



Research Article

Impact of *IRS1* (rs1801276) Gene Polymorphism on Type-2 Diabetes Mellitus in Bangladeshi Patients

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Abstract

The aim of this study was to investigate the possible effect of the insulin receptor substrate 1 (*IRS1*) gene (rs1801276) polymorphism on type-2 diabetes mellitus risk in Bangladeshi patients. Type-2 diabetes mellitus (T2DM) is a chronic metabolic condition defined by persistent hyperglycemia that is predominantly caused by hereditary and environmental factors. The *IRS1* (rs1801276) polymorphism has been linked to changes in the transmission of insulin and glucose metabolism which contribute to the development of type-2 diabetes. A cross-sectional study was carried out on 200 type-2 diabetes patients and 200 healthy controls. Genomic DNA was taken from blood samples, and the rs1801276 polymorphism was detected using PCR-RFLP. Statistical analysis was conducted to determine the relationship between the *IRS1* polymorphism and the prevalence of T2DM. To determine how the *IRS1* gene has an impact on type-2 diabetes we studied 200 case patients and 200 control individuals. Our investigation indicates that the *IRS1* gene polymorphism has no significant effect on the CG genotype [OR = 3.6283, 95% CI = (0.7443 to 17.6860, P=0.1108], GG genotype [OR = 1.0366, 95% CI = (0.0205 to 52.5059, P=0.9857] and the CG+GG genotype [OR = 3.6283, 95% CI = (0.7443 to 17.6860, P=0.1108] compared with wild type CC genotype. The *IRS1* (rs1801276) polymorphism is not significantly linked to Type-2 diabetes mellitus in Bangladeshi people. This finding emphasizes its potential for further studies with larger population to identify this polymorphism as a genetic marker for the diagnosis and treatment of T2DM patients in Bangladesh.

Keywords

IRS1, rs1801276, Type-2 diabetes mellitus, Bangladeshi population, genetic polymorphism, PCR-RFLP.

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Introduction

Type-2 Diabetes Mellitus (T2DM) is a group of complex metabolic disorders associated with an increased morbidity and mortality. As a multifactorial polygenic disease, T2DM rarely affects each person in the same way. It is diagnosed more commonly in late adulthood, and globally obese children and young adults have a higher prevalence of it. (Zajec et al., 2022). Prior research has shown that dysregulation of *IRS1* expression and function can affect the insulin signaling pathway, leading to the occurrence of IR and DM (Bedair RN et al., 2021). Past studies have also suggested a significant association between *IRS1* gene single nucleotide polymorphisms (SNPs) and T2DM risk (Atmodjo et al., 2021).

Insulin receptor substrate 1 (*IRS1*) gene, located at chromosome 2q36, is a member of the IRS protein family (Marushchak et al., 2021). Insulin receptor substrate 1 (*IRS1*), widely expressed in human tissues, is an endogenous substrate for insulin receptor (INSR) and a docking protein between the INSR and its downstream kinases. *IRS1* plays a key role in the transduction of insulin signaling, which in turn controls glucose and lipid metabolism. In fact, *IRS1* seriously participates in regulating insulin secretion by pancreatic β -cells, insulin action, peripheral insulin sensitivity, and modulating tissue response to insulin (Burguete-Garcia AI et al., 2010). Most individuals with IR remain unaware of their condition until they get T2DM, a genuine, deep-rooted ailment. If, early on, people discover that they have insulin resistance, they can improve their lifestyle and thus prevent or delay diabetes (Häring et al., 2014). *IRS* encodes a protein phosphorylated by the insulin receptor tyrosine kinase. Mutations in this gene are associated with T2DM and susceptibility to IR (Brown et al., 2015). During the acute phase of hyperglycemia, identifying genetic predictors for increasing IR can help treat T2DM.

Materials and Methods

Study Design

This research included 400 study subjects among which 200 patients were case and 200 patients in control groups, respectively. Patients who had type-2 diabetes and aged between 30-65 years and a confirmed diagnosis of T2DM at the City Hospital in Mohammadpur, Dhaka, Bangladesh. Blood was drawn from January 2024 to June 2024. Blood was extracted from patients by City hospital nurses who had been trained. Patients' ages, weights, glucose levels, and medication information were collected and maintained on file. The ethical committee of City hospital approved the study protocol, and the study was an observational study conducted in accordance with the

Declaration of Helsinki. Informed consent was acquired from the study subjects.

