

## Role of SNP (Rs12709426) In Ace Gene on the Onset of Hypertension

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### Abstract

ACE (Angiotensin 1 converting Enzyme) involved in regulating the blood pressure. Hypertension being one of the dreadful cause of several diseases and especially heart failures, require an at most attention to resolve or treat the condition. The current work involves the study of a reported SNP rs12709426 in ACE gene involved in effecting blood pressure. The current work involves blood sample collection from several diseased and control individuals. Extraction of the Genomic DNA from these samples followed by Agarose Gel electrophoresis to confirm the presence of the DNA. The sequence of ACE was retrieved and primers were designed to amplify specifically the SNP containing region. The amplified products are further subjected for agarose gel electrophoresis so as to confirm the quality of the amplicons. All the amplicons are further subjected for ABI sequencing in order to read the genomic DNA of the samples tested. The sequences obtained were further analysed using bioinformatics tools to identify the exact SNP that might be present in the samples. Based on the presence or absence of the selected SNP in the samples the importance of the SNP in the onset of the adverse condition can be identified.

### Keywords

Hypertension,

### Introduction

#### Hypertension

Hypertension also known as high blood pressure, is a long term medical condition in which the blood pressure is elevated. Long term high blood pressure is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease.( source : Wikipedia)

#### Symptoms

- With high blood pressure report headaches (particularly at the back of the head and in the morning), as well as light headedness, vertigo, tinnitus (buzzing or hissing in the ears), altered vision or fainting episodes
- On physical examination, hypertension may be associated with the presence of changes in the optic fundus seen by ophthalmoscopy.

#### Types of Hypertension

Essential hypertension (also called primary hypertension or idiopathic hypertension) is the form of hypertension that by definition has no identifiable cause. It is the most common type of hypertension, affecting 95% of hypertensive patients. Secondary hypertension (or, less commonly, inessential hypertension) is a type of hypertension which by definition is caused by an identifiable underlying secondary cause. It is much less common than the other type, called essential hypertension affecting only 5% of hypertensive patients.( source: Naish, Jeannette; Court, Denise Syndercombe (2014). Medical sciences (2 ed.). p. 562. ISBN 9780702052491.)

#### Primary hypertension:

Essential hypertension (also called primary hypertension or idiopathic hypertension) is the form of hypertension that by definition has no identifiable cause. It is the most common type of hypertension, affecting 95% of hypertensive patients, it tends to be familial and is likely to be the consequence of an interaction between environmental and genetic factors. Prevalence of essential hypertension increases with age, and individuals with relatively high blood pressure at younger ages are at increased risk for the subsequent development of hypertension. Hypertension can increase the risk of cerebral, cardiac, and renal events.

#### Secondary hypertension

Secondary hypertension (or, less commonly, inessential hypertension) is a type of hypertension which by definition is caused by an identifiable underlying secondary cause. It is much less common than the other type, called essential hypertension affecting only 5% of hypertensive patients. It has many different causes including kidney diseases and tumours. It also can be a side effect of many medication.

ACE gene is known to be involved in hypertension as analysed by gene cards data base. This gene is important in activating the Angiotensin by converting inactive angiotensin 1 to active angiotensin 2. This angiotensin two plays a major role in controlling blood pressure by its vasopressor activity.

In order to control the hypertension condition certain inhibitors of ACE are used that can inhibit the vasocompression and regulate the blood flow.

#### Materials and Methods

##### Sample Collection

Seven diseased and three control Blood samples of Homo sapiens were collected from a hospital Hyderabad, Telangana. All the samples were collected in EDTA vials and stored at 4<sup>0</sup> refrigeration until use.

