Original Article

Influence of Genetically Polymorphic GLTU4 on Insulin Remedy Response in Type 1 Diabetic Patients

Mohammed Suhail Abed and Mohammed Ibrahim Rasool

ABSTRACT

Objective: To investigate the association between the *SLC2A4* rs5435 (T>C) single nucleotide polymorphism and glycemic response to exogenous insulin in Iraqi patients with type 1 diabetes mellitus.
Study Design: A case–control study
Place and Duration of Study: This study was conducted at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kerbala University, Iraq from 1st December 2023 to 31st December 2024.
Methods: This study was conducted involving 100 patients with type 1 diabetes and 30 healthy controls assessed fasting serum glucose and HbA1c levels and genotyped the SLC2A4 rs5435 (T>C) polymorphism using allele-specific polymerase chain reaction. Genotype and allele frequencies were compared between groups and analyzed for associations with glycemic parameters.
Results: Among patients with type 1 diabetes, genotype frequencies were 89% for homozygous wild-type (TT), 6% for heterozygous mutant (TC), and 5% for homozygous mutant (CC). In the healthy control group, the corresponding frequencies were 90% (TT), 6.6% (TC), and 3.3% (CC). The SLC2A4 rs5435 (T>C) polymorphism showed no significant differences in genotype or allele distribution between patients and controls and was not associated with FSG or HbA1c levels, indicating it does not affect response to exogenous insulin therapy.
Conclusion: The SLC2A4 rs5435 (T>C) polymorphism does not affect glycemic control or response to exogenous insulin in Iraqi type 1 diabetes patients, though larger multicenter studies are needed to confirm these results and investigate genetic factors influencing insulin responsiveness.
Key Word: Glucose transporter type 4 (GLUT4), Polymorphism, Allele-specific polymerase chain reaction, Insulin response

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INTRODUCTION

Glucose is a central metabolic substrate produced by photosynthesis and is essential for energy metabolism in all forms of life. In higher organisms, it also functions as a signaling molecule that maintains energy balance by regulating hormones, enzymes, gene expression, and neural activity, with its effects on lipogenic and glycolytic genes mediated by the transcription factor ChREBP.[1]
Through the glucosamine route, glucose flux controls transcription factor activity by encouraging O-GlcNAcylation.[2]

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Through the generation of NAD+, glycolysis influences Sirt1 deacetylase activity, a key transcriptional regulator that has come to light.[3] Histone changes brought forth by glucose metabolism through the synthesis of Acetyl-CoA lead to epigenetic regulation of gene expression.[4] Rising glucose levels regulate glucose-sensitive neurons in the brain and insulin secretion from pancreatic β-cells, influencing feeding behavior, energy expenditure, and overall glucose balance. Glucose homeostasis primarily depends on cellular glucose uptake, which is controlled by glucose transporter expression on cell surfaces in most tissues (except hepatocytes and β-cells). The existence of multiple glucose transporter isoforms with different kinetic properties and regulated surface expression enables precise control of glucose uptake, metabolism, and signaling to maintain metabolic homeostasis.[5]
Since its identification as a distinct glucose transporter isoform by James et al. in the late 1980s[6], GLUT4 is a well-studied glucose transporter crucial for glucose homeostasis, with extensive research highlighting its complex regulation by insulin. Since the early 1980s, insulin has been known to promote GLUT4 translocation from intracellular compartments to the cell surface in adipocytes and skeletal muscle.[7-9] In the three decades that have passed, a great deal has been

discovered about this regulation's specifics.[10] GLUT4 is expressed in skeletal muscle, adipocytes, and cardiomyocytes and is the primary transporter responsible for insulin-stimulated glucose uptake in muscle and fat, with skeletal muscle accounting for most glucose disposal. Its expression and activity increase during exercise or after high-carbohydrate intake to meet elevated energy demands.[11] Insulin stimulates GLUT4 translocation through two main pathways: a PI3K-dependent pathway involving insulin receptor activation and IRS-1/IRS-2 phosphorylation, and a PI3K-independent pathway mediated by the c-Cbl/CAP complex that activates TC10 in lipid rafts. Both pathways promote GLUT4 movement to the plasma membrane in muscle and fat cells, and inhibition of the c-Cbl/CAP pathway reduces insulin-stimulated GLUT4 translocation in adipocytes.[11,12]

