Can J Diabetes 43 (2019) 128–135

Contents lists available at ScienceDirect

Canadian Journal of Diabetes

journal homepage: www.canadianjournalofdiabetes.com

Original Research

# Evaluation of the rs3088442 G>A SLC22A3 Gene Polymorphism and the Role of microRNA 147 in Groups of Adult Pakistani Populations With Type 2 Diabetes in Response to Metformin

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## Key Messages

- The present investigation demonstrates that the common variant, G>A (rs3088442), is associated with a clinical response to metformin in Pakistani patients who have type 2 diabetes.
- microRNA 147 expression was found to be increased in patients who were taking metformin (responders) compared with the nonresponder group and controls.
- mRNA expression of SLC22A3 was significantly reduced in patients taking metformin as compared to other groups.
- These findings add to the growing body of work on the pharmacogenetic and biologic role of SLC22A3.

ARTICLE INFO

*Article history:*
Received 15 July 2017
Received in revised form
3 July 2018
Accepted 9 July 2018

*Keywords:*
genotyping
metformin
microRNA 147
polymerase chain reaction (PCR)
sulfonylureas
SLC22A3
UTR

ABSTRACT

*Objectives:* Type 2 diabetes is a complex genetic disorder, and a large number of genetic polymorphisms may be involved in its pathogenesis. Pharmacologically, type 2 diabetes can be treated with 9 different approved classes of drugs, but metformin is suggested as the first line of therapy, followed by sulfonylureas.

*Methods:* This was a case-control study consisting of 300 metformin responders and 300 metformin nonresponders in patients with type 2 diabetes and 300 healthy Pakistani subjects. Genotyping of the SLC22A3 G>A polymorphism was performed by allele-specific polymerase chain reaction (PCR) for microRNA 147 expression; real-time polymerase chain reaction was used, and expressional analysis of SLC22A3 was done by semiquantitative polymerase chain reaction.

*Results:* GA and AA genotypes were highly significantly associated with the drug treatments when compared with controls. A statistically significant difference was observed in the distribution of the SLC22A3 A allele between healthy subjects and patients with type 2 diabetes. When odds ratios were adjusted for glycated hemoglobin levels and postprandial and fasting blood glucose levels, our findings showed that the overexpression of allele A of the rs3088442 G>A variant may act as a protective allele and is associated with the clinical response to metformin. microRNA 147 expression was found to be increased in patients who were metformin responders compared with the nonresponder group and controls. mRNA expression of SLC22A3 was significantly reduced in patients taking metformin as compared to other groups.

*Conclusions:* These results suggested that the SLC22A3 rs3088442 at position 2282 A allele may confer metformin clinical responses in patients with type 2 diabetes in the Pakistani population. Overexpression of microRNA 147 is associated with a downward expression of the SLC22A3 gene in patients who have type 2 diabetes.



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https://doi.org/10.1016/j.jcjd.2018.07.001

*Mots clés :*
génotypage
metformine
microARN 147
réaction en chaîne par polymérase (RCP)
sulfonylurées
SLC22A3
UTR (de l'anglais, *untranslated region* pour séquence non traduite)

RÉSUMÉ

*Objectifs :* Le diabète de type 2 est une maladie à prédisposition génétique complexe à laquelle un grand nombre de polymorphismes génétiques peuvent participer à sa pathogenèse. Sur le plan pharmacologique, 9 classes de médicaments approuvés conviennent au traitement du diabète de type 2, mais la metformine est considérée comme le traitement de première intention, et les sulfonylurées, comme le traitement de deuxième intention.
*Méthodes :* Il s'agissait d'une étude cas-témoins qui comptait 300 patients répondeurs à la metformine et 300 patients non répondeurs à la metformine, tous atteints de diabète de type 2, et 300 Pakistanais en santé. Nous avons réalisé le génotypage du polymorphisme G>A du SLC22A3 par réaction en chaîne par polymérase (RCP) spécifique d'allèle pour l'expression du microARN 147. De plus, nous avons utilisé la réaction en chaîne par polymérase en temps réel et nous avons effectué l'analyse de l'expression du SLC22A3 par réaction en chaîne par polymérase semi-quantitative.
*Résultats :* Nous avons noté une association très significative entre les patients porteurs des génotypes GA et AA et les traitements médicamenteux par rapport aux témoins. Nous avons observé une différence significative sur le plan statistique dans la distribution de l'allèle A du SLC22A3 entre les sujets en santé et les patients atteints du diabète de type 2. Lors de l'ajustement des rapports de risque approchés aux concentrations de l'hémoglobine glyquée et aux concentrations de la glycémie après le repas et à jeun, nos résultats ont montré que la surexpression de l'allèle A du variant rs3088442 G>A peut servir d'allèle protecteur et est associée à la réponse clinique à la metformine. Nous avons observé une augmentation de l'expression du microARN 147 chez les patients répondeurs à la metformine par rapport aux patients non répondeurs et aux témoins. Nous avons observé une diminution significative de l'expression du microARN du SLC22A3 chez les patients qui prenaient de la metformine, par rapport aux patients des autres groupes.
*Conclusions :* Ces résultats ont montré que l'allèle A du variant rs3088442 2282 du SLC22A3 peut conférer des réponses cliniques à la metformine chez les patients atteints de diabète de type 2 de la population pakistanaise. La surexpression du microARN 147 est associée à la diminution de l'expression du gène SLC22A3 chez les patients qui sont atteints du diabète de type 2.>END ABSTRACT>



## Introduction

Type 2 diabetes mellitus is characterized by the beta cells of the pancreas in the islets being unable to secrete insulin, or they turn out to be insulin resistant (1). Worldwide, Pakistan stands 6th in the incidence of type 2 diabetes. In year 2000, the prevalence of type 2 diabetes in Pakistan was estimated to be 5.2 million, and it was expected to rise to 13.9 million by the year 2030 (2). Type 2 diabetes is a metabolic disease that is a tremendously heterogeneous, multifactorial disorder. Both environmental and genetic factors contribute to disease pathogenesis (3).

Metformin, a biguanide agent, is considered to be the first-line therapy for the treatment of type 2 diabetes. It has the ability to decrease the levels of both basal and postprandial plasma glucose (4,5). The classical functional phenomenon of metformin is to reduce the hepatic energy by inhibiting the respiratory chain complex 1 of mitochondria. As a result, it activates adenosine 5'monophosphate protein kinase. Basically, it is a cellular metabolic sensor, thus reducing the glucose level in the blood. It can be used as a monotherapy or in a group with any other antidiabetes agents, including insulin, dipeptidyl peptidase 4 inhibitors, sulfonylureas, thiazolidinediones and alpha-glucosidase inhibitors as well as glucagon-like peptide-1 receptor agonists (6). Another commonly used class of antidiabetes agents is sulfonylureas. Approximately 80% to 90% of patients respond very well to such drugs, but 10% to 20% of treated patients fail to achieve enough glycemic control, even with the use of high dosages. About 5% to 10% of patients who respond very well to sulfonylureas in the beginning lose the capability of maintaining adequate control of glucose levels in the body with the passage of time (7). Malfunction of sulfonylurea therapy in patients might be associated with a number of factors, but the most predominant factor is the failure of beta-cell function (8).

