Research Article [Tiwari *et al.*, 5(10): Oct., 2014:3927-3931]
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Association of Aldosteron Synthase CYP11B2 C-344T) Gene Polymorphism Susceptibility to Essential Hypertension in a Central India Population

Sweta Tiwari[1*], Rashmi Arnold[2], Jai Prakash Pandey[1] and Samta Shukla[3]
1, Department of Biotechnology, A. P. S. University Rewa, (MP) - India
2, Department of Botany, Govt. Science College, Rewa, (MP) - India
3, Department of Microbiology, A. P. S. University Rewa, (MP) - India

Abstract

The aldosteron synthase gene (CYP11B2) is an important candidate gene region in essential hypertension it is the key enzyme in the biosynthesis of aldosteron. We therefore studied the association of -344 T/C polymorphism of the CYP11B2 gene with the presence and severity of hypertension in Case-Control study. We studied 440 individuals, of whome 210 were hypertensive patients and 230 controlled hypertensive individuals.
In this study $\chi 2$ and p value of overall genotype frequency was CC, CT and TT =3.560, (0.1687), Df-2 and the allele frequency was C and T=0.02810, (0.8669), Df-1 and Carriage Rate was C and T=0.001915, (0.9651), Df-1 for the total central Indian population in this study there was no significant variance in blood pressure among any of the three genotype.

Key-Words: Hypertension, RAAS, CYP11B2

Introduction

Essential hypertension is a multifactional disorder that is influenced by genetic and environmental factors[1]. The rennin-angotensin-aldosteron system (RAAS) is one of the key modulators of blood pressure in essencial hypertension [2]. The rennin-angiotensin-aldosteron system and other factors that influence the renal sodium handling, through the regulation of the secretion and action of aldosteron, are strong contributors to the development of hypertension [3]. Aldosteron hormone, screted by the adrenal cortex of the adrenal gland, is chiefly concerned with water-electrolytes balance. Aldosteron is synthesized by the aldosteron synthase enzyme, which is encoded by the CYP11B2 gene located on chromosome 8q22.[4-6]
Several polymorphisms have been identified in the CYP11B2 gene. Among them, the promoter region C-344T polymorphisms (rs id 1799998) is the most wiedly studied as it persuades the binding of steroidogenic factor-1.

*** Corresponding Author**
E-mail: swetawari49@gmail.com
Mob.: +91-8871376054

The transcriptional regulatory protein , Genes of the rennin-angiotensin-aldosteron-system (RAAS), including angiotensinogen (AGT), angiotensin 1-converting enzyme (ACE), type I angiotensin (ang)II receptor (ATIR) and CYP11B2, are natural candidate for sodium homiostasis and blood pressure regulation. Polymorphisms of these genes have been major targets for molecular analyses in association with hypertension. Polymorphisms such as AGT M235T, ACE I/D, and AT1R A1166C have been investigated in association studies with hypertension [7-10].
The present study was designed to investigate the association of -344T/C polymorphism of the CYP11B2 gene with genetic predisposition to essencial hypertension. Hence, we investigated the association between aldosteron synthase (CYP11B2 C-344T) gene polymorphism and susceptibility to essencial hypertension in central Indian population.

Material and Methods

Study population:- The study population was conducted from Jan, 2014 to June, 2014 in 210 unrelated essential hypertensive patients (136 males and 74 females) aged 35 to 65 who were resident of central area of India. Best-trained observers measured BP using standard mercury sphygmomanometers on the right arm of quietly seated participants after at least a 5-min. rest in the survey [11]. Three BP measurements were obtained from each participant. The diagnosis of hypertension was made according to a classification

based on blood pressure levels > 140/90 mmhg. Patients with history of diabetes mellitus, hyperlipidaemia, liver or renal disease, congestive cardiac failure and renal episode of myocardial infection were excluded. Patients with pregnancy and lactation and receiving medications for indications that could affect blood pressure were also excluded. The control group consist of 230(124 males and 106 females) aged between 30-65yr unrelated healthy volunteers. These subjects had no personal or family history of hypertension and other cardiovascular diseases in first-degree relatives and had SBP <130 mmhg and DBP<85 mmhg. Healthy volunteers from health camp and patients who visited the outpatient clinics with minor illness without hypertension, diabetes mellitus, hyperlipaemia and family history of hypertension in previous records were recruited as controls. Plasma lipid profile and blood glucose level were measured after overnight fasting in both hypertensives and normotensives to rule out diabetes and hyperdaemia. The questionnaire was prepared by the Shyamshah medical college, rewa (M.P.). In all subjects height was measured to the nearest centimeter and weight to the nearest 0.1 kg which were for calculation of BMI (kg/m^2). Blood pressure was measured after the subject had been resting for at least 5min. In the right arm using standard sphygmomanometer and the average reading was recorded.

