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Research Article

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Genetic Polymorphisms in Metformin Transporter Proteins OCT1 and OCT2 May Influence Metformin Efficacy in Type 2 Diabetes Patients

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Abstract

Background: Elucidation of the genetic variations in drug responses is very instrumental and for this genotyping of the polymorphisms in SLC22A1 (OCT1) gene, the R61C, G401S, M420del, G456R, M408V, P341L and polymorphisms in SLC22A2 (OCT2) gene A270S and T201M was done in healthy controls and the type 2 diabetes mellitus (T2DM) patients. This was done to demonstrate the potential effect of these SNPs in response to metformin in T2DM patients. **Material and Methods**: Two hundred newly diagnosed T2DM patients and healthy controls of Pakistani origin were recruited with age range of 18 to 77 years. Blood samples of the T2DM patients were collected before and three months after metformin therapy and measured through HPLC technique. Genotyping of the selected SNPs were done using various RMS-PCR, RFLP and Allele specific PCR. Patients were grouped as responders and non-responders on the basis of HBA1c levels before and after drug therapy. **Results**: Among the patients, 40.5 % non-Responders and 59.5% were Responders to metformin. Mutant genotypes of the M420del and A270s and heterozygous genotypes of G401, R61C and A270S showed significance effect towards the disease with p=0.021, p=0.000 and p=0.000, p=0.000 and p=0.000 respectively. Only the G/- genotype of M420del showed significantly lesser frequency in non-Responders demonstrating its role in better response of the patients towards metformin therapy. **Conclusion:** Genetic polymorphism of the transporter proteins in our study do not have significant effects on efficacy of metformin. But, need to explore variants of transcription factors as metformin disposition is controlled by multiple transporters.

Keywords: Genetic polymorphism; Type 2 diabetes mellitus; Single Nucleotide Polymorphism; Transporter proteins; Responders; Non-Responders.

Abbreviations: SLC: Solute Carrier; OCT: Organic Cation Transporter; T2DM: Type 2 Diabetes Mellitus; SNP: Single Nucleotide Polymorphism; A1C: Hemoglobin A1C; HPLC: High Performance Liquid Chromatography.

Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by hyperglycemia with elevated blood glucose levels. The disease develops mainly due to physiological anomalies, including insulin secretion, and manifests as a chronic illness over time. The increasing burden of DM is a major healthcare issue worldwide, attributed to modern lifestyles, obesity, changes in nutritional preferences, and lack of physical exercise. Approximately 463 million people had DM in 2019, with this number expected to rise to 700 million by 2045, posing a major threat of multi-organ failures, including heart diseases [1-3].

Typically, DM is classified as type 1 (5-10%), type 2 (~90%), and gestational diabetes (~5%). In type 2 DM, insulin response is reduced, often termed insulin resistance. Major risk factors include family history, lack of physical exercise, obesity, and unhealthy diet [3,4]. Studies show that both environmental and genetic factors contribute to DM. Genetic insights are gained through candidate gene approaches, familial investigations, and sequencing analyses. Understanding DM genetics is crucial for risk assessment, early interventions, and clinical outcomes [5,6].

In type 2 DM, genetic factors significantly influence disease development and progression. Linkage analysis and genome-wide association studies highlight various genes and loci involved in type 2 DM pathogenesis and drug response. Identifying novel genetic variants is vital for understanding type 2 DM pathophysiology and improving therapeutic responses [7-9].

Therapeutic modalities for type 2 DM include synthetic entities and natural compounds. Metformin, an insulin sensitizer, is a first-line drug demonstrating efficient glucose-lowering effects by targeting various organs and microbiomes. However, therapeutic responses vary among patients due to genetic variations/polymorphisms. Identifying genetic alterations, such as single nucleotide polymorphisms, is essential for assessing drug response [10-12]. This study investigates single nucleotide variations in SLC22A1 and SLC22A2genes in 200 type 2 DM patients, comparing results with healthy controls to understand genetic variants' role in metformin response.

