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ASSOCIATION OF CYP3A5 (6986G>A) GENE POLYMORPHSIM WITH ESSENTIAL HYPERTENSION AND BLOOD PRESSURE.

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ABSTRACT

Hypertension is the premier cause of the disease burden in developed as well as developing countries of the world. Hypertension affects approximately 37-55% population of the developed countries and the genetic susceptibility to it could be an outcome of the inherited differences in the capacity of sodium retention.CYP3A5 enzyme has been implicated in the regulation of blood pressure (BP) and thus, may serve as a potential risk factor for the development of hypertension. Carriers of the *CYP3A5*1* allele had high, whereas homozygous carriers of the *CYP3A5*3* allele exhibit low CYP3A5 expression in the kidney, where it represents the major isoform of the CYP3A family.

The aim of the present hospital based case control study was to investigate the association of the *CYP3A5*1* rs776746 (6986 *A*→) allele with BP in hypertension cases and healthy controls in a sample of Kashmiri population, a north India state. The study included 150 hypertension patients (78 males and 72 females; age (mean ± S.D) 60.55 ± 11.19 years) and normotensive controls (79males and 71 females; age (mean ± S.D) 60.11±9.92 years). The distribution of CYP3A5 genotype in hypertension cases and controls was assessed by PCR-RFLP method. Logistic regression was used to assess the relationship between CYP3A5 genotype and the risk of essential hypertension. Although CYP3A5*1 genotype was higher in cases (10%) than in controls (3.66%), however, it did not reach a statistically significance It was found that CYP3A5*1 carriers showed decrease in systolic blood pressure (SBP) by

6.02 mmHg and an increase in diastolic blood pressure (DBP) by 2.50 mmHg, but, the results were not statistically significant. So, there is no association of CYP3A5*1 with the risk of essential hypertension.

KEYWORDS: CYP3A5, SBP, DBP, gene polymorphism.

INTRODUCTION

Hypertension is the biggest health challenge in the modern world as it is most common, easily detectable and oftenly asymptomatic and lethal.[1] In developed countries, hypertension has been reported to be the 4th contributor of premature deaths whereas 7th in the developing countries.[2] According to worldwide data on Global burden of hypertension, there is 26.4% over all prevalence among the adult population whereas in India prevalence ranges between 20%-40% in urban and 12%-17% among rural adults.[3, 4]

Cytochrome P450 enzymes are organ specific with CYP3A5 highly expressed in kidneys.[5] As a glucocorticoid 6β-hydroxylase, the CYP3A5 enzyme converts cortisol or corticosterone to 6β-corticosterone, which may lead to increased sodium and water retention.[6, 7] The CYP3A5 gene has a common polymorphism (6986A→ G, rs776746) in the intron third. The G allele generates an aberrant RNA splicing site and results in a premature stop codon, which causes truncation of the CYP3A5 protein.[8, 9] In genetic epidemiology studies, CYP3A5*1 refers to the A allele (functional allele) and CYP3A5*3 refers to the G allele (nonfunctional allele). Recently, several studies have suggested a possible relationship between CYP3A5 gene polymorphisms and essential hypertension. Human CYP3A superfamily of the phase I enzymes is chiefly responsible for the elimination of various endogenous as well as exogenous compounds.[10] CYP3A subfamily of CytchromeP_{450} consists of CYP3A4, CYP3A5, CYP3A7, and CYP3A43, are chiefly expressed in human liver and intestines. Among these CYP3A4 and CYP3A5 are the two functional forms of CYP3A in human liver and intestine.[11,12,13] Large inter-individual differences in CYP3Aexpression levels in the small intestine and in human hepatocytes could greatly contribute to inter-individual variations in drug dosage. Several studies have intimated that 30–85% of the inter-individual have in constancy in CYP3A prevalence due to genetic factors, such as single nucleotide polymorphisms (SNPs).[14, 15]

The diversification of social and cultural behavior in India predicts the region wise difference in prevalence of hypertension. However, till date, no study has been carried out to evaluate

role of this polymorphism in relation to prevalence of essential hypertension in Kashmiri population, a north India state. This inadequacy forced us to conduct this study with the aim to investigate whether an association exists between selected polymorphism in CYP3A5 and essential hypertension. The significance of the proposed studies lies in their potential to legitimize or disproves a causative role for CYP3A activity in vertebrate renal physiology and to unmask possible molecular mechanisms underlying their effects.

