RESEARCH PAPER

Impact of TCF7L2 and KCNJ11 Variants on Glycemic Control in a Type 2 Diabetes Cohort – A Cross-Sectional Study

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ABSTRACTS

Background: Pharmacogenomic variation may contribute to differences in Glycemic Control and response to antidiabetic therapy. However, evidence regarding TCF7L2, KCNJ11, and PRKD1 variants in Indian patients with type 2 diabetes mellitus (T2DM) remains limited.

Objective: To characterize selected TCF7L2, KCNJ11, and PRKD1 variants and evaluate their associations with HbA1c, TCF7L2 genetic risk burden, age at diabetes onset, and sulfonylurea use in patients with T2DM.

Methods: A Cross-Sectional pharmacogenomic study was conducted in 144 patients with T2DM receiving metformin-based therapy at a tertiary care center in Navi Mumbai, India. Six variants in TCF7L2, KCNJ11, and PRKD1 were analyzed. Associations between individual genotypes and HbA1c were assessed using genotype-group comparisons. An unweighted genetic risk score (GRS) was calculated from four TCF7L2 variants. Multivariable linear regression was used to evaluate KCNJ11 rs5219 genotype, current sulfonylurea use, their interaction, and clinical covariates in relation to HbA1c.

Results: Mean HbA1c was 8.67 ± 2.10%. None of the six variants showed a significant unadjusted association with HbA1c. The TCF7L2 GRS was not significantly associated with HbA1c ($p = 0.258$). TCF7L2 rs7903146 heterozygotes had an approximately 2.84-year earlier age at diabetes onset than the reference group ($p = 0.034$), although the genotype pattern was non-monotonic. In the adjusted model, KCNJ11 rs5219 genotype was associated with HbA1c among participants not receiving sulfonylureas ($\beta = 1.321$, 95% CI 0.404–2.237; $p = 0.005$), with a genotype-by-sulfonylurea interaction ($\beta = -1.138$, 95% CI −2.225 to −0.050; $p = 0.040$).

Conclusion: The findings suggests a potential KCNJ11-sulfonylurea interaction and a genotype-specific association of TCF7L2 rs7903146 with age at diabetes onset. These findings require validation in larger prospective studies with pretreatment and longitudinal Glycemic measurements before clinical or causal pharmacogenomic interpretations can be made.

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1. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a complex, polygenic metabolic disorder characterized by progressive insulin resistance and relative insulin deficiency. Its global prevalence has reached epidemic proportions, with approximately 589 million adults affected worldwide as of 2024, projected to exceed 780 million by 2045. (1) India bears a disproportionate share of this burden, with over 77 million individuals affected, making it the second-largest diabetes-affected country globally. In this epidemiological context, optimizing pharmacological therapy assumes critical public health importance. (2)

Despite a vast therapeutic resource, achieving glycemic control target and sustaining it remains a subtle clinical milestone, with nearly two-thirds of glycemic controlled populations failing to meet optimal glycated hemoglobin thresholds. In general, clinical management traditionally anchors on behavioral modifications and trial-and-error pharmacotherapy. However, there is growing evidence that an individual's underlying genetic architecture and its interaction with environmental conditions heavily influences both the age of disease onset and subsequent therapeutic outcome.

Metformin remains the cornerstone of pharmacological management in T2DM, acting primarily by suppressing hepatic glucose production via activation of AMP-

activated protein kinase (AMPK) and modulation of the mitochondrial respiratory chain complex I.(3) Despite its established efficacy and safety, a substantial proportion of patients fail to achieve glycemic targets with metformin alone, necessitating the addition of second-line agents.(4) Sulfonylureas, DPP-4 inhibitors, and SGLT2 inhibitors constitute the most commonly used add-on therapies in clinical practice, particularly in low- and middle-income countries where cost considerations influence prescribing.(5)

Insulin resistance is a complex metabolic condition driven by heterogeneous molecular mechanisms. In addition, genetic mutations play critical roles in disrupting cellular metabolism and regulatory networks, thereby predisposing individuals to complex metabolic disorders, including T2DM. A well-recognized but underutilized dimension of antidiabetic pharmacotherapy is the pharmacogenomic variability in drug response. Individual differences in glycemic outcomes cannot be explained solely by clinical factors such as body mass index (BMI), diabetes duration, or treatment adherence. (5) Locus-specific genetic variation implicated in glucose homeostasis, beta-cell function, and drug metabolism constitutes an important source of this variability. (6,7)

Genome-wide association studies (GWAS) have successfully mapped over a hundred common single-nucleotide polymorphisms (SNPs) linked to an increased susceptibility to T2DM. Among the genetic loci most robustly associated with T2DM susceptibility, Transcription Factor 7-Like 2 (TCF7L2) occupies a central position. *TCF7L2* encodes a high-mobility group box transcription factor that operates as a key effector within the Wnt signalling pathway, and directly regulates pancreatic β-cell proliferation, survival, and proinsulin processing.(8) *TCF7L2*-variant rs7903146 (10q25.3; fourth intron in the TCF7L2 gene) have been identified as a potent T2DM risk variant across diverse ethnic groups. Additional *TCF7L2*-variants, such as rs12255372, rs10509970, and rs10885387 have been demonstrated to be thoroughly linked to impaired insulin secretion, accelerated primary or secondary failure of oral antidiabetic agents, and reported to influence incretin signalling. Incretin signaling pathway triggers release of a group of metabolic hormones by the gut after eating, including secretion of insulin from the pancreas. (9)

