Promoter region polymorphism of CYP11B2 (344 C>T) gene in healthy volunteers of South Indian Tamilian population

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CYP11B2 gene encodes aldosterone synthase enzyme, which produces aldosterone. The objective of study was to establish the allele and genotype frequency of CYP11B2 (344 C>T) gene polymorphism in healthy volunteers of South Indian Tamilian population. Authors carried out the study in 424 unrelated healthy volunteers including both male and female subjects. Genotyping was done by PCR-RFLP method. The observed genotype and allele frequencies were compared with other major ethnic groups. The allele frequency was 39.9 and 60.1% for wild type C allele and variant T allele, respectively. The diastolic blood pressure was found to be significantly higher in the variant TT homozygous genotypes in the total study subjects and also in the male subjects (p<0.05). Interethnic difference in the genotype and allele frequencies of CYP11B2 gene polymorphism was observed in the South Indian Tamilian population.

Keywords: Aldosterone, blood pressure, CYP11B2, genotype, polymorphism

Introduction

Aldosterone is a mineralocorticoid hormone secreted by the zona glomerulosa of the adrenal gland, which is chiefly concerned with water and electrolytes balance of the body. Aldosterone is synthesized by the enzyme aldosterone synthase (P450aldo) which is encoded by CYP11B2 gene, located on chromosome 8q22.4-6. Aldosterone is mainly stimulated by angiotensin II, potassium and ACTH with transient effect.7,8 The commonly reported polymorphic variation of CYP11B2 gene is the substitution of cytosine to thymidine at -344 position (rs id 1799998) located in the promoter region.9 The 344 C>T polymorphism increases aldosterone level, which leads to hypertension.6,10,11 It plays a vital role in the development of high blood pressure.12 Its association was also reported with low rennin hypertension,13 increased left ventricular size and dysfunction,14 progression of renal dysfunction14 and myocardial infarction.15 This polymorphism has been extensively studied in major ethnic groups with very few studies in Indian population,19,20 but hitherto there is no study reporting the frequency of this polymorphism in Tamilian (South Indian) population. Therefore, authors studied the genotype and allele frequency of CYP11B2 gene polymorphism (344 C>T). In addition, they also investigated the association of this polymorphism with blood pressure in normal healthy volunteers including males and females of South Indian Tamilian population.

Materials and Methods

Study Subjects

Volunteers were recruited from Pondicherry whose three generation have stayed in Pondicherry/Tamil Nadu and speak Tamil as their mother tongue. The total number of subjects included in the study was 510, among which 6 samples did not amplify for CYP11B2 (344 C>T) polymorphism. Eighty subjects with increased body mass index (BMI), blood pressure, biochemical values like lipid profile, blood glucose and receiving medications for other chronic illness were excluded from the study. Finally, the study consisted of 424 unrelated, healthy volunteers of both sexes (183 males and 241 females) aged 30-60 yr with systolic blood pressure less than 130 mm Hg and diastolic blood pressure less than 85 mm Hg with no personal or family history of hypertension and other chronic illness. Blood pressure was measured by using mercury sphygmomanometer after resting the subjects for 10 min. Blood pressure was recorded two times with 10 min interval and the average was taken.

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All the participants were interviewed using standardized questionnaire regarding the lifestyle, smoking, alcohol consumption and drug intake. Height and weight were measured and BMI was calculated. Biochemical analyses were done after overnight fasting which included measures of lipid profile and fasting plasma glucose level. Subjects with diabetes mellitus, hyperlipidemia and other chronic illness were excluded from the study. The study was approved by institutional ethics committee and written informed consents were obtained from all the participants.

