



Analysis of Genetic Polymorphism in TCF7L2 Causing Diabetes

*¹Sravanthi Anumandla, ²Sushma Rayabarapu

^{1,2} Kakathiya University, Warangal

*Email: sravanthi.a1401@gmail.com

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Abstract

The current Day research in the area of Medical molecular Biology and Diagnostics indispensably utilises the principles and applications of Bioinformatics tools and software's so as to complete the task accurately within a short span of time. In the present study the Genetic Polymorphism in the gene TCF7L2 of humans. Responsible for causing diabetes has been analysed using various insilico methodologies. The blood samples of the diabetic and control were collected and the genomic DNA extraction was performed. The Sequence of the gene TCF7L2 was retrieved from NCBI data base and a primer was designed for its amplification using Primer 3 Software. The standard primer is than synthesized and used for PCR amplification of the samples. The amplified products were then sent for sequencing. The results obtained after sequencing was further analysed using insilico tools and the polymorphism can be detected among the samples after multiple sequence alignment. The SNP position of TCF7L2 has been obtained from SNP database of NCBI.

Keywords

TCF 712, Polymorphism, SNP, MSA, PCR, Amplification etc

Introduction

Diabetes mellitus (DM), commonly referred to as **diabetes**, is a group of metabolic diseases in which there are high blood sugars levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications.

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin. It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Type 2 DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed

to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 DM is the most common type of diabetes mellitus. "Secondary" diabetes refers to elevated blood sugar levels from another medical condition. Secondary diabetes may develop when the pancreatic tissue responsible for the production of insulin is destroyed by disease, such as chronic pancreatitis (inflammation of the pancreas by toxins like excessive alcohol), trauma, or surgical removal of the pancreas.

Some of the symptoms of Diabetes include:

- **Increased appetite:** Diabetics are unable to utilize glucose for energy so they keep feeling hungry all the time since the cells are craving for glucose.
- **Increased thirst:** Increased blood sugar can make diabetics dehydrated, inducing thirst all the time.
- **Frequent urination:** Dehydration along with the constant feeling of thirst, diabetics tend to drink more water which is the reason they end up visiting the bathroom more often. Excess urination is a common sign of diabetes.
- **Sudden weight loss:** In diabetics, despite the intake of glucose, the body isn't able to utilize it for deriving energy. Therefore, the body utilizes fat to derive energy, causing weight loss, without exercising and dietary changes.
- **Poor wound healing:** Increased blood sugar causes hardening of blood vessels affecting blood circulation. When blood flow to injured tissues gets affected, blood clotting proteins and other factors that promote healing do not reach the site of injury. This causes wounds to heal slowly.

Numerous SNPs have each been associated with (slightly) increased risk for **type-2 diabetes**, but they only marginally improve the odds of predicting whether an individual will get **type-2 diabetes** based on the traditional clinical characteristics combining age, sex and weight. The SNP analysed in the current study is as follows:

- rs7903146, in the TCF7L2 gene, and 85% of the time equivalent to rs7901695

Materials and Methods

Sample collection:

The study includes the collection of blood samples from diabetic individuals to isolate the DNA from those samples and further analysis. The blood samples were collected from the diagnostic centre. 10 diabetic and 5 control samples were collected. The blood samples that are collected in the EDTA vials should be sterile and are used for the isolation of the DNA.

Isolation of the DNA from the samples

The DNA was isolated from the collected samples using the Bunce method. In the process of isolation of the DNA the anti-coagulated blood has to be taken for which it is collected in the EDTA vials. The chemical sodium acetate is added in order precipitate out the proteins that are present in the blood samples. The ice cold chloroform is added which acts as a decolorizing agent.

Addition of iso propyl alcohol:

The supernatant that is collected after the process of centrifugation and is washed with the equal volumes of ice cold isopropyl alcohol. The supernatant with the added alcohol is preserved over night at 4⁰C or at -20⁰C for 30 minutes. Here the alcohol that is added acts as a precipitating agent for the isolated DNA.

Washing with the ethyl alcohol

The supernatant along with the isopropyl alcohol is again centrifuged at 12000RPM for 5 minutes. From this the pellet is collected which is washed with the 70% of ethyl alcohol. Here the pellet is collected which is to be air dried.

