Title: Association of single nucleotide polymorphisms of the Angiotensin- Converting Enzyme (ACE) Gene with Hypertension in North Indian Population

Submitted in partial fulfilment of the requirement for the degree of

Master of Science (Biotechnology Integrated)

Dissertation

Submitted by:
Sahiba lall, (Registration No. 11109547)

Under the guidance of
M. Amin-ul Mannan, Ph.D.

SCHOOL OF BIOENGINEERING AND BIOSCIENCES,

LOVELY PROFESSIONAL UNIVERSITY, PHAGWARA,

PUNJAB-144411

DECLARATION

I hereby declare that the project entitled, **"Association of single nucleotide polymorphisms of the Angiotensin- Converting Enzyme (ACE) Gene with Hypertension in North Indian Population**" is an authentic record of my own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, for the partial fulfilment of the award of Master of Science in Biotechnology (Integrated.) under the guidance of Dr. Mohammad Amin-ul Mannan.

This work is my original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place: Lovely Professional university **Sahiba lall** (**11109547**)

Date:

CERTIFICATE

This is to certify that **Sahiba lall (Registration no. 11109547)** have completed the project, *entitled* **"Association of single nucleotide polymorphisms of the Angiotensin- Converting Enzyme (ACE) Gene with Hypertension in North Indian Population"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study.

No part of the report has ever been submitted for any other degree at any University. The report is fit for submission and the partial fulfilment of the conditions for the award of **M.Sc. Biotechnology (Integrated)**.

Date: (Supervisor Signature)

Acknowledgement

I want to thank my advisor Dr. Mohammad Amin-ul Mannan of Lovely Faculty of Technology and Sciences at Lovely Professional University. The way to Dr. Mohammad Amin-ul Mannan office was constantly open at whatever point I had a question with respect to my Research Project or composing. He enabled this project to be my work and given me the right direction to stroll on.

I might want to express gratitude toward Dr. Sanjeev Kumar Mittal, Cardiologist in SPS Hospital who had given me blood tests to take a shot at. He had likewise helped in the biochemical investigation of various patients. Also, he is caring in tuning into our venture and giving his help.

At long last, I should offer my exceptionally significant thanks to my folks and to my companions for offering help and persistent consolation during my time of study and through the way toward investigating and composing this postulation. This achievement would not have been conceivable without them.

Thank you!

Sahiba Lall

CONTENTS

ABSTRACT

The study was carried to find the association of single nucleotide polymorphisms of the angiotensin- converting enzyme (ACE) gene with hypertension, in the north Indian population of Punjab region. The ACE gene is part of the renin-angiotensin system, which regulates blood pressure, body fluids and salts in the body. Previous studies have shown that ACE is associated with high blood pressure and hypertension. However, the exact mechanism and its role in the context of the biochemical parameter were not clear. In this study, 39 patient's blood samples were collected from Ludhiana region and genetic polymorphism of ACE gene was carried out by PCR method. We got PCR amplification in only 24 samples. Based on PCR band size we infer that polymorphism in intron 16 (Alu) of ACE gene, there were propensity of deletion homozygote (DD) i.e., 71.4 % in hypertensive and 75 % in normotensive individuals, respectively. The insertion/deletion heterozygote (I/D) and insertion homozygote (I/I) were 14.3% in hypertensive and absent in normotensive individuals. Further samples might be needed to significantly draw any conclusions.

Chapter - 1

INTRODUCTION

Hypertension (HT), also called high blood pressure (HBP), is clinical phenotype harmful to human health (Kannel et al; 1999). High circulatory strain generally does not bring about symptoms, however, long-term hypertension is hazards and responsible for cardiovascular diseases, strokes, vision impairment and kidney diseases (Griendling KK et al; 1993), (Lindpaintner K et al; 1991). About 90–95% of individuals are affected by essential hypertension, which is due to sedentary lifestyle (high intake of dietary salt, obesity, smoking, and alcohol (Neuringer IR et al., 1993, Navar LG et al., 1994). Rest 5–10% individuals are affected by auxiliary hypertension, which leads to kidney disease and organ damage (Neuringer IR et al., 1993).

When the heart pumps the blood, the pressure exerted in blood vessels is called as systolic (left ventricle) and when the heart rests is called as diastolic (right ventricle), (Kannel et al .,1999). Usually normal systolic pressure is in the range of 100–140 millimeters mercury (mmHg) and diastolic pressure in the range of 60–90 mm Hg (Zitnay C et al., 1998). Populace suffering from high blood pressure the resting blood pressure is at or over 140/90 mmHg (Neuringer IR et al., 1993).Blood pressure can be controlled by lifestyle pattern change like weight reduction, low dietary salt intake, physical exercise, and a solid diet (Neuringer IR et al., 1993). In extreme cases, medicines intervention is required to control the blood pressure (Atiyeh BA et al., 1995).

Hypertension of unknown cause or essential hypertension has more than 90% case of hypertension, which leads to cluster in families & made thousands of genetic diseases or syndromes resulting, biochemical abnormalities (Miller SA et al .,1998). Although interaction with several genetic & environment factors leads to hypertension, RAS (rennin-angiotensin system) plays a significant role in controlling blood pressure (Gupta R et al., 2003).

Single nucleotide polymorphisms, often called SNPs are hereditary variants among individuals. SNP lead to nucleotide substitution such as cytosine (C) with the thymine (T) in a DNA (Mulder HJGH et al., 2003. Among renin-angiotensin-aldosterone (RAAS) qualities considered till date, *CYP11B2* encoding aldosterone synthase has developed much interest. To

date, two normal hereditary variations of the aldosterone synthase (*CYP11B2*) have been recognized as conceivable determinants of hypertension in patients (Takemoto Yujii et al., 1998). These are: (i) single nucleotide polymorphism in the promoter region of the gene at - 344T/C; (ii) a polymorphism including intron 2 of *CYP11B2* (Chobain AV et al., 2003)

Another SNP which is associated with hypertension is ACE gene. It directs the circulatory system and adjusts body fluids and salts. By cutting a protein called angiotensin I at a specific area, the angiotensin-changes to protein angiotensin II. Angiotensin II is responsible for secretion of aldosterone hormone which helps in maintaining the osmotic balance in body and kidneys (Manunta Paolo et al., 2002). The expanded measure of liquid in the body also builds blood pulse. The angiotensin-changing also affect bradykinin. Bradykinin causes veins to broaden (widen), which diminishes circulatory strain and thus reducing the blood pressure. (Thiol B et al., 2002).

