

Effects of *SLC22A2* (rs201919874) and *SLC47A2* (rs138244461) genetic variants on Metformin Pharmacokinetics in Pakistani T2DM patients

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Abstract

Objective: To determine the frequencies of single nucleotide polymorphisms rs201919874 and rs138244461 in genes *SLC22A2* and *SLC47A2* respectively in Pakistani diabetes patients in order to characterise the genetic variants and determine their association with the pharmacokinetics of metformin.

Methods: The case-control study was conducted at the International Islamic University, Islamabad, Pakistan, from June 2016 to June 2017, and comprised genotypes of diabetic cases and matching controls which were determined following allele-specific polymerase chain reaction. Cases were further divided into Group A and Group B. The former consisted of diabetics who were on monotherapy of metformin, while the latter consisted of diabetics treated with a combination of metformin and sulfonylureas. In-silico analysis was performed to verify the effect of single nucleotide polymorphisms rs201919874 and rs138244461 on the structure of genes. Association was statistically determined using SPSS 18.

Results: Of the 1200 subjects, 800(66.6%) were cases and 400(33.3%) were controls. Among the cases, 400(50%) each were in Group A and Group B. Significant difference was observed in the distribution of rs201919874 between Group A and controls ($p < 0.05$) and between Group B and controls ($p < 0.05$) for heterozygous genotypic frequency and for allelic frequency. Conversely, statistically significant difference was observed in rs138244461 ($p < 0.05$) for all genotypic and allelic frequencies. Genotypes were significantly associated with glycated haemoglobin, random and fasting glucose levels in Group A compared to Group B ($p < 0.05$). In-silico analysis showed that both single nucleotide polymorphisms were expected to create significantly damaging structural changes in domains and helix ($p < 0.05$ each).

Conclusions: Both exonic single nucleotide polymorphisms were found to be associated with the pharmacokinetics of metformin.

Key Words: Diabetes, Metformin, Pakistan, SNPs. (JPMA 69: 155; 2019).

Introduction

Metformin has been most commonly used as a first-line therapy for treatment of type 2 diabetes mellitus (T2DM) for decades due to both its good anti-hyperglycaemic effect and safety profile.¹ The pharmacological basis of how metformin lowers the glucose level is not completely clarified, but it has been established that its key function is to inhibit hepatic gluconeogenesis.² Metformin does

not undergo under any kind of metabolism by hepatic enzymes and is excreted unchanged by the kidneys. Transporters of metformin play a key role in its distribution to different tissues and in its elimination through renal passage.³ A considerable inter-individual variability in glucose-lowering response to metformin was reported previously along with reduction of glycated haemoglobin (HbA1c) values ranging from 0.8% to 3%. Furthermore, less than two-thirds of patients responded adequately to metformin and achieved a desired fasting blood sugar (FBS) level.⁴⁻⁷

Human organic cation transporters (OCT 1-3) are encoded

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by genes SLC22A1, SLC22A2 and SLC22A3. These are poly-specific transporters for small and hydrophilic organic cations like endogenous compounds serotonin and dopamine, toxic substances and clinically used drugs. Among more than 120 clinically used drugs that interact with different human OCTs, at least 20 are well-known being transported. These include the anti-diabetic drug metformin, antineoplastic platinum compounds, the antiviral drugs acyclovir, the histamine H2 receptor antagonist cimetidine, ganciclovir, lamivudine and zalcitabine, and the antiarrhythmic drug quinidine.⁸

All solute carrier superfamily (SLC22) proteins share a common membrane topology with 12 α -helical transmembrane domains. Mutational analysis and homology modelling of the steric structure of the proteins led to the conclusion that they possess a large cleft that is accessible from the aqueous phase. Located within this cleft is an inner cavity containing different interaction sites for different substrates.⁹ It is involved in the uptake of various xenobiotics from the bloodstream and takes them into renal epithelial cells.¹⁰ Kimura et al used Human embryonic kidney (HEK293) cells to check the expression of OCT2 and illustrated that metformin is a good substrate for this transporter.¹¹ Different functional variants have been identified in the gene SLC22A2 that encodes OCT2 transporter.¹²

