



Identification and Comparative Assessment of the SNP rs267600955 of Gene TNF ALPHA involved in Arthritis

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Abstract

Arthritis, one of the primitive but yet unsolved problem affecting majority of the population, has been considered in the current for the identification of the SNP involved. The SNP rs267600955 has been considered in the work. The blood samples from a total of 10 individuals were collected, of which 8 were affected with arthritis and two were normal used as controls. All the samples were subjected for blood DNA extraction and were purified with ethanol treatment and preserved in TE buffer. The quality of the DNA was tested by Agarose Gel electrophoresis. In order to sequence the TNF alpha gene (involved in arthritis), the sequence was retrieved from the databases and primer was designed using online tool Primer 3. Using the primer the product was amplified and the amplicons were sent for sequencing. The 10 sequences thus obtained were compared for the presence of SNP using Insilco analysis. The work concludes that from a total of 8 diseased samples 2 were showing the SNP under study. This indicates that there is only a limited percentage of mutation affecting the onset of the disease. However all the normal individuals were free from the SNP. However the final conclusion can be made with the increase in the number of the samples.

Keywords: Isolation, Extraction, Preservation, Electrophoresis, Polymerase chain reaction, Amplification, Sequencing and Alignment.

Introduction:

Arthritis is an autoimmune disorder which causes inflammation of one or more joints in the body. Normal wear and tear, inflammation, injury can cause inflammation of joints. TNF- α plays a dominant role in rheumatoid synovitis (Table 1). In cultures of synovial cells from patients with RA, blocking TNF- α with antibodies significantly reduced the production of IL-1, IL-6, IL-8, and GM-CSF.⁴ Hence, the blockade of TNF- α may have a more global effect on inflammation than the blockade of other cytokines present in high concentration in synovial fluids, such as IL-1. The gene coding for human TNF- α is located on the short arm of chromosome 6 within the major histocompatibility gene complex. A 1.7kb TNF messenger

ribonucleic acid (mRNA) codes for a 26-kDa type II transmembrane protein with 233 amino acids. The mature soluble TNF is generated by proteolytic cleavage of the C-terminal extracellular region of the molecule and consists of 157 amino acids. Human TNF- α is not glycosylated and contains a single intramolecular disulphide bridge. Tumor necrosis factor (TNF) has an extremely broad spectrum of biological activities. Cytotoxicity to tumour cell lines was one of the first functions to be discovered which led to its name, tumour necrosis factor.¹ TNF- α is produced mainly by monocytes and macrophages, but also by B-cells, T-cells and fibroblasts. It is one of the key cytokine molecules that causes inflammation in RA. It is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines, including interleukin-1 (IL-1), IL-6, IL-8 and granulocyte monocyte-colony stimulating factor (GM-CSF). TNF- α is also known to stimulate fibroblasts to express adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1).

Material and Methods

Sample collection: The study includes the blood samples of the Arthritis diseased individuals so as to obtain the DNA from those samples. The blood samples were collected from the nearby diagnostic center located at LB Nagar. The Collection was performed in EDTA Vials. 8 diseased samples and 2 normal samples were collected. All the samples were further subjected for Blood DNA Extraction protocol.

Isolation of the DNA from the samples:

Bunce method was employed for the extraction of DNA from the collected blood samples. Bunce method uses sucrose or glucose to provide density to the White Blood Cells from which the DNA can be isolated. Here Tris HCL has been used for regulating the pH and Chloroform is used to decolorize the redness of the samples due to hemoglobin. The DNA that gets precipitated when reacted with alcohol can be clearly visualized in the decolorized blood samples. The protein impurities and lipid particles are removed in the various steps of the protocol. The DNA thus obtained was purified with Ethanol washing and was preserved in TE Buffer. This can further be used for other analytical protocols.

Agarose Gel Electrophoresis for the Identification of DNA samples:

Once all the samples have been obtained extracted and purified the presence of the DNA in them can be confirmed by the appearance of the band in the gel. 0.8% agarose was used in TAE buffer to cast the gel and the samples were loaded with the loading dye. The gel was run for 20min and the bands were observed in UV Transilluminator.

Identification of the causative gene for Arthritis:

In spite of the several causes for Arthritis, the current study aimed to identify the causative gene responsible for the same. The gene was identified using Gene Cards database, and the sequence of it was retrieved. The gene was found to be TNF Alpha. Tumor necrosis factor alpha (TNF ALPHA) is present on the chromosome number 6 which encodes a multifunctional proinflammatory cytokine belonging to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.

Gene Sequence Retrieval:

NCBI data base was used to retrieve the gene sequence of TNH Alpha. The gene sequence was copied and the location on the chromosome was noted.

Identification of the SNP present in the selected gene:

SNP data base from NCBI has been used for the selection of the SNP falling within the selected gene. The SNP selected for the study was rs267600955.

Primer designing using Primer 3 and PCR Amplification:

From the complete genomic DNA isolated in the previous steps, it is essential to amplify the required gene of interest. Amplification can be performed only in the presence of a specific primer. Thus designing and synthesis of the primer are the two essential steps to be practiced before Gene Sequencing. The Suitable pair of Forward and Reverse Primers for the TNF Alpha gene was designed using Primer 3 Software.

The sequences thus obtained were synthesized by oligonucleotide Synthesis method. The Primers thus obtained were used for PCR Amplification.

Sequence Analysis:

The Amplicons obtained were further subjected for Sequencing of the selected gene. The gene sequence was obtained which was used for further analysis.

Multiple Sequence alignment for the Identification of SNP:

Using the sequence of all the 10 samples Multiple Sequence Alignment with Clustal W was performed. The alignment was studied to identify the presence or absence of SNP in the sequences and draw conclusion whether the SNP has a role in the disease onset.

Results and Discussion:

Extraction of DNA from the Blood Samples:

The 10 blood samples collected were subjected for Blood DNA Extraction and the Extracted DNA was subjected from Gel electrophoresis to confirm the presence of the DNA.

The Fig 1 Shows the Band patterns obtained in Gel Electrophoresis:



The above figure shows the 10 bands obtained for the 10 DNA samples extracted.

Sequence Retrieval of TNF Alpha from NCBI:

The gene sequence of TNF Alpha was retrieved from NCBI. The gene was found to be on the chromosome 6 and the position of the gene was found to be from 31575567 to 31578336 with its length 2769bp.

Primer designing using PRIMER 3:

The gene sequence thus obtained was used for primer designing. The tool used for the same was Primer3. The primers both forward and reverse were obtained.

size. This study can be used as a support to increase the sample size and extend the work.

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