Sample collection and storage

The laboratory assistants who performed the experiments were blind to the data of the subjects. Five millimeters of blood samples were taken into separate tubes containing EDTA as anti-coagulant and stored at 4°C until DNA isolation. Genomic DNA extraction from blood samples was carried out by using standard protocols with a genomic DNA isolation kit. DNA samples stored at -20 °C until use.

IRS1 polymorphism Pro512Ala genotyping

The *IRS1* genotyping was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Genomic DNA was amplified using the primers: forward-5'-GTGGGTAGGCAGGCATCATC-3' and reverse-5'-CGGTGAGGAGGAGCTAAGCA -3' to detect the genotypes of the *IRS1* rs1801276 variant. The PCR reaction mixture was prepared by mixing 20 μ L consisting of 1 μ L of the forward primer, 1 μ L of the reverse primer, 12.5 μ L of Master Mix, 6 μ L of template DNA, and 2 μ L of nuclease-free water. PCR cycling process was performed with the following condition included: (a) pre-denaturation at 95 °C for 5 min (b) 35 cycles of denaturing at 95 °C for 30s, annealing at 58.5°C for 30s, and extension at 72 °C for 30s (c) final extension at 72 °C for 5min. The amplified products were then analyzed by RFLP. After overnight digestion of the PCR product (308 bp) at 37 °C in an incubator with the restriction enzyme of HpaII, the RFLP products (308 bp, 136 bp, 110 bp and 62 bp) were electrophoresed on 3% agarose gel and stained with ethidium bromide for visualization using a UV transilluminator. In terms of *IRS1* (rs1801276) gene polymorphism, the undigested fragment (308bp) was found to be a wildtype homozygote (CC) and the digested fragments (308, 136, 110, 62 bp) were detected as polymorphic homozygotes (genotype GG). Moreover, both digested and undigested fragments (136, 110, 62 bp) were identified as heterozygotes (genotypes CG).

Statistical Analysis

Allele frequencies were also compared, logistic regression models adjusted for age, BMI, and gender were used to assess the association between *IRS1* polymorphism and diabetics. The Hardy-Weinberg equilibrium (HWE) for the *IRS1* gene Pro512Ala polymorphism was employed to verify using χ^2 test in the patient and control groups separately. This test was used to assess the possible difference in allele frequencies between the control and case groups too, with a p-value of <0.05 considered statistically significant. To appraise the strength of the associations,

we calculated the odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs).

Results

Our investigation indicates that the *IRS1* gene polymorphism has no significant association on the CG and CG+GG genotype compared with CC genotype.

Two hundred T2DM patients (cases) and two hundred healthy controls were examined for their genotypes and allele frequencies of the *IRS1*-rs1801276 polymorphism. The goal was to determine the relationship between this polymorphism and the risk of type-2 diabetes. Results from three analyses are presented here: genotype distribution (Table 1), impact on type-2 diabetes (Table 2), and allele comparison (Table 3).

Table 1. Genotype distribution of *IRS1*-rs1801276 among T2DM patients and controls

<i>IRS1</i> rs1801276	Case (n = 200)	X ²	P value	Controls (n = 200)	X ²	P-value
CC	191	0.0641	0.74478	198	0.0051	0.9433
CG	7			2		
GG	0			0		

Table 2. The impact of *IRS1* [rs1801276] gene polymorphism on type-2 diabetes

Genotype	Case (200)	Control (200)	Odd ratio 95% CI	P-value
CC	191	198	Reference	-
CG	7	2	3.6283 (0.7443 to 17.6860)	0.1108
GG	0	0	1.0366 (0.0205 to 52.5059)	0.9857
CG+GG	7	2	3.6283 (0.7443 to 17.6860)	0.1108

Table 3. The comparison of C and G Allele *IRS1* [rs1801276] polymorphism in case of type-2 diabetes

Allele	Case	Control	Odd ratio 95% CI	P-value
C	391	398	Reference	-
G	7	2	3.5627 (0.7355 to 17.2565)	0.1145

Genotype Distribution

Table 1 shows the genotype distributions of T2DM patients and healthy individuals. Key findings from the table were as follows:

The CC genotype was found to be very common among both controls and cases, appearing in 198 out of 200 controls (99%) and 191 out of 200 cases (95.5%). Statistical analysis showed no significant difference between the groups, with chi-square (X²) values of 0.0641 (p = 0.74478) for cases and 0.0051 (p = 0.9433) for controls. The CG genotype was observed in 2 controls (1%) and 7 cases (3.5%). Although this genotype appeared more frequently in cases, the difference was not statistically significant, likely due to the limited sample size. The GG genotype was not present in either group, suggesting that it is either very rare or nonexistent in the population under study.

