Sex-based Association of CYP11B2 (-344 C/T) Polymorphism in Indian Type 2 Diabetic Patients

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Abstract

Background: CYP11B2 gene association studies have been conducted on hypertension, T2DM, and T2DNH in subjects from Caucasian, Asian, and Indian populations. The -344 C/T variant is a commonly reported polymorphism of the CYP11B2 gene. The aim of the present study was to investigate the association between the CYP11B2 (-344 C/T) polymorphism and sex in type 2 diabetic patients in the Indian population.

Methods: The CYP11B2 (-344 C/T) polymorphism was identified by PCR-RFLP and sequencing.

Results: The CYP11B2 gene CC, CT, and TT genotypes accounted for 14.55%, 50.00%, and 35.45% of the male T2DM subjects, 20.55%, 34.25%, and 45.21% of the male controls, 11.46%, 54.17%, and 34.38% of the female T2DM subjects and 11.11%, 44.44%, and 44.44% of the female controls. The CT heterozygote was more frequent among the T2DM subjects than among the controls. The C allele was most frequent among the male T2DM subjects (39.55%), followed by the female T2DM subjects (38.54%), the male controls (37.67%), and the female controls (33.33%). The T allele was most frequent among the male controls (62.33%), followed by the female controls (66.67%), the male T2DM subjects (60.45%), and the female T2DM subjects (61.46%). Overall, the distribution of CYP11B2 genotypes and allele frequencies did not differ significantly.

Conclusion: We did not find any significant association of the CYP11B2 (-344 C/T) polymorphism with sex in the studied cohort.

Keywords: CYP11B2; Sex; T2DM; RFLP; Sequencing; Indian Population.

Introduction

Aldosterone is an important component of the Renin-Angiotensin-Aldosterone System (RAAS) and plays an important role in controlling blood pressure in the body [36]. RAAS component genes can be candidates for evaluating predisposition to development of hypertension, cardiovascular disease, and progression of renal disease in type 2 diabetes. The CYP11B2 gene encodes a steroid 11/18-beta-hydroxylase that functions in mitochondria in the zona glomerulosa of the adrenal cortex to synthesize the mineralocorticoid aldosterone, and its expression is regulated by angiotensin II and potassium [6]. The CYP11B2 gene contains 9 exons and 8 introns and is located on chromosome 8q22 [4, 11, 15, 18].

The -344 C/T variant is a commonly reported polymorphism of the CYP11B2 gene, which is located in the promoter region of the gene [2]. The CYP11B2 gene polymorphism is associated with serum aldosterone level and production [13, 24, 35], blood pressure [4, 30, 7, 14], left ventricular size and mass [15, 34], ischemic stroke [25], and essential hypertension. This association with risk of hypertension was found to be confined to male subjects in the south Indian Tamil population [22, 23].

CYP11B2 gene association studies have been conducted extensively in subjects who suffer from hypertension and come from Caucasian, Asian, and Indian populations [1, 4, 7, 10, 19, 22, 26, 29, 31, 32, 33] but only a few studies have been conducted in an Indian population with subjects suffering from type 2 diabetes.
Therefore the aim of the present study is to investigate the association between the CYP11B2 (-344 C/T) polymorphism and sex in type 2 diabetic patients in an Indian population.

**Materials & Methods**

**Ethics**

Ethical committee clearance was obtained from the respective medical institutions prior to the recruitment of subjects for this study. Informed consent was obtained from all the participants prior to their recruitment for the study.

**Subjects**

This is a sex-based case control study, consisting of 183 males (T2DM = 110, Con = 73) and 141 females (T2DM = 96, CON = 45). Registered T2DM subjects were recruited at two participating medical institutions, namely (a) Calcutta Medical College (Kolkata), (b) B.P. Poddar Hospital & Research Centre (Kolkata). Healthy unrelated controls were randomly selected and recruited from local community centers.

**Biochemical Analysis**

Venous blood (10 ml) was collected from each individual included in the study for biochemical (5 ml) and genetic analysis (5 ml). Biochemical analyses to determine glucose (mg/dl), cholesterol (mg/dl), triglyceride (mg/dl), HDL cholesterol (mg/dl), and LDL cholesterol was performed by using automated analyzer (EM 360, TRANSASIA).

