Research Article

Clinical and genetic association of insulin growth factor-2 gene in diabetes mellitus among south Indian population

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, abnormally elevated hepatic glucose production, and reduced glucose-stimulated insulin secretion. **Objective:** The aim of this study was to determine the possible association of single-nucleotide polymorphisms located in insulin growth factor-2 (IGF-2) gene on diabetes mellitus. **Material and Methods:** This case-control study assessed 100 T2DM patients and 100 controls. Genotyping was done by polymerase chain reaction-restriction fragment length polymorphism technique. **Results:** The mean age of the T2DM patients was 61.7 ± 8.8 years with a mean body mass index of 24.0 ± 3.3 kg/m^2. There was a significant difference in the levels of total cholesterol, high-density lipoprotein-C, and triglycerides between the T2DM patients and controls ($P < 0.05$) while as very low (VLDL) was not associated. **Conclusion:** We hypothesized that IGF-2 gene was associated with T2DM patients when compared to controls. Both the alleles were significantly associated ($P = 0.005$). IGF-2 Apa1 can be used as a biomarker for identifying individuals at a high risk of developing T2DM.

KEY WORDS: Diabetes Mellitus; Insulin; Cholesterol; Polymorphism; Polymerase Chain Reaction

INTRODUCTION

Diabetes mellitus is a global health problem with a tremendous impact on morbidity and premature mortality worldwide. It affects 366 million people worldwide (6.4% of the world's adult population aged between 20-79 years). This number will be increased to 552 million by 2030.[1] Diabetes, a disease associated with altered glucose homeostasis, is common in India. The international diabetes federation estimates that around 61.3 million people in India had diabetes in 2011, and projects that by 2030, this will go up to 101.2 million.[2] T2DM is recognized as chronic persistent hyperglycemia resulting from pancreatic dysfunction or insulin resistance, and this disease is assuming epidemic proportions.[3]

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Type 2 diabetes mellitus (T2DM) has been recognized as a heterogeneous group of metabolic and multifactorial disorders affecting the adult population. India ranks second in the world in diabetes prevalence, just after China.[2] Genetic and environmental factors play important roles in the progression of the disease.[4] Both longitudinal and cross-sectional studies have demonstrated that T2DM is influenced by several behavioral as well as lifestyle factors.[5] Clinical and epidemiological studies have indicated that obesity is a major risk factor for T2DM, associated with an increased risk of developing insulin resistance and impaired glucose tolerance. Impaired insulin secretion and insulin resistance; the two main pathophysiological mechanisms leading to T2DM have a significant genetic component.[6]

Insulin-like growth factors (IGFs) are regulators of processes such as growth and metabolism. IGF-1 and IGF-2 contribute

to pancreatic β-cell growth and development by regulating β-cell replication, renewal, and apoptosis.[7] Deregulation of balance between β-cell renewal and apoptosis due to alterations in IGF levels is potentially of great importance in the development of glucose intolerance, a major characteristic of diabetes. In addition, insulin-dependent glucose homeostasis may be affected by IGFs as they act via the insulin signaling pathway.[8] Defects in the IGF/insulin-signaling pathway affects birth weight and fat metabolism in both domestic animals and humans, which are known risk factor for development of type 2 diabetes (T2D).[9] IGF-2 polymorphisms have been associated with weight gain; body mass, obesity, and adiposity.[10] Hence, the aim of this study was to evaluate the association of IGF-2 Apa1 polymorphism in T2D patients.

METHODS AND MATERIALS

Sampling

Patients for the study were selected from the Princess Durru shehvar children's and General Hospital, Hyderabad. 3 ml of venous blood for DNA isolation was collected. Demographs was obtained along with information regarding clinical and family history in a well-designed pro forma. The salting out technique was used successfully to isolate large quantities of DNA from blood. DNA was extracted from peripheral blood lymphocytes using 300 µl of whole blood. The isolated DNA was amplified using polymerase chain reaction (PCR). PCR is an enzyme catalyzed biochemical reaction in which small amount of a specific DNA fragment is amplified into large amount of linear double strand DNA using gene specific oligonucleotide primers.

