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Original Article

Association of SLC22A1, SLC47A1, and KCNJ11 polymorphisms with efficacy and safety of metformin and sulfonylurea combination therapy in Egyptian patients with type 2 diabetes

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Abstract

Background and purpose: Multidrug and toxin extrusion transporter 1 (MATE1), encoded by the SLC47A1 gene and single nucleotide polymorphisms of organic cation transport 1, may impact metformin's responsiveness and side effects. Inward-rectifier potassium channel 6.2 (Kir 6.2) subunits encoded by KCNJ11 may affect the response to sulfonylurea. This study aimed to evaluate the association between SLC22A1 rs72552763 and rs628031, SLC47A1 rs2289669 and KCNJ11 rs5219 genetic variations with sulfonylurea and metformin combination therapy efficacy and safety in Egyptian type 2 diabetes mellitus patients.
Experimental approach: This study was conducted on 100 cases taking at least one year of sulfonylurea and metformin combination therapy. Patients were genotyped *via* the polymerase chain reaction-restriction fragment length polymorphism technique. Then, according to their glycated hemoglobin level, cases were subdivided into non-responders or responders. Depending on metformin-induced gastrointestinal tract side effects incidence, patients are classified as tolerant or intolerant.
Findings/Results: KCNJ11 rs5219 heterozygous and homozygous mutant genotypes, SLC47A1 rs2289669 heterozygous and homozygous mutant genotypes (AA and AG), and mutant alleles of both polymorphisms were significantly related with increased response to combined therapy. Individuals with the SLC22A1 (rs72552763) GAT/del genotype and the SLC22A1 (rs628031) AG and AA genotypes were at a higher risk for metformin-induced gastrointestinal tract adverse effects.
Conclusion and implications: The results implied a role for SLC47A1 rs2289669 and KCNJ11 rs5219 in the responsiveness to combined therapy. SLC22A1 (rs628031) and (rs72552763) polymorphisms may be associated with increased metformin adverse effects in type 2 diabetes mellitus patients.

Keywords: Metformin and sulfonylurea combination therapy; Single nucleotide polymorphisms; Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a major public health problem worldwide, with 537 million persons aged 20-79 having DM worldwide in 2021 (1), more than 90% of these cases are type 2 DM (T2DM) (2). The International Diabetes Federation (IDF) listed Egypt as the second most diabetes-affected nation in North Africa and the Middle East (1).

Treatment options for T2DM are many; however, guidelines recommend beginning with monotherapy. Nevertheless, in many cases, monotherapy is insufficient, and combination therapy should be provided (3,4). However, monotherapy is useful for controlling blood glucose for only a few years; then, the patient must use a combination therapy (4).

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Many international guidelines, including those of the American Diabetes Association (ADA), and the European Association for the Study of Diabetes (EASD), support metformin as T2DM treatment for the first-line (4). Randomized clinical research and meta-analyses have demonstrated that adding sulfonylurea to metformin improves glucose control without compromising safety or tolerability (5-7). Response to oral hypoglycemics is highly variable, and genetic build-up can account for as much as 40% of this variation (8). Metformin requires organic cation transporter 1 (OCT1), encoded by solute carrier family 22 member 1 (SLC22A1), to enter hepatocytes, where it performs its main activity (9). Metformin decreases hepatic gluconeogenesis; however, the precise mechanism for enhancing insulin sensitivity is uncertain (10). Furthermore, reduced metformin absorption by OCT1 was thought to lead to greater metformin concentrations in the intestine, increasing the risk of gastrointestinal tract (GIT) side effects (11). Single nucleotide polymorphisms (SNPs) of SLC22A1 impact the transporter function, causing inter-patient differences in metformin disposition and efficacy (12). Furthermore, rs628031 of the OCT1 gene (Met408Val, 1222A>G) was related to variability in metformin's therapeutic efficacy (13,14) and side effects (15).

