Angiotensin-Converting Enzyme Gene Insertion/Deletion (I/D) Polymorphism in Hypertensive Patients with Different Degrees of Obstructive Sleep Apnea

Jian ZHANG, Bin ZHAO, WESONGLUOBU, Yuhua SUN, Ying WU, Weidong PEI, Jue YE, Rutai HUI, and Lisheng LIU

To investigate the role of the angiotensin-converting enzyme gene (ACE) insertion (I)/deletion (D) polymorphism in hypertensive patients with different degrees of obstructive sleep apnea (OSA). A case-control study was performed. One hundred seventy four Chinese subjects were divided into four groups depending on the severity of OSA as follows: 1) normal control group (NC, n=68), 2) isolated hypertension group (HT, n=45), 3) hypertensive patients with mild OSA group (MO, n=27), and 4) hypertensive patients with moderate to severe OSA group (MSO, n=34). The distribution of ACE gene I/D allele and genotypes were analyzed in the subject population, as was an OSA pedigree. The study showed that the frequency of ACE gene I/D polymorphism differed significantly among the four groups. The frequency of I allele and II genotype were significantly higher in the MSO group than in the other groups (p<0.05). The distribution of I allele and II genotype showed no significant difference between any of the other groups (p>0.05, respectively). Meanwhile the higher frequency of I allele and II genotype was observed in the OSA pedigree. The higher frequency of ACE gene I allele and II genotype were closely associated with the hypertensive patients with MSO. The inherited factors played an important role in the pathogenesis of hypertensive patients with MSO. (Hypertens Res 2000; 23: 407-411)

Key Words: angiotensin converting enzyme polymorphism, obstructive sleep apnea, hypertension

Introduction

It is known that obstructive sleep apnea (OSA) and hypertension (HT) are common diseases in the middle-aged male population and in post-menopausal women, and the two disorders often co-exist in the same patient, in the same patient, with about 50% of OSA having HT and about 30% of HT patients having OSA (I). Cardiovascular events including stroke, acute myocardial infarction (AMI), fatal arrhythmia, and sudden death increase in patients with the two disorders (I-3). It is also known that renin-angiotensin converting enzyme gene (ACE) insertion (I)/deletion (D) polymorphism might be involved in the development of several cardiovascular diseases, but its role in human blood pressure regulation remains controversial (4). Plasma levels of angiotensin converting enzyme (ACE) in siblings have been reported to be similar, suggesting that there is a genetic influence (5). Further studies have demonstrated that there is a relationship between homozygous allele deletion of the ACE gene (DD genotype) and high plasma and tissue ACE activity (6). Several studies, however, have suggested that the angiotensin-converting enzyme system (RAS) was depressed in patients with OSA (I). Meanwhile, evidence of familial aggregation has been found in some OSA families, and
this facts suggest that the inheritance factor might play an important role in the pathogenesis of OSA (7). Accordingly, the present study has been undertaken to explore the association between the deletion (D)/insertion (I) polymorphism of the ACE gene and hypertensive patients who also have OSA.

**Method**

**Subjects and Classification**

One hundred-and-seventy-four eligible subjects were enrolled in the study in Beijing area from December 1995 to March 1998. Hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg and/or diastolic blood pressure (DBP) of ≥90 mmHg. Antihypertensive medications were withdrawn slowly over a period of 1 to 2 weeks. Clinical blood pressure (CBP) was measured on three different occasions, approximately 1 week apart. After the subjects rested quietly for 15 min, their CBPs measured once every minute for 3 min and averaged. Based on the results of the CBP measurement, the subjects were classified into either normotension or hypertension groups.

All the subjects then underwent consecutive polysomnography (PSG) monitoring. The subjects were allowed to perform their usual activities during the day, but after 10 PM bed rest was requested. During night (7 AM), PSG (Paradise 9600) was performed. Respiration was monitored with a thermistor at the nose and mouth to detect airflow and by a thoraco-abdominal strain belt to detect respiratory effort. Oximetry was recorded using a finger oximeter worn on the patient's first or second digit. According to the method of Rechtschaffen and Kales (8) electroencephalography (EEG), electro-oculography (EOG), and electromyography (EMG) were carried out to assess state of vigilance, e.g. wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. NREM sleep includes sleep stages I, II, III, and IV. To monitor circulation, electrocardiography (ECG) and heart rate (HR) were recorded continuously. Based on the results of the PSG, the subjects were further classified into either the non-OSA group, mild OSA group, or moderate to severe group. Apnea was defined as a cessation of airflow, as measured by a nasal/oral thermistor, for at least 10 s or more; hypopnea was defined as at least a 50% reduction of airflow for at least 10 s or more. At the same time, the apnea or hypopnea episodes were associated with at least a 4% drop in arterial oxyhemoglobin saturation or an arousal. Obstructive apnea (or hypopnea) was defined as the absence or reduction of airflow in the presence of rib cage and abdominal excursions. The hypopnea and apnea index (AHI) were established as the number of apneas + hypopneas per hour of sleep. 