Concurrently, polymorphisms within the *KCNJ11* gene, which encodes the pore-forming Kir6.2 subunit of the ATP-sensitive potassium (K_{ATP}) channel in pancreatic β-cells, play an essential role in dictating insulin release dynamics. The K_{ATP} channel serves as the molecular target of sulfonylurea drugs.(10) The missense mutation in the non-synonymous variant rs5219 (Glu23Lys, E23K), located in exon 1 of KCNJ11, alters the gating properties of the Kir6.2 channel and has been associated with differential β-cell sensitivity to sulfonylurea stimulation.(11) This variant in *KCNJ11* decreases β-cell sensitivity to ATP, causing the K_{ATP} channels to remain pathologically open, thereby hyperpolarizing the cell membrane and directly blunting glucose-stimulated insulin exocytosis. In a similar direction, protein kinase D1 (PRKD1), encoded by *PRKD1*, participates in insulin signaling cascades, lipid metabolism, and cellular stress responses. While less extensively studied in the context of T2DM pharmacogenomics, emerging evidence suggests that PRKD1 variants may contribute to interindividual variation in insulin sensitivity and glucose disposal. The rs57803087 variant in PRKD1 was included in the present analysis to explore its potential contribution to glycemic outcomes in a South Asian clinical cohort. (12)

Despite these clear mechanistic pathways, translated clinical application remains restricted. In Cross-Sectional managed environments, single-locus genetic evaluations frequently lose predictive power due to the overlapping noise of patient demographics, disease duration, and lifestyle components. To resolve this, modern genomic medicine utilizes a polygenic framework, aggregating individual risk variants into a cumulative Genetic Risk Score (GRS) to decode cumulative genetic liability. Genetic Risk Score (GRS) is defined as the unweighted or weighted sum of risk alleles across selected loci. GRS approaches have been explored for capturing polygenic contributions beyond single-SNP analyses, and have shown utility in predicting T2DM susceptibility and, in some studies, age of onset. However, evidence for their utility in predicting treatment response or glycemic control remains inconsistent, and their application in pharmacogenomics, particularly in South Asian populations remains unresolved. (13,14) Notably, an under-explored dimension of gene-treatment interactions in T2DM progression is critically limited. Additionally, metabolic outcomes are intrinsically tied to behavioral stressors. The psychological burden of disease management, quantified as diabetes-related distress, has been linked to a corresponding drop in medication adherence and self-care efficacy, establishing a complex bio-pscho-social feedback loop. (12)

The present study was designed to address this gap by conducting a Cross-Sectional pharmacogenomic analysis in a cohort of 144 Indian patients from Navi Mumbai, Maharashtra region with T2DM on metformin-based therapy. The specific objectives were: (i) to characterize the distribution of selected candidate variants; (ii) to assess individual SNP associations with glycemic control as measured by HbA1c; (iii) to evaluate the cumulative effect of an unweighted multi-locus GRS on HbA1c; and (iv) to investigate gene-drug interactions. Given the sample size constraints, the findings are intended to be hypothesis-generating to support future, larger-scale precision medicine initiatives.

2. METHODS

2.1 Study Population and Sample Size

This Cross-Sectional study evaluated an established cohort of 144 adult participants diagnosed with T2DM under active outpatient management. Ethical approval was granted by the institutional review board, and written informed consent was acquired from all participants prior to study enrolment for genetic investigation as well as publication of the result.

The required sample size was estimated assuming prevalence (p) of 50% due to lack of prior regional data of

type 2 diabetes had poor Glycemic control, defined as HbA1c ≥ 7%. (15,16) Accordingly, the sample size was calculated using the single-proportion formula:

$$N=Z^2pq/d^2$$

where p = 0.5 represents the expected proportion of patients with poor Glycemic control, $q = 1 – p = 0.5$, Z = 1.96 represents the standard normal deviate corresponding to a 95% confidence level, and d = 0.08 represents the allowable margin of error. Substitution of these values yielded an estimated minimum sample size of 151 participants. But during the study period, 144 individuals who met the predefined inclusion criteria and had consented for clinical and genetic data were enrolled and included in the final pharmacogenomic analysis.

2.2 Genotyping and Quality Control

Genomic DNA was isolated from peripheral whole-blood samples utilizing standard spin-column extraction kits according to manufacturer protocols. Six candidate SNPs across three genes were selected for targeted genotyping: *TCF7L2* (rs7903146, rs12255372, rs10509970, rs10885387), *PRKD1* (rs57803087), and *KCNJ11* (rs5219). Genotyping was performed using the Applied Biosystems™ Axiom™ Asia Precision Medicine Research Array Kit. Quality control was maintained by incorporating blind duplicate checks and non-template controls across all analytical plates, yielding a final call rate exceeding 99%.