Material and Methods

Ethical statement: This study was approved by ethical review committee of university. All procedures performed in study on human participants were un accordance with ethical standards for the institutional research and with 1964 Helsinki declaration. Initially a total of 260 patients with T2DM from Jinnah-Allama Iqbal Institute of Diabetes and Endocrinology (JAIDE), Jinnah Hospital, Lahore and 200 healthy controls were included in the study. However, because of inclusion criteria and later stage drop out of patients the sample size was reduced to 200 in each group. T2DM was diagnosed in patients according to the criteria of the American Diabetes Association [13]. Drug naïve patients were included in the present study with baseline A1C levels ranging between 7-9%. Patients having abnormal renal functions (raised creatinine levels ≥ 1.5 mg/dl in males and ≥ 1.4 mg/dl in females), cirrhosis of liver, congestive heart failure, pregnant females and patients with peptic ulcer disease or inflammatory bowel disease were excluded from the study.

All participating subjects gave a written consent to be included in the study. Metformin (Tab Glucophage) was given starting with low dose of 500mg twice daily followed by a relatively higher dose after two weeks titrated to optimum dose ranging between 1500-2000 mg according to their glycemic control. Adverse effects, if any of metformin therapy, were monitored continuously during the follow-up phase. Similarly, age and sex matched two hundred healthy individuals were also included in this study without any history of diabetes mellitus.

Following the blood sampling, A1C levels estimation was done by A1C analyzer (TD4611A TAI Doc). Baseline A1C was taken at the time of enrolment and second time sampling for A1C was done three months after treatment with metformin. As there is no accepted criterion in the clinical cut-off point to divide patients into Responders and non-Responders, we selected the criteria empirically, based on our clinical experiences and previous studies (Amani et al., 2024) as follows: Responders and non-Responders (patients whose A1C levels had decreased by≥ 0.8% or < 0.8% from the baseline within 3 months of metformin therapy respectively).

Further, the whole blood samples were processed for DNA isolation using TIANamp Genomic DNA Kit (Tiangen Biotech, China). Three months after metformin treatment; blood samples for estimation of the plasma levels were taken twice. First, the sampling was done 10-12 hours after the last dose of metformin at night and was considered as trough level. Second time sampling was done 3 hours after the morning dose of the drug referring as peak levels. Blood samples (5 ml) were collected in 0.5 M EDTA (ethylene diamine tetra acetate) containing vials and centrifuged for 15 minutes at approximately 5000 rpm. The plasma fraction was transferred to a labeled polypropylene tube and kept frozen in a freezer at -80O °C until assayed.

Genotyping and plasma metformin levels estimation

PCR ingredients and their concentrations used in the reactions are given in table 1. Genotyping for SNPs M408V, G401S, M420del, G465R of SLC22A1 gene was done using PCR-RFLP method as mentioned in supplementary table 2. SNPs R61C, P341L of SLC22A1 and T201M of SLC22A2gene were genotyped by ARMS-PCR as mentioned in supplementary table 3 and A270S was genotyped using allele specific PCR as mentioned in supplementary table 4. PCR profile is given in Figures 1-4 in supplementary data. PCR based amplified products were run on 2-3% agarose gel electrophoresis. Gel pictures representing the genotypes for the SNPs M408V, M420del, G401S, P341L, R61C and A270S are shown in Figures 5-10 respectively.

The concentration of metformin HCl in blood plasma was measured by a validated reversed phase-high performance liquid chromatography (RP-HPLC) method (LC-20AT VP pump, an SIL-20AC HT auto-sampler, MetaSil-Phenyl column (250 x 4.6) mm, 5 µm). The mobile phase was a mixture of sodium dihydrogen phosphate (0.02 M; pH= 7.0) and acetonitrile (70/30 v/v). A constant flow rate of 1 ml/min was maintained. The mobile phase was filtered by passing through a 0.45 µm membrane filter and vacuum-degassed before use. Detection was carried out at a wavelength of 236nm. A total of 500 µL plasma and 500 µL acetonitrile was added to an Eppendorf tube (1.5 ml) followed by vortex for 3 minutes and centrifuged at 10,000 rpm for 10 min. Upper layer was separated and filtered by using 0.2-micron sterile syringe filters and transferred into auto-sampler vial, 80 µL was subsequently injected into HPLC system. The assays were validated by performing different procedures. Calibration standards were prepared in human plasma by spiking a known amount of metformin to control plasma (drug-free) samples to produce. Calibration curves were generated by measuring the detector response as peak-area versus concentration of the drug. The accuracy and precision were assessed by measuring the intraday and inter-day Coefficient of Variation (CV) at different Quality Control (QC) concentrations: low, medium and high. Recovery was also measured14.

