Association of the C-344T polymorphism of CYP11B2 gene with essential hypertension in Hani and Yi minorities of China

Wenru Tang, Hongyan Wu, Xuhong Zhou, Baowen Cheng, Yongli Dong, Li He, Haijing Yu, Lin Xu, Jing Lu, Kaiyuan Li, Chunjie Xiao*

Laboratory for Conservation and Utilization of Bio-resources and Human Genetics Center of Yunnan University,
#2 N. Cuihu Rd. Kunming, Yunnan 650091, PR China

Received 3 March 2005; received in revised form 6 July 2005; accepted 6 July 2005
Available online 26 August 2005

Abstract

Background: Aldosterone synthase (CYP11B2) is a key enzyme in the biosynthesis of aldosterone. Recently, a C-344T polymorphism in the promoter region of the CYP11B2 gene has been reported to be in association with high blood pressure. We investigated the association between this polymorphism and essential hypertension in Hani (n = 305 individuals) and Yi (n = 233 individuals) minorities of China.

Methods: CYP11B2 genotyping with polymerase chain reaction–restriction fragment length polymorphism was performed in 267 normotensive subjects and 271 essential hypertensive subjects. At the same time, the T(-344)C polymorphism detection in 33 subjects was also performed by sequencing.

Result: The frequency of CYP11B2 C-344T genotype in normotensive controls and essential hypertensive cohort in Hani population were TT: 0.729 vs. 0.610; CT + CC: 0.271 vs. 0.390, respectively. The frequency of CYP11B2 C-344T genotype in normotensive controls and essential hypertensive cohort in Yi population were TT: 0.612 vs. 0.475; CT + CC: 0.388 vs. 0.525, respectively. The frequency of CC + CT genotype in the essential hypertensive group was significantly higher than that in the normotensive controls in both Hani and Yi populations (P < 0.05).

Conclusion: The -344C allele of the CYP11B2 may play a role in genetic predisposition to developing essential hypertension in Hani and Yi minorities of China.

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Keywords: Essential hypertension; Polymorphism; Aldosterone synthase; Hani minority; Yi minority

1. Introduction

Essential hypertension is a multifactional disorder that is influenced by genetic and environmental factors [1]. Genes of the rennin-angiotensin-aldosterone system (RAAS), including angiotensinogen (AGT), angiotensin I-converting enzyme (ACE), type I angiotensin (Ang) II receptor (AT1R) and CYP11B2, are natural candidates for sodium homeostasis and blood pressure regulation. Polymorphisms of these genes have been major targets for molecular analyses in association with hypertension. Polymorphisms such as AGT M235T, ACE I/D, and AT1R A1166C have been investigated in association studies with hypertension [2–5]. Aldosterone synthase, a mitochondrial P450 oxidase, has steroid 11β-hydroxylase activity as well as the 18-hydroxylase and 18-oxidase activities required for the terminal steps of aldosterone biosynthesis. Expression of this enzyme is limited to the adrenal zona glomerulosa, where it is principally regulated by serum levels of potassium and angiotensin II [6,7]. CYP11B2 gene encoding aldosterone synthase was mapped to chromosome 8q24.3 [8]. To date, 3 common polymorphic variants of CYP11B2, C-344T at promoter region, a mutation in intron 2, and K173R in
enxon 3 have been identified [9–12]. The disequilibrium between C-344T polymorphism and a mutation in intron 2 or K173R mutation have been described [9–12]. Many researchers studied the relationship between C-344T polymorphism and hypertension. There is apparent discrepancy among results in these association studies [11–21]. Moreover the frequency of the C is significantly different in the people from different ethnic groups [13]. In our study, the distribution of CYP11B2-344 site and the relationship to essential hypertension have been compared in groups of essential hypertensive and normotensive subjects of 2 minority groups (Hani and Yi) in China.

2. Materials and methods

2.1. Subjects

The 538 subjects aged 30–70 years of Hani and Yi populations were residents of the remote rural area of Yunnan province. Best-trained observers measured BP using standard mercury sphygmomanometers on the right arm of subjects. Blood pressure of normotensive and hypertensive subjects

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Hani population</th>
<th>Yi population</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Control</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Male/female</td>
<td>67/66</td>
<td>102/70</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.6±9.7</td>
<td>52.2±10.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5±2.6</td>
<td>22.8±2.8*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>104.3±6.1</td>
<td>159.9±15.7*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>69.2±4.1</td>
<td>99.6±8.6*</td>
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</tbody>
</table>

*P<0.01.

