Association of aldosterone synthase (CYP11B2 C-344T) gene polymorphism & susceptibility to essential hypertension in a south Indian Tamil population

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Background & objective: 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 and essential hypertension in south Indian Tamil population.

Methods: 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.

Results: 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 hypertensives (OR=1.8; 95% CI: 1.0-3.6, P=0.05) but not in female subjects.

Interpretation & conclusion: 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.

Key words Aldosterone - CYP11B2 - genotype - hypertension - polymorphism

Essential 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 8q22. Several polymorphisms have been identified in the CYP11B2 gene. Among them, the promoter region C-344T polymorphism (rs id 1799998) is the most widely
studied as it persuades the binding of steroidogenic factor-1, the transcriptional regulatory protein. This polymorphism either increases aldosterone to renin ratio (ARR) in essential hypertensives 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 association 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.

**Material & Methods**

**Study population:** The study was conducted from June 2005 to April 2008 in 406 unrelated essential hypertensive patients (200 males and 206 females) aged 30-60 yr who were residents of Tamil Nadu and Pondicherry for at least three generations. The subjects were selected randomly and the sample size was calculated using PS-Power and sample size calculation version 3.0 software. They were recruited from the outpatient clinics of hypertension and internal medicine of Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) hospital, Puducherry in south India. Patients, who were receiving antihypertensive medications for more than 3 months or newly diagnosed hypertensive patients with systolic blood pressure (SBP) more than 140 mmHg and/or diastolic blood pressure (DBP) more than 90 mmHg on two or more consecutive visits were considered as hypertensives. Patients with history of diabetes mellitus, hyperlipidaemia, liver or renal disease, congestive cardiac failure and recent episode of myocardial infarction were excluded. Patients with pregnancy and lactation and receiving medications for other indications that could affect blood pressure were also excluded. The control group consisted of 424 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 the health camp and patients who visited the outpatient clinics with minor illness without hypertension, diabetes mellitus, hyperlipidaemia and family history of hypertension in previous records were recruited as controls. None of the subjects in the control group was receiving antihypertensive therapy, treatment for heart disease or hormone-replacement therapy during the time of the study. Plasma lipid profile and blood glucose level were measured after overnight fasting in both hypertensives and normotensives to rule out diabetes and hyperlipidaemia. All the participants were interviewed using standardized questionnaire with regard to their lifestyle, smoking, alcohol consumption and drug intake. The questionnaire was prepared according to the British Hypertension Society guidelines by the clinicians in the department of medicine and hypertension clinic, JIPMER and Institute ethics committee approval was obtained. In all subjects, height was measured to the nearest centimeter and weight to the nearest 0.1 kg which were used for calculation of BMI (kg/m²). Blood pressure was measured two minutes apart three times in the right arm using standard sphygmomanometer after the subjects rested for 10 min and the average reading was recorded. The study was approved by the institutional ethics committee and written informed consent was obtained from all the participants.

**Genotyping:** Five milliliters of venous blood was collected from the participants using ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and the genomic DNA was extracted using phenol-chloroform method. The CYP11B2 C-344T polymorphism was detected using PCR-RFLP method. PCR reaction was performed in mastercycler gradient (Eppendorf, AG, Hamburg, Germany) using 25 µl reaction mixture containing 100 ng of DNA, 200 µM of dNTPs, 0.2 µM of each primers (Alpha DNA, Montreal, Canada) (Sense-5’-CAGGAGGAGACCCCATGTGAC -3’; antisense-5’-CCTCCA CCCTGTTCAGCCC-3’), 1.5 mM of MgCl₂ and 0.5 U of Taq DNA polymerase. This was subjected to 35 cycles with initial denaturation at 94°C for 5 min, cyclic denaturation at 94°C for 60 sec, followed by annealing at 67°C for 60 sec, extension at 72°C for 60 sec and a final extension at 72°C for 5 min. The amplification was checked in horizontal gel electrophoresis unit (Aplex, Massy cedex, France) using 1 per cent agarose gel followed by restriction digestion of the 538 bp PCR product with HaeIII endonuclease (New England Biolabs inc., IA, USA) for 2 h at 37°C. The digested product was analyzed in vertical electrophoresis system (Shelton scientific inc., USA) using 8 per cent polyacrylamide gel electrophoresis (PAGE) which resulted in 203, 138,
The mean age was significantly higher in controls when compared to cases \( P<0.001 \). There was a significant difference in SBP, DBP, smoking, alcohol intake, total cholesterol, LDL, and VLDL cholesterol level among the cases and controls (Table I). Confounding factors that revealed significant difference, except BP, were taken for multiple logistic regression analysis.

**Results**

The different restriction fragments of \( CYP11B2 \) C-344T gene variant are shown in the Fig. The homozygous variant genotype T/T was significantly higher in hypertensive cases when compared to the controls (OR-1.8; 95% CI: 1.1-2.8, \( P=0.02 \)) after adjusting the confounding factors. The variant allele T was also higher in the cases when compared to the controls (66.6 vs. 60.1%, \( P=0.007 \)) (Table II). The observed and expected genotype frequencies were in concordance with the Hardy-Weinberg equilibrium.

Gender-wise analysis showed, the frequency of homozygous variant genotype T/T (OR-1.8; 95% CI: 1.0-3.6, \( P=0.05 \)) and variant allele T (66.1 vs 58.2%) was significantly higher in male cases when compared to male controls, but there was no difference among the female cases and controls (Table III).