It has been revealed that rs72552763 of the OCT1 gene (M420del, 1260GAT>del) is linked with altered metformin pharmacokinetics (16) and also with metformin-induced gastrointestinal adverse effects (17,18). Metformin exposure and hepatic distribution were considerably reduced in carriers with M420del variant (19). Metformin is eliminated into the urine *via* multidrug and toxin extrusion transporter 1 (MATE1) which is expressed by the solute carrier family 47 member 1 (SLC47A1) gene (20). The rs2289669 G>A of SLC47A1 is related to variability in metformin treatment efficacy (21,22). On the other hand, SUs are insulin secretagogues that stimulate the closure of ATP-sensitive potassium channels (KATP) in pancreatic β-cell (23). The KATP channel consists of four sulfonylureas receptor 1 subunits and four pore-forming inward-rectifier potassium channel 6.2 (Kir 6.2) subunits (24). KCNJ11 encodes Kir 6.2 subunits, and its polymorphism has been linked to sulfonylurea responsiveness (25). KCNJ11 rs5219 E23K has been associated with the therapeutic effect of SUs (26).

Insufficient research has been done on Egyptian genetics. Numerous studies categorize Egyptians as Caucasians. However, some researchers have discovered differences in the frequency of specific genes between Egyptians and Caucasians (27-29). Therefore, this study aims to examine the relationship between SLC22A1 rs72552763 and rs628031, SLC47A1 rs2289669 and KCNJ11 rs5219 genetic variants with the metformin and sulfonylurea combination therapy efficacy and safety in Egyptian T2DM patients.

PATIENTS AND METHODS

Study design

This cross-sectional observational research was approved by the committee of medical ethics of Zagazig University (IRB No. 5234) and conducted in accordance with the Declaration of Helsinki. participants provided their informed consent in writing.

Patient selection

Between August 2021 and July 2022, 100 people receiving T2DM chronic treatment were recruited consecutively from the diabetes outpatient clinic of Zagazig University Hospital, Egypt.

Participants were to be at least 18 years old, unrelated Egyptians with sulfonylurea / metformin dual medication for a year before the study. Individuals who were pregnant or breastfeeding, had type 1 DM, chronic liver disease, malignancies, or chronic renal disease, were undertaking monotherapy with insulin, metformin, or triple treatment, or taking any drugs that interact with the OCT1 receptor were left out of the study.

Data collection

The required demographic information and patient data, including a complete medical and medication history, age, gender, weight, height, family history, physical activity, and duration of treatment, were collected. Subsequently,

anti-diabetic medications were supplied to each patient, and their daily doses were obtained from the patient's profile or an interview conducted during a monthly visit.

In conjunction with metformin, glibenclamide, glimepiride, and gliclazide as sulfonylureas were prescribed. For compliance evaluation, patients were questioned about their dose instructions and treatment regimen for their given medicine.

Patient classification

Patients were categorized into responders (glycated hemoglobin (HbA1C) < 7%) and non-responders (HbA1C >7%) based on HbA1C value, as the ADA recommends a cutoff of 7% (30).

Furthermore, they were divided into metformin-tolerant and intolerant patients. Intolerant individuals had at least one of the following symptoms within a year after starting treatment: diarrhea, nausea, flatulence, abdominal pain, and vomiting.

Blood sampling and HbA1C measurement

Briefly, 5 mL of blood was divided into two ethylenediaminetetraacetic acid (EDTA) tubes, one for glycated hemoglobin measurement and the other for DNA extraction. Then, HbA1C was determined using a Roche/Hitachi cobas c311 analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland) that uses a turbidimetric inhibition immunoassay method and according to the manufacturer's instructions.

DNA extraction and genotyping

Using the Geneaid Genomic DNA Mini Kit (blood/cultured cell; Geneaid Biotech Ltd., New Taipei City, Taiwan), DNA was extracted. However, the samples were stored at -80 °C until genotyping. Genotyping for the four SNPs: rs72552763 (31), rs628031 (32), SLC47A1 rs2289669 (33), and KCNJ11 rs5219 (34) was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Amplification was accomplished using a PERKIN ELMER DNA thermal cycler 480 (Norwalk, CT 06856, USA). The PCR reaction mixtures (20 µL) consisted of 5 pmol of each primer (Table 1; Beijing SBS Genetech Co., Ltd., Beijing, China), 500 ng of genomic DNA, and 2X TOPsimple™ DyeMIX-nTaq PCR master mix (10 µL; Enzynomics, Daejeon, Republic of Korea).

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Table 1. PCR conditions and primers for genotyping of SLC22A1, SLC47A1, and KCNJ11.

