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Association of angiotensin-converting enzyme 2 gene A/G polymorphism and elevated blood pressure in Chinese patients with metabolic syndrome

JIAN ZHONG, ZHENGCHEN YAN, DAIYAN LIU, YINXING NI, ZHIGANG ZHAO, SHANJUN ZHU, MARTIN TEPEL, and ZHIMING ZHU

CHONGQING, P.R. CHINA

To establish whether angiotensin-converting enzyme 2 (ACE2) gene A/G single nucleotide polymorphism is associated with hypertension in Chinese patients with metabolic syndrome. The study was conducted in 353 patients with metabolic syndrome. The alleles of the ACE2 A/G polymorphism, which is located on the X chromosome, were detected using polymerase chain reaction and subsequent cleavage by Alu I restriction endonuclease. G allele frequencies in patients with metabolic syndrome were 36.6% in female subjects and 43.4% in male subjects, respectively. Female patients with metabolic syndrome who carry the GG genotype had a significantly higher diastolic blood pressure compared with other genotypes. Multivariate logistic regression showed that female gender (P = 0.019) and carrying only the G allele (odds ratio 2.83 [95% CI 1.36 to 5.91]; P = 0.005) were significantly associated with increased diastolic blood pressure. It is concluded that the ACE2 A/G polymorphism is associated with hypertension in patients with metabolic syndrome. (J Lab Clin Med 2006;147:91–95)

Abbreviations: ACE1 = angiotensin-converting enzyme type 1; ACE2 = angiotensin-converting enzyme type 2; ANOVA = analysis of variance; BMI = body mass index; CI = confidence interval; OR = odds ratio; PCR = polymerase chain reaction; SD = standard deviation; WHO = World Health Organization

ACE is a key enzyme in the renin-angiotensin system. ACE converts angiotensin I to angiotensin II, which is a potent vasoconstrictor, growth modulator, and proinflammatory peptide. Re- recently, the classic view of the renin-angiotensin system has been challenged by the discovery of the enzyme, ACE2, which is also known as the functional receptor of the SARS coronavirus.2–6 ACE2 has 42% homology with ACE1 at the metalloprotease catalytic domain, but it differs from ACE1 in having only one enzymatic site. ACE2 is a carboxypeptidase that converts angiotensin I into angiotensin-(1–9). It also converts angiotensin II into angiotensin-(1–7), which has vasodilatory, antiproliferative, and natriuretic effects.3,7–11 ACE2 transcripts have been identified in the heart, kidney, endothelial cells, and vascular smooth muscle cells.7,12,13 ACE2 has been shown to be involved in the pathogenesis of diabetic complications. In diabetic rats prone to diabetic nephropathy, the ACE2 protein expression was significantly reduced in renal tubules.14 In diabetic (db/db) mice, which showed obesity and hyperglycemia, but no nephropathy, an increased ACE2 protein expression was considered to be renoprotective.15 Furthermore, from several studies the hypothesis seems that
ACE2 modulates blood pressure in the mammalian organism. In the Sabra rat model of salt-sensitive hypertension ACE2, mRNA and protein levels are reduced in the hypertension-prone strain compared with the hypertension-resistant strain. ACE2 expression was also reduced in spontaneously hypertensive rats and spontaneously hypertensive stroke-prone rats compared with normotensive Wistar Kyoto rats. In addition, the upregulation of ACE2 by all-trans-retinoic acid reduced blood pressure in spontaneously hypertensive rats. These data indicate that reduced ACE2 is associated with elevated blood pressure probably due to reduced generation of vasodilatory angiotensin-(1–7).

Therefore, we hypothesized that ACE2 gene is a candidate gene for hypertension in patients with metabolic syndrome. One single nucleotide polymorphism has been found in intron 3 of the ACE2 gene and may affect protein function. The current study showed for the first time that patients with metabolic syndrome carrying the G allele of the ACE2 gene A/G polymorphism had an increased risk to develop hypertension.