For genetic association analyses, variants were coded according to an additive genetic model based on the number of copies of the prespecified effect allele, with values of 0, 1, and 2 representing zero, one, and two copies of the effect allele, respectively. For KCNJ11 rs5219 (Glu23Lys), the Lys allele was designated as the effect allele and genotypes were represented as Glu/Glu, Glu/Lys, and Lys/Lys. The Hardy-Weinberg equilibrium (HWE) was evaluated for each genotyped variant. Exact testing was used for variants for which the expected genotype counts were insufficient for reliable application of the asymptotic chi-square approximation. HWE assessment was used as a component of genotyping quality control and was not interpreted as evidence excluding genotyping error or population stratification.

Blood samples were analyzed for HbA1c, total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL), and LDL-cholesterol (LDL). BMI (kg/m^2) was calculated. Glycemic Control was categorized as poor if HbA1c ≥ 7.0% or good if HbA1c < 7.0%. Information regarding current medication use, a family history of diabetes and the age of initial diagnosis was obtained from patient records.

2.3 Calculation of Cumulative Genetic Risk Score (GRS)

To capture polygenic architecture and additive gene-gene effects, an unweighted, cumulative Genetic Risk Score (GRS) was established for each patient across the six candidate markers. Genotypes at each locus were coded additively based on the presence of the globally recognized metabolic risk allele, such as 0 represented homozygous wildtype, 1 represented heterozygous, and 2 represented homozygous risk. The continuous, cumulative GRS was calculated as the simple sum of risk alleles across all six successfully genotyped loci, generating a theoretical score ranging from 0 (minimum genetic risk) to 12 (maximum genetic risk).

2.4 Statistical Analysis

All statistical analyses were performed using Python version 3.13.5 with the pandas, SciPy, and statsmodels libraries. Continuous variables were summarized as mean ± standard deviation (SD), with minimum and maximum values, while categorical and genotype variables were summarized as frequencies and percentages. Distributional characteristics and assumptions of parametric analyses were assessed before modelling. For linear regression, residuals were examined for approximate normality, linearity, independence, and homoscedasticity. Primary regression analyses used HbA1c (%) as the outcome variable to retain clinically interpretable effect estimates.

Associations between individual SNPs and HbA1c were examined using genotype-group comparisons. Genotypes were additionally coded as 0, 1, or 2 according to the number of copies of the prespecified effect allele for genetic association analyses. The association of TCF7L2 rs7903146 genotype with age at diabetes onset was assessed using genotype-specific comparisons. An unweighted Genetic Risk Score (GRS) was calculated by summing the number of prespecified TCF7L2 risk alleles across the four analyzed variants, and its association with HbA1c was assessed using linear regression. The association of KCNJ11 rs5219 genotype and current sulfonylurea use with HbA1c was evaluated using multivariable linear regression. The model included genotype dosage, sulfonylurea use, their interaction term, age, BMI, duration of T2DM, and number of concurrent antihyperglycemic medications. In this model, the genotype coefficient represents the genotype-associated difference in HbA1c among non-users of sulfonylureas, while the interaction coefficient represents the difference in this association among sulfonylurea users. The study's Cross-Sectional design and absence of pretreatment HbA1c measurements precluded assessment of treatment response or causal effects.

All statistical tests were two-sided, and nominal p-values <0.05 were considered statistically significant. Given the exploratory nature of the genetic association and gene-treatment interaction analyses, statistically significant findings were interpreted cautiously and considered to require validation in larger, independent cohorts.

3. RESULTS

3.1 Cohort Characteristics

The final pharmacogenomic cohort comprised 144 participants with T2DM receiving metformin-based therapy. The mean age was 49.42 ± 7.95 years, mean BMI was 25.76 ± 3.85 kg/m², and mean duration of diabetes was 6.92 ± 6.06 years. The mean age at diabetes onset was 42.19 ± 6.68 years, while the mean HbA1c was 8.67 ±

2.10%. The mean total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol concentrations were 183.10 ± 41.09 mg/dL, 175.19 ± 89.19 mg/dL, 48.48 ± 11.46 mg/dL, and 103.28 ± 33.36 mg/dL, respectively.

Based on the predefined HbA1c threshold of 7.0%, 49 participants were classified as having good Glycemic Control and 95 as having poor Glycemic control. The clinical characteristics of the study cohort are summarized in Table 1. The distributions of the major clinical and genetic variables are shown in Figure 1.

Table 1. Summary of continuous clinical and demographic variables in the study cohort (N = 144). Values reported as Mean ± Standard Deviation.

Variable	Mean	SD	Min	Max
Age (years)	49.42	7.95	30	63
BMI (kg/m²)	25.76	3.85	19.6	40
Age of Onset (years)	42.19	6.68	26	59
Duration of T2DM (years)	6.92	6.06	1	26
HbA1c (%)	8.67	2.10	5.6	14.8
Total Cholesterol (mg/dL)	183.10	41.09	98	276
Triglycerides (mg/dL)	175.19	89.19	69	619
HDL-C (mg/dL)	48.48	11.46	26	74
LDL-C (mg/dL)	103.28	33.36	34	210