Polymorphic site	Primers' sequences	PCR conditions	Restriction enzyme and fragment size (bp)
SLC22A1 rs628031 A>G	(F) 5' TTTCTTCAGTCTCTGACTCATGCC 3' (R) 5' AAAAAACTTTGTAGACAAAGGTAGCACC 3'	35 Cycles: 30 s at 94 °C, 45 s at 63 °C, 1 min at 72 °C	MscI AA (mutant type): 397 GG (wild type): 210 and 187; AG (heterozygotes): 397, 210, and 187
SLC22A1 rs72552763 GAT>del	(F) 5'AGGTTCACGGACTCTGTGCT 3' (R) 5'AAGCTGGAGTGTGCGATCT 3'	35 Cycles: 45 s at 93 °C, 45 s at 58 °C, 35 s at 72 °C	BspHI GAT/GAT (wild type): 403 and 197 del/del (mutant type): 600 GAT/del (heterozygotes): 600, 403, and 197
SLC47A1 rs2289669 G>A	(F) 5′ TCAGTTTCCACAGTAGCGTCG 3′ (R) 5′ GACACTGGA AGCCACACTGAA 3′	35 Cycles: 30 s at 95 °C, 30 s at 57 °C, 30 s at 72 °C	TaqI GG (wild type) 211 AA (mutant type): 190 GA (heterozygotes): 190 and 211
KCNJ11 rs5219 C>T, E23K	(F) 5′ GAATACGTCCTGACACGCCT 3′ (R) 5′ GCCAGCTGCACAGGAAGGACAT 3′	35 Cycles: 1 min at 95 °C, 1 min at 71 °C, 1 min at 72 °C	BanII EE (wild type): 178 KK (mutant type): 218 EK (heterozygotes): 218 and 178

PCR, polymerase chain reaction; bp, base pair.

Then, 1 µL of restriction endonuclease (New England Biolabs, Ipswich, Massachusetts, United States) was used to digest PCR products for 1 h at 37 ℃. Then, 2% agarose gel dyed with ethidium was used to separate the PCR product. Subsequently, the gel was examined with an ultraviolet transilluminator. The used restriction enzymes were MscI for rs628031, BspHI for rs72552763, TaqI for rs2289669, and BanII for rs5219. Table 1 summarizes the precise locations of SNPs, primer sequences, and restriction enzymes.

Statistical analysis

Numerical variables were expressed as mean ± SD, while qualitative variables were represented as numbers and percentages. T-test and Chi-square (χ^2) were used to compare groups' demographics. The Hardy-Weinberg equilibrium of genotype frequencies was examined, and any divergence was assessed for statistical significance using the χ^2 test. Odd ratios (ORs) and 95% confidence intervals (CIs) were computed to assess the association between genetic variants and response or side effects. A *P*-value < 0.05 was regarded as statistically significant. Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 25.

RESULTS

Patient characteristics

Sixty-two percent of patients responded to combined therapy (62 patients). Except for the HbA1C level, which was lower in the responders group than in the non-responders group (6.56% versus 8.69%, $P < 0.001$), there were no statistically significant differences between the two groups regarding all other patient characteristics. Metformin side effects were noticed in 64 patients (64%). There were no significant variations in patient characteristics between the tolerant and intolerant groups. All patients' demographics are summarized in Table 2. The genotype and allele frequencies of the studied genes in the study population are illustrated in Table 3. The results of the allele variants of SLC22A1, SLC47A1, and KCNJ11 genes obtained from PCR-RFLP are shown in Figs. 1-4.

Table 2. Patients' characteristics.

