energy level. This depletion of energy is responsible for the activation of the 5-adenosine monophosphate kinase (AMPK) protein. The activation of AMPK leads to an increase in the catabolic reactions that produce energy, such as β-oxidation, glycolysis, or autophagy, and an inhibition of anabolic reactions that require energy, thus ensuring the restoration of cellular energy balance. Additionally, AMPK promotes the transcription of mitochondrial genes and inhibits the expression of genes involved in lipogenesis and of anabolic pathways that consume ATP, such as protein synthesis.[3-6]

Clinically, variation in the response to metformin is very high, and more than 36% of individuals with T2D treated only with metformin have unacceptable glucose levels. Genetic and non-genetic characteristics have been related to modifying the metformin response; for example, age, gender, and body weight are partially responsible for this variation. In addition, studies in native population of the American continent have shown less control in blood glucose concentrations in comparison to European or Asian populations, who tend to have better metabolic

control.[7,8] Several studies have provided evidence that the effectiveness of metformin is affected by genetic variants in the transporters OCT1 (*SLC22A1*), OCT2 (*SLC22A2*)[1,2], and MATE-1 (*SLC47A1*).[9] In particular, OCT1 plays an important role in the hepatic uptake of metformin, and their genetic variants in *SLC22A1* have been widely studied and associated with decreased the efficacy of metformin. Among these variants, rs622342, rs628031, and rs594709 have been shown to decrease the effectiveness of metformin in several populations.[10-12] On the other hand, OCT2 and MATE-1 are mainly expressed on the kidney and facilitate the excretion of metformin from tubular cells into urine.[9] Studies in patients and in cell lines identified that the single nucleotide polymorphisms (SNPs) rs316019 and rs2289669 in the *SLC22A* and *SLC47A1* genes, respectively, could exert an effect on the distribution and elimination of metformin.[13] However, opposite results have been reported, without a significant impact of these variants on the glycemic response to metformin.[14]

The purpose of this study was to analyze the relationship between SNPs in the *SLC22A1*, *SLC22A2*, and *SLC47A1* genes and blood glucose control in diabetic patients after 6 and 12 months under treatment with metformin alone.

Methods

Study participants

A cohort study was conducted in 308 individuals aged than 25 years, residents of Mexico City, with a diagnosis of fewer than 3 years type-2 diabetes (T2D). All participants received only metformin monotherapy during follow-up. Follow-up of the participants was carried out for 1 year, performing clinical measurements at the beginning of the study (T1), at 6 months (T2), and at 12 months (T3). The protocol was approved by the National Committee and the Ethics Committee Board of the Mexican Institute Social Security (IMSS) and was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from each participant. Anthropometry, blood pressure, and the dose of metformin used in the patients' treatment were recorded for each patient. Patients who withdrew from the study, who did not follow the treatment, who had micro- or macroalbuminuria, and those who were treated with some other antidiabetic drug during follow-up were excluded from the study.

Anthropometric and biochemical measurements

Participants were scheduled for their clinical evaluation. Each one was weighed with a digital scale, waist and hip circumference was measured with an anthropometric tape, and height, with a portable stadiometer. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercury sphygmomanometer. Blood samples were obtained after a 12-h fast for biochemical studies. Concentrations of high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), transaminases (alanine and aspartate amino transferase), creatinine, and albumin were measured using the ILab 350 (Instrumentation Laboratory, Spain). Percentage of glycosylated hemoglobin (HbA1c) was measured using the VARIANT II system (Bio-Rad Laboratories, Inc., CA, USA). Serum concentrations of creatinine and microalbuminuria were analyzed to rule out individuals with impaired kidney function.

Genotyping

Genomic DNA extraction from peripheral blood leukocytes was performed using the automated FLEX STAR system (AutoGen, Inc., MA, USA). optical density (OD) and the 260/280 ratio were measured in order to assess DNA concentration and quality. SNPs rs622342, rs628031, rs594709 (*SLC22A1* gene); rs316019 (*SLC22A2* gene), and rs2289669 (*SLC47A1* gene) were genotyped using TaqMan probes and the 7900HT real-time PCR system (Applied Biosystems, Inc., CA, USA).

