ANALYSIS OF CODON 972 (GLY → ARG) POLYMORPHISM IN IRS-1 GENE IN TYPE 2 DIABETIC POPULATION

Robby Kumar¹, Nishant Sharan²

¹Department of Biochemistry, S.S.R Medical College, Belle Rive, Mauritius
²SRF, National Bureau of Animal Genetic Resources, Karnal, India

Summary: Polymorphism of Insulin Receptor Substrate-1, especially the GGG→AGG (Gly-Arg) substitution at codon 972, is one of the major factors leading to the development of type 2 diabetes mellitus. This defect in IRS-1 causes insulin resistance along with many other consequences. It generally impairs insulin signalling via the phosphatidylinositol-3 (PI3)-Kinase pathway. In this study, the heterozygous Gly→Arg substitution at codon 972 of the IRS-1 gene was found in 2 of the 43 control Indian subjects, which is higher than normal when compared with the other population. The prevalence of the codon 972 GGG→AGG substitutions was found to be around 4.6%, which may be due to a predisposition factor. In diabetic subjects, on the other hand, 5 out of 43 showed substitution at codon 972, with a percent prevalence of 14%, establishing the role of the polymorphism of IRS-1 codon in the prevalence of diabetes mellitus.

Keywords: insulin, diabetes, polymorphism

Introduction

Diabetes mellitus is defined as a metabolic disorder characterized by relative or absolute insulin deficiency. The insulin receptors have intrinsic protein tyrosine kinase activity located in the cytoplasmic domain. When a ligand binds to the receptor a series of activities occur (1). Insulin acts through a cell surface receptor that belongs to a subfamily of growth factor receptor tyrosine kinases. There are two main pathways that propagate the signal generated through insulin: the insulin receptor substrate (IRS)/phosphatidylinositol (PI) 3-kinase pathway, and the Ras/MAP kinase pathway. The IRS/PI 3-K pathway leads to the activation of a cascade of PI-dependent kinases. These include PDK1, PKC isoforms and the serine/threonine kinase AKT. AKT phosphorylates glycogen synthase kinase 3 (GSK3), cGMP-inhibitable phosphodiesterase and FKHR transcription factors, leading to the stimulation of glycogen synthesis and inhibition of lipolysis and gene expression, respectively. There is also evidence linking the activation of AKT to the stimulation of glucose transport. The Ras/MAP kinase pathway can be activated by insulin through the formation of complexes between the exchange factor SOS and GRB2. GRB2 can be activated by IRS or SHC, which are direct substrates of the IR kinase. It appears that the acute metabolic effects of insulin require activation of the IRS/PI 3-K

ANALIZA POLIMORFIZMA U 972. KODONU (GLY→ARG) U GENU IRS-1
KOD OBOLELIH OD DIJABETESA TIPA 2

Kratak sadržaj: Polimorfizam supstrata insulinskog receptora 1, posebno supstitucija GGG→AGG (glicin-arginin) u 972. kodonu, jedan je od glavnih faktora koji dovode do razvoja dijabetes melitusa tipa 2. Ovaj defekt u IRS-1 izaziva insulinsku rezistenciju kao i brojne druge posledice. U najvećem broju slučajeva očituje se insulinsku signalizaciju preko putanje fosfradiinositol-3 (PI3)-kinaza. U ovoj studiji, heterozigotna supstitucija glicin arginin u 972. kodonu gena IRS-1 pronađena je kod 2 od 43 kontrolnih subjekata, što je više od normalnog nivoa u poređenju s drugom populacijom. Utvrđeno je da je prevalenca supstitucija GGG→AGG u 972. kodonu oko 4,6%, što može biti posledica faktora predispozicije. S druge strane, 5 od 43 subjekata sa dijabetesom imalo je supstituciju u 972. kodonu, uz prevalencu od 14%, što ukazuje na to da polimorfizam kodona u IRS-1 ima ulogu u prevalenciji dijabetes melitusa.

