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Association of *GRK5* variant rs10886471 with the therapeutic effect of repaglinide in patients of type 2 diabetes mellitus in Peshawar, Pakistan

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Abstract: Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder with a rising global prevalence. The primary objective of this study was to explore the relationship between the *GRK5* variant (rs10886471) and the therapeutic effect of repaglinide in patients of T2DM in Peshawar, Pakistan. A quasi-experimental study was designed. The study group consisted of patients with Type 2 Diabetes Mellitus (T2DM) categorized into responders and non-responders based on their HbA1c level reduction in response to repaglinide treatment. After ethical approval, and consent from the participants, sociodemographic and clinical data was collected from 60 T2DM patients. Blood samples were collected followed by DNA extraction and quantification with UV-Vis Spectroscopy. Genotyping for the *GRK5* variant rs10886471 was done using the PCR-based method. Among socio-demographic factors family history and BMI showed significant association ($P<0.05$) with the therapeutic response to repaglinide. The Statistical analyses, including chi-square tests and logistic regression of *GRK5* variant rs10886471 exhibited a significant association with the therapeutic response. Variant allele exhibited significant association (OR: 1.2, $p=0.049$) with the therapeutic response to repaglinide. The study demonstrated a significant relationship between the *GRK5* variant (rs10886471) and the therapeutic response to repaglinide in patients of T2DM of Peshawar, Pakistan.

Keywords: Type 2 diabetes mellitus, *GRK5* variant, pharmacogenomics.

INTRODUCTION

Diabetes mellitus is a diverse and progressive metabolic disorder characterized by insulin resistance and inadequate insulin secretion. It arises from a complex interaction of genetic predisposition and environmental factors, particularly sedentary lifestyles, and excessive caloric intake (Rachdaoui, 2023). Over the past few decades, T2DM has emerged as a global health concern, with its occurrence increasing rapidly in both developed and developing countries (Liu *et al*., 2022). Over 90% of diabetes Mellitus cases are of T2DM. Pakistan, as a developing nation, is experiencing a significant burden of T2DM, which has far-reaching health, social, and economic implications (Jan *et al*., 2023). As per International Diabetes Federation latest data, diabetes has emerged as a global health crisis, affecting a staggering 537 million adults between the ages of 20 and 79 in 2021, representing a prevalence of 1 in 10 individuals worldwide (L'heveder *et al*., 2013). Both genetic and environmental factors cause T2DM. One such genetic element that has drawn attention in the context of diabetes management is the G-protein-coupled receptor Kinase 5 (*GRK5*) gene (Lappano and Maggiolini, 2011). *GRK5* encodes a protein kinase that plays a role in regulating G-protein-coupled receptor (GPCR) signaling pathways. GPCRs are integral membrane proteins that transmit signals from extracellular stimuli to intracellular responses, playing a critical role in different physiological mechanisms, including insulin secretion, glucose metabolism, and lipid homeostasis (Gurevich *et al*., 2012). As such, alterations in the functioning of *GRK5* can have a profound impact on these cellular pathways, potentially contributing to the development and progression of T2DM (Feng and Astell-Burt, 2017). The significance of *GRK5* in the management of diabetes is emphasized by its interactions with essential elements of insulin signaling pathways. Insulin, a hormone central to glucose homeostasis, Given the complex nature of T2DM, a personalized approach to treatment and management is becoming increasingly important (Jones *et al*., 2018). A study utilizing genome-wide association analysis revealed a connection between *GRK5* (rs10886471) and the susceptibility to T2DM. Notably, around 20 genes and 60 genetic loci have been implicated in influencing T2DM vulnerability (Ji *et al*., 2013).

Repaglinide falls within the category of oral anti-diabetic agents classified as meglitinides. This medication is administered to help in the regulation of blood glucose concentrations among individuals diagnosed with T2DM. Repaglinide is recognized for its unique mechanism of action, which sets it apart from other antidiabetic medications. Enhancing initial-phase insulin secretion elicited by meals, an essential milestone in the trajectory of T2DM as acknowledged by Dorn Horst (2001), it

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stimulates insulin release and is intended to be used with meals. When ingested before eating, it induces a swift insulin reaction in response to the meal (Upadhyay *et al*., 2018). In comparison to sulfonylureas, the medication has a shorter half-life, which lowers the risk of hypoglycaemia if a patient skips a meal, according to Dorn Horst (2001). It also appears that its activity on beta cells is glucose-dependent, as it has no effect on insulin secretion in the absence of glucose. The lower risk of hypoglycemia and the resulting more adaptable eating habits may have significant therapeutic benefits (Krentz and Bailey, 2005).

