



Identification And Comparative Assessment of the SNP rs281865419 of Gene TNF ALPHA Involved In Arthritis

*¹Sheela, ²Annamala Bhavani

^{1,2}Koti Woman's College, Osmania University, Hyderabad

Email: annamalabhavani143@gmail.com

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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 study for the identification of the SNP involved. The SNP rs281865419 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 1 was showing the SNP under study. This indicates that there is only a limited percentage of mutation affecting the onset of the disease. Also, 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. 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 mess 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.

Materials and Methods:

Collection of Diseased and Control Blood samples:

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 centre located at LB Nagar. The Collection was performed in EDTA Vials. 8 diseased samples and 2 normal samples were collected.

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 haemoglobin. 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 in the samples

Once all the samples have been obtained DNA was extracted and purified, the presence of DNA in those samples can be confirmed by the appearance of the band in the Agarose 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 Trans illuminator.

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. Tumour necrosis factor alpha (TNF ALPHA) is present on the chromosome number 6 which encodes a multifunctional pro inflammatory cytokine belonging to the tumour 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/TNFR2. 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 rs281865419

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 for Gel electrophoresis to confirm the presence of the DNA. The results indicated that the DNA was successfully obtained from all the samples subjected for the run.

Fig 1: The DNA bands obtained for the samples processed



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

SNP Analysis

SNP NAME: rs281865419 [Homo sapiens]

The pattern is
GGGGCAGCTCCAGTGGCTGAACCGC[C/T]GG
GCCAATGCCCTCCTGGCCAATGG

Original Base: C, Altered base: T
Chromosome:6, Gene: 31577157

individuals were reported with the SNP. However this can be concluded only with the increase in the sample size. This study can be used as a support to increase the sample size and extend the work.

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