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GENETIC ASSOCIATION OF KCNJ11 POLYMORPHISM AND TYPE 2 DIABETES SUSCEPTIBILITY: A CASE-CONTROL APPROACH

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ABSTRACT

Diabetes is globally one of the leading non-communicable diseases that pose significant public health challenges. The Potassium Inwardly Rectifying Channel Subfamily J Member 11 (KCNJ11) gene polymorphism has been implicated in beta-cell dysfunction and altered insulin secretion through potassium-gated channels, potentially influencing type 2 diabetes mellitus (T2DM) susceptibility. Genotyping was performed using restriction fragment length polymorphism, with verification through Sanger Sequencing. The association between KCNJ11 genotypes and T2DM risk was evaluated using odds ratios and confidence intervals. Biochemical and clinical variables were compared between patients and controls using Student's t-test and Chi-square (χ^2) tests. Statistical analysis was conducted using SPSS software. The frequency distribution of KCNJ11 (rs5210) SNP (A/G) differed significantly between the control group and T2DM patients ($X^2 = 31.7$). The dominant genetic model (p-value <0.00; OR = 4.41) showed its strong association with T2DM. Drug response analysis highlighted those patients with the GG genotype responded better to metformin + sulfonylurea (M + S) compared to lantus + gliptin (L + G), indicating genotype-specific therapeutic efficacy. This study confirms that the rs5210 genetic variant of KCNJ11 gene significantly increases susceptibility to T2DM. Furthermore, the GG genotype may influence the effectiveness of antidiabetic therapies, providing a potential basis for personalized treatment strategies. These findings underscore the importance of genetic screening in diabetes management.

Keywords: Antidiabetic drugs, Genetic variation, KCNJ11, polymorphism, type 2 diabetes mellitus

Abbreviations: RFLP - Restriction fragment length polymorphism; RS - Restriction site; PPS - Post-prandial sugar; MS - Metformin sulphonylurea, FBS - Fasting blood sugar, DBP - Diastolic blood pressure; SBP - Systolic blood pressure; KCNJ11- Potassium inwardly-rectifying channel subfamily J member 11; PCR - Polymerase chain reaction; SNP - Single nucleotide polymorphism; T2DM - type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that pose severe global health challenge, particularly among the individuals with pre-existing conditions (Gong *et al.*, 2012; Aida *et*

al., 2020). Unlike type 1 diabetes mellitus (T1DM), which is insulin-dependent, T2DM is insulin-independent but significantly more prevalent worldwide (Muftin and Jubair, 2019). Furthermore, gestational diabetes mellitus (GDM) raises the risk of long-term consequences such as weight gain, poor glucose utilization, and heart failure.

These complications may eventually cause T2DM in both the mother and offspring (Sathya *et al.*, 2024). The rapid lifestyle changes during the past few decades and poor dietary habits have led to a sharp increase in T2DM cases globally (Muhammad and Agussalim, 2018). T2DM is more common now than it was a few decades ago, with approximately 250 million cases worldwide (Ramteke *et al*., 2024). By 2035, the prevalence of diabetes in India is predicted to rise to 10.1% (Awasthi *et al*., 2020). This dramatic increase is partly attributed to the impaired insulin secretion, which is regulated by key genes such as Potassium Inwardly-Rectifying Channel Subfamily J member 11 (KCNJ11) gene and the Sulfonylurea Receptor-1 (SUR1) gene (Aka *et al.*, 2021). Specifically, single nucleotide polymorphisms (SNPs) in KCNJ11 reportedly alter insulin secretion by affecting the ATP-sensitive potassium (KATP) channels, which regulate insulin release (Engwa *et al.*, 2018; Liu *et al.*, 2019). These KATP channels is composed of Kir6.2 and SUR1 subunits, are crucial for pancreatic beta-cell viability and normal insulin secretion (Hansen *et al.*, 2005). Mutations in KCNJ11 gene is not only associated with T2DM but also causes the conditions such as neonatal diabetes and persistent hyperinsulinemic hypoglycemia (Cejkova *et al.*, 2007). Several studies have confirmed that loss-of-function mutations in KCNJ11 increase the risk of T2DM (Makhzoom *et al.*, 2019; Khan *et al*., 2020).