Statistical analysis

Genotype frequencies were estimated for each SNP and genotypic comparison was done across the patients and healthy people and between Responder group and non-Responder group within the diabetic patients and their corresponding ODDs ratios were also calculated using SPSS 27. Peak and trough levels of plasma metformin were measured in the Responders and Non-Responders and the difference in the means within the two groups was measured by independent t-test using GraphPad Prism 6.

Results

Cohort Summary

Out of 200 patients, 40% (n=81) patients were categorized as non-Responders and 60% (n=119) as Responders on the basis of variation in A1C three months after treatment with metformin. The median age of the patients was 49 years whereas in Responders it was approximately 50 years and in non-Responders it was approximately 49 years. Comparatively, more females (69%, n=138) were affected in the present study as compared to males (31%, n=62). Amongst all the females, 88 (64%) were Responders and 50 (36%) were non-Responders. In case of males, 52% (n=32) were Responders and 48% (n= 30) were non-Responders. Though, the females were more in the study group but the gender distribution was not different in the two response groups significantly. Study showed that 54% of the patients had positive family history of Diabetes whereas 36% patients lacked the family history. It was found that patients responded to the metformin irrespective of the fact that they have diabetes in their relatives or not. More patients (65.5%) had diabetes from time duration of less than 5 years and those who had diabetes of more than 5 years responded well to metformin therapy. It was also shown that patients responded to the metformin drug therapy regardless of the time duration from which they had diabetes.

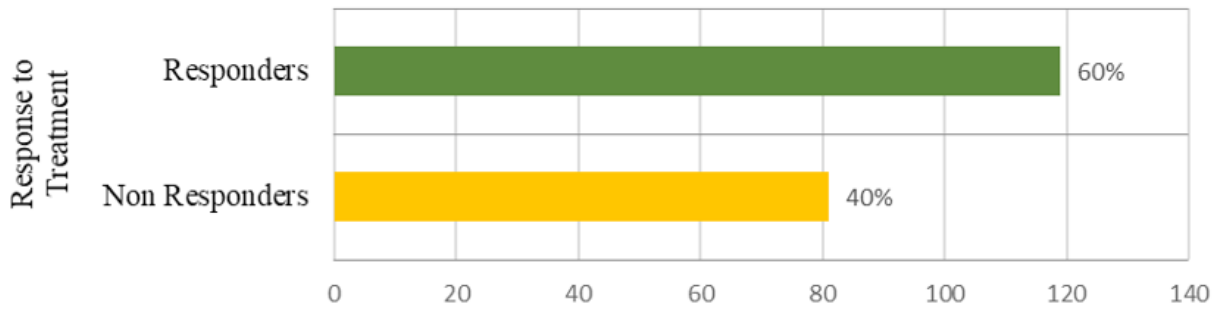

Figure 1: Patients distributions between the two groups i.e. Responders and Non-Responders on the basis of response to metformin therapy.