**Table I.** Demographic details and biochemical parameters of the study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=406)</th>
<th>Controls (n=424)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male : Female)</td>
<td>200 : 206</td>
<td>183 : 241</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>44.1 ± 0.4&quot;</td>
<td>47.3 ± 0.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 ± 0.3</td>
<td>23.1 ± 0.2</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>152.6 ± 0.8&quot;</td>
<td>118.0 ± 0.5</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>97.2 ± 0.5&quot;</td>
<td>78.1 ± 0.3</td>
</tr>
<tr>
<td>Smoking Yes</td>
<td>71 (17.5%)**</td>
<td>34 (8.0%)</td>
</tr>
<tr>
<td>No</td>
<td>335 (82.5%)</td>
<td>390 (92%)</td>
</tr>
<tr>
<td>Alcohol Yes</td>
<td>109 (26.8%)*</td>
<td>84 (19.8%)</td>
</tr>
<tr>
<td>No</td>
<td>297 (73.2%)</td>
<td>340 (80.2%)</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>175.6 ± 1.4&quot;</td>
<td>170.2 ± 1.6</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)</td>
<td>118.0 ± 2.3</td>
<td>115.6 ± 2.5</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td>40.6 ± 0.4</td>
<td>41.1 ± 0.5</td>
</tr>
<tr>
<td>Serum LDL (mg/dl)</td>
<td>110.6 ± 1.3&quot;</td>
<td>106.6 ± 1.4</td>
</tr>
<tr>
<td>Serum VLDL (mg/dl)</td>
<td>24.3 ± 0.5&quot;</td>
<td>22.5 ± 0.4</td>
</tr>
</tbody>
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Values are expressed as mean ± SEM or numbers and percentages

\( ^*P<0.05, \quad ^{**}<0.001 \) compared to controls

**Fig.** Representative PAGE picture of \( CYP11B2 \) C-344T polymorphism. Lanes 1- 2 log DNA ladder; Lane 2- Undigested PCR product; Lanes 3 and 6- Homozygous mutant (TT); Lanes 4 and 7- Wild type (CC), Lanes 5 and 8- Heterozygous (CT).
Discussion

Hypertension, a major risk factor for cardiovascular and renal disease, is predicted to increase cardiovascular deaths by 111 per cent in India by 2020, much more than in any other Asian country. Hypersecretion of aldosterone was authenticated to cause hypertension. The variations in CYP11B2 gene, influences the aldosterone synthesis. In our study, the homozygous variant TT genotype and variant allele T was significantly higher in hypertensive cases than the controls. Several studies have investigated the association between CYP11B2 polymorphism and essential hypertension among different ethnic groups, with conflicting results.

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. Another Indian study reported that T allele was highly prevalent in highlanders as compared to lowlanders of north India. In accordance with this study, ours showed a higher prevalence of T allele in both hypertensives and normotensives. A case-control study conducted in French and Scottish Caucasian population showed that the variant allele T was highly prevalent in hypertensive cases and significantly associated with hypertension.

When the influence of C-344T polymorphism was analyzed separately for male and female participants, variant allele T was found to be significantly higher and the homozygous genotype T/T had a marginal significant association in male hypertensive cases, but not in the female subjects. Further, the present study did not show any gene-sex interaction. A study conducted in Chinese population did not find any gender-specific association with hypertension, whereas a study done in Australian population showed that female hypertensive cases with C allele were at increased risk of hypertension. The underlying cause to develop hypertension was limited to female cases, suggesting that this polymorphism could interact with the Y-chromosome.
Our study suggests that the risk was confined to male hypertensives and the reason for this has to be further evaluated.

A larger community-based study in Japanese population revealed that C-344T polymorphism was not associated with blood pressure levels in either sex. Variations in CYP11B2 C-344T have been shown to be associated with increased blood pressure levels, plasma and urinary levels of aldosterone, and plasma renin levels. We could not analyze the mean blood pressure and plasma aldosterone levels in cases since many of our subjects were on antihypertensive medication which might influence the study. When the blood pressure level was analyzed in healthy volunteers, significant difference was not observed among the genotypes (data not shown). However, the results of our study are in agreement with the Caucasian study that did not show significant association between blood pressure and C-344T genotypes. There was a linear increase in diastolic blood pressure in 344T allele carriers with increase in age in the Italian population. Another study conducted in elderly Caucasian population reported that systolic blood pressure was higher in TT genotypes, compared to CC genotypes.

Association of genetic polymorphisms with complex polygenic disorders such as hypertension is further strengthened by haplotype and linkage disequilibrium analysis. CYP11B2 C-344T
polymorphism was found to be in linkage disequilibrium with other CYP11B2 Int2W/C and K173R polymorphisms. Aldosterone-renin ratio was found to be significantly higher in hypertensive cases with haplotype combination of variant alleles 344T and Int2C when compared to other haplotype combinations.

Inter-ethnic variation exists in the polymorphism of C-344T polymorphism. A meta-analysis was carried out to examine the prevalence of C-344T polymorphism among different ethnicities (Table IV). There is significant heterogeneity among the reports, with some studies showing association with T allele and others with C allele. The difference could be attributed to variations in environmental factors, apart from the differences in the selection of cases and controls, sample size, age, BMI, and other ecological factors.

In conclusion, the present study shows an association between C-344T polymorphism and essential hypertension in a south Indian Tamil population, and the risk has been found to be a predisposing factor in males. 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.

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References