MATERIALS AND METHODS

Screening of subjects

By using the WHO software that builds upon the principles outlined by S.K. Lwanga and Lameshow, the sample size for a Quasi-Experimental Study was determined using the formula n = Z^2 * (1-α/2) * P * (1-P) / d^2, with considerations for a 95% confidence level and a margin of error set at 5% (*Rashid M, et al ; 2019).* Approval was obtained from the KMU-Ethics board (NO: KMU/IBMS/IRBE/6th meeting/2023/9960-K, dated 12/06/2023 annexure II), enabling the selection of 60 patients from tertiary care facilities in Peshawar, Khyber Pakhtunkhwa.

Using a sterilized syringe, 3 ml of whole blood was drawn into EDTA tubes with care, appropriately labelled, and stored at a temperature of -20^{o}C. The inclusion and exclusion of study participants were determined based on a specified set of criteria (Moonsarn *et al*., 2023). Inclusion criteria were Patients with a genetic predisposition to T2DM, irrespective of their gender, patients with T2DM, having HBA1c > 7%, patients with age above 30 years, patients using repaglinide. Exclusion criteria were insulin dependent diabetic patients, gestational diabetes.

A total of 60 patients who were prescribed repaglinide (1 mg thrice a day, PO before each meal daily) were enrolled for eight consecutive weeks with proper full follow-up Blood sampling for HbA1c estimation was done twice in the study period, first at the beginning of the repaglinide therapy and the second one; eight weeks after repaglinide therapy. Based on HbA1c reduction from the baseline by repaglinide, patients were categorized into responders (35) and non-responders (25).

The HbA1c Fast test kit (version WIF22-S-17) was used to analyze HbA1c, using the Getein1100 testing machine. A DNA extraction kit (WizPrepTM gDNA Mini Kit) was used for DNA extraction. The DNA quality and quantity that were extracted were evaluated using the UV-Spectrophotometry technique (Bulla *et al*., 2016). After the spectrophotometry procedure, the DNA was identified using the electrophoresis technique (Kuhn *et al*., 2017). DNA presence was confirmed by extracting the setup and validating it through exposure to UV light using the Life Technologies E-Gel® Imager (Heija, 2016).

Genotyping

PCR, specifically PCR-RFLP, was utilized for genotyping GRK5 (Garner and Revzin, 1981) using the GRK5*10 SNP rs10886471 sequence sourced from NCBI. Primers (forward: AAGTTCTTCCCTGCTAGAGAA, reverse: CTCTTTTTGTTCTAAGTGAAAAC) were designed with Primer 3 web and validated through UCSC insilico PCR. The 25µL master mix, comprising Dream Taq Green Master Mix (Thermo Scientific), included an initial denaturation at 95^{o}C for 5 minutes, followed by 35 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 60^{o}C, and extension at 72^{o}C. A final extension occurred at 72^{o}C for 5 minutes (Obeid *et al*., 2003). The 329 bp PCR products were then analyzed on a 2% agarose gel, with a 100 bp hyperladder for comparison (Figure 1).

Results of gel electrophoresis of GRK5 variant (rs10886471) after applying restriction enzyme

The identification of polymorphism within the *GRK5* gene was accomplished utilizing a 1% agarose gel, in conjunction with a 50 bp ladder and the restriction enzyme BanII. The outcome of the PCR procedure yielded fragments of 197 and 132 base pairs post-digestion for the variant allele (C>T) . Electropherograms illustrating the genetic variations of the *GRK5* Single Nucleotide Polymorphisms (SNPs) are portrayed (fig. 2).

Data evaluation

The statistical analysis of the influence of the *GRK5* variant (rs10886471) on the therapeutic effect of repaglinide in T2DM patients was conducted using SPSS version 26. Descriptive statistics as regard to various demographic and clinical characteristics and genotype frequencies were calculated.