KCNJ11 gene, located on chromosome 11p15.1, has a single exon thus, making it unique in its structural simplicity (Isalova *et al.*, 2019). The major SNPs in KCNJ11, including rs5210, rs5215, and rs5219 have been studied for their association with T2DM (Alqadri, 2022). These SNPs, located in both 3' untranslated region (UTR) and coding regions may result in amino acid substitutions that affect the function of KATP channels (Alqadri, 2022). Although the studies in South Asia have identified an association between rs5215 variant and T2DM, the role of rs5210, especially in other ethnic groups, remains less conclusive (Haghvirdizadeh *et al.*, 2015; Bhargave *et al.*, 2023). The present study aimed to assess the association of rs5210 polymorphism in KCNJ11 gene with T2DM in East-Northern Indian population, a region that has been under represented in genetic studies of T2DM. Furthermore, this study evaluated the response of different anti-diabetic drug combinations *viz*. metformin + sulfonylurea (M + S) and lantus + gliptin (L + G) [Lantus is produced by strain of *Escherichia coli* (K12) and gliptin is chemically synthesized compound also known as dipeptidyl peptidase-4 (DPP-4) inhibitors. The plant *Galega officinalis*, sometimes referred to as French lilac or goat's rue, is the source of metformin and para-substituted arylsulfonamides are known as sulfonylureas. They are created by replacing a nitrogen residue in the urea moiety with the para-position on the benzene ring] in the patients with different KCNJ11 genotypes. Given the increasing evidence for genotype-specific drug efficacy, understanding the interaction between KCNJ11 polymorphisms and drug response could help in optimizing the treatment strategies for T2DM.

MATERIALS AND METHODS

Study design

This study was conducted at O.P. Chaudhary Hospital & Research Centre, Lucknow, Uttar Pradesh, with 250 participants comprising of 125 patients with type 2 diabetes mellitus (T2DM) and 125 healthy controls. The participants were aged between 25-60 years. T2DM patients were diagnosed as per the World Health Organization (WHO) guidelines (Makhzoom *et al.*, 2019) and a comprehensive set of biochemical parameters - including fasting glucose, postprandial glucose, systolic and diastolic blood pressure; very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) etc. were recorded. This was done as per the enzyme-based approach (glucose oxidase peroxidase) and ElZA

kit was used for insulin measurements. The sample size was determined using the Quanto software (*https://www.quantoretail.com/*) based on previous studies (Sakamoto *et al.,* 2007). The study design ensured equal representation of T2DM patients and healthy controls for robust comparative analysis. Participants were selected based on strict inclusion and exclusion criteria. T2DM patients were included if they had fasting blood glucose levels >100 mg dL^{-1}, postprandial glucose >140 mg dL^{-1}, random glucose levels >200 mgdL^{-1}, and HbA1c levels >6.5%. Participants with severe comorbidities, such as cardiac disease or nephropathy, were excluded from the study. Healthy controls were selected based on normal blood glucose levels and absence of family history of diabetes.

Sample collection and analysis

Ethical approval for the present study was obtained from the Ethical Committee of Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow, Uttar Pradesh (India) vide reference number RP/PhD/01/170922/IEC/SPPGIDMS dated September 17, 2022. Prior informed consent was obtained from all the participants and the blood samples were collected in accordance with the World Medical Association's Code of Ethics. Blood samples were drawn from January 2020 onwards in the Pathology Department of O.P. Chaudhary Hospital & Research Centre. For each participant, 6 ml of venous blood was collected in EDTA vials. Serum was separated for biochemical analysis, and the remaining blood used for DNA extraction. DNA was isolated using the phenol-chloroform method (Khan *et al*., 2019) and both blood samples and extracted DNA were stored at -20°C until use. Key biochemical parameters, including fasting and postprandial glucose levels, HbA1c, HDL, VLDL, and blood pressure were measured as per enzyme-based approach (glucose oxidase peroxidase) and ElZA kit was used for insulin measurement. The Cholestech LDX™ analyzer (Abbott Rapid Dx North America LLC) was used for the measurement of biochemical parameters.

