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

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Abstract

Objective

To investigate the possible effect of the insulin receptor substrate 1 *(IRS1)* gene rs1801276 polymorphism risk on Bangladeshi Patients with Type-2 Diabetes Mellitus.

Background

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. The purpose of this study is to investigate the association between the IRS1 (rs1801276) gene polymorphism and T2DM in the Bangladeshi population.

Design and Methods

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 analyses were conducted to determine the relationship between the IRS1 polymorphism and the prevalence of T2DM.

Results

To determine how the IRS1 gene has an impact on insulin resistance in 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 = 4.6649, 95% Cl =(0.9950 to 21.8700, P=0.0507] , GG genotype [OR = 1.0366, 95% Cl =(0.0205 to 52.5059, P=0.9857] and the CG+GG genotype [OR = 4.6649, 95% Cl =(0.9950 to 21.8700, P=0.0507] compared with wildtype CC genotype.

Conclusions

The IRS1 (rs1801276) polymorphism is not linked to Type-2 diabetes mellitus in Bangladeshi people. This finding emphasizes its potential as a genetic marker for the diagnosis and treatment of T2DM in this population.

Keywords

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

Introduction

Type 2 DM (T2D) is a group of complex metabolic disorders associated with an increased morbidity and mortality. As a multifactorial polygenic disease, T2D 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 IRS-1 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 IRS-1 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.

Study Background and Methodology

Study Design: This research included 200 patients (case) and 200 controls, in total which is 400 samples. 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 until June 2024. Blood was extracted from patients by hospital nurses who had been trained. Patients' ages, weights, glucose levels and medication information were collected and maintained on file. The ethical committee of the relevant 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 both control and case groups.

Sample collection and storage

All the laboratory assistants who performed the experiments were blind to the data of the subjects. Five millimeter 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 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 μL consisting of 1 μL of the forward primer, 1 μL of the reverse primer, 12.5 μL of Master Mix, 6 μL of template DNA, and 2 μL 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 ºC for 30s (c) final extension at 72 ºC for 5min. The amplified products were then analyzed by RFLP. After overnight digestion of the PCR product (308 bp) at 37 ºC 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

To determine how the IRS1 gene has an impact on insulin resistance in type 2 diabetes we studied 200 cases of patients and 200 control individuals. Our investigation indicates that the IRS1 gene polymorphism has no significant association on the CG and CG+GG genotype compared with CC genotype.

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

IRS1 rs1801276	Case (n = 200)	X²	P value	Controls (n = 200)	X²	P value
CC	191	0.0641	0.74478	198	0.0051	0.9433
CG	7			2		
GG	0			0		

Table: The Impact of IRS1 [rs1801276] Gene Polymorphism on type-2 Diabetes

Genotype	Case (200)	Control (200)	Odd ratio 95% Cl	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: The comparison of C and G Allele IRS1 [rs1801276] polymorphism in case of type-2 Diabetes

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

Two hundred T2DM patients (cases) and two hundred healthy controls had their genotypes and allele frequencies of the IRS1-rs1801276 polymorphism examined. Determining the relationship between this polymorphism and the risk of type 2 diabetes was the goal. Results from three analyses are presented here: allele comparison, influence on type 2 diabetes, and genotype distribution.

Genotype Distribution

Key findings from the genotype distribution investigation were as follows:

• CC Genotype: o Very common in controls (198 out of 200; 99%) and cases (191 out of 200; 95.5%).

o The results of statistical analysis showed no significant difference between the groups, with chi-square (X2) values for cases and controls being 0.0641 (p = 0.74478) and 0.0051 (p = 0.9433), respectively.

• CG Genotype: o Found in 2 controls (1%), and 7 cases (3.5%).

o Although there were more examples of this genotype, statistical significance is not possible due to the limited sample size.

• GG Genotype: o Present in both cases and controls, suggesting that this genotype is uncommon or nonexistent in the population under study.

Impact of Genotype on T2DM Risk

To evaluate the possible risk of T2DM linked to various genotypes, odds ratios (ORs) were computed:

The reference group is the CC Genotype.

• CG Genotype: p-value = 0.1108; OR = 3.6283 (95% CI: 0.7443 to 17.6860).

Although the link is not statistically significant (p > 0.05), it does point to a possible trend of elevated T2DM risk.

• GG Genotype: p-value = 0.9857; OR = 1.0366 (95% CI: 0.0205 to 52.5059).

Although the lack of this genotype restricts interpretation, it shows no connection with type 2 diabetes.

• CG+GG Group: p-value = 0.1108; OR = 3.6283 (95% CI: 0.7443 to 17.6860).

Allele Distribution

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.

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. These results are not statistically significant, though. 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 a number of 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. 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.

IRS-1 polymorphism might be a contributing risk factor for the development of type 2 Diabetics. 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.

Acknowledgments

Funding information

Conflict 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.

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.

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