The SLC22A3 gene encodes a 62-kilodalton transmembrane protein known as the organic cation transporter 3. It is essential to the excretion and elimination of various drugs and bioamines, such as histamine, metformin and dopamine (9). In recent times, growing evidence has demonstrated that SLC22A3 plays a crucial role in the pathogenesis of type 2 diabetes. Previous studies have demonstrated that SLC22A3 plays an important role in the clinical response to metformin. The presence of genetic variants within this transporter was linked with several variations in the pharmacokinetics of metformin and sulfonylureas that hinder the antidiabetes activity of the drug (10–12). Studies have been conducted concerning the role of transporter SLC22A3 and the clinical activities of metformin and sulfonylureas (13), and genetic variants of this gene within the coding region and in the vicinity of the promoter region have been linked significantly with type 2 diabetes (14). Expression of SLC22A3 is present in many tissues, including the brain, skeleton, muscle, heart and placenta. Numerous genome-wide association studies have associated SLC22A3 with a risk for loci of prostate cancer and coronary artery disease (15,16).

MicroRNAs are noncoding, small RNAs that regulate the expression of genes and various biologic processes, such as cell cycle, development, differentiation and metabolism (17–19). Therefore, they have been involved in multiple diseases, including cancer, diabetes and immune or neurodegenerative disorders (20,21). They contribute to the expressional regulation of various target genes by binding to the region located in 3'UTR. About 30% of human genes could be regulated by several microRNAs, as reported by earlier studies. Thus, single nucleotide polymorphisms (SNPs) that are present in close vicinity to the microRNA target sites often influence the role of microRNAs. As we know, most of the microRNAs downregulate gene expression; thus, they are becoming 1 of the promising therapeutic agents used to fight cancer and diseases in which protein is upregulated (13,22,23).

So far, limited studies have been conducted concerning the relationship between SLC22A3 and metformin response. Thus, similar studies of variants of SLC22A3 must be conducted in a variety of ethnic populations. Hence, this study was designed to find the association between the rs3088442 G>A polymorphism and metformin response and also the distribution of genotypes of this variant in

Pakistani patients with type 2 diabetes. Further, we analyzed the expression of microRNA 147 and mRNA expression of SLC22A3.

## Methods

### *Blood collection*

Blood samples of 600 unrelated patients with type 2 diabetes and 300 (ethnically and age-matched) healthy individuals were recruited into this study with their consent. They were recruited from different hospitals and clinics of Islamabad and Rawalpindi, Pakistan. Cases were divided into 2 groups: metformin responders (n=300) and metformin nonresponders (taking sulfonylureas in addition to metformin) (n=300). Metformin responders were patients with type 2 diabetes who had been taking metformin for 6 months and were responding to it. Metformin nonresponders were patients with type 2 diabetes who were not responding to metformin in monotherapy, such that sulfonylureas were added to their treatment after 6 months. All the subjects (patients and controls) were of Pakistani origin.

### *Exclusion and inclusion criteria*

Patients who failed to meet the criteria of the drugs were excluded from the study. Pregnant women and patients with type 1 diabetes, maturity-onset diabetes of the young and gestational diabetes were excluded as well. Written informed consent was given by all the individuals before the study was initiated. The research work followed the guidelines of the declaration of Helsinki and was formally approved by the ethics committee of the International Islamic University Pakistan.

Detailed clinical data were collected from all patients with type 2 diabetes and from healthy individuals during blood sampling, including random glucose, fasting blood glucose and glycated hemoglobin (A1C) levels, systolic blood pressure, diastolic blood pressure and total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride levels (Supplementary Table S1).

### *DNA extraction and genotyping*

Genomic DNA was isolated from white blood cells using the phenol chloroform method. The concentration of DNA was confirmed by using NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, United States). Genotype analysis was performed by allele-specific polymerase chain reaction (PCR). The primer sequence for the G>Ars3088442 SNP was 5'-TTCTCTGCTCACCTTGGTTTCCG-3' (forward primer wild), 5'-TTCTCTGCT CACCTTGGTTTCCA-3' (forward primer mutant) and AACATGTAGGCTTCTCCCCAAGACA (reverse primer). The PCR product was electrophoresed in 2% agarose gel and visualized using the gel documentation system.

### *In silico analysis of microRNAs targeting SLC22A3 gene 3'UTR*

*The SNP info web server* (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) was used to find the candidate microRNA that targets the SLC22A3 3'UTR region. On the basis of pairing between seed regions and binding calculation affinity, we chose microRNA 147 (GUGUGUGGAAAUGCUUCUGC) as a promising candidate for further testing of microRNA.

### *microRNA isolation and real time PCR of microRNA 147*

microRNAs were isolated from blood by using a mirVana microRNA isolation kit (Ambion, Foster City, California, United States, #AM1560) according to the manufacturer's procedure. A TaqMan microRNA reverse transcription kit (Applied Biosystems, Foster City, California, United States, #4366596) was used for the synthesis of cDNA from microRNAs that were extracted from blood by using specific primers present in TaqMan microRNA assay (Applied Biosystems, #4366596). microRNA147 quantitative real-time PCR was performed in duplicate and for normalization of the data; let-7a, a microRNA precursor, was used as an internal control.

### *RNA isolation, cDNA synthesis and semiquantitative PCR*

Total RNA from blood samples were extracted by using the TRIzol reagent (Ambion). Concentration of RNA was confirmed by using NanoDrop (Thermo Fisher Scientific). The extracted RNA was first reverse-transcribed to synthesize cDNA using the SuperScript First-Strand Synthesis System for RT-PCR (Thermo Fisher Scientific, #11904-018). In the second step, this cDNA was amplified using gene-specific primers (SLC22A3) followed by the expressional analysis of the respective gene in controls and case samples. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Amplified PCR products were fractionated onto a 2% agarose gel. Data obtained were analyzed by using ImageJ software (National Institutes of Health, Bethesda, Maryland, United States). Sequences of primers were for SLC22A3: Forward 5'-ATCGTCAGCGAGTTTGACCT-3', Reverse 5'-TTGAATCACGATTCCCACAA-3' (324 bp fragment); and for GAPDH: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3' (226 bp fragment).

### *Statistical analysis*

Statistical analysis was performed using Statistical Practices for Social Sciences (SPSS v. 18.0, IBM, Armonk, New York, United States). Median ranges were used to describe the central tendency and variability of continuous variables, while frequencies were used to describe the distribution of categorical variables. Mean values were compared using the t test. The Fisher exact test or the nonparametric Mann-Whitney test was used to compare clinical characteristics among the various patient groups. The chi-square test was used to assess the deviation from Hardy-Weinberg equilibrium. Odds ratios were calculated assuming the A allele to be dominant in the variant studied. According to quasicomplete separation of data, logistic regression was used to calculate the odds ratios for the interaction effects. The level of statistical significance was set at $p<0.05$. For the calculation of the relative expression of microRNA 147 among groups, the comparative threshold cycle method was used. Statistical analysis was conducted by using the nonparametric 2-tailed Mann-Whitney U test; $p<0.05$ was considered statistically significant.