SUBJECTS AND METHODS

Study population. This study was approved by the ethics committee of our hospital. Written informed consent was obtained from all participants. The study was performed as a cross-sectional study, in which 353 patients with metabolic syndrome were analyzed. The metabolic syndrome was defined according to the proposed Asia-Pacific criteria of the WHO 1999 Consultation on definition, diagnosis, and classification of diabetes mellitus and its complications. We classified subjects with the metabolic syndrome by WHO criteria according to the following schema: impaired fasting glucose and/or impaired glucose tolerance and/or insulin resistance and/or type 2 diabetes mellitus and two or more of the following: (1) blood pressure ≥ 140/90 mm Hg or treated hypertension; (2) central obesity, waist-hip-ratio > 0.9 for men and > 0.85 for women; or BMI > 25 kg/m²; (3) microalbuminuria ≥ 30 mg/24 h on at least two different occasions, or more advanced nephropathy; (4) plasma triglycerides ≥ 150 mg/dL (≥ 1.70 mmol/L) or HDL cholesterol < 35 mg/dL (< 0.9 mmol/L) for men and < 39 mg/dL (< 1.0 mmol/L) for women. Sitting blood pressure was measured twice to the nearest 2 mm Hg after a 5-minute rest using a standard mercury sphygmomanometer (phases I and V of Korotkoff). The mean value from three separate measurements was calculated for systolic and diastolic blood pressure. Hypertension was defined as blood pressure levels ≥ 140/90 mm Hg or the use of antihypertensive medication. BMI was calculated by weight divided by height squared. Each subject received a detailed interview about personal disease history and smoking history. All study subjects were of Han Chinese origin, without any known ancestors of another ethnic origin, and were living in the same region at the time of the study. All patients underwent complete physical examinations and routine biochemical analyses of blood and urine as well as an assessment of the presence and extent of macrovascular or microvascular diabetic complications. The anthropometric parameters required to calculate BMI and waist-to-hip ratio were measured. Plasma triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and glucose were determined by standard methods on a Beckman LX20 analyzer (Beckman Instruments, Inc., Fullerton, Calif).

ACE2 A/G polymorphism. Genomic DNA was prepared from peripheral blood. The A/G polymorphism at nucleotide 8790 in intron 3 was tested using PCR restriction fragment length polymorphism analysis. The primer pairs used and the annealing temperature were as follows: forward 5'-TTCTCCCTGCTCTATACCCG-3' and reverse 5'-TTCTTCATGTCTCTGTGGCCTTA-3', which amplify the intron 3 region where the A/G polymorphism is located. PCR amplification products were obtained using 25-μL reactions (0.5-pg genomic DNA, 500 pmol of primers, 0.5 mmol/L each of deoxy-ATP, -GTP, -CTP, and -TTP, 1.5-mmol/L MgCl₂, 0.5 units Taq DNA polymerase (Takara Bio. Inc. Japan), 50-mmol/L KCl, 0.001% gelatin, and 10-mmol/L Tris-HCl; pH 8.3) with 4 minute denaturation at 94°C, followed by 35 cycles of 50 seconds at 94°C, 50 seconds at 52°C, and 50 seconds at 72°C in a thermal cycler (PTC-200 Peltier Thermal cycler, MJ Research, Watertown, Mass). The reaction was terminated at 72°C for 10 minutes. The PCR products were digested for 4 hours at 37°C with Alu I endonuclease on a 1.2% agarose gel. Alleles of the A/G polymorphism of the ACE2 gene in nine patients with metabolic syndrome are shown. The expected products after digestion were 817 bp for GG homozygous, 589 bp and 228 bp for AA homozygous, and 817 bp, 589 bp, and 228 bp for GA heterozygous. M denotes marker.

Statistical analysis. Parametric data are expressed as means ± SD. Group differences of continuous variables were compared using unpaired the Student t-test or ANOVA as appropriate. The Bonferroni correction for multiple comparisons was applied. To assess the extent to which the allele frequencies and risk factors were associated with hypertension, we estimated ORs and the corresponding 95% CI by multiple logistic regression analysis using a stepwise approach. All tests were two-sided, and a P value less than 0.05 was considered statistically significant. All statistical analyses
were performed using the Statistical Package for Social Science program (SPSS for Windows, version 10.0; SPSS, Chicago, Ill).

RESULTS

The study was performed as a cross-sectional study, in which 353 patients of Han Chinese origin with metabolic syndrome were analyzed. Table I shows the clinical and biochemical characteristics of the patients with metabolic syndrome. Genotype and allele frequencies for the A/G polymorphism of the ACE2 gene are presented in Table II. G allele frequencies in patients with metabolic syndrome were 36.6% in female subjects and 43.4% in male subjects, respectively. As shown in Table III, the clinical and biochemical parameters of patients with metabolic syndrome were analyzed according to their genotype in women and in men. No significant differences in age, BMI, waist circumference, and waist-to-hip ratio according to genotype could be observed. In women carrying the GG genotype, the diastolic blood pressure was significantly higher compared with the GA or AA genotype (92 ± 3 mm Hg vs 84 ± 1 mm Hg or 84 ± 2 mm Hg; P < 0.01). In men who carry the G allele, the diastolic blood pressure was not significantly different compared with those who carry the A allele (87 ± 2 mm Hg vs 85 ± 2 mm Hg; P = n.s.).