Genotyping: - 5 ml. of blood was collected in 0.5M ethylene diamine tetra acetic acid (EDTA) tubes from each hypertensive patients as well as from healthy controls. These samples were stored frozen at -80·c until DNA was extracted from them. Genomic DNA was extracted from whole blood by the modification of Salting out procedure described by Miller and Coworkers [12]. The CYP11B2 C-344T polymorphism was sought using a PCR-RFLP method. 25 µl of PCR reaction mixture contained 2 µl template DNA (Final Concentration 75-100ng/microlitre), 1.5mMof $MgCl_2$ and 1u of taq DNA polymerase, 200 µM $dNTP_S$, 0.75 µl of 10 pm/microl of forword and reverse primers (Sence-5'-CAGGAGGAGACCCCATGTGAC-3';
Antisence-5' CCTCCA CCCTGTTCAGCCC-3'), specific for CYP11B2 C-344T and sterile water to set up the volume of restriction mixture to 25 µl. Thermal profile used for the amplification ofdesired segment of gene was as follows: Initial denaturation at 94^0C for 5 min. and 35 cycles of denaturation at 94^0C for 1 min. annealing at 65^0C for 1 min. and extension at 72^0C for 1 min., followed by final extension at 72^0C for 5 min. The amplification was checked in horizontal gel electrophoresis unit using 1 percent agarose gel followed by restriction digestion of the 538 bp pcr product with Hae III endonuclease for 4h at 37^0C. The digested product was analyzed in vertical electrophoresis unit using 8 percent polyacrylamide gel electrophoresis (PAGE) with resulted in 203, 138, 126, 71 bp for wild type, 274,203,138,126 and 71 bp for heterozygous variant and 274,138 and 126 bp for homozygous variant.

Statstical Analysis: - Statstical analysis was done by using Student t test and P values obtained suggest the level of significant change here. The descriptive data and comparision of anthropometric and biochemical parameters of hypertensive patients versus controls are presented by The age, sex BMI, WHR, Systolic Blood pressure (mmhg), Disystolic blood pressure (mmhg), HbA1, HDL-C, LDL-C, TG(mg/Dl), Blood urea, Creatinine are also presented by t-test. The genotype frequency of each gene in each study group were tested to be in accordance with Hardy-Weinberg equilibrium using $\chi 2$ test for independence and Odds ratio & Cl presented by Fisher exact test. Statistical results show the no significant level of change has been seen in overall distribution of CYP11B2 C-344T genotype in HC group as Compared to Hypertensive group.

Results and Discussion

We determined the Age, Sex, BMI, WHR were the parameter as expected the hypertensive patients and markedly higher levels of weight of women ($P<0.0001^{***}$), Men (0.0012^{**}) and BMI of women (0.0003^{**}). Other results were not found significantly different between case and control group. As expected the hypertensive patients has markedly higher levels of systolic blood pressure (0.0354*) and HbA1c (P<0.0001***) Nominal difference was also observed for LDL-C (0.0018**), triglyceride (0.0015**). Creatine value, blood urea level (0.0023**), HDL-C level and diastolic pressure was not significantly different between two groups. The distribution of the polymorphisms of CYP11B2 was consistent with Hardy-Weinberg equilibrium in healthy controls. The observed genotype frequencies, allele frequencies and Carriage rates for CYP11B2 C-344T polymorphism are depicted in table-1.

No significant level of change has been seen in overall distribution of CYP11B2 C-344T genotype in HC group as compared to disease group although HC group showed little increase in 'CC' genotype as compared to patients of Hypertension (17% vs 12.2%). Similarly, mutant type 'TT' genotype was present in low frequency in Hypertensive patients group 38.3 % and also in control group 41.73% but difference was nominal and statistically nonsignificant 3.560,(0.1687). An odds ratio of CC genotype is 0.6920 which

indicates little protective effect whereas an odds ratio of CT genotype is of Hypertensive group respectively indicate little or no effect and association of this mutant genotype with the hypertension susceptibility. Overall allele 'C' was found little lower frequency in disease group as compared to HC group whereas allele '*T'* was present in slightly high frequency in the disease group but the difference was nominal and not significant 0.02810 (0.8669). Carriage rate of allele 'T' was slightly high in hypertensive group as compared to healthy control (87.6% Vs 83.04%) whereas carriage rate of allele '*C'* was approximately similar in both control and disease group and no significant level of change has been seen depicted in table-2 and table -3. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests CYP11B2 is not significantly associated with hypertension in our population.