Multidrug and toxin extrusion (MATE) transporters are encoded by genes *SLC47A1* and *SLC47A2*. They are involved in the efflux of several lipophobic organic cations, including metformin. These transporters contain 400 to 550 amino acid residues and span 12 transmembrane domains.^{13,14} Genes of these transporters are located on the short arm of the 17th chromosome, 17p11.2.¹⁵ Two isoforms of MATE2 have been identified one of which is MATE2K.¹⁶ Like MATE1, MATE2K has been involved in the transport of several structurally distinct compounds, including metformin.¹⁷ Up till now, only few genetic variants have been identified in MATE2K and very few have been analysed with respect to metformin.¹⁸

Genetic variation in the genes SLC22A2 and SLC47A2 that encodes OCT2 and MATE2K transporters have been found to be linked with therapeutic efficacy of metformin in T2DM.^{10,19} Both single nucleotide polymorphisms (SNPs) are present in exon so it was hypothesized that any changes in these sequences may reduce transcription rates and thereby reduced OCT2 and MATE2K expression leading to a decreased transport of metformin into the

kidney and its elimination from the organ. Hence, the present study was planned to evaluate the occurrence of *SLC22A2* (rs201919874G>A) and *SLC47A2* (rs138244461 C>T) genetic polymorphisms in T2DM patients that were on monotherapy of metformin and those taking combination therapy along with sulfonylureas.

Material and Methods

The case-control study was conducted at the International Islamic University, Islamabad, Pakistan, from June 2016 to June 2017, and comprised genotypes of diabetic cases and matching controls which were determined following allele-specific polymerase chain reaction. Cases were further divided into Group A and Group B. The former consisted of diabetics who were on monotherapy of metformin, while the latter consisted of diabetics treated with a combination of metformin and sulfonylureas. Sample size was calculated by using online calculator²⁰ by considering confidence level 95% and confidence interval (CI) in line with literature.²¹ Patients in group A were taking 1500mg of metformin per day for 6 months or more, while those in Group B was on 1000mg of metformin and 80mg of sulfonylureas per day for one year or more. From each individual, 3-5 ml blood sample was taken and collected in ethylenediaminetetraacetic acid (EDTA) tubes.

Cases were defined as subjects of either gender aged 35-80 years. Diagnosis of T2DM was based on the World Health Organisation (WHO) / American Diabetes Association (ADA) definition.^{22,23} Patients with other types of diabetes, co-treatment with other anti-diabetic drugs, and pregnant women were excluded. Written informed consent was taken from all individuals prior to the study which was approved by the Pakistan Institute of Medical Sciences (PIMS) Hospital, Islamabad, Pakistan. Blood sampling from T2DM patients was done in outpatient clinics of endocrinology at PIMS. Detailed demographic and clinical data was collected from each individual. Unrelated healthy volunteers of either gender were enrolled through non-probability consecutive sampling.

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes using standard phenol chloroform method and stored at -20°C, until use. Genotyping was done using allele-specific polymerase chain reaction (PCR) and amplification was done by using 2700 Applied Biosystems. The primers sets, two forward primers and one reverse primer, were used to amplify

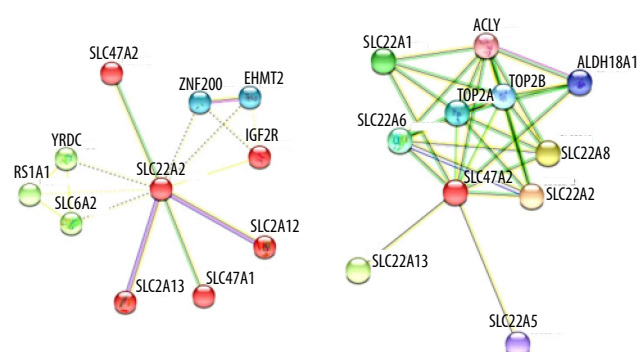


Figure-1: Gene network of SLC22A2 and SLC47A2 predicted by STRING.

SLC22A2 rs201919874 and SLC47A2 rs138244461 variants (rs201919874-F1: GCGAAAAGTTAATCCACGTATAGG rs201919874-F2: GCGAAAAGTTAATCCACGTATAGA and rs201919874-R: CTGAAAACCTTACACATAGTGTGCTG; rs138244461-F1: CCG CCT CCA GAG CAG TCC CAC rs138244461-F2: CCG CCT CCA GAG CAG TCC CAT and rs138244461-R: GGTGGTCAATCTAGGTTCCCG). Amplified products were visualised via 2% agarose gel electrophoresis.