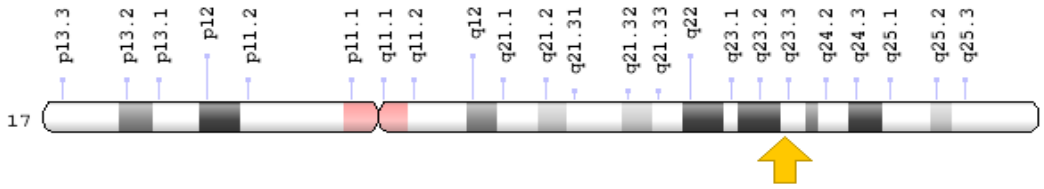


Fig 1: Chromosome location of ACE Gene on 17q23.3 as shown by the arrow. *(Source Homo sapiens Annotation Release 108, GRCh38.p7) (NCBI)*

ACE genotypes includes I/D (Isoleucine to aspartic acid substitution) allele of 287 bp Alu repeating intron 16 sequence of 3 genotypes (D/D= deletion homozygote I/I insertion homozygote, and I/D insertion/deletion heterozygote) (Duru K et al., 1994, Pamies AE et. al 1999) As I/D polymorphism of ACE gene region (intron 16) correlative with circulating ACE plasma activity, higher ACE plasma activity observed in ACE-D/D genotype. The increase in plasma ACE results in the production of angiotensin II, ACE I/D polymorphism result in diseases alike diabetic nephropathy, hypertension, tuberculosis & coronary heart disease.

The present investigation is to study the association of ACE gene polymorphism with essential hypertension in north Indian population. Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase, present on the surface of endothelial & epithelial cells. This enzyme turned up

mainly in lungs which convert Angiotensin I to Angiotensin II.ACE is a central enzyme in the renin-angiotensin-aldosterone (RAAS) and Kallikrein-kinin systems playing an essential function in blood pressure (BP) regulation and electrolyte balance.(Alaatin Y et al.,2000, Dazida G et al .,2001)

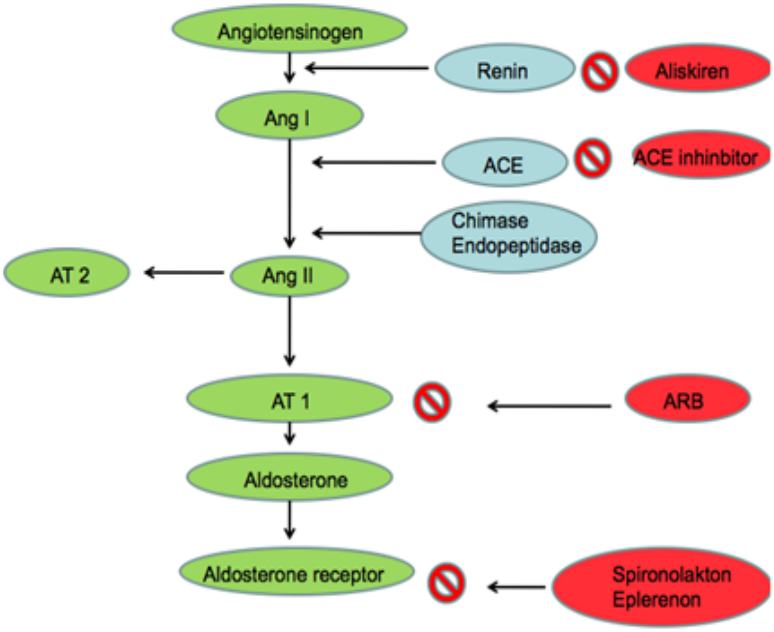


Fig 2A- Renin-Angiotensin System.

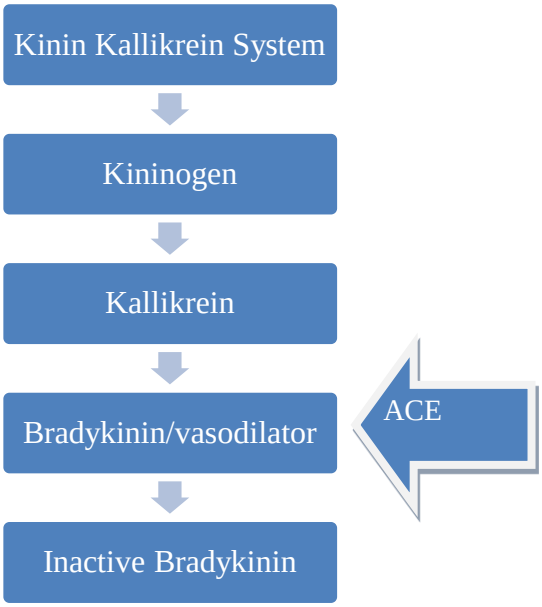


Fig 2B- Kinin-Kallikrein System.

Chapter - 2

REVIEW OF LITERATURE

Hypertension is one of the major factors for high mortality rate in cardiovascular diseases Hypertension also leads to the increased risk of stroke, heart failure, and development of other heart diseases (Duru K et.al., 1994). The normal blood pressure is in the range of 140/90 mm Hg, above this range, the hypertension treatment is required. The extent of the effect of hypertension on the body depends on the targeted organ or the organ which is being affected that is heart, brain, and kidneys. The treatment of the hypertension is the main measure which should involve the understanding of the risk associated with the resultant problem and its effective measure should be analyzed (Chiang FT et al., 1996).

All-important hypertension or hypertension of unknown reasons are more than 90% case of hypertension. It runs to bunches in families and represents a group of genetically based disease or syndrome (Nanko Y et al., 1998). It was calculated that in the United States 43% people have hypertension and ~24% of the adult population acquiring antihypertensive drugs. This ratio alters with race, higher in African American (32.4%) than Caucasians (23.3%) (Morise T et al., 1994. Blood pressure rises in the age between 55 to 60 years. It is mainly increasing in the elderly people due to systolic hypertension.

It was reported in 2000 that around a billion adults had hypertension and it is estimated that in 2025 it will rise from 1 billion to 1.56 billion. The prevalence of hypertension ranges between 22% and 25%. The recent report was given by WHO state that 57 million global deaths were estimated in 2008 and 36 million because of non-communicable diseases. This was estimated that the median prevalence of total hypertension in 2009 was 37.6% in men and 40.1% in women (Morise T et al., 2004). Risk factors for hypertension include age over 60, race, heredity, salt sensitivity, obesity, inactive lifestyle, alcohol consumption. (Nanko Y et al., 1998). Organ damage is caused by continuing raising of blood pressure in the long term and causes higher death rate.