MATERIALS AND METHODS

Subjects

This hospital based cross sectional study was conducted over a period of seventeen months starting from May, 2012 to September, 2013, following approval by the ethical committee of Government Medical College (GMC), Srinagar, India. Informed consent was obtained from all the subjects. Demographic characteristics of essential hypertension cases and controls are mentioned in Table 1.

Table 1. Demographic characteristics of essential hypertension cases and controls

Demographic Features	**HTN Cases (n=150)**	**Controls (n=150)**
Age (Years mean ± S.D)	60.55±11.19	60.11±9.92
Gender		
Male	78 (52%)	79 (52.6%)
Female	72 (48%)	71 (47.3%)
Systolic pressure (mmHg)	139.7 ± 17.47	120.3 ± 7.98
	(90.00 - 200.0)	(100.0 - 130.0)
Diastolic pressure (mmHg)	89.51 ± 10.71	80.93 ± 6.27
	(60.00-120.0)	(60.00-100.0)
Smoking	45(45%)	22(14.66%)
Rural	54(36%)	82(54.66%)
Urban	96(64%)	68(45.33%)

Genomic DNA isolation

Five ml of venous blood was collected from each subject in a sterile EDTA coated vials and were subsequently stored at -80^{o}C for future use. Genomic DNA was isolated from the blood samples following Phenol-Chloroform method.[16] Integrity of the isolated genomic DNA was checked by running 5µl of the sample on 1% agarose gel.

*Genotyping of CYP 3A5 *3*

CYP3A5*3 polymorphism in intron 3rd was analyzed through PCR-RFLP (Restriction Fragment Length Polymorphism) method. PCR reactions contained 100 ng of DNA, a 0.25 mM dNTPs (Sigma-Aldrich), 1× PCR buffer, 2.5 units of Taq polymerase (Fermentas), and

15 pmol of forward 5/-CATGACTTAGTAGACAGATGAC-3/ and reverse 5/-GGTCCAAACAGGGAAGAAATA-3/ primers in a total volume of 25 µl. PCR cycling conditions included a 5-min initial denaturation step at 95°C followed by 35 cycles of the following: 50 s at 95°C, 50 s at an appropriate annealing temperature, 50 s at 72°C, and a final extension step at 72°C for 5 min. Quality of amplicons was assessed by running it in 2% agarose gel. The PCR products so obtained were subjected to restriction digestion using SspI enzyme (Fermentas). The digested products of each subject were genotyped by running on 3% agarose gel (Fig1).CYP3A5*3 wild type allele possess the restriction site and thus produces three fragments of size 148, 125, and 20-bp whereas mutant allele lacks restriction site and on digestion produces two fragments of size 168 and 125-bp.(fig 1)

