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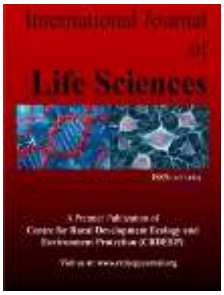

Full Length Research Paper

Genetic Polymorphism of *CYP11B2 G*ene and its Association with Hypertension in Vindhyan Population OF Madhya Pradesh

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ABSTRACT

*In hypertension, the aldosterone synthase gene (CYP11B2) plays a significant role. Aldosterone synthesis is affected by both hereditary and environmental factors. Aldosterone synthase (encoded by the CYP11B2 gene) controls the conversion of deoxycorticosterone to aldosterone and, thus, has genetic influences. Polymorphisms in the CYP11B2 gene are linked to an increased risk of hypertension. Components of the renin-angiotensin system (RAS) and factors that affect regulation of secretion and action of aldosterone are strong contenders. Excess production of aldosterone such as occurs in primary or idiopathic hyperaldosteronism or in the inherited form, glucocorticoid-suppressible hyperaldosteronism (GSH), results in sodium retention, hypokalemia, and suppression of the RAS, a metabolic alkalosis, and hypertension. The Hypertension patients had markedly higher levels of HDL-C(P<o.oooi***), LDL-C (P<o.oooi***), TG(P<o.oooi***), Systolic BP (P<o.oooi***), Diastolic BP (P<o.oooi***) compared to that of control subject. Thus P-Value of HDL-C, LDL-C, TG, Systolic BP and Diastolic BP revealed association significantly with Hypertension. Significant level of change has been seen in overall distribution of CYP11B2 (C344T)genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'CC' genotype as compared to Patients of Hypertension (54.46% vs35.41%). Similarly, mutant type 'TT' genotype was present in low frequency in Hypertension patients group 12.50% and also in control group 09.82% (χ^2 = 7.643, P= o.0219*). 'CT' genotype is higher in patients group then control group (52.08% vs35.71%) allele frequency was also significantly different (χ^2=5.544, P=o.0185*) whereas carriage rate were not significantly associated (χ^2 = 2.513, P= o.1129ns). Our findings suggest the association of CYP11B2 gene polymorphism with hypertension in our population.*

Introduction:

Hypertension, a major risk factor for cardiovascular disease, is a multifactorial and polygenic disorder, predisposed by genetic and environmental factors. The renin-angiotensin-aldosterone system (RAAS) is one of the key modulators of blood pressure in essential hypertension. Aldosterone hormone, secreted by the adrenal cortex of the adrenal gland is chiefly concerned with water-electrolytes balance. Aldosterone is synthesized by the aldosterone synthase enzyme, which is encoded by the *CYP11B2* gene located on chromosome 8q224-6[2].Several polymorphisms have been identified in the *CYP11B2* gene. Among them, the promoter region C-344T polymorphism is the most widely factor-1, the transcriptional regulatory protein8. This polymorphism either increases aldosterone to renin ratio (ARR) in essential hypertensive or decreases aldosterone production, leading to sodium wasting and decreased excretion of potassium. Studies on C-344T polymorphism have shown positive as well as negative association15-18 with hypertension and other cardiovascular parameters. These studies were conducted extensively in Caucasians and Orientals but studies in Indian population are rare. A case-control study on hypertension in highlanders with high salt intake is perhaps the only association study on *CYP11B2* gene C-344T polymorphism in Indian population. Hence, we investigated the association between aldosterone synthase (*CYP11B2* C-344T) gene polymorphism and susceptibility to essential hypertension in south Indian Tamil population [1-3].

Essential hypertension is a complex disorder. Although environmental factors are major determinants of the rise in blood pressure (BP), a significant genetic contribution is now generally accepted. For example, young offspring of hypertensive parents have a