Isolation of DNA and Genotype Analysis

Genomic DNA was isolated from the peripheral blood of subjects using salting out method. The DNA was stored at −20°C until processing. Genotyping for the IGF-2 Apa1 gene polymorphism (rs680) was performed by PCR with the use of specific published primers. Forward primer: 5'-CTTGGACTTTGAAGTCAAATTGG-3'; Reverse primer: 5'GGTCGTGCCAATT ACATTTCA-3' synthesized from Sigma-Aldrich Chemical Pvt. Limited (Bangalore, India), followed by restriction fragment length polymorphism analysis. A three-step PCR was performed using XP thermal cycler. Briefly, the PCR conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 68°C for 45 s, final extension at 68°C for 5 min. The 292 bp amplified PCR product was digested with Apa1 enzyme at 37°C for 2 h and electrophoresed on 2% agarose gel with ethidium bromide. Bands of 229 bp were observed in case of GG genotype, 292 bp and 229 bp in AG genotype and an undigested 292 bp band in AA genotype. Restriction enzyme digested PCR products were imaged and analyzed by documentation in UVI Tech gel documentation system (UVI Tech Ltd. Cambridge, United Kingdom).

Statistical Analysis

Genotype and allele frequencies were calculated. The groups were compared using the $\chi 2$ test to analyze the statistical significance of the difference in allelic distribution of various polymorphisms in patients and controls. Values of $P < 0.05$ were considered statistically significant. Odds ratio was performed using MedCalc for Windows, version 7.4.1.0 (MedCalc Software, Mariakerke, Belgium).

RESULTS

Clinical Characteristics

A total of 200 subjects with 100 T2DM patients and 100 healthy volunteers were enrolled in this study. The demographic characteristics of risk factor variables for T2DM patients and control subjects are presented in Table 1. The mean age of the T2DM patients was 61.7 ± 8.8 years with a mean body mass index (BMI) of 24.0 ± 3.3 kg/m^2. The average age of the control subjects was 63.2 ± 5.4 years with a mean BMI of 23.8 ± 2.2 kg/m^2. In this study, we found 65% of patients and only 35% in controls were smokers. 30% of patients was alcoholic and only 5% of controls were alcoholic.

The mean and standard deviation values of total cholesterol was (197 ± 25) and (135 ± 10), LDL (130 ± 17) and (112 ± 13), high-density lipoprotein (HDL) (37 ± 10) and (40 ± 8), VLDL (22 ± 10) and (20 ± 6), and triglyceride (175 ± 15) and (138 ± 10) among T2DM patients and controls, respectively, as shown in Table 2. There was a significant difference in the levels of total cholesterol, HDL-C, and triglycerides between the T2DM patients and controls ($P < 0.05$) while as VLDL was not associated.

Genotyping for Apa1 polymorphism revealed AA genotypes in 48% and 70% of T2DM and controls, respectively. AC was present in 42% and 28% in T2DM and control group, respectively. CC was seen in 10% of T2DM cases and 2% of the controls. The AA genotype was significantly associated with T2DM (OR, 0.39; 95% CI: 0.22 to 0.70, P 0.001). AC genotype was found more in patients than controls and was associated with T2DM (OR, 1.86; 95% CI: 1.03 to 3.35, P 0.03) and CC genotype was also associated with T2DM (OR, 5.45; 95% CI: 1.16 to 25.52, P 0.03). Frequency of A allele was 0.69 in T2DM and 0.84 in controls (OR, 0.42; 95% CI: 0.26 to 0.68) while as the frequency of C allele was 0.31 in T2DM patients and 0.16 in controls (OR, 2.35; 95% CI: 1.45 to 3.82). Both the alleles were significantly associated ($P = 0.005$) with T2DM patients when compared to controls (Table 3).

Table 1: Demographic details of T2D patients and controls of this study

Parameters	T2D (*n*=100)	Controls (*n*=100)
Age (years)	61.7±8.8	63.2±5.4* (*P*=0.092)
Male	65	35
Female	45	55
Smoker	60	40
Alcoholic	30	5
Nonalcoholic	70	95
BMI	24.0±3.3	23.8±2.2* (*P*=0.504)

Age and BMI is shown in mean and standard deviation, BMI: Body mass index, T2DM: Type 2 diabetes mellitus

