Angiotensinogen M235T, β2 Adrenergic Receptor Arg16Gly and Aldosterone Synthase C-344T Gene Polymorphisms and Essential Hypertension among Han Population Living at High Altitude in China

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Received date: April 05, 2016; Accepted date: April 27, 2016; Published date: May 07, 2016

Abstract

We explored the association of angiotensinogen (AGT) M235T, β2 adrenergic receptor (β2-AR) Arg16Gly and aldosterone synthase (CYP11B2) C-344T genes polymorphisms with essential hypertension (EH) in Han population living at high altitude in China. A total of 390 hypertensive subjects (males, 207; females, 183) and 424 normal healthy individuals (males, 251; females, 173) were enrolled in this study based on the inclusion and exclusion criteria. The polymorphisms of AGT M235T, β2-AR Arg16Gly, CYP11B2 C-344T genes were analyzed by Snapshot mini sequencing method. The frequencies of CC genotype and C allele of AGT M235T in EH group were higher than in control (p<0.05), gender wise analysis revealed that the genotype and allele patterns between patients and controls were found to be significantly different (p<0.05). However, genotype and allele frequencies of the distribution of β2-AR Arg16Gly and CYP11B2 C-344T between EH patients and normotensive controls were not significantly different (p>0.05, respectively); in gender-specific analysis, the differences were also not found (p>0.05, respectively). This finding suggests that the polymorphism of AGT M235T was correlated with EH in Han population living at high altitude in China, however, β2-AR Arg16Gly and CYP11B2 C-344T polymorphism were unlikely to be associated with hypertensive subjects of this population.

Keywords: AGT M235T; β2-AR Arg16Gly; CYP11B2 C-344T; Polymorphism; Essential hypertension; Han population

Introduction

Essential hypertension (EH) is one of the most common diseases of human being, and also is an important cause for the myocardial infarction, cerebral apoplexy, and some serious nephropathy etc. which is a complex multifactorial condition influenced by both genetic and environmental factors [1]. Epidemiological and family based studies in many geographically and ethnically distinct populations indicate that EH is a multifactorial disorder with a familial tendency, while it is influenced by race, gender, diet and so on [2]. As there is a complex interaction between a variety of genetic and environmental factors in EH, the precise cause has not been determined [3]. Renin angiotensin (RA) system is a powerful regulatory system with an important role in the regulation of BP by the RA system. The M235T mutation of AGT gene is a single base pair substitution of thymine with cytosine at nucleotide 704 (T704C) in exon 2 of the AGT gene. It is known that due to the polymorphism of AGT M235T, the level of circulating angiotensinogen is increased and the individuals are hypertensive [4,5].

The sympathetic nervous system plays a major role in BP regulation. β2-AR have a pivotal role in the sympathetic nervous system, it is a G-protein coupled receptor that, upon activation by catecholamine, increases the intracellular second messenger cyclic adenosine monophosphate (cAMP) [6]. β2-AR Gly16Arg polymorphism is localized in the extracellular amino terminus region of the protein, which has been characterized that arginine substituted by glycine at nucleotide 16 [7]. Studies have demonstrated that the Gly16Arg genotypes appear to influence the degree of agonist induced receptor desensitization, the functional importance of the Arg16Gly polymorphism has been studied in mediating the vasodilatation that is important in blood pressure control. Other blood pressure regulating effects of the β2 adrenoceptor include renal sodium handling and control of renin release [8-10].

CYP11B2 gene encodes for a cytochrome P450 enzyme, involved in the terminal steps of aldosterone synthesis, aldosterone acts on the distal nephron to regulate sodium resorption, potassium excretion, an intravascular volume [11]. Aldosterone levels are also associated with polymorphic variation in the CYP11B2 gene, the C-344T polymorphism have been identified to be mediate sodium balance and arterial pressure by influencing intravascular volume and arterial thickness which associated with EH in different population groups [12-14].