Hypertension is the most common risk factor for the development of cardiovascular diseases (i. e. stroke and myocardial infarction) which are among the major causes of global morbidity and mortality. Despite this, it is inadequately controlled worldwide. Blood pressure (BP) is a highly quantitative trait and therefore it has been difficult and somewhat arbitrary to define specific levels at which high blood pressure becomes too high, i.e. hypertension.

The rennin-angiotensin system (RAS) or the renin-angitensin-aldosteron system (RAAS) is a hormone system that reglates blood pressure and water fluid balance.Genes of the rennin-agiotensin-aldosterone system (RAAS), including angiotensinogen (AGT), angiotensin I-convertingenzyme (ACE), type I angiotensin (Ang) II receptor (AT1R) and CYP11B2, are natural candidates for sodium homeostasis and blood pressure regulation. Polymorphisms of these genes have been major targets for molecular analyses in association with hypertension.

The position of -344 of CYP11B2 gene is located in the promoter region. The potential influence of the C-344T variant on the promoter activity of CYP11B2 was analysed in a few studies. In our study, No significant level of change has been seen in overall distribution of CYP11B2 C-344T genotypes in HC group as compared to disease group although HC group showed little increase in' CC' genotype as compared to Patients of Hypertension. Similarly, mutant type 'TT' genotype was present in low frequency in Hypertensive patients group and also in control group but difference was nominal and statistically nonsignificant. An odds ratio of CC genotype is which indicates little protective effect whereas an odds ratio of CT genotype is of Hypertensive group respectively indicate little or no effect and association of this mutant genotype with the hypertension susceptibility. Overall allele 'C' was found little lower frequency in disease group as compared to HC group whereas allele '*T'* was present in slightly high frequency in the disease group but the difference was nominal and not significant. Our study is consistent with a case-control study conducted among Indians living in highland, with smaller sample size, did not find significant association between C-344T polymorphism and hypertension. The study showed a significant association with BMI but the subjects had mean BMI below 25 kg/m2 which is not considered as a risk factor for hypertension[13]. A larger community-based study in Japanese population revealed that C-344T polymorphism was not associated with blood pressure levels in either sex. Some studies suggest stronger association of C-344T polymorphism in some populations such as South Indian Tamil population[14].

Conclusion

In conclusion, the present study shows no association between C-344T polymorphism and essential hypertension in a Central Indian population. Since hypertension is a polygenic disorder influenced by multiple genes, further association studies and screening of other candidate gene polymorphisms is required to elucidate the precise genetic susceptibility of essential hypertension.

References

1. Lifton, R. P., Gharavi, A. G. and Geller, D. S. 2001. Molecular mechanisms of human hypertension. *Cell*. 104(4):545–56.
2. Poch, E., Gonzalez, D., Giner, V., Bragulat, E., Coca, A. and de La Sierra, A. 2001. Molecular basis of salt sensitivity in human hypertension. Evaluation of rennin-angiotensin-aldosteron system gene polymorphisms. *Hypertension*. 38: 1204-9.
3. Strazzullo, P., Galletti, F. and Barba, G. 2003. Altered renal handling of sodium in human hypertension: short review of the evidence. *Hypertension*. 41:1000–1005.
4. Freet, E. M. and Connel, J. M. 2004. Mechanisms of hypertension: the expainding role of aldosteron. *J Am Soc Nephrol*. 15: 1993-2001.
5. Hilgers, K. F. and Schmidt, B. M. 2005. Gene varients of aldosteron synthase and hypertension. *J Hypertens*. 23: 1957-1959.
6. Kupari, M., Hautanen, A., Lankinen, L., Koskinen, P., Virolainen, J. and Nikkila H. 1998. Associations between human aldosteron synthase (*CYP11B2)* Gene polymorphisms