Statistical analysis

The sociodemographic and clinical characteristics of the participants were summarized, the qualitative variables were in frequencies, and the quantitative variables, in medians and 25th–75th percentiles. To compare frequencies or medians between groups or SNPs, Chi-square (X^2), Mann-Whitney *U*, or Kruskal-Wallis tests were used. Hardy-Weinberg equilibrium (HWE) was verified using the X^2 test with one degree of freedom. To identify the effect of the polymorphisms studied on the biochemical parameters, for each biochemical parameter the delta variable (Δ) was generated, which represents the difference between the measurement obtained at the beginning of the study (T1) and the measurement after 6 months (T2) or at the end of the study (T3). Generalized linear models adjusted were evaluated to identify possible associations between the SNP and the

change (Δ) generated in the biochemical parameters. A value of $p \leq 0.05$ was considered significant. Statistical analysis was performed using STATA v.13 statistical software.

Results

To identify how many participants were under good glucose control using metformin monotherapy, we classified the 308 participants as controlled and uncontrolled using glycosylated hemoglobin (HbA1c) levels of <7% and ≥7%, respectively, in agreement with the recommendations of the American Diabetes Association. We found that 48.7% of the patients were uncontrolled, with ≥7% of HbA1c values. A significant increase in age, years of evolution of T2D, waist circumference, diastolic blood pressure, and triglycerides was observed in the uncontrolled group, as well as reduced levels of HDL-c and creatinine with respect to the controlled group (Table 1). At the end of the study, a total of 155 uncontrolled patients were detected, among whom 116 had low glycemic control from the beginning, and 39 were controlled at the start of the study.

Genotype frequencies were consistent with the Hardy-Weinberg equilibrium (HWE) test, except for rs628031, that was very near the HWE (p = 0.046). In order to identify differences between genotypes with changes in biochemical measurements during follow-up, the Δ value was generated (Δ is the difference between the biochemical values obtained at T2 or T3 with the values obtained at T1). No significant differences were observed between biochemical Δ values and the SNP evaluated at 6 months of follow-up (data not shown). Nevertheless, when Δ values for 12 months of follow-up were evaluated, we identified higher Δ values for HbA1c in individuals carrying the CC (Table 2) and AA (Table 3) genotypes for rs622342 and rs628031 in the *SLC22A1* gene, respectively. However, no significant differences were found for SNP rs594709 (Table 4), rs316019 (Table S1), and rs2289669 (Table S2).

On the other hand, when we evaluated generalized linear models adjusted for gender, age, years of diabetes evolution, and waist circumference, we identified a significant association of the CC-rs622342 (β=1.36; p <0.001), AA-rs628031 (β=0.98; p = 0.032), and GG-rs594709 (β=1.21; p = 0.016) genotypes with the Δ values of HbA1c during the follow-up period (Table 5). Additionally, we identified a significant effect of the CGA and CAG haplotypes on the Δ value in HbA1c compared with the highest frequency haplotype (AGA). It is noteworthy that the CAG haplotype is constituted by the risk alleles for the three SNPs in the *SLC22A1* gene (Table 6).

Furthermore, we found linkage disequilibrium (LD) between rs628031 with rs594709 in the *SLC22A1* gene, with a Lewontin statistic (D′) value of 0.539 and a *p* value of <0.001.

Discussion

Metformin is widely used for the treatment of type-2 diabetes, which, unlike other drugs, does not cause hypoglycemia or hyperinsulinemia and its main action comprises the reduction of glucose and fatty acid synthesis, the reduction of intestinal absorption of glucose, and the improvement of insulin sensitivity in peripheral tissues. OCT play an important role in the membrane transport of metformin in different tissues. Metformin is not metabolized by hepatic enzymes and is excreted unchanged by the kidneys. Variants in the *SLC22A1*, *SLC22A2*, and *SLC47A1* genes have been identified that modify the function of the transporters that encode and that possess an important role in the pharmacodynamics of metformin.[15] Shu *et al*.[16] functionally characterized 15 protein-altering variants of the human liver OCT1; these authors observed that five variants exhibit decreased function and one had increased function. In addition, they found that genetic variants in the SLC22A1 gene contribute to a wide variation in response to metformin.[17]