Ključne reči: insulin, dijabetes, polimorfizam
pathway, whereas the Ras/MAP kinase pathway may play a role in certain tissues to stimulate the actions of insulin on growth and proliferation. However, based on the genetic evidence described below, it is likely that pathways other than the IRS-PI 3-kinase must be very important for insulin signalling, as indeed is becoming increasingly clear (2–5). Disruption of IRS-1 causes growth retardation and insulin resistance associated with hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation. Furthermore, a recent study has shown that islets from mice with disruption of IRS-1 exhibit marked insulin secretory defects in response to glucose and arginine (6, 7). The polymorphism of Insulin Receptor Substrate-1 (IRS-1) plays an important role in insulin resistance and hence causes type 2 diabetes mellitus. The possible role of IRS-1 mutations in the development of NIDDM (Non-Insulin Dependent Diabetes Mellitus or Type 2 Diabetes Mellitus) has been shown by recent studies. Several amino acid polymorphisms have been identified in the IRS-1 gene. The most prevalent among them is the GGG→AGG (Gly-Arg) substitution at codon 972. The GGG→AGG substitution at codon 972 creates a novel BstO1 restriction site (8–10). It has been reported that insulin secretion is impaired in subjects having the GGG→AGG (Gly-Arg) polymorphism (5). More evidently, the molecular scanning of the IRS-1 gene in normal subjects and patients with type 2 diabetes has revealed several amino acid polymorphisms, the most common of which is the codon 972 Glycine to Arginine change. This amino acid substitution has functional consequences, causing impairment of the IRS-1-associated PI 3-kinase activity due to their defective interaction, thus leading to diabetes mellitus (11–19).

Materials and Methods

The blood samples were collected and fasting blood glucose was estimated in both control and diabetic subjects. Only those subjects who were freshly diagnosed with type 2 diabetes mellitus were selected, while the patients who were already taking the treatment were ruled out. For control, healthy individuals were taken with no present or past or family history of diabetes mellitus. Genomic DNA was isolated from whole blood (250 μL) using the Bioserve DNA extraction kit. Quality of the isolated DNA was checked by measuring the OD at 260 and 280 nm. The sample showing a 260/280 ratio of 0.7–0.8 was used for the PCR analysis. The quantity of the DNA in the sample was assessed on agarose gel by comparing the intensity of the band with the known standard DNA samples (Genei, India). Polymerase chain reaction was done for the amplification of the IRS-1 gene fragment from the extracted DNA. The amplified gene fragment was eluted from the gel by a Bioserve gel elution kit. This extraction kit was designed to extract and purify a DNA fragment from the agarose gel based on the binding of DNA to the silica-based membranes in chaotropic salts and elution with average recoveries of 50 to 80% of 100 bp to 10 Kb DNA fragments. The DNA was eluted and stored at −200 °C, after which the Restriction of eluted gene fragment was done with the help of restriction enzyme BstO1, that has the property to cut DNA at specific sites. The reaction mixture was prepared by adding buffer, BSA, DNA fragment, Restriction enzyme and sterile water. The mixture was incubated at 600 °C for 3–4 h. After incubation the restricted product was visualized on 3% agarose gel. The primers used for PCR were: a) IRS-1 (Forward): 5’-GCAGGCTG-GCAGGAGAG-3’ and b) IRS-1 (Reverse)→5’-CTCA-CCTCCTCTGCAGC-3’.

Results and Discussion

A total of 86 blood samples collected from individuals including 43 type 2 diabetic subjects and 43 age and sex matched control subjects (Table I) were analyzed for the IRS polymorphism at codon 972 (Gly→Arg). Figure 1 presents isolated genomic DNAs from different patients or control subjects on 0.8%
agarose gel, which shows a characteristic band of genomic DNA near the well side. After assessing the quality, DNA samples showing a 260/280 ratio of 0.7–0.8 were used for further experimentation.

The genomic DNA from different subjects was amplified by polymerase chain reaction using primers targeted against the human IRS gene containing codon 972. Polymerase chain reaction is an in vitro method for replicating the desired DNA fragment so that its amount increases exponentially. PCR amplification of the human genome using primers of the IRS gene, as mentioned above, yielded a fragment of 221 bp. Figure 2 shows a representative gel photograph of the amplified DNA product on 2% agarose gel.