significant predisposition to hypertension. Recent studies in humans have drawn attention to genes that influence renal sodium handling [5]. Components of the renin-angiotensin system (RAS) and factors that affect regulation of secretion and action of aldosterone are strong contenders. Excess production of aldosterone such as occurs in primary or idiopathic hyper aldosteronism or in the inherited form, glucocorticoid-suppressible hyper aldosteronism (GSH), results in sodium retention, hypokalemia, and suppression of the RAS, a metabolic alkalosis, and hypertension. Although this constellation of changes does not occur inessential hypertension, BP is reported to correlate positively with body sodium and negatively with body potassium, a situation not found in normotensive subjects. Moreover, whereas plasma aldosterone concentration is by definition within the normal range, it is on average abnormally high for the concurrent plasma renin level. That is, aldosterone secretion may be slightly more sensitive than normal to its principal agonist, angiotensin II (Ang II); there are experimental data that strengthen this conclusion. Expression of the gene-encoding aldosterone synthase (CYP11B2) is regulated by Ang II and potassium; a chimeric rearrangement of this gene with the adjacent geneencoding11b-hydroxylase (CYP11B1) is known to result in GSH. This locus is, therefore, an important candidate region in other forms of hypertension [4-7]. *We have selected CYP11B2 gene polymorphism to study its distribution pattern and association with hypertension.*

Materials and Methods:

Study population:

The study population consisted of 210 unrelated subjects comprising of 90 Hypertension patients and 120 ethnically matched controls of central Indian population were included in this study. In this region Hindu, Muslim and some Sikh peoples are mainly living but most people's belong to Hindu religion in this region.

Inclusion and Exclusion criteria for Cases

Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology Regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi.

Anthropometric and Biochemical Measurements:

Anthropometry

Height and Weight were measured in light clothes and without shoes in standing position as per standard guidelines. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in standing position midway between iliac crest and lower costal margin and hip circumference was measured at its maximum waist to hip ratio (WHR) was calculated using waist and hip circumferences. Systolic and diastolic blood pressures were measured twice in the right arm in sitting position after resting for at least 5 minute using a standard sphygmomanometer and the average of the two reading was used.

Biochemical Analysis

Biochemical parameters related to type 2 diabetes were estimated for both cases and controls subjects. Measurement of Serum levels of Total cholesterol (TC), Triglycerides (TG), HbA1c, High density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C) and Urea were measured based on spectrophotometric method using automated clinical chemistry analyzerCobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany).

Blood collection and plasma/serum separation

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C

Molecular Laboratory Analysis

Method for DNA isolation:

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*. 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mMTris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was than allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of deionized autoclaved water and mixed thoroughly on vortexer. The mixture was centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µl.of proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl. of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl.of saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) was added to remove most of the non nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated

by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transferring of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mMTris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization.

Determination of quality and quantity of isolated DNA

The isolated DNA is to be used for PCR based study. Therefore its suitability for PCR along with its size heterogeneity is among the most important criterion for purity. As a matter of general practice all DNA preparations were tested for quality and quantity measures, as described in the following paragraphs.

Quantitation by UV spectrophotometry

The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. The absorbance at 260 nm was used to calculate the amount of DNA, using the relationship that double stranded DNA at 50µg/ml concentration has an absorbance= 1.0 at 260 nm.

Agarose Gel Electrophoresis

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangalore, India) was used. In brief, 4-5 µl of each genomic DNA was loaded on 0.8 agarose (0.8 % w/v, Sigma) containing ethidium bromide solutions (0.5 µg/ml) and electrophoresis was done at 80 V in 1x TAE buffer (40 mMTris, 20 mM acetic acid, 1 mM EDTA). Lambda DNA *Eco*RI / *Hind* III double digest (Bangalore Genei, Bangalore, India) was used as molecular weight marker after completion of electrophoresis, the DNA bands were visualized and photographed using an UV trans illuminator (312 nm) and gel documentation system (VilberLourmate, Cedex 1, France) respectively.

Polymorphism screening

In general, the genomic DNA extracted from peripheral blood of healthy individuals and diseased individuals was subjected to PCR followed by restriction digestion and electrophoresis to genotype both the groups for relevant gene of interest. All the PCRs were carried out in a PTC 200 thermal cycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Due to the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the trace amounts of DNA, which could serve as an unwanted template. Appropriate negative control was included in each PCR run carried out for each gene, to monitor this contamination of PCR mix to avoid any false positive results. The negative control used for PCR contained whole PCR reaction mix except target DNA which was replaced by HPLC purified water free of RNase, DNase, and any contamination from any other source resembling the gene sequence. Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. A typical digestion includes one unit of enzyme per microgram of starting DNA. One enzyme unit is usually defined as the amount of enzyme needed to completely digest one microgram of double stranded DNA in one hour at the appropriate temperature. Their biochemical activity of the restriction enzyme is the hydrolysis of phosphodiester backbone at specific sites in a DNA sequence. Precaution was taken to avoid star activity of restriction enzymes. When DNA is digested with certain restriction enzymes under non-standard conditions, cleavage can occur at sites different from the normal recognition sequence. Such aberrant cutting is called “star activity” which can be due to high pH (>8.0) or low ionic strength, extremely high concentration of enzyme (>100 U/µgof DNA) and presence of organic solvents in the reaction (e.g. ethanol, DMSO). The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their size. Gels were stained with ethidium bromide solution (0.5µg/ml) and subsequently visualized and photographed under UV trans illuminator.

