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Hypertension and hypertensive left ventricular hypertrophy are associated with ACE2 genetic polymorphism

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ABSTRACT

Aims: Renin-angiotensin system modulates cardiac structure independent of blood pressure. The present study aimed at investigating whether single nucleotide polymorphism (SNP) and haplotype of angiotensin converting enzyme 2 (ACE2) could influence blood pressure and the susceptibility to hypertensive left ventricular hypertrophy (LVH).

Subjects and methods: A total of 647 patients (347 females and 300 males) with newly diagnosed mild to moderate essential hypertension were enrolled in a blood pressure matched, case-control study. Four ACE2 tagSNPs (rs2074192, rs4646176, rs4646155 and rs2106809) were genotyped and major haplotypes consisting of these four SNPs were reconstructed for all subjects.

Key findings: In females, minor alleles of ACE2 rs2074192 and rs2106809 respectively conferred a 2.1 and 2.0 fold risk for LVH. ACE2 haplotype TCGT increased the risk for LVH while another haplotype CCGC decreased the risk in females. The covariates-adjusted mean left ventricular mass index was 11% greater in TCGT haplotype carriers than in noncarriers in women. In females, the covariates-adjusted mean systolic blood pressure was 3.4 mm Hg lower in CCGC haplotype carriers than in noncarriers. In males, the covariates-adjusted mean systolic blood pressure was 2.4 mm Hg lower in CCGC haplotype carriers than in noncarriers.

Significance: ACE2 tagSNPs rs2074192 and rs2106809 as well as major haplotypes CCGC and TCGT may serve as novel risk markers for LVH in hypertensive patients.

1. Introduction

Essential hypertension is a critical risk factor for worldwide cardiovascular disease morbidity and mortality [1]. It is a kind of multifactorial diseases which is attributable to both genetic and environmental factors. Although some aggressive interventions have been taken to reduce the influence of hypertension, a large percentage of patients are still unable to achieve recommended blood pressure control [2]. Therefore, a novel treatment strategy, genetic factors and precision medicine, should be considered to improve the control and management of hypertension.

The activated renin-angiotensin system (RAS) plays an important role in the occurrence and development of essential hypertension. Recently, a novel component in RAS, angiotensin converting enzyme 2 (ACE2), has been reported to counterbalance the effect of activated RAS and provides a new insight into the treatment of hypertension [3,4]. ACE2 is a kind of human angiotensin converting enzyme (ACE) homologue. Although ACE2 has considerable homology to human ACE (40% identity and 61% similarity), it is profoundly different from ACE in physiological function [3,4]. As a carboxypeptidase, ACE2 converts angiotensin II to angiotensin-(1-7) [Ang-(1-7)], which binds with high affinity to the Mas receptor to exert a variety of beneficial effects, including antifibrosis, antihypertrophy and vasodilatation [3–5]. Angiotensin II, in turn, downregulates ACE2 activity and induces deleterious cardiovascular effects [6].

Left ventricular hypertrophy, a common form of target organ damage in patients with essential hypertension, predicts the long-term incidence of cardiovascular events [7]. Essential hypertension modulates left ventricular structure through hemodynamic factors including blood pressure and nonhemodynamic factors including renin-angiotensin system (RAS). The involvement of ACE2 in cardiac remodeling has been confirmed by previous studies [8–11]. The genetic deletion or pharmacological inhibition of ACE2 exacerbates cardiac hypertrophy and myocardial dysfunction [8,9] while ACE2 overexpression ameliorates left ventricular remodeling and dysfunction [10]. Ang II-induced myocardial hypertrophy and fibrosis were exacerbated by ACE2

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deficiency whereas recombinant human ACE2 (rhACE2) attenuated Ang II– and pressure-overload–induced adverse myocardial remodeling [11]. Another critical RAS component Ang-(1-7) also prevents the development of cardiac remodeling. Antagonist Ang-(1-7) A779 prevented the antihypertrophic effects of rhACE2 [12]. Infusion of Ang-(1-7) attenuates myocyte hypertrophy and interstitial fibrosis [5,13], which is not mediated through modulation of blood pressure [13].