Characteristics	Responders, n = 62 (62%)	Non-responders, n = 38 (38%)	*P*-value	Tolerant group, n = 36 (36%)	Intolerant group, n = 64 (64%)	*P*-value
Gender: number (%)						
Male	21 (33.9%)	14 (36.8%)	0.76	12 (33.3%)	23 (35.9%)	0.79
Female	41 (66.1%)	24 (63.2%)		24 (66.7%)	41 (6.1%)	
Age (year) Mean ± SD	54.45 ± 8.35	52.89 ± 9.08	0.38	51.83 ± 8.73	55.00 ± 8.429	.078
Family history: number (%) (first-degree relatives)						
Yes	43 (69.4%)	24 (63.2%)	0.52	28 (77.8%)	39 (60.9%)	0.08
No	19 (30.6%)	14 (36.8%)		8 (22.2%)	25 (39.1%)	
Physical activity: number (%)						
Active	17 (27.4%)	6 (15.8%)	0.18	5 (13.9%)	18 (28.1%)	0.10
Inactive	45 (72.6%)	32 (84.2%)		31 (86.1%)	46 (71.9%)	
Treatment duration (y)	2.39 ± 1.39	2.83 ± 1.22	0.11	2.33 ± 1.07	2.69 ± 1.46	0.20
BMI (Kg/m^2)	31.57 ± 3.24	32.14 ± 1.19	0.21	32.39 ± 2.16	31.45 ± 2.86	0.09
Metformin dose (mg/day)	1341.9 ± 479.4	1506.58 ± 430.61	0.09	1422.2 ± 474.4	1394.5 ± 465.05	0.78
Sulfonylurea: number (%)						
Glimepiride	54 (87.09%)	26 (68.42%)	0.07	30 (37.5%)	50 (62.5%)	
Gliclazide	5 (8.06%)	6 (15.78%)		4 (36.4%)	7 (63.6%)	0.66
…Glibenclamide	3(4.83%)	6 (15.78%)		2 (22.2%)	7 (77.8%)	
Sulphonylurea dose (mg/day)						
Glimepiride	3.518 ± 0.66	3.7692 ± 0.51	0.07	3.7 ± 0.59	3.54 ± 0.64	0.27
Gliclazide	42 ± 16.43	45 ± 16.43	0.77	52.5 ± 15	38.5 ± 14.63	0.17
Glibenclamide	4.166 ± 1.44	4.5833 ± 1.02	0.63	3.75 ± 1.76	4.6429 ± 0.944	0.34
HbA1C (%)	6.56 ± 1.47	8.69 ± 0.62	< 0.001	7.03 ± 1.06	7.56 ± 1.61	0.05

Table 3. Allele and genotype frequencies of SLC22A1, SLC47A1 and KCNJ11 polymorphisms in the study participants.

Polymorphism	Genotype frequency; n (%)			Allele frequency; n (%)	
SLC22A1 (rs628031)	GG 39 (39)	AG 44 (44)	AA 17 (17)	G 122 (61)	A 78 (39)
SLC22A1 (rs72552763)	GAT/GAT 71 (71)	GAT/del 24 (24)	del/del 5 (5)	GAT 166 (83)	del 34 (17)
SLC47A1 (rs2289669)	GG 38 (38)	GA 40 (40)	AA 22 (22)	G 116 (58)	A 84 (42)
KCNJ11 (rs5219)	EE 40 (40)	EK 47 (47)	KK 13 (13)	E 127(63.5)	K 73 (36.5)

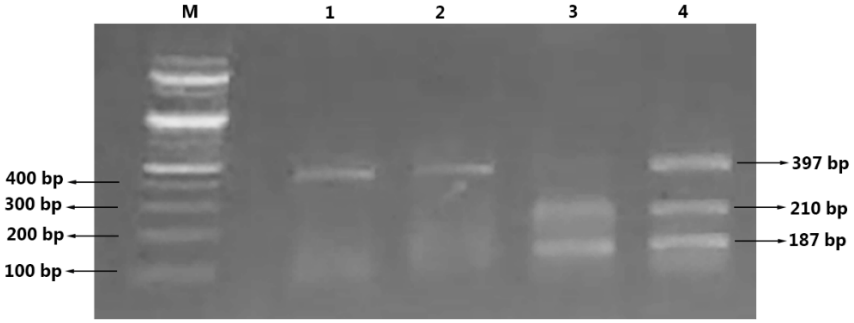

Fig. 1. Visualization of gel electrophoresis of SLC22A1 (rs628031) under UV light. Lane M: 100 bp ladder; lane 1: undigested sample (397 bp); lane 2: AA (homozygous mutant type, 397 bp); lane 3: GG (homozygous wild type, 210 bp, 187 bp); lane 4: AG (heterozygotes, 397 bp, 210 bp and 187 bp). bp, Base pair.