To assess the extent to which the G allele and other risk factors were associated with hypertension, a multivariate logistic regression was performed using a stepwise approach. Multivariate logistic regression showed that female gender was significantly associated with diastolic hypertension. In addition, patients who carry the GG genotype had significantly higher diastolic blood pressure (OR 2.83 [95% CI 1.36 to 5.91]; P = 0.005; Table IV).

DISCUSSION

This study showed a strong association of the ACE2 gene A/G polymorphism at nucleotide 8790 in intron 3 to hypertension in female Chinese patients with metabolic syndrome. The metabolic syndrome is thought to be attributable to genetic predisposing factors in combination with environmental factors. Several candidate genes are involved in the metabolic syndrome, including genes for adrenergic receptors, lipoprotein lipase, peroxisome proliferator-activated receptor, and insulin receptor substrate-1.23 Now we show that female patients with metabolic syndrome who carry the GG genotype had significantly higher diastolic blood pressure. In male patients who carry the G allele, diastolic blood pressure was not significantly different compared with those patients who carry the A allele.

In the current study, hypertension was defined as blood pressure levels ≥ 140/90 mm Hg or the use of antihypertensive medication. Evaluation of blood pressure data may therefore underestimate the magnitude of blood pressure increase due to a certain genotype. However, a significant elevation of diastolic blood pressure could be observed in female patients who carry the GG genotype. Furthermore, a multivariate logistic regression analysis was performed using hypertension as dependent variable. This multivariate logistic regression showed that female gender and carrying the GG genotype represented a significant risk factor for hypertension in patients with metabolic syndrome, whereas elevated blood lipids did not show a significant association.

Several lines of evidence indicate that an impaired ACE2 function is related to hypertension probably because of the impaired generation of angiotensin-(1–7), which has vasodilatory and natriuretic effects.9 Studies in animal models showed that the rat ACE2 maps to a quantitative trait locus with a significant logarithm-of-the-odds score for hypertension in three models of hypertension—the Sabra salt-sensitive rat, the spontaneously hypertensive rat, and the stroke prone spontaneously hypertensive rat.2 In these hypertensive rats, both ACE2 mRNA and protein were significantly reduced. It has been proposed that the elevated blood pressure in these three strains of rats may result from the increase in angiotensin II and reduced angiotensin-(1–7) as a result of decreased ACE2 activity.24 However, it should be noted that mice with targeted deletion of the ACE2 gene develop heart failure and finally hypotension.2 One might suggest that the A/G polymorphism of the ACE2 gene may determine differences in structure or activity of the ACE2, thereby promoting hypertension. In addition, reduced ACE2 is associated with upregulation of hypoxia-inducible genes and com-

| Characteristic               | MS   |
|------------------------------|------|
| n (Male/Female)              | 353 (166/187) |
| Age (years)                  | 59.5 ± 0.8 |
| Waist circumference (cm)     | 89 ± 1 |
| Waist-to-hip ratio           | 0.94 ± 0.01 |
| Body mass index (kg/m²)      | 24.9 ± 0.2 |
| Systolic blood pressure (mm Hg) | 152 ± 1 |
| Diastolic blood pressure (mm Hg) | 86 ± 1 |
| Fasting blood glucose (mmol/L) | 10.1 ± 0.4 |
| Total cholesterol (mmol/L)   | 4.96 ± 0.08 |
| Triglycerides (mmol/L)       | 2.08 ± 0.14 |
| HDL-cholesterol (mmol/L)     | 1.23 ± 0.02 |
| LDL-cholesterol (mmol/L)     | 3.08 ± 0.06 |

BMI was calculated by weight divided by height squared. Data are mean ± SD.
pensatory responses including the apelin system. Poly-
morphisms of the ACE2 gene were associated with
familial predisposition to intracranial aneurysms in a
Japanese cohort, probably indicating its effect on vas-
cular modeling. The current study showing that fe-
male patients with metabolic syndrome who carry the
GG genotype have significantly higher diastolic blood
pressure is observational. The functional significance of
the polymorphism, eg, abnormal levels of downstream
metabolites of the renin-angiotensin system, is yet un-
known in these patients. The allele frequencies for the
G allele reported in the current study in Chinese pa-
tients with metabolic syndrome of Han Chinese origin
were slightly higher compared with the allele frequency
reported in Australian subjects of white Anglo-Celtic
origin. In that study, the allele frequency of the G
allele was about 18% in women and about 20% in men.
However, patients with metabolic syndrome were not
investigated in that study. As classifications of the
metabolic syndrome may vary among different societ-

