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Association of single nucleotide polymorphism within angiotensin II receptor type 1 (AGTR 1) gene with Hypertension in North Indian Population.

Dissertation

SUBMITTED BY

-

Shivani

11103453

To

Department of Biotechnology

In partial fulfilment of the requirement for the award of the degree of

Master of Science in Biotechnology Integrated

Under the guidance of

Dr. Gyanesh Singh

9 May, 2017

TOPIC APPROVAL PERFORMA

School of Bio Engineering and Bio Sciences

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Supervisor Name : Dr. Gyanesh B Singh **UID :** 15278 **Designation :** Associate Professor

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SPECIALIZATION AREA : Molecular Biology and Genetic Engineering

PROPOSED TOPIC : Association of single nucleotide polymorphism within angiotensin II receptor type 1 (AGTR 1) gene with Hypertension in North Indian Population.

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	**Parameter**	**Rating (out of 10)**
1	Project Novelty: Potential of the project to create new knowledge	6.50
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	8.00
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	8.00
5	Social Applicability: Project work intends to solve a practical problem.	8.50
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	8.00

PAC Committee Members		
PAC Member 1 Name: Dr. Ashish Vyas	UID: 12386	Recommended (Y/N): Yes
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PAC Member 4 Name: Dr. Umesh Goutam	UID: 14691	Recommended (Y/N): NA
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Association of single nucleotide polymorphism within angiotensin II receptor type 1 (AGTR 1) gene with Hypertension in North Indian Population.

Final Topic Approved by PAC:

Overall Remarks: Approved

07 Nov 2016

Approval Date:

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CERTIFICATE

This is to certify that **Shivani** has completed M.Sc. Dissertation II titled "Association of single nucleotide polymorphism within angiotensin II receptor type 1 (AGTR 1) 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 project has ever been submitted for any other degree or diploma. The project is fit for the submission and the partial fulfilment of the conditions for the award of M.Sc. biotechnology.

Date 9 May, 2017

Signature of Thesis Advisor
Dr. Gyanesh Singh
UID 15278

DECLARATION

I hereby declare that the project entitled, Association of single nucleotide polymorphism within angiotensin II receptor type 1 (AGTR 1) gene with Hypertension in North Indian Population**,** for the M.Sc. Degree is entirely my original work and all ideas and references have been duly acknowledged. It does not contain any work for the award of any other degree or diploma.

Date 9 May, 2017

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Abstract

Hypertension leads to the increasing risk of the stroke, heart failure and development of other heart diseases. AGTR1 is an important gene involved in essential hypertention. The AGTR1 gene is placed on chromosome 3q21-25 and is greater than 55kb long. The +1166 A/C polymorphism has been associated with the extreme form of crucial high blood strain.

Aim : Association of single nucleotide polymorphism within angiotensin Il receptor type 1 (AGTR 1) gene with Hypertension in North Indian Population.

Method: DNA extraction was carried out using standard protocols.. PCR was done using AGTR1 primers. PCR amplified DNA was digested with DDE I restriction enzyme followed by agarose gel electrophoresis.

Result: Both AC, and CC genotypes of the AGTR1 gene appear to be associated with hypertension.

Conclusion: AC and CC genotypes of AGTR1 turned out to to be more in hypertension patients.

Chapter-1

INTRODUCTION

Hypertension is the main cause of several cardiovascular disorders (Schuer, 2008). The normal blood pressure in humans is usually 120 mmHg systolic and 80 mmHg diastolic (abbreviated as 120/80 mmHg). Higher blood pressure is categorized as ≥140 mmHg systolic blood pressure or ≥ 90 mmHg diastolic blood pressure. The extent of the effect of hypertension on the body tissues depends on various factors but it mainly affects heart, brain and kidneys.

Treatment of hypertension can be complex as it involves the understanding of risks associated with different organs (Foex and Sear, 2004, Sear, 2004). Multiple genes are known to be involved in causing hypertension (john son et al., 2002).

It is estimated that more than fourty three percent people in the united states have hypertension or are adopting antihypertensive medication including is twenty four percent of the adult population (Alabousi et al.,. 2015) .).This ratio changes with (1) race, existing higher in blacks (32.4%) and lower in whites (23.3%).Blood pressure rises in the age between 55 to 60 years. It is mainly increases in the elderly people due to systolic hypertension. In the late nineties and early twenty-century the prevalence of hypertension is changed among different studies in India. This range varies from 2-15% in urban Indian population and 2-8% in rural Indian population. In developing countries, this is the seventh highest contributor that leads to premature death.