The Institute Ethics Committee of the International Islamic University and the Pakistan Institute of Medical Sciences hospital approved the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

## Results

### *Demographic and clinical features of subjects*

A total of 600 unrelated patients with type 2 diabetes (300 patients with type 2 diabetes who were taking metformin and 300 who were taking metformin plus sulfonylureas) were age-matched with 300 healthy individuals without histories of type 2 diabetes or cardiovascular disease. The clinical characteristics of

**Table 1**
Genotypic and allelic frequencies of SLC22A3 G>A in patients with type 2 diabetes (metformin responders and metformin-plus nonresponders) and in healthy individuals

| Genotypes (n=300) | Metformin (responders) (n=300) | Metformin nonresponders (n=300) | Healthy individuals (n=300) | Metformin responders vs. metformin nonresponders OR; CI; p value | Metformin responders vs. healthy individuals OR; CI; p value | Metformin nonresponders vs. healthy individuals OR; CI; p value |
|---|---|---|---|---|---|---|
| GG | 36 (12.0%) | 26 (9.0%) | 114 (38.0%) | 1.12; 0.6–1.9 p>0.05 | 2.7; 2.19–3.52 **p<0.05** | 0.2; 0.1–0.3 **p<0.05** |
| GA | 106 (35.0%) | 170 (57.0%) | 98 (33.0%) | 0.56; 0.4–0.8 **p<0.05** | 1.5; 1.1–2.1; p>0.05 | 2.9; 2.1–4.1 **p<0.05** |
| AA | 158 (53.0%) | 104 (34.0%) | 88 (29.0%) | 1.8; 1.2–2.5 **p<0.05** | 2.1; 1.5–2.9 **p<0.05** | 1.2; 0.9–1.7 **p<0.05** |
| Alleles (n=600) | | | | | | |
| G | 178 (30.0%) | 222 (37%) | 326 (54.0%) | 1.5; 0.9–2.1 | 2.8; 2.16–3.32 | 2.04(1.62–2.5) |
| A | 422 (70.0%) | 378 (63%) | 274 (46.0%) | **p=0.0005** | **p=0.00001** | **p=0.00001** |

*Note:* p<0.05 (in boldface) is considered a positive association.

controls and patients with type 2 diabetes are presented in Supplementary Table S1. No statistically significant difference was observed between patients with type 2 diabetes and controls with respect to gender, age or height. Both patient groups with type 2 diabetes showed higher levels of A1C, fasting blood glucose, postprandial blood glucose, total cholesterol, low-density lipoprotein and triglycerides when compared to control individuals. No significant differences were observed between the group with type 2 diabetes treated with metformin and those treated with sulfonylureas with respect to weight, high-density lipoprotein levels or systolic and diastolic blood pressure; however, a significant association was observed when these factors were compared with those of healthy individuals.

*Analysis of genotypic and allelic frequencies*

The genotype and allele distribution of the study variant are presented in Table 1. There was no deviation from the Hardy-Weinberg equilibrium for the rs3088442 G>A variant. As presented in Table 1, allele A-containing genotypes of the rs2292334 G>A variant were over-represented in the patients with type 2 diabetes who were taking metformin (metformin responders) in comparison to the patients with type 2 diabetes who were taking sulfonylureas (metformin nonresponders) and the control individuals, resulting in a higher frequency of the minor allele A in the patients with type 2 diabetes who were taking metformin than in the other 2 groups.

The frequency of the homozygous GG genotype was 36/300 (12.0%) in the metformin-responder group, 26/300 (9.0%) in the nonresponder group and 114/300 (38.0%) in healthy individuals. The homozygous GG genotype was found to be statistically significant when both groups were compared with healthy individuals (OR 2.7, CI 2.19 to 3.52; p<0.05; and OR 0.2, CI 0.1 to 0.3, p<0.05), whereas no significant association was found when both treated groups were compared (OR 1.12, CI 0.6 to 1.9; p>0.05). The frequency of the heterozygous GA genotype was 106/300 (35.0%) in the metformin responders group, 170/300 (57.0%) in the nonresponders group and 98/300 (33.0%) in healthy individuals. The heterozygous GA genotype was found to be statistically significant when the responders were compared with the nonresponders (OR 0.56, CI 0.4 to 0.8; p<0.05) and when nonresponders were compared with healthy individuals (OR 2.9, CI 2.1 to 4.1; p<0.05). No significant association was found when the responders were compared with healthy individuals (OR 1.5, CI 1.1 to 2.1; p>0.05). The frequency of the homozygous AA genotype 158/300 (53.0%) was significantly higher in responders and nonresponders: 104/300 (34.0%) compared with controls 88/300 (29.0%). From the analysis of allele frequency, we found that minor allele A frequency was significantly higher in patients responding to metformin than the major allele G frequency in relation to the other groups (Table 1).

Changes in the study variables after 6 months of metformin monotherapy in responders and metformin plus sulfonylureas combined therapy in metformin nonresponders, according to the 2 genotype groups (GG homozygotes and A carriers) of SLC22A3 G>A, are presented in Supplementary Tables S2 and S3. In the present study, we further analyzed the strength of the relationship between the G>A variant, with type 2 diabetes stratified by the 2 genotype groups (GG homozygotes and A carriers) according to different clinicopathologic parameters, including lipid profiling and A1C levels (Supplementary Table S4). When we analyzed the study parameters with respect to metformin response according to SLC22A3 G>A genotypes, our results showed a statistically significant difference between responders and nonresponders with respect to the majority of the parameters of this study. As shown in Supplementary Table S2, most of these parameters, including weight, fasting blood glucose, postprandial blood glucose, A1C and total cholesterol levels, were significantly reduced after 6 months of treatment by monotherapy in responders with allele A. The average change in A1C (in %), postprandial blood glucose and fasting blood glucose levels per SLC22A3 genotype is presented in Supplementary Table S5. Lipid profiling is presented in Supplementary Table S6.

*In silico analysis of microRNAs targeting SLC22A3 gene 3'UTR*

In silico analysis suggested that the G>A polymorphism of SNP rs3088442 formed a putative binding site in 3'UTR for microRNA 147 in the mRNA of the SLC22A3 gene. Therefore, overexpression of microRNA 147 might downregulate the expression of SLC22A3 (Figure 1).

*Expression of microRNA 147 by real-time PCR*

By using quantitative real-time PCR, we analyzed the expression of microRNA 147 in the 2 groups with diabetes (metformin responders and metformin nonresponders) and in the 1 control group. The expression of microRNA 147 in treated groups was evaluated relative to controls and was calculated as 1. We found significantly increased (p<0.05) expression of microRNA 147 in both treated groups—those taking metformin (a 76.98±10.36-fold increase) and those taking metformin along with sulfonylureas (a 29.61±11.54-fold increase) as compared to healthy individuals (1.00±0.62) (Figure 2A). Next, we compared the group taking metformin with the group taking metformin plus sulfonylureas. A significant increase