Detection of CYP11B2(C-344T) Single Nucleotide Polymorphism

Polymorphism in CYP11B2 is located in the 59 flanking region of the gene, 344 nucleotides upstream from the start of translation within a binding site for the transcription factor steroidogenic factor-1 (SF-1); this position may be either a C or T nucleotide (-344C and -344T alleles).

Primer sequence: The oligonucleotides sequences (primers) used [8].

Forward primer: 5'- CAGGAGGAGACCCCATGTGAC -3'; Reverse Primer: 5' - CCTCCA CCCTGTTCAGCCC--3'

PCR Mix

The PCR was carried out in a final volume of 25 µl, containing 50-100 ng of genomic DNA(4-5 µl), 2.5 µl of 10X *Taq* polymerase buffer (10 mMTrisHCl pH 8.8, 50 mMKCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration

1X; Genetix Biotech Asia Pvt.Ltd., India), 1 µl of 10 mMdNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for and 1 µl of unit of 1U/ µl Red Taq DNA polymerase (Bangalore genei).

PCR Thermal Program

After an initial denaturation of 5 min at 95˚C, the samples were subjected to 35 cycles at 95˚C for 1 min, at 58˚C for 45 s, and 72˚C for 45 s, with a final extension of 10 min at 72˚C in a thermal cycler. A 100bp ladder with amplified product has been run under 2.5 % agarose gel electrophoresis.

Restriction digestion

PCR amplification of *CYP 11B2*Gene with specific primers gave 538-bp undigested product which was digested with *HaeIII* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (CC) was digested as 203, 138, 126, and 71 bp whereas the mutated homozygous genotype (TT) was cut as 274, 138 and 126 bp. The heterozygous genotype (TC) was represented as fragments of 274, 203, 138, 126, and 71 bp of DNA fragments in the gel. The digestion products were then separated by electrophoresis on a 2.5% agarose gel. The results were documented by digital camera and further saved by gel documentation system.

Results

Anthropometric results:

The descriptive data and comparison of anthropometric and biochemical parameters of Hypertension patients versus controls are presented in Table no. 4.1. The age, sex, BMI, WHR were the parameters. As expected the Hypertension patients had markedly higher levels of high age group (P<0.0001***), weight of women (P<0.0001***) then men (P=0.5045ns) and BMI of Women (P<0.0001***) then Men (P=0.1913ns). The parameter Waist circumference (cm) and Hip (cm) determine the WHR. Our statistical data from Waist circumference (cm) and Hip (cm) was not significant different between Hypertension patients group and healthy control (HC) group. WHR was not significantly different between patient and healthy population. Thus WHR in Women (P=0.2904ns) and Men (P=0.1912ns) were not found were not found association with Hypertension.

Table 1: Comparison studies of anthropometric parameters between Hypertension patientsand healthy controls.

Characteristics	Cases (96)	Controls(112)	P-value
n(Men/Women)	96(54/42)	112(62/50)	
Age(years)	59.6±12.4	52.5±12.5	P<0.0001***
Height(m)	162.50±11.3	161.2±12.4	0.4333,ns
Weight (Kg)			
Women	69.5 ±4.7	60.6 ± 4.5	P<0.0001***
Men	68.4±5.6	67.8±7.1	0.5045,ns
BMI (kg/m^2)			
Women	25.6±3.1	22.1 ± 4.3	P<0.0001***
Men	24.6±4.7	23.8± 4.1	0.1913,ns
Waist circumference (cm)			
Women	92.5±6.2	93.6±6.7	0.2233,ns
Men	90.0±7.0	89.0±6.0	0.2685,ns
Hip (cm)			
Women	95.9±2.4	96.1±2.2	0.5315,ns
Men	90.8±4.3	91.2±1.5	0.3579,ns
WHR			
Women	0.97±0.05	0.98±0.08	0.2904,ns
Men	0.98±0.08	0.99±0.01	0.1912,ns

