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Epidemiological Study of AGT Gene Polymorphism among Chinese Subjects with Primary Hypertension

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Abstract

Background: Polymorphisms in the promoter region of the angiotensinogen (AGT) gene may affect AGT transcription and thus blood pressure. We determined the frequency of the AGT A-20C polymorphism in Chinese patients with primary hypertension. Using a molecular epidemiology approach, we also determined the relationship between primary hypertension and environmental-AGT A-20C polymorphism interactions.

Methods: 912 subjects diagnosed with primary hypertension were analyzed from Shenyang Chinese Populations and their samples were genotyped using A-20C polymorphism within angiotensinogen (AGT) gene by Restriction Fragment Length Polymorphism methods (PCR-RFLP)

Results: PCR and restriction fragment length polymorphism techniques were used to determine the distribution frequency of AGT A-20C alleles. Frequencies of 84.7% and 15.3% were observed for the A and C alleles, respectively. The polymorphism was in Hardy-Weinberg equilibrium according to a χ^2 test (χ^2=0.58, P>0.05). Linear regression of AGT genotype and blood pressure revealed that systolic blood pressure was significantly higher for AC and CC genotypes compared to the wild-type AA genotype in females (P<0.05).

Conclusion: This is the first report on the frequency of AGT A-20C polymorphism in a Chinese population. Compared to the AA genotype, the effects of genotypes AC and CC on blood pressure were mainly manifest as significantly higher systolic blood pressure in females.

Keywords: AGT gene; Polymorphism; Hypertension

Background

Screening for various genes linked to hypertension susceptibility has become a major focus in @lood Nressure (BP) research. Recent global studies have revealed that some polymorphic loci on the angiotensinogen gene (AGT) are associated with hypertension [1,2]. Angiotensinogen is a substrate precursor of angiotensin-converting enzyme and is an important limiting factor for the renin-angiotensin system. Studies have revealed that polymorphisms in the AGT promoter region (A-20C, C-18T and G-6A) may affect AGT transcription, thus altering AGT levels in the body and influencing the efficacy of antihypertensive therapy [3,4]. We conducted epidemiological surveys and determined the frequency of AGT gene A-20C polymorphism for 912 patients with primary hypertension from Anhui province in China to determine whether interactions between AGT A-20C variants and environmental factors increase the risk of primary hypertension

Methodology

Subjects

A total of 912 subjects diagnosed with primary hypertension (systolic BP 140–179 mm Hg and/or diastolic BP 90-109 mm Hg) were recruited for the study. Patients were aged 26–62 years and had not taken any antihypertensive drugs in the previous 2 weeks. Subjects with secondary hypertension, severe arrhythmia, coronary artery disease, stroke, hepato-renal insufficiency, tumors, bilateral renal artery stenosis, hyperkalemia, history of gastroduodenal surgery, recent history of heart failure or any drug allergy were excluded, as well as pregnant or breastfeeding mothers. All patients gave informed consent before participating in the study and approved by the ethics committee of China Medical University.

The following information was obtained from all subjects using questionnaires: sociodemograpic characteristics such as race, age, educational level, marital status, and place of birth; health status, including general health status, history of allergy, diseases and treatment; daily food intake (semi-quantitative food frequency); history of smoking and alcohol intake; living conditions, home expenses, animal/pet infections, toxic exposure and daily stress; occupational activity and exposure; and family history, including health information for first- and second-degree relatives.

Each patient's BP was measured according to a multi-interval protocol while the patient was seated (mean of three measurements, accuracy 2 mm Hg). A small blood was taken for genotype analysis.