This is reported that in 2000 around 1billion adults had hypertension and it is estimated that in coming years this increase from 1billion to 1.56 billion by 2025. The prevalence of hypertension is ranges between 22% and 25%. The recent report given by WHO that is

57million global deaths was estimated in 2008 and 36 million because of non-communicable diseases. This is estimated

that the median prevalence of total hypertension in 2009 was 37.6% in men and 40.1% in women. (Sarkar et al., 2015).

Risk factors for hypertension includes, age over 60, race, heredity, salt sensitivity, obesity, inactive lifestyle, alcohol consumption. (Cutler. ja 1996). Primary (or essential) and secondary hypertension are two wide categories of hypertension .Patients diagnosed with hypertension approximately 90-95% have primary hypertension. There is no known cause of primary hypertension, unlike secondary hypertension; therefore, its diagnosis is made after excluding known causes of so called secondary hypertension. The fluctuations in BP are genetically checked and it will be called inherited BP .Essential hypertension is acquired to be a distinctive complex disease.

This is regulated by both genes and environment. 30-50% of the individual changes of BP is heritable. There are multiple physiological pathways which are involved in hypertension and these are called as biological processes. These pathways are may be influenced by multiple gene products. (Dong feng gu et al., 2006) The main focus of the study is on rennin angiotensin-aldosterone.

Water and salt homeostasis is regulated by renin-angiotensin-aldosterone system. In regulation of BP, RAS plays an important role and pathogenesis of essential hypertension. This is a main hazard agent for cardiovascular and cerebrovascular diseases .From the polymorphisms in the RAS genes extensively potential factors are investigated and these are involved in essential hypertension.

Polymorphisms in genes writing for elements of the and α -adducin (*ADD1*) and renin-angiotensin system (RAS) have been accounted to be linked with blood pressure changes and responses to antihypertensive agents in various studies.

These genetic agents are angiotensinogen II type –r eceptor (AGTR1) and, angiotensin-converting enzyme (ACE).The main focus of the study is on AGTR1.The human AGTR1gene represents to chromosome 3q24 and make up five exons. It converts a membrane protein with 359 residues. AGTR1 encodes the type I receptor and this intermediate the cardiovascular effects. AGTR1 acts as an important role in controlling BP and volume in the cardiovascular system. This works as an effector controller. Angiotensin II acts majorly via the angiotensin II type 1 receptor (*AGTR1*) as an intense vasoconstrictor that modulates systemic blood pressure and vascular tone. Moreover, angiotensin II is implied in cardiac and vascular growth works.

Genetic mutations in elements of the renin-angiotensin system (RAS), the enzymatic shower that regulates angiotensin II, have been linked with cardiovascular disease including hypertension. Several form of essential hypertension has been linked with the 1166 A/C polymorphism in various studies (yang et al., 2015)

Chapter-2

Review of literature

Hypertension is the factor behind the increase in the death rate due to the cardiovascular diseases (Schuer, 2008). Hypertension leads to the increasing risk of the stroke, heart failure and development of other heart diseases.

The normal blood pressure which is considered recommended ranges from 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 (Foex and Sear, 2004).

Hypertension is very much usual in the up and preponderance is powerfully modulated by the age. All-important hypertension or hypertension of strange reason reports more than 90% case of hypertension. It runs to bunches in families and represents a group of genetically based disease or syndrome. (John son et al., 2002).

It is calculated that in United States 43% people have hypertension or in which ~24% of the adult population acquiring antihypertensive medicinal Drug. This ratio alters with (1) race, existing higher in blacks (32.4%) and smaller in whites (23.3%).Blood pressure rises in the age between 55 to 60 years. It is mainly increases in the elderly people due to systolic hypertension. In the late nineties and early twenty-century the prevalence of hypertension is changed among different studies in India.

This is reported that in 2000 around 1billion adults had hypertension and it is estimated that in coming years this increase from 1billion to 1.56 billion by 2025. The prevalence of hypertension is ranges between 22% and 25%. The recent report given by WHO 57million global deaths was estimated in 2008 and 36 million because of non-communicable diseases. This is estimated that the median prevalence of total hypertension in 2009 was 37.6% in men and 40.1% in women. (Taposh sarkar et al., 2015). Risk factors for hypertension includes, age over 60, race, heredity, salt sensitivity, obesity, inactive lifestyle, alcohol consumption. (cutler. JA 1996). 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 raising in younger age radical, although in order patients increased systemic vascular resistance and increased stiffness of the vasculature play a prevalent role. The final pathway begins when there is an increase 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 mass termed vascular. The fluctuations in BP are genetically checked and it will be called inherited BP .while yet, this is not find that which gene is responsible to alter BP. Obesity ,high salt intake, alcohol consumption are the various factors which give rise to BP as these factors are called as hypertension causing factors (Oscar A et al., (2000)

Types of hypertension

- **Primary hypertension**: primary hypertension also called as essential hypertension. In primary hypertension with every visit BP is frequently increased with equal rates, no symptoms occurred there but frequent headache tiredness, nose bleeds occurs. Factors which involved in primary hypertension are obesity, smoking, alcohol, diet, hereditary.