The associations between the genetic variations in these genes and hypertension would thus be of significant interest. However, to date, associations with these variants are found to be contradictory in different populations. Gannan district of Gansu province is located in the northeastern margin of the Qinghai-Tibet Plateau of China; the average altitude of this area is about 3,500 m above sea. In this study, we aim to investigate the AGT M235T, β2-AR Arg16Gly and CYP11B2 C-344T genes polymorphism in EH patients and healthy controls among Han populations who resided in this area by using Snapshot
minisequencing method, through comparing the genotype and allele frequencies, to assess whether the polymorphism of above genes are associated with genetic predisposition to EH.

Materials and Methods

Subjects

A total of 814 participants aged 18 to 70 years old who resided in Gansu province were randomly selected and completed the survey. The subjects were divided into hypertensive (patients) and normotensive (controls) individuals. The survey was conducted in May to September of 2014, we used ways of concentrated investigation and household visits, informed consent were obtained from each patient.

The first measurement of BP after 5 min time of rest was taken on the right arm of each participant who is in a seated position, and then research team performed three successive measurements with at least a 1 min time interval between measurements. The average of the three measurements of BP was used for analysis. A standardized mercury sphygmomanometer was used. Patients were diagnosed in accordance with JNC 7 guidelines and hypertension was defined as systolic BP (SBP) ≥ 140 mm Hg and/or diastolic BP (DBP) ≥ 90 mm Hg, and/or self-reported treatment of hypertension with anti-hypertension medication taken in the past 2 weeks.

The exclusion criteria were individuals with secondary hypertension (due to renovascular disease, renal failure, pheochromocytoma, aldosteronism or other causes of secondary hypertension). The normotensive subjects who had BP<140/90 mm Hg, no history of EH, no diabetes mellitus or other systemic diseases were never been treated with antihypertensive medication. In this case-control study, a total of 390 EH subjects and 424 controls were enrolled.

Genotype determination

The blood samples were taken into EDTA-containing containers and stored in -20°C, genomic DNA was extracted using a DNA extraction kit (Sangon Biotech Shanghai, China) based on the manufacturer’s protocol, and stored at -70°C.

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The blood samples were taken into EDTA-containing containers and stored in -20°C, genomic DNA was extracted using a DNA extraction kit (Sangon Biotech Shanghai, China) based on the manufacturer’s protocol, and stored at -70°C. The AGT M235T, β2-AR Arg16Gly and CYP11B2 C-344T genes polymorphism was determined by SnaPshot minisequencing. The primers of three genes used were as Table 1.

Statistical Analysis

The statistical software package SPSS18.0 (Version 18.0; SPSS, Chicago) was used. Categorical variables were expressed as proportions (%) and continuous variables as mean ± standard deviation. All comparisons between EH group and the control group for continuous variables were performed by independent t-test, and comparisons among genotypic groups were analyzed with one way ANOVA. The chi square test was performed to compare genotype/allele frequency, P<0.05 was considered statistically significant.

Results

Characteristics of subjects

All the individuals belonged to the general population, baseline clinical characteristics of subjects enrolled (Table 2). The age and sex matched individuals were included in the study, the SBP, DBP and BMI were higher among the patients as compared to controls (p<0.05).

Table 1: The primers and reaction fragments of AGT M235T β2-AR Arg16Gly and CYP11B2 C-344T.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Primer</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT</td>
<td>M235T</td>
<td>CTTGGGAGCTGAGGACTACTAC</td>
<td>273 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse CACCTTGTCAGCATTCCGGTTTG</td>
<td></td>
</tr>
<tr>
<td>β2-AR</td>
<td>Arg16Gly</td>
<td>Forward ATGAGGCTTCAGCGCTC</td>
<td>230 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse GATGAGAGACATGGATGCC</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Male/Female</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>390</td>
<td>207/183</td>
<td>46.6 ± 9.7</td>
<td>23.3 ± 2.6*</td>
<td>155.8 ± 13.3*</td>
<td>100.6 ± 8.8*</td>
</tr>
<tr>
<td>NT</td>
<td>424</td>
<td>251/173</td>
<td>48.0 ± 10.6</td>
<td>21.0 ± 1.5</td>
<td>124.2 ± 14.1</td>
<td>83.4 ± 7.2</td>
</tr>
</tbody>
</table>
Table 2: Baseline characteristics of subjects.