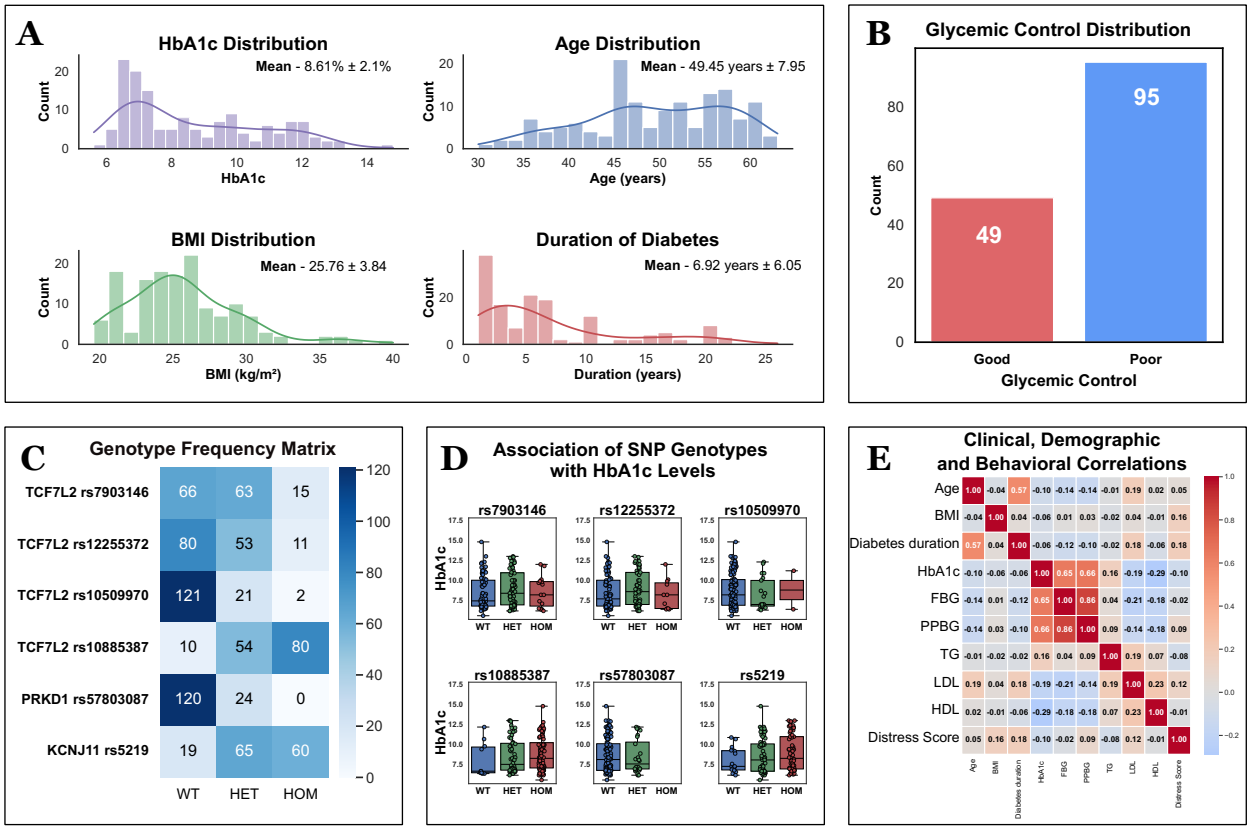


Figure 1. Overview of the clinical, genetic and phenotypic characteristics of the study cohort.
A. Distribution of key continuous clinical variables in the pharmacogenomic cohort, including HbA1c, age, body mass index (BMI), and duration of diabetes. Histograms depict variable distributions with corresponding mean ± SD values; **B.** Distribution of Glycemic Control categories showing a higher proportion of participants with poor Glycemic Control (n = 95) compared with good Glycemic Control (n = 49); **C.** Genotype frequency matrix showing the distribution of wild-type (WT), heterozygous (HET), and homozygous (HOM) genotypes across the six analyzed SNPs in the study cohort; **D.** Genotype-wise distribution of HbA1c across the six candidate variants, showing the distribution of HbA1c values across genotype groups; E. Correlation matrix showing relationships among clinical, biochemical, and behavioral variables included in the study; **E.** Heatmap Correlation matrix showing relationships among clinical, biochemical, and behavioral variables included in the study.

Histograms in Figure 1A depict variable distributions with corresponding mean ± SD values. This bar chart in Figure 1B shows stratification of glycemic boundaries. A vast majority of participants (66%, 95 individuals) showed poor glycemic control, highlighting therapeutic failure and a high clinical need in this group, and only 49 patients

(34%) displayed successfully controlled blood glucose levels. Genotype frequency matrix in Figure 1C has displayed the counts of patients carrying specific genetic variants across six SNPs related to diabetes genes (*TCF7L2*, *PRKD1*, and *KCNJ11*): Wild Type (WT) (normal or most common genetic variant), Heterozygous (HET) (carrying one normal and one mutated copy of the gene, and Homozygous (HOM) (carrying two mutated copies of the gene). Some mutations, such as *PRKD1 rs57803087* and *TCF7L2 rs10509970*, are predominantly wild type (WT) in this population, while others like *KCNJ11 rs5219* show a high frequency of mutated copies (homozygous, HOM).