*(*denotes level of significant change between case and control)*

Biochemical and clinical findings

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p value suggests the level of significant were changed here. The descriptive data and comparison of biochemical parameters of Hypertension patients versus healthy controls are presented in Table no. 2. As expected the Hypertension patients had markedly higher levels of HDL-C(P<0.0001***), LDL-C (P<0.0001***), TG(P<0.0001***), Systolic BP (P<0.0001***), Diastolic BP (P<0.0001***) compared to that of control subject. Thus P-Value of HDL-C, LDL-C, TG, Systolic BP and Diastolic BP revealed association significantly with Hypertension. Whereas rest of all parameters such as Post-Prandial Glucose (mg/Dl), HbA1C(%),and Blood Urea(mg/dL) were not significantly different between patient and healthy population **.**

Table 2. Comparison of Biochemical and clinical findings of Hypertension patients and Healthy controls.

Characteristics	Cases (96)	Controls(112)	P-value
Post-Prandial Glucose (mg/Dl)	117.7±12.4	118.4±11.6	0.6747,ns
HbA1C(%)	5.8±0.7	5.7±0.8	0.3424,ns
HDL-C(mmol/L)	108.8±12.2	109.3±11.6	0.7625,ns
LDL-C (mg/dL)	62.1±2.6	41.8±3.7	P<0.0001***
TG(mg/dL)	145.9±13.2	126.2±12.2	P<0.0001***
Systolic BP (mmHg)	165.4±8.1	124.8±5.7	P<0.0001***
Diastolic BP (mmHg)	97.1±5.8	82.5±6.2	P<0.0001***
Blood Urea(mg/dL)	18.1±1.7	17.8±1.8	0.2204,ns

(denotes the level of significant change between case and control)*

Detection of Genetic Polymorphism in CYP11B2 Gene:

PCR amplification of *CYP 11B2*Gene with specific primers gave 538-bp undigested product which was digested with *HaeIII* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (CC) was digested as 203, 138, 126, and 71 bp whereas the mutated homozygous genotype (TT) was cut as 274, 138 and 126 bp. The heterozygous genotype (TC) was represented as fragments of 274, 203, 138, 126, and 71 bp of DNA fragments in the gel.

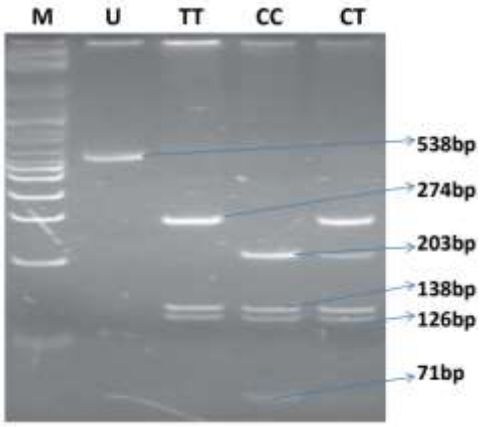


Fig 1: Representative gel picture of *CYP 11B2* (C344T) polymorphism. Lane M represents 50 bp molecular marker, Lane U represented as undigested PCR product, Lane CC Wild type genotype, Lane CT heterozygous genotype and Lane TT variant genotype.

The distribution of the polymorphisms of *CYP 11B2* (C344T)was consistent with Hardy- Weinberg equilibrium (HWE) in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for *CYP11B2* (C344T)polymorphism are depicted in table 4.4 and table 4.5 and Graph 1,.2, 3. Significant level of change has been seen in overall distribution of *CYP11B2* (C344T)genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'CC' genotype as compared to Patients of Hypertension (54.46% *vs*35.41%). Similarly, mutant type 'TT' genotype was present in low frequency in Hypertension patients group 12.50% and also in control group 09.82% (χ^2 = 7.643, P= 0.0219*). 'CT'genotype is higher in patients group then control group (52.08% *vs*35.71%) allele frequency was also significantly different (χ^2=5.544, P=0.0185*) whereas carriage rate were not significantly associated (χ^2 = 2.513, P= 0.1129ns).