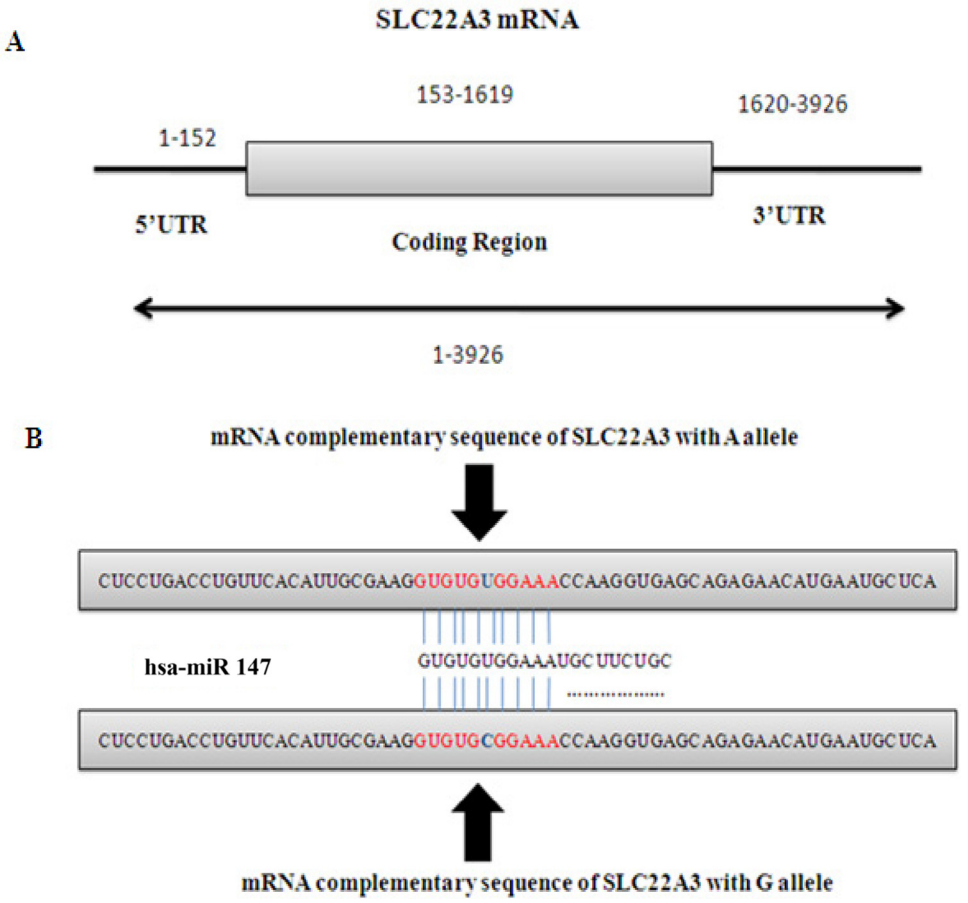


**Figure 1.** microRNA 147 targets 3'UTR of the *SLC22A3* gene. A, Diagrammatic representation of the *SLC22A3* gene and sequence alignments among *SLC22A3* 3'UTR and hsa-miR-147. B, In silico prediction of *SLC22A3* mRNA and microRNA 147 interactions shows changes in binding in the seed region. The sequence in black shows the mRNA region of *SLC22A3*. The sequence in red indicates the binding site. Nucleotide, in blue, indicates the polymorphism site. *hsa,* Homo sapiens. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

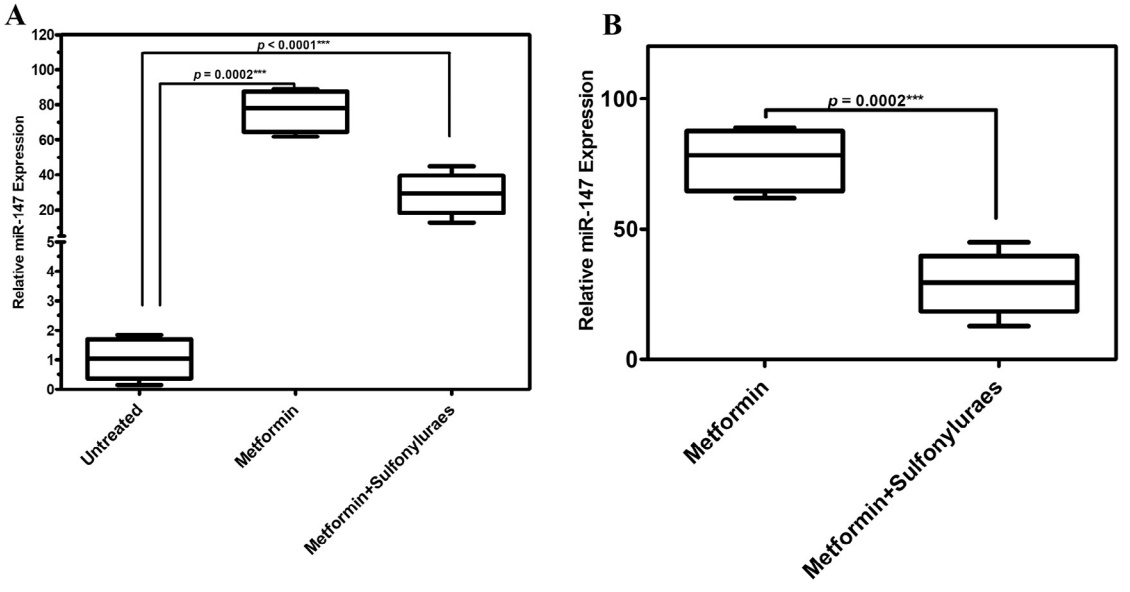


**Figure 2.** Relative expression of microRNA 147 as determined by TaqMan-based assay. A, Real-time quantitative polymerase chain reaction study of microRNA 147 (miR 147) in peripheral blood cells from patients with type 2 diabetes and healthy individuals. B, Real-time quantitative polymerase chain reaction study of microRNA 147 in peripheral blood cells from patients with type 2 diabetes taking metformin and patients with type 2 diabetes taking metformin plus sulfonylureas. Data are presented as mean ± SEM. Statistical analysis was conducted using the nonparametric 2-tailed Mann-Whitney U test. $p<0.05$ was considered statistically significant.

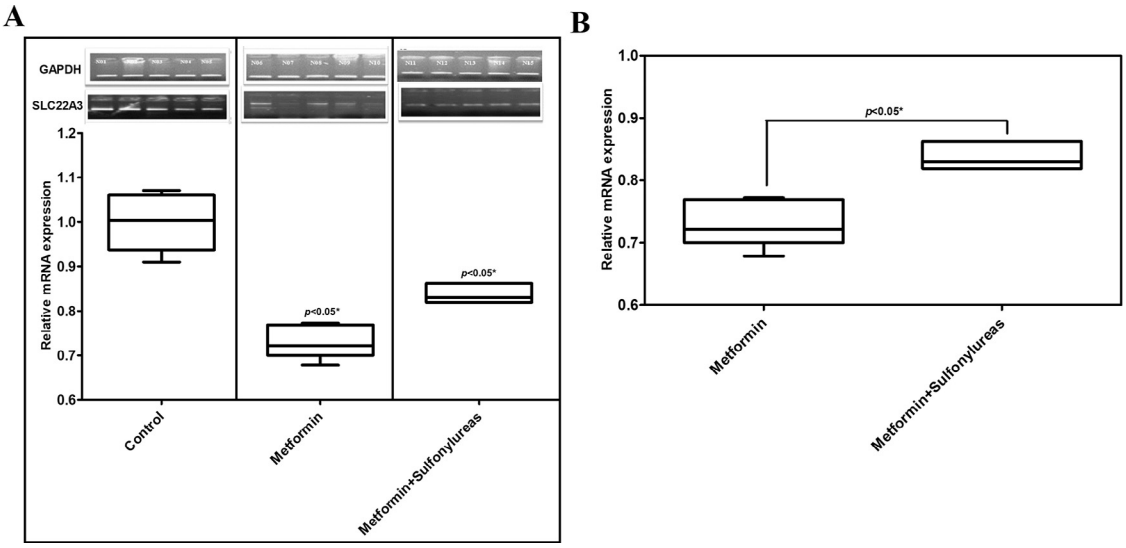


**Figure 3.** mRNA expression of the SLC22A3 gene was determined by semiquantitative polymerase chain reaction (PCR). A, Semiquantitative PCR analysis of SLC22A3 in peripheral blood cells from patients with type 2 diabetes and healthy individuals were analyzed by ImageJ software. B, Semiquantitative PCR analysis of the SLC22A3 gene in peripheral blood cells from patients with type 2 diabetes taking metformin and patients taking metformin plus sulfonylureas. Data are presented as mean ± SEM. Statistical analysis was conducted using the nonparametric 2-tailed Mann-Whitney U test. p<0.05 was considered statistically significant.