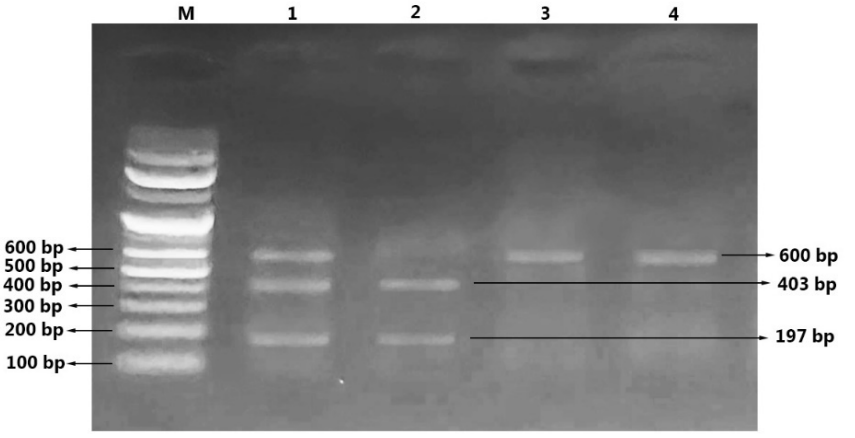

Fig. 2. Visualization of gel electrophoresis of SLC22A1 (rs72552763) under UV light. Lane M: 100 bp ladder; lane 1: GAT/del (heterozygotes, 600 bp, 403 bp, and 197 bp); lane 2: GAT/GAT (homozygous wild type, 403 bp and 197 bp); lane 3: del/del (homozygous mutant type, 600 bp); lane 4: undigested sample (600 bp). bp, Base pair.

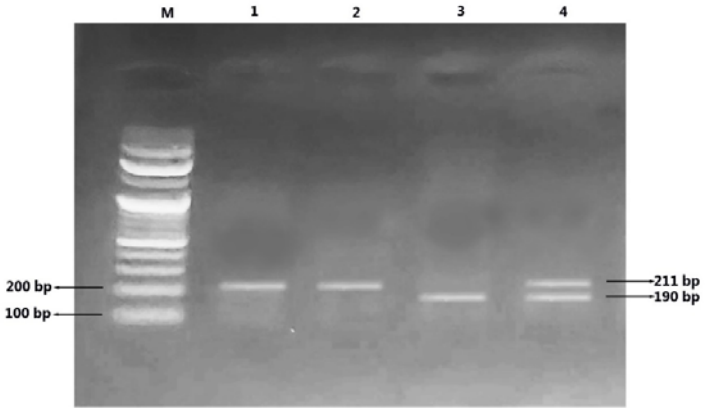

Fig. 3. Visualization of gel electrophoresis of SLC47A1 (rs2289669) under UV light. Lane M: 100 bp ladder; lane 1: undigested sample (211 bp); lane 2: GG (homozygous wild type, 211 bp); lane 3: AA (homozygous mutant type, 190 bp); lane 4: GA (heterozygotes, 190 bp, 211 bp). bp, Base pair.

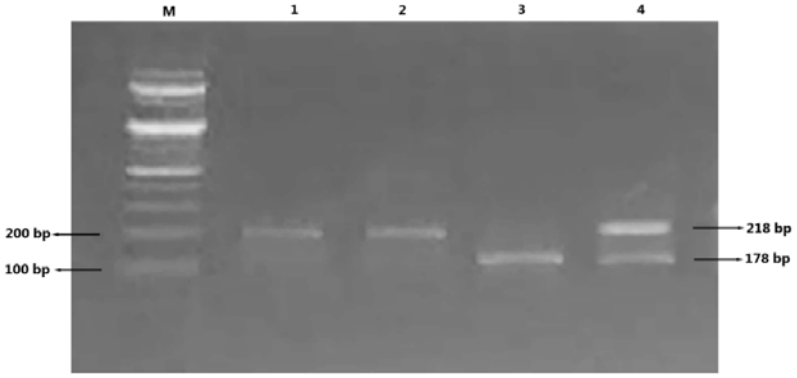

Fig. 4. Visualization of gel electrophoresis of KCNJ11 (rs5219) under UV light. Lane M: 100 bp ladder; lane 1: undigested sample (218 bp); lane 2: KK (homozygous mutant type, 218 bp); lane 3: EE (homozygous wild type, 178 bp); lane 4: EK (heterozygotes, 218, 178). bp, Base pair.

Association between SLC22A1, SLC47A1 and KCNJ11 gene polymorphisms and response

Table 4 provides the genotype and allele frequencies of gene polymorphisms for responders and non-responders. In each study group, all genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. The findings suggest that the SLC47A1 rs2289669 and KCNJ11 rs5219 genetic variants are associated with a greater response to combination therapy. Compared to the rs2289669 GG genotype carriers, the GA and AA genotype carriers responded better to combination therapy. Relative to the rs5219 EE genotype carriers, the EK and KK genotype carriers responded better to combination therapy. The genetic variants SLC22A1 rs628031 and SLC22A1 rs72552763 were not associated with responsiveness to combination therapy.