Impact of genotype on T2DM risk

Table 2 highlights the impact of the genotype on the susceptibility of T2DM. To evaluate the possible risk of type 2 diabetes mellitus (T2DM) associated with different genotypes, odds ratios (ORs) were calculated using the CC genotype as the reference group. For the CG genotype, the analysis yielded a p-value of 0.1108 and an OR of 3.6283 (95% CI: 0.7443 to 17.6860). Although this result is not statistically significant (p > 0.05), it suggests a potential trend toward an increased risk of T2DM due to its high odds ratio. For the GG genotype, the p-value was 0.9857 with an OR of 1.0366 (95% CI: 0.0205 to 52.5059), indicating no significant association with T2DM; however, interpretation is limited due to the rarity or absence of this genotype in the study population. When CG and GG genotypes were combined into a single group (CG+GG), the results remained the same as for CG alone, with a p-value of 0.1108 and an OR of 3.6283 (95% CI: 0.7443 to 17.6860), again pointing to a possible but statistically non-significant trend of increased T2DM risk.

Allele Distribution

Table 3 demonstrates allele distribution in the studied population. The following was revealed by comparing the C and G alleles: In both cases (391 out of 400; 97.75%) and controls (398 out of 400; 99.5%), the C allele is dominant. G Allele: Found in 2 controls (0.5%) and 7 cases (1.75%). P-value = 0.1145; OR = 3.5627 (95% CI: 0.7355 to 17.2565). Although this conclusion is not statistically significant, it does point to a possible trend of elevated risk associated with the G allele as the odd ratio is higher.

Biological Relevance

The *IRS1*-rs1801276 polymorphism is found in a gene that is crucial for insulin signaling. Variations in this gene may theoretically impact insulin sensitivity and glucose metabolism, raising the risk of type-2

diabetes. However, the CC genotype and C allele are common in the community, and there is no appreciable difference between patients and controls in our study. The CG genotype and G allele suggest a propensity for elevated risk for type-2 diabetes, despite the small sample size limiting statistical power and precision.

According to the data, there is no significant correlation between the *IRS1*-rs1801276 polymorphism and the incidence of type-2 diabetes in the population under study. While the G allele and CG genotype may indicate a trend toward greater risk, the CC genotype and C allele are dominant. Though the odds ratio is comparatively higher, these results are not statistically significant due to smaller sample size and low frequency of the polymorphism. The possible involvement of this polymorphism in T2DM susceptibility needs to be confirmed by more research including bigger and more varied populations.

Discussion

Insulin is a key factor in preserving glucose homeostasis and is a significant regulator of early growth. When insulin, which is released by beta (β) cells in the pancreas, binds to insulin receptors (IR) in target tissues, it activates several signaling molecules and interferes with their pathways. Tyrosine kinase domain autophosphorylation on the receptor's cytoplasmic surface is the principal effect of insulin-receptor contact, followed by phosphorylation of three tyrosine residues of cytosolic insulin receptor substrates (IRS). The recruitment of additional signaling molecules, such as growth factor receptor-bound protein 2 (GRB2) and phosphatidylinositol 3-kinase (PI3-kinase), subsequently activates the Akt signaling pathway, which mediates numerous metabolic effects, including glucose and lipid metabolism, and the mitogen-activated protein (MAP) kinase pathway, which mediates cell proliferation, respectively (Wing, 2008).

The structure of IRS proteins is similar, with a variable-length COOH-terminal tail after a phosphotyrosine binding (PTB) domain next to an NH₂-terminal pleckstrin homology (PH) domain. For IR–*IRS1* interactions to be effective, the PH domain is necessary. Conversely, the PTB domain has a direct interaction with the insulin receptors' juxtamembrane (JM) domain (Voliovitch et al., 1995). There are roughly 20 tyrosine (Tyr) phosphorylation sites in the COOH terminus of the IRS proteins, which act as on/off switches to draw in downstream effector molecules (Arikoglu et al., 2014).