Different bioinformatics tools were used to perform in-silico analysis on SNPs SLC22A2 rs201919874 and SLC47A2 rs138244461. Gene network was predicted by STRING 10.5²⁴ (Figure 1). Sequence features rely on the physiochemical properties (hydrophobicity, flexibility and rigidity, evolutionary conservation and volume) of amino acids. The structural features included the impact of mutations on the structure of the protein and protein stability. These characteristics are essential to look at, as mutation in the conserved region disrupts the structure of protein in the same way as if when a small amino acid is replaced by a large amino acid and eventually protein stability is affected, hence drug ligand binding activity gets affected too.²⁵

We used SNP nexus tool for the functional annotation of the SNPs. For carrying out the sequence features analysis, Suspect algorithm was used.²⁶ It is a web server where users can submit individual mutations. Three-dimensional (3D) structure of the protein was predicted using.²⁷ After structure prediction the impact of SNP polymorphisms on the 3D structure of SLC22A2 and SLC47A2 proteins were evaluated. For this, initially STRUM²⁸ was used to check the stability of protein after mutations and then MutPred version 1²⁹ was used to determine the gain and loss of structure and function upon mutation.

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. Fisher exact test or non-parametric Mann-Whitney test was used to compare clinical characteristics between different patient groups. Chi-square test was used to assess the deviation from Hardy-Weinberg equilibrium (HWE). The level of statistical significance was set at $p < 0.05$. Data was analysed using SPSS 18.

Results

Of the 1200 subjects, 800(66.6%) were cases and 400(33.3%) were controls. Among the cases, 400(50%) each were in Group A and Group B. The mean age of Group A was 46.995 ± 12.60 years with 180(45%) females, and in Group B it was 53.09 ± 12.39 years with 188(47%) females. The mean age of the healthy controls was 47.5 ± 12.71 years and there were 196(49%) females. All other parameters were also noted (Table 1).

Genotype and allele frequency distribution of SLC22A2 rs201919874 and SLC47A2 rs138244461 polymorphisms in T2DM patients and healthy controls were summarised separately (Table 2). There was a significant statistical difference in the allele frequencies of rs201919874 and rs138244461 polymorphisms between T2DM patients in both groups and healthy controls ($p < 0.005$), indicating that the two SNPs had a significant influence on the clinical efficacy of drug in T2DM. The heterozygous genotype GA of SLC22A2 rs201919874 was associated with metformin

Table-1: Characteristics of diabetes patients and healthy individuals (n=400).

Parameters	Healthy Individuals	Group-A Metformin Responders	Group-A Metformin Non-Responders
Gender			
Male (%)	51%	55%	53%
Female (%)	49%	45%	47%
Age (Years) ($\pm$ SD) ^a	47.5 ± 12.71	46.995 ± 12.60	53.09 ± 12.39
Weight ($\pm$ SD) ^a	78.10 ± 9.96	78.21 ± 11.14	78.68 ± 12.61
Height ($\pm$ SD) ^a	5.61 ± 0.38	5.67 ± 0.311	5.65 ± 0.47
BP systolic ($\pm$ SD) ^a	126.25 ± 7.54	131.7 ± 10.65	135.002 ± 10.84
BP Diastolic ($\pm$ SD) ^a	81.93 ± 3.34	83.64 ± 4.388	84.42 ± 4.405
Fasting Blood Glucose (mg/dl) ($\pm$ SD) ^a	0	130.015 ± 78.29	146.335 ± 68.24
Random Blood Glucose (mg/dl) ($\pm$ SD) ^a	0	116.3 ± 20.13	216.4 ± 59.02
HbA1c (%)	0	7.66	8.37
Total Cholesterol (mg/dl) ($\pm$ SD) ^a	0	187.225 ± 25.62	213.365 ± 36.71
LDL (mg/dl) ($\pm$ SD) ^a	0	131.66 ± 33.7	142.43 ± 20.808
HDL (mg/dl) ($\pm$ SD) ^a	0	43.59 ± 11.98	47.45 ± 8.307
TAG (mg/dl) ($\pm$ SD) ^a	0	153.8 ± 48.44	167.08 ± 27.77

BP: Blood pressure, SD: Standard deviation, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TAG: Triacylglycerol

Table-2: Comparisons of allelic frequencies of SLC22A2 and SLC47A2 polymorphisms in T2DM patients.