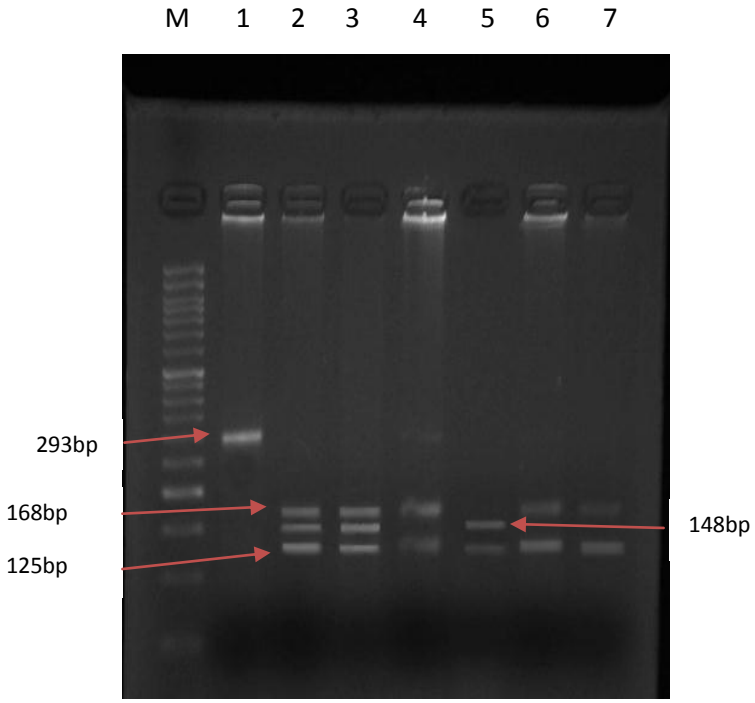

Figure1 PCR-restriction fragment length polymorphism analysis (PCR-RFLP) of CYP3A5*3 polymorphism.

M- 50 bp DNA ladder.

Lane 1- Untreated PCR amplicon of 293 bp

Lane 5 -Homozygous wild genotype

Lane2, 3 - Heterozygous genotype

Lane 4, 6 and 7 - Homozygous mutant genotype

Table 2. Genotypic frequency of CYP3A5 in essential hypertension patients and controls

Polymorphism	Cases	Controls	OR (95% CI)	*P* value -
	n (%)	*n* (%)		
	150	150		
A/A (1*/1*)	9 (6.00)	3 (2.00)	1	
A/G(1*/3)	12 (8.00)	5 (3.33)	1.25(0.23-6.57)	0.8
G/G(3*/3)	129 (86.00	142 (94.66)	3.30(0.87-12.47)	0.063

RESULTS

In the present study 150 confirmed essential hypertension cases belonging to the Kashmir division were analyzed for polymorphism in intron 3rd of the CYP3A5 gene. In these HTN cases the genotypic frequency for homozygous wild type (A/A) was found to be 6% (9/150). The genotypic frequencies observed for heterozygous genotype (A/G) and homozygous variant (G/G) were 8% (12/150) and 86 % (129/150) respectively as shown in the Table (2). The frequency of wild allele in HTN patients was 0.1 (30/300) and mutant allele frequency was 0.9 (270/300). Therefore the percentage calculated for wild and mutant allele of CYP3A5 in HTN patients was 10 and 90 respectively (Table 3).

Table 3. The distribution of CYP3A5 allele in essential hypertension patients and controls

Polymorphism	Alleles	Cases	Control
		n^s (%)	n^s (%)
		300	300
CYP3A5*3	A (*1)	30(10.00)	11(3.66)
6986A G	G (*3)	270(90.00)	289(96.33)

n^s = Number of Alleles

Statistically significant P<0.05.

An equal number of normotensive controls were screened for the intron 3rd polymorphism of the CYP3A5 gene. The genotypic frequency observed in controls for homozygous wild (A/A) was 2% (3/150). The frequencies analyzed for heterozygous genotype (A/G) and homozygous mutant (G/G) were 3.33% (5/150) and 94.66 (142/150) respectively, (Table 2). The distribution of the three genotypes for the CYP3A5*3 polymorphism confirmed well to the predictions of Hardy–Weinberg law. The frequency of wild allele in 150 healthy individuals taken as controls was 0.036 (11/300) and mutant allele frequency was 0.96 (289/300). Therefore the percentage for wild and mutant allele of CYP3A5 in controls was 3.66 and 96.33 respectively (Table 3).We observed that the frequency of CYP3A5*1 wild

allele was higher in cases as compared to controls but the difference was not statistically significant.

Association of CYP3A5*1 with Blood Pressure and hypertension

Overall we observed that the presence of the CYP3A5*1 allele exhibited no effect on systolic blood pressure (SBP) and diastolic blood pressure (DPB). The observed adjusted difference between CYP3A5*1/*1+CYP3A5*3/*1 individuals compared with CYP3A5*3/*3 individuals for SBP and DBP was not statistically significant (Table 4).

Table 4. Association of genotype with systolic and diastolic blood pressure

CYP3A5 genotype	*3/*3	*3/*1	*1/*1	P value
(n)	132	10	8	
SBP (mmHg)	140.0 ± 17.60	142.0 ± 21.50	131.3 ± 3.536	0.07
DBP (mmHg)	89.21 ± 10.89	91.00 ± 11.97	92.50 ± 4.629	0.5469

n= no. of individuals

Value represents ± SD

Students't test (Unadjusted) comparingCYP3A5*3/*3 individuals with CYP3A5*3/*1 +CYP3A5*1/*1 individuals

Statistically significant P<0.05.