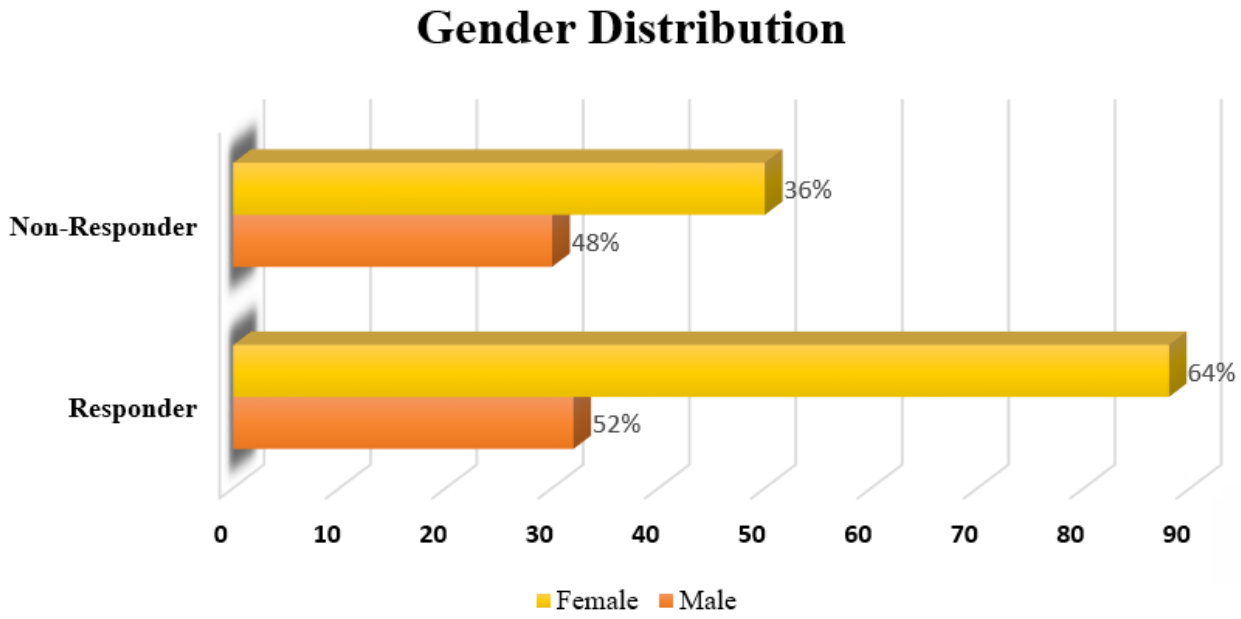

Figure 2: Gender Distribution of the patients between the two response groups i.e. Responders and Non-Responders.

Genotypic and Allelic Distribution

Basic information of the candidate SNPs of SLC22A1 and SLC22A2gene and their minor allele frequency in the Responder and the non-Responder group of the diabetic patients is given in the Table 1.

SNP *ID*	Gene	Position	Base Change	Role	MAF		OR [95% CI]	*p*-Value
					Responders	Non-Responders		
rs34059508	***SLC22A1***	160154805	G/A	Exon 8	0.0	0.0	---	---
rs628031		160139813	A/G	Exon 7	0.29	0.32	0.87[0.56-1.34]	0.53
rs72552763		160139851	3del G	Exon 7	0.29	0.25	0.78 [0.50-1.24]	0.30
rs34130495		160139792	2G/A	Exon 7	0.31	0.25	0.74 [0.47-1.16]	0.19
rs2282143		160136611	6C/T	Exon 6	0.1	0.09	0.84 [0.42-1.67]	0.62
rs12208357		160122116	4C/T	Exon 1	0.13	0.14	0.91 [0.50-1.66]	0.77
rs316019	***SLC22A2***	160249250	5G/T	Exon 4	0.35	0.35	1.03 [0.67-1.57]	0.88
rs145450955		160250619	C/T	Exon 3	0.00	0.00	---	---

Table 1: Basic information and the minor allele frequency of the corresponding SNPs under study in the Response groups.

G465R and T201M showed no variations in genotypes so these were not proceeded for further analysis. None of the allele of the rest corresponding SNPs showed any association with response to metformin. Analysis of carrier genotypes (genotypes having polymorphic allele of a particular SNP) showed the effect of presence of even a single allele in the genotype.

The genotype frequencies of the SNPs in the controls and T2DM patients and their ODDs ratios along with their association are given in table 2. The results showed that the deletion of M420del and the heterozygous genotypes of G401S and R61C A270S has a significant association with the development of disease with ODDs of 2.51 with p=0.021, 5.02 p=0.000 and 3.72 p=0.000 respectively. Whereas, the heterozygous and mutant genotypes of A270S showed protective effect of the genotypes toward the disease. This SNP may reduce the risk of the development of the disease in combination with other factors.