Genotype	Healthy Individual	T2DM patients Metformin	T2DM patients sulfonylureas	Healthy controls vs T2DM on Metformin		Healthy controls vs T2DM on Sulfonylureas	
				Or ^a (95% CI) ^b	<i>p</i>	Or ^a (95% CI) ^b	<i>p</i>
Genotypes of OCT2							
GG	319 (79.8%)	138 (34.5%)	220 (55.0%)	1(Ref)		1(Ref)	
GA	80 (20.0%)	259 (64.8%)	178 (44.5%)	7.48(5.43-10.31)	P<0.05	3.226(2.35-4.42)	P<0.05
AA	1 (.2%)	3 (.8%)	2 (.5%)	6.94(0.715-67.15)	P>0.05	2.90(.261-32.1)	P>0.05
GA+AA	81(20.2%)	262(65.5%)	180(45.0%)	1.919(1.263-2.915)	P<0.05	3.22(2.35-4.409)	P<0.05
Alleles of OCT2							
G	718 (89.8%)	535(66.875%)	618(77.25%)	4.337(3.305-5.69)	P<0.05	2.57(1.94-3.41)	P<0.05
A	82 (10.2%)	265(33.125%)	182(22.75%)				
Genotypes of MATE2K							
CC	314 (78.5%)	64 (16.0%)	186 (46.5%)	1(Ref)		1(Ref)	
CT	66 (16.5%)	221 (55.2%)	42 (10.5%)	16.42(11.18-24.13	P<0.05	4.39(3.14-6.16)	P<0.05
TT	20 (5.0%)	115 (28.8%)	214(53.5%)	28.21(16.34-48.68)	P<0.05	3.54(2.02-6.22)	P<0.05
CT+TT	86(21.5%)	336(84.0%)	544 (68.0%)	13.32(8.78-20.20)	P<0.05	4.20(3.084-5.72)	P<0.05
Alleles of MATE2K							
C	694 (86.8%)	349 (43.6%)	544(68%)	8.461(6.60-10.83)	P<0.05	3.08(2.39-3.96)	P<0.05
T	106 (13.2%)	451 (56.4%)	256(32%)				

T2DM: Type 2 diabetes mellitus, P Values < 0.05 (in bold) are considered positive associations., a Odds ratio., b Confidence interval.

Table-3: The influence of SLC22A2 and SLC47A2 polymorphisms on weight and lipid profile of patients that were on Metformin.

Polymorphism	Weight median (25–75%)		<i>p</i>	Total cholesterol median (25–75%)	<i>p</i>	HDL cholesterol median (25–75%)	<i>p</i>	LDL cholesterol median (25–75%)	<i>p</i>	TAG median (25–75%)	<i>p</i>
SLC22A2											
rs201919874	GG	67(59-78)		167(158-178)		55(45-58)		126(123-128)		135(130-145)	
	GA	81(78-89)	<i>P</i> <0.05	240(239-243)	<i>P</i> <0.05	43(35-45)	<i>P</i> <0.05	156(148-160)	<i>P</i> <0.05	180(167-199)	<i>P</i> <0.05
	AA	91(67-99)		248(238-249)		43(34-45)		156(81-165)			
SLC47A2											
rs138244461	CC	76(62-89)		167(157-178)		47(45-58)		125(123-128)		138(131-145)	
	CT	80(76-89)	<i>P</i> <0.05	237(178-240)	<i>P</i> <0.05	45(37-48)	<i>P</i> <0.05	147(128-156)	<i>P</i> <0.05	159(142-179)	<i>P</i> <0.05
	TT	89(88-99)		242(240-248)		42(34-45)		160(153-163)		199(176-210)	

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TAG: Triacylglycerol.

response in both patient groups ($p<0.05$), whereas, in case of SLC47A2 rs138244461 polymorphism CT and TT, both were significantly associated with clinical response of metformin in both groups ($p<0.05$) (Figures 2-3).

A significant genotype interaction was found with respect to weight and lipid profile in the two treated groups (Tables 3-4). Similarly, most of the clinical characters showed significant association with rs201919874 and rs138244461 polymorphisms (Table 5). GA and AA genotypes were associated with the clinical efficacy of

metformin in Group A patients as they failed to attain normal levels of HbA1c along with fasting and random blood glucose levels ($p<0.05$). In case of Group B patients, association was established only with random blood glucose levels ($p<0.05$). CT and TT genotypes were significantly associated with random glucose level in both the treated groups ($p<0.05$) (Table 6). With respect to lipid profile, significant association was observed in both the treated groups for Total cholesterol (TC), low-density lipoprotein (LDL) and triacylglycerol (TAG), but not for

Table-4: The influence of *SLC22A2* and *SLC47A2* polymorphisms on weight and lipid profile of patients that were on Metformin + Sulfonylureas.

