



## Cytochrome P450 genetic variants and hypertension risk in South Indian population

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### ABSTRACT

**Introduction:** Hypertension is a result of interaction between complex environmental and genetic factors. The genes cytochrome (*CYP*) *2C8*, *CYP2C9*, and *CYP2J2* are involved in the metabolism of arachidonic acid, which releases endothelium derived hyperpolarizing factors responsible for mediating the smooth muscle relaxation. **Aim:** The study is aimed to analyze the association of *CYP2C8*, *CYP2C9*, and *CYP2J2* gene polymorphisms with the risk of hypertension in the South Indian population. **Methods and Results:** The case-control study consisted of 279 hypertensive patients and 321 healthy controls aged ranges between 30 and 60 years. The deoxyribonucleic acid was extracted using standard phenol-chloroform method. Genotyping was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism and real-time-PCR methods. Multiple-logistic regression analysis was used to analyze the association between polymorphisms and the risk of hypertension. We did not observe any association of *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3*, and *CYP2J2\*7* with hypertension. **Conclusion:** Our results suggest that *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3*, and *CYP2J2\*7* are not associated with hypertension in South Indian population.

**Key words:** Cytochrome genes, South Indian population, hypertension

### INTRODUCTION

Hypertension (HTN) is a result of interaction between complex environmental and genetic factors. The cardiovascular diseases and chronic kidney disease could

be the presenting manifestation of hypertension. Various factors such as usage of tobacco, alcohol, excess intake of salt, physical inactivity and genetic predisposition ultimately leads to hypertension.<sup>1</sup> In 1980, there were about 600 million HTN patients, which rose to 1 billion in 2008. Approximately, 40% of individuals aged 25 and above were diagnosed with HTN worldwide.<sup>2</sup> Every year HTN account for 9.4 million deaths worldwide.<sup>3</sup>

The epoxygenases of cytochrome *CYP2J2* and *CYP2C* subfamilies catalyze oxidative metabolism of arachidonic acid into metabolites, which may have an influential role in hypertension.<sup>4,5</sup> Endothelial *CYP* enzymes are shown

Access this article online	
Journal Sponsor	Website: www.jyoungpharm.org
	DOI: 10.5530/jyp.2014.1.4

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to play a prime role in regulation of blood pressure by acting as endothelium derived hyperpolarizing factor (EDHF) synthase.<sup>5</sup> The metabolites of arachidonic acid such as epoxyeicosatrienoic acid (EETs) and 20 hydroxyeicosatetraenoic acid are referred as EDHF, which also contributes to endothelium dependent vasodilation in addition to NO and prostacyclins (prostaglandin 12).<sup>6</sup> The role of CYP epoxygenases are demonstrated by many studies. Inhibition of CYP epoxygenases decreased renal sodium excretion and increased blood pressure in rats fed with high salt diet. It is shown that EETs have potent vasodilatory actions in various vascular beds such as coronary and renal. The activation of calcium dependent potassium channels and hyperpolarization of smooth muscle cells are attributed for vasodilatory actions of EETs.<sup>7,8</sup> The EETs also play a role in regulation of natriuresis by inhibiting the reabsorption of sodium in the proximal tubule and sodium and water in collecting ducts.<sup>9</sup> Further, inhibitors of soluble epoxide hydrolases, which hydrolyse EETs to corresponding dihydroxyeicosatrienoic acid are shown to reduce blood pressure and progression of renal injury in rodent models of hypertension.<sup>10,11</sup>

The allelic frequency of *CYP2C8*, *CYP2C9*, and *CYP2J2* genes varies between ethnic groups.<sup>12,13</sup> The *CYP2J2* gene is located on chromosome 1, *CYP2C8* and *CYP2C9* genes on chromosome 10 respectively. So far 17 variant alleles for *CYP2J2*,<sup>14</sup> 57 variant alleles for *CYP2C9* and 13 variant alleles for *CYP2C8* gene has been identified out of which *CYP2J2\*7*, *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*2*, and *CYP2C9\*3* are most common polymorphisms. All these polymorphisms lead to decreased enzyme activity.<sup>15,16</sup> Therefore, due to the decreased enzyme activity, there may be decreased formation of EDHF, which may lead to disease susceptibility. Although there are many studies, which showed the influence of genes in regulation of HTN, identification of the candidate genes still remain unclear.<sup>17,18</sup> Literature search also shows that there are no studies involving these polymorphisms on the susceptibility of HTN in South Indian population. Thus, the present study aimed to explore the influence of these functional polymorphisms on development of HTN in our population.

## METHODS

### Subjects

The case-control study consisted of 279 hypertensive patients and 321 healthy controls. All the study subjects were unrelated ethnic Tamilians from families who were residing in South India for at least three generations and spoke any

one of the four South Indian languages. Subjects were aged between 30 and 60 years of either gender recruited from inpatients and outpatients ward of Jawaharlal Institute of Postgraduate Medical Education and Research Hospital, Pondicherry, India. Standardized datasheet was used to know about the status of their smoking, alcohol, drug intake, and lifestyle. The investigation was approved by Institute Human Ethics Committee. The subjects were explained with study procedure in detail and written informed consent was obtained.