Preservation of the DNA

The pellet which is air dried should be dissolved in the 50 micro liter of the TE buffer. The TE buffer that is added acts as a preservation agent for the DNA. Later it is stored at 4C temperature.

Gene Selection and Sequence Retrieval

In case of Diabetes the most commonly studied gene include Insulin. However the current study is focused to analyze the genetic polymorphism in TCF7L2. The gene has been selected from Gene Cards database and the role of this gene in diabetes has been studied from general literature and PubMed.

The SNP database has been used to select the SNP possible for the selected gene. The SNP selected was further analyzed.

Primer Designing for Amplification of Given SNP

The primer 3 tool has been used to design a standard primer following the primer designing laws so as to amplify the selected gene of interest from the complete human genome. The tool can be obtained at <http://bioinfo.ut.ee/primer3-0.4.0/> the result obtained was 5 pairs of possible primers with different lengths, and amplification products. Depending on the need and the location of the SNP best primer set (forward and reverse) are selected. The primer designed above is sent for synthesis in an oligonucleotide synthesizer. This would provide ready to use primers. The primers can be used in PCR after proper dilution with either distilled water or TE Buffer.

PCR Amplification of the Gene TCF7L2

In order to amplify the specific gene region containing the SNP all the samples are amplified using the above designed Primer. The PCR set up was designed and the programme was run for a cycle no 30 to get a good quantity of the amplicons which can be further used for sequencing and analysis. The amplicons were further sent for sequencing using ABI 3500 sequencer which utilizes sequencing PCR and Capillary electrophoresis techniques to provide the sequence of the amplicons. The result is obtained in the form of an ABI chromatogram which can be read only in specified softwares. Thus the sequence is then converted into normal sequence and saved.

Sequence Analysis

The amplicon sequences thus obtained are further analysed to check the difference between the diseased and the normal samples.

All the sequences obtained after sequencing are subjected for multiple sequence alignment. For this purpose Texshade tool from SDSC Biology workbench has been used. The texshade would align all the sequences as per their similar regions and would highlight the non-similar or variational regions. The tool can be obtained from <http://workbench.sdsc.edu/>

Results and Discussion

Fig 1: Extraction of DNA From collected blood samples



The above figure shows the processing of the blood samples for the extraction of genomic DNA.

Fig 2: DNA bands obtained for the samples on Agarose Gel



SNP Selection and Data collection

The SNP that falls in the above gene range has to be selected. The data base used for the same is NCBI SNP data base. The following SNP has been selected. [rs7903146](#) [*Homo sapiens*].

The SNP causes a change in the base **C/T** at the position 112998590 on chromosome 10.

Table 1: Primer designing for the amplification of the above region of SNP

	Sequence	Length	GC Content	Melting Temperature	Product Size
Left Primer	TGCCGGACCAAA GAGAAGAT	20 bp	50	59.02	229 bp
Right Primer	GCCTTCCCTGTAA CTGTGGT	20 bp	55	59.60	

Fig 3: Multiple Sequence Alignment using Texshade to identify the regions of similarity and variation



The above figure clearly depicts the change in the single nucleotide C/T in the pattern “**T TATATAA**” In the above results 4 out of total 15 samples had the mutant allele C instead of wild allele T. All the four showing the SNP are disease and 6 of diseased and 5 normal showed the wild allele T at the same position

Conclusion

The aim of the current research was to analyse the Single Nucleotide polymorphism among the disease and the normal samples of Diabetic individuals with respect to the gene TCF7L2 and the SNP C/ T. The gene sequence of the same was retrieved from NCBI and the SNP was selected from SNP database. Primer3 tool has been employed to design the primer for amplification of the selected gene region containing the SNP. The DNA samples were than amplified with the specific primers using PCR. The amplicons were sent for ABI sequencing. The sequences obtained were further compared using texshade tool of SDSC Biology workbench. The results showed that only 4 samples of 10 disease showed the presence of SNP C/T at the location and all the others including the normal showed the wild allele. This indicates that the presence of SNP leads to diabetes, however all diabetic cases are not only due to the presence of SNP. This study can further be extended to develop a prognostic or diagnostic approach for the early detection of diabetic condition based n sequencing.

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