An odds ratio of CC genotype was calculated as 0.4585 which indicates little protective effect of CC genotype from disease and indicate significantly differences between patient and healthy group (P=0.0079**). An odds ratio of TC and TT genotype was not indicating as protective effect. Significant difference in Allele frequency between Hypertension patients group and healthy control group indicate association with Hypertension (P=0.0211*) but not show protective effect whereas carriage rate was not significantly different. Overall allele 'C' was found little lower frequency in disease group as compared to HC group (61.45% Vs 72.32%) whereas allele '*T'* was present in little high frequency in the disease group was significantly different. Carriage rate of allele 'T' was slightly high in Hypertension group as compared to healthy control (42.46% Vs 33.55%) whereas carriage rate of allele '*C*' was nominal different in both control and disease group but no significant level of change has been seen. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests *CYP11B2* (C344T) polymorphism is significantly associated with Hypertension in our population.

Table 3. Frequency distribution and association of Genotype, allele frequency and carriage rate of *CYP11B2*gene polymorphism in population of Vindhyan region using Chi Square Test

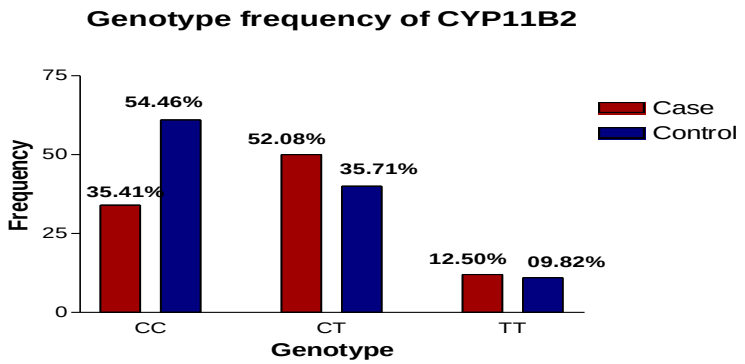
***CYP 11B2* gene**	**Case N= 96**		**Control N=112**		**Chi square value X^2 (p value)**
	N	**%**	**N**	**%**	
Genotype					
CC	34	35.41	61	54.46	
CT	50	52.08	40	35.71	
TT	12	12.50	11	09.82	7.643(0.0219*)
Allele					
C	118	61.45	162	72.32	
T	74	38.54	62	27.67	5.544 (0.0185*)
Carriage Rate					
C	84	57.53	101	66.44	
T	62	42.46	51	33.55	2.513 (0.1129ns)

(- denotes the level of significant association between case and control.)*
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)

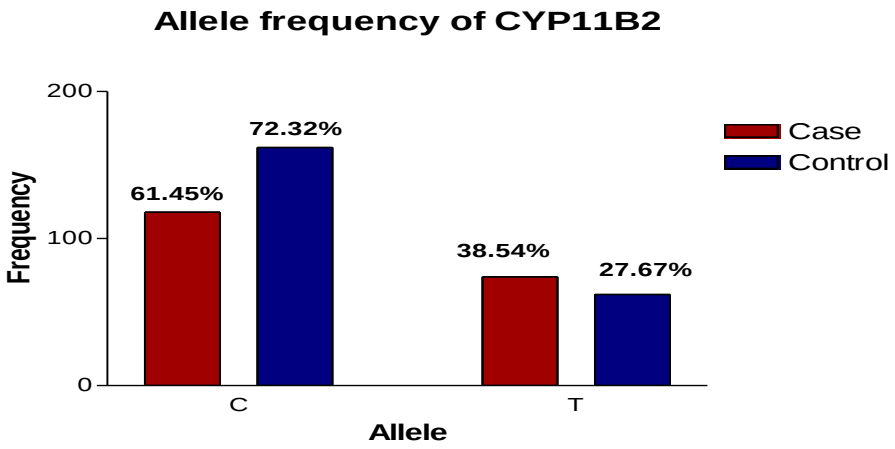
Table 4. Fisher Exact Test values of *CYP 11B2* gene polymorphism

***CYP 11B2* gene**	**Case N= 96**		**Control N=112**		**P value**	**Odds ratio (95% confidence interval)**
	N	**%**	**N**	**%**		
Genotype						
CC	34	35.41	61	54.46	0.0079**	0.4585(0.2619to 0.8025)
CT	50	52.08	40	35.71	0.0245*	1.957 (1.121 to 3.414)
TT	12	12.50	11	09.82	0.6584ns	1.312 (0.5506 to 3.125)
Allele						
C	118	61.45	162	72.32		0.6103 (0.4040 to 0.9220)
T	74	38.54	62	27.67	0.0211*	1.639 (1.085 to 2.475)
Carriage Rate						
C	84	57.53	101	66.44		0.6841 (0.4275 to 1.095)
T	62	42.46	51	33.55	0.1218ns	1.462 (0.9133 to 2.339)