According to studies involving various populations and meta-analyses regarding type-2 diabetes, numerous polymorphisms identified in the *IRS1* gene, which is located in 2q36–37, particularly the Gly972Arg substitution, have been linked to insulin resistance thus far (Aileen et al., 2005; Burguete-Garcia

et al., 2010; Celi et al., 2000; Jellema et al., 2003; Martínez-Gómez et al., 2011; Zaman Huri et al., 2012). There have been reports linking the *IRS1* gene's single nucleotide polymorphisms (SNPs) Gly972Arg, Pro170Arg, and Met209Thr to decreased phosphatidylinositol 3 kinase (PI3K) activity (Yoshimura et al., 1997) and the subsequent emergence of insulin resistance (Armstrong et al., 1996; Garcia et al., 1993; Yoshimura et al., 1997).

Furthermore, it has been observed that the polymorphisms Gly972Arg and Ala513Pro, which are situated close to the Tyr-Met-X-Met (YMXM) motifs around Tyr987 and Tyr612, affect insulin resistance, hyperinsulinemia, and the fatty acid composition of muscles (Garcia et al., 1993).

According to our findings, there is no significant correlation between the *IRS1* (rs1801276) polymorphism and type-2 diabetes mellitus in Bangladeshi people. However, having a higher odds ratio suggests a potential trend toward an increased risk of T2DM. Hyperglycemia and metabolic dysfunction may potentially be caused by the G allele through impairing *IRS1*-mediated insulin signaling. These findings align with previous studies on other populations, emphasizing the role of *IRS1* as a critical genetic marker for T2DM. Additionally, the results underscore the importance of genetic screening in populations with high diabetes prevalence. Early identification of at-risk individuals can facilitate targeted interventions, such as lifestyle modifications or insulin-sensitizing therapies. However, the study's cross-sectional design limits the ability to infer causation. Future longitudinal studies with larger sample sizes are required to validate these findings.

IRS1 polymorphism might be a contributing risk factor for the development of type 2 diabetes. Our findings indicate that the *IRS1* gene polymorphism has no substantial influence in Bangladeshi type-2 diabetes patients. Identifying these genetic variations can help predict disease susceptibility and guide personalized treatment strategies. While genetic factors play a critical role in the disease's pathogenesis, the complex nature of Type-2 diabetes requires further research to fully understand the interplay of genetic and environmental factors. Our data suggest that focusing on early-onset diabetes, which is characterized by a stronger genetic background, may be part of such a strategy.

The analysis suggests that the *IRS1*-rs1801276 polymorphism is not significantly associated with T2DM risk in the studied population. The CC genotype and C allele are dominant, while the G allele and CG genotype may show a trend of increased risk. However, these findings are not statistically significant. Further studies with larger and more diverse populations are needed to confirm the potential role of this polymorphism in T2DM susceptibility.

Conclusion and Future Direction

The study found no significant association between the IRS1 (rs1801276) polymorphism and Type-2 diabetes mellitus (T2DM) in the Bangladeshi population. Although the CC genotype and C allele were dominant, the CG genotype and G allele showed a non-significant trend toward increased T2DM risk. Due to a comparatively higher odds ratio, authors recommend further research with larger, more diverse populations and longitudinal studies to confirm these findings and explore genetic and environmental factors, especially in early-onset cases, to aid in disease prediction and personalized treatment.

List of Abbreviations

IR: Insulin resistance
PCR: Polymerase chain reaction
RFLP: Fragment length polymorphism analysis
T2D: Type 2 diabetes
IRS1: Insulin Receptor Substrate 1
NH: Normal homozygous
MH: Mutant homozygous
HE: Mutant Heterozygous
DNA: Deoxyribonucleic acid
OR: Odd Ratio
EDTA: ethylenediaminetetraacetic acid
TAE: Tris-acetate-EDTA
SNP: Single nucleotide polymorphism
CI: Confidence intervals

Data Availability Statement

Data relevant to the study is already included in the article or attached in the supplements. Raw data will be provided on reasonable request upon contacting with the corresponding.