Results of PCR products and SnaPshot minisequencing

The PCR products (Figure 1), a typical SNaPshot minisequencing of samples (Figure 2). The different color of product peaks denoted different alleles SNP locus, and the fragment size of alleles among different SNP loci was different.

Figure 1: The PCR products.

Figure 2: A typical SNaPshot minisequencing of samples.

Analysis of the genotypes and alleles

A total of 798 venous blood samples were collected from the subjects 722 in AGT M235T, 708 in β2-AR Arg16Gly and 701 in CYP11B2 C-344T in study were successfully analyzed, respectively. The analysis of the genotypes and alleles (Table 3).

Table 3: Genotype and allele frequencies of AGT M235T, β2-AR Arg16Gly and CYP11B2 C-344T polymorphism in EH and control group.

It can been found that the CC genotype and C allele of AGT M235T were higher in the EH group compared to control group (p<0.05), in gender-wise analysis of the AGT M235T polymorphism revealed that the genotype and allele patterns between patients and controls groups were found to be significantly different both in male and female populations (p<0.05) (Table 4). However, the frequencies of genotype and allele were not significant difference between EH group and control group in β2-AR Arg16Gly and CYP11B2 C-344T polymorphisms analysis (p>0.05), on gender wise analysis, the differences were also not found (p>0.05).
Discussion

EH is a polygenic disorder. It is widely accepted that EH subjects appear to have inherited an aggregate of genes related to hypertension and/or to have been exposed to exogenous factors that predispose them to hypertension. Epidemiological studies suggested that genetics accounts for 30 to 40% of BP changes, studies on candidate gene polymorphisms and their association with EH can help take up proper interventional/treatment strategies [15]. Genes of AGT, β2-AR and CYP11B2 are natural candidates for BP regulation which have been observed in various previous studies. The association between the polymorphism of AGT M235T, β2-AR Arg16Gly and CYP11B2 C-344T polymorphism in high altitude areas are scarce.

The potential role of the molecular variants M235T of AGT gene in hypertension was originally explored by Jueneimaire group through linkage and association study in the causation of human EH in Utah and French populations [16]. A series of studies among the UK, Malaysian, and south Indian supported the former finding [17-19], several meta-analyses concluded that the coding polymorphism of AGT M235T was associated with increased risk of hypertension [6,20]. However, subsequent association studies found a negative association in Japanese, German, African-Americans and North India [21-23]. In China, Cai et al. have found that a nonfunctioning M235T, increased the plasma AGT level via regulation of AGT gene transcription, and was involved in the pathogenesis of the hypertension [24].

The β2-AR is a G protein-coupled receptor that increase vascular smooth muscle resistance, resulting in vasodilatation, which in turn lowers the peripheral resistance and hence lowers BP [25]. Other effects include renal sodium handling and renal sodium handling and hence lowers BP [26].