**CYP11B2 gene analysis**

Approximately 5ml of venous blood was drawn from each of the subjects in EDTA vials and genomic DNA was extracted from whole fresh blood using standard salting out method using phenol-chloroform [17]. The CYP11B2 (-344 C/T) polymorphism was identified by PCR-RFLP and sequencing. Subjects were genotyped using primers CAGGAGGAGACCCCATGTGA (sense) and CCTCCACCCCTGTTCCAGCCC (antisense). PCR amplification was performed in a final volume of 10μL reaction mixture containing 50ng of genomic DNA, 20 pmol of each primer, 10X Taq PCR buffer, 25 mM MgCl2, 100 mM of each dNTPs and 0.5 U/μL of Red Taq polymerase. PCR amplification was performed in a DNA thermo cycler (Bio-Rad). PCR was carried out with an initial denaturing time at 95°C for 5 min. Then the DNA was amplified for 35 cycles with denaturation at 94°C for 1 min, annealing at 69°C for 1:30 min and extension at 72°C for 1:30 min and final extension 72°C for 10 min. The PCR products were checked by 1% agarose gel electrophoresis with ethidium bromide staining and directly visualized in UV light. Restriction fragment length polymorphism (RFLP) analysis was performed by restriction endonuclease Hae III and by incubating at 37°C for 3 h and 30 min. Electrophoresis of the digested samples was done in 2.5% agarose gel with ethidium bromide staining and analyzed under UV light. The C alleles were detected as fragments of 202 bp and the T alleles as fragments of 273 bp plus smaller fragments (138bp, 125bp, and 71 bp) in each case. The genotypes were confirmed by direct sequencing (DNA Analyzer 3730 ABI, Applied Bio system, USA) (Figure 1).

**Statistical Analysis**

Data were analyzed using SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as Mean±SD. Student’s t-test and ANOVA test were used to determine differences in means and significance levels. Genotype and allele frequencies of the CYP11B2 gene polymorphism were compared between males and females in type 2 diabetic patients (T2DM) and controls using

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*Figure 1. Sequence chromatogram for rs1799998 (-344 C/T). Y represents the presence of C and T, Heterozygous CT (Line 1 and 4), line 2 represents Homozygous CC and line 3 represents Homozygous TT. Color coding of the peaks: green, adenine (A); blue, cytosine (C); black, guanine (G); red, thymine (T).*
χ²-test. To compare the genotype and allelic distributions between two study groups, the Pearson χ²-test, Odds Ratio (OR), and Risk Ratio (RR) with 95% confidence interval (CI) were also calculated using an online calculator (http://www.vassarstats.net/odds2x2.html). Statistical significance was assumed at the P < 0.05 level.

Results and Discussion

Clinical characteristics of all subjects

Baseline clinical characteristics of males and females with type 2 diabetes and healthy controls are presented in Table 1. The DBP and HDL values were significant between total male and female subjects (p < 0.05), whereas only DBP was significant between T2DM male and T2DM female subjects and HDL was significant between male and female controls (p < 0.05). In contrast, no significant difference was observed for age, BMI, SBP, glucose, cholesterol, triglycerides and LDL between males and females with T2DM and controls.

Genotypic and allelic distribution of the CYP11B2 gene polymorphism

Sex-wise distribution of the CYP11B2 gene genotype along with allele frequencies among subjects with Type 2 diabetes and healthy controls are presented in Table 2. The frequencies of CYP11B2 gene CC, CT, and TT genotypes were 14.55%, 50.00%, and 35.45% for males with T2DM and 20.55%, 34.25%, and 45.21% for male controls. Genotype distributions of the CYP11B2 polymorphism differ between T2DM male subjects and male controls but are not statistically significant (χ² = 4.485, p < 0.106). The CC, CT, and TT genotypes account for 11.46%, 54.17%, and 34.38% of the T2DM female subjects, and 11.11%, 44.44%, and 44.44% of the female controls (χ² = 1.397, p < 0.497). This table shows that the CT heterozygote occurs more often in the T2DM subjects than in the controls.