The box plots show how individual SNPs relate to long-term blood sugar levels (HbA1c) in Figure 1D. The height of the boxes (interquartile range) and the spread of the whiskers reflect variance within each group. Each plot compares HbA1c levels across the WT, HET, and HOM groups for a specific SNP. Variations in box heights and median lines suggest whether carrying a specific mutated allele (HET or HOM) significantly increases or decreases HbA1c levels compared to the wild type. For certain variants, such as those within the TCF7L2 gene (*rs7903146* and *rs12255372*), the boxes show a progressive upward or downward shift from WT to HOM. A clear shift in the median lines indicates that carrying the mutated risk allele is associated with a distinct change in average HbA1c levels. In variants like *PRKD1 rs57803087*, the WT group contains a massive sample size (as seen in Figure 1C), resulting in a tightly defined box, whereas the lack of HOM individuals leads to wide or missing statistical representation for the mutant type. When the median line of a mutant type (HET or HOM) sits completely outside the interquartile range of the WT, it suggests a statistically significant genetic influence on how effectively a patient processes glucose.

The heat-map in Figure 1E evaluated the relationships between demographic, metabolic, and behavioural variables. The colours indicate the direction and strength of these relationships (Deep Red = Strong Positive Correlation, Deep Blue = Strong Negative Correlation). The correlations can be broken down into three main health axes, such as the glycemic axis (blood sugar markers), the metabolic & lipid axis, and the behavioural axis (distress score). A strong positive clustering with a dense block of deep red intersections appeared between HbA1c, FBG (Fasting Blood Glucose), and PPBG (Postprandial Blood Glucose). This is biologically relevant, as higher daily fasting and post-meal spikes directly drive up cumulative HbA1c percentages. The BMI & cardiovascular markers showed noticeable positive correlations between BMI and lipid profiles like TG (Triglycerides**)** and LDL cholesterol. Conversely, it displayed a negative correlation (shifting toward blue) with HDL cholesterol. Variation was observed in age and diabetes duration with lipid profiles and overall metabolic degradation. The Distress Score measured the diabetes-related emotional burden. It showed distinct positive correlations with higher HbA1c and poor glycemic control. This highlights a clinical behavioural loop: higher emotional distress often correlates with lower treatment adherence, resulting in poorer physiological blood sugar outcomes.

3.2 Hardy-Weinberg Equilibrium (HWE) and Genotypic Quality Control

The genotype distributions of the six analysed variants are presented in Figure 1. The observed genotype counts were 66, 63, and 15 for TCF7L2 rs7903146; 80, 53, and 11 for TCF7L2 rs12255372; 121, 21, and 2 for TCF7L2 rs10509970; 10, 54, and 80 for TCF7L2 rs10885387; 120, 24, and 0 for PRKD1 rs57803087; and 19, 65, and 60 for KCNJ11 rs5219, respectively, corresponding to the three genotype categories.

The observed effect-allele frequencies were 0.323 for TCF7L2 rs7903146, 0.260 for rs12255372, 0.087 for rs10509970, 0.743 for rs10885387, 0.083 for PRKD1 rs57803087, and 0.642 for KCNJ11 rs5219. The low frequencies observed for TCF7L2 rs10509970 and PRKD1 rs57803087 resulted in very small numbers of homozygous carriers.

HWE testing did not identify statistically significant deviation from equilibrium for the analysed variants. Because of the low-frequency variants and small homozygous genotype counts, these findings were interpreted as a component of genotyping quality control and not as evidence excluding population stratification or genotyping error. The allele frequencies and HWE results are summarized in Table 2.

Table 2. Genotype distribution, effect allele frequencies, and Hardy–Weinberg equilibrium for the six genotyped SNPs (N = 144).

SNP	WT Allele Freq (p)	Risk/ Effect Allele Freq (q)	HWE p-value
TCF7L2 rs7903146	0.677	0.323	0.9999
TCF7L2 rs12255372	0.740	0.260	0.867
TCF7L2 rs10509970	0.913	0.087	0.630
TCF7L2 rs10885387	0.257	0.743	0.977
PRKD1 rs57803087	0.917	0.083	0.552
KCNJ11 rs5219	0.358	0.642	0.978

Note: *Allele frequencies were calculated from the observed genotype distributions. The effect allele frequencies for KCNJ11 rs5219 and TCF7L2 rs10885387 were 0.642 and 0.743, respectively, indicating relatively high representation of these effect alleles in the study cohort. HWE was assessed as a genotype quality-control measure and was not interpreted as evidence excluding population stratification or genotyping error.*

3.3 Individual SNP Associations with HbA1c

In unadjusted genotype-group analyses, none of the six candidate variants showed a statistically significant association with HbA1c. The corresponding F-statistics

ranged from 0.22 for PRKD1 rs57803087 to 1.62 for KCNJ11 rs5219. For TCF7L2 rs7903146, rs12255372, rs10509970, and rs10885387, the respective p-values were 0.497, 0.425, 0.604, and 0.592. The p-values for PRKD1 rs57803087 and KCNJ11 rs5219 were 0.643 and 0.202, respectively. Thus, the unadjusted analyses did not provide evidence of an association between any individual candidate SNP and HbA1c in the study cohort. The complete results are presented in Table 3.

Table 3. One-way ANOVA results comparing mean HbA1c across genotype groups for the six analysed SNPs. No significant associations were identified.