Genotyping of rs5210 via PCR-RFLP

The rs5210 SNP in KCNJ11 gene was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Alqadri, 2022). Genomic DNA was extracted from the blood samples using manual phenol-chloroform method (Khan *et al*., 2019), followed by elution in 50 µL autoclaved Milli-Q water. The quality and quantity of DNA were verified by using 1% agarose gel electrophoresis (Lee *et al.,* 2012) using Genei mini horizontal gel instrument unit. The extracted DNA was stored at -20°C until further use in PCR analysis. The PCR amplification for KCNJ11rs5210 region was done by using a set of primers (FP: 5'- ATCCAGGGTGTTACAAGGCA-3', RP: 5'-TTTCAGGGACCAAGTAGAGCTG-3') (N S 2 Enterprises) as per Khan et al. (2019) with the condition of initial denaturation at 95°C for 5 min,3 5 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and final extension step at 72°C for 5 min. A 20 µL reaction mixture was prepared with 1 µL each of forward and reverse primers, 1 µL template DNA, 10 µL master mix (GENEI), and 7 µL autoclaved Milli-Q water. The 316 bp PCR-amplified product was confirmed by 2% agarose gel electrophoresis. For genotyping, restriction digestion of PCR product was carried out using an appropriate restriction enzyme (*Hpy*188III, NEB) followed by analysis on an agarose gel to identify the allelic variants of rs5210 polymorphism.

Statistical analysis

All statistical analyses were performed using SPSS software. The collected data, which included both genotypic and clinical information, were first stored in MS. Excel and were then converted to SPSS files for analysis. Various tests, including the Hardy-Weinberg Equilibrium (HWE) test, Student's t-test, chi-square test and genotypic analysis were conducted to compare the results between diabetic patients and healthy controls. Multiple nominal regression analyses were conducted for diabetic patients and continuous variables were expressed as means and standard deviations. Allelic substitution frequencies were reported as percentages, and significant results between the two groups were considered at a p-value of <0.05.

RESULTS AND DISCUSSION

This study revealed a significant association between KCNJ11 rs5210 polymorphism and susceptibility to type 2 diabetes mellitus (T2DM) in the East-Northern Indian population. The rs5210 SNP involves a nucleotide substitution from A to G. The G allele considered the disease causing variant and was significantly more prevalent in patient group as compared to the control group, confirming its role in T2DM risk. Its frequency was notably higher in T2DM patients that are 41.2%. In case of control group comprising of 125 people, 79 (63.2%) were wild (AA), 37 (29.6%) were heterozygous (AG), and 9 (7.2%) were mutant (GG). On contrary, out of 125 people in the patient group, 35 (28%) were homozygous wild type (AA), 77 (61.6%) were heterozygous type (AG), and 13 (10.4%) were homozygous mutant type (GG). The genotypic variation between control and patient groups is highlighted in Table 1. In line with the studies from other population group, particularly those from East Asia, our work adds more proof and correlates with their research and proves that the rs5210 polymorphism is linked to an elevated risk of type 2 diabetes (Yang *et al*., 2012). Further, our investigation identified KCNJ11 rs5210 polymorphism as a significant genetic marker, aligning with findings from other population groups, including the Finnish and Japanese cohorts (Odgerel *et al*., 2012; Kostov *et al*., 2020). Several genes have been screened by genome-wide association studies (GWAS), and more than 60 polymorphic variants have been shown to have enhanced susceptibility and be linked to the disease. Out of various genes and associated SNP's that cause T2DM some of the major targeted genes and associated SNPs are - Peroxisome proliferator activated receptor gamma2 (PPARG2 gene associated Pro12Ala SNP), Fat mass and obesity associated (FTO gene associated SNP is rs9939609), Solute carrier family 30 member 8 (SLC30A 8 gene associated SNP rs13266634), Transcription factor 7-like 2(TCF7L2 gene associated SNP rs7903146) (Jamil *et al.*, 2020).

Our findings showed that G allele was more common in T2DM patients than in controls (Table 1) which emphasized that rs5210 polymorphism increases the risk of developing the disease in the targeted population. Consistent with previous studies, our findings indicate that patients with G allele are more likely to have elevated levels of low-density lipoprotein (LDL) and are at greater risk for developing T2DM. In a prior study, the frequency of the G allele was reported to be 16% (Khan et al., 2015), whereas in our study it was 10.4%.