The C allele was a little more frequent among the T2DM male subjects (39.55%) than among the male controls (37.67%) but the T allele was more frequent among the male controls (62.33%) than among the T2DM male subjects (60.45%). The table shows that the C allele was also a bit more frequent among the T2DM female subjects (38.54%) than among the female controls (33.33%). Conversely, the T allele was more frequent among the female controls (66.67%) than among the T2DM female subjects (61.46%). Overall, neither the frequencies of the CYP11B2 genotypes nor their allele frequencies differed significantly among the different study groups.

In this cross-sectional study, we could not observe any significant differences in genotype and allele frequency of the CYP11B2 (-344 C/T) polymorphism between males and females in T2DM subjects and controls (p < 0.05). Several studies have found an association between this polymorphism and hypertension [5, 27, 30] left ventricle size and mass [15, 28], and myocardial infarction [34, 9]. Studies on the CYP11B2 (-344 C/T) polymorphism have shown positive [4, 7, 33, 16, 31] and negative associations [26, 32, 10, 37] with hypertension and other cardiovascular parameters. [8] reported a significant association between the CYP11B2 gene polymorphism and renal insufficiency in hypertensive subjects, and an association with hypertension has been reported in south Indian male subjects [22, 23]. A recent study from Europe in a French population has reported that the CYP11B2 (-344 C/T) polymorphism is associated with T2DM and hypertension and that metabolic syndrome varies by gender [3], whereas Purkait et al., [2013] have reported no association between the CYP11B2 gene polymorphism, T2DNH, and T2DM subjects in an Indian population, probably due to the small sample size.

Conclusion

We did not find any statistically significant association of CYP11B2 (-344 C/T) polymorphism with Sex (males and females) in Indian T2DM subjects. It is also possible that type 2 diabetic patients with a high risk CYP11B2 genotype may be underrepresented in the present study because of premature cardiovascular-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total Study Group (N=324)</th>
<th>T2DM (N=206)</th>
<th>CONTROL (N=118)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Male (N=182)</td>
<td>Female (N=142)</td>
<td>Male (N=109)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age (Year)</td>
<td>51.87 ± 10.62</td>
<td>52.63 ± 8.77</td>
<td>54.76 ± 11.50</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.55 ± 3.80</td>
<td>24.86 ± 4.58</td>
<td>24.84 ± 4.15</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>136.66 ± 23.46</td>
<td>135.01 ± 25.98</td>
<td>143.57 ± 24.36</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86.23 ± 11.36</td>
<td>83.51 ± 11.54*</td>
<td>88.02 ± 12.07</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>141.29 ± 66.91</td>
<td>139.64 ± 46.09</td>
<td>169.75 ± 85.91</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>164.99 ± 45.21</td>
<td>172.88 ± 40.31</td>
<td>168.44 ± 50.96</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>166.53 ± 87.47</td>
<td>161.84 ± 68.52</td>
<td>169.95 ± 93.72</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>45.06 ± 16.02</td>
<td>50.14 ± 17.36*</td>
<td>49.87 ± 17.59</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>95.16 ± 29.70</td>
<td>97.66 ± 26.17</td>
<td>94.21 ± 33.78</td>
</tr>
</tbody>
</table>

*Variables are expressed as the mean± SD values; *P<0.05 between males and females.

Table 2. Sex-based distribution of genotype and allele frequencies of the *CYP11B2* (-344 C/T) polymorphism in T2DM subjects and controls.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2DM</td>
<td>CON</td>
</tr>
<tr>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>CC</td>
<td>16 (14.55)</td>
<td>15 (20.55)</td>
</tr>
<tr>
<td>CT</td>
<td>55 (50.00)</td>
<td>25 (34.25)</td>
</tr>
<tr>
<td>TT</td>
<td>39 (35.45)</td>
<td>33 (45.21)</td>
</tr>
</tbody>
</table>