Figure 2 PCR amplification of the human genome using primers targeted against the IRS-1 gene. Lane 1, 100 bp DNA ladder; lane 2–7, amplified products from different genomic DNA samples.

Figure 3 Restriction pattern of the IRS-1 gene fragment with BstO1. Lane 1, 100 bp DNA ladder; lanes 2, 4, 6, 8, 10, eluted gene fragment; lanes 3, 5, 7, 9, 11, eluted fragment after restriction with BstO1; lane 12, genomic DNA; lane 13, restricted human genomic DNA.

The amplified gene product was eluted from the gel and restricted with the restriction enzyme BstO1, which is an endonuclease and restricts the DNA at 5’-CC▼(A/T) GG-3’ and 3’-GG ▼(T/A) CC-5’ site. In case of diabetes a single nucleotide mutation (G→A) at codon 972 leads to the generation of a restriction site for BstO1. When the eluted fragment of different subjects was restricted with BstO1, only 4 showed restriction yielding an extra band of ~190 bp (Figure 3 and 4).

Figure 4 Restriction pattern of the IRS-1 gene fragment with BstO1. Lane 1, 100 bp DNA ladder; lanes 2 and 4, eluted gene fragment; lanes 3 and 5, eluted fragment after restriction with BstO1; lane 7, genomic DNA; lane 8, restricted human genomic DNA.

Figure 5 Restriction pattern of the IRS-1 gene fragment with BstO1. Lane 1, 100 bp DNA ladder; lanes 2–9, eluted gene fragment; lanes 10 and 11, eluted fragment after restriction with BstO1; lane 12, genomic DNA; lane 13, restricted human genomic DNA.

Genetic variance in the insulin receptor substrate-1 (IRS-1) is thought to play a key role in the insulin resistance that characterizes type 2 diabetes. Transfection studies have demonstrated that the most common IRS-1 variant, Arg972, which involves a Gly→Arg substitution at codon 972, impairs insulin signalling via the phosphatidylinositol-3 (PI3)-Kinase pathway, and in some (but not all) studies this variant was found with an increased frequency among type 2 diabetic patients (20–29). In this study, the heterozygous Gly972→Arg substitution of the IRS-1 gene was found in 2 of the 43 control subjects. The prevalence of the codon 972 GGG→AGG substitutions was found around 4.6%. In the diabetic subjects, 6 out of 43 showed substitution at codon 972, with a prevalence of 14%, while other studies have found this percent to be 25 (30). The codon 972 Gly→Arg substitution was found in 4.6% of Indian subjects with normal glucose tolerance. The prevalence was nearly equal to that reported for the Caucasian population, and slightly greater than reported in the Japanese and Taiwanese population. A significant difference was observed in the frequency of the variant allele between the normal control and diabetic subjects.
The modest association between the mutation and NIDDM, which was statistically significant only when all the available studies were pooled, suggests that this uncommon mutation may contribute to the etiology of NIDDM in a small subgroup of cases. One possibility is that the mutation is not a sufficient cause, but can interact with obesity to cause diabetes. Specifically, given the low prevalence of the mutation together with the modest relative risk of diabetes associated with it, we estimate that about 7.6% of cases of diabetes are attributed to the mutation in the IRS-1. Although the subgroup analyses within the limited data available from our study must be interpreted with caution, they suggest a plausible hypothesis that can be tested with a study of modest size. The clustering of overweight together with diabetes in the carriers of the mutation suggested that excess body weight interacts with the mutation to increase the risk of diabetes (5, 27–29).

Results indicate that the Indian population has a much higher incidence of the polymorphism of codon 972 GGG→AGG (Gly-Arg), and a higher incidence of the polymorphism is also seen in the control subjects, suggesting a high predisposition among them.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

Arg IRS-1 Variant Is Associated With Type 1 Diabetes in Continental Italy. Diabetes 2003; 52(3): 887–90.