The Fig. 1 shows biochemical parameters along with the participant's demographic data and substantial correlation existing between them. Predisposition of genetic, biochemical, lifestyle and

Table 1: Genetic variations of KCNJ11 (rs5210) gene between T2DM patients and controls

Variables	Patients (n = 125)	Controls (n = 125)	OR*(95% CI)	*P* value
Genotype				
AA	35 (28.0%)	79(63.2%)	Reference	
AG	77 (61.6%)	37(29.6%)	4.69 (2.68-8.21)	0.00
GG	13 (10.4%)	9 (7.20%)	3.26 (1.27-8.33)	0.00
χ^2 value			31.7	
Dominant model				
AA	35 (28.0%)	79 (63.2%)	Reference	
GG + AG	90 (72.0%)	46 (36.8%)	4.41 (2.59-7.52)	0.00
Recessive model				
AA + AG	112 (89.6%)	116 (92.8%)	Reference	
GG	13 (10.4%)	9 (7.2%)	1.49 (0.61-3.63)	0.37
Alleles				
A	147(58.8%)	195 (78.0%)	Reference	
G	103 (41.2%)	55 (22.0%)	2.48 (1.68-3.67)	0.00

*OR - The likelihood that an event will occur in one group of persons as opposed to another is measured by an odds ratio (OR). Clinical research frequently uses it to assess how strongly a risk variable and the result are related.

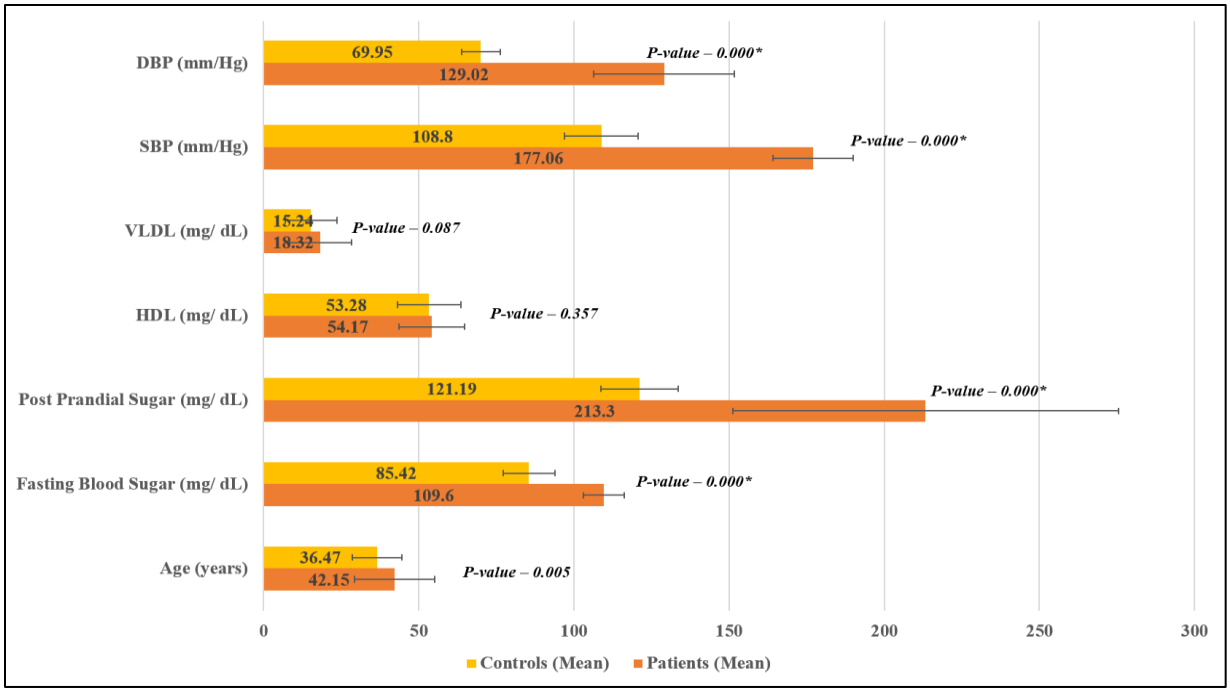

Fig. 1: Comparative biochemical parameters of T2DM patients and healthy controls

environmental associated parameters are primarily involved in the rise of T2DM. This has been shown in our study supported with valid highly significant P-value of SBP, DBP and FBS (P- value: 0.000*, 0.000*, 0.000*, respectively), but involvement of HDL and VLDL didn't show any significant correlation with T2DM. Similar observations were also found in a study done in Thailand (Rattanatham *et al*., 2021). Fig. 2. shows the PCR amplification of targeted gene of this study that is KCNJ11 rs5210 having amplicon size of 310 bp.