Polymorphism	Weight median (25–75%)	<i>p</i>	Total cholesterol median (25–75%)	<i>p</i>	HDL cholesterol median (25–75%)	<i>p</i>	LDL cholesterol median (25–75%)	<i>p</i>	TAG median (25–75%)	<i>p</i>	
SLC22A2											
rs201919874	GG	76(62-80)		164(158-199)		49(41-58)		99(188-138)		101(98-177)	
	GA	88(78-90)	<i>p</i> <0.05	200(198-217)	<i>p</i> <0.05	36(30-40)	<i>p</i> <0.05	156(142-178)	<i>p</i> <0.05	190(167-216)	<i>p</i> <0.05
	AA	80(61-99)		180(160-201)		42(40-45)		126(88-165)		182(167-198)	
SLC47A2											
rs138244461	CC	76(62-80)		167(157-178)		47(45-58)		125(123-128)		138(131-145)	
	CT	80(76-89)	<i>p</i> <0.05	237(178-240)	<i>p</i> <0.05	54(37-48)	<i>p</i> <0.05	147(128-156)	<i>p</i> <0.05	159(142-179)	<i>p</i> <0.05
	TT	89(88-99)		242(240-248)		42(34-45)		160(153-163)		199(176-210)	

p-values <0.05 (bold) are considered positive associations, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TAG: Triacylglycerol.

Table-5: The clinical characteristics per *SLC22A2* rs201919874 and *SLC47A2* rs138244461 genotypes in Group A and Group B treated group.

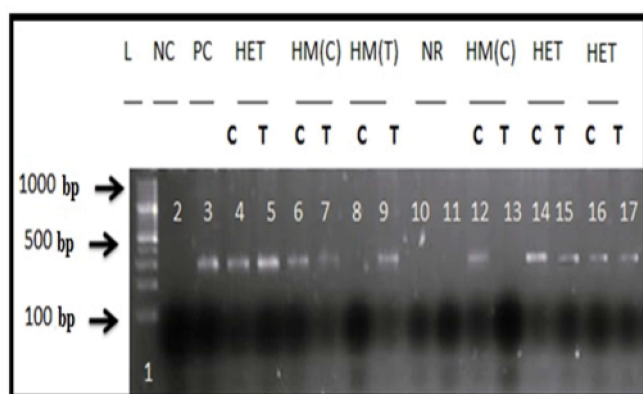
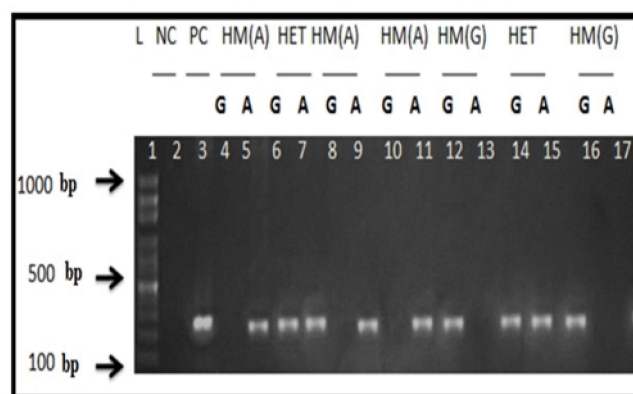
Parameters	SLC22A2 rs201919874			SLC47A2 rs138244461		
Metformin Responders	GG	GA+AA	<i>p</i> -value	CC	CT+TT	<i>p</i> -value
Weight	88(78-90)	76(62-80)	<i>p</i><0.05	76(62-80)	88(77-90)	<i>p</i><0.05
Height	5.6(5.4-5.7)	5.7(5.5-6)	<i>p</i> >0.05	5.7(5.5-6)	5.6(5.4-5.8)	<i>p</i> >0.05
BP Systolic	135(120-140)	135(120-140)	<i>p</i> >0.05	135(120-141)	134(122-145)	<i>p</i> >0.05
BP Diastolic	82(80-90)	85(80-90)	<i>p</i><0.05	81(80-90)	82(80-90)	<i>p</i> >0.05
Fasting Blood Glucose (mg/dl)	125(120-130)	125(120-130)	<i>p</i> >0.05	125(120-130)	125(120-130)	<i>p</i> >0.05
Random Blood Glucose (mg/dl)	110(99-132)	117(99-132)	<i>p</i><0.05	112(99-136)	119(98-130)	<i>p</i><0.05
HbA1c (%)	7.5(7.5-8)	8.1(7.5-8)	<i>p</i><0.05	7.5(7.5-8)	8.6(7.5-8.1)	<i>p</i><0.05
Total Cholesterol (mg/dl)	164(158-199)	200(198-217)	<i>p</i><0.05	166(158-198)	200(190-216)	<i>p</i><0.05
HDL Cholesterol (mg/dl)	49(41-58)	48(41-59)	<i>p</i> >0.05	49(41-58)	38(30-44)	<i>p</i><0.05
LDL Cholesterol (mg/dl)	99(88-138)	156(140-178)	<i>p</i><0.05	99(88-139)	148(136-170)	<i>p</i><0.05
TAG (mg/dl)	110(98-177)	190(167-216)	<i>p</i><0.05	101(99-167)	189(156-210)	<i>p</i><0.05
Metformin Non-Responders	GG	GA+AA	<i>p</i> -value	CC	CT+TT	<i>p</i> -value
Weight	67(59-78)	81(78-89)	<i>p</i><0.05	76(62-80)	88(77-90)	<i>p</i><0.05
Height	5.7(5.5-6.1)	5.6(5.4-5.8)	<i>p</i> >0.05	5.7(5.5-6)	5.6(5.4-5.8)	<i>p</i> >0.05
BP Systolic	130(120-137)	136(130-145)	<i>p</i><0.05	135(120-140)	135(120-140)	<i>p</i> >0.05
BP Diastolic	81(80-84)	87(82-90)	<i>p</i><0.05	81(80-90)	82(80-90)	<i>p</i> >0.05
Fasting Blood Glucose (mg/dl)	139(135-145)	145(140-150)	<i>p</i><0.05	121(115-133)	126(120-139)	<i>p</i> >0.05
Random Blood Glucose (mg/dl)	194(178-230)	233(194-245)	<i>p</i><0.05	146(123-160)	212(101-215)	<i>p</i><0.05
HbA1c (%)	7.8(7.5-8)	8.8(8-9)	<i>p</i><0.05	7.5(7.5-8)	8.5(7.9-9)	<i>p</i><0.05
Total Cholesterol (mg/dl)	167(158-178)	240(239-243)	<i>p</i><0.05	166(158-198)	200(190-216)	<i>p</i><0.05
HDL Cholesterol (mg/dl)	55(45-48)	55(45-48)	<i>p</i> >0.05	52(40-60)	37(32-47)	<i>p</i><0.05
LDL Cholesterol (mg/dl)	126(123-128)	156(147-161)	<i>p</i><0.05	118(89-142)	152(139-175)	<i>p</i><0.05
TAG (mg/dl)	135(130-145)	180(167-199)	<i>p</i><0.05	108(99-157)	192(140-212)	<i>p</i><0.05