- **Secondary hypertension**: The main cause are bad arteries that supply blood to kidneys.. Over the counter medication such as ibuprofen and pseudoephedrine can cause secondary hypertension, reason is found and can be verified.

Isolated hypertension: Isolated hypertension recorded in two numbers that is the upper one this is important then lower one and called as systolic pressure. This BP is extended during heartbeat. Lower called diastolic BP, in this the heart is resting between beats. Normal 120/180. Upper above 180, lower below 90.common 65 and above. Danger of

cardiovascular disease for older person.

Malignant hypertension: 1% of people are having malignant hypertension. Distributed among younger adults, African-American men, pregnant women toxemia.

Numbness in arms and legs, blurred vision, confusion, chest pain and headache are the indications for

extremely rise in blood flow rates.

Resistant: resistant hypertension includes three different types: antihypertensive medications and increase in BP. Diabetes or kidney diseases are common in older, obese, female, African-American and underlying illness.

PATHOGENESIS AND PATHOPHYSIOLOGY OF ESSENTIAL HYPERTENSION

Population surveys advocate that, the blood stress is a continuous variable without an absolute dividing line amongst every day and normal values. coronary heart sickness, stroke and kidney failure as a result of immoderate Blood strain .Systolic blood strain is extra correlated than diastolic BP in immoderate BP. There is an incorrect concept that hypertension is an unmarried sickness that can be dealt with a single formula.

Hypertension is a heterogeneous sickness wherein patients can be stratified with the useful resource of pathophysiologic kinds. Pathological way of critical hypertension is multifactorial and quite complicated. A couple of elements modify Blood strain for identical tissue perfusion. those elements are Humoral mediators, vascular reactivity, circulating blood quantity, vascular quality ,blood viscosity, Cardiac output, Blood vessel elasticity, Neural stimulation. Hypertension has been attributed to abnormalities in plenty of elements far not going that each one of these elements are operative in any given affected person but more than one hypotheses may additionally show to be correct, understanding those complex mechanisms has essential implications for the focused on of antihypertensive remedy to achieve blessings past reducing blood strain. The development of essential hypertension starts with prehypertension in people aged 10-30 (with the aid of multiplied cardiac output) years and after that this come on in people aged 20 - forty years (in which improved peripheral resistance is distinguished) and after this founded in elderly 30-50years, and in the end this got here across the persons aged forty- 60. (L Michael Prisant., 2015)

GENETICS

Hypertension is a complicated trait with both genetic and environmental epitopes. From the research of families and twins, it is calculated that the heritability of blood stress is 30 - 50%.Accordinglly from the examine it end that a range of genes with smaller consequences (that is a single hypertension gene) reviews for the heritability of this complex illness. . Furthermore, genetic elements moreover have an effect on behavioral patterns, which may result in BP elevation. For instance, a bent towards weight troubles or alcoholism can be inspired via each genetic and environmental elements; because of this the percentage of BP variability because of inheritances hard to decide and can variety in distinctive populations. Blood Hypertension is a complicated trait with each genetic and environmental epitopes .From the research strain is regulated via way of two genetics variations of various types.

Secondary hypertension is due to segregation of rare mutations in households, inside the absence of different risk elements that is monogenic hypertension ,there are uncommon mutations that lowers blood pressure and consequently guard toward the development of hypertension .primary hypertension is regulated environmental factors including age, frame mass index ,sex , salt, intake, and others (Georg B Ehret, 2016).

Mutations in at the least 10 genes were shown to elevate or decrease BP via common pathway by means of manner of developing or reducing salt and water re absorption through way of the nephron. Essential mutations had been recognized with the usage of this approach. The importance of genes within the hypertension is derives from the observation, it's miles finds that hypertension is 2.4 times more not unusual inside the ones instances who have hypertensive mother and father.

That could be a sturdy empirical show that tells the significance of genes within the hypertension.

a diffusion of candidate genes have been investigated, consisting of loci concerning the α-adducin, rennin angiotensin aldosterone device, sodium epithelial channel, catecholaminergic/adrenergic characteristic, the renal kallikrein kinin device, and others associated with lipoprotein metabolism, hormone receptors, and growth elements(Oscar Aet.,al.,2000)

RENIN–ANGIOTENSIN SYSTEM

The primary popularity of the check is on rennin angiostensin-aldosterone system (RAAS). RAAS controls water and salt balance. RAS performs an essential function in regulating Bp and pathogenesis of essential hypertension.