Blood pressure results because of cardiac output and vascular resistance. It assumes that patients with an increase in cardiac output and systematic vascular resistance or both can cause arterial

hypertension. The cardiac output is often rising in younger age, although in order patients increased systemic vascular resistance and increased the stiffness of the vasculature play a prevalent role. The final pathway begins when there is an increased level of cytosolic calcium in vascular smooth muscle and it can cause vasoconstriction. Various growth agents, including angiotensin and endothelins, stimulate an increase in vascular smooth muscle. Although high blood pressure is contributed by obesity, high dietary salt intake, alcohol consumption, the genetic causes are still need to be validated (Morshed M et al., 2002)

Types of hypertension

- **Primary hypertension**: primary hypertension also called as essential hypertension. Headaches, lethargy, nose bleeding are some of the symptoms.
- **Secondary hypertension**: abnormalities in blood supply, nausea, sleep deprivation, hormonal imbalance are some of the symptoms associated with secondary hypertension. Usage of drugs like ibuprofen and pseudoephedrine are also linked with secondary hypertension

Besides this hypertension can also be categorized as

- Isolated systolic hypertension
- Malignant hypertension
- Resistant hypertension

GENETICS

Hypertension is a complicated trait with both genetic and environmental factors. It was calculated that the heritability of blood stress is 30-50%. Genetic determinants have an effect on behavioral patterns and in combination with environmental factors, it may lead to high blood pressure. For instance, a bent towards weight troubles or alcoholism can be inspired via genetic and environmental elements.

Primary hypertension is regulated by environmental factors including age, body mass index, sex, salt, intake, and others (Carretero Oa et al., 2000). Secondary hypertension is due to segregation of rare mutations. Mutations in at the least 10 genes were shown to elevate or decrease BP via common pathway by means of the manner of developing or reducing salt and water reabsorption

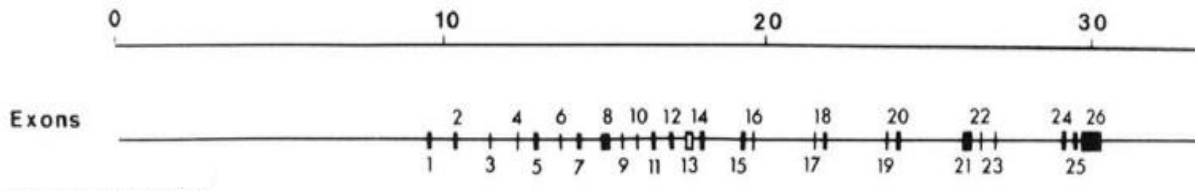
through the way of the nephron. The candidate genes and systems involved in hypertension are α-adducin, rennin-angiotensin system, aldosterone, sodium epithelial channel, catecholaminergic/adrenergic characteristic, the renal kallikrein-kinin system, and others associated with lipoprotein metabolism, hormone receptors, and growth elements (Morshed Mahboob et al .,2002)

RENIN–ANGIOTENSIN SYSTEM

Rennin-Angiotensin system– essential function in regulating BP and pathogenesis of essential hypertension. The classical renin-angiotensin device (RAS) includes renin, angiotensin-converting enzyme, and angiotensinogen (Alaatin Y et al., 1994). Renin is synthesized within the kidney and secreted in blood to create a reaction to hemodynamic, neurogenic, and ionic signals. Renin, an aspartic protease cleaves substrate angiotensinogen. Angiotensinogen is a 58 KiloDaltons (KD) protein synthesized and secreted from the liver. Renin cleaves angiotensinogen at amino terminal decapeptide angiotensin I (AGTR I). Angiotensin-changing enzyme (ACE), expressed by endothelial cells, cleaves Ang I to release the two carboxy-terminal amino acids. The ensuing octapeptide is unique angiotensin II (Ang II). Ang II is a robust vasoconstrictor even in absence Ang I is biologically inactive.

ACE

Angiotensin I converting enzyme major protein in the renin-angiotensin-system (RAS). It cleaves angiotensin I to form angiotensin II and plays important roles in sodium homeostasis and blood pressure control.



Human ACE gene organization: The top line shows the scale of 30 Kb and bottom lines shown the26 numbered exons, exon 13 is specific for testicular ACE (adapted from Hubert et al; JBC 266 (23), 1991.

Out of 26 exons, the endothelial ACE is encoded by 25 exons and the testicular ACE is constituted by 14 exons whose 13 are common with the endothelial enzyme. Molecular cloning of ACE endothelial and subsequently of testicular cDNA showed that the structure of the gene resulted from gene duplication (Hubert et al; 1991).

Table 1. Angiotensin-converting enzyme (ACE) and its homologous proteins in different species.

Species	Designation for the enzyme	Other Aliases	Chromosome (Location)
Homo sapiens	ACE somatic isoform	ACE 1	17
	Carboxycathepsin	CD143	17q23
	C143 antigen	DCP	
	Dipeptidyl carboxypeptidase-I	DCP1	
	Dipeptide hydroxylase	HGNC:2707	
	Kininase II	MGC26566	
	Peptidase P	PDH	
	Peptidyl-dipeptidase A		
	Peptidyl dipeptidase-4		
	Peptidyl- dipeptide hydrolase		
Mus musculus	Dipeptydyl peptidase	CD143 somatic	11
		MGI: 87874	65 cm
Pan troldytes	Dipeptidyl carboxypeptidase-I	DCP1	17
	Similar to ACE		
Rattus norvegicus	Dipeptidyl carboxypeptidase-I	DCP1	10
	Kininase II	RGD:2493	10q32.1
		StsRR92	
Drosophila	CG8827-PA, isoform A	ACE	2L
	CG8827-PB, isoform B	AnCE	34E2
		Anon-EST:fe3D10	
		BG:DS08220,3	
		Br3t.I234Eb	
		CG8827	
		Race	
Gallus gallus	ACE		27
	Peptidyl-dipeptidase A	-	
Apis melidera	Similar to ACE	-	1.GS
Bos taurus	Similar to ACE	-	Un
Canus tamilaris	ACE	DCP1	9

Chapter - 3

SCOPE OF STUDY

To find the association of ACE gene polymorphism with essential hypertension in Punjab region population.

ACE gene polymorphism and hypertension

ACE, zinc metallopeptidase allotted at the surface of endothelial & epithelial cells. ACE enzyme an inactive decapeptide, angiotensin I convert to octapeptide angiotensin II, that's a most important active product of RAS. Renin is launched in kidneys by juxtaglomerular cells under conditions of volume loss, sympathetic activation, or salt. Angiotensin II effective vasoconstrictor acts on adrenal cortex launch of aldosterone stimulate tubules in kidneys allows reabsorbing greater sodium and water from urine as a consequence affecting direct boom blood fluid, volume loss & blood pressure growth.