In our study, we identified that SNPs in the *SLC22A1* gene are related with changes in HbA1c levels, in that individuals carrying the CC-rs622342 genotype and the AA-rs628031 genotype exhibited a significant increase in HbA1c levels after 12 months of follow-up compared with individuals carrying the AA-rs622342 and GG-rs628031 genotypes, respectively. Analyzing generalized linear models that had been adjusted, we identified a significant association among the CC-rs622342 (p <0.001), AA-rs628031 (p = 0.032), and GG-rs594709 (p = 0.016) genotypes in the *SLC22A1* gene with the increase of HbA1c levels in diabetic patients treated with metformin alone at 12 months of follow-up.

Similar results were reported in the follow-up study of T2D patients in South India, indicating that diabetic patients carrying the AA-rs622342 genotype had a 5.6 times better chance of response to metformin treatment compared with patients carrying the CC genotype.[18] For their part, Becker et al.[15] reported a significant association among individuals carrying the CC-rs622342 genotype, with an average increase of 0.02% in HbA1c levels. This same working group in 2010 identified a significant association between the interaction of the CC-rs622342 genotype and the A-rs2289669 allele with the change in HbA1c levels (-0.68; 95% CI: -1.06 to -0.30; p = 0.005) in

incident metformin users.[19] In order to clarify the effect of the rs622342 polymorphism of the *SLC22A1* gene in the transport of drugs by OCT1, Becker et al.[10] conducted an analysis using data from the Rotterdam cohort study of incident levodopa users, irrespective of whether the Rotterdam group used other anti-parkinsonian drugs. Becker et al. concluded that subjects with the minor allele (C) were associated with higher prescribed doses of anti-parkinsonian drugs and shorter survival time after starting levodopa therapy, suggesting that patients with the AC or CC genotype exhibit a lesser response to these drugs and more severe symptoms. This result suggests that the SNP rs622342 could be related with affecting the functionality of OCT1, reducing its capacity for the transport of drugs such as metformin, consequently reducing their action on glycemic control. On the other hand, Tkáč et al.[20] found no association between the rs622342 and rs316019 polymorphisms with HbA1c levels at 6 months follow-up of patients with T2D treated with metformin alone. Similar results to those of Tkáč et al. were reported in the study conducted in Danish population.[21]

For the rs628031 SNP, results similar to ours were reported in the cohort of Han Chinese subjects with T2D incident metformin treatment, indicating that patients with the GG-rs628031 genotype had a greater reduction in postprandial plasma glucose compared with those with the AA-rs628031 genotype (p <0.010).[11] Opposite results have been reported in Iranian,[22] Indian,[23] Caucasian[15] and Japanese[24] populations, where no association was found between the rs628031 polymorphism and the response to treatment with metformin. These data reveal that there is a substantial variation in the contribution of rs628031 to the efficacy of metformin. It is possible that the rs622342 polymorphism found in an intron is in linkage disequilibrium (LD) with a functional polymorphism, although the probability that it exerts a direct effect on splicing or gene expression should not be ruled out.[10] For its part, the rs628031 polymorphism causes a change in exon 7 of OCT1, which consists of an amino-acid substitution at position 408 of the protein (Met408Val).[15]

The rs628031 is a common polymorphism in the Caucasian population and it tend to decrease to expression of the mRNA of the *SLC22A1* gene in enterocytes, which leads to a decrease in the intestinal uptake of metformin, therefore to its accumulation.[25] Furthermore, it has been reported that rs628031 is in LD with rs36056065, which has been reported to cause an insertion of eight nucleotides at the extreme of exon 7; this insertion may modify the splicing of mRNA, consequently altering the functionality of OCT1.[26]

On the other hand, we found that the GG genotype on rs594709 had significant increase in HbA1c values, similar to results reported by Xiao et al.,[12] where the authors identified that individuals carrying the A allele of rs594709 presented better efficacy to treatment with metformin, improving insulin sensitivity. Also, a study in European population reported a significant association between allele G-rs594709 and lower serum acylcarnitine levels (p <0.001). Acylcarnitines are intermediate metabolites that participate in mitochondrial oxidation processes, and it has been proposed that these metabolites are exported from hepatocytes by OCT1, suggesting that the OCT1 function is affected by variants in this gene.[27]

