Gene as a risk factor for type 2 diabetes mellitus and its related complications

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Abstract

Diabetes has turned out to be pandemic towards the end of the century; unfortunately there are many people who are still unaware of the fact that they are having diabetes even if they have already developed various complications related to it. Patients with long duration of Type 2 Diabetes Mellitus have a significantly increased risk of developing related complications. This ignorance is due to similar manifestations for two related health problems like that of heart problem which may or may not be related to diabetes. Finding genetic variants in genes is a pretty good method for assessment of the risk for type 2 diabetes mellitus and the complications related to it. These genetic predisposing factors can foretell the susceptibility of a person to the disease or become the reason behind diabetes in the person. The present case control study intends to associate the reported single nucleotide polymorphisms in seven genes viz. Calpain10 (CAPN10), Adiponectin (ADIPOQ), Apolipoprotein C-III (APOC III)], Angiotensinogen (AGT), Apolipoprotein E (APOE) and Toll-Like Receptor 4(TLR4) genes as risk factors for Type 2 Diabetes and its related Complications in the discrete diabetic population of Andhra Pradesh and Telangana states in India. The results showed the association some of the single nucleotide polymorphisms in the candidate genes which increased the risk of development of type 2 diabetes mellitus and progression in the form of complications.

The results showed the presence of new Single Nucleotide Polymorphism in the Type 2 Diabetes cases. Further extension of analyses work on a larger sample size is to be undertaken in our study population.

Introduction

Diabetes mellitus is a heterogeneous group of metabolic disorder characterized by dysregulated glucose metabolism. Its development and advancement in form of complications are due to several risk factors. Uncontrolled Diabetes or elevated blood glucose levels for prolonged time lead to long term and short term complications like [1] microvascular (damage to small blood vessels) and macrovascular (due to damage to the arteries). The high blood glucose levels for prolonged time affect both the quality and length of life [2,3]. This condition is attributed to impairment of glucose utilization. The insulin resistance [4,5] or deficiency [6] is responsible for this condition. The prevalence of T2DM is increasing on daily basis in most of the world wide countries [7] with a significant effect on the adult population [8]. It is expected to rise to 529 million by 2035 (Diabetes Atlas-update-2014). The increase is more in especially developing countries [3] like India. India is designated as "Diabetic capital of the World" for it has the largest increase of 80% diabetic subjects than any other country. (The International Diabetes Federation) The Characteristic "Asian Indian Phenotype" makes them more susceptible to the disease [9]. Among them the people living in southern states are more prone due to their life style [10]. The people who have well-controlled blood sugar levels have less common and less severe in complications of diabetes mellitus [11]. Several risk factors can foretell the susceptibility of a person to the type 2 diabetes mellitus and its related complications. They are grouped into Genetic, Environmental and Biochemical risk factors [12]. This factors either individually or on interaction with one another to cause the disease. The structural variations in the genes are responsible for variation in phenotype [13,14] is known to be the genetic risk factors.

Calpain 10 (CAPN10)

Calpain10 gene [15,16] encoded by cysteine protease is the first but not the last candidate gene with susceptibility for T2DM. It was identified through a positional cloning and genome wide screening [17]. Its role regulation of the glucose is known. Studies on CAPN 10 for T2DM among different ethnic groups in wide range of population showed uneven results [18-23] due to genetic heterogeneity between populations. Some confirming the original finding that haplotype combination comprising three intronic CAPN10 single-nucleotide polymorphisms (UCSNP-43, -19, and -63) was associated with increased risk of T2DM but some do not.

Adiponectin (ADIPOQ)

ADIPOQ gene is located on Chromosome 3q27 is responsible for the major adipocyte secretory protein. It has a potent role in the metabolism of fat and the regulation of glucose. It includes insulin sensitivity in muscle and liver, regulating energy homeostasis and glucose tolerance. The low circulating levels of adiponectin are associated with obesity and diabetes [24,25].

Dickkopf WNT signaling pathway inhibitor 1(DKK1)

This gene encodes a cysteine rich glycoproteins and is a member of the dickkopf family. Elevated levelsof DKK1 in bone marrow plasma and peripheral blood are associated with the presence of osteolytic bone lesions .It is involved in glucose metabolism [26].