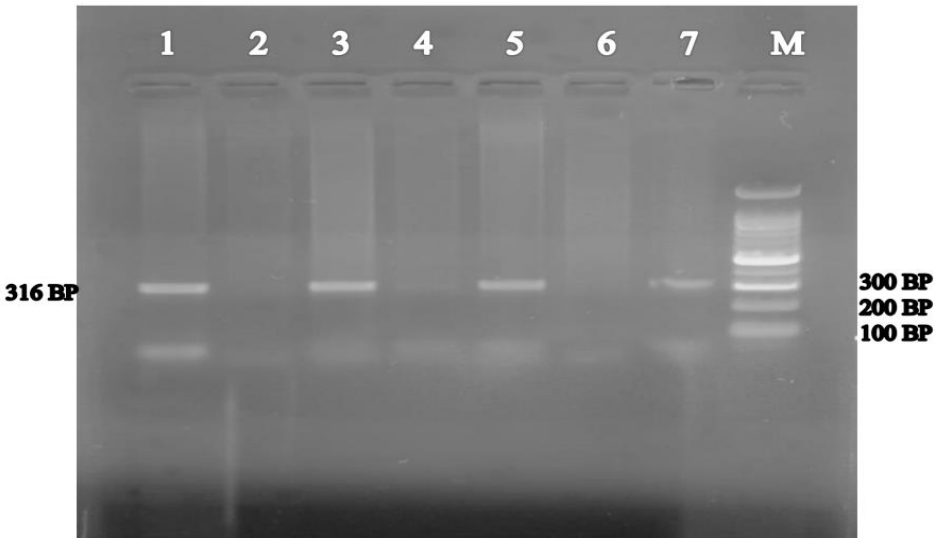

Fig. 2: PCR amplification of KCNJ11 rs5210 gene (Lane M: 100 bp DNA ladder; Lane 1, 3, 5, and 7: 316 bp amplified PCR products)

The present study further emphasized the significance of dietary practices as a genetic risk factor and also correlated with the findings of Chiu *et al*. (2023) which showed that people who have non-vegetarian food preferences were more likely to develop type 2 diabetes. Table 3 shows a highly

significant (P-value of 0.00001*) correlation with the individuals having a non-vegetarian diet and their susceptibility of having T2DM.

Genotyping and restriction digestion analysis

Genotyping was conducted using the restriction enzyme *Hpy*188III (NEB), which specifically recognizes the sequence 5'-TCNNGA-3', with rs5210 SNP resulting from a G-to-A substitution at this site. The restriction digestion was performed with the reaction mixture consisting of 0.5 µL *Hpy*188III enzyme (2.5 units), 6 µL PCR product, 1 µL cut smart buffer, and 2.5 µL autoclaved Milli-Q water, followed by incubation at 37°C for 16 h. The resulting fragments were visualized on a 3% agarose gel as depicted in Fig. 3.