### Table II. Genotype and allele frequency for ACE2 A/G polymorphism in patients with metabolic syndrome (MS)

| n     | GG (%) | GA (%) | AA (%) | χ²  | P  | G (%)  | A (%)  | χ²  | P  |
|-------|--------|--------|--------|-----|----|--------|--------|-----|----|
| Female| 187    | 26 (13.9)| 85 (45.5)| 76 (40.6)| 1.89| 0.39 | 137 (36.6)| 237 (63.4)| 1.94| 0.16 |
| Male  | 166    | —      | —      | —       | —   | —    | 72 (43.4)  | 94 (56.6) | 0.47| 0.50 |

As ACE2 is located on the X chromosome (one copy), it is inappropriate to present genotype data in male subjects.

### Table III. Clinical and biochemical parameters in patients with metabolic syndrome (MS) according to their ACE2 genotype in females or males according to their ACE2 A/G allele in males

#### Female

| MS     | n   | GG      | GA      | AA      | Age (years) | Waist circumference (cm) | Waist-to-hip ratio | Body mass index (kg/m²) | Systolic blood pressure (mm Hg) | Diastolic blood pressure (mm Hg) | Fasting blood glucose (mmol/L) | Total cholesterol (mmol/L) | Triglycerides (mmol/L) | HDL-cholesterol (mmol/L) | LDL-cholesterol (mmol/L) |
|--------|-----|---------|---------|---------|-------------|--------------------------|-------------------|--------------------------|-----------------------------|----------------------------|--------------------------|-----------------------|------------------------|------------------------|------------------------|
| Female | 26  | 26 (13.9)| 85 (45.5)| 76 (40.6)| 61.8 ± 2.0  | 91 ± 2                   | 0.96 ± 0.01       | 24.1 ± 1.0               | 159 ± 6                     | 92 ± 3**                   | 8.86 ± 0.81              | 5.25 ± 0.23            | 2.11 ± 0.24            | 1.40 ± 0.15            | 3.25 ± 0.21            |
|        | 76  |          |         |         | 62.1 ± 2.0  | 88 ± 1                   | 0.94 ± 0.01       | 25.2 ± 0.4               | 159 ± 3                     | 84 ± 1                     | 10.72 ± 0.69             | 5.23 ± 0.23            | 2.07 ± 0.23            | 1.34 ± 0.04            | 3.19 ± 0.15            |

#### Male

| MS     | n   | G      | A       | Age (years) | Waist circumference (cm) | Waist-to-hip ratio | Body mass index (kg/m²) | Systolic blood pressure (mm Hg) | Diastolic blood pressure (mm Hg) | Fasting blood glucose (mmol/L) | Total cholesterol (mmol/L) | Triglycerides (mmol/L) | HDL-cholesterol (mmol/L) | LDL-cholesterol (mmol/L) |
|--------|-----|--------|---------|-------------|--------------------------|-------------------|--------------------------|-----------------------------|----------------------------|--------------------------|-----------------------|------------------------|------------------------|------------------------|
| Male   | 72  | 72 (43.4)| 94 (56.6)| 59.0 ± 2.0  | 89 ± 2                   | 0.93 ± 0.01       | 25.3 ± 0.51              | 147 ± 3                     | 87 ± 2                     | 9.73 ± 0.79              | 4.53 ± 0.18            | 1.97 ± 0.25            | 1.06 ± 0.05            | 2.72 ± 0.14            |

*P < 0.01 vs GA or AA group.*
ies, further research is necessary to confirm that the current findings on ACE2 polymorphism also apply to non-Chinese populations.

In conclusion, in women, the presence of the GG allele of ACE2 gene and the presence of diastolic hypertension in patients with metabolic syndrome

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Table IV. Multivariate logistic regression analysis assessing the independent association of the G allele of ACE2 gene and the presence of diastolic hypertension in patients with metabolic syndrome

| OR (95%CI) | P |
|-----------|---|
| Age 0.98 (0.95–1.00) 0.084 | |
| Gender 0.43 (0.22–0.87) 0.019 | |
| BMI 1.06 (0.97–1.15) 0.200 | |
| Total cholesterol 0.83 (0.53–1.25) 0.370 | |
| Triglycerides 1.10 (0.91–1.32) 0.343 | |
| HDL-cholesterol 1.54 (0.59–4.03) 0.383 | |
| LDL-cholesterol 1.35 (0.81–2.24) 0.246 | |
| ACE2 polymorphisms, carrying G allele 2.83 (1.36–5.91) 0.005 | |

Gender indicates 0 = females and 1 = males.