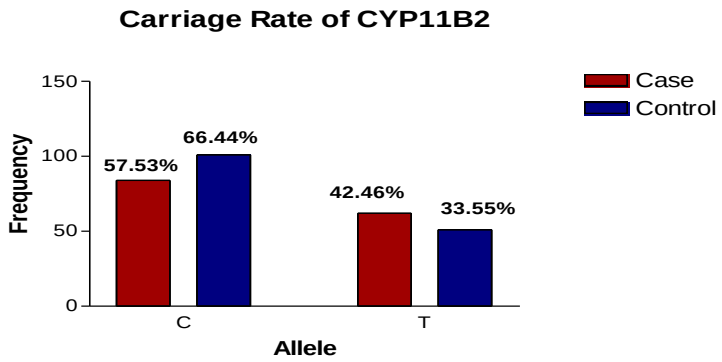
(- denotes the level of significant association between case and control.)*
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)



Graph 1: Genotype Frequency of *CYP 11B2* gene.



Graph 2: Allele Frequency of *CYP 11B2* gene.



Graph 3: Carriage rate of *CYP 11B2* gene

Discussion

The heritability of hypertension (HTN) is widely recognized and as a result, extensive studies ranging from genetic linkage analyses to genome-wide association studies are actively ongoing to elucidate the etiology of both monogenic and polygenic forms of HTN. Due to the complex nature of essential HTN, however, single genes affecting blood pressure (BP) variability remain difficult to isolate and identify and have rendered the development of single gene targeted therapies challenging [10]. The roles of other causative factors in modulating BP, such as gene–environment interactions and epigenetic factors are increasingly being brought to the forefront. In this review, we discuss the various monogenic HTN syndromes and corresponding pathophysiologic mechanisms, the different methodologies employed in genetic studies of essential HTN, the mechanisms for epigenetic modulation of essential HTN, pharmacogenomics and HTN, and finally, recent advances in genetic studies of essential HTN in the pediatric population [9-11].

The prevalence of hypertension is increasing in India as well as in the world. The average prevalence of hypertension in India is 25-30%. The median prevalence of total hypertension in 2009 was 37.6% in men and 40.1% in women in U.S. Hypertension is a major risk factor for majority of patients with cardiovascular, cerebrovascular and renal morbidity and mortality. Environmental factors as well as genetic factors account for regulation of blood pressure and its control. Understanding of genetic factor may not only help in recognizing those at risk but also help in treatment [13]. Discovering hypertension susceptibility genes would help recognizing those at risk for developing the disease before the expression of clinical symptoms. Genetic and epidemiological studies have suggested that essential hypertension is a polygenic and multifactorial disorder that results from genetic and/or environmental factors. In India awareness, treatment and control status of hypertension is low, with only half of the urban and a quarter of the rural hypertensive individuals being aware of its presence. In this review we have discussed epidemiology and genetics of hypertension, both the monogenic and polygenic forms[14].The silent killer or essential hypertension, is an important risk factor for cardiovascular disease, it affects 20% to 30% of the population worldwide and will alarmingly rise to 1.5 billion. Its heritability is around 31 to 68%, besides affecting environmental factors. Comparing to the last years, there have been a substantial progress in the understanding of the Blood pressure and HTN etiology. We provide an overview of the current findings of the GWAS aiming to contribute in the understanding of the pathophysiology of Blood pressure and HTN. From the fact that only a fraction of the phenotypic variability of BP can thus far be explained by the recently discovered common genetic polymorphisms from GWAS, therefore, we tried to highlight the major role of rare and structural variants, epigenetic in the missing heritability of HTN[12-15].