(p<0.05) in microRNA 147 expression in the group taking metformin as compared to the group taking metformin plus sulfonylureas was discerned (Figure 2B).

*Expression analysis of potential target gene (SLC22A3)*

Semiquantitative PCR was used to assess expression levels of the SLC22A3 gene between controls and patients with diabetes treated with 2 different drugs. Expression of GAPDH was observed to be uniform in all samples of controls and those with diabetes, whereas expression of SLC22A3 varied (Figure 3). In the majority of samples, SLC22A3 mRNA levels appeared to be reduced in the patients with diabetes who were taking metformin as compared to controls (p<0.05) and to patients who were taking metformin plus sulfonylureas (p<0.05). *SLC22A3* was extremely downregulated in patients who were taking metformin alone as compared to the patients who were using sulfonylureas as well (p<0.05). We found significant downregulation (p<0.05) of SLC22A3 in treated groups taking metformin (0.73±0.04) and metformin plus sulfonylureas (0.84±0.02) as compared to healthy individuals (Figure 3A). Next, we compared patients with type 2 diabetes taking metformin with the patients taking metformin plus sulfonylureas and found a significant decrease in expression of SLC22A3 in the metformin group, as shown in Figure 3B.

## Discussion

An SNP (rs3088442) from the 3'UTR region of the SLC22A3 gene was analyzed for an association with patients treated with 2 drugs in a case-control sample of 900 Pakistanis. The advancements in genetics have led to the recognition of numerous risk loci and variants, not only for monogenetic disorders but also for other complex diseases. However, because of their complex etiology, the multifactorial traits have not only ethnicity-specific variations in genetic predisposition but might also share most, if not all, of the susceptibility loci.

In the present study, we have presented a novel mechanism conducted by the rs3088442 G>A polymorphism of SLC22A3, which may possibly involve a clinical response to metformin in patients with type 2 diabetes. Here, we had numerous lines of indications to support this hypothesis. In this study, we have provided the first evidence that the minor allele A of rs3088442 G>A, as a polymorphic variant in the SLC22A3 gene, has a protective effect in the susceptibility to type 2 diabetes in the Pakistani population. First, we found that the minor allele A of SNP rs3088442 might be associated with the clinical response to metformin in patients with type 2 diabetes. Second, our results suggested that G>A conversion creates a binding site for microRNA 147. Third, upregulation of microRNA 147 leads to downregulation of SLC22A3. Taken collectively, this may suggest that overexpression of microRNA 147 leads to the inhibition of SLC22A3 mRNA expression, thus, eventually, leading to a positive clinical response to metformin in patients with type 2 diabetes.

Increasingly, evidence has recognized organic cation transporter 3 as the important antidiabetes and anticancer drug transporter (9). The outcomes of the present study have several essential impacts on the function of SLC22A3 in clinical responses to the antidiabetes drug metformin. It is 1 of the most widely used drugs for the treatment of type 2 diabetes (13). In the Pakistani population, the association of the SLC22A3 G>A polymorphism with type 2 diabetes has not been analyzed before. Therefore, we hypothesized that the G>A polymorphism of SLC22A3 might have a functionally important role in the clinical response to metformin in Pakistani patients with type 2 diabetes.

According to our results, rs3088442 G>A was generally associated with a decreased risk for type 2 diabetes. A considerably higher frequency of homozygous genotype (AA) was observed in patients who were taking metformin, which demonstrated that the AA genotype may play a crucial role in the clinical response to metformin in patients with type 2 diabetes. Moreover, we also observed that the minor allele A has a remarkably higher allele frequency in patients following the same lifestyles and associations. From these results, it was demonstrated that minor allele A might be a protective allele, whereas major allele G may have a negative influence on the clinical response to metformin in Pakistani patients with type 2 diabetes. It can be suggested that populations with the genotype AA for this SLC22A3 polymorphism are more likely to have good clinical responses to metformin than those with the GG genotype.

In the present study, we compared the frequency of G and A alleles in rs308844 G>A variant. The frequency of the minor allele A was 49% in a healthy Japanese population (24) and 47% in healthy Caucasians in the United States (25). Accordingly, it appears that the frequency of this allele in our healthy individuals (46.0%) is lower than that of these 2 populations, which suggests that the healthy Pakistani population may be more prone to develop type 2 diabetes.

Here, we have provided the very first evidence that the major allele of rs3088442 G>A as a polymorphic variant in the SLC22A3 gene has a protective effect on susceptibility to type 2 diabetes in the Pakistani population. When further analysis was performed via a logistic regression model in which the odds ratios were adjusted for A1C, postprandial and fasting blood glucose levels, along with lipid profiling, it was revealed that the rs3088442 G>A variant has a highly significant correlation with a decreased risk for type 2 diabetes.

microRNAs belong to a class of small, 22-nucleotide endogenous noncoding RNAs that contribute to the expression regulation of several target genes by binding to the region of 3'UTR. Our in silico analysis showed that microRNA 147 acts as a potential negative regulator of SLC22A3, based on its ability to bind to the 3'UTR of SLC22A3 mRNA. To our knowledge, this is the first study analyzing the expression profiles of SLC22A3 in patients with type 2 diabetes with respect to microRNA 147 in the Pakistani population. Here, we have presented a high-affinity binding of microRNA 147 to the minor allele A of SNP rs3088442 (Figure 1). Consistent with the study of Nies et al, the SLC22A3 G>A rs3088442 polymorphism was associated with the downregulation of SLC22A3 mRNA expression in hepatocytes (13). Our study demonstrated that the SNP rs3088442 recruited microRNA 147 to inhibit SLC22A3 mRNA expression. Moreover, the case-control study indicated that the minor allele A of rs3088442 was associated with a decreased expression of SLC22A3 in patients. We showed a significant downregulation of SLC22A3 mRNA expression in patients who were taking metformin as compared to patients who were taking sulfonylureas. Our results demonstrated a possible mechanism by which microRNA 147 is involved in the clinical response to metformin in SLC22A3.

Our hypothesis was in accordance with the findings of Li et al, who suggested that a G>A substitution in rs3088442 creates a binding site for microRNA 147 in the mRNA of the gene SLC22A3, and that eventually decreases its expression. As a result, it contributed to the protective mechanism against inflammatory responses and decreased risk for coronary heart disease (26). This substitution, whose mechanisms may affect susceptibility to type 2 diabetes, has not been fully elucidated but may include the following factors.

First, gene silencing of SLC22A3 may cause the inhibition of the production of proinflammatory mediators such as interleukin-6 (26). Associations between insulin resistance and cytokines such as interleukin-6 and the development of type 2 diabetes have been confirmed in several studies (27,28). Investigations have also suggested that proinflammatory cytokines play a crucial role in the pathophysiology of diabetic nephropathy and diabetic retinopathy (29,30). Furthermore, it has been proposed that interleukin-6 affects the homeostasis and metabolism of glucose in skeletal muscle cells, adipocytes, pancreatic cells, hepatocytes and neuroendocrine cells (31). Second, in the individuals with the AA genotype of the rs3088442 G>A polymorphism, adhesion of peripheral blood mononuclear cells decreases significantly compared with individuals with the genotype GG. Third, the SLC22A3 gene silencing may cause impairment of the leukocyte-endothelial interaction (26). Several studies have suggested that leukocyte-endothelial interactions increase in animal models of diabetes mellitus (31). Fourth, gene silencing of SLC22A3 may cause the suppression of monocyte infiltration (26). It has been demonstrated that obesity is associated with macrophage infiltration and activation, so if infiltration develops, it will lead to insulin resistance and, eventually, type 2 diabetes will be developed (32,33). Our findings provide evidence for the protective role of the variant rs3088442 G>A in susceptibility to type 2 diabetes in the Pakistani population.