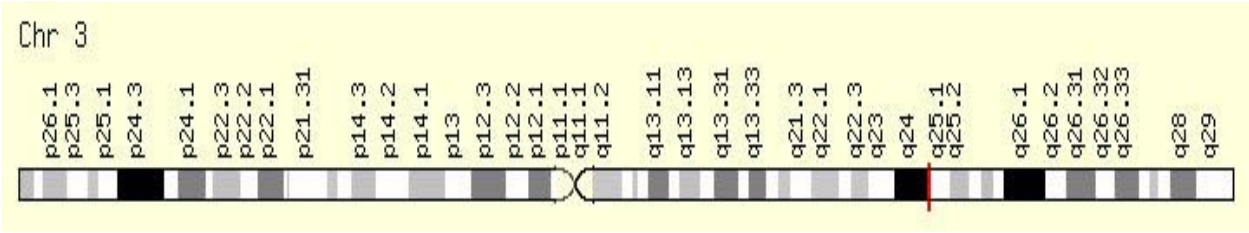
Renin is made in kidney, saved there, and released in response to blood, nervous or ion-channel signals. Renin, an aspartic protease, has a totally excessive specificity for its substrate.

Ang I is cleaved thru cathepsin G, chymostatin-sensitive Ang-II-producing enzyme (CAGE) or chymase to shape Ang II. Ang II is also appeared to be generated with the aid of the movement of cathepsin D, tonin and tissue plasminogen activator (t-PA) on angiotensinogen. Ang II is similarly acted upon by means of the use of amino peptidase A and amino peptidase N sequentially to provide angiotensin III (Ang III) and angiotensin IV (Ang IV) respectively. Angiotensin II increases blood strain by way of diverse mechanisms. Nearby production of angiotensin II in diverse tissues, alongside the blood vessels, coronary coronary heart, adrenals, and brain, is controlled by means of way of ACE and different enzymes, collectively with the serine proteinase chymase.

The hobby of nearby renin–angiotensin structures an d possibility pathways of angiotensin II formation may also moreover make an critical contribution to remodelling of resistance vessels and the development of organ damage.

AGTR1

From the investigation it finds that AGTR1 is extensively potential genetic factor involved in essential hypertention.AGR1are commonly prescribed antihypertensive medication and it also shown that AGTR1 reduce the risk of other cardiovascular endpoints and this independent upon their antihypertensive effects.



AGTR1 gene refer as an extraordinary candidate within the aetiology of hypertension and different cardiovascular diseases(CVD).The AGTR1 gene is placed on chromosome 3q21-25 and is greater than 55kb long .4 transcription initiation sites had been determined. The gene coding for AGTR1 receptor is on human chromosome III (q22 band) and pair approximately 60kb consisting of five exons and 5 introns. The AGTR1 detected mostly in vascular.

AGTR1 gene polymorphism and hypertension

The human AGTR1 gene has a period of about 60 kb, is compiled of 5 exons and four introns and has been found to be enormously polymorphic. in particular, a single nucleotide polymorphism (SNP) has been defined wherein there may be both an adenine (A) or a cytosine (C) base (A/C

trans version) in feature 1166 inside the three′ untranslated place of the gene at least 14 AT-1

polymorphisms have been defined inside the gene AGTR1; specially the +1166 A/C polymorphism has been related to the excessive form of crucial immoderate blood pressure. The A1166C polymorphism has been moreover associated with progressed aortic stiffness, left ventricular hypertrophy, pregnancy- related HT, early coronary ailment and exaggerated vasoconstriction (Stankovic et al., 2003) placed a massive association amongst high blood strain and A1166C polymorphism of AGTR1 gene in male subjects, but not in girls. The A1166C polymorphism end up related to topics beneath prolonged-term remedy and/or with family history of HT (Bonnardeaux et al., 1994) (Benetos et al., 1996) or subjects with hypercholesterolemia (Morisawa et al., 2001; Stankovic et al., 2003).

In cutting-edge studies, however, its association with excessive blood strain become hooked up only in subjects with extreme, early onset, form of this disease (Denser and Schunkert, 2000; Frishberg etal., 1998) did no longer discover any distinction for AGTR1 gene polymorphism in normotensive controls and subjects with resistant essential high blood stress however excessive values of systolic blood stress were associated with the C allele in older and obese sufferers. Jean-Jacques (Mourad et al., 2002) have established that the best-of-a-type presence of the C allele of the AT1-receptor A1166 C gene polymorphism contributes to a higher increase of BP with age. A boom of aortic stiffness have become referred to in hypertensive topics bearing the C allele.