Association between objectively assessed obesitymarkers, salt intake and hypertension were investigated. Estimates of 24 h sodium and potassium excretion from a single morning spot urine specimen were calculated and used as surrogate for salt intake. The association between overweight/obesity and hypertension in different age-groups was assessed in multilevel logistic regression

models. Further associations between salt intake and hypertension were analyzed. Measures of systolic and diastolic blood pressure as well as proportion of overweight/obesity and hypertension both increased with age. Overweight and obesity were significantly associated with hypertension in adults. Moreover, thinness seems to be associated with hypertension as well. We observed a significantly reduced chance of hypertension for higher urinary sodium-to-potassium compared to a lower ratio in children. Overweight/obesity and hypertension were highly prevalent (>47% of adults >40 years are overweight or obese and >69% are hypertensive in the same age group) in our sample. Weight status was confirmed as a correlate of high blood pressure in our sample from Zanzibar, Tanzania. To early and effectively prevent related severe cardiovascular outcomes, screening strategies but also monitoring strategies are required for this population [16-19].

Our descriptive data and comparison of anthropometric and biochemical parameters of Hypertension patients versus controls are presented in Table no. 4.1. The age, sex, BMI, WHR were the parameters. As expected the Hypertension patients had markedly higher levels of high age group (P<0.0001***), weight of women (P<0.0001***) then men (P=0.5045ns) and BMI of Women (P<0.0001***) then Men (P=0.1913ns). The parameter Waist circumference (cm) and Hip (cm) determine the WHR. Our statistical data from Waist circumference (cm) and Hip (cm) was not significant different betweenHypertension patients group and healthy control (HC) group. WHR was not significantly different between patient and healthy population. Thus WHR in Women (P=0.2904ns) and Men (P=0.1912ns) were not found were not found association with Hypertension. Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p value suggests the level of significant were changed here. The descriptive data and comparison of biochemical parameters of Hypertension patients versus healthy controls are presented in Table no. 4.2. As expected the Hypertension patients had markedly higher levels of HDL-C(P<0.0001***), LDL-C (P<0.0001***), TG(P<0.0001***), Systolic BP (P<0.0001***), Diastolic BP (P<0.0001***) compared to that of control subject. Thus P-Value of HDL-C, LDL-C, TG, Systolic BP and Diastolic BP revealed association significantly with Hypertension. Whereas rest of all parameters such as Post-Prandial Glucose (mg/Dl), HbA1C(%),and Blood Urea(mg/dL) were not significantly different between patient and healthy population.

The adjusted dietary assessment questionnaire was used to determine dietary habits of medical students which were related to biochemical and anthropometric markers of studied cohort. Thirty-seven young and healthy volunteers aged 19–28 years old entered the protocol and were divided according to sex and according to residence. Subjects were given questionnaires for tracking food/beverage consumption. Venous blood samples were taken after overnight fast (n = 32). Nutrient status and energy consumption were determined and analyzed [22]. Study population had normal weight and body mass index (BMI). Biochemical characteristics were within normal reference range, while some participants had lipid profile disbalance. Men had significantly higher BMI than women. Average BMI was significantly higher in participants with elevated cholesterol levels compared to participants with normal cholesterol levels. Majority of participants consumed less than five meals per day with no major differences between students according to residence and sex. Men had significantly higher protein intake and consumed at least four meals daily compared to woman who had three or less meals daily with no differences in intake according to residence. Students with normal lipid profile consumed more carbohydrates than students with increased cholesterol. Results suggest that students with bad dietary habits have potentially higher risk for future cardiovascular problems, even before the onset of adverse effects[20-23].