p-values <0.05 (bold) are considered as positive associations, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TAG: Triacylglycerol, HbA1c: Glycated haemoglobin.

Table-6: The average change in HbA1c level (in %), Random and Fasting Blood Glucose per OCT2 and MATE2K genotype.

Polymorphism		HbA1c median (25–75%)	p-value	Random Blood Glucose median (25–75%)	p-value	Fasting Blood Glucose median (25–75%)	p-value
Metformin SLC22A2							
rs201919874	GG	7.8(7.5-8)	p<0.05	194(178-230)	p<0.05	139(135-145)	p<0.05
	GA	8.6(8-9)		233(195-245)		145(140-150)	
	AA	7.8(7-9)		245(170-249)		139(130-150)	
SLC47A2							
rs138244461	CC	7.5(7.5-8)	p<0.05	138(134-142)	p<0.05	189(178-210)	p<0.05
	CT	8.5(7.8-9)		145(138-149)		218(189-243)	
	TT	8.7(8-9)		147(140-150)		236(210-245)	
Sulfonylureas SLC22A2							
rs201919874	GG	7.5(7.5-8)	p>0.05	125(120-130)	p>0.05	110(99-132)	p>0.05
	GA	7.5(7.5-8)		125(120-130)		116(98-130)	
	AA	8(8-8)		127(125-130)		124(120-128)	
SLC47A2							
rs138244461	CC	7.5(7.5-8)	p>0.05	125(120-130)	p>0.05	115(99-136)	p>0.05
	CT	7.5(7.5-8)		125(120-130)		110(98-130)	
	TT	7.5(7.5-8)		126(119-130)		122(100-131)	

HbA1c: Glycated haemoglobin.