Similarly, the presence of the C allele come to be related, in hypertensive populations, with correction of the progressed aortic stiffness through prolonged-time period antihypertensive treatment concerning angiotensin-changing enzyme inhibition. Liu Y, Shan G showed that A1166C allele of the AGTR1 gene can be a predisposing issue for critical excessive blood stress in Tibetan. There may be scanty statistics available from India on dating among gene

polymorphism particularly AGTR1 Gene and essential excessive blood strain. Research are had to bring about a few changes in information genetic basis of excessive blood stress.

Table 1: Genetic polymorphism evaluated in AGTR1 (Dong feng gu et. al 2006)

Pathway	Gene	Symbol	Chromosome	Polymorphisms	Allele	MAF †	Description and potential function ‡

RAAS	Angiotensin II type I receptor	AGTR	13q21-25	A-1138T	A/T	0.16	Perfect With T-810AS
				C-521T	C/T	0.21	Associated with platelet all binding
				A1166C	A/C	0.06	3’ UTR

Chapter-3

Scope of Study

The purpose of the present study will to investigate the association of AGTR1 gene polymorphism with essential hypertension. The main scope of the study is to find out the contribution of AGTR1 gene polymorphism in essential hypertension.

AGTR1 gene polymorphism and hypertension

The human AGTR1 gene is approximately 60 kb, has five exons and 4 introns and has been located to be noticeably polymorphic. specially, a SNP has been described in which there can be both an

adenine (A) or a cytosine (C) base (A/C trans version) in feature 1166 inside the 3′ untranslated area of the gene.at the least fourteen AT-1 polymorphisms have been known within the gene AGTR1; mainly the +1166 A/C change.

The A1166C polymorphism has been also associated with expanded aortic stiffness, left ventricular hypertrophy, pregnancy- related HT, early coronary illness and exaggerated vasoconstriction (Stankovic et.,2003) discovered a widespread affiliation among high blood pressure and A1166C polymorphism of AGTR1 gene in male topics, however now not in women. The A1166C polymorphism turn out to be related to topics beneath lengthy-term treatment and/or with circle of relative's records of hypertension (Bonnardeaux et al., 1994) (Benetos et al., 1996).

In current research, but, its association with high blood strain changed into mounted quality in topics with immoderate, early onset, form of this sickness (Danser and Schunkert, 2000; Frishberg etal., 1998) did not discover any difference for AGTR1 gene polymorphism in normotensive controls and topics with resistant vital high blood pressure however high values of systolic blood pressure had been associated with the C allele in older and obese sufferers.

Jean-Jacques Mourad et al (2002) have proven that the one of a kind presence of the C allele of the AT1-receptor A1166 C gene polymorphism contributes to a better growth of BP with age.

Liu Y, Shan GL et al8showed that A1166C allele of the AGTR1 gene can be a predisposing

issue for critical high blood strain in Tibetan, there is scanty data to be had from India on courting between gene polymorphism specifically AGTR1 Gene and critical hypertension. Studies are had to result in some adjustments in know-how genetic foundation of high blood pressure. A growth of aortic hardening has been reported to be associated with C allele.

Chapter-4

Objectives:

1. **Isolation of DNA for SNP AGTR1 gene**

2. **Amplification of gene by PCR**

3. **Identification of SNP using restriction digestion and gel electrophoresis.**

Chapter-5

Material And Methods

Materials:

Labware used :

- Beaker
- Conical flask
- Eppendorf (2ml, 1.5ml)
- Refrigerator (-20^{0}C)
- Autoclave
- Microwave oven
- Gel Docking equipment
- PCR tubes
- Centrifuge 10,000rpm
- Electrophoresis
- Micropipette (1000ul, 100ul, 10ul)
- Falcon tubes

Chemicals used:

- Tris- HCl
- EDTA
- NaCl
- SDS
- Phenol:Chloroform:isoamylalchol
- Isopropanol

- Lysis Buffer
- RNAse A
- Proteinase K
- Sodium Acetate
- NaOH
- Ammonium Chloride
- Potassium Bicarbonate
- Potassium Chloride

Methodology

Collection of Buccal Swab Sample

Volunteers were chosen (age range, 38-68 years), and records including age, etc were collected. The volunteers decided on have been requested to rinse their mouth with clean water 30s earlier than sampling of buccal swabs. For each man or woman, each facets of buccal mucosa had been wiped with a cotton swabs for 15 sec, and a total of 4 samples were taken in 500µ l of buffered solution containing 10mM EDTA, 2% SDS. Isolation of DNA from cotton swabs were carried out as given below.