SNP	F-statistic	p-value
TCF7L2 rs7903146	0.70	0.497
TCF7L2 rs12255372	0.86	0.425
TCF7L2 rs10509970	0.51	0.604
TCF7L2 rs10885387	0.53	0.592
PRKD1 rs57803087	0.22	0.643
KCNJ11 rs5219	1.62	0.202

3.4 TCF7L2 rs7903146 and Age of Diabetes Onset

Evaluation of the chronological metrics revealed that high-impact variants significantly dictate the timing of primary disease manifestation. The box plot with an overlaid swarm plot (Figure 2) demonstrated the relationship between the *TCF7L2* rs7903146 genotype and the Age of Diabetes Onset (Years). While individual genotypes showed no correlation with current cumulative glycemic levels, this figure uncovers a crucial finding that the genetic risk architecture strongly dictates the timing of disease manifestation. There is a clear, progressive downward shift in the median age of diabetes onset as the number of risk alleles increases. Wildtype non-carriers (0, indicated in Blue) exhibited the highest median age of onset at approximately 45 years. In comparison, in heterozygous carriers (1, indicated in Green), the median drops noticeably to approximately 41 years; while homozygous risk carriers (2, indicated in Red) exhibited the earliest biological collapse, presenting a median age of onset at approximately 38 years. The individual data points (circles) reveal that while wildtype individuals (0) frequently develop diabetes later in life (stretching up to 54 years), homozygous risk carriers (2) cluster tightly at much younger onset ages, with very few individuals developing the disease past age 45. The green heterozygous group features a prominent late-onset outlier near 59 years, highlighting that while genetics heavily influence the baseline timeline, external clinical or environmental protective factors can still delay onset in rare individuals. Notably, heterozygous (1) individuals for the TCF7L2 rs7903146 risk allele demonstrated an earlier age of onset by 2.84 years compared to wildtype (genotype 0) (p=0.034). Collectively, analysis of *TCF7L2* rs7903146 demonstrated a distinct, progressive gene-dosage effect on the age of diabetes diagnosis.

This finding has explained a vital dimension to a key scientific question: if these SNPs don't significantly change current HbA1c levels (Table 3), why are they considered major risk markers? The *TCF7L2* rs7903146 risk allele did not display how poor or good a patient's glycemic control is once they are diagnosed and under care; rather, it accelerated the chronological timeline of pancreatic β-cell exhaustion. Individuals carrying one or two copies of the risk allele experience an earlier biological onset of metabolic dysfunction. This explains why homozygous risk carriers are developing T2DM nearly a decade earlier than non-carriers, presenting a serious long-term clinical burden due to the extended lifetime duration of the disease.

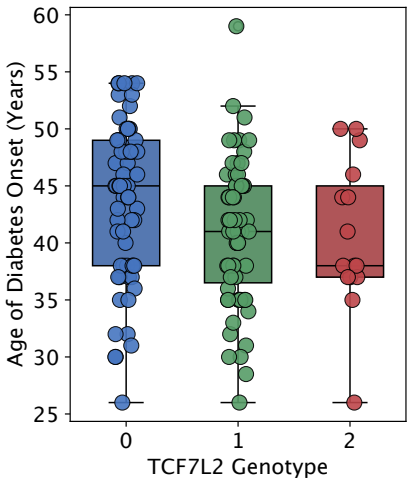


Figure 2: Distribution of age at diabetes onset across TCF7L2 rs7903146 genotype groups (0 = wildtype, 1 = heterozygous, 2 = homozygous). Heterozygous individuals showed an earlier age at diabetes onset than the wild-type reference group; the homozygous group did not show a statistically distinguishable difference from the reference group.

3.5 Genetic Risk Score and HbA1c: Cumulative Genetic Burden Predicts Glycemic Status

To account for potential polygenic architecture and additive gene-gene effects, a cumulative Genetic Risk Score (GRS) was calculated across the six candidate loci of the three genes. In contrast to individual single-locus assessments, the aggregated GRS demonstrated a clear association with patient metabolic outcomes. Participants with poor glycemic control exhibited a modestly higher cumulative genetic burden compared to those with good control, as evidenced by a rightward shift in median GRS distribution (Figure 3A). Furthermore, linear regression analysis confirmed a distinct positive trend between an increasing GRS and worsening glycemic status (Figure 3B).

By shifting from a single-locus approach to an aggregated polygenic risk architecture, the present data demonstrated that while individual variants hold weak predictive power on their own, their cumulative genetic burden significantly drives glycemic worsening. The result perfectly reconciles the apparent paradox between the negative single-SNP ANOVA results (Table 3) and the high clinical burden of poor glycemic control (Figure 3B).