## Conclusions

Collectively, our study demonstrated that SLC22A3 is an essential factor in the clinical response to metformin. In summary, the present investigation demonstrates that the common variant G>A (rs3088442) is associated with better clinical response to metformin in Pakistani patients with type 2 diabetes. These findings add to the growing body of work concerning the pharmacogenetic and biologic role of SLC22A3.

## Supplementary Material

To access the supplementary material accompanying this article, visit the online version of *Canadian Journal of Diabetes* at https://www.canadianjournalofdiabetes.com.

## Acknowledgments

We are grateful to the patients and their families for their participation in this study. The authors thank the Department of Medicine, Pakistan Institute of Medical Sciences, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad**,** Pakistan, for providing samples of type 2 diabetes; International Islamic University, Islamabad, Pakistan; and the Institute of Biomedical and Genetic Engineering, G9/1, Islamabad, Pakistan, for providing laboratory facilities.

## Funding

Present study was funded by International Islamic University.

## Author Disclosures

Conflicts of interest: None.

## Author Contributions

SM contributed to sample collection, DNA and RNA extraction, primer designing, genotyping, cDNA synthesis, analysis and interpretation of PCR data, statistical analysis, preparation of graphs and figures and revision of the manuscript; SKR contributed to the analysis and interpretation of qPCR data, statistical analysis, preparation of graphs and figures and revision of the manuscript; NM contributed to analysis and interpretation of the PCR data, statistical analysis, preparation of graphs and figures and revision of the manuscript; NK contributed to bioinformatics analysis and interpretation of PCR data and revision of the manuscript; MAA and RN

helped in designing sample collection; SK contributed to analysis and interpretation of PCR data, statistical analysis, preparation of graphs and figures and revision of the manuscript.

## References


1. Stumvoll M, Goldstein BJ, Van Haeften TW. Type 2 diabetes: Principles of pathogenesis and therapy. Lancet 2005;365:1333–6.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047–53.
3. Brunetti A, Chiefari E, Foti D. Recent advances in the molecular genetics of type 2 diabetes mellitus. World J Diabetes 2014;5:128–40.
4. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: An overview. Clin Sci 2012;122:253–70.
5. Scarpello JH, Howlett HC. Metformin therapy and clinical uses. Diab Vasc Dis Res 2008;5:157–67.
6. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: Pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012;22:820–7.
7. Topic E. The role of pharmacogenetics in the treatment of diabetes mellitus. J Med Biochem 2014;33:58–70.
8. Holstein A, Hahn M, Stumvoll M, Kovacs P. The E23K variant of KCNJ11 and the risk for severe sulfonylurea-induced hypoglycemia in patients with type 2 diabetes. Horm Metab Res 2009;41:387–90.
9. Chen L, Pawlikowski B, Schlessinger A, et al. Role of organic cation transporter 3 (SLC22A3) and its missense variants in the pharmacologic action of metformin. Pharmacogenet Genomics 2010;20:687–99.
10. Kimura N, Masuda S, Tanihara Y, et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. Drug Metab Pharmacokinet 2005;20:379–86.
11. 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;117:1422–31.
12. Becker ML, Visser LE, Van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: A preliminary study. Diabetes 2009;58:745–9.
13. Nies AT, Koepsell H, Winter S, et al. Expression of organic cation transporters OCT1 (SLC22A1) and OCt3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. Hepatology 2009;50:1227–40.
14. Lazar A, Walitza S, Jetter A, et al. Novel mutations of the extraneuronal monoamine transporter gene in children and adolescents with obsessive-compulsive disorder. Int J Neuropsychopharmacol 2008;11:35–48.
15. Tregouet DA, Konig IR, Erdmann J, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nat Genet 2009;41:283–5.
16. Tomlins SA, Mehra R, Rhodes DR, et al. Integrative molecular concept modeling of prostate cancer progression. Nat Genet 2007;39:41–51.
17. Ramirez CM, Rotllan N, Vlassov AV, et al. Control of cholesterol metabolism and plasma HDL levels by miRNA-144. Circ Res 2013;112:1592–601.
18. Liu G, Friggeri A, Yang Y, Park YJ, Tsuruta Y, Abraham E. miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. Proc Natl Acad Sci USA 2009;106:15819–24.
19. Wang L, Jia XJ, Jiang HJ, et al. MicroRNAs 185, 96, and 223 repress selective high-density lipoprotein cholesterol uptake through posttranscriptional inhibition. Mol Cell Biol 2013;33:1956–64.
20. Li P, Liu Y, Yi B, et al. MicroRNA-638 is highly expressed in human vascular smooth muscle cells and inhibits PDGF-BB-induced cell proliferation and migration through targeting orphan nuclear receptor NOR1. Cardiovasc Res 2013;99:185–93.
21. Martin MM, Buckenberger JA, Jiang J, et al. The human angiotensin II type 1 receptor: 1166 A/C polymorphism attenuates microRNA-155 binding. J Biol Chem 2007;282:24262–9.
22. Hoekstra M, Van der Lans CA, Halvorsen B, et al. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochem Biophys Res Commun 2010;394:792–7.
23. Wu C, Gong Y, Sun A, et al. The human MTHFR rs4846049 polymorphism increases coronary heart disease risk through modifying miRNA binding. Nutr Metab Cardiovasc Dis 2013;23:693–8.
24. Aoyama N, Takahashi N, Kitaichi K, et al. Association between gene polymorphisms of SLC22A3 and methamphetamine use disorder. Alcohol Clin Exp Res 2006;30:1644–9.
25. Hengen N, Lizer MH, Kidd RS. Evaluation of genetic variations in organic cationic transporter 3 in depressed and nondepressed subjects. ISRN Pharmacol 2011;2011:161740.
26. Li L, He M, Zhou L, et al. A solute carrier family 22 member 3 variant rs3088442 G/A associated with coronary heart disease inhibits lipopolysaccharide-induced inflammatory response. J Biol Chem 2015;290:5328–40.
27. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004;27:813–28.
28. Jagannathan-Bogdan M, McDonnell ME, Shin H, et al. Elevated proinflammatory cytokine production by a skewed T cell compartment requires monocytes and promotes inflammation in type 2 diabetes. J Immunol 2011;186:1162–72.
29. Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol 2011;7:327–40.
30. Krady JK, Basu A, Allen CM, et al. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. Diabetes 2005;54:1559–65.
31. Kristiansen OP, Mandrup-Poulsen T. Interleukin-6 and diabetes: The good, the bad, or the indifferent? Diabetes 2005;54(Suppl. 2):S114–24.
32. Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. Diabetes 2003;52:1256–64.
33. Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? Diabetologia 2005;48:1038–50.