Heritability accounted for 67% of the phenotypic variation in circulating ACE2 [14]. Thus, genetic variants of ACE2 gene have a potential to influence the occurrence and development of cardiovascular diseases. In fact, more and more studies on this subject have been performed and reported [15–18]. A number of studies conducted in different races and ethnicities revealed that genetic variants of ACE2 were related to essential hypertension, coronary heart disease and some other cardiovascular events [15–18]. A research reported that the relationship might be due to the effect of ACE2 genetic variants on RAS hormone level [19]. It is reasonable to speculate that ACE2 genetic variants may also affect the cardiac structure.

Thus, we hypothesized that genetic factors in ACE2 gene might influence the susceptibility to hypertensive left ventricular hypertrophy. To test the hypothesis, we performed a blood pressure matched, double-blind, case–control studies and aimed at investigating the association of ACE2 single nucleotide polymorphism (SNP) and haplotype with blood pressure level and the risk for LVH in untreated hypertensive patients.

2. Subjects and methods

2.1. Study population

In this clinical study both male and female Chinese Han patients who met the following criteria were included: 18 years ≤ age ≤ 79 years; patients with newly diagnosed essential hypertension (without any antihypertensive medication); 140 ≤ systolic blood pressure (SBP) ≤ 179; 90 ≤ diastolic blood pressure (DBP) ≤ 109. The exclusion criteria were as follow: secondary hypertension, like known renal artery stenosis and idiopathic hyperaldosteronism; congestive heart failure; a documented history of unstable angina pectoris within the past 6 months or myocardial infarction within the past year; clinically important cardiac arrhythmia; valvular heart disease; uncontrolled diabetes mellitus; any clinically important abnormal laboratory finding, such as serum aspartate transaminase or creatinine level twice the upper limit of normal; a history or suspicion of alcohol or drug abuse; pregnancy or lactation in women; high-salt intake (estimation of the amount of daily salt intake > 6 g); the use of any agent that may cause an alteration of blood pressure and RAS hormone levels, such as direct renin inhibitors, angiotensin converting enzyme inhibitor, Ang II receptor blockers, aldosterone antagonists, beta blockers, diuretics and calcium channel blockers. The study complies with the Declaration of Helsinki. All procedures were reviewed and approved by the institutional review board of each participating institution (Ethical approval number: Ethic Committee of the First Affiliated Hospital of Shantou University No.2018013), and written informed consents were obtained from all subjects.

2.2. Echocardiographic measurement

Two skilled physicians independently measured the transthoracic echocardiographic parameters of all subjects using a Vivid 7 ultrasound system (GE Vingmed, Horten, Norway) with a 2.5-MHz probe. If the measurements of the echocardiographic parameters differed by > 10%, a third physician reevaluated the echocardiograms. The measurement was done blindly with respect to other clinical data and, in particular, to the genotyping of all subjects. Left ventricular end-diastolic diameter (LVEDD), septal wall thickness (SWT) and posterior wall thickness (PWT) were measured according to recommendations of the American Society for Echocardiography. Left ventricular mass (LVM) was calculated with the formula $1.04 \times [(\mathrm{LVEDD} + \mathrm{SWT} + \mathrm{PWT})^3 - (\mathrm{LVEDD})^3] - 14$ as described by Devereux [20]. LVMI was calculated as LVM in grams divided by body surface area in square meters. LVH was defined as LVMI ≥ 120 g/m^2 in men and LVMI ≥ 110 g/m^2 in women.