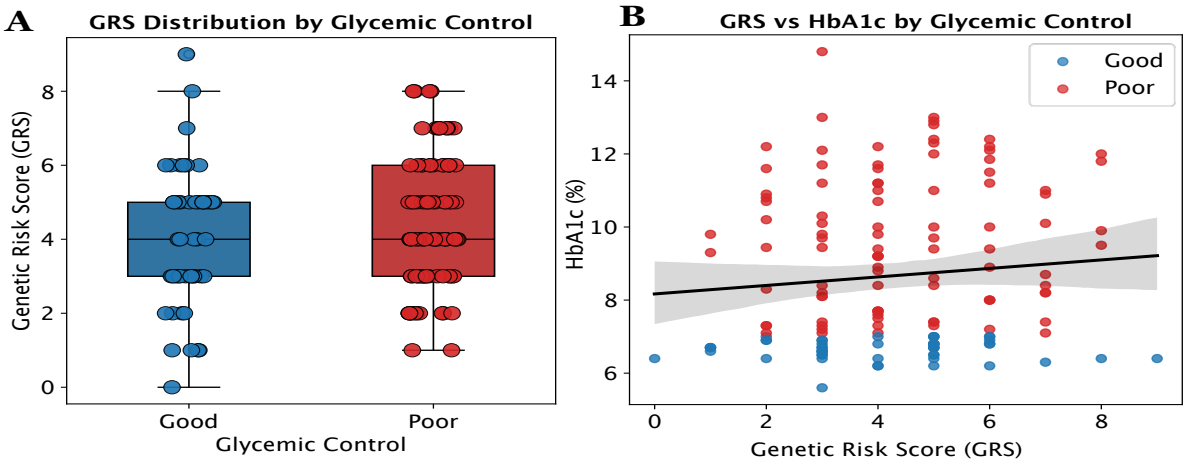


Figure 3: Genetic risk score (GRS) and Glycemic status in the study cohort.
A. Distribution of cumulative TCF7L2 genetic risk scores among participants with good and poor Glycemic control. **B.** Relationship between TCF7L2 genetic risk score and HbA1c levels, with individual observations stratified by Glycemic-control group and fitted regression line with 95% confidence interval. The GRS was not significantly associated with HbA1c ($p = 0.258$).

The figure 3A boxplots reveal a clear, rightward shift (modestly higher median and interquartile range) in cumulative genetic risk scores among participants in the poor glycemic control group (indicated in red) compared to those with good glycemic control (indicated in blue). This confirms that individuals who fail to maintain stable blood sugar levels harbour a structurally heavier concentration of risk-associated variants. The overlapping individual data points (overlaid observations) underscore that while a high GRS strongly predisposes patients to poorer outcomes, genetics alone are not completely deterministic, leaving room for lifestyle and behavioural modifiers. In figure 3B the upward-sloping fitted regression line demonstrates a direct positive trend between an increasing cumulative genetic burden and worsening baseline HbA1c levels. The dual-colour stratification (blue for good control, red for poor control) visually anchors the clinical reality: as a patient's GRS climbs along the X-axis, data points shift predominantly from blue to red, moving upward along the Y-axis. The narrow 95% confidence interval band indicates that this cumulative linear trend is highly stable across the cohort. Altogether, as individual genetic liability was accumulated, a corresponding rise in mean HbA1c levels was observed across the cohort, with data points transitioning predictably from the good control threshold (blue) to the poor control cluster (red). These findings indicate that while individual variant effect sizes are too subtle to establish significance in isolation, their combined, polygenic intersection serves as a robust linear predictor of glycemic vulnerability.

3.6 Pharmacogenetic Interaction: KCNJ11 rs5219 × Sulfonylurea (SU) Interaction

To decode the precise biological mechanisms behind the data, a multiple linear regression model incorporating drug history was built. The model isolated a highly significant, independent pharmacogenetic interaction ($G \times E$) between the *KCNJ11* rs5219 genotype and active sulfonylurea (SU) therapy (Table 4). Among patients not receiving SU therapy, the *KCNJ11* rs5219 Lys genotype was independently associated with an increase in HbA1c (β=1.321, 95% CI: 0.404–2.237, p=0.005). This indicates a remarkably large clinical effect size, with each additional Lys allele increasing HbA1c by 1.32 percentage points. Simultaneously, sulfonylurea use was associated with higher HbA1c (β=1.894, p=0.024). It is critical to note that this association is most plausibly interpreted as confounding by indication. Because baseline pre-initiation HbA1c data is unavailable, sulfonylurea use in this model functions as a proxy for more severe, refractory disease, rather than the cause of elevated blood glucose.

Despite this confounding in patients actively receiving SU agents, this genetic liability was effectively neutralized. The KCNJ11 rs5219 × sulfonylurea interaction term was statistically significant and counterweighed the genetic penalty by 1.138% per risk allele (β=-1.138, 95% CI: -2.225 to -0.050, p=0.040). This interaction suggests that sulfonylurea therapy may attenuate the genotype-

associated elevation in HbA1c by 1.14 percentage points. Given the complexity of this model (incorporating 7+ predictors for N=144), these estimates must be viewed as exploratory. Standard baseline covariates, including age, known disease duration, BMI, and total drug numbers, held no predictive value (p>0.28).

Table 4. Multiple linear regression model for HbA1c, incorporating KCNJ11 rs5219 genotype, sulfonylurea use, their interaction, and clinical covariates. Genotype coded additively (0 = Glu/Glu, 1 = Glu/Lys, 2 = Lys/Lys). Significant terms in bold (p < 0.05).