It play basic capacity in each other hormonal device, kinin-kallikrein course, uses bradykinin (Biron P et al ., 1991)'despite the way that the position of ACE in worsening those proteins changed into not persistently repeated in vivo, those discoveries hastened to inquire about on neurological afflictions including Parkinson disease, despairing, and distinctive emotional issue. The position of ACE in CNS, be that as it may, isn't limited to those neurotransmitters. it has been tried that ACE debases amyloid beta peptide in vitro, one of the essential negative organic retailers ensnared in Alzheimer sickness (advertisement) pathogenesis(wilsonFH et al .,2001).

Chapter – 4

AIM & OBJECTIVE

Association of nucleotide polymorphisms of the Angiotensin- changing Enzyme (ACE) Gene with hypertension in North Indian population.

Objective:

- To amplify ACE gene by PCR.
- To study the relationship between ACE gene polymorphism and hypertension.
- To study the various biochemical parameters and its association with ACE polymorphism.

Chapter – 5

MATERIAL & METHODS

Materials:

Labware used:

Beaker, Conical flask, microfuge tubes (2ml, 1.5ml), Refrigerator (-20^{0}C), Autoclave Microwave oven, Gel documentation equipment, PCR tubes, Centrifuge, Electrophoresis apparatus, Micropipette (1000µl, 100µl, 10µl), Falcon tubes, Water bath, Gloves, pH meter.

Chemicals used:

Tris- HCl, EDTA (Ethylene Diamine Tetra Acetic Acid), NaCl (Sodium Chloride), SDS (Sodium Dodecyl Sulfate), Phenol:Chloroform: Isoamylalcohol, Isopropanol, RBC Lysis Buffer, RNase A, Proteinase K, Sodium Acetate, NaOH, Ammonium Chloride, Potassium Bicarbonate, Potassium Chloride, Cell lysis buffer.

Methodology:

DNA isolation

People from different age groups were randomly selected (30-60 years) and blood samples were collected from Dr. Sanjeev's Heart Center, Ludhiana. 5-6 ml of the blood test were taken from hypertension patients into EDTA vials. Blood was immediately stored at -20°C. Half of the blood was used for the estimation of biochemical parameters.

Table: 2 Biochemical Parameters of patients with regard to their age, lifestyle, smoking, alcoholic consumption and serum general LDL cholesterol and blood pressure.

	HYPERTENSION	NORMAL
Number	28	4
Age	30-70	30-70
SBP (mmHg)	162 ± 4.3 (SD)	115± 3.4 (SD)
DBP (mmHg)	96 ± 2.6 (SD)	76 ± 3.22(SD)
LDL (mg/dl)	107 ± 21.53 (SD)	69 ± 3.51 (SD)
HDL (mg/dl)	55 ± 3.2 (SD)	44 ± 2.53 (SD)

SD stands for standard deviation, SBP-Systolic blood pressure, DBP-Diastolic blood pressure, LDL-Low density cholesterol, HDL-High density cholesterol

Genetic analysis-

A) **Genomic DNA isolation protocol:**

1x lysis buffer + 1ml Blood (twice)

↓ 37°C / Overnight incubation

Vortex + centrifuge at 7,000 rpm

↓

Give minimum 2 washes

(Till pellet gets white/brown)

↓

Add 405ul per sample of solution (Cell Lysis solution) + 5ul Proteinase K (10mg/ml)

+ 25ul SDS (10%) + 375ul 0.2M Sod. Acetate (2M stock)

↓

Vortex to mix

↓

Keep at 56^0C for 30 mins./ overnight

↓

I → Add PCI 120ul each falcon (25:24:1)

↓

Mix gently make emulsion, Centrifuge at 10,000 rpm

↓

Transfer the supernatant in fresh Falcons

↓

Repeat once

↓

Mix & centrifuge, Transfer to 1.5 ml microfuge tubes

↓

The volume of supernatant add isopropanol (1ml chilled), incubate for 1hour in -20^{0}C.

Centrifuge at 10,000 rpm

↓

Discard the supernatant

↓

Add 500 µl of 70% ethanol, Centrifuge Discard supernatant, Keep at 37^{0}C wait until smell goes

↓

Add 100 µl of 1X TE, Store at -20^{0}C

B) PCR Amplification

ACE gene specific primers forward 5'-CTGGAGACCACTCCCATCCGTTCT-3' and reverse 5'-GATGTGGCCATCACACATTCGTCAGAT-3'. PCR amplification for 30 cycles of the gene made using denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C completed and confirmed on the basis of size on Agarose gel electrophoresis.

PCR Setup-

94^0C	1 min	
94^0C	15 sec	
58^0C	30 sec	Repeat 30 cycles
72^0C	25 sec	
72^0C	5 min	

C) STATISTICAL Analysis

Statistical analysis of genotypic genotype II, DD & ID percentage of ACE gene in normal control and hypertension patients was calculated, after observing bands on 2% agarose gel and a significant association was observed between the DD>II>I/D polymorphism association of ACE gene and hypertension in north Indian Population as shown in table 3.

Table 3: Showing Genotype percentage of ACE gene in normal control and hypertension patients

Genotype	Hypertension	Normal	Total
II	03 (14.3 %)	-	3
DD	15 (71.4%)	03 (75 %)	18
ID	03 (14.3%)	-	03

Chapter – 6 RESULT & DISCUSSIONS

Result- The genomic DNA were isolated by Phenol: Chloroform method as described in the methods section. Out of 39 samples, we couldn't get DNA in 7 samples (N1, N3, N5, N9, H5, H26 and H27, Fig 3, Fig 7). The DNA was also degraded in samples (H22 and H23, Fig 7). Out of 32 samples, we got PCR amplification in 24 samples, in 8 samples (H11, H12, H13, H22, H23, H26, H27, N30) due some technical reasons didn't get PCR amplification. The concentration and purity of DNA samples were estimated by a spectrophotometer as shown in table 4. The concentration was calculated by following formula.