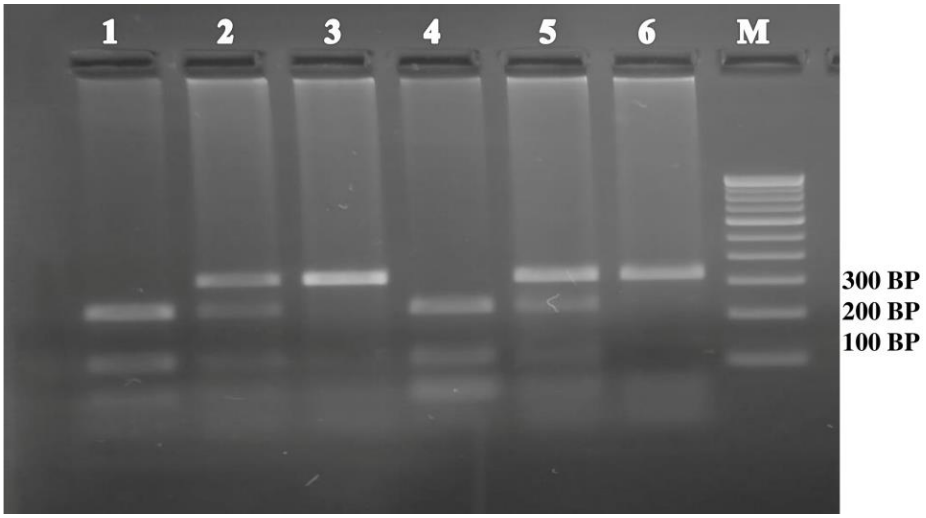

Fig. 3: Restriction digestion of KCNJ11 rs5210 with RE Hpy188III (Lanes 1, 4: homozygous GG; lanes 2, 5: heterozygous AG; lanes 3, 6 homozygous AA; lane M 100 bp DNA marker)

Post-digestion, the three genotypes were observed: Wild-type (AA): A single band of 316 bp; Heterozygous (AG): Three bands of 316 bp, 218 bp, and 98 bp; Mutant (GG): Two bands of 218 bp and 98 bp. For better understanding of association between Genotypes and T2DM four different model based highlights are present in Table 2.

Table 2: Different genotypic models and their relation with T2DM

S. No.	Model	Odd ratio (OR)	Class interval	P- value
1.	Homozygous model (GG vs. AA)	3.2603	1.2755–8.3337	0.01
2.	Heterozygous model (AG vs. AA)	4.6973	2.6867–8.2125	0.0001
3.	Dominant model (GG + AG vs. AA)	4.41	2.59–7.52	0.00
4.	Recessive model (GG vs. AG + AA)	1.49	0.61–3.63	0.37

The dominant model showed the strongest association, with an odds ratio of 4.41, suggesting that the individuals carrying either the G allele (GG or AG) are significantly more likely to develop T2DM as compared to those with the AA genotype. The recessive model, however, did not yield any significant results (P = 0.37), indicating that the GG genotype alone may not fully explain T2DM susceptibility in this population.

Comparative genotypic analysis

A comparative genotypic analysis was done and verified through Sanger sequencing, further validating the accuracy of the restriction digestion results. Sequencing results for each genotype are shown in Fig. 4, which clearly illustrates the presence of AG (Fig.4a), GG (Fig.4b), and AA (Fig.4c) genotypes.

