Study of Alpha Adducin Gene Polymorphism in Young Essential Hypertensive North Indians

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ABSTRACT

Introduction: Hypertension is a major risk factor for cardiovascular disorders such as stroke, heart failure, vascular disease, and end-stage renal disease, and is one of the leading causes of morbidity and mortality worldwide. The polymorphism of α adducin (ADD1) gene and their expression in the related tissue can reveal the mystery of essential hypertension and this can help in diagnosis, prognosis and management of essential hypertension. This gene analysis would provide a better approach for identifying the genotype-phenotype correlations. **Objective:** This study was designed to examine the relationship between α-adducin gene polymorphism (Gly460Trp) and essential hypertension in North Indian population. **Methods:** The subjects were recruited randomly including 101 patients of essential hypertension and 151 healthy controls. Genotyping was performed using polymerase chain reaction (PCR)-restriction fragment length polymerase (RFLP). **Results:** We found that frequencies of the alpha adducin Trp alleles in hypertensive and control groups were 0.21 and 0.11, respectively. There was approximately double increase in the 460-Trp allele frequency in the North Indian hypertensives compared with the normotensives. **Conclusion:** The rs 4961 polymorphism of the ADD1 gene is associated with essential hypertension. We found a significant association between the Gly460Trp gene polymorphism of the alpha adducin and hypertension in a North Indian population. Furthermore, the study indicated that alpha adducin might be a susceptible gene to essential hypertension in North Indian population.

Key words: Cardiovascular, Coronary heart disease, α adducing, Essential hypertension, Stroke.

INTRODUCTION

Hypertension is the most prevalent cardiovascular risk factor in the industrialized world. Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients. It is a complex trait resulting from the interaction of multiple genetic and environmental determinants. Essential hypertension is directly responsible for 57% of all stroke deaths and 24% of coronary heart disease death in India.[1] Moreover not only genetic but also epigenetic inheritance plays a significant role in essential hypertension. One can speculate that hypertension develops as a consequence of "errors" in well coordinated regulatory systems of blood pressure. Errors in the cascade of molecular, biochemical and genetic processes, which regulate blood pressure, have finally enough potential to result in hypertension. These errors could not be huge, but their determination is sometimes difficult.

Identification of genetic markers for the predisposition to hypertension may permit focused intervention on such hypertension ogenic factors. Numerous genetic markers have been identified in the regulation of blood pressure and essential hypertension. One such marker that has drawn

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substantial attention is α-adducin (ADD1) gene. Adducin is a heterodimeric cytoskeleton protein and consists of an α-subunit (Mr 103 kDa) and either a β-(Mr 97 kDa) or γ-subunit (Mr 90 kDa). Three genes (ADD1, ADD2, and ADD3, or Add1, Add2, and Add3, human and rat genes, respectively) that map to different chromosomes encode these subunits. Adducin promotes the organization of the spectrin–actin lattice by favoring the spectrin–actin binding and controlling the rate of actin polymerization as an end-capping actin protein. Its function is calcium- and calmodulin dependent. It is phosphorylated by protein kinases A and C, tyrosine, and ϱ-kinases. It is a member of the myristoylated alanine-rich C kinase substrate protein family, which is involved in signal transduction, cell-to-cell contact formation, and cell migration.[2] Numerous environmental factors surrounding the organism during its developmental period should influence the expression of genetic information. However despite the considerable research efforts, it is still difficult to identify all genes and/or other genetic determinants leading to essential hypertension and other cardiovascular diseases.[3] The purpose of our study is to bring a piece of light on gene environmental interactions and characterization of aberrations in gene function leading to the disease. As only a small proportion of the sequence variations in our genomes will probably have functional impacts, identifying their subset of the sequence variants will be one of the major challenges of the next decade.

There is no doubt that not only genetic but also environmental factors are very important for the regulation and maintenance of blood pressure. There is growing evidence that complex interactions among multiple genes and multiple environmental factors play an important role in determining an individual's risk of various common diseases including hypertension.

Probably more perspective and effective should be the recommendation for the modification of life style. Maintenance of appropriate body weight, higher physical exercise, restriction of stress, augmented food and vegetables consumption etc should be a standard recommendation to each physician to their patients. Not only patients but the whole population must be motivated for life style changes because most of "civilization" diseases including hypertension have their roots in the earlier stages of ontogeny. Finally one can say that if "Sensitive" genome enters "toxic" environment during particular critical developmental period, there is very high probability that these will develop.