**Figure-2:** Electropherogram of Ethidium Bromide Stained 2% Agarose Gel Showing Allele Pattern obtained for SLC22A2 rs201919874 in Diabetic Patients: lane 1:ladder (100bp), lane 2: negative control, lane 3: positive control, lane 4 and 5: sample1 (heterozygous CT), lane 6 and 7: sample 2 (homozygous for C), lane 8 and 9: sample3 (homozygous for T), lane 10 and 11: sample 4 (NA: no results), lane 12 and 13: Sample 5 (homozygous for C), lane 14 and 15: sample 6 (heterozygous CT), lane 16 and 17: sample 7 (heterozygous CT).**Figure-3:** Electropherogram of Ethidium Bromide Stained 2% Agarose Gel Showing Allele Pattern obtained for SLC47A2 rs138244461 in Diabetic Patients: lane 1:ladder (100bp), lane 2: negative control, lane 3: positive control, lane 4 and 5: sample1 (homozygous for A), lane 6 and 7: sample 2 (heterozygous GA), lane 8 and 9: sample3 (homozygous for A), lane 10 and 11: sample 4 (homozygous A), lane 12 and 13: Sample 5 (homozygous for G), lane 14 and 15: sample 6 (heterozygous GA), lane 16 and 17: sample 7 (homozygous GA).

high-density lipoprotein (HDL) in Group A for SLC22A2 rs201919874 polymorphism.

In-silico analysis showed a significant damaging impact on the structure of respective proteins ($p<0.05$). The evolutionary conservation of changed amino acids was

analysed and scores were checked on position 199 and 578. The score ranges from 0 to 100 and 100 is considered being the highest. The mutation of T199I had a score of 84 and R578H showed a score of 32. Since the threshold was 25, therefore both SNPs were expected to create

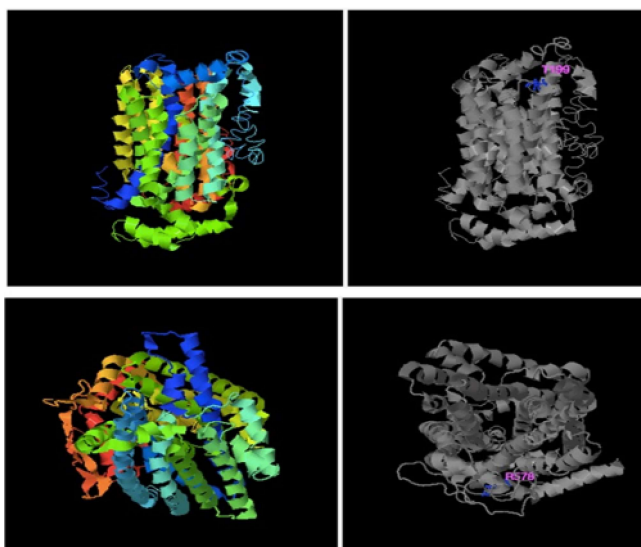


Figure-4: 3D structural model of OCT (*SLC22A2*) and MATE2K (*SLC47A2*) predicted by I-Tasser, (A) Wild structure of *SLC22A2*, (B) Mutant structure of *SLC22A2*, (C) Wild structure of *SLC47A2*, (D) Mutant structure of *SLC47A2*.

significantly damaging structural changes in domains and helix, indicating that I and H both residues were less favourable in position-specific scoring matrix (PSSM).

Five models for each protein were generated and we selected the model with the highest c-score (Figure 4). Both SNPs showed negative value (-2.89 and -0.31). Mutations of *SLC22A2* rs201919874 and *SLC47A2* rs138244461 polymorphisms are associated with the damaging of OCT2 and MATE2K protein structure ($p < 0.05$). Due to *SLC22A2* rs201919874 polymorphism gain of catalytic residue takes place along with loss of loop ($p = 0.0804$), loss of glycosylation ($p = 0.1275$), gain of helix ($p = 0.132$) and gain of molecular recognition features (MoRF) of binding site ($p = 0.1934$). Due to *SLC47A2* rs138244461 polymorphism gain of catalytic residue takes place along with loss of sheet ($p = 0.1907$), loss of methylation ($p = 0.0071$), gain of helix ($p = 0.2059$) and loss of MoRF binding site ($p = 0.0229$).