## Supplementary Material

**Table S1**
Clinical features of healthy individuals and patients with type 2 diabetes included in the single nucleotide polymorphism association analysis

| Parameters | Healthy Individuals n=300 | Metformin-treated group (responders) n=300 | Metformin + sulfonylureas group (nonresponders) n=300 | p value | | |
|---|---|---|---|---|---|---|
| | | | | Metformin responders vs. metformin nonresponders | Metformin responders vs. healthy individuals | Metformin nonresponders vs. healthy individuals |
| Gender | | | | | | |
| Male (%) | 51 | 43 | 54 | >0.05 | >0.05 | >0.05 |
| Female (%) | 49 | 57 | 46 | | | |
| Age (years) (mean ± SD) | 45.78±12.60 | 46.995±12.60 | 47.09±12.39 | >0.05 | >0.05 | >0.05 |
| Weight (kg) (mean ± SD) | 70.5±11.2 | 78.21±11.14 | 78.68±12.61 | >0.05 | **<0.05** | **<0.05** |
| Height (feet) (mean ± SD) | 5.67±0.498 | 5.67±0.311 | 5.65±0.47 | >0.05 | >0.05 | >0.05 |
| Systolic BP (mmHg) (mean ± SD) | 122.67±9.190 | 135.002±10.84 | 131.7±10.65 | >0.05 | **<0.05** | **<0.05** |
| Diastolic BP (mmHg) (mean ± SD) | 80.25±0.377 | 87.42±4.405 | 84.64±4.388 | >0.05 | **<0.05** | **<0.05** |
| Fasting blood sugar (mg/dL) (mean ± SD) | 97.40±0.46 | 153.1±22.6 | 148.1.015±21.0 | **<0.05** | **<0.05** | **<0.05** |
| Postprandial blood glucose (mg/dL) (mean ± SD) | 140.78±1.182 | 237.77.4±31.0 | 211.6±27.0.13 | **<0.05** | **<0.05** | **<0.05** |
| A1C (%) (mean ± SD) | 6.89±0.41 | 8.9±3.17 | 7.7±2.18 | **<0.05** | **<0.05** | **<0.05** |
| Total cholesterol (mg/dL) (mean ± SD) | 176.20±1.77 | 213.365±36.71 | 187.225±25.62 | **<0.05** | **<0.05** | **<0.05** |
| LDL (mg/dL) (mean ± SD) | 90.0±0.68 | 143.8±0.60 | 120.66±1.14 | **<0.05** | **<0.05** | **<0.05** |
| HDL (mg/dL) (mean ± SD) | 56.30±0.68 | 43.54±0.4 | 43.58±0.6 | >0.05 | **<0.05** | **<0.05** |
| TAG (mg/dL) (mean ± SD) | 139.11±1.03 | 167.08±1.38 | 154.62±2.41 | **<0.05** | **<0.05** | **<0.05** |

*Note:* p<0.05 (in boldface) is considered a positive association.
*A1C*, glycated hemoglobin; *BP*, blood pressure); *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *TAG*, triglycerides.

**Table S2**
Change in the study variables after 6 months of metformin in metformin responders according to the genotypes (GG homozygotes and A carriers) of SLC22A3 G>A

| Parameters | GG | | | GA+AA | | |
|---|---|---|---|---|---|---|
| | Baseline median (25%–75%) | After 6 months median (25%–75%) | p value | Baseline median (25%–75%) | After 6 months median (25%–75%) | p value |
| Age (years) | 48 (40–60) | 47 (40–60) | >0.05 | 48 (40–60) | 47(41–60) | >0.05 |
| Weight (kg) | 77 (62–80) | 76 (62–80) | >0.05 | 78 (57–70) | 62 (56–71) | **<0.05** |
| Systolic BP (mmHg) | 135 (130–145) | 135 (130–145) | >0.05 | 137 (121–140) | 135 (120–140) | >0.05 |
| Diastolic BP (mmHg) | 89 (82–90) | 88 (82–90) | >0.05 | 89 (82–85) | 84 (80–85) | **<0.05** |
| Fasting blood glucose (mg/dL) | 155 (140–152) | 153 (139–150) | **<0.05** | 148 (140–150) | 144 (130–140) | **<0.05** |
| Postprandial blood glucose (mg/dL) | 231 (210–246) | 230 (210–245) | >0.05 | 193 (180–215) | 189 (178–210) | **<0.05** |
| A1C (%) | 9.6 (8.5–9.5) | 9.5 (8–9.5) | >0.05 | 9.6 (8.5–9.4) | 8 (7.5–8) | **<0.05** |
| Total cholesterol (mg/dL) | 243 (190–243) | 242 (189–241) | **<0.05** | 226 (161–242) | 221(158–240) | **<0.05** |
| HDL (mg/dL) | 44 (35–46) | 43 (35–46) | >0.05 | 42 (40–50) | 47(43–58) | **<0.05** |
| LDL (mg/dL) | 143 (134–150) | 143 (134–150) | >0.05 | 127 (94–141) | 127(93–140) | >0.05 |
| TAG (mg/dL) | 177 (149–190) | 176 (149–190) | >0.05 | 180 (138–105) | 170(135–198) | **<0.05** |

*Note:* p<0.05 (in boldface) is considered a positive association.
*A1C*, glycated hemoglobin; *BP*, blood pressure); *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *TAG*, triglycerides.

**Table S3**
Change in the study variables after 6 months of metformin plus sulfonylureas therapy in metformin nonresponders according to the genotypes (GG homozygotes and A carriers) of SLC22A3 G>A

| Parameters | GG | | | GA+AA | | |
|---|---|---|---|---|---|---|
| | Baseline median (25%–75%) | After 6 months median (25%–75%) | p value | Baseline median (25%–75%) | After 6 months median (25%–75%) | p value |
| Age (years) | 48 (45–58) | 47 (45–60) | >0.05 | 45 (45–57) | 45 (45–60) | >0.05 |
| Weight (kg) | 60 (54–80) | 59 (54–80) | >0.05 | 65 (53–85) | 60 (56–80) | **<0.05** |
| Systolic BP (mmHg) | 136 (120–140) | 135 (120–140) | >0.05 | 136 (120–140) | 135 (120–140) | >0.05 |
| Diastolic BP (mmHg) | 85 (80–90) | 84 (80–90) | >0.05 | 85 (80–90) | 84 (80–90) | >0.05 |
| Fasting blood glucose (mg/dL) | 154 (161–200) | 155 (159–200) | >0.05 | 165 (165–200) | 156 (158–200) | **<0.05** |
| Postprandial blood glucose (mg/dL) | 220 (102–150) | 220 (100–150) | >0.05 | 230 (110–160) | 220 (100–150) | **<0.05** |
| A1C (%) | 8 (7.5–8) | 8 (7.5–8) | >0.05 | 7.9 (7.5–8) | 7.5 (7.5–8) | **<0.05** |
| Total cholesterol (mg/dL) | 199 (167–210) | 199 (167–210) | >0.05 | 185 (159–210) | 175 (159–200) | **<0.05** |
| HDL(mg/dL) | 44 (37–44) | 44 (37–44) | >0.05 | 44 (37–44) | 44 (37–44) | >0.05 |
| LDL(mg/dL) | 120 (92–147) | 120 (92–147) | >0.05 | 122 (92–147) | 122 (92–147) | >0.05 |
| TAG(mg/dL) | 146 (120–200) | 145 (120–200) | >0.05 | 146 (120–200) | 145 (120–200) | >0.05 |

*Note:* p<0.05 (in boldface) is considered a positive association.
*A1C*, glycated hemoglobin; *BP*, blood pressure); *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *TAG*, triglycerides.