DNA Concentration (µg/ml) = (A260 reading/A280 studying) ×Dilution Factor (DF) × 50

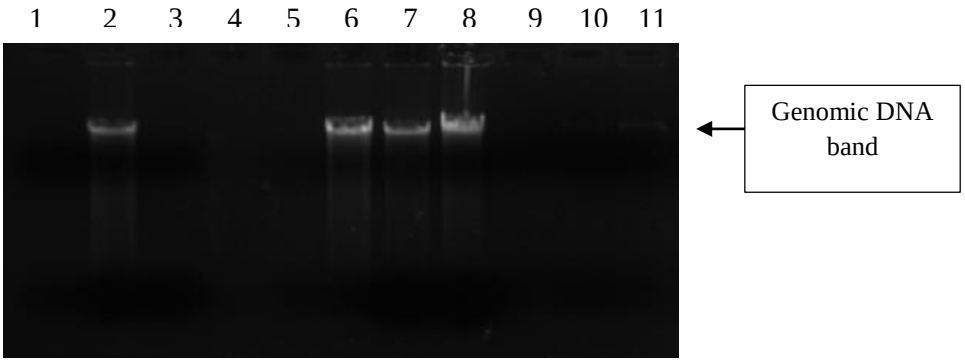


Fig 3: Agarose gel electrophoresis of isolated genomic DNA- Lane 1- N1, Lane 2- N2, Lane 3- N3, Lane 4- N4, Lane 5- N5, Lane6- N6, Lane 7- N7, Lane8- N8, Lane9- N9, Lane10- N10, Lane11-N11

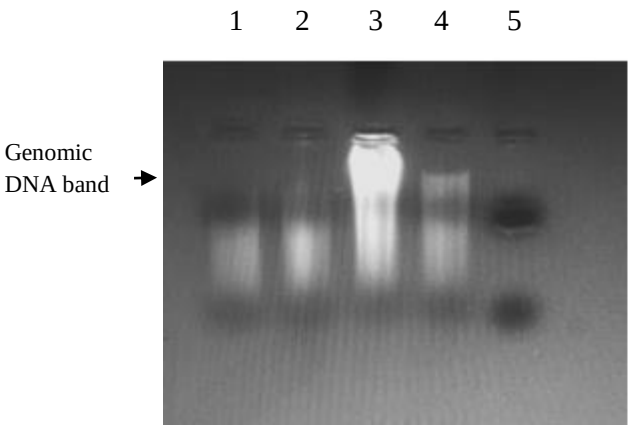


Fig 4: DNA on 1% agarose gel, lane 1 H1, lane 2 – H2, lane3- H3, lane 4- H5, Lane 5- H5.

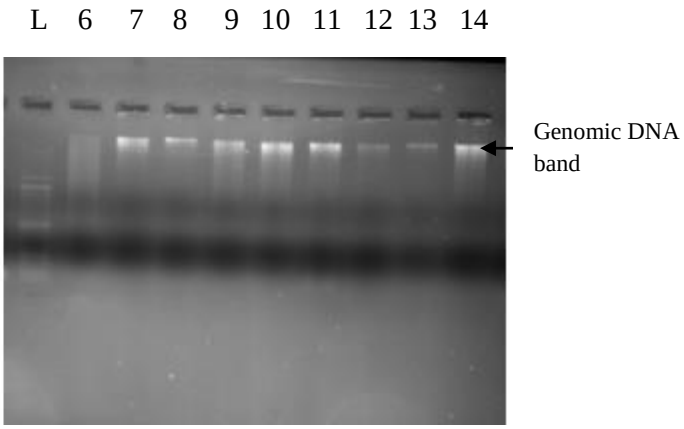


Fig 5: DNA on 1% agarose Lane1- 50 bp ladder, Lane2- H6, Lane 3- H7, lane 4- H8, Lane 5- H9, Lane 6- H10, Lane7- H11, Lane 7- H12, Lane 8- H13, Lane 9- H14.

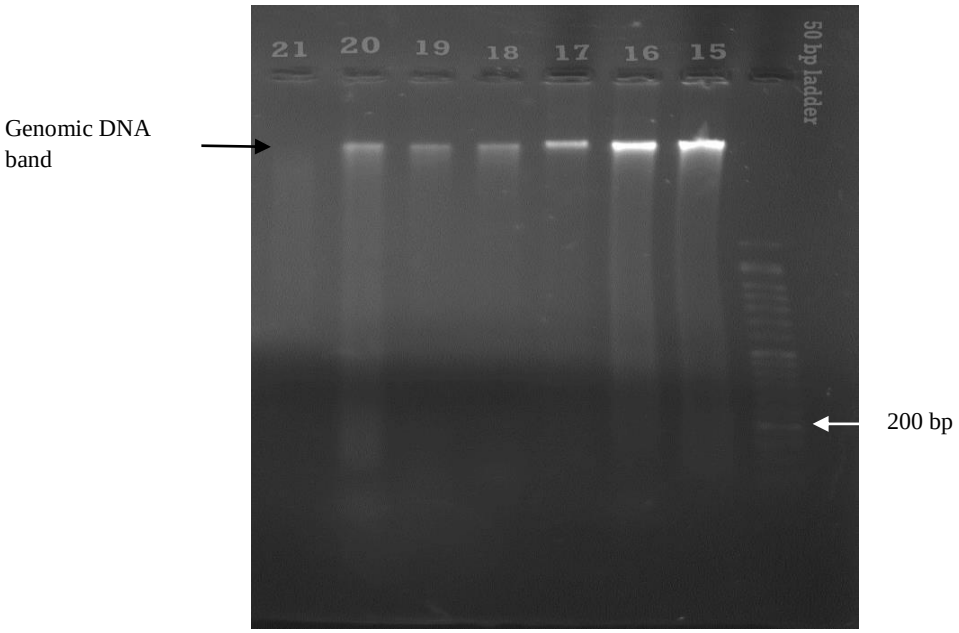


Fig 6: DNA on 1% gel - Lane 15- H15, Lane 16- H16, Lane 17- H17, Lane 18- H18, Lane 19- H19, Lane 20- H20, Lane 21- H21.