A chi-square test was conducted to assess the difference between responders and non-responders genotypesotype. Logistic regression was carried out to assess outcome VS intervention with regard to the effect of *GRK5* variant (rs10886471) on glycemic control. Reduction in HbA1c level was taken as dependent variable and GRK5 variant (rs10886471) was taken as an independent variable. A value of P<0.05 was considered statistically significant (Shang *et al*., 2018).

RESULTS

Demographic Characteristics

The details of various *Demographic Characteristics* are shown in table 1. There were 82% male and 18% female. There was significant difference (P=0.043) among responders and non-responders with respect to BMI.

Table 1: Demographics characteristics distribution of patients by response to repaglinide

Variables	Responders	Non-responders	P-values
BMI category			0.043*
Lean	0	1	
Normal	11	6	
Overweight	14	5	
Obesity	10	13	
Change in HbA1c Level			0.001*
Responders(>20%)	20	-	
Non responders(<10%)	-	17	
Non responders (20%)	-	8	
Family History			0.05*
Yes	31	23	
No	4	2	

Table 2: Allele frequency comparison between responders and non-responders.

Genotype	Responders N (%)	Non-responders N (%)	Unadjusted OR (95% CI)	P-value
CC	26 (74.2%)	3 (12%)	Ref	
CT	3 (9%)	10 (40%)	0.750 (0.932-1.7002)	0.046*
TT	6 (17%)	12 (48%)	1.2 (0.988-7.895)	0.049*

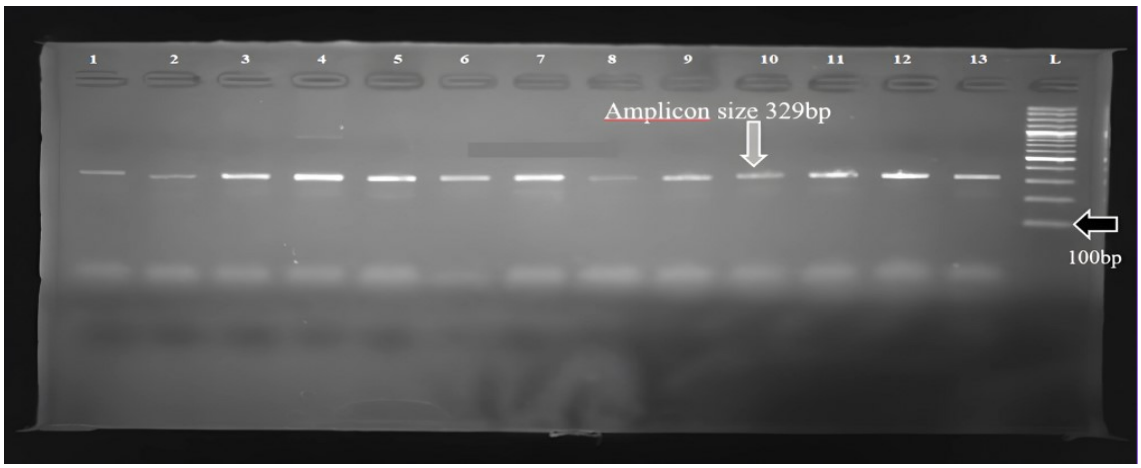

Fig. 1: Visualization of PCR product through Gel electrophoresis using Ladder 100bp

Fig. 2: Electropherogram of PCR products following the application of a restriction enzyme BanII for *GRK5* variant utilizing a 50 bp Ladder.

The chi-square test indicated a significant association between the change in HbA1c levels and the response to repaglinide (p=0.001*). Patients who responded positively to repaglinide treatment demonstrated larger reductions in HbA1c levels compared to non-responders.

Association of GRK5 variant (rs10886471) with repaglinide therapy

The results of the *GRK5* variant (rs10886471) association with repaglinide response is given in table 2. Among individuals who responded positively to the treatment, the wild type (CC genotype) was identified in 26 out of 35 cases (74.2%). Conversely, among non-responders, this genotype was found in only 3 out of 25 cases (12%). The heterozygous variant (CT genotype) was observed in 40% of non-responder patients (10 individuals out of 25), compared to 9% among responders (3 individuals out of 35). Notably, there was a statistically significant association between repaglinide therapy and this variant allele (odds ratio: 1.2, p-value: 0.049).