#### **Extraction of DNA from the samples:**

All the samples were subjected for DNA extraction by Bunce method which includes buffer solution containing sucrose, MgCl<sub>2</sub> and Tris HCl which were used for collecting the WBC cell pellet. The cell pellet was further treated with solution containing Tris HCl, EDTA and NaCl followed by incubation. This was further proceeded towards incubation at 65 degrees for 20 minutes to break open the cell wall and separate out the cellular components including the DNA. Here the shaking with chloroform was necessary to decolorize the red pigment due to haemoglobin. Later the centrifugation was performed to separate out the clear supernatant. The supernatant was supposed to contain the DNA. The DNA was then precipitated from its dissolved state by adding chilled isopropyl alcohol. This step causes the water molecules to bond with alcohol and free the DNA to get precipitated. The precipitated DNA was then separated and washed with 80% ethanol to remove the impurities present. The excess ethanol was then removed and DNA pellet was kept for complete air dry. The dried and purified DNA was stored in TE buffer for further analysis. All the DNA samples extracted in the above step were subjected for Agarose Gel run to confirm the presence of the DNA in these samples. 0.7% Agarose gel was used and the bands obtained were considered to be successful. Absence of band at this step requires to redo the DNA extraction step.

#### **Selection of SNP for Hypertension**

For studying the effect of gene mutations on the onset of the disease condition ie hypertension, selection of correct SNP was the most important step. The SNP related to the disease has to be selected from NCBI sub data base SNP. The SNP selected here was rs12709426. Its position on chromosome was 17:63484395. Its functional consequence was identified as missense, upstream variant 2KB. Its wild allele was recognized as 'A' and alternate was 'G'. The SNP database of NCBI not only provides the details about the Gene SNP but it also provides other information like the location of SNP, Its Biological effect, Its importance in the health condition etc.

#### **Primer Designing For Amplification:**

To compare the sequences of the control and the diseased samples amplification must be performed before Sequencing. It is a free online tool and user friendly. For the same primer 3 tool had been used. The tool would provide a perfect set of forward and reverse primers parameters and can amplify the

given gene region. The tool can used for obtaining more than one set of primers for a single gene region.

#### **Amplification using PCR**

Using the specific primers obtained in the previous step and produced by oligonucleotide synthesis, the samples had to be amplified so as to make the replicas of the selected gene region. The amplified product can be further used for sequencing. The ABI sequencer had been used for the same. The PCR protocol used was as per the standards. The amplification was performed for all the samples with the same primer.

#### **Sequencing**

The amplified products were sent for sequencing using ABI 3500 sequencer. Here the same set of primers were used and sequencing PCR was performed. To purify the product obtained an Exo-sap purification was performed followed by sequencing. The major technique involved in sequencing was the capillary gel electrophoresis of denatured DNA fragments. The time required for complete sequencing was 2 days. The result of the sequencer was obtained in the form of a chromatogram which can be converted to the basic sequence file.

#### **Comparison of Sequences using Clustalw**

All the sequences that are obtained after sequencing including both diseased and control were compared to detect the presence of SNP in the sample. This technique of comparison is done using an online bioinformatics tool name Clustal W that would align many sequences, compare them to obtain similar and non-similar regions on a group of sequences.

#### **Results and Discussion**

##### **Isolation of DNA and Agarose Gel Electrophoresis:**

The DNA isolated for controlled and diseased blood samples of Homo sapiens using BUNCE method is depicted in the below Fig1.1 and the DNA BANDS obtained using Agarose Gel Electrophoresis was viewed under UV-TRANSILLUMINATOR and the last lane in the gel indicates the marker are shown in fig 1.2.