DNA extraction from Buccal Swab

- The buccal swab were suspended in 500µ l lysis buffer (10mM Tris (pH 8), 10mM EDTA, and 2% SDS. The samples were incubated for 1 h at 56°c until the tissue dissolved, then added equal quantity of phenol: chlroform: isomyl alcohol (25: 24: 1) and mixed slowly.

- The samples were spun for 10 min at ten thousand rpm and the supernatant was transferred to a fresh microfuge tubes. Required amount of isopropanol and

sodium acetate was added to this for DNA precipitation. Precipitated DNA was air dried and resuspended in 10mM Tris –HCL, 10mM EDTA, pH7.6 (TE) buffer for further storage at -20 °c (Souvik Ghatak et al., 2013).

Quantification and Quality Checking of Genomic DNA:

Isolated DNA (10µl) was diluted in 990 µl of TE buffer and integrity of DNA samples was checked by Agarose gel electrophoresis and UV spectrophotometry. Concentration was calculated from the value of A_{260} (A_{260} = 1 = 50µ g/ml of dsDNA). Purity of nucleic acid preparation was determined from A_{260} /A_{280} ratio. Ratios of 1.8-1.9 indicate highly purified preparation of DNA, free from protein (Sambrook et al., 2001).

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

Sample	BP	LDL (mg/dl)	HB (g)	Lifestyle (Smoking/Drinking)	Age
H1	130/80	157	13	No	61
H2	170/80	146	12.6	No	35
H3	170/90	172	13.2	No	49
H4	150/80	108	13	Yes	45
H5	80/120	142	11.7	No	42
H6	150/90	118	13.2	Yes	43
H7	160/90	123	12.6	No	52
H8	170/100	111	12.2	No	60
H9	160/100	108	12.8	Yes	68
H10	150/90	147	11.8	Yes	61
H11	130/100	157	13	No	52
H12	150/100	112	12	No	68
H13	150/90	134	13	No	64
H14	160/100	111	11.8	No	56
H15	80/100	108	12	No	49
H16	170/100	132	12.8	No	57
H17	160/90	124	13.4	Yes	38
H18	130/80	109	11	No	43

H19	170/100	125	11.5	Yes	54
H20	150/90	130	13	Yes	52
H21	80/120	117	12.7	Yes	44
H22	160/100	112	12.2	No	48
H23	130/80	132	12.2	No	59
H24	140/80	172	12.2	No	60
H25	130/80	157	11.7	No	38
H26	150/80	145	11	No	45
H27	160/90	114	12.4	No	42
H28	170/100	123	12.8	Yes	52
H29	160/100	108	11.8	Yes	58
H30	150/90	111	12	No	42

Agarose Gel Electrophoresis :

The gel-casting tray (Bangalore Genie, India) was cleaned properly and its open ends were sealed tightly using tape. A comb was used to make wells as per manufacturer instruction. Usually 1-2 gram (according to need) agarose was boiled in 100ml of 1X TAE (Tris-Acetate EDTA) or TBE buffer. Afterwards, 0.5µ g/ml of ethidium bromide will be added to boiled agarose solution, and poured into the gel-casting tray. Upon solidification, the comb was removed, sample DNA was loaded, and. electrophoresis was done in 1X TAE or TBE buffer (pH 8.0).

Isolated DNA sample were mixed with loading dye and loaded in each well for electrophoresis. DNA bands were visualized in Gel doc system. Fluorescent band appearing very near to the wells, represented high molecular weight i.e. good quality of DNA.

PCR Amplification :

Following PCR conditions were used

94^0C	5 min
94^0C	30 sec
55^0C	40 sec
72^0C	30 sec
72^0C	6 min

RFLP :

Restriction digestion done using Dde1 enzyme, which gives two bands of 220 bp and 140 bp for CC genotypic; and yields a single band of 360 bp for AA genotypic. In case of AC genotype, three bands 360 bp, 220 bp & 140 bp should be visible.

Chapter – 6

Results & Discussion

Result – DNA was isolated from several blood or buccal swab samples, and quantity and quality of DNA was found to be good for PCR.