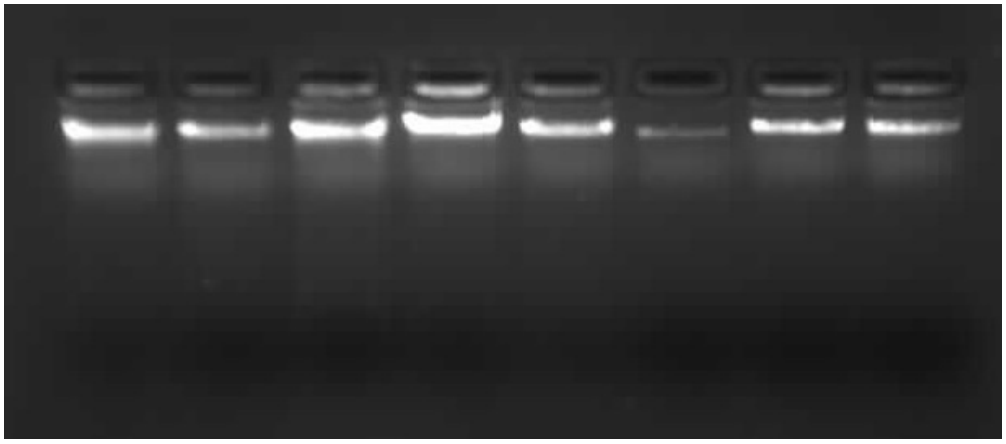

Figure 1: 1.0% Agarose gel showing the bands of genomic DNA

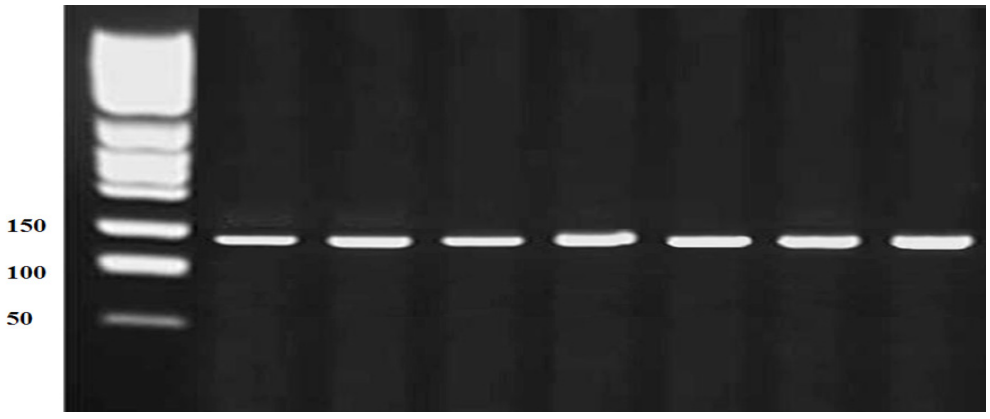

Figure: 2% Agrose gel showing the amplification of PCR product

AIMS AND OBJECTIVE

The aim of this study is to determine the genetic polymorphisms of ADD1 gene in North Indian Essential hypertensive subjects.

METHODOLOGY

Sample Collection

2 ml of blood sample was taken for each participant. Blood was collected in EDTA vials stored at -20°C for polymorphism study (for DNA isolation and PCR analysis).

Study Design

It is a case control study. The cases were hypertensive individuals and controls were normotensive individuals.

A total of 101 untreated hypertensive and 151 normal controls. North Indian patients residing in the same geographic region with a similar socioeconomic level were included in the study. No any patients have been receiving any antihypertensive drugs before these blood testing. Informed consent for participation was obtained from each subject. The participants completed a standard questionnaire on demographic characteristics, smoking, consumption, previous history of myocardial infarction (MI) and stroke. The body mass index (BMI) was calculated from height and weight measurements. The BMI was defined as high when greater than 25 kg/m^2. On three consecutive visits, arterial blood pressure was measured from the left arm following a five minutes rest in a supine position at the first and fifth korotkoff phases by an arm cuff of appropriate size. Hypertension was defined according to JNC VII Criteria.

Inclusion criteria

Subjects were recruited in two groups. Both male and female individuals belonging to age of 20 to 55 years were included. A total of 252 hypertensive and normotensive Indian patients residing in the same geographical region with a similar age sex and socioeconomic level were included in the study. Control Subjects (n=151) were normotensive (< 120/80 mm Hg), and none of them have been receiving any antihypertensive therapy, treatment for heart disease, or hormone replacement therapy.