In skeletal muscle, exercise stimulates GLUT4 translocation through a PI3K-independent pathway in which muscle contraction activates AMPK, leading to increased GLUT4 movement to the plasma membrane and enhanced glucose uptake to meet elevated energy demands.[11,13] Insulin resistance can be assessed at the cellular level without liver involvement, and defects in insulin signaling do not always reduce glucose uptake due to compensatory regulation of SLC2A4 expression and GLUT4 translocation. Decreased glucose uptake in specific tissues may not impair whole-body glucose clearance because of tissue mass compensation. Whole-body insulin resistance depends on total GLUT4 content across tissues, with skeletal muscle as the primary site in non-obese individuals and adipose tissue playing a larger role in obesity. Many studies emphasize membrane GLUT4 alone, underscoring the need to consider total cellular GLUT4 for a complete understanding of glucose clearance.[14]

METHODS

This case-control study was conducted at Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kerbala University, Iraq from 1st December 2023 to 31st December 2024 vide letter No. 4545/QM/Approval/ KD8399JD Date: November 11, 2023 analyzed the rs5435 SNP in 100 type 1 diabetic patients (56 females, 44 males; aged 6–18) on basal-bolus insulin and 30 age-matched healthy controls (12 females, 18 males). Venous blood samples were collected in EDTA tubes for DNA extraction and SNP analysis. Fasting serum glucose was measured using a UV-based enzymatic method, where glucose is converted to glucose-6-phosphate by hexokinase, then oxidized to gluconate-6-phosphate by glucose-6-phosphate dehydrogenase, producing NADPH, which is quantified photometrically and is proportional to glucose concentration.[15]

Hemoglobin A1c was measured from EDTA blood using the Cobas Integra 400+ analyzer with a TTAB-based hemolyzing reagent to prevent leukocyte interference. The assay detected all beta-chain N-terminal glycated hemoglobin variants, forming soluble antigen-antibody complexes, while excess antibodies were bound by polyhapten reagents into insoluble complexes quantified by turbidimetry.[15]

The sample was agitated for a minimum of 10 minutes at room temperature using a rotisserie shaker. A microcentrifuge tube was prepared with 20 µL of proteinase K (PK) solution. Then, 200 µL of the blood sample was added to the tube containing the PK solution and briefly mixed. Next, 200 µL of cell lysis buffer (CLB) was added, and the mixture was vortexed for at least 10 seconds. Blood samples were incubated at 56 °C by using a water bath. After taking the tube out of the water bath, 250 µL of binding buffer (BB) was added, and the mixture was vortexed for a minimum of 10 minutes. The content of the tube was transferred to the binding column, and it was centrifuged for 1 minute at maximum speed (15000 RPM) to enhance filtration (passage of lysate from the binding tube to the collection tube). The collection tube containing the lysate was removed and discarded. The binding column was transferred to a new collection tube. 500 µL of column wash solution (CWS) was added to the binding column, which was then centrifuged at maximum speed for 3 minutes. The flow through was discarded. Step 10 was repeated two more times, resulting in a total of three washes. The binding column was placed in a clean microcentrifuge tube. 75 µL of nuclease-free water was added to the binding column, followed by centrifugation at maximum speed for 1 minute. The binding column was discarded, and the eluate was saved.

Polymerase chain reaction was used to amplify the SLC2A4 rs5435 gene with primers designed via Primer-BLAST and obtained from Macrogen, Korea. Lyophilized primers were dissolved to 100 pmol/µl stocks, diluted to 10 pmol/µl working solutions, and stored at –20 °C.[16] PCR conditions were optimized through multiple trials to determine the ideal annealing temperature, DNA and primer concentrations, and number of cycles.

Agarose Gel Electrophoresis.[17] To prepare the agarose gel, 1.5 grams of agarose powder were mixed with 70 milliliters of TBE (Tris-Borate EDTA) buffer (pH 8) and heated to boiling until the agarose was completely dissolved. The gel solution was stirred to ensure it was well mixed and free of bubbles, resulting in a clear solution. The solution was then allowed to cool to a temperature of 50-60°C. 10 µL of red-safe nucleic acid stain was added to the gel. A comb was placed at one end of the tray to form wells for loading the PCR product samples. The agarose solution was poured into the tray and left to solidify at room temperature for 30 minutes, after which the comb was carefully removed from the gel. The gel was installed in a gel electrophoresis tank. TBE buffer was added to the tank

until it rose three to five millimeters above the gel's surface. Five microliters of DNA ladder were put into one agarose gel well, and five microliters of each PCR product were put into the remaining wells. The voltage of the electrophoresis device was adjusted to produce an electrical field of five volts for every centimeter that separated the cathode and anode. After the run was completed, a UV transilluminator set to 360 nm was used to visualize the bands. A digital camera was then used to capture an image of the gel. The data was entered and analyzed through SPSS-25.