Genotyping
Blood (5 mL) was collected from the volunteers in 15 mL PP centrifuge tubes with EDTA as anticoagulant. The genomic DNA was extracted from the blood sample using standard phenol-chloroform method. The CYP11B2 (344 C>T) polymorphism was identified by PCR-RFLP method. Standard PCR reaction was performed in a 25 µL reaction mixture containing 100 ng of DNA, 200 µM of dNTPs, 0.2 µM of each primers (sense-5′-CAGGAGGA GACCCCATGTGAC-3′; antisense-5′-CCTCCACCC TGTTCAAGCCC-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 by 1% agarose gel electrophoresis. The amplified 538 bp PCR product was digested with HaeIII restriction enzyme for 2 h at 37°C and analyzed on 8% polyacrylamide gel electrophoresis (PAGE).

Statistical Analysis
Data were analyzed using Statistical Package for Social Sciences statistical software (SPSS windows version release 13). The demographic details of study subjects with continuous variables were compared by student unpaired t test and categorical variables by x² test. Continuous variables were expressed as mean ± SEM values. Allele frequencies were calculated from the genotypes of the subjects. Differences in allele frequencies and genotype distributions were compared by x² and Fishers exact test. Effect of genotypes on blood pressure was analyzed by one-way ANOVA followed by Tukey multiple comparison test. 95% CI was analyzed by CIA version. The observed genotype frequencies were compared with the expected genotype frequencies to check for the Hardy-Weinberg equilibrium; p<0.05 was used as the level of significance.

Results
The anthropometric characteristics and the biochemical parameters of the total study subjects according to the genotypes were shown in Table 1. There were no significant differences in sex distribution, age, BMI, serum lipid parameters, as well as prevalence of smokers and alcoholics between the genotypes of CYP11B2 (344 C>T) polymorphism. However, diastolic blood pressure was found to be significantly higher among the TT homozygous variant genotype when compared to homozygous wild type CC genotype (p<0.05), but did not differ in systolic blood pressure.

The genotype frequencies of wild type homozygous CC, heterozygous variant CT and homozygous variant TT genotypes were 13.2, 53.3% respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total subjects (n=424)</th>
<th>CC (n=56)</th>
<th>CT (n=226)</th>
<th>TT (n=142)</th>
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</thead>
<tbody>
<tr>
<td>Sex (Male:Female)</td>
<td>183:241</td>
<td>31:25</td>
<td>91:135</td>
<td>61:81</td>
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<tr>
<td>Age (yr)</td>
<td>44.1 ± 8.1</td>
<td>44.3 ± 8.0</td>
<td>44.0 ± 8.1</td>
<td>44.1 ± 8.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 3.6</td>
<td>22.2 ± 3.1</td>
<td>23.3 ± 3.7</td>
<td>23.1 ± 3.5</td>
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<tr>
<td>SBP (mm Hg)</td>
<td>118.0 ± 8.8</td>
<td>116 ± 11.3</td>
<td>117 ± 8.6</td>
<td>118.3 ± 8.0</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>78.1 ± 5.2</td>
<td>76.2 ± 6.3</td>
<td>77.6 ± 5.1</td>
<td>78.6 ± 4.7</td>
</tr>
<tr>
<td>Smokers - n (%)</td>
<td>34 (8.0%)</td>
<td>6 (10.7%)</td>
<td>15 (6.6%)</td>
<td>13 (9.1%)</td>
</tr>
<tr>
<td>Alcoholics - n (%)</td>
<td>84 (19.8%)</td>
<td>12 (21.4%)</td>
<td>43 (19.0%)</td>
<td>29 (20.4%)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>170.2 ± 33.9</td>
<td>178.9 ± 35.0</td>
<td>169.9 ± 35.6</td>
<td>167.3 ± 29.9</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>115.6 ± 51.6</td>
<td>117.4 ± 48.0</td>
<td>116.3 ± 55.2</td>
<td>113.9 ± 47.0</td>
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<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>41.1 ± 9.8</td>
<td>42.2 ± 9.3</td>
<td>41.2 ± 9.6</td>
<td>40.5 ± 10.2</td>
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<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>106.6 ± 28.3</td>
<td>114.4 ± 28.9</td>
<td>106.3 ± 29.0</td>
<td>103.9 ± 26.7</td>
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<tr>
<td>VLDL cholesterol (mg/dL)</td>
<td>22.5 ± 10.7</td>
<td>22.3 ± 9.7</td>
<td>22.6 ± 11.5</td>
<td>22.9 ± 9.5</td>
</tr>
</tbody>
</table>