Exclusion criteria

The exclusion criteria was uncontrolled hypertension, blood pressure more than 140/90 mmHg or malignant hypertension, patients on oral contraceptives, the presence of seceondary hypertension, severe vulvular heart disease, autoimmune disease, inflammatory arthritis, chronic or acute infectious disease endocrinal disorder and other chronic debilitating disease and cancer.

Genotyping of alpha adducing gene polymorphism

A two ml of blood sample was taken into an EDTA-anticoagulated tube by standard venipuncture method (used by technician) for each participant. Genomic DNA was extracted from EDTA anticoagulated blood samples by the standard phenol chloroform methods. DNA Concentration was determined by the standard phenol chloroform methods. DNA concentration was determined by the spectrophotometer by measuring OD (Figure 1).

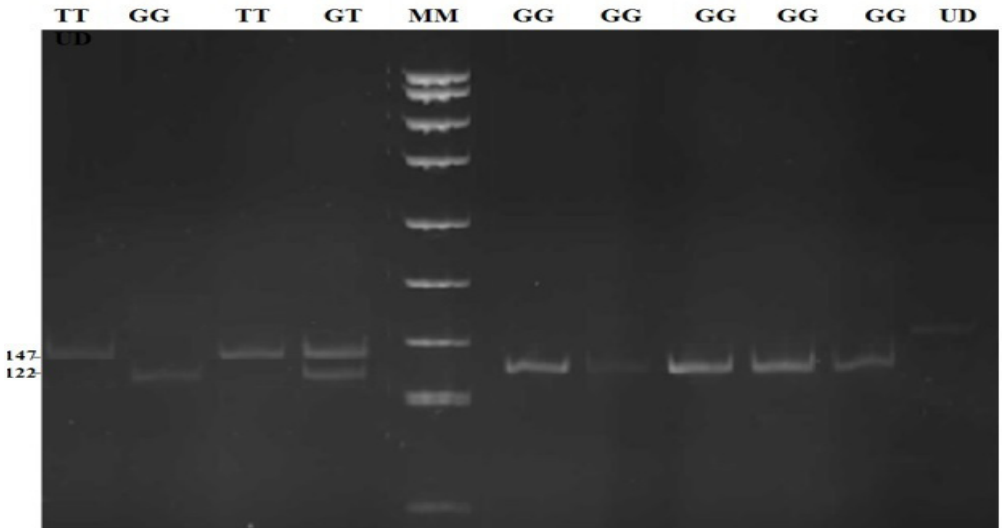

Figure 3: Polyacrylamide Gel (15%) showing genotypes of -460G/T polymorphism in adducin gene using Sau 961 Digest, UD: Undigested

Alpha Adducin -460G/T						
Genotype frequencies						
	Controls		**Patients**		***P*-value**	**X^2**
	Count (n=151)	**Frequency (%)**	**Count (n=101)**	**Frequency (%)**		
GG	120	78.75	69	61.96	**0.007**	**9.798**
GT	28	19.98	21	33.51		
TT	3	1.27	11	4.53		
All	**151**	**100.00**	**101**	**100.00**		
Allele Frequencies						
	Controls		**Patients**		***P*-value**	**Odd's Ratio (95%CI)**
	Count (n=304)	**Frequency (%)**	**Count (n=202)**	**Frequency (%)**		
G	268	(88.74)	159	(78.71)	**<0.003**	**0.469 (0.287-0.766)**
T	34	(11.26)	43	(21.29)		

Figure 4: Distribution of genotypes and alleles of adducin (G460T) gene polymorphism in the study subjects

Primer Sequence

Gene	*Position*	*Primers*	*Melt. Tm*	*RE*
ADD1	-460G/T	F-5'-CTCCTTTGCTAGTGACGGTGATTC-3' R-5'-TTGGGACTGCTTCCATTCGGCC-3'	61.0°C	*Sau 961*

The primers were selected according to the published article of J. Kalita *et.al.* (2011).[22] Polymerase chain reaction (PCR) and Restriction fragment length polymorphism (RFLP) methods were created to genotype the study subjects for the Polymorphism. The method was used to determine the genotype. G to T substitution, which polymorphism resulting in the gene variant of amino acid residue 460 (Gly460Trp) is located at nucleotide position 614 of exon 10 of the alpha adducin gene. Briefly, two allele specific primers and their non-selective complementary strand primer were mixed and used for the PCR amplication in a single reaction. Deliberate differences were introduced into the allele specific primers in addition to the base substitution, and they were able to drastically reduce cross reactions between two allelic PCRs in a mixed reaction.