Discussion

In spite of a lot of advances in its treatment, the rise of T2DM is becoming a health burden globally. So far, nine different classes of drugs have been identified for the treatment of T2DM, but metformin is still considered the first line of therapy worldwide including Pakistan. Genetic polymorphisms in the genes coding for OCT2 and MATE2K have been found to be linked with an altered glycaemic

response to drug metformin.³⁰ In the present study two SNPs *SLC22A2* (OCT2) rs201919874 and *SLC47A2* (MATE2K) rs13824446 were studied with respect to their association with two groups of T2DM.

Different nutrient transporters, including OCT1 and MATE1, could change nutrient homeostasis through several ways.³¹ Various studies have reported that genetic variations in the genes of OCT1 or MATE1 have an impact on their function regarding drug transportation with respect to T2DM.^{32,33} *SLC22A1* rs651154 and *SLC47A1* rs2453583 have been reported to be linked with T2DM.³¹ Therefore, in the current study the distributive characteristics of SNPs *SLC22A2* rs201919874 and *SLC47A2* rs138244461 were investigated in T2DM patients who were on metformin monotherapy and combination therapy with sulfonylureas in comparison with healthy subjects. A statistical difference was found between groups with respect to HbA1c, fasting and random glucose levels. A previous study investigated the influence of rs201919874 (T199I) to the disposition of metformin in healthy individuals and reported that it was significantly associated with increased metformin plasma concentration and reduced renal clearance.³⁴ These findings for rs201919874 (T199I) polymorphism were consistent with this study. The current study found that allele A in *SLC22A2* gene was associated with the therapeutic efficacy of metformin. Significant associations were found in metformin T2DM patients with respect to HbA1c, random blood glucose and fasting glucose whereas no significant association was found in T2DM patients who were on combination therapy along with sulfonylureas. Significant difference was observed in genotypes with respect to HbA1c level between metformin treated patients and those treated with combination therapy.

Recently, a study found that the non-synonymous MATE2K variant Gly211Val was associated with a complete loss of transport activity, mainly because of a decrease in MATE2K protein expression.³⁵ No study has been conducted so far on SNP rs138244461 of MATE2K with respect to clinical response of Metformin in T2DM patients. So the data generated by this study will be novel in its orientation. The effect of *SLC47A2* rs138244461 polymorphism on pharmacokinetics of metformin was also evaluated in T2DM patients. We found that allele T in *SLC47A2* gene is associated with the therapeutic efficacy of metformin. Significant associations were found in metformin T2DM patients with respect to HbA1c, random glucose and

fasting glucose whereas no significant association was found in T2DM patients who were on combination drugs. Similarly, significant difference was observed in genotypes with respect to HbA1c level between Group A and B patients. Thus, there are chances that the effect of the rs138244461 polymorphism gets reduced by having combination therapy of sulfonylureas along with metformin in T2DM patients.

Furthermore, significant differences were observed between genotypes regarding TC, LDL, HDL and TAG values. Patients who were homozygous for AA&TT and heterozygous GA&CT had high levels of cholesterol. When combined effect of GA&AA genotypes were studied together, then HDL was not significant in Group A patients, but significantly associated in Group B patients. In case of CT&TT genotypes, a significant correlation with HDL was found. Recently, through a genome-wide association study, new novel variant (rs11212617) was identified in gene ataxia telangiectasia mutated (ATM).³⁶ This study demonstrated a strong link with the glycaemic control of metformin drug. Thus, a more detailed and comprehensive pharmacogenetic study and research is needed with respect to metformin in T2DM. Any kind of genetic variations in the transporter genes might lead to 'loss-of-function' or 'gain-of-function' thus eventually alter the function of the efflux transporters.

Metformin has been considered the first choice and absolute 'reference drug' for the treatment of T2DM, according to the European Association for the study of Diabetes (EASD), American Diabetes Association (ADA) and International Diabetes Federation (IDF) and guidelines.³⁷ The search to unveil the complex genetic architecture of T2DM is still in the pipeline and the pharmacogenetics of the majority of the oral anti-diabetic drugs that are available for T2DM treatment must be evaluated for better clinical response in patients. In terms of limitations, the sample size was not large enough. Future large-scale studies are needed for the identification of novel loci affecting treatment response, especially considering the fact that the biology of metformin working mechanism is not fully understood. SLC22A2, which encodes OCT2, is reported to be associated with significantly decreased transport activity compared to the reference protein.

Conclusion

SLC22A2 and SLC47A2 variants play an important role in

the clinical efficacy of metformin in T2DM patients. Additional investigations may identify the ethnic variability in the SLC22A2 and SLC47A2 genes and the inter-individual differences in response to metformin.

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