The degree of the OSA was determined by AHI. The subjects with AHIs of less than 5 were considered to have no OSA; with AHIs between 5 to 20 were considered to have mild OSA; with AHIs between 21 to 50 were considered to have moderate OSA; with AHIs more than 50 were considered to have severe OSA.

Based on the results of the subjects’ CBPs and PSGs of the subjects, we divided the subjects into four groups as follows: normal control (NC); hypertension group (HT); hypertensive patients with mild OSA group (MO), and hypertensive patients with moderate to severe OSA group (MSO).

Body mass index (BMI) was calculated by dividing body weight (in kilograms) by the square of height (in meters).

**Experimental Procedures**

A blood sample was collected from each subject, and leukocyte DNA was extracted according to standard protocols (9). The I/D polymorphism of the ACE gene was detected by polymerase chain reaction (PCR) as described by Rigat et al. (10), and a Perkins-Elmer thermal cycler (model 9600) was used. The PCR primers were 5'-CTGGAGACCACCTCCCATCTTTCT-3' (sense primer) and 5'-GATGTGGCCATCACATTCGTCAGAT-3' (antisense primer). The PCR process involved denaturing at 95°C for 1 min, annealing at 57.5°C for 1 min, and extension at 72.5°C for 1 min. In total, 30 cycles with a final extension of 10 min were performed. The alleles I (490 bp) and D (190 bp) were determined by 2% agarose gel electrophoresis and ethidium bromide stains. Each sample in DD genotype was amplified twice in the presence of 5% dimethylsulphoxide that increases the specificity of the amplification reaction, since D fragment compared with the I fragment could lead to the erroneous classification of the I/D samples as DD (11).

**Statistical Analysis**

Data analysis was performed using a SPSS 7.5 statistical package. Variables are presented as means±SD. The χ² test was used to compare genotype distribution with the expectation of the alleles being in Hardy-Weinberg equilibrium. Unpaired Student's t-test was used for parametric comparison. A p value<0.05 was considered significant.

**Results**

*The Baseline Characteristics of the Subjects*

The sex, age, body mass index (BMI) AHI, sleep parameters and some biochemistry parameters are shown in Tables 1 and 2.
Results of PCR
The distribution of the polymorphism of the ACE gene is shown in Table 3.

Familial Aggregation of OSA
Figure 1 shows a pedigree of hypertension co-existing with moderate to severe OSA.

Discussion
The main findings of the current study are as follows: 1) The frequency of I allele and II genotype was significantly higher in the hypertensive patients with moderate and severe OSA than in the control. 2) There were no signif-
cant differences among the hypertensive patients with mild OSA, the HT group and the control. However, accompanying the decrease of the AHI, the frequency of I allele and II genotype decreased as well. 3) The pedigree of the hypertensive patients with moderate and severe OSA showed familial aggregation.

It is known that the renin-angiotensin system (RAS) plays an important role in blood pressure regulation and cardiovascular homeostasis. ACE is an important speed-limiting enzyme in RAS, which converts angiotensin I (Ang I) into angiotensin II (Ang II), with the concentration of Ang II in plasma depending mainly on ACE genotype (5, 6). Subjects with II genotype with the lowest Ace activity may also be prone to the lowest Ang II concentration, while in contrast, subjects with the DD genotype showing the highest Ace activity may be prone to the highest Ang II concentration (12). A meta-analysis (4) shows the role of the ACE D allele and DD genotype in blood pressure regulation remains controversial, but might be involved in the development of several cardiovascular diseases. The ECTIM study suggested that the ACE D allele was a risk factor for myocardial infarction (13). The PEGASE study demonstrated that the ACE D allele is associated with an increased risk of both cardiac and of cerebrovascular ischemic complications in a population of high-risk hypertensive individuals. The deleterious effect of the ACE D allele seen in the present study is compatible with the result reported by Morris et al. (14), showing that the prevalence of the ACE D allele declines with age in a population of severe hypertensives whose parents are both hypertensives. This decline could be explained in terms of the selective loss from the death of hypertensives carrying the D allele. In our study, we demonstrated that the ACE I allele and II genotype had higher prevalence in the hypertensive patients with moderate and severe OSA. Previous studies have demonstrated that plasma renin activity, angiotensin, and aldosterone are all suppressed in patients with OSA (15, 16). Our observation supports this notion. Recently, Xiao et al. (17) reported that OSA was related to the increased frequency of ACE gene I allele; the results were similar to ours. It is unknown whether patients with OSA who develop hypertension do so because of the insufficiency of the reduction of their RAS activity. Based on our results, we speculate that the elevation of the blood pressure of hypertensive patients with OSA may derive from a different mechanism. Meanwhile, the pedigree of the hypertensive patients with moderate and severe OSA in the study shows familial aggregation, and the II genotype also has a higher prevalence.

In conclusion, the higher frequency of ACE gene I allele and II genotype were closely associated with the hypertensive patients with moderate to severe OSA. The inherited factors played an important role in the pathogenesis of hypertensive patients with moderate to severe OSA.

Acknowledgements

We are grateful to Prof. Xu Shouchun, one of the mentors of Zhang Jian, for his guidance. We would also like to acknowledge Zhang Chunlin, and Zhu Xilin for their expert technical assistance.

References