In a study conducted in Chinese population reporting significant decrease in serum lipid levels in individuals carrying the GG genotype of rs2289669, and for the rs316019 SNP, it has been reported that carriers of the GT genotype are associated with greater renal clearance of metformin in healthy volunteers, both European and African.[12] Recently, it was reported that SNP rs2289669 in the *SLC47A1* gene exerts an effect on the response to metformin treatment, with highest HbA1c reduction in patients with the A allele.[28] However, we did not find a significant association between SNP rs316019 or rs2289669 with HbA1c levels or with their change (Δ) at 12 months of follow-up. Similar results were obtained in Indian population, where a significant association was not found between rs2289669 polymorphisms and changes in HbA1c levels in T2D patients with metformin monotherapy after 3 months of follow-up.[29] This suggested that these variations could be due to differences in the population size of the studies or differences in allele frequencies due to genetic diversity among populations.

Additionally, we identified a significant effect of CGA and CAG haplotypes of SNP rs622342, rs628031, and rs594709 in the *SLC22A1* gene on the increase of HbA1c levels at 12 months of follow-up compared with the AGA haplotype. These genetic variations could be involved in a poor response to the action of metformin and decreased insulin sensitivity in peripheral tissues. It is possible that the carriers of the variants mentioned require a higher dose of metformin to improve their glycemic control. Future studies are required to evaluate the dose response to treatment with metformin in individuals carrying these genotypes and with poor glycemic control, and how the presence of the rs628031, rs622342, and rs594709 SNP could affect the molecular interaction between OCT1 and metformin, which could facilitate the development of new metformin analogues that are refractory to these variants.

Conclusion

In summary, in this study we identified a significant association between variants in the *SLC22A1* gene with the increase in glycosylated hemoglobin levels at 12 months of follow-up of T2D patients who were incident metformin users, identifying that metformin therapy is less effective in the glycemic control of patients with T2D carriers of the CC-rs622342, AA-rs628031, and GG-rs594709 genotypes. This information that may contribute to the identification of patients with an altered response to metformin prior to the initiation of therapy.

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References

1. Kleinberger JW, Pollin TI. Personalized medicine in diabetes mellitus: current opportunities and future prospects. *Ann N Y Acad Sci*. 2015;1346(1):45-56.
2. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics.* 2012;22(11):820-827.
3. Daugan M, Dufaÿ Wojcicki A, d'Hayer B, Boudy V. Metformin: An anti-diabetic drug to fight cancer. *Pharmacol Res*. 2016;113(Pt A):675-685.
4. Foretz M, Viollet B. Regulation of hepatic metabolism by AMPK. *J Hepatol*. 2011;54(4):827-829.
5. Krishan S, Richardson DR, Sahni S. Adenosine monophosphate-activated kinase and its key role in catabolism: structure, regulation, biological activity, and pharmacological activation. *Mol Pharmacol*. 2015;87(3):363-377.
6. Zhou T, Xu X, Du M, Zhao T, Wang J. A preclinical overview of metformin for the treatment of type 2 diabetes. *Biomed Pharmacother*. 2018;106:1227-1235.
7. Herman WH, Ma Y, Uwaifo G, et al. Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. *Diabetes Care*. 2007;30(10):2453-2457.
8. Jablonski KA, McAteer JB, de Bakker PI, et al. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. *Diabetes*. 2010;59(10):2672-2681.
9. Wagner DJ, Hu T, Wang J. Polyspecific organic cation transporters and their impact on drug intracellular levels and pharmacodynamics. *Pharmacol Res*. 2016;111:237-246.
10. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. OCT1

polymorphism is associated with response and survival time in anti-Parkinsonian drug users. *Neurogenetics*. 2011;12(1):79-82.