RESULTS

Fasting serum glucose levels in patients varied widely (57–400 mg/dl) with a high mean of 208.28 mg/dl, whereas control participants showed a narrower range (91–118 mg/dl) and a much lower mean of 104.96 mg/dl (Fig. 1). HbA1c ranged from 8.5% to 15.1%, with a mean of 10.77% for patients while for control participants, HbA1c ranged from 4.1% to 5.7% with a mean of 4.97% (Fig. 2). The rs5435 T>C gene polymorphism produced a distinct 355-bp band, with the amplicon size confirmed by comparison to a 100–1500 bp DNA ladder (Fig. 3).

Patients were grouped into three SLC2A4 rs5435 T>C genotypes: TT, TC, and CC. Among the 100 patients, the TT genotype was predominant (89%), followed by TC (6%) and CC (5%). Control participants were categorized into TT, TC, and CC genotypes for the SLC2A4 rs5435 T>C polymorphism. As indicated in Table 8, among the 30 controls, the TT genotype was predominant (90%), followed by TC (6.6%) and CC (3.3%) [Table 1].

Table 2 displays LSD among biochemical parameters means for SLC2A4 rs5435 genotypes of study participants. Table 3 displays the HWE for the SLC2A4 rs5435 genotypes among control participants. Table 4 displays the HWE for the SLC2A4 rs5435 genotypes among the patients. Table 5 displays the odds ratio of SLC2A4 rs5435 genotypes among study participants.

Table No. 1: Allele frequencies of SLC2A4 rs5435 T > C gene polymorphism among type 1 diabetic and healthy controls patients

Genotype	Type t diabetic n=100)		Healthy controls (n=30)	
	No.	%	No.	%
TT	89	89.0	27	90.0
TC	6	6.0	2	6.6
CC	5	5.0	1	3.4

Table No. 2: LSD among biochemical parameter means for SLC2A4 rs5435 genotypes of study participants

Variable		Genotypes	N	Mean	Std. Deviation	Std. Error
HbA1c	Control	TT	27	4.96b	0.43	0.08
		TC	2	5.00b	0.14	0.10
		CC	1	5.20b	0.00	0.00
	Patients	TT	89	10.81a	1.82	0.19
		TC	6	10.80a	2.12	0.86
		CC	5	10.10a	1.44	0.64
LSD				2.56		
FSG	Control	TT	27	104.33b	7.93	1.52
		TC	2	112.50b	4.94	3.50
		CC	1	107.00b	0.00	0.00
	Patients	TT	89	209.22a	89.05	9.43
		TC	6	212.66a	82.05	33.49
		CC	5	186.20a	97.22	43.47
LSD				88.22		

Table No. 3: HWE for SLC2A4 rs5435 genotypes among control participants

Genotypes	TT	TC	CC	P-value
Observed	27	2	1	
Expected	26.13	3.73	0.13	0.011
HWE-freq.	87.11%	12.44%	0.44%	
Allele freq.	T=56 (93.3%)		C=4 (6.67%)	

The null hypothesis is rejected because the population is at H-W equilibrium

Table No. 4: HWE for SLC2A4 rs5435 genotypes among the patients

Genotypes	TT	TC	CC	P-value
Observed	89	6	5	
Expected	84.64	14.72	0.64	<0.0001
HWE-freq.	84.64%	14.72%	0.64%	
Allele freq.	T=184 (92%)		C=16 (8%)	

The null hypothesis is rejected because the population is at H-W equilibrium

Table No. 5: Odds ratio of SLC2A4 rs5435 genotypes among study participants

Genotypes	Control	Patient	Odds Ratio (95%CI)	P-value
TT	27	89	Reference=1	
TC	2	6	0.91 (0.17-4.77)	0.91
CC	1	5	1.52 (0.17-13.55)	0.71
Genotypes/Female	Control	Patient	Odds Ratio (95%CI)	P-value
TT	11	51	Reference=1	
TC	0	2	1.12 (0.05-24.86)	0.94
CC	1	3	0.65 (0.06-6.82)	0.71
Genotypes/male	Control	Patient	Odds Ratio (95%CI)	P-value
TT	16	38	Reference=1	
TC	2	4	0.84 (0.14-5.07)	0.85
CC	0	2	2.14 (0.10-47.13)	0.62