Concentration was calculated by standard spectrophotometric methods:

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

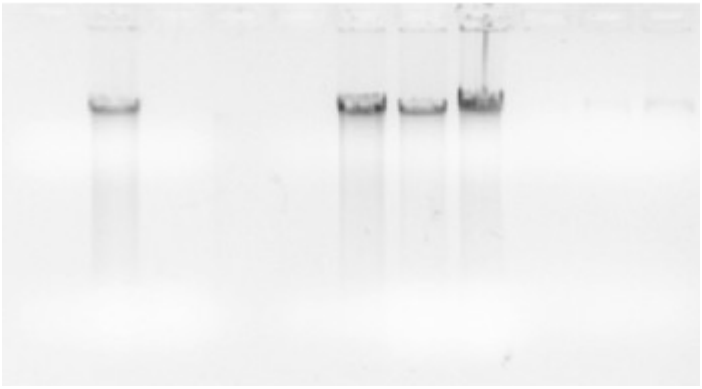
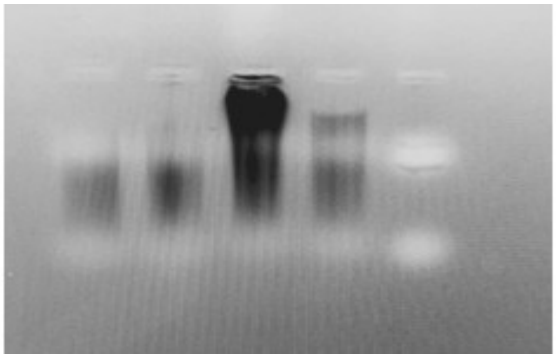


Figure 1- Isolated DNA on agarose gel.



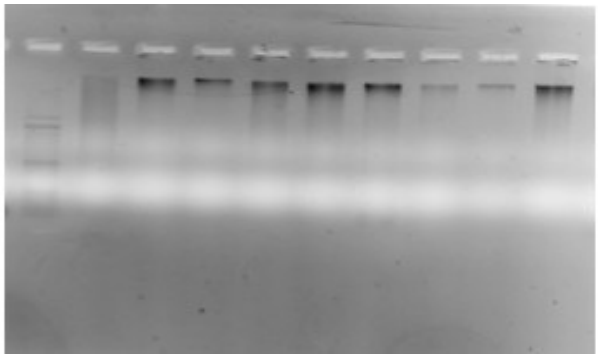


Figure 2: DNA on 1% agrose gel, lane 1 H1, lane 2 – H2, lane3- H3, lane 4- H5, Lane 5- H5.

Fig 4: DNA on 1% agrose Lane1- 50bp 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.

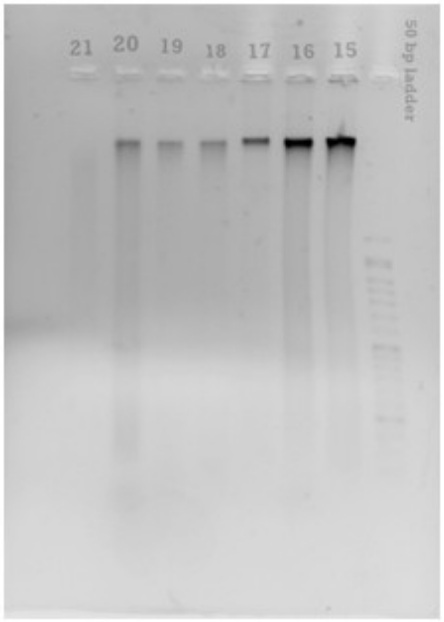


Figure. 3: Lane 1- H21, Lane 2- H20, Lane3- H19, Lane4- H18, Lane 5- H17, Lane 6- H16, Lane 7- H16, Lane 8- H15, Lane 9- 50bp Ladder.

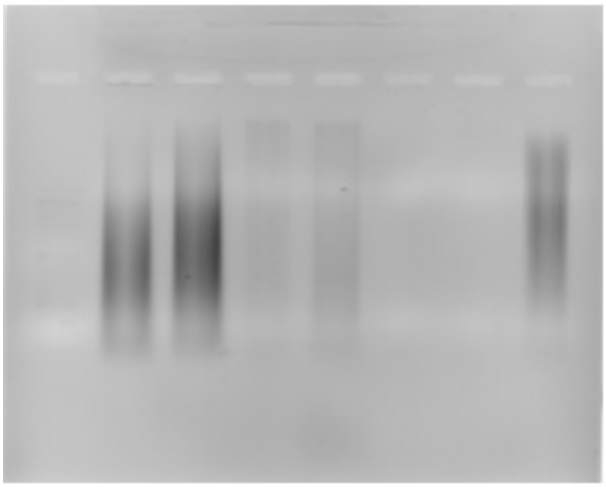


Figure 4: lane 1- 50 bp ladder, lane 2- H22, Lane 3- H23, Lane4- H24, Lane 5- H25, Lane 6- H26, Lane 7- H27, Lane 8- H28.

Table1: Spectrophotometric analysis of DNA (absorbance at 260 and 280).

