Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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

JIAN ZHONG, ZHENGCHEN YAN, DAOYAN 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 index; 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.[1] 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

From the Center for Hypertension and Metabolic Diseases, Department of Hypertension and Endocrinology, Daping Hospital, Third Military Medical University, Chongqing, P.R. China.

Supported by Grant 30270537 from the National Natural Science Foundation of China.

Submitted for publication May 17, 2005; revision submitted August 21, 2005; accepted for publication October 5, 2005.

Reprint requests: Dr. Zhiming Zhu, Center for Hypertension and Metabolic Diseases, Department of Hypertension and Endocrinology, Daping Hospital, Third Military Medical University, Chongqing 400042, P.R. China; e-mail: zhuzm@yahoo.com.

0022-2143/$ – see front matter

doi:10.1016/j.lab.2005.10.001

ACE2 modulates blood pressure in the mammalian organism.[9] 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.[2] In addition, the upregulation of ACE2 by all-trans-retinoic acid reduced blood pressure in spontaneously hypertensive rats.[16] 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 nulceotide polymorphism has been found in intron 3 of the ACE2 gene and may affect protein function.[17,18] 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.[19,20] 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/m^2; (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.[21] 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).

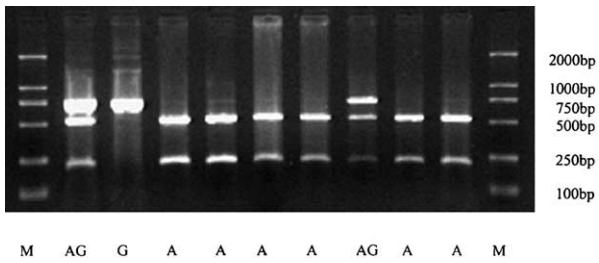

Fig 1. Electrophoretic separation of PCR fragments containing A/G polymorphic sequences after digestion with Alu I endonuclease on a 1.2% agarose gel. Alleles of the A/G polymorphism of the ACE2 gene in nine patients with metabolic syndrome are shown. The expected products after digestion were 817 bp 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.

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.[22] For the ACE2 A/G polymorphism, the primer pairs used and the annealing temperature were as follows: forward 5′-TTCTCCCTGCTCCTATACTACCG-3′ and reverse 5′-TTCATTCATGTCCTTGCCCTTA-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 $MgCl_2$, 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 enzyme, electrophoresed on a 1.2% agarose gel, and stained with ethidium bromide. The expected products after digestion were 817 bp for the G allel, 589 bp and 228 bp for the A allel, and 817 bp, 589 bp, and 228 bp for GA heterozygous (Fig 1). Genotyping was performed in a blinded fashion.

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

Table I. Clinical and biochemical characteristics of patients with metabolic syndrome (MS)

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/m^2)	24.9 ± 0.2
Systolic blood pressure (mm Hg)	152 ± 1
Diastolic blood presure (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.

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 risk for increased 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 regresssion 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-

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

		Genotype Frequencies					Allele Frequencies			
	n	GG	GA	AA	χ^2	*P*	G	A	χ^2	*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

	MS		
Female	**GG**	**GA**	**AA**
n	26	85	76
Age (years)	61.8 ± 2.0	62.4 ± 1.4	60.7 ± 1.3
Waist circumference (cm)	91 ± 2	88 ± 1	88 ± 1
Waist-to-hip ratio	0.96 ± 0.01	0.94 ± 0.01	0.95 ± 0.01
Body mass index (kg/m^2)	24.1 ± 1.0	25.2 ± 0.4	24.4 ± 0.5
Systolic blood pressure (mm Hg)	159 ± 6	159 ± 3	153 ± 3
Diastolic blood pressure (mm Hg)	92 ± 3**	84 ± 1	84 ± 2
Fasting blood glucose (mmol/L)	8.86 ± 0.81	10.72 ± 0.69	10.73 ± 0.93
Total cholesterol (mmol/L)	5.25 ± 0.23	5.23 ± 0.23	5.15 ± 0.14
Triglycerides (mmol/L)	2.11 ± 0.24	2.07 ± 0.23	1.84 ± 0.17
HDL-cholesterol (mmol/L)	1.40 ± 0.15	1.34 ± 0.04	1.31 ± 0.04
LDL-cholesterol (mmol/L)	3.25 ± 0.21	3.19 ± 0.15	3.24 ± 0.13
	MS		
Male	**G**	**A**	
n	72	94	
Age (years)	59.0 ± 2.0	55.8 ± 1.8	
Waist circumference (cm)	89 ± 2	89 ± 1	
Waist-to-hip ratio	0.93 ± 0.01	0.94 ± 0.01	
Body mass index (kg/m^2)	25.3 ± 0.51	25.1 ± 0.44	
Systolic blood pressure (mm Hg)	147 ± 3	146 ± 3	
Diastolic blood pressure (mm Hg)	87 ± 2	85 ± 2	
Fasting blood glucose (mmol/L)	9.73 ± 0.79	9.19 ± 0.66	
Total cholesterol (mmol/L)	4.53 ± 0.18	4.78 ± 0.14	
Triglycerides (mmol/L)	1.97 ± 0.25	2.40 ± 0.41	
HDL-cholesterol (mmol/L)	1.06 ± 0.05	1.13 ± 0.04	
LDL-cholesterol (mmol/L)	2.72 ± 0.14	3.02 ± 0.10	