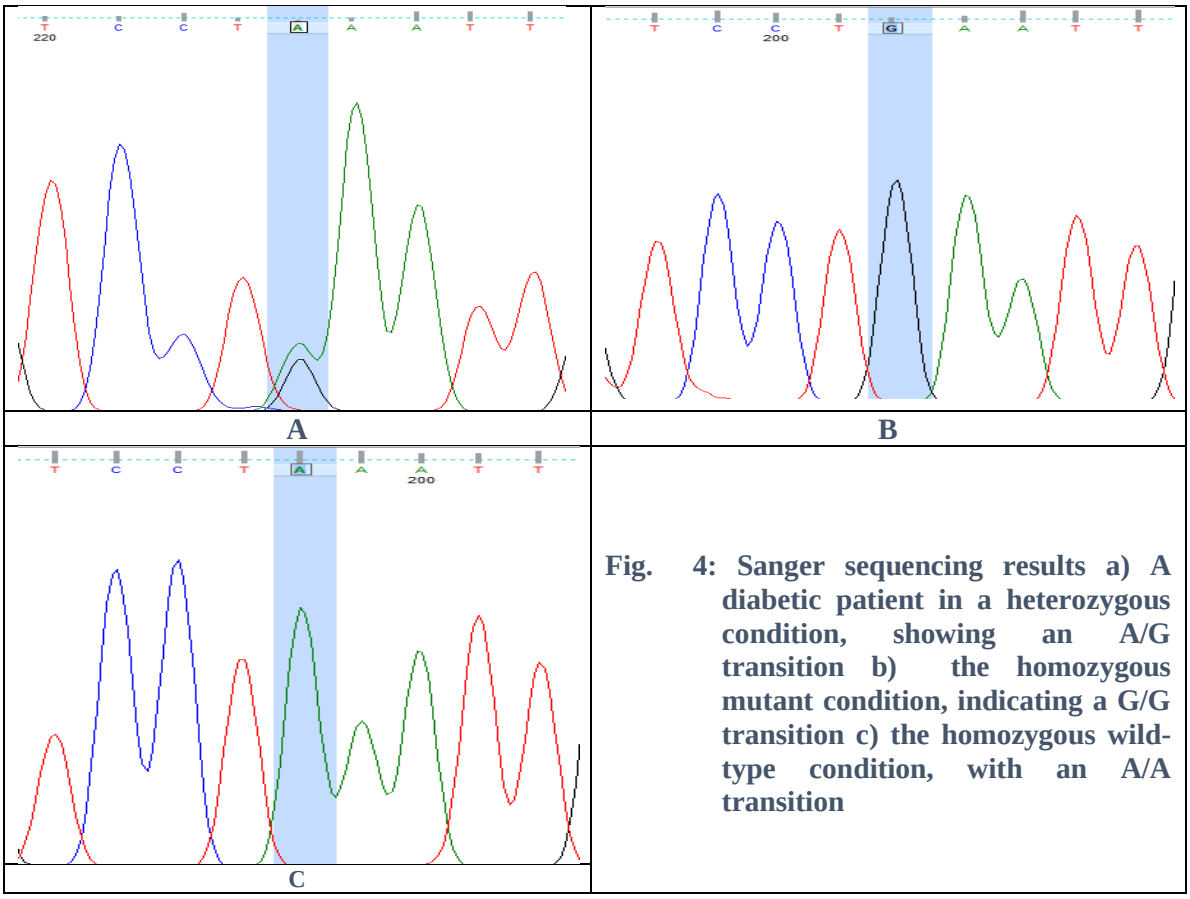

Fig. 4: Sanger sequencing results a) A diabetic patient in a heterozygous condition, showing an A/G transition b) the homozygous mutant condition, indicating a G/G transition c) the homozygous wild-type condition, with an A/A transition

Pharmacogenetic analysis

In addition to genotypic association with disease risk, the effectiveness of various antidiabetic drug combinations was assessed in patients with different genotypes. The combination of metformin + sulfonylurea (MS) was found the most effective treatment across all genotypes (Fig. 5). The GG genotype showed a complete response rate of 61.5%, whereas AA genotype showed 45.7% complete response rate. This is in line with a previous study on the pharmacogenetic effects of antidiabetic medications which showed that genotype-specific therapy improves medication efficiency (Sethi *et al.*, 2015).

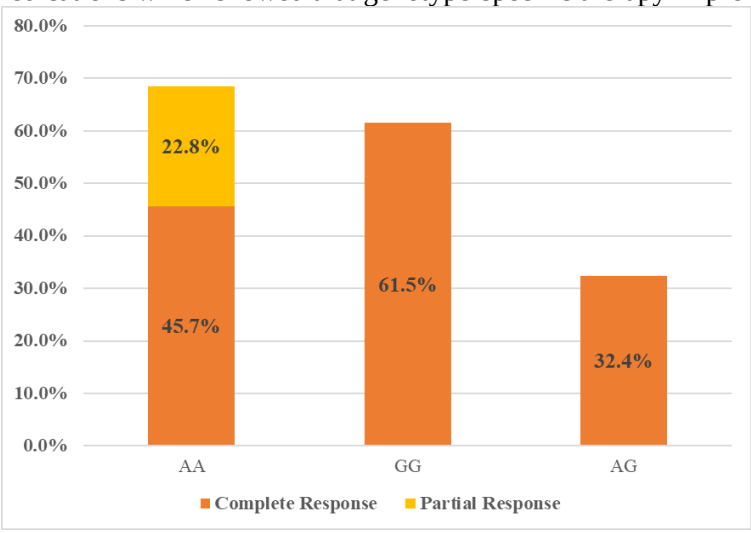

Fig. 5: Combined drug responses on different genotype group

- In patients with AA genotype, the MS combination resulted in 45.7% complete response and 22.8% partial response.
- Among those with GG genotype, the MS combination demonstrated even greater efficacy, with 61.5% complete response.
- In AG genotype group, the MS combination showed a 32.4% complete response, indicating a more moderate therapeutic effect in heterozygous individuals.

Out of the 125 control samples, with a 29.6% complete response; the AG genotype displayed a more varied reaction, suggesting that individualized treatment plans may be necessary for heterozygous individuals to attain the best results. These results indicate that the metformin + sulfonylurea (MS) combination was particularly effective for the patients carrying GG allele, suggesting a potential pharmacogenetic relationship between KCNJ11 rs5210 polymorphism and drug response in T2DM patients.