Genotype	Controls	Cases	OR	*p-value*
	n (%)	n (%)	[95% CI]	
Genotypes				
rs628031 (M408V)				
A/A	27 (14%)	19 (10%)	1.00	
A/G	90 (46%)	85 (42%)	1.34 (0.70-2.59)	0.419
G/G	81 (40%)	97 (48%)	1.70 (0.88-3.28)	0.113
M420del				
G/G	134 (68%)	112 (56%)	1.00	
G/-	54 (27%)	68 (34%)	1.51 (0.97-2.33)	0.056
-/-	10 (5%)	21 (10%)	**2.51 (1.14-5.56)**	**0.021***
G401S				
G/G	140 (71%)	100 (50%)	1.00	
G/A	24 (12%)	86 (43%)	**5.02 (2.98-8.44)**	**0.000***
A/A	34 (17%)	15 (7%)	0.62 (0.32-1.19)	0.167
P341L				

C/C	171 (86%)	164 (82%)	1.00	
C/T	27 (14%)	35 (17%)	1.35 (0.78-2.33)	0.261
T/T	0 (0%)	2 (1%)	NA (0.00-NA)	0.999
R61C				
C/C	182 (92%)	150 (75%)	1.00	
C/T	16 (8%)	49 (24%)	**3.72 (2.03-6.80)**	**0.000***
T/T	0 (0%)	2 (1%)	NA (0.00-NA)	0.999
A270S				
C/C	14 (7%)	84 (42%)	1.00	
C/A	94 (47%)	93 (46%)	**0.16 (0.09-0.31)***	**0.000***
A/A	90 (46%)	24 (12%)	**0.04 (0.02-0.09)***	**0.000***

Table 2: Genotypic distribution and association with the diseased.

The table 3 was utilized to explore the genotypic of the genetic variants among patients diagnosed with diabetes, focusing on their correlation with levels of glycemic control. The genotype frequencies of the SNPs in the Responders and Non-Responders are given in table 3. None of the SNP showed any association with the response of the patients toward the metformin therapy but the G/- heterozygous genotype of the M420del as shown in the table 3. Table 4 represents the carrier genotypic distribution of the patients with respect to glycemic controls of the patients.

Genotype	**Responders**	**Non-Responders**	***p-value***	**OR**
	n (%)	**n (%)**		**[95% CI]**
Genotypes				
rs628031 (M408V)				
A/A	11 (9.2%)	8(9.9%)		1.00
A/G	47 (39.5%)	37 (45.7%)	0.944	0.964 (.344-2.698)
G/G	61 (51.3%)	36 (44.4%)	0.623	1.291 (.466-3.577)
M420del				
G/G	60 (50.4%)	51 (63.0%)		1.00
G/-	49 (41.2)	19 (23.5%)	**0.021***	**2.179 (1.127-4.214)**
-/-	10 (8.4%)	11 (13.6%)	0.365	0.634 (0.236-1.702)
G401S				
G/G	54 (45.4%)	45 (55.6%)		1.00
G/A	56 (47.1%)	30 (37.0%)	0.133	0.625(0.339-1.153)
A/A	9 (7.6%)	6 (7.4%)	0.864	0.905(0.291-2.815)
P341L				
C/C	96 (80.7%)	67 (82.7%)		1.00
C/T	21 (17.6%)	14 (17.3)	0.498	1.420 (0.515-3.911)
T/T	2 (1.7%)	0 (0.0%)	1.000	NA
R61C				
C/C	87 (73.1%)	62 (76.5%)		1.00
C/T	32 (26.9%)	17 (21.0%)	0.498	0.788(0.396-1.569)
T/T	0 (0.0%)	2 (2.5%)	0.999	NA
A270S				
C/C	51 (42.9%)	33 (40.7%)		1.00

C/A	53 (44.5%)	39 (48.1%)	0.838	1.067(0.572-1.991
A/A	15 (12.6%)	9 (11.1%)	0.882	0.930(0.355-2.432)

Table 3: Genotypic distribution and association of SNPs with the response of the T2DM patients towards metformin therapy.