11. Zhou Y, Ye W, Wang Y, et al. Genetic variants of OCT1 influence glycemic response to metformin in Han Chinese patients with type-2 diabetes mellitus in Shanghai. *Int J Clin Exp Pathol*. 2015;8(8):9533-9542.
12. Xiao D, Guo Y, Li X, et al. The impacts of *SLC22A1* rs594709 and *SLC47A1* rs2289669 polymorphisms on metformin therapeutic efficacy in Chinese type 2 diabetes patients. *Int J Endocrinol*. 2016;2016:4350712.
13. Zolk O. Disposition of metformin: variability due to polymorphisms of organic cation transporters. *Ann Med*. 2012;44(2):119-129.
14. Dujic T, Zhou K, Yee SW, et al. Variants in pharmacokinetic transporters and flycemic response to Metformin: a metgene meta-analysis. *Clin Pharmacol Ther*. 2017;101(6):763-772.
15. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J*. 2009;9(4):242-247.
16. Shu Y, Leabman MK, Feng B, et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc Natl Acad Sci U S A*. 2003;100(10):5902-5907.
17. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest*. 2007;117(5):1422-1431.
18. Umamaheswaran G, Praveen RG, Damodaran SE, Das AK, Adithan C. Influence of SLC22A1 rs622342 genetic polymorphism on metformin response in South Indian type 2 diabetes mellitus patients. *Clin Exp Med*. 2015;15(4):511-517.

19. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. *Pharmacogenet Genomics*. 2010;20(1):38-44.
20. Tkac I, Klimcakova L, Javorsky M, et al. Pharmacogenomic association between a variant in SLC47A1 gene and therapeutic response to metformin in type 2 diabetes. *Diabetes Obes Metab*. 2013;15(2):189-191.
21. Christensen MMH, Hojlund K, Hother-Nielsen O, et al. Steady-state pharmacokinetics of metformin is independent of the OCT1 genotype in healthy volunteers. *Eur J Clin Pharmacol.* 2015;71(6):691-697.
22. Shokri F, Ghaedi H, Ghafouri Fard S, et al. Impact of ATM and SLC22A1 polymorphisms on therapeutic response to metformin in Iranian diabetic patients. *Int J Mol Cell Med*. 2016;5(1):1-7.
23. Sur D. A tale of genetic variation in the human *SLC22A1* gene encoding OCT1 among type 2 diabetes mellitus population groups of West Bengal, India. *IMPACT: IJRANSS*. 2014;2(5):97-106.
24. Chen L, Takizawa M, Chen E, et al. Genetic polymorphisms in organic cation transporter 1 (OCT1) in Chinese and Japanese populations exhibit altered function. *J Pharmacol Exp* Ther. 2010;335(1):42-50.
25. Semiz S, Dujic T, Causevic A. Pharmacogenetics and personalized treatment of type 2 diabetes. *Biochem Med (Zagreb)*. 2013;23(2):154-171.
26. Seitz T, Stalmann R, Dalila N, et al. Global genetic analyses reveal strong inter-ethnic variability in the loss of activity of the organic cation transporter OCT1. *Genome Med*. 2015;7(1):1-23.
27. Kim HI, Raffler J, Lu W, et al. Fine mapping and functional Analysis reveal a role of

SLC22A1 in acylcarnitine transport. *Am J Hum Genet*. 2017;101(4):489-502.

28. Mousavi S, Kohan L, Yavarian M, Habib A. Pharmacogenetic variation of *SLC47A1* gene and metformin response in type2 diabetes patients. *Mol Biol Res Commun*. 2017;6(2):91-94.
29. Raj GM, Mathaiyan J, Wyawahare M, Priyadarshini R. Lack of effect of the *SLC47A1* and *SLC47A2* gene polymorphisms on the glycemic response to metformin in type 2 diabetes mellitus patients. *Drug Metab Pers Ther*. 2018;33(4):175-185

Table 1. Demographic and clinical characteristics of the patients with T2D stratified by glycemic control at the beginning of the study