Table 3: Clinicopathological parameters of diabetic patients based on KCNJ11gene allele models

Parameters	Total	Wild (AA)	Carrier (AG + GG)	*p*- value
Age (year)				
≤ 50	82 (65.6%)	22 (17.6%)	49 (39.2%)	0.60
> 50	43 (34.4%)	15 (12.0%)	41 (32.8%)	
Diet (No.)				
Vegetarian	52 (41.6%)	30 (24%)	36 (28.8%)	0.00001*
Non-vegetarian	73 (58.4%)	5 (4%)	54 (43.2%)	
SBP (mm Hg^{-1})				
≤ 120	67 (53.6%)	21 (16.8%)	33(26.4%)	0.01
> 120	58 (46.4%)	14 (11.2%)	57 (45.6%)	
DBP (mm Hg^{-1})				
≤ 80	53 (42.4%)	23 (18.4%)	39 (31.2%)	0.024
> 80	72 (57.6%)	12 (9.6%)	51 (40.8%)	
Smoking				
Yes	73 (58.4%)	29 (23.2%)	40 (32%)	0.0002
No	52 (41.6%)	6 (4.8%)	50 (40%)	
FBS (mg dL^{-1})				
≤ 100	47 (37.6%)	17 (13.6%)	23 (18.4%)	0.23
> 100	78 (62.4%)	18 (14.4%)	67 (53.6%)	
PPS (mg dL^{-1})				
≤ 140	58 (46.4%)	14 (11.2%)	18 (14.4%)	0.02
> 140	67 (53.6%)	21 (16.8%)	72 (57.6%)	
HDL (mg dL^{-1})				
≤ 40	53 (42.4%)	22 (17.6%)	22 (17.6%)	0.000054
> 40	72 (57.6%)	13 (10.4%)	68 (54.4%)	
VLDL (mg dL^{-1})				
≤ 30	31 (24.8%)	11 (8.8%)	30 (24%)	0.83
> 30	94 (75.2%)	24 (19.2%)	60 (48%)	

The results of our pharmacogenetic analysis further highlight the clinical relevance of KCNJ11 rs5210 (Table 4). Notably, patients with the GG genotype demonstrated a significantly higher response to the metformin + sulfonylurea (MS) combination therapy compared to other genotypic groups. This finding suggests that lifestyle interventions, alongside genetic screening, may play a

Table 4: Genotypic variations and responses to anti-diabetic drug combinations

Genotype	Drug combination	Respondent (%)	Partial response (%)	No response (%)
AA (n = 35)	Metformin + Sulfonylurea	16 (45.7)	8 (22.8)	-
	Lantus + Gliptin	4 (11.4)	6 (17.1)	1 (2.8)
GG (n = 13)	Metformin + Sulfonylurea	8 (61.5)	-	1 (7.6)
	Lantus + Gliptin	2 (15.3)	1 (7.6)	1 (7.6)
AG (n = 77)	Metformin + Sulfonylurea	25 (32.4)	21 (27.2)	5 (6.4)
	Lantus + Gliptin	3 (3.8)	13 (16.8)	4 (5.1)

pivotal role in preventing and managing T2DM in genetically susceptible populations. While our study provides valuable insights, it is not without limitations. The relatively small sample size may limit the generalize ability of our findings. Larger cohort studies are needed to validate these results and explore additional SNPs within the KCNJ11 gene.

Conclusion: This study establishes the rs5210 variant of the KCNJ11 gene as a significant marker of T2DM susceptibility in the East – Northern Indian population. The association between genetic predisposition and the efficacy of specific antidiabetic therapies, such as the metformin + sulfonylurea combination, underscores the potential for personalized medicine in managing T2DM. However, the influence of environmental factors such as diet cannot be overlooked, and future research should aim to develop integrated approaches that combine genetic and lifestyle interventions for more effective disease prevention and management.

Conflict of interest: All the authors declare to no conflict of interest.

Ethical approval: Ethical Approval was taken from Institutional Ethical committee (IEC) of Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow to carry out this study. RP/PhD/01/170922/IEC/SPPGIDMS

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