Patient	260	280	260/280
H1	0.647	0.273	2.369

H2	1.726	0.921	1.874
H3	0.902	0.638	1.413
sH4	0.512	0.281	1.822
H5	1.040	0.779	1.335
H6	1.832	0.972	1.884
H7	1.251	2.140	0.584
H8	1.837	0.998	1.840
H9	1.271	0.935	1.359
H10	0.638	0.935	0.682

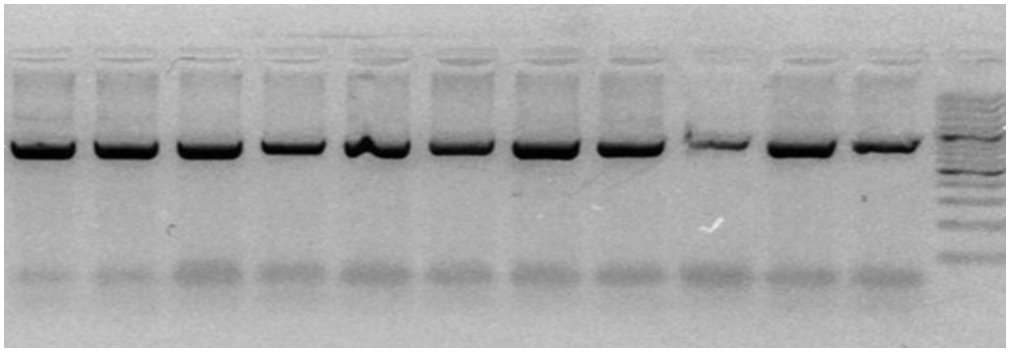


Figure 5: PCR amplified 360 bp fragment of AGTR1 gene. (PCR product was run on 2% agarose gel).

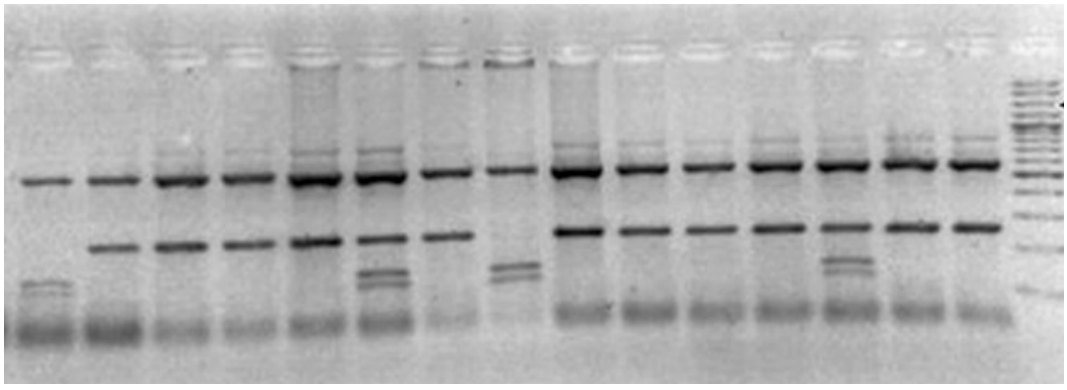


Figure 6: AGTR1 gene polymorphism of A1166C :Dde 1 digestion of PCR product : Right to Left :lane 1(50 bp ladder), lanes 2-8 (AC), lane 9 (unknown), lane 10 (CC), lane 11-12 (AC), lanes 13-15 (CC) ,lane 16 (unknown).

Polymorphism in AT1R gene is known to be associated with multiple cardiovascular ailments (Wenquan Niu and Yue Q, 2010). More importantly, change of an adenine to cytosine (A to C) at 1166 nucleotide is mainly associated with these problems (Ciuffo GM et al.). The present study is designed to detect polymorphism in this gene. A study performed in the Tamil people of South India suggest that AT1R A1166C polymorphism is not significantly related with basic hypertension. (Sudhir chandra et al, 2014). Similarly, another study performed in Nigeria also suggest that AT1R 1166 (A to C) polymorphism is not related with basic hypertension. (P Lava Kumar et al , 2014).

We observed that AC genotype is prominently found over AA and CC genotype. Though we get CC genotype, which is represented by 220 bp and 140 bp band upon PCR and RFLP (Figure 6). Although our study need to be duplicated with higher number of samples, this preliminary observation indicate association of 1166 (A to C) polymorphism with hypertension. Interestingly, two samples in our study did not show the typical pattern of AGTR1 genotypes (Figure 6). These two samples might have unknown type of polymorphisms, however, this need to be confirmed using higher sample size.

Chapter - 7

Conclusion and Future Scope

Conclusion:

Our analysis suggest that C allele of A1166C polymorphism in the angiotensin II type 1 receptor is related with fundamental hypertension.

Future scope:

Two samples in our study did not show the typical A to C polymorphism in our case. This could be a new type of polymorphism, which need to be investigated.

Chapter-8

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