Methods

DNA was extracted by a high-salt precipitation method. EDTA-anticoagulated peripheral blood samples (10 ml) were centrifuged at 3000 rpm at 4°C for 10 min and the separated blood were preserved. RBC lysis buffer (30 ml; Gentra Systems Inc., USA) was added to the cell pellet and slowly shaken. The sample was left to stand at room temperature and periodically shaken for complete lysis of the red blood cells. The sample was centrifuged at 3000 rpm at 4°C for 10 min and the supernatant was removed. The residue was spread evenly using a rotary vibrato and. 30 µl of Protease K and 50 µl of RNase (both from Qiagen, Germany) were added. The solution was thoroughly mixed and 15 ml of

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WBC lysis buffer (Gentra Systems) was added, stirred and incubated in a water bath at 37°C for 20 min. The sample was cooled and then 4 ml of cold protein residual liquid (Gentra Systems) was added, thoroughly mixed, frozen at -20°C for 5 min and then centrifuged at 3000 rpm at 4°C for 10 min. The supernatant was transferred to a 50-ml centrifuge tube containing 15 ml of isopropyl alcohol and mixed gently until DNA flocks separated out. Separated DNA flocks were then transferred to a 1.5-ml Eppendorf tube containing 75% ethanol and shaken, and the liquid portion was then discarded. The residue in the Eppendorf tube was then poured onto filter paper and dried, and 1-1.5 ml of liquid DNA (Gentra Systems) was added. The product was placed on a shaking table overnight and then stored -80°C for further use.

To determine AGT genotypes, the target gene sequence was amplified by PCR and the products were cleaved by restriction enzyme *Eco 0109 I*. Genotypes were identified from the cleavage point, i.e. the length of the restriction fragments.

PCR was carried out on a sample volume of 10 µl containing 20 ng of DNA, 1 µl of 10µl PCR buffer (Qiagen), 0.6 µl of 25 mmol/l MgCl, and 0.1 µl each of the following primers (Research Genetics Inc., USA): forward, 5′-AGA GGT CCC AGC GTG AGT GTC-3′; and reverse, 5′-AGC CCA CAG CTC AGT TAC ATC-3′. Reactions were out on DTC-225 PCR system (MJ Research Inc., USA) using the following conditions: preheating at 94°C for 5 min; then heating at 94°C for 1 min and 64 °C for 1 min and extension at 72°C for 1 min over 30 cycles. The restriction endonuclease *Eco 0109 I* (New England Biolabs, USA) was used to digest the amplified samples at 37°C. The data was collected and analyzed by SPSS13.0 software package, t-tests was used in quantitative data, and qualitative data analysis was used by chi-square test, the impact of factors that influence blood pressure use of multiple linear regression analysis. Testing the level was $P < 0.05$

Results

The study subjects were Chinese individuals with primary hypertension aged 26-62 years (mean 47.63 ± 7.96), with 410 males and 502 females. According to the genotype results, 664 of the 912 subjects were wild-type (AA), 227 were heterozygous for the A→C mutation (AC) and 21 were homozygous (CC). The allele frequency was 84.9% A and 15.1% C, and a χ^2 test revealed that the variants were in Hardy-Weinberg equilibrium (χ^2=0.551, p>0.05; Table 1). There were no significant differences in height, age, body weight or BMI among subjects of different genotype. Moreover, there was no significant difference in systolic or diastolic BP (p>0.05; Table 2), after correction for age, height, weight, BMI and other factors, excluding smoking habits. Table 3 reveals that BP, especially systolic BP, was higher in individuals with the AGT mutation (genotypes AC and CC) compared to those with the AA genotype. This difference was significant among female subjects. Both genotypes affecting systolic BP showed significant differences (P=0.0368 and 0.0222 for the total and female populations, respectively; Table 3).

Discussion

Primary hypertension, also known as Essential Hypertension (EH), is a polygenetic inherited disease influenced by multiple environmental factors. Understanding the disease in terms of molecular genetics may assist in preventing and diagnosing EH and enhancing the effectiveness of drug therapy. Aside from its roles in the renin angiotensin system in regulating salt metabolism, vascular permeability and sympathetic nervous system activity, AGT also plays an important role in the pathogenesis of hypertension [5,6]. Studies have focused on searching for the relationship between AGT polymorphism and susceptibility to hypertension [7,8]. Several studies have focused on different single nucleotide polymorphisms in the AGT promoter region, including -20 A→C, -18 C→T and -6 G→A substitutions. AGT gene expression is mainly controlled by the transcriptional regulatory region. In 1996, Yanai et al. [7] discovered a nuclear factor (AGCF1) that binds to AGCE1 in the AGT transcription initiation site between positions -25 and ¬-1. Ishigami et al. [8] reported that A-20C polymorphism is closely associated with EH and plasma AGT levels. Using human liver tumor cells, Zhao et al. [9] observed that A→C substitution at position -20 in the AGT promoter led to an increase in AGT transcription. Therefore, we predicted that increased expression of

Genotype	Frequency (n)	
	Actual	Expected
AA	664	657
AC	227	234
CC	21	21
Total	912	912

Table 1: AGT allele frequencies for hypertensive subjects.