The 2344C allele of a 2-allele (C or T) polymorphism in the promoter of the gene encoding aldosterone synthase (CYP11B2) is associated with increased left ventricular size and mass and with decreased baroreflex sensitivity, known risk factors for morbidity and mortality associated with myocardial infarction (MI). We hypothesized that this polymorphism was a risk factor for MI. a nested case-control design to investigate the relationships between this polymorphism and the risk of nonfatal MI in 141 cases and 270 matched controls from the Helsinki Heart Study, a coronary primary prevention trial in dyslipidemic, middle-aged men. There was a non-significant trend of increasing risk of MI with number of copies of the 2344C allele[24].However, this allele was associated in a gene dosage– dependent manner with markedly increased MI risk conferred by classic risk factors. Whereas smoking conferred a relative risk of MI of 2.50 (*P*50.0001) compared with nonsmokers in the entire study population, the relative risk increased to 4.67 in 2344CC homozygous smokers (relative to nonsmokers with the same genotype, *P*50.003) and decreased to 1.09 in 2344TT homozygotes relative to nonsmokers with this genotype. Similar joint effects were noted with genotype and decreased HDL cholesterol level as combined risk factors. Smoking and dyslipidemia are more potent risk factors for nonfatal MI in males who have the 2344C allele of CYP11B2[24-26]. Aldosterone synthase gene (CYP11B2) -344C/T polymorphism has been reported to be associated with serum aldosterone level, urinary aldosterone excretion, blood pressure, and left ventricular size and mass. The aim of this study was to evaluate the relation between CYP11B2 polymorphism and end-stage renal disease (ESRD) in the Korean population and the association with CYP11B2 polymorphism and cardiovascular morbidity in ESRD patients on hemodialysis. Genotyping was performed in 134 control subjects and 271 ESRD patients for CYP11B2 polymorphism using polymerase chain reaction through subsequent cleavage with restriction enzyme. Also current blood pressure, demographic, anthropometric and biochemical variables were investigated. The genotype distribution did not differ between ESRD patients and controls and there were no significant differences in blood pressure, use of antihypertensive medication, left ventricular hypertrophy and cardiovascular disease among the three genotypes in ESRD patients on hemodialysis [27].

Our data from *CYP 11B2*Gene polymorphism revealed association with hypertension. The PCR amplification of *CYP 11B2*Gene with specific primers gave 538-bp undigested product which was digested with *HaeIII* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (CC) was digested as 203, 138, 126, and 71 bp whereas the mutated homozygous genotype (TT)

was cut as 274, 138 and 126 bp. The heterozygous genotype (TC) was represented as fragments of 274, 203, 138, 126, and 71 bp of DNA fragments in the gel. The distribution of the polymorphisms of *CYP 11B2* (C344T)was consistent with Hardy- Weinberg equilibrium (HWE) in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for *CYP11B2* (C344T) polymorphism are depicted in table 4.4 and table 4.5 and Graph 4.1, 4.2, 4.3. Significant level of change has been seen in overall distribution of *CYP11B2* (C344T) genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'CC' genotype as compared to Patients of Hypertension (54.46% *vs*35.41%). Similarly, mutant type 'TT' genotype was present in low frequency in Hypertension patients group 12.50% and also in control group 09.82% (χ^2 = 7.643, P= 0.0219*). 'CT'genotype is higher in patients group then control group (52.08% *vs*35.71%) allele frequency was also significantly different (χ^2=5.544, P=0.0185*) whereas carriage rate were not significantly associated (χ^2 = 2.513, P= 0.1129ns).

Our data from odds ratio of CC genotype was calculated as 0.4585 which indicates little protective effect of CC genotype from disease and indicate significantly differences between patient and healthy group (P=0.0079**). An odds ratio of TC and TT genotype was not indicating as protective effect. Significant difference in Allele frequency between Hypertension patients group and healthy control group indicate association with Hypertension (P=0.0211*) but not show protective effect whereas carriage rate was not significantly different. Overall allele 'C' was found little lower frequency in disease group as compared to HC group (61.45% Vs 72.32%) whereas allele '*T'* was present in little high frequency in the disease group was significantly different. Carriage rate of allele 'T' was slightly high in Hypertension group as compared to healthy control (42.46% Vs 33.55%) whereas carriage rate of allele '*C*' was nominal different in both control and disease group but no significant level of change has been seen. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests *CYP11B2* (C344T) polymorphism is significantly associated with Hypertension in our population.

Conclusion

The Renin-angiotensin aldosterone system (RAAS) plays an important role in the regulation of blood pressure. Aldosterone, synthesized by aldosterone synthase in the adrenal cortex is encoded by the *CYP11B2* gene. In this case-control study we examined the association between *CYP11B2* C-344T polymorphism. The study was conducted in 406 hypertensive cases and 424 healthy controls from Tamil population. Genotyping was performed by PCR-restriction fragment length polymorphism method. Statistical analysis was performed by logistic regression analysis. The 344TT homozygous variant genotype (OR-1.8; 95% CI: 1.1-2.8; *P*=0.02) and T allele (*P*=0.007) were found to be significantly associated with hypertension. In gender based analysis, the risk was significantly higher in male hypertensive (OR-1.8; 95% CI: 1.0-3.6, *P*=0.05) but not in female subjects. A significant association between *CYP11B2* gene polymorphism and essential hypertension was observed and the risk was confined to male subjects in south Indian Tamil population [28-30].

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