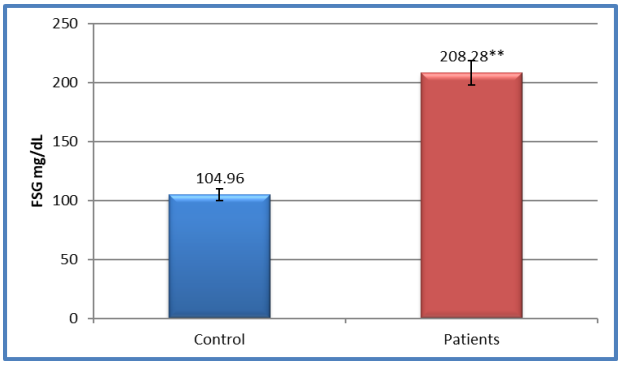

Figure No. 1: Mean of FSG of patients and control samples

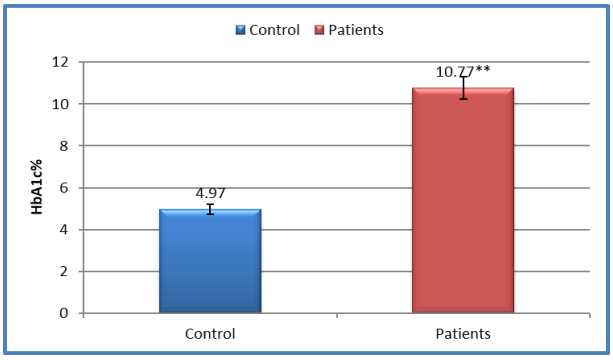

Figure No. 2: Mean of HbA1c of patients and control samples

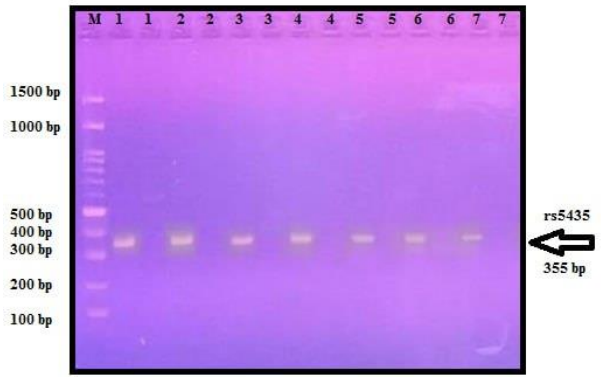

Figure No. 3: Genotyping of rs5435 genetic polymorphism

DISCUSSION

Fasting serum glucose (FSG) and HbA1c levels were evaluated in 100 patients and 30 healthy controls. The least significant differences (LSD) between patients and controls were 46.38 for FSG and 0.96 for HbA1c. For SLC2A4 rs5435 genotypes, the LSD values were 88.22 for FSG and 2.56 for HbA1c. As shown in Table 9, the observed differences in FSG and HbA1c across all rs5435 genotypes exceeded the corresponding LSD values, indicating statistically significant differences in biochemical parameters among genotypes ($P<0.05$). These findings were similar to a previous studies.[18,19] Regarding the analysis of odds ratio, for SLC2A4 rs5435 mutant genotypes for the patients; heterozygous genotype TC has an odd ratio (0.91 with a confidence interval of 0.17–4.77) lower than 1, indicating it is approximately at the same risk for increased FSG and HbA1c levels with the TT genotype. P=0.91. Whereas homozygous genotype CC has an odd ratio (1.52 with a confidence interval of 0.17–13.55) higher than 1, indicating it is at higher risk for increased FSG and HbA1c levels than the TT genotype. However, the results revealed that the odd ratio for SLC2A4 rs5435 mutant homozygous genotype CC is at risk, but the value of the odd ratio was not significant (P=0.71). Odds ratio statistical test that had been employed in this study was concurrent with Kalra.[20]

CONCLUSION

SLC2A4 gene polymorphism rs5435 was detected with variable frequencies and different genotypes in Iraqi patients. The SNP of the SLC2A4 gene that was detected in Iraqi type 1 diabetic patients was noted to not significantly affect the response to exogenous insulin.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mohammed Suhail Abed, Mohammed Ibrahim Rasool
Drafting or Revising	Mohammed Suhail

Critically:	Abed, Mohammed Ibrahim Rasool
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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