<table>
<thead>
<tr>
<th>Gene/SNP</th>
<th>Genotype/Allele</th>
<th>Male</th>
<th>Female</th>
<th>χ²</th>
<th>p</th>
<th>Male</th>
<th>Female</th>
<th>χ²</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>EH</td>
<td>NT</td>
<td></td>
<td></td>
<td>EH</td>
<td>NT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGT/M235T</td>
<td>CC</td>
<td>140 (75.3)</td>
<td>149 (67.1)</td>
<td>7.79</td>
<td>0.01</td>
<td>120 (74.6)</td>
<td>105 (68.6)</td>
<td>5.36</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>38 (20.4)</td>
<td>55 (24.8)</td>
<td></td>
<td></td>
<td>34 (21.1)</td>
<td>37 (24.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>8 (4.3)</td>
<td>18 (8.1)</td>
<td></td>
<td></td>
<td>7 (4.3)</td>
<td>11 (7.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>318 (85.5)</td>
<td>353 (79.5)</td>
<td>4.68</td>
<td>0.03</td>
<td>274 (85.1)</td>
<td>247 (80.7)</td>
<td>4.73</td>
<td>0.03</td>
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<tr>
<td></td>
<td>T</td>
<td>54 (14.5)</td>
<td>91 (20.5)</td>
<td></td>
<td></td>
<td>48 (14.9)</td>
<td>59 (19.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2-AR/Arg16Gly</td>
<td>AA</td>
<td>56 (30.4)</td>
<td>67 (29.5)</td>
<td>2.16</td>
<td>0.34</td>
<td>49 (31.4)</td>
<td>42 (29.8)</td>
<td>1.29</td>
<td>0.25</td>
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<tr>
<td></td>
<td>AG</td>
<td>97 (52.7)</td>
<td>123 (54.2)</td>
<td></td>
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<td>84 (53.8)</td>
<td>77 (54.6)</td>
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<tr>
<td></td>
<td>GG</td>
<td>31 (16.8)</td>
<td>37 (16.3)</td>
<td></td>
<td></td>
<td>23 (14.7)</td>
<td>22 (15.6)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>A</td>
<td>209 (56.8)</td>
<td>257 (56.6)</td>
<td>0.06</td>
<td>0.8</td>
<td>182 (58.3)</td>
<td>161 (57.1)</td>
<td>0.16</td>
<td>0.68</td>
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<tr>
<td></td>
<td>G</td>
<td>159 (43.2)</td>
<td>197 (43.4)</td>
<td></td>
<td></td>
<td>130 (41.7)</td>
<td>121 (42.9)</td>
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<tr>
<td>CYP11B2/C-344T</td>
<td>CC</td>
<td>23 (12.7)</td>
<td>26 (11.8)</td>
<td>0.63</td>
<td>0.72</td>
<td>21 (13.4)</td>
<td>17 (11.9)</td>
<td>1.6</td>
<td>0.21</td>
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<tr>
<td></td>
<td>CT</td>
<td>76 (42.0)</td>
<td>90 (40.9)</td>
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<td>67 (42.7)</td>
<td>59 (41.3)</td>
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<td>69 (43.9)</td>
<td>67 (46.8)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>122 (33.7)</td>
<td>142 (32.3)</td>
<td>0.09</td>
<td>0.75</td>
<td>109 (34.7)</td>
<td>93 (32.5)</td>
<td>2.11</td>
<td>0.34</td>
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<tr>
<td></td>
<td>T</td>
<td>240 (66.3)</td>
<td>298 (67.7)</td>
<td></td>
<td></td>
<td>205 (65.3)</td>
<td>193 (67.5)</td>
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</tr>
</tbody>
</table>

Table 4: Gender wise comparison of genotype and allele frequencies of AGT M235, β2-AR Arg16Gly and CYP11B2 C-344T polymorphism in EH and control group.
control of renin release. Numerous studies have attempted to determine the association of the β2-AR variants with hypertension and related conditions. The relation -ships between the polymorphisms of the β2-AR gene and cardiovascular phenotypes have been assessed in different populations, but the results were lacking an apparent consistency [27-29]. The β2-AR gene locus has been linked to both systolic and diastolic BP in large populations studies in United States [30], similar association was also found in Chinese and in Caribbean populations [28,31]. In vitro studies demonstrated that the Gly16 variant showed enhanced agonist-promoted down regulation [32]. Masuo et al. demonstrated that individuals with Gly16 alleles showed higher BP and plasma norepinephrine than Arg16 carriers in a cohort study [33]. In contrast, there was no association between β2-AR variants and BP in Japanese and black African populations [34,35].

The negative association between the Gly16Arg and EH in our study was different from previous studies. In a meta-analysis of 15 independent studies, Kato N, Sugiyama T, Morita H, Kurihara H, Sato T, et al. (2004) found that there was a significant association between the Gly16Arg variant and EH in the Japanese and South Korean populations [36]. However, in our study, the Gly16Arg variant was not significantly associated with EH. A possible reason for this discrepancy may be the genetic differences between Han populations living at high altitude and other populations.

We sincerely thank all the investigators and the subjects participating in this study.

### Acknowledgements

We sincerely thank all the investigators and the subjects participating in this study.

### Conflict of interest

The authors declare that there are no conflicts of interest.

### References