### Genotyping

A volume of 5 ml of blood was collected from all subjects in polypropylene tubes with anticoagulant (100 µl of 10% ethylenediaminetetraacetic acid). Lipid profile was done from plasma obtained after centrifugation. The deoxyribonucleic acid was extracted by standard phenol–chloroform method and was stored at –20°C. The genotyping of *CYP2C8\*2* and *CYP2C9\*2* alleles was done by polymerase chain reaction (PCR)–restriction fragment length polymorphism method. Amplification of *CYP2C8\*2* was done by forward F5'AAAGTAAAAGAACACCAAGC3' and reverse primers R5'AAAATCCTTAGTAAATTACA3'. Similarly, forward and reverse primers for *CYP2C9\*2* were F5'TACAAATACAATGAAAATATCATG3' and R5'CTAACAACCAGACTCATAATG3'. The PCR products of *CYP2C8\*2* and *CYP2C9\*2* were digested using MboI and AvaII restriction enzymes. Agarose gel (1%) was used to check for amplified PCR product and enzyme-digested products of *CYP2C8\*2* and *CYP2C9\*2* were electrophoresed on 8% and 12% polyacrylamide gels, respectively. Genotype was identified using the band pattern (Table 1). Real-time-PCR allelic discrimination method was performed for genotyping of *CYP2C8\*3*, *CYP2C9\*3* and *CYP2J2\*7*. The kits for amplification, allele discrimination and the real-time-thermocycler were purchased from Applied Biosystems, Foster City, CA, USA.

### Statistical analysis

The analysis of genotype data was carried out with the SPSS software; version 16. Student's *t*-test was used to compare the demographic details with continuous variables

**Table 1:** Restriction digestion pattern of *CYP2C8\*2* and *CYP2C9\*2* alleles by PCR-RFLP method

Gene	SNP	Product size (bp)	Band patterns	
			Major allele (1)	Minor allele (2)
<i>CYP2C8</i>	805A>T	167	69, 65, 33	98, 69
<i>CYP2C9</i>	430C>T	690	521, 169	690

PCR-RFLP: Polymerase chain reaction–restriction fragment length polymorphism, bp: Base pairs. Band patterns observed under ultra violet light

and Chi-square test was used to compare the dichotomous variables of the study groups (cases and healthy controls). The differences in genotype and allele frequencies were compared using Fisher's exact test. The confounding factors were adjusted and the risk of HTN was estimated by performing logistic regression analysis with low risk genotype as the reference groups.  $P < 0.05$  was considered as statistically significant.

## RESULTS

The demographic details of the study subjects are shown in Table 2. The study participant's age, body mass index, systolic blood pressure, diastolic blood pressure, smoking behavior, total cholesterol, triglycerides, high density lipoproteins, and low density lipoproteins cholesterol were significantly different between the cases and healthy controls ( $P < 0.05$ ). Smokers were higher in cases when compared with the healthy controls (15.8% vs. 7.2%).

The multi-logistic regression odds ratio (OR) of *CYP2J2\*7*, *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3* polymorphisms, and HTN compared between cases and healthy controls are shown in Table 3. The table also shows the genotype and allele frequency of the polymorphisms in the South Indian population. The *CYP2C9\*2* CT + TT genotype between the cases and the controls was significantly different in their distribution ( $P = 0.04$ ) and the crude OR showed 2.4-fold risk for HTN. The risk further increased to 3.2-fold after adjusting for the confounding factors, but there was no statistical

significance. The *CYP2C9\*2* T allele also showed a significant difference when compared between the cases and the healthy controls ( $P = 0.04$ ). Similarly, *CYP2C9\*3* and *CYP2C8\*2* showed increased risk after adjusting for confounding factors, but not significantly associated with HTN.

## DISCUSSION

This is the first study to explore the association of CYP polymorphisms and hypertension in South Indian population. CYP allelic variants were not significantly associated with hypertension. However, the previous studies have shown varied results of association of CYP gene polymorphisms with hypertension. Dreisbach *et al.* demonstrated that lack of association between *CYP2C8*, *CYP2C9*, and *CYP2J2* polymorphisms with of risk of HTN in the African American population.<sup>4</sup> Whereas, King *et al.* showed that *CYP2J2\*7* was significantly associated with hypertension in Caucasian males and Caucasians without a family history of hypertension.<sup>19</sup> However, they did not include *CYP2C9* gene polymorphisms in their analysis. Further this study also showed no association between *CYP2C8* and hypertension. But, in the present study, although *CYP2C9\*2*, *CYP2C9\*3* and *CYP2C8\*2* showed 3.2-, 1.2- and 2.4-fold risk for HTN it did not attain statistical significance.