Statistical analysis

All the statistical analyses were performed with SPSS version 15. 0. (Statistical Package for the Social Science). Chi square test was put in use for comparison of the allele and genotypic frequency variations between different populations. The divergences from the Hardy–Weinberg equilibrium for allele and genotype frequencies for the various SNPs were assessed by Fisher's Exact Test. The 95% confidence intervals were enumerated for all observed allele frequencies. The $P < 0.05$ was considered statistically significant.

DISCUSSION

CYPs are a superfamily of monooxygenases that are responsible for the oxidative metabolism of more than half the drugs currently available. CYP3A5 is abundantly expressed in the liver and small intestine, but only in the 30% of whites and 70% of blacks who possess ≥1 CYP3A5*1 allele. Research into the role of the CYP3A5 polymorphism in BP regulation was

stimulated by an initial study in a small group of African–Americans in which higher BPs were reported in individuals carrying the CYP3A5*1 allele.[17] Subsequently, additional studies have analyzed the role of CYP3A5 on blood pressure in different populations and have produced controversial results.[18,19,9] In this regard, it is important to consider that the frequency of CYP3A5*1 carriers, i.e. high expressers' of CYP3A5, shows extreme variations across human populations and is significantly correlated with the distance from the equator, with the highest frequency of approx. 70% observed in Africans and the lowest, with approx. 10%, found in European Caucasians.[20] CYP3A expression in the kidney is moderate as compared to the liver and is predominantly CYP3A5[5]. There are several lines of evidence consistent with an association between CYP3A enzyme activity and blood pressure or sodium retention. As early as 1975, it was shown that 6α-hydroxycortisol and 6 –β hydroxycortisol were higher by an average of 48% in patients with essential hypertension compared with normotensive subjects[21] Thus a hospital based case-control study was devised which was aimed to evaluate the association of the CYP3A5*3 rs776746 (6986 $A \rightarrow G$) allele in hypertensive cases and healthy controls of Kashmir valley, this study consists of one hundred fifty confirmed essential hypertension cases (78 males and 72 females; age (mean ± S.D) 60.55 ± 11.19 years) and also equal number of age and gender matched normotensive controls (79 males and 71 females; age (mean ± S.D) 60.11±9.92 years). We observed a higher representation of hypertensive cases in the age group between 40 and 60. The highest number of the hypertension cases turned out from the urban area 96 (64%) and 54 (36%) from rural areas. The wide difference in the incidence rate across different regions of the Kashmir valley suggests the role of some environmental exposure of the local population.[22] So from our results, the frequency of wild allele in cases is 10% which is not statistically significant so there is no possible association with essential hypertension **.**Our results showing high prevalence (90 .00 %) of the defective CYP3A5 *3 allele in Kashmiri population is in agreement with the other studies conducted in India and elsewhere.[23,24,25] In the present study, we analyzed the relevance of CYP3A5 polymorphism for blood pressure levels. Overall analysis revealed that the presence of the CYP3A5*1 allele exhibited no effect on systolic blood pressure (SBP) and diastolic blood pressure (DPB). The observed adjusted difference between CYP3A5*1/*1+ CYP3A5*3/*1 individuals compared with CYP3A5*3/*3 individuals for SBP and DBP was not statically significant. Our results substantiate few early findings in which no significant differences between CYP3A5 gene with blood pressure and essential hypertension was seen in African- Americans and Caucasians.[26, 9]

CONCLUSION

The present study concludes that there is no association of CYP3A5*1 with the risk of essential hypertension. The reason for contrary results obtained from several studies remains ambiguous and might be attributed to differences in ethnic background and the selection of population studied, difference in sample size and gene-environment interaction. As the CYP3A5 gene has been implicated in BP regulation in some ethnic groups, so additional epidemiological, biological and clinical studies in Kashmir population are required to further investigate the role of CYP3A5 in the pathogenesis of hypertension.

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