Characteristic	Controlled <7 % of HbA1c n=158 (51.3%)	Uncontrolled ≥7 % of HbA1c n=150 (48.7%)	*p*
Age (years)	55 (49-63)	54 (47-59)	0.008†
Gender, n (%)			
Masculine	43 (27.3)	53 (35.3)	0.124‡
Feminine	115 (72.8)	97 (64.7)	
Years of T2D evolution	2 (1-3)	2 (1.2-3)	0.004†
Waist circumference (cm)	97 (90-105)	100 (92-108)	0.023†
BMI, kg/m^2	29.2 (27.1-32.7)	31.1 (27.1-35)	0.115†
Systolic BP, mmHg	125.7 (115-135.3)	126 /117.3-133.7)	0.946†
Diastolic BP, mmHg	76.2 (70-81)	78.9 (73-84)	0.004†
Creatinine, mg/dL	0.7 (0.6-0.8)	0.6 (0.5-0.8)	0.003†
TG, mg/dL	159 (117-204)	188 (142-253)	<0.001†
HDL-c, mg/dL	45.5 (38-54)	41 (36-49)	0.004†
LDL-c, mg/dL	141 (121-162)	143 (123-161)	0.956†

Data are reported as medians and percentiles (25th-75th percentiles), or as noted in table. † Mann-Whitney *U* test; ‡ Chi-square test. HbA1c: Glycosylated hemoglobin; BMI: Body mass index; BP: Blood Pressure; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol.

Table 2. Relationship of the SNP rs622342 in the *SLC22A1* gene with baseline levels and with change (Δ) of the biochemical variables in diabetic patients treated with metformin

Variable	Genotypes			*p*†
	AA	AC	CC	
	n=116 (37%)	n=153 (50%)	n=39 (13%)	
HbA1c, %	7 (6.3, 8.1)	6.8 (6.3, 8.2)	7.1 (6.1, 7.9)	0.841
TG, mg/dL	158 (126, 213)	173 (127, 222)	188 (144, 270)	0.270
HDL-c, mg/dL	45 (38, 53)	43 (37, 52)	43 (38, 50)	0.429
LDL-c, mg/dL	144 (132, 161)	139 (115, 59)	144 (125, 172)	0.076
Creatinine, mg/dL	0.6 (0.5, 0.7)	0.7 (0.6, 0.8)	0.7 (0.7, 0.8)	0.002
Δ HbA1c, %	-0.1 (-0.6, 0.4)	0.1 (-0.4, 0.5)	0.5 (-0.1, 2.5)	0.003
Δ TG, mg/dL	-9.5 (-56, 36)	-8 (-50, 28)	0 (-68, 61)	0.545
Δ HDL-c, mg/dL	2 (-2, 8)	3 (-1, 8)	1 (-4, 6)	0.306
Δ LDL-c, mg/dL	0 (-23, 18.5)	1 (-13, 18)	0 (-31, 24)	0.601
Δ Creatinine, mg/dL	0 (-0.1, 0.1)	0 (-0.1, 0.1)	0 (-0.2, 0)	0.012

Data are reported as medians (25th – 75th percentiles). † Kruskal-Wallis test. HbA1c: Glycosylated hemoglobin; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; Δ: Difference between measurement T3 and measurement T1.

Table 3. Table 3. Relationship of the SNP rs628031 in the SLC22A1 gene with the basal levels and with change (Δ) of the biochemical variables in diabetic patients treated with metformin

Variable	Genotypes			*p*†
	GG	GA	AA	
	n=215 (70%)	n=78 (25%)	n=15 (5%)	
HbA1c, %	7 (6.2, 8.1)	6.7 (6.3, 8.4)	6.8 (6.4, 7.2)	0.822
TG, mg/dL	173 (129, 237)	169 (119, 203)	173 (122, 217)	0.390
HDL-c, mg/dL	44 (37, 51)	46 (38, 53)	47 (37, 56)	0.373
LDL-c, mg/dL	143 (121, 163)	139 (123, 159)	139 (129, 162)	0.842
Creatinine, mg/dL	0.6 (0.6, 0.8)	0.7 (0.6, 0.8)	0.6 (0.5, 0.9)	0.834
Δ HbA1c, %	0.1 (-0.4, 0.7)	-0.1 (-0.8, 0.3)	0.6 (0, 2.7)	0.004
Δ TG, mg/dL	-13 (-63, 33)	-1 (-47, 59)	2 (-54, 36)	0.224
Δ HDL-c, mg/dL	3 (-1, 8)	1 (-3, 6)	5 (-1, 11)	0.370
Δ LDL-c, mg/dL	0 (-20, 17)	2.5 (-18, 19)	4 (-7, 26)	0.817
Δ Creatinine, mg/dL	0 (-0.1, 0.1)	0 (0, 0.1)	0 (-0.1, 0)	0.076