	AA	AA+AC	*p*
	(n=664)	(n=248)	
Age (years)	47.24 ± 7.28	46.12 ± 8.75	0.1024
Height (cm)	165.20 ± 6.99	166.58 ± 7.67	0.1297
Weight (kg)	63.87 ± 10.48	63.66 ± 11.01	0.4087
BMI (kg/m^2)	24.86 ± 3.58	24.63 ± 3.53	0.8460
SBP (mm Hg)	151.93 ± 16.58	154.25 ± 18.28	0.1107
DBP (mm Hg)	93.48 ± 9.19	94.90 ± 8.44	0.1820

Table 2: Subject characteristics by genotype.

Population	β ± SE	*p*
Total		
SBP	2.9819 ± 1.4247	0.0368
DBP	1.5680 ± 0.8002	0.0505
Male		
SBP	0.9019 ± 2.2543	0.6895
DBP	1.1520 ± 1.3053	0.3784
Female		
SBP	4.1895 ± 1.8239	0.0222
DBP	1.7999 ± 1.0152	0.0770

Table 3: Regression results for mutant genotype (AC+CC) and multiple factors affecting BP After correction for age, height, weight, BMI

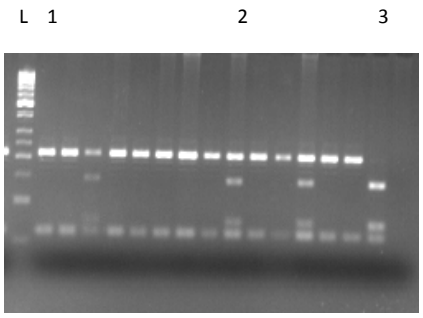

Figure 1: Genotyping of the AGT gene within A-20C polymorphism by *Eco 0109I* FRLP L, DNA size marker (50bp ladder). PCR fragments containing 1.wild type: AA (265bp) are digested in two fragments (205bp,60bp). 2. Heterozygotes: AC (470bp) PCR fragments are digested into four fragments (205bp、137 bp、68 bp、60bp). 3. Homozygous: CC (260bp) PCR fragments are digested into three fragments (137bp、68 bp、60 bp).

AGT and higher AGT levels in plasma due to AGT mutation would lead to increases in angiotensin II production, inducing hypertrophy of cardiac cells and an increase in heart contractility, indirectly leading to an increase in systolic BP. It is still not known why females have a higher incidence of this phenomenon, but it is possible that estradiol plays a role in regulating plasma AGT levels [10]. Transfection studies have revealed that A-20C mutation in the AGT promoter leads to higher AGT transcription. Therefore, A-20C mutation may lead to changes in plasma AGT levels [5,11]. Thus, we hypothesized that AGT A-20C mutation could lead to an increase in BP. Our results revealed differences in BP, especially systolic BP, for different AGT genotypes, although the difference was only significant for females, but only the regression results were significant; no actual differences in BP were significant. Even though no direct conclusions were possible, analysis of AGT-20 A-C polymorphism may further our understanding of EH and its prevention from an epidemiological point of view. Besides, as the study of pharmacogenomics advances, standardized personal antihypertensive therapy may be possible. Plasma AGT levels were not measured in the present study. Moreover, the genetic heterogeneity of hypertension, complicated genotypes, and artificial characteristics for the definition of hypertension, fluctuating blood pressure levels, and the effects of interactions between environmental and genetic factors on hypertension could have affected the study outcome. However, gene polymorphism and therapeutic efficacy do no correlate with patient prognosis, and this might be linked to other genetic loci, thus providing further opportunities for study. This is the first report on AGT-20 A-C polymorphism in a Chinese population. In the future, our aim is to identify further correlations between EH and genetic polymorphism-environmental interactions.

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