There are studies in Han Chinese, Russian, Caucasian, and Saudi Arabian populations, which reported that *CYP2J2\*7* was associated with risk of hypertension.<sup>19-22</sup> This is contradicting with the present study and the study done in African American population.<sup>4</sup> This probably may be due to differences in ethnicity,<sup>12</sup> which is an important factor attributed to clinical outcomes. Pharmacogenetic variations in genes encoding CYP enzymes are increasingly important as it elucidates inter- and intra-ethnic differences owing to the therapeutic response, adverse events, prognosis, and disease susceptibility. Apart from ethnic differences the factors such as dietary habits, family history, and socioeconomic status may also play a major role in HTN. Our finding is of prime importance in hypertensives given that CYP genes are also involved in metabolism of antihypertensive drugs.<sup>23</sup> Thus CYP genetic polymorphisms involved in varied response to antihypertensives may not be associated with hypertension. This is in accordance with previous studies.<sup>4,19</sup> However, similar studies in larger and different populations must be carried out to conclude the role of CYP genes in association with hypertension.

**Table 2: Demographic detail of study subjects**

Parameter	Hypertensive cases (n=279)	Healthy controls (n=321)
Gender male/female	116/163	132/189
Age (years)	49.6±0.5	44.5±0.5*
BMI (kg/m <sup>2</sup> )	24.24±0.3	22.2±0.2*
SBP (mmHg)	144.4±1.1	117.7±0.5*
DBP (mmHg)	91.2±0.6	77.1±0.3*
Alcohol users n (%)		
Current	58 (20.8)	63 (19.6)
Never	221 (79.2)	258 (80.4)
Smokers n (%)		
Current	44 (15.8)	23 (7.2)
Never	235 (84.2)	298 (92.8)*
TC (mg/dL)	191.2±2.3	160.1±2.2*
Triglycerides (mg/dL)	141.9±4.0	105.7±2.8*
HDL cholesterol (mg/dL)	40.4±0.4	36.5±0.6*
LDL cholesterol (mg/dL)	122.2±1.9	102.6±1.7*

TC: Total cholesterol, SEM: Standard error of the mean, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, BMI: Body mass index, HDL: High density lipoprotein, LDL: Low density lipoprotein. Values are mean±SEM and numbers. \* $P < 0.05$

**Table 3:** Association between *CYP2J2*\*7, *CYP2C8*\*2, *CYP2C8*\*3, *CYP2C9*\*2, *CYP2C9*\*3 polymorphisms and hypertension compared between cases and healthy controls

Gene and SNP	Genotype	Hypertensive cases	Healthy control	OR (95% CI)	P value	OR (95% CI) adjusted	P value
<i>CYP2J2</i> *7 -76G>T	GG	260 (93.2)	286 (89.1)				
	GT + TT	19 (6.8)	35 (10.9)	0.6 (0.3-1.0)	0.08	0.7 (0.2-1.8)	0.4
	G	538 (96.4)	606 (94.4)				
	T	20 (3.6)	36 (5.6)	0.6 (0.4-1.0)	0.1		
<i>CYP2C8</i> *2 805A>T	AA	269 (96.4)	310 (96.6)				
	AT + TT	10 (3.6)	11 (3.4)	1.0 (0.4-2.5)	1.0	2.4 (0.5-11.5.2)	0.3
	A	548 (98.2)	631 (98.3)				
	T	10 (1.8)	11 (1.7)	1.0 (0.4-2.5)	1.0		
<i>CYP2C8</i> *3 1196A>G	AA	260 (93.2)	304 (94.7)				
	AG + GG	19 (6.8)	17 (5.3)	1.3 (0.7-2.6)	0.5	0.9 (0.2-4.1)	0.9
	A	539 (96.6)	625 (97.4)				
	G	19 (3.4)	17 (2.6)	1.3 (0.7-2.5)	0.5		
<i>CYP2C9</i> *2 430C>T	CC	261 (93.5)	312 (97.2)				
	CT + TT	18 (6.5)	9 (2.8)	2.4 (1.1-5.4)	0.04	3.2 (0.6-15.9)	0.1
	C	540 (96.8)	633 (98.6)				
	T	18 (3.2)	9 (1.4)	2.3 (1.0-5.2)	0.04		
<i>CYP2C9</i> *3 1075A>C	AA	236 (84.6)	275 (85.7)				
	AC + CC	43 (15.4)	46 (14.3)	1.1 (0.7-1.7)	0.7	1.2 (0.5-2.7)	0.6
	A	511 (91.6)	593 (92.4)				
	C	47 (8.4)	49 (7.6)	1.1 (0.7-1.7)	0.7		

Covariates included in the regression analysis were age, gender, BMI, SBP, DBP, smoking, alcohol, TC, triglycerides, HDL and LDL. OR (95% CI): Odds ratio (95% confidence interval), OR (95% CI) adjusted: Odds ratio obtained after performing logistic regression analysis, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, HDL: High density lipoproteins, LDL: Low density lipoproteins

## CONCLUSION

Our study results show that *CYP2C8*, *CYP2C9*, and *CYP2J2* gene polymorphisms were not associated with the risk of hypertension in South Indian population.

## ACKNOWLEDGMENTS

This work was funded by Department of Science and Technology (DST), Government of India, Grant no: 95996.

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