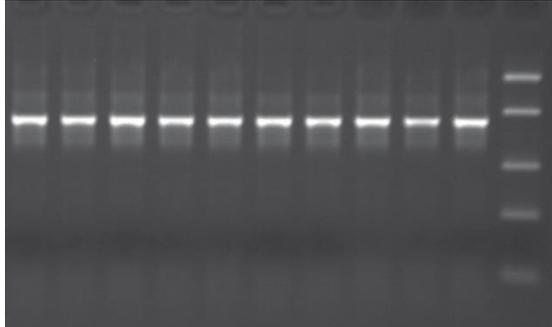
**Fig 1.1 Isolation of DNA from blood samples using BUNCE Method**



**Agarose Gel Electrophoresis**

To confirm the presence of DNA in the extracted samples band pattern identification was done using agarose gel electrophoresis. The clear bands indicates pure DNA

**Fig 1.2. View of DNA BANDS under the UV-TRANSILLUMINATOR as obtained from GELDOC**



**SNP Selection for the study**

The SNP under consideration is selected from NCBI’s SNP database. The selected SNP is shown below:

**rs12709426 [ Homo sapiens]**  
 GTGCTGAAGGACATGGTCCGGCTTAG[A/G]T  
 GCCCTGGATGCCAGCCGCTGCTC  
 CHROMOSOME: 17:6484395  
 FUNCTIONAL CONSEQUENCE: missense,  
 upstream variant 2KB.  
 CLINICAL SEQUENCE: Likely benign.  
 GLOBAL MAF:G=0.0190/95  
 The above data shows the importance of this mutation and its position on the chromosome.

**Randomly Selected FASTA Sequence Containing the SNP**

ATTGGGGGGCGGAAGTGGCCAGGGGCATG  
 TGGGCCGGGTCCAGGAGCAGACTCCAGCC  
 TGAGTCCCCTGTGCCATGGTACCCACTCT  
 GCCACCAGGAAGGTGCTGCAGGCTGGCTC  
 CTCCAGGCCCTGGCAGGAGGTGCTGAAGGA  
 CATGGTCCGGCTTAGATGCCCTGGATGCCCA  
 GCCGCTGCTCAAGTACTTCCAGCCAGTCAC  
 CCAGTGGCTGCAGGAGCAGAACCAGCAGA  
 ACGGCGAGGTCTCTGGGCTGGCCGAGTACC  
 AGTGGCACCCGCCGTTGCCTGACAACTACC  
 CGGAGGGCATAGGTAAGCCCTGAGTGAG  
 GATGGTGTGGGGCTAAGGTGGTCTCTCA

**Primer 3 output**

The below information is provided by the Primer 3 tool for selection of the best primer for amplification

No mispriming library specified Using 1-based sequence positions

**OLIGO**                      start len tm gc% any  
3' seq

**LEFT PRIMER**      136 20 60.12 55.00 4.00  
 2.00 GAGGTGCTGAAGGACATGGT

**RIGHT PRIMER**    329 20 59.28 55.00 5.00  
 0.00 TCCTCACTCAGGGCTTTACC

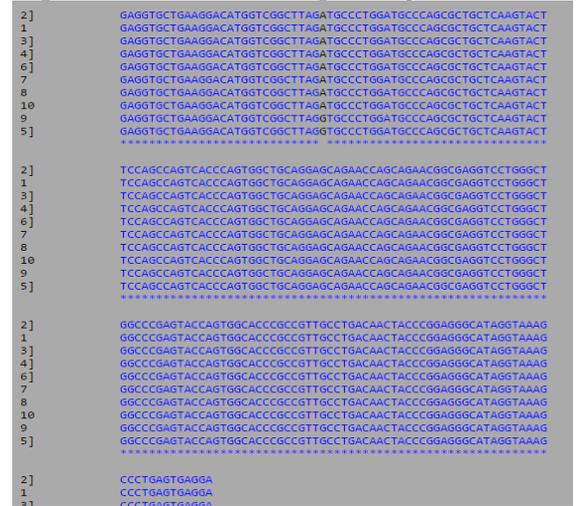
**SEQUENCE SIZE:** 355  
**INCLUDED REGION SIZE:** 355

**PRODUCT SIZE:** 194, **PAIR ANY COMPL:**  
 4.00, **PAIR 3' COMPL:** 3.00

**Sequencing Result:**

After the amplification the amplicons were subjected for sequencing using the above primers and the result obtained as a chromatogram than can be further converted into a sequence file. All the sequences obtained were subjected for multiple sequence alignment to identify the regions of similarity and variation. The SNP if any can be identified in the same result.