Apolipoprotein C-III (APOC III)

Apolipoproetin is the product of this gene. It induces the development of hypertriglyceridemia by delaying the catabolism of triglyceride-rich particles [27].

Angiotensinogen (AGT)

This gene belongs to a family of SERPIN. This produces protein angiotensin protein that is part of the renin-angiotensin system that regulates blood pressure [28].

Apolipoprotein E (APOE)

The APOE gene provides instructions for making a protein called apolipoprotein E. It mediates the binding, internalization, and catabolism of lipoprotein particles [29].

Toll-Like Receptor 4(TLR4)

The protein encoded by this gene is a member of the Toll-like receptor (TLR) family which plays a fundamental role in the diseases associated with protein coding TLR4 include endotoxin hypo responsiveness and macular degeneration [30].

Materials and methods

Enrolment of cases and controls

The discrete population of Andhra Pradesh and Telangana states in India was selected for the case control study. For this a total of 200 individuals visiting diagnostic centres were enrolled. They were primarily screened for diabetes using glucose oxidase peroxidase method [31] in accordance to WHO criteria. Out of which a total of 180 individuals (96 females and 84 males) of 15-85 years of age were selected after the exclusion of those with other serious disorders.Those with Fasting Blood Sugar (FBS) value of more than 126 mg/dl or $\geq$ 7.0 mmol/L and Post Prandial Blood Sugar (PPBS) value of more than 200 mg/dl or $\geq$ 11.1 mmol/L (WHO diagnostic criteria for diabetes) are cases and those with no family history of diabetes with FBS and PPBS value less than above values served as controls.

Collection of the Blood Sample

Before the collection of sample the subjects of the study were subjected to simple questionnaire for the details of family history, habits, and duration of diabetes before the procurement of blood sample. Thereafter in accordance to protocol 3ml of the left over blood samples was collected with 5ml syringe in the EDTA [32] vacutainers for the SNP study. A total of 51 samples (41 diabetic cases and 10 controls) were processed for all the seven genes.

Selection of polymorphism

We limited our selection of genetic variants to the previously reported Single nucleotide polymorphisms in recent epidemiological studies for all the seven candidate genes for T2DM and its related complications at the time of initiation of our studies. We selected a set of SNPs with reference sequence *rs2975760* [33] and *rs3792267* [34] in the *CAPN10*, and *rs3774261* [35] in *ADIPOQ* for T2DM, *rs1501299*

[35] and *rs2241767* [35] in ADIPOQ. The other SNPs of candidate genes with proven association with T2DM complications are *rs1569198* [36] in DKK1for Musculoskeletal Disorders, *rs121918381* [37] in APOC III for Dyslipidemia; *rs699* [38] in AGT for Hypertension; *rs429358* [39] in APOE for Alzheimer's disease [40] and *rs1927911* [41] in TLR 4 for diabetic Foot Ulcer were selected.

Genotyping

The Leucocyte component of the blood samples were processed for extraction and purification of Genomic DNA using modified Sambrook et al protocol [42,43]. The isolated and purified DNA was amplified in single nucleotide Polymorphism specified region by the Polymerase chain reaction [44,45] using specially designed primers and synthesized by synthesized by Bioserve, Hyderabad, India. The primers were designed by using Primer blast tool [44]. The PCR conditions were optimized to get the maximum yields of target DNA sequences. PCR was performed with reaction mixture of 20 µl volume. The reaction mixture consists of the primers, (forward and reverse), Taq polymerase enzyme, reaction buffer, Master mix (dNTP, MgCl$_2$), DNA and water. The ethidium bromide stained amplified product of PCR was qualitatively checked on 1.2% agarose gel electrophoresis [36,37]. The gel was visualized and photographed in a transilluminator (Figure 1).

The amplicons were then sequenced using Sanger sequencing method [38]. Mutations in all the genes were screened by different computational tools. They were checked for the validation of mutations in our population.