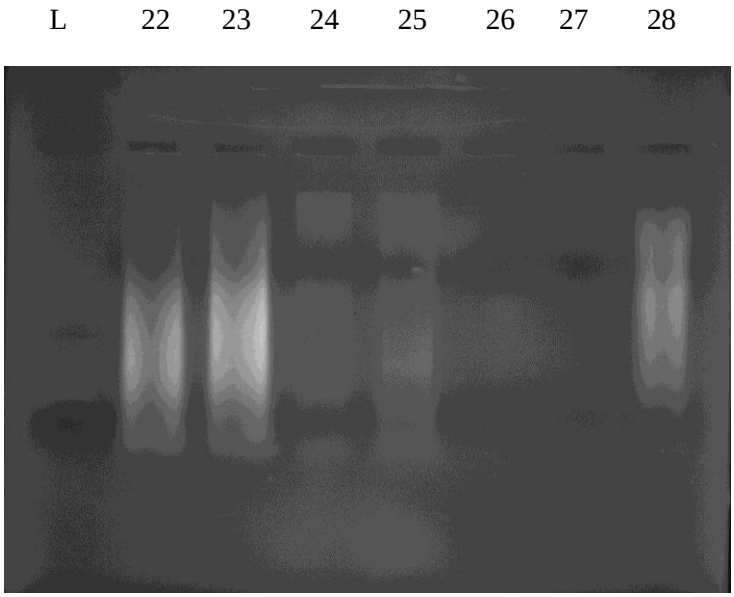


Fig 7: DNA on 1% gel - lane 1- 50 bp ladder, lane 22- H22, Lane 23- H23, Lane24- H24, Lane 25- H25, Lane 26- H26, Lane 27- H27, Lane 28- H28.

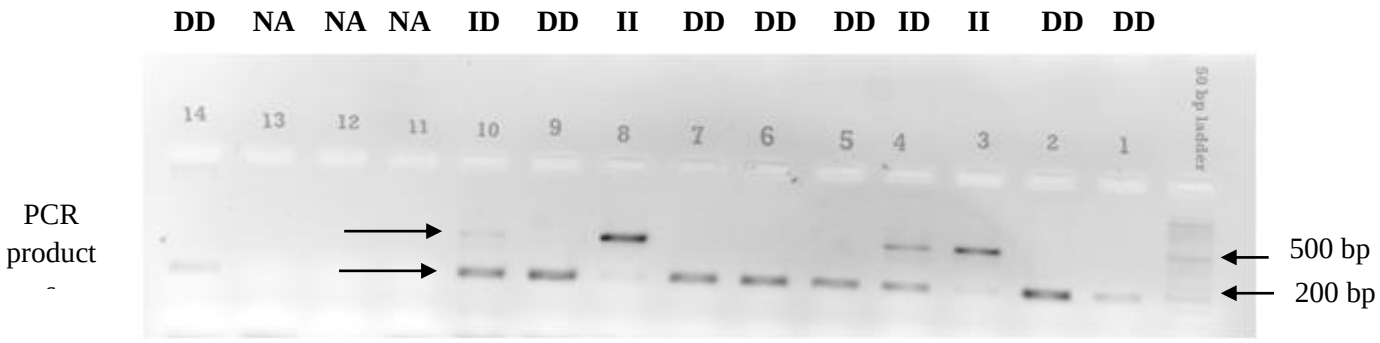


Fig 8: PCR amplification of genomic DNA from hypersensitive patients - lane 1 H1, lane 2- H2, Lane 3- H3, lane 4- H4, Lane 5- H5, Lane 6- H6, Lane 7- H7, Lane8 –H8, Lane 9- H9, Lane 10- H10, Lane 11- H11, Lane 12- H12, Lane 13- H13, Lane 14- H14

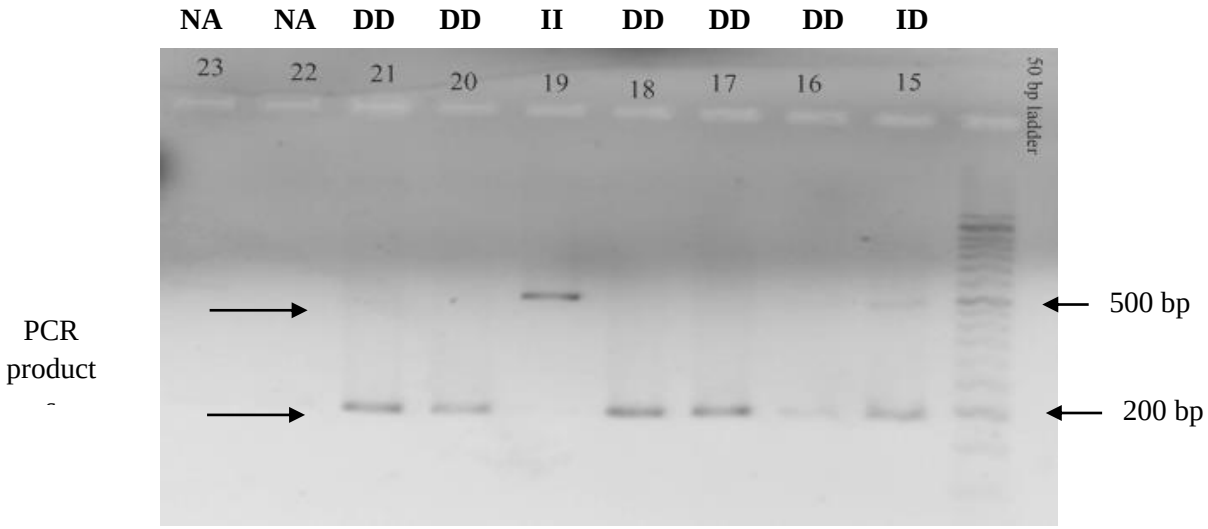


Fig 9: PCR on 2% gel - lane 23- H23, lane 22- H22, lane21- H21, Lane 20- H20, Lane 19- H19, Lane 18- H18, Lane 17- H17, Lane 16- H16, Lane 15- H15

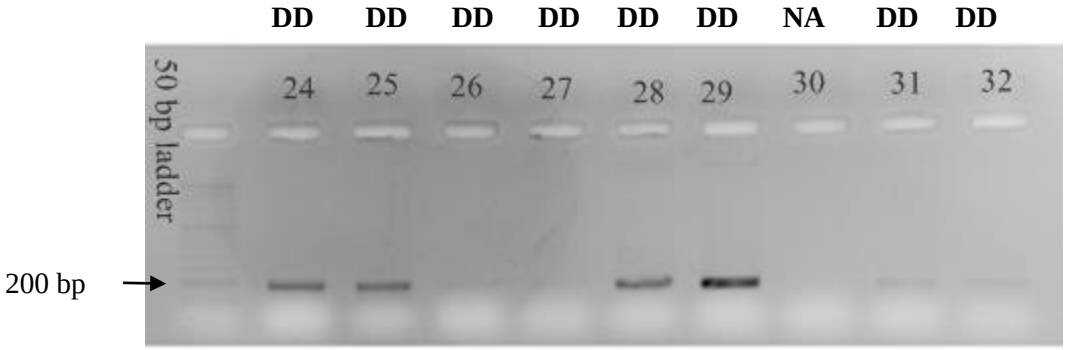


Fig 10: PCR on 2% gel - Lane24- H24, Lane 25- H25, Lane 26- H26, Lane27- H27, Lane28- H28, Lane 29- N29, Lane 30- N30, Lane 31- N31, Lane 32- N32.