Genotyping of ADD1 in a reaction mixture of 25 µl containing 20 pmol primers and 1U of Taq DNA polymerase enzyme using 200 µM dNTPs. The cycling conditions comprised a hot start at 95°C for 30 Sec, 61°C for 30 Sec and 72°C for 30 Sec, followed by one elongation step at 72°C for 5 min (Figure 2).

Rectriction digestion for ADD1 polymorphism was carried out in 5 µl volume with restriction enzyme Sau 961. 5 µl of product was loaded per well onto a 15% polyacrylamide gel (Figure 3). Restriction digestion products was run on polyacrylamide gel for 7 hour at constant 200 V on ethidium bromide staining and exposure to UV transilluminator (or placed in Gel Doc) for detecting band pattern. The PCR product was 147 and 122 bp for the 460Gly and 460Trp alleles, respectively (Figure 4).

Statistical analysis

Variables are presented as percentage or means ± SD. The graphpad prism 5 software was used in this study. The expected frequencies of the ADD1 G460T genotypes were tested for the Hardy-Weinberg Equilibrium. Statistical differences for the distribution of the genotypes G460T between EH and Controls groups were assessed by X2 test. The relationship of ADD1 genotypes with the clinicopathalogic parameters of patient were tested by t-test. Statistical significance was set at the $p<0.05$ level.

RESULTS

A total of 252 individuals were genotyped. In the hypertensive group, hypertension duration was 5 + 3 years. The clinical characteristics of the hypertensive patients and controls are represented in Table 1. Body Mass Index (BMI) SBP and DBP differed significantly between the hypertensive and normotensive groups. In our study group normotensive were significantly younger than the hypertensives, and females are more affective to hypertension as comparison to male subjects.

The genotype and allele distributions of the Gly-460-Trp polymorphism are reported in Table 2. Genotype frequencies in both the normotensives and the hypertensives were not significantly different from the values of the Hardy-Weinberg expectation. In the hypertensive group, 69 (61.96%) were GG homozygotes (GG), 21 (33.51%) were GT heterozygotes (GT), and 11 (4.33%) were TT homozygotes (TT). In the control group, 120 (78.75%) were GG, 28 (19.98%) were GT, and 3(1.27%) were TT. The frequency of the T allele was 0.11 in normotensives

Table 1: Statistical analysis of age (Years), diastolic blood pressure (mmHg) and biochemical parameter in control (N=151) and essential hypertensive (N=101) groups

	CONTROLS (n=151) Mean ± SD	PATIENTS (n=101) Mean ± SD	P Values
AGE (year)	32.63 + 7.75	34.08 + 8.46	0.1621
BMI (kg/m²)	23.56 + 6.69	25.14 + 7.37	0.2750
SBP (mmHg)	114.90 + 7.30	149.40 + 13.92	0.0001
DBP (mm/Hg)	73.8 + 5.01	86.14 + 6.29	0.0119
CHOLESTEROL (mg/dl)	176.28 + 20.22	205.95 + 48.20	0.0001

Data are expressed as percentage or mean ± SD. Significance of differences between the hypertensives and controls was examined using Mann Whitney U test.

and 0.21 in hypertensives (p=0.007). There was a double increase in the T allele frequency in the according to sex, age, body mass index by logistic regression, the ADD-1 Gly460Trp gene polymorphism was still associated with essential hypertension significantly.

DISCUSSION

Essential hypertension is multi-factorial disease associated with the interaction of genetic and environmental factors and genes contribute 20-40% of the pathogenesis of Essential hypertension.[2] Polymorphisms in several genes are associated with blood pressure levels.[4] One of these genes is alpha adducin polymorphism. Results of examining the association between rat alpha adducin gene polymorphism and blood pressure regulation in Studies of the Milan hypertensive supported the observation that the adducin gene variant affects renal function by modulating the overall capacity of tubular epithelial cells to transport ions modifying the assembly of the actin cytoskeleton. In the first study performed in humans (in the Italians and the French) by Cusi *et al*,[5] they revealed that the Trp allele of alpha adducin 460 polymorphism is not only associated with an increased risk of hypertension but it also associated with increased sensitivity to salt and treatment with hydrochlorothiazide in the white population. Additional analysis was performed in Caucasians,[6] Japanese[7] and South African[8] populations, and positive results were also found in these studies. Francesco *et al*.[9] report for the first time that the 460 Trp allele of the

Table 2: Allele and genotype frequencies of the α -adducin gene (ADD1) in the hypertensive and control groups

	PATIENTS (n=101)	CONTROLS (n=151)	
ADD-1 genotypes	-	-	X^2=9.798, df=2 p=0.007
GG	69 (61.96%)	120 (78.75%)	-
GT	21 (33.51%)	28 (19.98%)	-
TT	11 (4.53%)	3 (1.27%)	-

The frequency of alleles and genotypes in the Hypertensive and Normotensive group were compared using X2 test. Statistical significance was set at the p < 0.05 level.