Genotype	Responders	Non-Responders	OR	*p-value* adjusted*
	n (%)	n (%)	[95%CI]	
Genotypes				
RS628031 (M408V)				
A/A	5 (9.1%)	6 (13.3%)	0.59	1.00
A/G & G/G	50 (90.9%)	39 (86.7%)		0.70 (0.19-2.62)
G401S				
G/G	30 (54.5%)	28 (62.2%)	0.54	1.00
G/A & A/A	25 (45.5%)	17 (37.8%)		0.76 (0.33-1.79)
M420Del				
G/G	34 (61.8%)	33 (73.3%)	0.28	1.00
G/-&-/-	21 (38.2%)	12 (26.7%)		0.61 (0.25-1.50)
R61C				
C/C	49 (89.1%)	42 (93.3%)	0.46	1.00
C/T & T/T	6 (10.9%)	3 (6.7%)		0.58 (0.13-2.58)
A270S				
C/C	32 (58.2%)	24 (53.3%)	0.52	1.00
C/A & A/A	23 (41.8%)	21 (46.7%)		1.33 (0.57-3.11)
P341L				
C/C	41 (74.5%)	37 (82.2%)	0.45	1.00
C/T & T/T	14 (25.4%)	8 (17.8%)		0.68 (0.25-1.87)
*p-value after adjustment with age and gender				

Table 4: Carrier genotypes, their frequency and ODDs ratio of the candidate SNP under study in Response groups.

Plasma Metformin Levels in Response Groups

Plasma levels of metformin are given in table 5 with their p-values. The peak plasma levels of metformin were statistically different in both groups whereas, trough plasma level of metformin was not different between the two groups.

	Responder	Non-Responder	*P-value*
	(Mean ±SEM)	(Mean ±SEM)	
Peaks plasma drug level (ng/ml)	975.89±41.75	1049.22±50.73	**0.048***
Trough plasma drug level (ng/ml)	221.25±14.39	248.34±28.01	0.55
Difference between the peak and trough within the two groups	750.82±41.28	800.12±50.51	.690
*p-value significant <0.05			

Table 5: Plasma levels of metformin in diabetic patients three and ten hours of metformin intake.

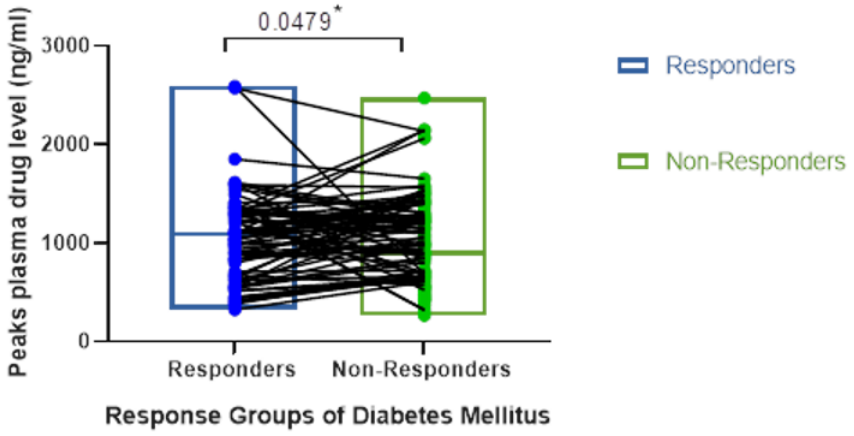

Figure 3: Peak plasma levels of the drug in the T2DM patients of the two response groups.

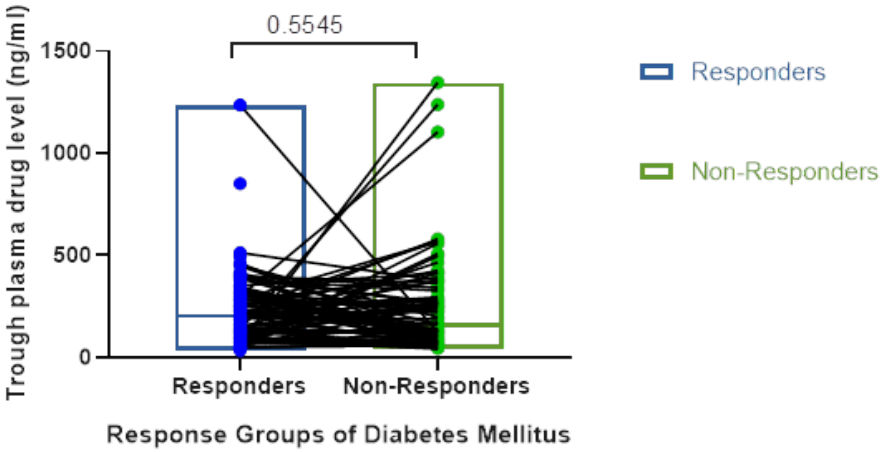

Figure 4: Trough plasma levels of the drug in the T2DM patients of the two response groups.