**Table S4**
The relationship of the variant rs3088442 with the clinical response of metformin stratified by the 2 genotype groups' homozygotes GG and carriers according to clinical-pathologic parameters

| Parameters | SLC22A3 rs3088442 | | |
|---|---|---|---|
| | GG<br>Median (25%–75%) | GA+AA<br>Median (25%–75%) | p value |
| **Healthy controls (n=300)** | | | |
| Age (years) | 50 (40–63) | 45 (40–60) | >0.05 |
| Weight (kg) | 55 (50–60) | 70 (57–70) | **<0.05** |
| Systolic BP (mmHg) | 120 (120–130) | 120 (120–135) | >0.05 |
| Diastolic BP (mmHg) | 80 (80–81) | 81 (80–82) | >0.05 |
| Fasting blood glucose (mg/dL) | 93 (89–99) | 96 (89–95) | **<0.05** |
| Postprandial blood glucose (mg/dL) | 144 (130–160) | 145 (132–156) | >0.05 |
| A1C (%) | 6 (6–7) | 7.7 (7–8) | **<0.05** |
| Total cholesterol (mg/dL) | 167 (155–190) | 167 (156–190) | >0.05 |
| HDL (mg/dL) | 56 (45–67) | 56 (45–57) | >0.05 |
| LDL (mg/dL) | 95 (90–99) | 95 (90–99) | >0.05 |
| TAG (mg/dL) | 135 (133–145) | 135 (134–145) | >0.05 |
| **Metformin (responders) (n=300)** | | | |
| Age (years) | 47 (40–60) | 47 (41–60) | >0.05 |
| Weight (kg) | 76 (62–80) | 62 (56–71) | **<0.05** |
| Systolic BP (mmHg) | 135 (130–145) | 135 (120–140) | >0.05 |
| Diastolic BP (mmHg) | 88 (82–90) | 84 (80–85) | **<0.05** |
| Fasting blood glucose (mg/dL) | 153(139–150) | 144(130–140) | **<0.05** |
| Postprandial blood glucose (mg/dL) | 230(210–245) | 189(178–210) | **<0.05** |
| A1C (%) | 9.5 (8–9.5) | 8 (7.5–8) | **<0.05** |
| Total cholesterol (mg/dL) | 242 (189–241) | 221 (158–240) | **<0.05** |
| HDL (mg/dL) | 43 (35–46) | 47 (43–58) | **<0.05** |
| LDL (mg/dL) | 143 (134–150) | 127 (93–140) | **<0.05** |
| TAG (mg/dL) | 176 (149–190) | 170 (135–198) | >0.05 |
| **Sulfonylureas (nonresponders) (n=300)** | | | |
| Age (years) | 47 (45–60) | 45 (45–60) | >0.05 |
| Weight (kg) | 59 (54–80) | 60 (56–80) | >0.05 |
| Systolic BP (mmHg) | 135 (120–140) | 135 (120–140) | >0.05 |
| Diastolic BP (mmHg) | 84 (80–90) | 84 (80–90) | >0.05 |
| Fasting blood glucose (mg/dL) | 156 (158–200) | 155 (158–200) | >0.05 |
| Postprandial blood glucose (mg/dL) | 220 (100–150) | 220 (100–150) | >0.05 |
| A1C (%) | 8 (7.5–8) | 7.7 (7.5–8) | **<0.05** |
| Total cholesterol (mg/dL) | 199 (167–210) | 175 (159–200) | **<0.05** |
| HDL (mg/dL) | 44 (37–44) | 44 (37–44) | >0.05 |
| LDL (mg/dL) | 122 (92–147) | 120 (92–147) | >0.05 |
| TAG (mg/dL) | 145 (120–200) | 135 (100–140) | **<0.05** |

*Note:* p<0.05 (in boldface) is considered a positive association.
*A1C*, glycated hemoglobin; *BP*, blood pressure); *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *TAG*, triglycerides.

**Table S5**
The average change in A1C (%), random blood glucose and fasting blood glucose levels according to the SLC22A3 genotype

| Polymorphism | | A1C (%)<br>median (25%–75%) | p value | Postprandial blood glucose (mg/dL)<br>median (25%–75%) | p value | Fasting blood glucose (mg/dL)<br>median (25%–75%) | p value |
|---|---|---|---|---|---|---|---|
| Healthy individuals | | | | | | | |
| rs3088442 | GG | 7.0 (6.0–7.0) | **<0.05** | 122 (119–130) | >0.05 | 93 (89–99) | >0.05 |
| | GA | 8.0 (7.0–8.0) | | 125 (120–130) | | 94 (89–90) | |
| | AA | 8.0 (7.0–8.0) | | 125 (115–130) | | 95 (89–99) | |
| Metformin (responders) | | | | | | | |
| rs3088442 | GG | 9.0 (7.5–9.0) | **<0.05** | 234 (212–245) | **<0.05** | 153 (138–150) | **<0.05** |
| | GA | 8.0 (7.8–8.0) | | 219 (195–243) | | 140 (139–150) | |
| | AA | 7.0 (7.0–7.5) | | 185 (170–213) | | 140 (139–151) | |
| Sulfonylureas (nonresponders) | | | | | | | |
| rs3088442 | GG | 7.5 (7.5–8.0) | >0.05 | 210 (170–210) | | 148 (95–150) | |
| | GA | 8.0 (7.5–8.0) | | 211 (170–210) | >0.05 | 148 (97–150) | >0.05 |
| | AA | 8.0 (7.5–8.0) | | 211 (170–210) | | 148 (97–150) | |

*Note:* p<0.05 (in boldface) is considered a positive association.
*A1C*, glycated hemoglobin.

**Table S6**
The influence of SLC22A3 polymorphisms on lipid profiles in healthy individuals and in patients with type 2 diabetes

| Polymorphism | | Total cholesterol (mg/dL) median (25%–75%) | p value | HDL (mg/dL) median (25%–75%) | p value | LDL (mg/dL) median (25%–75%) | p value | TAG (mg/dL) median (25%–75%) | p value |
|---|---|---|---|---|---|---|---|---|---|
| Healthy individuals | | | | | | | | | |
| rs3088442 | GG | 170 (156–120) | >0.05 | 56 (45–67) | >0.05 | 90 (90–94) | >0.05 | 135 (131–146) | >0.05 |
| | GA | 170 (155–120) | | 57 (50–67) | | 90 (90–95) | | 134 (132–145) | |
| | AA | 170 (155–120) | | 56 (45–67) | | 90 (90–95) | | 140 (133–145) | |
| Metformin (responders) | | | | | | | | | |
| rs3088442 | GG | 211 (172–230) | **<0.05** | 43 (42–47) | **<0.05** | 145 (93–159) | **<0.05** | 176 (135–198) | **<0.05** |
| | GA | 221 (158–240) | | 46 (37–57) | | 137 (94–140) | | 167 (145–189) | |
| | AA | 175 (159–200) | | 47 (43–58) | | 130 (92–135) | | 129 (100–181) | |
| Sulfonylureas (nonresponders) | | | | | | | | | |
| rs3088442 | GG | 180 (159–200) | >0.05 | 43 (42–47) | >0.05 | 145 (100–167) | **<0.05** | 160 (100–181) | >0.05 |
| | GA | 184 (159–203) | | 43 (42–47) | | 139 (98–166) | | 164 (100–181) | |
| | AA | 185 (160–204) | | 43 (42–47) | | 122 (92–147) | | 166 (100–200) | |

*Note:* p<0.05 (in boldface) is considered a positive association.
*HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *TAG*, triglycerides.