DISCUSSION

The results of this study provide valuable insights into the complex interplay of genetic, demographic, and clinical factors that influence the therapeutic response to repaglinide in patients with T2DM in Peshawar, Pakistan. The exploration of various variables within the demographic and clinical domains has shed light on the multifaceted nature of T2DM treatment, offering implications for personalized medicine and advancing our understanding of the factors shaping treatment outcomes.

The investigation into demographic variables revealed that gender, marital status, socioeconomic status, education, lifestyle, family history, residence, weight, height, and Body Mass Index (BMI) demonstrated varying degrees of association with repaglinide response. These findings highlight the heterogeneity of T2DM patients and the need to consider individual characteristics when designing treatment strategies. The observed associations suggest that tailoring treatment based on demographic profiles could lead to improved treatment outcomes and patient satisfaction (Brunetti *et al*., 2014).

One of the most significant findings of this study was the association between the *GRK5* variant (rs10886471) and the response to repaglinide therapy. The analysis revealed a significant association in our study population, indicating that the presence of the *GRK5* variant plays a role in determining repaglinide response. Individuals carrying the variant allele TT exhibited a decreased response to repaglinide compared to the wild CC genotype. This effect can be explained by the *GRK5* variant's effect on insulin signaling pathways that leads to altered treatment outcomes. *GRK5* encodes a protein kinase that plays a role in regulating G-protein-coupled receptor (GPCR) signaling pathways. GPCRs are integral membrane proteins that transmit signals from extracellular stimuli to intracellular responses, playing a critical role in different physiological mechanisms, including insulin secretion, glucose metabolism, and lipid homeostasis (Gurevich *et al*., 2012). As such, alterations in the functioning of *GRK5* can have a profound impact on these cellular pathways, potentially contributing to the development and progression of T2DM (Feng and Astell-Burt, 2017). A study utilizing genome-wide association analysis revealed a connection between *GRK5* (rs10886471) and the susceptibility to T2DM (Ji *et al*., 2013). Another study has highlighted a notable disparity in T2DM susceptibility between Chinese Han populations, including East Asian groups, and their Western counterparts (31). Furthermore, a study conducted among the Chinese population demonstrated an association between a *GRK5* variant (rs108864 and the therapeutic response to repaglinide in individuals with T2DM (Koh *et al*., 2014).

This finding adds to the growing body of evidence suggesting that genetic factors can significantly influence how patients respond to antidiabetic medications. Further research is required to unravel the specific mechanisms through which the *GRK5* variant affects the repaglinide response. Understanding these mechanisms could potentially lead to tailored treatment strategies for individuals with this genetic predisposition. The results align with the notion that genetic variations, even at a single nucleotide level, can impact signaling pathways and molecular interactions that influence drug metabolism, transport, and receptor interactions.

In addition to demographic and genetic factors, clinical variables such as pre-treatment HbA1c levels, post-treatment HbA1c levels, and the difference in HbA1c were estimated. These parameters offered insights into the relationship between initial glycemic control and treatment response. The observed patterns highlight the importance of baseline glycemic status in predicting the effectiveness of repaglinide therapy. These clinical insights can aid clinicians in identifying patients who are more likely to benefit from repaglinide treatment.

The collective findings from this study underscore the potential of personalized medicine in the management of T2DM. These findings underline the importance of personalized medicine approaches, where an individual's genetic makeup is considered to optimize treatment regimens (Bitar *et al*., 2021).

CONCLUSION

The study showed a significant association (OR= 1.2 (0.988-7.895), P-value= 0.049*) between the *GRK5* variant (rs10886471) and the therapeutic response to repaglinide in patients of T2DM in Peshawar, Pakistan.

Pharmacogenomic studies hold the potential to revolutionize diabetes management by identifying patients who might benefit from alternative treatment options or dosage adjustments based on their genetic profile. By considering an individual's genetic profile, demographic characteristics, and clinical status, clinicians can optimize treatment plans, minimizing adverse effects, and maximizing therapeutic outcomes. Such studies hold the potential to open avenues for pharmacogenomic-guided interventions, enhancing treatment precision and patient care.

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