Association between SLC22A1, SLC47A1 and KCNJ11 gene polymorphisms and metformin side effects:

Table 4 presents gene polymorphisms genotype and allele frequencies in tolerant and intolerant groups. AG alleles carriers and AA alleles carriers of rs628031 variants were more susceptible to metformin side effects than GG allele carriers. GAT/del allele carriers were more susceptible to metformin adverse effects than GAT/GAT allele carriers of rs72552763 variants. MATE1 rs2289669 did not show any association with metformin side effects.

Table 4. Genotype and allele frequencies of SLC22A1, SLC47A1 and KCNJ11 genetic variants.

Genotype and allele	Responder n (%)	Non responder n (%)	OR (95% CI)	*P*-value	Tolerant group n (%)	Intolerant group n (%)	OR (95% CI)	*P*-value
rs628031								
GG	22 (35.5)	17 (44.7)	1 (reference)		22 (61.1)	17 (26.6)	1 (reference)	
AG	32 (51.6)	12 (31.6)	2.1 (0.824 - 5.1)	0.122	10 (27.8)	34 (53.1)	4.4 (1.7-11.3)	0.002
AA	8 (12.9)	9 (23.7)	0.687 (0.219 - 2.1)	0.520	4 (11.1)	13 (20.3)	4.2 (1.1 - 15.2)	0.029
Allele								
G	76 (61.3)	46 (60.5)	1 (reference)		54 (75)	68 (53.3)	1 (reference)	
A	48 (38.7)	30 (39.5)	0.968 (0.540 - 1.7)	0.914	18 (25)	60 (46.9)	2.6 (1.4-5.0)	0.003
rs72552763								
GAT/GAT	43 (69.4)	28 (73.7)	1 (reference)		31 (86.1)	40 (62.5)	1 (reference)	
GAT/del	18 (29.0)	6 (15.8)	1.95 (0.69 - 5.5)	0.207	4 (11.1)	20 (31.3)	3.87(1.2 - 12.5)	0.023
del/del	1 (1.6)	4 (10.5)	0.163 (0.017 - 1.5)	0.113	1 (2.8)	4 (6.3)	3.10(.33 - 29.1)	0.322
Allele								
GAT	104 (83.9)	62 (81.6)	1 (reference)		66 (91.7)	100 (78.1)	1 (reference)	
del	20 (16.1)	14 (18.4)	.852(.402- 1.8)	0.676	6 (8.3)	28 (21.9)	3.08(1.2- 7.8)	0.018
rs2289669								
GG	16 (25.8)	22(57.9)	1 (reference)		17 (47.2)	21 (32.8)	1 (reference)	
GA	29 (46.8)	11 (28.9)	3.6 (1.4 - 9.3)	0.008	12 (33.3)	28 (43.8)	1.88 (0.74- 4.7)	0.180
AA	17 (27.4)	5 (13.2)	4.6 (1.4 - 15.3)	0.011	7 (19.4)	15 (23.4)	1.73 (0.57 - 5.2)	0.327
Allele								
G	61 (49.2)	55 (72.4)	1 (reference)		46 (63.9)	70 (54.7)	1(reference)	
A	63 (50.8)	21 (27.6)	2.7 (1.5 - 4.9)	0.001	26 (36.1)	58 (45.3)	1.46 (0.81 - 2.6)	0.207
rs5219								
EE	31 (50.0)	9 (23.7)	1 (reference)					
EK	26 (41.9)	21 (55.3)	0.35(.14 - 0.91)	0.033				
KK	5 (8.1)	8 (21.1)	0.18 (0.047- 0.69)	0.013				
Allele								
E	88 (71.0)	39 (51.3)	1 (reference)					
K	36 (29.0)	37 (48.7)	0.43 (0.23 - 0.78)	0.006				

n, Number; OR, odds ratio; CI, confidence interval.