and left ventricular size mass and function. *Cirulation.* 97: 569-75.
7. White, P. C. and Rainey, W. E. 2005. Polymorphisms in *CYP11B2* genes and 11-hydroxylase activity. *J Clin Endocrinol Metab*. 90: 1252-5.
8. Basset, M. H., Zhang, Y., Clyne, C., White, P. C. and Rainey, W. E. 2002. Differential regulation of aldosteron synthase and 11 betahydroxylase transcription by steroidogenic factor-1. *J Molendocrinol*. 28: 125-35.
9. Sagnella, G. A., Rothwell, M. J. and Onipinla, A. K.1999. A population study of ethnic variations in the angiotensin-converting enzyme I/D polymorphism: relationships with gender, hypertension and impaired glucose metabolism. *J Hypertens*. 17(5):657– 64.
10. Liu, Y., Zhuoma, C. and Shan, G. 2002. A1166C polymorphism of the angiotensin II type 1 receptor gene and essential hypertension in Han Tibetan and Yi populations. *Hypertens Res*. 25(4):515– 21.
11. Gao, L., Yan, W. and Yang, Z. L. 2004. A survey on prevalence of hypertension in Hani Tribe in Yunnann Province. *Chin J Hypertens*. 12(4): 362– 4.
12. Komiya, I., Yamada, T., Takara, M., Asawa, T., Shimabukuro, M. and Nishimori, T. 2000. Lys(173)Arg and -344T/C varients of *CYP11B2* in Japanese patients with low-renin hypertension. *Hypertension.* 35: 699-703.
13. Siani, A., Russo, P. and Paolo Cappuccio, F. 2004. Combination of renin-angiotensin system polymorphisms is associated with altered renal sodium handling and hypertension. *Hypertension*. 43(3): 598– 602.
14. White, P. C. and Slutsker, L. 1995. Haplotype analysis of CYP11B2. *Endocr Res*. 21(1-2):437–42.

Table 1: Anthropometric Parameters and Biochemical Parameters of the Hypertensive Patients and Controls

Characteristics	Cases	Controls	P-value
n(Men/Women)	210(136/74)	230(124/116)	
Age(years)	55.5±12.5	56.0±14.2	0.6964
Height(m)	165.50±13.40	167.2± 12.00	0.1611
Weight (Kg)			
Women	64.5 ±5.70	62 ± 4.50	P<0.0001***
Men	70±5.60	68.0±7.1	0.0012**
BMI (kg/m^2)			
Women	27.4±3.1	26.1 ± 4.3	0.0003**
Men	25.6±4.7	25.1± 5.1	0.2869
Systolic BP (mmHg)	131.20±8.1	129.8±5.7	0.0354*
Diastolic BP (mmHg)	88.1±5.8	87.5±6.0	0.2877
HbA1C (%)	7.2±0.8	5.6±0.6	P<0.0001***
HDL-C(mmol/L)	114.2±14.8	112.8±11.6	0.2679
LDL-C (mg/dL)	41.1±4.3	42.3±3.7	0.0018**
TG(mg/dL)	132.1±13.2	127.9±14.2	0.0015**
Blood Urea(mg/dL)	9.5±1.6	9.0±1.8	0.0023**
Creatinine(mg/dL)	2.0±0.14	1.08±0.10	P<0.0001***

Table 2: Frequency distribution and association of Genotype, allele frequency and carriage rate of CYP11B2 C-344T polymorphism

CYP11B2 Genotype	Case N= 210		Control N=230		Chi Square Value χ^2 (P Value)
	N	%	N	%	
CC	26	12.2	39	17	3.560,(0.1687) Df 2
CT	104	49.5	95	41.3	
TT	80	38.3	96	41.73	
Allele C	156	37.14	173	37.6	0.02810,(0.8669) Df-1
T	264	62.85	286	62.17	
Carriage Rate C	130	61.9	134	58.3	0.001915,(0.9651) Df-1
T	184	87.6	191	83.04	

Table 3: Fisher Exact Test values of CYP11B2 polymorphism

CYP11B2 Genotype	Case N= 190		Control N=210		P Value	Odds Ratio (CI)
	n	%	n	%		
CC	26	12.2	39	17	0.1820	0.6920 (0.4049 to 1.183)
CT	104	49.5	95	41.3	0.0855	1.394(0.9563 to 2.033)
TT	80	38.3	96	41.7	0.4954	0.8590 (0.5859 to 1.259)
Allele C	156	37.14	173	37.6	0.7807	0.9535(0.7257 to 1.253)
T	264	62.85	286	62.8	0.7807	1.049 (0.7981 to 1.378)
Carriage Rate C	130	61.9	134	58.3	1.0000	1.007(0.7349 to 1.380)
T	184	87.6	191	83.04	1.0000	0.9930(0.7246 to 1.361)

How to cite this article

Tiwari T., Arnold R., Pandey J.P. and Shukla S. (2014). Association of Aldosteron Synthase CYP11B2 C-344T) Gene Polymorphism Susceptibility to Essential Hypertension in a Central India Population. *Int. J. Pharm. Life Sci.*, 5(10):3927-3931.

Source of Support: Nil; Conflict of Interest: None declared

Received: 27.09.14; Revised: 01.10.14; Accepted:10.10.14