Table 4: Spectrophotometric analysis of DNA samples.

Sample	A260nm	A280nm	Ratio 260/280	Concentration (ng/μl)
H1	0.64	0.27	2.36	129.4
H2	1.72	0.92	1.87	161.0
H3	0.90	0.63	1.41	52.8
H4	0.51	0.28	1.82	46.2
H5	1.04	0.77	1.33	52.2
H6	1.83	0.97	1.88	172.0
H7	1.25	2.14	0.58	177.8
H8	1.83	0.99	1.84	167.8
H9	1.27	0.93	1.35	67.2
H10	0.63	0.93	0.68	59.4
H11	1.85	0.98	1.89	92.7
H12	1.32	1.50	0.87	36.4
H13	1.14	0.56	2.01	115.4
H14	2.61	1.78	1.46	166.0
H15	0.94	0.32	2.92	124.2
H16	0.85	0.64	1.32	42.4
H17	0.77	0.44	1.72	65.2
H18	1.04	0.72	1.44	64.4
H19	0.75	1.18	0.63	85.6
H20	0.87	0.65	1.33	45.4
H21	1.23	1.00	1.22	45.2
H22	1.11	1.77	0.62	131.2
H23	2.56	1.64	1.55	183.8
H24	1.85	0.98	1.88	173.4
H25	1.38	0.94	1.46	87.6
H26	1.41	1.05	1.34	73.0
H27	0.86	0.46	1.86	79.8
H28	1.27	0.86	1.47	82.2
H29	1.16	0.97	1.19	37.2
H30	1.03	0.87	1.17	30.8

Discussion-

The I/D polymorphism in the ACE gene has been accounted for 47% of the aggregate phenotypic fluctuation of serum ACE that causes renin- angiotensin hypertension [10]. A few reviews have shown the significance of ACE I/D polymorphisms in the pathogenesis of

hypertension. In our result, we found that I/D and I/I polymorphism is 14.3 % and DD is 71.4 % in hypertension patients. The serum ACE levels are usually associated with following the order of genotype DD>I/D>II. The D polymorphism is associated with coronary artery diseases, I/D with coronary heart disease and II genotypes with an intermediate phenotype (Right B et al). Our study significantly showed that there is the higher propensity of DD genotype in hypertension patients thus the individuals might be affected with coronary artery diseases (CAD). CAD occurs when the arteries are blocked due to cholesterol or plaques on the inner wall of the heart muscle. We also found the hypertensive patients are having high lipid cholesterol table 2. Most of the studies so far have focused on I/D marker for ACE gene polymorphism, our study highlights that DD marker is also a significant marker for identifying population prone to heart disease. We have also shared the data with Dr. Sanjeev Kumar Mittal from Dr. Sanjeev Heart Center, College Road, Maya Nagar, Ludhiana, so that he can consulate patients for probable risks.

Chapter – 7

CONCLUSION & FUTURE SCOPE

Hypertension is mainly caused by high blood pressure. Apart from genotype, the lifestyle of individuals, age, high intake of fat and oil reach food, and high dietary intake of sodium are some of the environmental factors contributing to high blood pressure. We have collected blood samples from 29 hypertensive and 11 samples from normotensive individuals to study the association of ACE gene polymorphism. Based on above data we surmise that DD polymorphism of ACE gene is associated with hypertension. The outcome recommends that patients should be informed of the potential threat of heart ailment.

To increase the significance of data larger populace from a different region of Punjab and nearby states should be taken into consideration. Other biochemical factors like lipid and liver profile should also be incorporated.

Chapter – 8

BIBLIOGRAPHY

1. Kannel WB et al. Cardiovascular risk factors and hypertension. In: IzzoJl, Black H, editors. Hypertension primer (Second edition): The essentials of high blood pressure.1999; 335:765–774.
2. Lindpaintner K. · Ganten D. Tissue renin-angiotensin system and their modulation: the heart as a paradigm for new aspects of converting enzyme inhibition. Cardiology 1991; 79 Suppl: 32–44.
3. Griendling KK, Murphy TJ, Alexander RW. Molecular biology of the renin-angiotensin system. Circulation 1993; 87: 1810–28.
4. Neuringer JR, Brenner BM.Hemodynamic theory of progressive renal disease—a 10 yr updates in brief review. Am J Kidney Dis 1993; 22:98–104.
5. Navar LG, Lewis L, Hymel A, Braam B, Mitchell KD.Tubular fluid concentrations and kidney contents of angiotensins I and II in anesthetized rats. J AmSoc Nephrol. 1994; 5:1153–8.Table 3 Comparison between various genotypes (D/D, I/D, I/I)Genotypes Odd's ratio 95 % Confidence IntervalD/D vs I/I 3.85 1.66–8.93D/D vs I/D 4.32 2.11–8.84I/D vs I/I 1.12 0.51–2.47268 Ind J ClinBiochem (July-Sept 2012) 27(3):265–269123
6. Atiyeh BA, Arant BS Jr, Henrich WL, Seikaly MG.In vitro production of angiotensin II by isolated glomeruli. Am J PhysiolRenal.1995; 268: F266–72.
7. H Urata, H Nishimura, D Ganten. Mechanism of angiotensin IIformation in humans. Eur Heart J. 1995;16: 79–85.
8. Zitnay C, Siragy HM.The action of angiotensin receptor subtypes on the renal tubules and vasculature: implications for volume homeostasis and atherosclerosis. Miner Electrolyte Metab.1998; 24:362–70.Siragy HM. AT1 and AT2 receptors in the kidney: role in disease and treatment. Am J Kidney Dis. 2000; 36: S4–90.
9. T. Sipahi, M. Budak, S. Şen, A. Ay & S. Şener Association between ACE geneI/D polymorphism and primary hypertension in Turkish patients of Trakya region. Biol Chem. 1991; 266:15377–83.