DISCUSSION

Metformin with sulfonylureas is one of the most frequently prescribed combination therapies for T2DM (7). Owing to genetic factors, oral anti-diabetic medication treatment outcomes vary considerably between individuals (8). This research studied the influence of two polymorphisms of SLC22A1 (rs628031 and rs72552763) on glycemic response and side effects of sulfonylurea/metformin combination therapy. No statistically significant association between SLC22A1 rs628031 and HbA1C reduction in response to sulfonylurea/metformin combination therapy has been identified in this study. Similar results were obtained in Caucasians (35), Iranians (36), Japanese (12), and Indians (37). *In vitro* tests have shown that the rs628031 polymorphism has normal metformin uptake (16). Furthermore, it has no impact on OCT1 expression (38) which supports the results of the current study.

In contrast, Chinese patients with the rs628031 AA genotype saw a higher decrease in HbA1c after metformin therapy (13). Shikata *et al.* showed that rs628031 polymorphism is a weak positive predictor for metformin efficacy in Japanese patients (14). Furthermore, Reséndiz-Abarca *et al.* demonstrated that Mexican patients with the AA-rs628031 genotype showed higher HbA1c levels after

metformin therapy (39). Nevertheless, Seitz *et al.* hypothesize that rs628031 is in linkage disequilibrium with another SNP in the same gene (rs36056065), which could explain the rs628031 effect (40). Compared with the G allele, the A allele at the SLC22A1 rs628031 locus was associated with metformin-induced GIT adverse effects in the current study. Similarly, in a study involving 246 Latvian patients, Tarasova et al. explored that the rs628031 A allele was significantly associated with metformin's adverse effects (15).

Enterocyte membranes express OCT1 receptors (41,42). And if OCT1 plays a role in metformin absorption, inhibiting it will raise intestinal metformin levels (18). Meanwhile, the current study showed no association between SLC22A1 rs72552763 polymorphism and sulfonylurea/metformin combination therapy response. Similar results have been reported using the GoDARTS cohort in 1,531 T2DM patients (43). Additionally, Davis *et al*. revealed that the reduced function allele of rs72552763 SNP was not associated with metformin response in 171 diabetic patients (44). Recently, Ortega-Ayala *et al*. demonstrated that rs72552763 polymorphism was associated with neither HbA1c control nor plasma metformin concentrations in 103 Mexican patients taking either metformin alone or metformin and glibenclamide (45). What confirms the results of this study is that Christensen *et al*. found no impact of this polymorphism on metformin steady-state pharmacokinetics in 34 healthy individuals (46).

On the contrary, Shu *et al.* reported that metformin was less effective in lowering glucose surges following an oral glucose tolerance test in 12 healthy individuals with the M420del variant than it was in 8 individuals with the reference allele (16). In a second pharmacokinetic investigation, these same people have higher serum metformin concentrations and lower volume of distribution (47) which in mice is explained by a reduction in hepatic uptake (16).

The previous study examined healthy people who took only two doses of metformin and were tested for oral glucose response, whereas the current research was conducted on T2DM cases, and HbA1C was determined in conjunction with prolonged metformin and sulfonylurea usage. This study found that GAT/del genotype carriers had a higher risk for metformin-induced GIT side effects compared to GAT/GAT carriers. Christensen *et al*. found that carriers of the GAT/del allele are characterized by lower metformin trough concentration, which is explained by several factors, including the lack of metformin intestinal absorption, as a result of having the reduced function allele, which supports our results (48). Similar results were obtained in the Genetics of Diabetes Audit and Research (GoDART) study, where subjects with two reduced-function alleles (G465R, G401S, R61C, M420del, C88R) had a greater risk of GIT intolerance than subjects with one or no defective allele (18). Additionally, a study involving 92 individuals conducted in Bosnia and Herzegovina revealed that each OCT1 reduced function allele at R61C and M420del increased the likelihood of GIT adverse effects by more than twofold (17).

On the contrary, a study of Latvian patients proved no association between the M420del genotype and metformin-induced GIT side effects (15). Differences in the results between prior and current research concerning the association between SLC22A1 polymorphisms (rs628031 and rs72552763) and the response to metformin may be because most studies involved metformin-naive patients on metformin monotherapy. In contrast, this study covered patients already receiving the combination of metformin and sulfonylurea. The current study found that the A allele in MATE1 rs2289669 SNP was associated with a better metformin/sulfonylurea treatment response. These results are in line with research by Becker *et al*. which is a population cohort of 116 subjects and concluded that each A allele in the MATE1 rs2289669 SNP was associated with a 0.30% greater decline in HbA1c (49).