$\chi^2 = 4.485, p = 0.106$  
$\chi^2 = 1.397, p = 0.497$


<table>
<thead>
<tr>
<th>Study Group</th>
<th>Statistics</th>
<th>C Vs T</th>
<th>CC Vs TT</th>
<th>CT Vs TT</th>
<th>CC Vs CT</th>
<th>(CC+CT) Vs TT</th>
<th>CC Vs (CT+TT)</th>
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<tbody>
<tr>
<td>T2DM Male Vs CON</td>
<td>$\chi^2$</td>
<td>0.59</td>
<td>0.00</td>
<td>4.91</td>
<td>2.74</td>
<td>3.14</td>
<td>0.89</td>
</tr>
<tr>
<td>P</td>
<td>0.439</td>
<td>1.000</td>
<td>0.267</td>
<td>0.098</td>
<td>0.076</td>
<td>0.345</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.8776 (0.6301 - 1.2224)</td>
<td>1.0063 (0.5106 - 1.9831)</td>
<td>0.571 (0.3475 - 0.9392)</td>
<td>1.7613 (0.8968 - 3.4593)</td>
<td>0.659 (0.4151 - 1.0461)</td>
<td>1.353 (0.7217 - 2.5366)</td>
<td></td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>0.9522 (0.8419 - 1.0769)</td>
<td>1.0017 (0.8321 - 1.2059)</td>
<td>0.7438 (0.5762 - 0.9601)</td>
<td>1.1534 (0.9605 - 1.3851)</td>
<td>0.7782 (0.5921 - 1.0226)</td>
<td>1.0463 (0.9493 - 1.1531)</td>
<td></td>
</tr>
<tr>
<td>FEMALE T2DM Male Vs CON</td>
<td>$\chi^2$</td>
<td>0.71</td>
<td>0.22</td>
<td>1.39</td>
<td>0.03</td>
<td>1.32</td>
<td>0.00</td>
</tr>
<tr>
<td>P</td>
<td>0.399</td>
<td>0.639</td>
<td>0.238</td>
<td>0.499</td>
<td>0.251</td>
<td>1.000</td>
<td></td>
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<tr>
<td>OR (95% CI)</td>
<td>0.7973 (0.4712 - 1.349)</td>
<td>1.108 (0.4767 - 2.5749)</td>
<td>0.5372 (0.2771 - 1.0416)</td>
<td>2.0625 (0.8833 - 4.8176)</td>
<td>0.6658 (0.3639 - 1.2863)</td>
<td>1.5194 (0.6988 - 3.3037)</td>
<td></td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>0.9699 (0.8221 - 1.1443)</td>
<td>1.0314 (0.7992 - 1.331)</td>
<td>0.7292 (0.5251 - 1.1026)</td>
<td>1.2394 (0.9435 - 1.5075)</td>
<td>0.7843 (0.5489 - 1.237)</td>
<td>1.055 (0.9352 - 1.1531)</td>
<td></td>
</tr>
<tr>
<td>T2DM Male Vs Female</td>
<td>$\chi^2$</td>
<td>0.04</td>
<td>0.21</td>
<td>0.13</td>
<td>0.53</td>
<td>0.03</td>
<td>0.43</td>
</tr>
<tr>
<td>P</td>
<td>0.841</td>
<td>0.647</td>
<td>0.718</td>
<td>0.467</td>
<td>0.862</td>
<td>0.512</td>
<td></td>
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<tr>
<td>OR (95% CI)</td>
<td>0.9587 (0.6446 - 1.4257)</td>
<td>0.8125 (0.3314 - 1.992)</td>
<td>0.6346 (0.2974 - 1.3542)</td>
<td>1.1818 (0.3645 - 3.8316)</td>
<td>0.6548 (0.3177 - 1.3496)</td>
<td>0.9659 (0.3145 - 2.9622)</td>
<td></td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>0.9837 (0.8426 - 1.1484)</td>
<td>0.9357 (0.723 - 1.2157)</td>
<td>0.7765 (0.5158 - 1.1688)</td>
<td>1.0317 (0.8226 - 1.2941)</td>
<td>0.7734 (0.5462 - 1.1865)</td>
<td>0.9961 (0.8783 - 1.1297)</td>
<td></td>
</tr>
</tbody>
</table>

related mortality.

Authors' Contribution

Conceived and designed the experiment: PP, BNS, JMN. Performed the experiment: PP, PCS and AGR. Analyzed the data: PP. Wrote the paper: PP, KH. Collected Sample: PP, PR, SB.

Acknowledgement

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References