Locus	SNP	Allele change	Effect size (SE)	P	Variance explained (%)
SLC22A1	M408V	A→G	-0.183 (0.97)	0.061	2
	M420 del	3del G	-0.045 (0.108)	0.681	0.1
	G401S	2G→A	-0.083 (0.104)	0.423	0.4
	P341L	6C→T	-0.195 (0.161)	0.227	0.7
	R61C	4C→T	0.056 (0.136)	0.683	0.1
SLC22A2	A270S	5G→T	-0.067 (0.110)	0.544	0.3

Table 6: Assessment of the association between the SLC22A1 and SLC22A2 genes polymorphisms and T2DM HBA1c linear regression in comparison to controls.

Polymorphisms of SLC22A1 and SLC22A2 were subjected to the multiple regression analysis to predict the change in HBA1c levels after the treatment with metformin therapy and glycemic control in T2DM patients. Statistical model was constructed and the model accounted approximately 3.6% of the variance in HBA1c i.e. the variance by all of the mentioned SNPs in table 5 is 3.6%. The R61C received the strongest weight in impacting the HBA1c level in the model followed by M420del, A270S, G401s, M408V and P341L (β=0.136, 0.108, 0.110, 0.104, 0.97, 0.161 respectively). The unique variance explained by each in predicting the change in HBA1c levels after the treatment is given in the table 5. In the presence of the mutant alleles of the SNPs M408V, M420 del, G401S, P341L, R61C and A270S, there is a change of 0.056, -0.045, -0.067, -0.083, -0.183 and -0.195 unit in the HBA1c levels when treated with the metformin therapy respectively. These all findings were not statistically significant but addition of more samples in analysis may bring the significance of these SNPs in the response of patients with metformin therapy.

Discussion

Genetic variants in the transporter proteins play a vital role in drug response and contribute in differential glycemic response to metformin. SLC22A1 and SLC22A2genes are highly polymorphic, and several polymorphisms have been reported in different populations. These variations are responsible for the differences in transporter function eventually leading to variation in response towards metformin exposure [15-17]. Considering this, the study was aimed to identify association of SLC22A1 and SLC22A2 polymorphism and response towards metformin in type 2 DM patients.

The research aimed to detect the polymorphisms of M408V, M420del, G401S, P341L, R61C and G465R of transporter protein gene SLC22A1 and T201M and A270S of SLC22A2 by genotyping and to see the association of these SNPs with the response of metformin. Only M420del SNP showed association with the response of patients towards metformin. Various studies have showed association of these polymorphisms with the response of the DM patients towards metformin therapy. It is shown that Indian individuals carrying the 'GG' genotype or 'G' allele for SLC22A1 gene variant M408V (rs628031) G/A are better responders for Metformin monotherapy (Singh et al., 2023). While other populations showed no effect of this polymorphism with response of patients [18,19].

An association was observed between the response of metformin in Responders and non-Responders, it was shown that the G/- genotype of M420del showed significantly lesser frequency in the non-Responders group showing its part in better response of the patients towards metformin therapy. Presence of rare genotype except G/- genotype of M420del showed significant effect on altering the transport efficiency of the studied OCTs. Similar findings have been reported by the scientific community where researchers have found association of genetic variations in the gene and response towards metformin treatment in diabetic patients [20-22]. No other variant has showed any influence over decreasing the plasma levels of metformin.

Regarding the two SNPs of SLC22A2 gene; firstly, in the A270S, the heterozygous CA genotype and the rare AA genotype were present more in the control group with statistically significant difference whereas none of the genotypes showed any association amongst Responders and non-Responders. In reported studies, the researchers have demonstrated the links between genetic variables of SLC22A2 gene and drug response [23,24]. Secondly, the genotyping results for the T201M showed no variation in the two study groups i.e. patients with type 2 diabetes and healthy controls. Wild type genotype (GG) was observed in the two groups (100%) and was not further proceeded for analysis. Polymorphism of T201M has also been investigated by the researchers to explore the link between this genetic variation, diabetes and the treatment response [25,26].