10. Domnita Crisan and Jeanne Carr. ACE gene is on the chromosome. Cytogenet Cell Genet. 1989; 51:1041–5.
11. Rigat B, Hubert C, Corvo P and Soubrier F (1992) PCR detection of the insertion/deletion polymorphism of the. Nucleic Acids Res 20: 1433–1433
12. Rigat B, Hubert C, Alhenc-Gelas F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86:1343–6.
13. Jeffers BW, Estacio RO, Raynolds MV, Schrier RW. Angiotensin-converting enzyme polymorphism in non-insulin dependent diabetes mellitus and its relationship with diabetic nephropathy. Kidney Int.1997; 52:473–7.
14. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, Dusselier L, Kahal Z, Chaillous L, Halimi S, Muller A, Sackmann H, Bauduceau B, Bled F, Passa P, Alhenc-Gelas F. Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complication in IDDM. J Clin Invest. 1997; 99:1585–95.
15. H J G H Mulder, P P van Geel, M J Schalij, W H van Gilst, A H Zwinderman, and A. V. G Bruschke DD ACE gene polymorphism is associated with increased coronary artery endothelial dysfunction.Heart. 2003; 89:557–8.
16. Yuji Takemoto, Mitsunori Sakatani, Seijyu Takami, Teruo Tachibana, Jitsuo Higaki, Toshio Ogihara, Tetsuro Miki, Tomohiro Katsuya, Tessei Tsuchiyama, Akira Yoshida, Hisahiro Yu, Yoshiro Tanio, Einosuke Ueda. Association between AII receptor gene polymorphism and serum ACE activity in patients with sarcoidosis. Thorax. 1998; 53:549–62.
17. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The Seventh report of the joint national committee on prevention, detection, evaluation and treatment of high blood pressure. The JNC & report. JAMA. 2003; 289: 2560–72.
18. S A Miller, D D Dykes, and H F Polesky A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1998; 16:1215.

19. Gupta R. Trends in hypertension. Epidemiology in India. Hum Hypertens. 2004; 18:73–8.
20. Chaturvedi N. Ethnic differences in cardiovascular disease. Heart. 2003; 89:681–6.
21. Manunta Paolo. Manunta P, Bianchi G. Are the new single nucleotide polymorphisms relevant for hypertensive populations? J Hypertens.2002; 20(12):2335–6.
22. Thiol B, Thiel B, Weder AB Genes for essential hypertension. Hype, help or hope? J Clin Hypertens (Greenwich). 2002;2: 187–93.
23. Duru K, Farrow S, Wang JM, Lockette W, Kurtz T. Frequency of deletion polymorphism in the gene for ACE is increased in African Americans with hypertension. Am J Hypertens. 1994; 7:759–62.
24. Chiang FT, Chern TH, Lai ZP, Tseng CD, Hsu KL, Lo HM, Tseng YZ. Age and gender dependent association of an ACE gene with essential hypertension in a Chinese population. J Hum Hypertens. 1996; 10:823–6.
25. Robert Y.L. Zee.Yi-Kun Lou.Lyn R. Griffiths. Brian J. Morris. e. ACE polymorphism and essential hypertension. Lancet. 1994; 343:125.
26. Nakno Y, Oshima T, Hiraga H. Genotype of ACE is a risk factor for early onset of essential hypertension Japanese population. J Lab Clin Med. 1998; 13:502–506. doi: 10.1016/S0022-2143(98)90058-0.
27. Morshed M, Khan H, Akhteruzzaman S. Association between ACE polymorphism and hypertension in selected individuals of Bangladeshi populations.J Biochem Mol Biol. 2002; 35(3):251–4.
28. Yildiz A, Akkaya V, Hatemi AC, Cine N, Tükek T, Görçin B, Demirel S, Türk S, Sever MS. No association between the deletion type ACE gene polymorphism and left ventricular hypertrophy in hemodialysis patients. Nephron. 2000; 84:130–5.
29. Maguchi M. ACE polymorphism in essential hypertensive patients in the Japanese population. Angiology. 1996; 47(7):643–648. doi: 10.1177/000331979604700702.
30. Pamies AE, Palmero PC, Garcia LR. Effect of angiotensinogen M235T and the ACE I/D polymorphism on arterial hypertension and cardiovascular risk factors. Med Clin. 1999; 113(51):164–168.
31. Dazida G, Sobatyl J, Puzniak A. Polymorphism of ACE and Ang II receptor type I gene in essential hypertension in the Polish population. Clin Res. 2001; 7:1236–1241.

32. Carretero OA, Oparil S. Essential hypertension. Part I: definition and etiology. Circulation. 2000; 101:329-35. [PMID: 10645931]
33. . Calhoun DA, Bakir SE, Oparil S. Etiology and pathogenesis of essential hypertension. In: Crawford MH, DiMarco JP, eds. Cardiology. London: Mosby International; 2000:3.1-3.10.
34. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell. 2001; 104:545-56. [PMID: 11239411]
35. Johnson RJ, Herrera-Acosta J, Schreiner GF, Rodriguez-Iturbe B. Subtle acquired renal injury as a mechanism of salt-sensitive hypertension. N Engl J Med. 2002; 346:913-23. [PMID: 11907292]
36. Guyton AC. Blood pressure control—special role of the kidneys and body fluids. Science. 1991; 252:1813-6. [PMID: 2063193]
37. Feinleib M, Garrison RJ, Fabsitz R, Christian JC, Hrubec Z, Borhani NO, et al. The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. Am J Epidemiol. 1977; 106:284-5. [PMID: 562066]
38. Longini IM Jr, Higgins MW, Hinton PC, Moll PP, Keller JB. Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. Am J Epidemiol. 1984; 120:131-44. [PMID: 6741914]
39. Biron P, Mongeau JG, Bertrand D. Familial aggregation of blood pressure in 558 adopted children. Can Med Assoc J. 1976;115:773-4. [PMID: 974967]
40. Wilson FH, Disse-Nicode`me S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, et al. Human hypertension caused by mutations in WNK kinases. Science. 2001; 293:1107-12. [PMID: 11498583].
41. Hsueh WC, Mitchell BD, Schneider JL, Wagner MJ, Bell CJ, Nanthakumar E, et al. QTL influencing blood pressure maps to the region of PPH1 on chromosome 2q31-34 in Old Order Amish. Circulation. 2000; 101:2810-6. [PMID: 10859286]
42. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, and Pathogenesis of Hypertension Review Evidence for a gene influencing blood pressure on chromosome Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham heart study. Hypertension. 2000; 36:477-83. [PMID: 11040222]

43. Kristjansson K, Manolescu A, Kristinsson A, Hardarson T, Knudsen H, Ingason S, et al. Linkage of essential hypertension to chromosome 18q. Hypertension. 2002; 39:1044-9. [PMID: 12052839].
44. Hunt SC, Ellison RC, Atwood LD, Pankow JS, Province MA, Leppert MF. Genome scans for blood pressure and hypertension: the National Heart, Lung, and Blood Institute Family Heart Study. Hypertension. 2002; 40:1-6. [PMID: 12105129]