Table 2: Shows the mean levels of lipid profile in the cases of T2D and controls

Lipid profile	Subjects with T2DM (*n*=100)	Subjects without T2DM (*n*=100)	*P*
Mean total cholesterol (mg/dl)	197±25	135±10	0.001
Mean LDL cholesterol (mg/dl)	130±17	112±13	0.001
Mean HDL cholesterol (mg/dl)	37±10	40±8	0.02
Mean VLDL cholesterol (mg/dl)	22±10	20±6	0.08
Mean TG	175±15	138±10	0.001

Data is shown as Mean, Standard deviation and P- value, HDL: High-density lipoprotein, VLDL: Very low density lipoprotein, T2DM: Type 2 diabetes mellitus

Table 3: Genotype and allele frequency of IGF2 gene in T2DM patients and controls

Genotype	T2D (*n*=100)	Controls (*n*=100)	Odds ratio	95% CI	*P*
AA	48 (48)	70 (70)	0.39	0.22-0.70	0.001
AC	42 (42)	28 (28)	1.86	1.03-3.35	0.03
CC	10 (10)	2 (2)	5.45	1.16-25.52	0.03
Allele					
A	138 (0.69)	168 (0.84)	0.42	0.26-0.68	0.005
C	62 (0.31)	32 (0.16)	2.35	1.45-3.82	0.005

IGF2: Insulin-like growth factor 2, T2DM: Type 2 diabetes mellitus

DISCUSSION

This study was conducted to explore the association between T2DM patients and healthy controls by investigating single nucleotide polymorphism, demographs, and clinical factors among South Indian population. In this study, we found 65% of patients and only 35% in controls were smokers. 30% of patients was alcoholic and only 5% of controls were alcoholic. There was a significant difference in the levels of total cholesterol, HDL-C, and triglycerides between the T2DM patients and controls ($P < 0.05$) while as VLDL was not associated. We hypothesized that IGF-2 gene was associated with T2DM patients when compared to controls. The AA genotype was significantly associated with T2DM (OR, 0.39; 95% CI: 0.22 to 0.70, $P = 0.001$). AC genotype was found more in patients than controls and was associated with T2DM (OR, 1.86; 95% CI: 1.03 to 3.35, $P = 0.03$) and CC genotype was also associated with T2DM (OR, 5.45; 95% CI: 1.16 to 25.52, $P = 0.03$). Frequency of A allele was 0.69 in T2DM and 0.84 in controls (OR, 0.42; 95% CI: 0.26 to 0.68) while as the frequency of C allele was 0.31 in T2DM patients and 0.16 in controls (OR, 2.35; 95% CI: 1.45 to 3.82). Both the alleles were significantly associated ($P = 0.005$) with T2DM patients when compared to controls (Table 3).

There is considerable evidence that IGF-2 regulates cell growth, differentiation, and metabolism.[11] Quantitatively IGF-2 is the predominant circulating IGF present in adults at a concentration of ~700 ng/ml,[12] three times that of IGF-1. Concentrations are similar in both genders. In common with insulin and IGF-1, binding of IGF-2 to the IGF-1R activates a receptor tyrosine kinase (RTK) associated with the b-subunit leading to an intracellular response.[13-15] Autophosphorylation of the b-subunit by the RTK recruits insulin receptor substrates (IRS) 1-4. Phosphatidylinositol 3-kinase then binds to IRS1 via its regulatory subunit and is activated, in turn activating Akt (protein kinase B). This has a number of intracellular effects, which ultimately promote cell survival and mitogenesis.

Previously, it was shown that polymorphisms in the IGF-1 and IGF-2 genes are associated with features of the metabolic syndrome.[16,17] Gene variants in the IGF-2 gene were found to be associated with IGF-2 levels and BMI.[18,19] Gomes et al.[20] (2006) studied the association between IGF-2 Apa1 polymorphism and the BMI, however, did not find any significant association. A previous study of IGF-2R as a susceptibility gene for T2DM has similarly implicated an insertion/deletion variant in the 3'UTR region of IGF-2R.[21] The insertion/deletion polymorphism is likely to result in the change in IGF-2R expression.[22]

The major limitations of this study were small sample size was and only a single single-nucleotide polymorphisms was selected from IGF-2 gene.

CONCLUSION

In conclusion, this study showed that IGF-2 Apa1 polymorphism is associated with T2DM in south Indians and it can be used as a biomarker for identifying individuals at a high risk of developing T2DM. Limited use of smoking and alcohol may reduce the incidence of T2DM.

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