Variable	Coef.	Std. Err.	t	p-value	[0.025	0.975]
Intercept	8.147	1.939	4.202	< 0.001	4.313	11.982
KCNJ11 rs5219 genotype	1.321	0.464	2.849	0.005	0.404	2.237
Sulfonylurea use	1.894	0.831	2.280	0.024	0.251	3.536
KCNJ11 rs5219 × SU (interaction)	−1.138	0.550	−2.069	0.040	−2.225	−0.050
Age (years)	−0.023	0.026	−0.908	0.365	−0.074	0.027
Duration of T2DM (years)	−0.036	0.034	−1.065	0.289	−0.104	0.031
BMI (kg/m²)	−0.000	0.046	−0.008	0.994	−0.090	0.090
Number of drugs	−0.019	0.173	−0.110	0.913	−0.361	0.323

4. DISCUSSION

This study provides a pharmacogenomic characterization of TCF7L2, KCNJ11, and PRKD1 variants in an Indian T2DM cohort. Individual SNP analyses did not demonstrate significant associations with HbA1c, while the adjusted model identified an association between KCNJ11 rs5219 genotype and HbA1c together with a genotype-by-sulfonylurea interaction. The KCNJ11 rs5219 variant encodes the Kir6.2 component of the pancreatic K-ATP channel, which is a molecular target of sulfonylureas, providing biological rationale for investigating a potential genotype-treatment interaction. (17,18)

The KCNJ11 genotype coefficient was 1.32 (95% CI: 0.40–2.24, p=0.005); however, because the model included an interaction term, this coefficient represents the estimated genotype-associated difference in HbA1c among participants not receiving sulfonylureas. The negative interaction coefficient (β = −1.14, 95% CI: −2.23 to −0.05, p=0.040) indicates that the genotype-associated slope differed according to current sulfonylurea use. Among sulfonylurea users, the estimated genotype-associated slope was approximately 0.18 HbA1c percentage points per additional Lys allele. Thus, the finding should be interpreted as statistical effect modification rather than evidence that sulfonylureas therapeutically attenuate the genetic effect. Previous pharmacogenomic studies have reported associations between KCNJ11 variation and sulfonylurea response, supporting the rationale for further investigation of this interaction. (18,19)

The association between sulfonylurea use and higher HbA1c should also be interpreted cautiously. In a Cross-Sectional cohort, current sulfonylurea use may partly reflect treatment escalation in patients with inadequate Glycemic control, resulting in confounding by indication. Because pretreatment HbA1c and longitudinal treatment-response data were unavailable, the present analysis cannot establish a causal effect of sulfonylurea therapy or directly quantify treatment response.

An exploratory association was also observed between TCF7L2 rs7903146 genotype and age at diabetes onset, with heterozygous carriers showing an approximately 2.84-year earlier onset than the reference group (p=0.034). However, the absence of a clear additive pattern and the small number of homozygous carriers warrant caution. Previous studies have investigated the relationship between TCF7L2-related genetic burden and age of diabetes onset, but the present finding requires validation in larger cohorts. (20,21)

The unweighted TCF7L2 genetic risk score was not significantly associated with HbA1c (p=0.258). This lack of association suggests that the cumulative number of selected TCF7L2 risk alleles did not show a detectable linear relationship with current Glycemic control in this cohort. The limited sample size and use of an unweighted score may have reduced the ability to detect modest cumulative effects, which are generally difficult to capture in small clinical cohorts.

Several limitations should be considered. The final cohort comprised 144 participants, slightly below the calculated target of 151, and the available sample size limited precision, particularly for low-frequency variants and interaction analyses. The relatively complex regression model also requires cautious interpretation given the available sample size. Furthermore, the study was conducted at a single clinical center in Navi Mumbai and therefore cannot be assumed to represent the broader genetic and clinical diversity of Indian or South Asian populations. Population stratification was not formally assessed. The Cross-Sectional design, absence of pretreatment HbA1c measurements, and lack of longitudinal treatment-response data prevented assessment of causal treatment effects. Larger, multi-center studies incorporating longitudinal HbA1c measurements, pretreatment Glycemic data, detailed treatment histories, and appropriate assessment of population structure are required to validate these observations.

5. CONCLUSION

This study characterized selected TCF7L2, KCNJ11, and PRKD1 variants in an Indian cohort of patients with type 2 diabetes mellitus receiving metformin-based therapy. None of the six analyzed variants showed a significant unadjusted association with HbA1c, and the unweighted TCF7L2 genetic risk score was not significantly associated with Glycemic control. A genotype-specific association between TCF7L2 rs7903146 and age at diabetes onset was observed, although the non-monotonic pattern and limited number of homozygous carriers warrant cautious interpretation. The adjusted analysis identified a KCNJ11 rs5219 genotype-by-sulfonylurea interaction, indicating that the genotype-HbA1c association differed according to current sulfonylurea use. Given the Cross-Sectional design, modest sample size, absence of pretreatment HbA1c measurements, and lack of longitudinal treatment-response data, these findings should not be interpreted as evidence of a causal pharmacogenomic treatment effect. Larger, prospective, multi-centre studies with longitudinal Glycemic measurements and detailed treatment histories are required to validate the observed associations and determine their potential clinical relevance.

Declarations

Ethical Approval: The study was approved by the Ethics Committee for Research on Human Subjects, Re-Registration No. ECR/457/Inst/MH/2013/RR-20, MGMIHS, Navi Mumbai, India (Approval No. MGMIHS: RES: 02: 2022: 646).

Conflict of Interest: Authors declare that there are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Competing interests: None

Consent for publication: Written informed consent was obtained from each patient. This study was conducted in accordance with the principles of the Declaration of Helsinki. Prior approval of Institutional Ethics Committee, Hospital Superintendent and from the Head of the Medicine department was obtained.

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