- 70 °C. Genotypes of C-344T polymorphism in the CYP11B2 gene were determined by a PCR-based method. Segments of CYP11B2 were amplified from each DNA sample by PCR in 15 µl reactions volume containing 0.5 U Taq DNA polymerase (TaKaRa Taq), 1 × concentration of the buffer supplied, 0.2 mmol/l concentration of each deoxynucleotide triphosphate and 10 pmol of both primers (CAGGAGGAGACCCATGTGAC sense and CCTCCA-CCTGTTCAACCC antisense). PCR conditions were: initial denaturation at 94 °C for 3 min; then, 35 cycles at 94 °C for 30 s, at 67 °C (annealing) for 30 s, at 72 °C (extension) for 30 s; final extension at 72 °C for 5 min. A PCR product of 538 bp was amplified. And the fragments were digested with HaeIII restriction enzyme (NEB) by incubating at 37 °C for 2 h, followed by separation of the fragments on a 2.5% agarose gel, ethidium bromide stained, and analyzed with GeneGenius systems (SYNGENE, America). Since the -344T allele lacks a HaeIII site (GGCC) present in the -344C allele, the -344T alleles were detected as fragments of 274, 138, and 126 bp, and -344C alleles as fragments of 203, 138, 126 and 71 bp. At the same time, the T(-344)C polymorphism detection in 33 subjects was also performed by sequencing with ABI PRISM BigDye Terminator V3.1 Sequencing Kit using ABI PRISM 377 DNA sequencer.

2.3. Statistical analysis

The statistical software package SPSS11.0 was used. All analyses were performed separately for the 2 minorities. Frequencies of genotypes were studied in cases and controls by a χ² test. Numerical data were analyzed by a one-way ANOVA. P<0.05 was considered statistically significant.

3. Results

We determined the sequences corresponding to the promoter region of CYP11B2 in 33 subjects. The C-344T and C-470T polymorphisms were found in the sequenced promoter region of the CYP11B2 gene. Sequence analysis in 12 CC (-344) and 12 TT (-344) subjects revealed that C-344T and C-470T polymorphisms were in complete linkage disequilibrium. Only the T-344C genotype was analyzed further. In addition, since the CC genotype occurred at a low frequency, the CT and CC genotypes were combined.

As show in Table 2, these frequencies were in agreement with those predicted by Hardy–Weinberg equilibrium. The frequencies of CYP11B2 C-344T genotypes in normotensive controls and essential hypertensive cohort in Hani population were TT: 0.729 vs. 0.610 and CT + CC: 0.271 vs. 0.390, respectively. Allele frequencies were T: 0.861 vs. 0.785 and C: 0.139 vs. 0.215. There was signification difference in C-344T genotype distribution and allele frequency between
normotensives and hypertensives in Hani population ($P<0.05$). The frequencies of CYP11B2 C-344T genotypes in normotensive controls and essential hypertensive cohort in Yi population were TT: 0.612 vs. 0.475 and CT+CC: 0.388 vs. 0.525, respectively. Allele frequencies were T: 0.772 vs. 0.702 and C: 0.228 vs. 0.298. The frequency of -344C allele in normotensive controls and essential hypertensive cohort in Yi population were no significant difference. However, the frequency of CC+CT genotype in the normotensive controls was significantly lower than that of the essential hypertensive group ($P<0.05$). The frequency of C allele in either EH or NH group of Hani population was significantly lower compared with that of Yi population respective ($P<0.05$). Table 3 showed that diastolic blood pressure tended to be higher in subjects with the TC+CC genotype than in subjects with the TT genotype in the 2 populations ($P<0.05$).