**Fig: 1.3 showing the Clustal W analysis of the sequence obtained after sequencing**



From the above result it can be clear that there is a variation in the amino acid at the SNP location where 2 sequences out of 10 showed a mutant allele G instead of wild allele A. This indicates that out of the 7 diseased samples 3 were mutant. In these individuals the cause of the hypertension may be the mutation. In case of control samples there was no SNP which indicates a normal sample is also normal with respect to sequence.

**Conclusion**

The current work was undertaken to analyse the effect of mutations on the onset of Hypertension. For this several blood samples were collected and the known SNP region was targeted and the Primers were developed. The targeted region was amplified using PCR and the amplicons were sent for sequencing using ABI sequencer. The results of the sequencing were represented as chromatograms

which were converted to sequence file format and the sequences were compared using CLUSTAL W software from SDSC biology workbench. The results indicated that out of the total 7 diseased samples collected and tested 3 showed the change in the amino acids from A to G. However there was no effect in any other region on the sequence. Regarding the normal samples all the 3 sequences were normal without showing any SNP. Thus the study can be concluded that the onset of hypertension may be either due to genetic cause or other environmental factors as all the diseased samples did not show the SNP.

#### Reference

1. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ (2013). "Effect of lower sodium intake on health: systematic review and meta-analyses". *BMJ*. **346**: f1326. doi:10.1136/bmj.f1326. PMC 4816 261 PMID 23558163.
2. Addison WL (March 1928). "The Use of Sodium Chloride, Potassium Chloride, Sodium Bromide, and Potassium Bromide in Cases of Arterial Hypertension which are Amenable to Potassium Chloride". *Can Med Assoc J*. **18** (3): 281–85. PMC 1710082 . PMID 20316740.
3. Arsenic Author: Frances M Dyro, MD, Chief of the Neuromuscular Section, Associate Professor, Department of Neurology, New York Medical College, Westchester Medical Center
4. atsd-medical management guidelines for arsenic trioxide
5. James, PA.; Oparil, S.; Carter, BL.; Cushman, WC.; Dennison-Himmelfarb, C.; Handler, J.; Lackland, DT.; Lefevre, ML.; et al. (Dec 2013). "2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults: Report From the Panel Members Appointed to the Eighth Joint National Committee (JNC 8)". *JAMA*. **311**(5): 507–20. doi:10.1001/jama.2013.284427. PMID 24352797)
6. Semlitsch, T; Jeitler, K; Berghold, A; Horvath, K; Posch, N; Poggenburg, S; Siebenhofer, A (2 March 2016). "Long-term effects of weight-reducing diets in people with hypertension.". *The Cochrane database of systematic reviews*. **3**: CD008274. doi:10.1002/14651858.CD008274.pub 3. PMID 26934541. Retrieved 9 March 2016.
- 7 Silverberg DS, Iaina A, Oksenberg A (January 2002). "Treating Obstructive Sleep Apnea Improves Essential Hypertension and Quality of Life". *American Family Physician*. **65** (2): 229–36. PMID 11820487.
8. Williams B et al.; British Hypertension Society; Michael Sutters, MD (2006). "Secondary Hypertension". *Hypertension Etiology & Classification - Secondary Hypertension*. Armenian Medical Network. Retrieved 2007-12-02.
9. Yokoyama, Yoko; Nishimura, Kunihiro; Barnard, Neal D.; Takegami, Misa; Watanabe, Makoto; Sekikawa, Akira; Okamura, Tomonori; Miyamoto, Yoshihiro (2014). "Vegetarian Diets and Blood Pressure". *JAMA Internal Medicine*. **174** (4): 577–87. doi:10.1001/jamainternmed.2013.14547. ISSN 2168-6106. PMID 24566947.