Data are reported as medians (25th – 75th percentiles); † Kruskal-Wallis test. HbA1c: Glycosylated hemoglobin; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; Δ: Difference between measurement T3 and measurement T1.

Table 4. Relationship of the SNP rs594709 in the SLC22A1 gene with the baseline levels and with change (Δ) of the biochemical variables in diabetic patients treated with metformin

Variable	Genotypes			*p*†
	AA	AG	GG	
	n=208 (68%)	n=87 (28%)	n=13 (4%)	
HbA1c, %	6.95 (6.2, 8.1)	6.7 (6.4, 8)	6.8 (6.4, 7.1)	0.751
TG, mg/dL	170 (125, 224)	173 (131, 229)	184 (122, 211)	0.902
HDL-c, mg/dL	44 (38, 51)	44 (36, 52)	49 (40, 55)	0.256
LDL-c, mg/dL	142 (122, 163)	136 (118, 157)	153 (142, 165)	0.098
Creatinine, mg/dL	0.6 (0.6, 0.8)	0.7 (0.6, 0.8)	0.6 (0.5, 0.9)	0.589
Δ HbA1c, %	0.1 (-0.4, 0.6)	0 (-0.5, 0.5)	0 (-0.4, 3.5)	0.287
Δ TG, mg/dL	-7 (-58.5, 44.5)	-19 (-54, 24)	15 (-24, 36)	0.339
Δ HDL-c, mg/dL	2 (-2, 7.5)	3 (-1, 9)	-1 (-2, 8)	0.621
Δ LDL-c, mg/dL	1 (-20, 21)	-1 (-15, 12)	3 (-18, 19)	0.587
Δ Creatinine, mg/dL	0 (-0.1, 0.1)	0 (-0.1, 0.1)	0 (-0.1, 0.1)	0.299

Data are reported as medians (25th – 75th percentiles); † Kruskal-Wallis test. HbA1c: Glycosylated hemoglobin; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; Δ: Difference between measurement T3 and measurement T1.

Table 5. Association among the SNP rs622342, rs628031, and rs594709 in the SLC22A1 gene with the increase of HbA1c levels in diabetic patients treated with metformin

Genotypes	Δ HbA1c (%)	β (95% CI) †	p
rs622342			
AA	0.52 ± 0.15	Ref	
AC	0.63 ± 0.12	0.34 (-0.08, 0.75)	0.116
CC	1.61 ± 0.39	1.36 (0.73, 2.00)	<0.001
rs628031			
GG	0.67 ± 0.12	Ref	
GA	0.64 ± 0.17	-0.44 (-0.90, 0.02)	0.060
AA	0.98 ± 0.51	1.0 (0.09, 1.92)	0.032
rs594709			
AA	0.63 ± 0.12	Ref	
AG	0.69 ± 0.18	-0.12 (-0.57, 0.32)	0.581
GG	3.1 ± 0.62	1.21 (0.22, 2.19)	0.016

Data are reported as geometric mean ± standard error. β: regression coefficient; CI: Confidence interval. †Generalized linear models adjusted for gender, age, years of diabetes evolution, and waist circumference.

Table 6. Effect of SLC22A1 gene haplotypes on the increase of HbA1c levels

	SNP				Δ HbA1c, %	
Haplotype	rs622342	rs628031	rs594709	Frequency	β (95% CI)†	*p*
1	A	G	A	0.475	Ref	
2	C	G	A	0.275	0.7 (0.3, 1.0)	< 0.001
3	A	A	G	0.056	0.2 (-0.5, 0.8)	0.630
4	C	A	G	0.053	0.9 (0.2, 1.5)	0.008
5	A	G	G	0.047	-0.1 (-0.8, 0.6)	0.810
6	A	A	A	0.047	0.2 (-0.5, 0.9)	0.610
7	C	G	G	0.028	0.9 (-0.1, 1.8)	0.087
8	C	A	A	0.020	-0.8 (-2.0, 0.3)	0.160

†Generalized linear models adjusted for gender, age, years of T2D evolution, and waist circumference.