Variables are expressed as the mean ± SD values and percentage

*p<0.05 by one-way ANOVA followed by Tukey test between CC vs TT genotypes
and 33.5%, respectively. The overall frequency of C and T allele were 39.9 and 60.1%, respectively. The genotype and allele frequency were almost equally distributed and did not differ significantly among the male and female study subjects (data not shown). The variant allele frequencies were 58.2 and 61.6% for males and females, respectively. The observed and expected genotype frequencies were in Hardy-Weinberg equilibrium.

The effect of genotypes on blood pressure was compared for male and female subjects as shown in Table 2. There was no significant difference for SBP among the genotypes but T/T genotypes showed a significant higher diastolic blood pressure in males ($p<0.05$) when compared to C/C genotypes in male subjects.

**Discussion**

The present study reports the genotype and allele frequency of CYP11B2 gene (344 C>T) polymorphism in the South Indian Tamilian population. The influence of this polymorphism on blood pressure and other anthropometric variables are expressed as the mean ± SD values.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Genotypes</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>114.9 ± 11.3</td>
<td>117.3 ± 11.4</td>
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<td></td>
<td>116.6 ± 10.0</td>
<td>117.1 ± 7.6</td>
<td>76.9 ± 5.9</td>
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<tr>
<td></td>
<td>117.6 ± 9.6</td>
<td>118.9 ± 6.5</td>
<td>78.8 ± 5.3*</td>
</tr>
</tbody>
</table>

Variables are expressed as the mean ± SD values

*p<0.05 by one-way ANOVA followed by Tukey test between CC vs TT genotypes

**Table 2—Effect of CYP11B2 (344 C>T) polymorphism and sex on blood pressure**

When the influence of blood pressure was analyzed in relation to genotypes, the diastolic blood pressure was found to be higher in CYP11B2 TT homozygotes. Similarly, the diastolic blood pressure was higher in TT genotypes in males but similar trend was not observed in female subjects. The CYP11B2 344 C>T polymorphism revealed positive association and negative association in relation to cardiovascular disorders. Moreover, C allele was associated with high BMI in highlanders accustomed to high salt intake, increased left ventricular size and mass in young Finnish. However, T allele was predominantly seen in the highlanders of Himalayans.

A significant frequency of homozygous wild type CC of our population was similar to North Indians, Japanese, Scottish and Chinese but did not match with French, Germans and Africans (Table 3). The frequency of heterozygous variant genotype CT of our study was different from Japanese, Chinese and Africans but similar to Caucasians. The frequency of homozygous variant genotype TT of Tamilian population was similar to Germans and Scottish but different from Japanese, French, Chinese and Africans.

The frequency of variant T allele of our population was found to be different from other major ethnic populations like Japanese, Caucasians, Chinese and Africans (Table 3).

When the influence of blood pressure was analyzed in relation to genotypes, the diastolic blood pressure was found to be higher in CYP11B2 TT homozygotes. Similarly, the diastolic blood pressure was higher in TT genotypes in males but similar trend was not observed in female subjects. The CYP11B2 344 C>T polymorphism revealed positive and negative association in relation to cardiovascular disorders. Moreover, C allele was associated with high BMI in highlanders accustomed to high salt intake, increased left ventricular size and mass in young Finnish. However, T allele was predominantly seen in the highlanders of Himalayans.

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association of the -344T allele with hypertension was noted in French population.

In conclusion, the present study imparted the genotype and allele frequencies of CYP11B2 (344 C>T) polymorphism in South Indian Tamilian population. Moreover, increase in diastolic blood pressure was observed in homozygous TT individuals in the total study subjects and as well as in the male subjects. The reason for the increase in diastolic blood pressure is unknown and it has to be elucidated.

Acknowledgement
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References
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