Shu et al in 2003 employed site directed mutagenesis strategy in order to see the effect of M420del, M408V and G465R variants on transport efficacy of OCT1. Study exhibited increased area under the curve (AUC) of metformin showing its altered pharmacokinetics in diabetic patients. On the other hand, it was shown that in the presence of minor allele of M420del, R61C and G401S, efficiency of OCT1 was reduced leading to a decreased metformin uptake [27]. Tarasova and her co-workers in 2012 showed M420del, M408V and A270S had no negative influence on the function of OCT1 neither genotypic nor allelic on Latvian population [28].

Ligong Chen and his co-workers in 2010 analyzed seven variants of SLC22A1 gene including M420del, M408V, R61C G401S and G465R of OCT1 through site directed mutagenesis technique to find the influence of these variants over the transport activity of OCT1. They found consistent results with the present study i.e., these variants exerted no influence on the transport activity of OCT for metformin and had no association of these variants with the response of the patients [29].

Kerb and his co-worker did a site directed mutagenesis experimental work showing the effect of M420del, R61C, G401S and G465R as functional variant OCT1. They showed the concordant results as the present study i.e., no changes in substrate affinity and selectivity were detected after mutagenesis inserting the rare variant allele of M420del, R61C and G401S. However, it was further demonstrated that G465R may alter the metformin effect in T2DM [30]. Similarly, in study with 189 Caucasian patients using metformin for approximately 160 days, association of R61C, G401S, M420Del, and G465R with relative A1C change was not

replicated [31].

Overall, this study demonstrates that genetic variants of transporter proteins do not play substantial role in efficacy of metformin. Though, this is the first study done related to the pharmacogenetics of metformin in Pakistani population, but we can clearly say that glycemic response of metformin is not dependent on genetic polymorphism.

The trough plasma concentrations of metformin tended to be higher in non-Responders than those in the Responders suggesting that they may have a larger pharmacodynamics response to metformin. However, this preliminary study displayed paradoxical effects. So, we need to work on further pharmacokinetic studies which might affect the clinical response of metformin. This drug is not metabolized in-vivo, the gastrointestinal absorption, hepatic uptake and renal excretion of metformin are mediated by OCTs and multidrug and toxin extrusion proteins (MATEs) and other proteins. Metformin disposition is governed by multiple transporters rather than a single transporter.

Recently, it was revealed that metformin is not only involved in AMPK activating mechanism but also inhibits the mitochondrial respiratory chain complex-I. According to some researchers this complex is the primary target of metformin therefore, we assume that defects at this level may lead to failure to achieve adequate glycemic response with metformin. OCT2 is the best predictor of metformin clearance; studies on the clinical relevance of these findings are warranted to see its association with genetic polymorphism of SLC22A2 gene.

Further research is required in order to authenticate the findings of present study with larger sample size and more pharmacokinetics parameters along with other transporter protein polymorphisms. Furthermore, as genetic testing becomes more accessible and cost effective; a shift may be seen from prescribing certain drugs based on clinical characteristics of certain populations towards a more tailored therapy taking into account individual genetic variations. Patient networking and database registration should be optimized in clinical care setups to improve follow up for future studies.

Conclusion

Genetic polymorphism of transporter proteins based on our study does not have significant effects on efficacy of metformin rather we believe that response is more dependent on the variants of transcription factors as metformin disposition is controlled by multiple transporters which control the signaling pathway of metformin. The role of genetic variations with respect to therapeutic outcomes must be further verified via clinical trials while leading to the concept of personalized treatment. There is a need for more research in sizable cohorts of previously examined patients from various ethnic/genetic and cultural backgrounds. Future development of specialized tools for improved therapy will be made possible by a greater knowledge of metformin metabolomics, T2DM, pharmacogenetics, and gene mutations in T2DM patients.

Disclosure

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Declarations

Ethical Approval and Consent to participate: All the participants provided written informed consent and the study was duly approved by Ethical review committee of University of Health Sciences, Lahore.

Consent for publication: The participants also provided the consent for the publication/s of data obtained from the study.

Availability of supporting data: Will be provided on demand

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