Tkáč *et al*. provide support for this hypothesis as they identified an association between the degree of HbA1c reduction and MATE1 rs2289669 A allele in 148 metformin-treated patients (22). In addition, He *et al*. found that individuals with a variant genotype (AA) experienced a significantly higher decline in HbA1c levels and that these individuals had a

greater area under the plasma concentration versus time curve but a poorer renal clearance (21). However, this may be because patients with the MATE1 rs2289669 AA genotype have decreased efflux of metformin in the kidney owing to a malfunctioning of the MATE1 transporter which will result in higher plasma concentration of metformin and perhaps to a greater reduction in blood glucose level and have a decreased efflux from the liver cells which leads to an increase in the concentration of metformin in it and a more potent suppression of the gluconeogenesis (49). On the contrary, Klen *et al*. found no association between HbA1c values and MATE1 rs2289669. The study was conducted in Slovenia and comprised 135 patients with a combination of sulfonylurea and metformin treatment for at least six months (50).

In accordance with a previous study, Xiao *et al*. did not establish a correlation between MATE1 rs2289669 polymorphism and metformin's glucose-lowering effect (51). In contrast to prior studies, the study design, sample size, treatment duration, and ethnicity were all potential factors that could have led to different outcomes. This study showed no association between the rs2289669 genotype and metformin-induced GIT side effects. Consistent with the study of Latvian patients, there was no association between the MATE1 rs2289669 genotype and metformin-induced GIT side effects (15). This study found an association between the KCNJ11 rs5219 E23K variant and a reduced response to metformin/sulfonylurea combination therapy. In accordance with a study comparing 43 patients with sulfonylurea-induced severe hypoglycemia to a control group of 54 patients revealed that the K allele was related to a greater HbA1c level and a decreased likelihood of severe hypoglycemia (52). El-Sisi *et al*. found that a 23K variant was associated with a higher incidence of secondary failure to sulfonylurea in Egyptian patients, which is consistent with the present investigation findings (53). This association was repeated in 525 Caucasian patients with T2DM (54).

A study by Nielsen et al. associated the E23K polymorphism with a reduced ability of glucose to trigger the release of insulin (55). In accordance with this study, Sesti *et al*. suggest that E23K polymorphism may alter insulin secretion in response to sulfonylureas especially if the cells are previously stressed by a high glucose exposure which is the case in T2DM patients (54). Biochemical studies confirmed these results with the COS-1 cell line, which demonstrated that the E23K variant increased the openness of the KATP channels, decreased their sensitivity to the ATP and raised the threshold concentration for insulin secretion (56). Other studies explained the association between hyperglycemia and this polymorphism by decreased hyperglycemia-induced inhibition of glucagon secretion (57). In contrast, The United Kingdom Prospective Diabetes Study (UKPDS) included 364 participants and proved that there is no association between KCNJ11 polymorphisms and sulfonylurea responsiveness (58). This result was confirmed in a study comprising 156 individuals treated with sulfonylurea monotherapy or sulfonylurea/metformin combination therapy (59).

The variability in current and previous results may be raised from the different methods of outcome evaluation, type of sulfonylurea, treatment duration, different races, and clinical characteristics of patients. Notably, the small sample size, the diversity in treatment duration among the included patients, and the use of different sulfonylurea medications are among the work limitations.

CONCLUSION

SLC22A1 (rs628031 and rs72552763) polymorphisms are associated with increased risk for metformin-induced GIT side effects while SLC47A1 rs2289669 and KCNJ11 rs5219 polymorphisms are associated with the responsiveness of metformin/sulfonylurea combination therapy in Egyptian patients with T2DM. Further research with a bigger sample size and additional SNPs in these genes are required to confirm the influence of these genetic variations on metformin/sulfonylurea combination therapy in Egyptian patients and to provide more compelling evidence for their value as indicators of anti-diabetic drug response.

Conflicts of interest statement

The authors declared no conflicts of interest in this study.

Authors' contributions

A. Ahmed drafted the manuscript and helped with the literature search; H. Elnahas contributed to the conception and design of the study; S.M. Shalaby conducted all experiments analyzed and interpreted data; H.M. Elsadek contributed to data interpretation and critical revision of the manuscript. All authors read and approved the finalized article.

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