β: regression coefficient; CI: Confidence interval.

Table S1. Relationship of the SNP rs316019 in the SLC22A2 gene with the basal levels and with change (Δ) of the biochemical variables in patients with T2D treated with metformin

Variable	Genotypes			*p*†
	GG	GA	AA	
	n=281 (91%)	n=26 (8.6%)	n=1 (0.4%)	
HbA1c, %	7.4 (6.3, 8.1)	7.9 (6, 7.5)	6 (6, 6)	0.269
TG, mg/dL	193 (125, 226)	185 (149, 198)	264 (264, 264)	0.403
HDL-c, mg/dL	45.1 (37, 52)	45.1 (40, 49)	45 (45, 45)	0.968
LDL-c, mg/dL	143 (121, 162)	140 (129, 155)	183 (183, 183)	0.354
Creatinine, mg/dL	0.67 (0.6, 0.8)	0.66 (0.6, 0.8)	0.5 (0.5, 0.5)	0.426
Δ HbA1c, %	0.19 (-0.4, 0.6)	-0.01 (-0.8, 0.2)	0.2 (0.2, 0.2)	0.368
Δ TG, mg/dL	-13.12 (-54, 38)	-26 (-70, 26)	-116 (-116, -116)	0.144
Δ HDL-c, mg/dL	3.18 (-2, 8)	5.76 (-1, 11)	2 (2, 2)	0.668
Δ LDL-c, mg/dL	-1.59 (-18, 18)	2.88 (-15, 24)	-30 (-30, -30)	0.460
Δ Creatinine, mg/dL	0.01 (-0.1, 0.1)	-0.01 (-0.1, 0.1)	0.1 (0.1, 0.1)	0.600

Data are reported as medians (25th – 75th percentiles); † Kruskal-Wallis test. HbA1c: Glycosylated hemoglobin; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; Δ: Difference between measurement T3 and measurement T1.

Table S2. Relationship of the SNP rs2289669 in the SLC47A1 gene with the baseline levels and with change (Δ) of the biochemical variables in patients with T2D treated with metformin

Variable	Genotypes			*p*†
	GG	GA	AA	
	n=78 (25%)	n=150 (49%)	n=80 (26%)	
HbA1c, %	7.3 (6.2, 8.2)	7.5 (6.3, 8)	7.3 (6.3, 8.1)	0.757
HDL-c, mg/dL	45.8 (39, 54)	44.6 (36, 51)	45.3 (38, 51)	0.621
LDL-c, mg/dL	142 (124, 162)	142 (120, 161)	144 (124, 163)	0.771
TG, mg/dL	186.9 (125, 222)	192 (128, 223)	200 (127, 230)	0.628
Creatinine, mg/dL	0.66 (0.6, 0.8)	0.68 (0.6, 0.8)	0.67 (0.5, 0.8)	0.861
Δ HbA1c, %	0.23 (-0.5, 0.4)	0.12 (-0.4, 0.6)	0.21 (-0.5, 0.7)	0.676
Δ HDL-c, mg/dL	4.3 (-1, 10)	3.1 (-2, 7)	2.9 (-3, 6.5)	0.262
Δ LDL-c, mg/dL	-0.89 (-18, 19)	-0.21 (-15, 18)	-3.7 (-21, 17.5)	0.824
Δ TG mg/dL	-15.5 (-70, 33)	-12.3 (-45, 36)	-17.6 (-55.5, 38)	0.475
Δ Creatinine, mg/dL	0.21 (-0.1, 0.1)	0.01 (-0.1, 0.1)	-0.01 (-0.1, 0.1)	0.652

Data are reported as medians (25th – 75th percentiles); † Kruskal-Wallis test. HbA1c: Glycosylated hemoglobin; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; Δ: Difference between measurement T3 and measurement T1.