Authors Contributions

Md. Siddiquil Islam designed and supervised the project. Tahmina Foyez, Abu Syed Md. Mosaddek, Salma Parvin, and Kazi Asharaful Islam contributed in sample collection and report writing. Yesmin Begum, Israt Jahan Bulbul contributed in lab work. Tanvir Mamun Romy, Peyas Ahmed, Ferdin Ehsan, Md. Shihad Al Shariar, Umme Rokaya Keya, and Jubayer Hosen collected the samples and conducted the lab work.

Conflicts of Interest

The authors declare no conflicts of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Zajec A, Trebušak Podkrajšek K, Tesovnik T, Šket R, Čugalj Kern B, Jenko Bizjan B, et al. Pathogenesis of type 1 diabetes: Established facts and new insights. *Genes*. 2022;13(4):706. doi:10.3390/genes13040706.
2. Bedair RN, Magour GM, Ooda SA, Amar EM, Awad AM. Insulin receptor substrate-1 G972R single nucleotide polymorphism in Egyptian patients with chronic hepatitis C virus infection and type 2 diabetes mellitus. *Egypt Liver J*. 2021;11(2). doi:10.1186/s43066-020-00069-1.
3. Atmodjo WL, Larasati YO, Jo J, Nufika R, Naomi S, Winoto I. Relationship between insulin-receptor substrate 1 and Langerhans' islet in a rat model of type 2 diabetes mellitus. *In Vivo*. 2021;35(1):291–7. doi:10.21873/invivo.12258.
4. Marushchak M, Krynytska I. Insulin receptor substrate 1 gene and glucose metabolism characteristics in type 2 diabetes mellitus with comorbidities. *Ethiop J Health Sci*. 2021;31(5):1001–10. doi:10.4314/ejhs.v31i5.12.
5. Burguete-Garcia AI, Cruz-Lopez M, Madrid-Marina V, Lopez-Ridaura R, Hernández-Avila M, Cortina B, et al. Association of Gly972Arg polymorphism of IRS1 gene with type 2 diabetes mellitus in lean participants of a national health survey in Mexico: A candidate gene study. *Metabolism*. 2010;59:38–45. doi:10.1016/j.metabol.2009.07.007.
6. Häring HU, Merker L, Seewaldt-Becker E, Weimer M, Meinicke T, Broedl UC, et al. Empagliflozin as add-on to metformin in patients with type 2 diabetes: A 24-week, randomized, double-blind, placebo-controlled trial. *Diabetes Care*. 2014;37(6):1650–9. doi:10.2337/dc13-2105.
7. Brown GR, Hem V, Katz KS, Ovetsky M, Wallin C, Ermolaeva O, et al. Gene: A gene-centered information resource at NCBI. *Nucleic Acids Res*. 2015;43 (D1):D36–42. doi:10.1093/nar/gku1055.

8. Arikoglu H, Hepdogru MA, Kaya DE, Asik A, Ipekci SH, Iscioglu F. IRS1 gene polymorphisms Gly972Arg and Ala513Pro are not associated with insulin resistance and type 2 diabetes risk in non-obese Turkish population. *Meta Gene*. 2014;2:579–85. doi:10.1016/j.mgene.2014.07.008.
9. Wing SS. The UPS in diabetes and obesity. *BMC Biochem*. 2008;9(Suppl 1):S6. doi:10.1186/1471-2091-9-S1-S6.
10. Voliovitch H, Schindler DG, Hadari YR, Taylor SI, Accili D, Zick Y. Tyrosine phosphorylation of insulin receptor substrate-1 in vivo depends upon the presence of its pleckstrin homology region. *J Biol Chem*. 1995;270(30):18083–7. doi:10.1074/jbc.270.30.18083.
11. Myers MG Jr, White MF. Insulin signal transduction and the IRS proteins. *Annu Rev Pharmacol Toxicol*. 1996;36:615–58. doi:10.1146/annurev.pa.36.040196.003151.
12. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α - and obesity-induced insulin resistance. *Science*. 1996;271(5249):665–70. doi:10.1126/science.271.5249.665.
13. Reiss K, Del Valle L, Lassak A, Trojanek J. Nuclear IRS-1 and cancer. *J Cell Physiol*. 2012;227(8):2992–3000. doi:10.1002/jcp.24019.
14. Tanti JF, Gremeaux T, Van Obberghen E, Le Marchand-Brustel Y. Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J Biol Chem*. 1994;269(8):6051–7. doi:10.1016/S0021-9258(17)37568-3.