Results

The results showed difference in mean and standard deviation of Fasting and post-prandial blood glucose levels in T2DM cases and controls. The values for blood glucose are shown in Table 1. The graphical representation of the Blood sugar values is shown in Graphs 1 and 2.
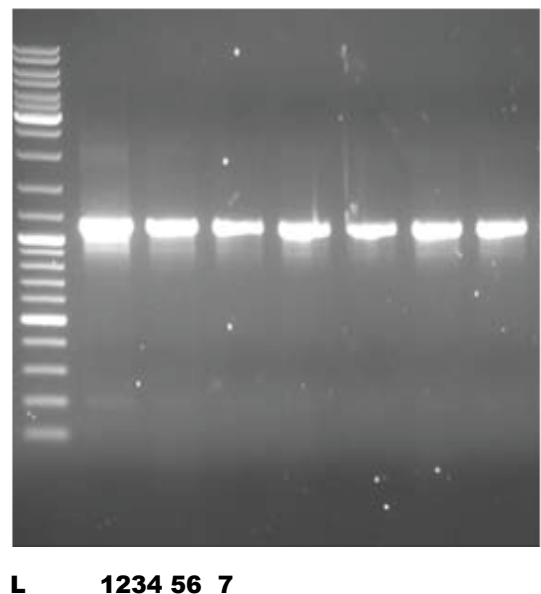

L   1234 56 7

Figure 1. Photographic image of PCR product on Agarose Gel
(L= MW Ladder, 1=CAPN10,2=ADIPOQ,3=DKK1, 4=AGT,5=APOC III ,6=APOE,7= TLR4 DNA Samples)

Table 1. Fasting and Postprandial Blood sugar Levels.

Parameter	T2DM Cases n=90	Controls n=90	Normal Range mg/dl	P value
FBS	152 ± 92.8	92 ± 11.7	70-110	0.8
PPBS	229.3 ± 67.6	131.2 ± 18.8	170-200	0.7

Graph 1. Comparison of FBS levels in T2DM Cases and Control.

Graph 2. Comparison of PPBS levels in T2DM Cases and Control.

Table 2. Distribution of reported and Novel SNP in Diabetic cases.

GENE	Reported SNP	Reported SNP type	Amplicon sequence	% of subjects with reported SNP	New SNP details (Position)
CAPN10	rs2975760 rs3792267	C/T A/G	240591700-240592223	48.7% 48.7% 31.7%	240592222
ADIPOQ	rs3774261 rs1501299 rs2241767	A/G A/C(REV) A/G	186853309-186853804	41.4% 43.9% 46.3%	
DKK1	rs1569198	A/G	52316013-52316520	63.41% 48.7%	52316022
APOC III	rs121918381	A/G	116832368-116832880	43.9%	
AGT	rs699	C/T(REV)	230710528-230710030 (REV)	48.7%	
APOE	rs429358	C/T 44908684	44908662 -44909173	60.9%	
TLR4	rs1927911	C/T 11707776 G/A	11707736-11708239	31.7% 21.7%	11708205

The sequencing of amplified portion of the two genes revealed the presence of previously reported SNPs in the diabetic population are shown below in the Table 2. The SNPs *rs2975760, rs3792267* in *CAPN10* and *rs3774261* ADIPOQ genes are reported for diabetes are present in 48.7% and 41.4% respectively. The previously SNPs reported for complications are present in 43.9% and 46.3% in (*rs1501299,*

Table 3. BOX SHADE showing Position of Novel SNPs.

rs2241767) ADIPOQ, 63.41% in (*rs1569198*) DKK1, 43.9% in (*rs121918381*) APOC III, 48.7% in AGT, 60.9% in (*rs429358*) APOE, 31.7% in (*rs1927911*) TLR4.

The BOX SHADE (Table 3) of the amplified gene shows the presence of SNPs in new position in *CAPN10*, *DKK1* and *TLR4* genes along with the previously reported SNPs in south Indian population. Only the SNP reported in *DKK1* in the world wide population could not be found in any of the test samples.

Conclusion

The association of the reported SNPs for diabetes and related complications are successfully replicated in our discrete study population. A normal individual carrying these gene variants may face a greater risk of developing diabetes if having uncontrolled blood sugar levels that exceeds the values of WHO recommendation criterion for diabetes diagnosis.

We conclude that genetic variation in the genes influences blood glucose levels in making non-diabetic subjects susceptible to diabetes and its progression to complications This shows the combined effect of more than one factor for outcome of diabetes.

The SNP found in new position could be new biomarkers for type 2 diabetes mellitus. However, further analyses on a larger sample size are required to establish a conclusive association of the novel SNPs with diabetes and its related complications to diabetic population of Andhra Pradesh and Telangana states in India.

Acknowledgements

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