*$P < 0.01$ vs GA or AA group.

pensatory responses including the apelin system.[8] Polymorphisms of the ACE2 gene were associated with familial predisposition to intracranial aneurysms in a Japanese cohort, probably indicating its effect on vascular modeling.[25] The current study showing that female 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 unknown in these patients. The allele frequencies for the G allele reported in the current study in Chinese patients with metabolic syndrome of Han Chinese origin were slightly higher compared with the allele frequency reported in Australian subjects of white Anglo-Celtic origin.[22] 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 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.

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 genotype of ACE2 polymorphism is associated with an increased risk for hypertension in Chinese patients with metabolic syndrome.

REFERENCES

1. Chua DY, Bakris GL. Clinical implications of blockade of the renin-angiotensin system in management of hypertension. Contrib Nephrol 2004;143:105–16.
2. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002;417:822–8.
3. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 2002;275:33238–43.
4. Carey RM, Siragy HM. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. Endocrinol Rev 2003;24:261–71.
5. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426:450–4.
6. Turner AJ, Hiscox JA, Hooper NM. ACE2: from vasopeptidase to SARS virus receptor. Trends Pharmacol Sci 2003;25:291–4.
7. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ Res 2000;87:E1–9.
8. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 2002;277:14838–43.
9. Yagil Y, Yagil C. Hypothesis. ACE2 modulates blood pressure in the mammalian organism. Hypertension 2003;41:871–3.
10. Rice GI, Thomas DA, Grant PJ, Turnerm AJ, Hooper NM. Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. Biochem J 2004;383:45–51.
11. Zhu Z, Zhong J, Zhu S, Liu D, van der Giet M, Tepel M. Angiotensin-(1–7) inhibits angiotensin II-induced signal transduction. J Cardiovasc Pharmacol 2002;40:693–700.
12. Zisman LS, Keller RS, Weaver B, Lin Q, Speth R, Bristow MR, et al. Increased angiotensin-(1–7)-forming activity in failing human heart ventricles evidence for upregulation of the angiotensin-converting enzyme homologue ACE2. Circulation 2003;108:1707–12.
13. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203:631–7.
14. Tikellis C, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvanis J, et al. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. Hypertension 2003;41:392–7.
15. Ye M, Wysocki J, Naaz P, Salabat MR, LaPointe MS, Batlle D. Increased ACE 2 and decreased ACE protein in renal tubules from diabetic mice: a renoprotective combination? Hypertension 2004;43:1120–5.
16. Zhong JC, Huang DY, Yang YM, Li YF, Liu GF, Song XH, Du K. Upregulation of angiotensin-converting enzyme 2 by all-trans retinoic acid in spontaneously hypertensive rats. Hypertension 2004;44:907–12.
17. Chiu RW, Tang NL, Hui DS, Chung GT, Chim SS, Chan KC, et al. ACE2 gene polymorphisms do not affect outcome of severe acute respiratory syndrome. Clin Chem 2004;50:1683–6.
18. Prabakaran P, Xiao X, Dimitrov DS. A model of the ACE2 structure and function as a SARS-CoV receptor. Biochem Biophys Res Commun 2004;314:235–41.
19. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetes Med 1998;15:539–53.
20. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of WHO consultation, Part 1: diagnosis and classification of diabetes mellitus. Geneva: WHO/NCD/NCS/99.2; 1999.
21. World Health Organization, International Society of Hypertension. Guidelines for the management of hypertension. Guidelines subcommittee. J Hypertens 1999;17:151–83.
22. Benjafield AV, Wang WY, Morris BJ. No association of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms with essential hypertension. Am J Hypertens 2004;17:624–8.
23. Groop L. Genetics of the metabolic syndrome. Br J Nutr 2004;83(suppl 1):S39–84.
24. Ferrario CM, Trask AJ, Jessup JA. Advances in the biochemical and functional roles of angiotensin converting enzyme 2 and angiotensin-(1–7) in the regulation of cardiovascular function. Am J Physiol Heart Circ Physiol 2005. [Epub ahead of print].
25. Yamada S, Utsunomiya M, Inoue K, Nozaki K, Inoue S, Takenaka K, et al. Genome-wide scan for Japanese familial intracranial aneurysms: linkage to several chromosomal regions. Circulation 2004;110:3727–33.