Gly 460 Trp polymorphism of ADD-1 is associated with blunted endothelium dependent vasodilatation. Deleterious alterations of endothelial physiology, also referred to as endothelial dysfunction, not only represent a key early step in the development of atherosclerosis, but also appeareared to be involved in plaque progression and the occurrence of atherosclerotic complications. Although the products of many genes are involved in renal sodium handling, a mutation in only one or a few of these genes may be sufficient to alter salt sensitivity in any one individual.[10] A Single gene polymorphism underlies a very heterogenous syndrome such as primary hypertension, affecting up to 40% of the adult population of industrialized countries.[11] J. Kalita,[22] Li-na Zhang[13] Yan-yan Li,[14] Zhang LN[15] and Lifang Wang *et al*[16] also supports our study. In a Scottish population, a study involving parents and offspring with blood pressures in either the upper or bottom 30% of the population distribution revealed that the alpha adducin Trp allele was not related to blood pressure and did not affect whole-body or cellular sodium metabolism.[17] In the present study, we investigated the association between polymorphism of the alpha adducin gene and hypertension in North Indian population. In the case–control sample of 252 subjects, the 460Trp allele of alpha adducin gene was found to have significantly been associated with essential hypertension. We found a significant interaction between the Gly460Trp polymorphism and hypertension. Our results disagree with what was previously reported by Kato *et al*.[18] who reported lack of association between the alpha adducin locus and essential hypertension in 507 Japanese subjects. Kamitani *et al*[17] also reported no association between blood pressure levels and the presence of the 460Trp allele of the alpha adducing gene. There are a number of possible explanations for this inconsistency. The major consideration may be the ethnic differences.

Another possibility is population differences in environmental factors such as diet, behavior, and lifestyle. Since the alpha adducin gene is thought to be related to salt-sensitive hypertension, sodium intake is of particular concern. The exact mechanism by which alpha adducing increases blood pressure

is not known. ADD-1 is thought to regulate ion transport via changes in the actin cytoskeleton.[19] Adducin is thought to stimulate Na^+-K^+-ATPase, promoting sodium re-absorption by renal tubular cells.[20,21] Trp/Trp individuals with this alteration in renal sodium handling will have increased sensitivity of blood pressure to sodium intake and are at increased risk for developing low renin hypertension.[7] Total 265 samples were taken to our study but results of only 252 samples are presented in this thesis. Rest of the samples shows different problems (haemolization, degradation and smearing etc). G allele frequency in North Indian patients was approximately similar (78.71%) to Caucasians (77.4%),[12] Turkish population of Emin[21] and North Indian Stroke patients of J. Kalita (76.7%),[22] but higher than Han Chinese patients in two different studies of 2014 and 2007 (54.22, 64.8%).[23,24]

Tryptophan allele frequency (21.29%) is intermediate between Scandinavian[25] 17% and Caucasians[2] 23.2% but it was lower from the Koreans[26] 65.15%, Caucasian[18] 53%, Chinese in different studies[27,28] 48%, 58% and in Han Chinese[24] 35%.

CONCLUSION

In conclusion, we found a significant association between the Gly460Trp gene polymorphism of α-adducin and hypertension in a North Indian population. Genetic tests are employed to confirm diagnosis of a genetic condition and to help in the management of the disease. Uncertainty often leads to stress and the genetic test results can provide a much needed sense of relief. Genetic testing and counseling help people to make decisions about managing their health care. Testing negative for an abnormality helps to avoid unnecessary checkups. Testing positive for a mutation enables a person to look for preventive or treatment options.

By this we will be able to establish the relationship between phenotype and genotype in the north Indian population and develop certain prognostic markers for clinical purpose. The present study will provide a lead to the contribution of alpha adducin and angiotensinogen gene heterogeneity to the susceptibility and development of Essential hypertension.The purpose of our study is to bring a piece of light on gene environmental interactions potentially implicated in the pathogenesis of essential hypertension.

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ABBREVIATION

RFLP : Restriction fragment length polymorphism
PCR : Polymerase chain reaction
MI : Myocardial Infarction
BMI : Body Mass Index
ADD1 : Alpha adducin gene 1

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