### 4. Discussion

The position of -344 of CYP11B2 gene is located in the promoter region. The potential influence of the C-344T variant on the promoter activity of CYP11B2 was analyzed in a few studies. White et al. [9] found that the -344C allele of the CYP11B2 promoter binds the steroidogenic transcription factor (SF-1) 4 times than the -344T allele in gel shift assays. Bassett et al. [24] also demonstrated that the C allele binding SF-1 more strongly than the T allele, but SF-1 was not a major regulator of -344T allele in gel shift assays. However, Clynnet et al. [25] reported that 2 different elements at positions -71/ -64 and -129/ -114 that consist of SF-1 and COUP-TF binding sites, respectively, were required for both basal and Ang II- or K+-stimulated CYP11B2 transcription. It was possible that another unknown orphan-nuclear receptor binding to SF-1 site might be an import regulator of CYP11B2 gene [26]. Though the mechanisms whereby C-344T polymorphism influences hypertension might be further investigated, the polymorphism has been considered to influence the mineralocorticoid synthesis, and been the main target for molecular analysis in association with hypertension [11–21].

This study first reported on genetics polymorphisms of CYP11B2 in Hani and Yi minorities of China. As shown in Table 2, in the 2 present study populations, the CT+CC genotype was significantly more frequent in hypertensives (Hani: 0.390; Yi: 0.525) than in normotensives (Hani: 0.271; Yi: 0.388) ($P<0.05$). Moreover, diastolic blood pressure tended to be higher in subjects with the C allele than in subjects with the TT genotype ($P<0.05$), suggesting an association of -344C allele with essential hypertension. However, the association between the C-344T polymorphism of the CYP11B2 gene and hypertension has been investigated by several researchers. The results were conflicting, with some studies showing a positive association with the C allele with hypertension [11,14–16], some are associated with the T allele [12,13,17–19], and others are not associated [20,21]. Tamaki et al. [11] reported that the frequency of the TC+CC genotypes in the normotensive group was significantly lower than in the hypertensive in Japanese, in line with our results. A similar association with the C allele had been reported by other groups [14–16], 2 with their results confined to females [15,16]. However, Komiya et al. [12] reported an association of the T allele and low-rennin hypertension in Japanese. Brand et al. [12,13,18,19] also confirmed T allele and hypertension. Pojoga et al. [20] detected a significant association of the C-344T polymorphism and plasma aldosterone levels, but no association with hypertension. No association was also found in a large Japanese population [21]. These discrepancies might be due to race [27], age [28], gender [29], sampling methods [21], genetics epitasis [30] and environmental factors such as salt intake [31].

In summary, we reported that -344C allele in CYP11B2 gene was associated with hypertension in Hani and Yi populations of China. For essential hypertension was recognized as a polygenic syndrome, further investigation in a large population might be necessary to confirm our results.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Hani population</th>
<th></th>
<th>Yi population</th>
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</tr>
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<tr>
<td></td>
<td>Control</td>
<td>Hypertension</td>
<td>$p$</td>
<td>Control</td>
</tr>
<tr>
<td>$n$</td>
<td></td>
<td></td>
<td></td>
<td>$n$</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
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<td></td>
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<tr>
<td>TT</td>
<td>133</td>
<td>172</td>
<td></td>
<td>134</td>
</tr>
<tr>
<td>CT+CC</td>
<td>97 (0.729)</td>
<td>105 (0.610)</td>
<td></td>
<td>82 (0.612)</td>
</tr>
<tr>
<td>Allele C</td>
<td>0.139</td>
<td>0.215</td>
<td></td>
<td>0.228</td>
</tr>
<tr>
<td>Frequency</td>
<td>T 0.861</td>
<td>0.785</td>
<td>$&lt;0.05$</td>
<td>0.772</td>
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### Table 3

<table>
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<th>Variable</th>
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<th>CT+CC</th>
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<tr>
<td>Male/female</td>
<td>106/96</td>
<td>60/3/35/5</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>133.3±30.1</td>
<td>140.2±30.2</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84.5±16.6</td>
<td>90.0±17.5</td>
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<td>$n$</td>
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<td>104</td>
<td></td>
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<tr>
<td>Male/female</td>
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<td>43/8/45/8</td>
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<tr>
<td>SBP (mm Hg)</td>
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<td>127.8±29.5</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>79.8±15.9</td>
<td>84.6±18.5</td>
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References