2.3. TagSNPs selection and haplotype reconstruction

The CHB (Han Chinese in Beijing) SNP database was downloaded from International HapMap Project website (NIH DHHS USA.gov Copyright, available at: http://hapmap.ncbi.nlm.nih.gov/) and the SNP data was analyzed by tagger software Haploview (version 4.2; Daly Lab, Broad Institute Cambridge, MA, USA, available at: https://www.broadinstitute.org/haploview/). Four tagSNPs (rs2074192, rs4646176, rs4646155 and rs2106809) were selected because they could capture the information of 25 ACE2 SNPs with a minor allele frequency equal to or > 1% in the HapMap database and Ensembl project (European Molecular Biology Laboratory, European Bioinformatics Institute, Cambridge, UK, available at: http://www.ensembl.org/). We used the program PHASE (version 2.1; University of Washington, Seattle, USA, available at: http://stephenslab.uchicago.edu/software.html) to reconstruct haplotypes. The program PHASE implements a Bayesian statistical method to reconstruct haplotypes from population genotype data.

2.4. DNA extraction and genotyping

DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification kit (Promega, WI, USA) according to the standard protocol. ACE2 genotyping was performed by direct sequencing after polymerase chain reaction (PCR) amplification of DNA. PCR primers were designed using Primer3 software (Biology Workbench version 3.2; San Diego Supercomputer Center, University of California, San Diego, CA, USA, available at: http://www.simgene.com/Primer3). The primer pairs used were as follows:

rs2074192
  Forward primer: 5′- GTAAGCCATTTCCCATCCC-3′;
  Reverse primer: 5′- ATTGTGCCACTGCCCTCTA-3′;
rs4646176
  Forward primer: 5′- GTTCTGGGGTATGGAGTCA-3′;
  Reverse primer: 5′- TTCAGGAAAGGTAAGGGGG-3′;
rs4646155
  Forward primer: 5′- AAAGTAGGTTGGGGTTGTG-3′;
  Reverse primer: 5′- CATTCAGATGGGTGTAGGG-3′;
rs2106809
  Forward primer: 5′- GCAGAGAAAGGGAGAGAAC-3′;
  Reverse primer: 5′- TTGGGTGAGGCTGGACTTG-3′;

The amplified PCR purpose fragment was purified by Wizard PCR Preps DNA Purification Resin (Promega, WI, USA) and then sequenced using the BIG DYE dideoxy-terminator chemistry (Perkin-Elmer, CA, USA) on an ABI 3100 DNA sequencer.

2.5. Statistic analysis

Females and males were analyzed separately since the ACE2 gene is located on the X chromosome. Allele frequencies were calculated according to the genotypes of all patients. Hardy–Weinberg equilibrium was assessed by chi-square-test. Continuous data were presented as mean ± standard deviation. Chi-square-test was used to compare qualitative parameters and one-way analysis of variance was used to compare quantitative parameters between groups. The associations of SNPs and haplotypes with LVH were analyzed using a multivariate logistic regression, adjusted by conventional cardiovascular risk factors, and were expressed as an OR and 95% CI. The associations of SNPs and

Table 1
Clinical characteristics of all subjects.

Characteristics	Females (n = 347)		Males (n = 300)	
	LVH	Control	LVH	Control
Number	162	185	127	173
Age (year)	61.6 ± 9.5	58.3 ± 8.9*	58.0 ± 8.9	55.8 ± 9.1*
HR (beats/min)	78.7 ± 7.9	77.2 ± 5.9*	79.5 ± 8.4	77.5 ± 5.7*
SBP (mm Hg)	162.9 ± 9.7	161.6 ± 9.2	163.9 ± 9.2	158.6 ± 9.9*
DBP (mm Hg)	96.5 ± 5.1	95.7 ± 5.0	98.0 ± 5.8	94.6 ± 5.5*
BMI (kg/m2)	24.8 ± 3.6	23.8 ± 3.3*	25.3 ± 3.4	24.2 ± 3.2*
Creatinine (umol/L)	72.6 ± 15.4	68.6 ± 14.6*	90.7 ± 18.9	82.6 ± 15.2*
Sodium (mmol/L)	143.5 ± 1.7	143.9 ± 1.6*	143.6 ± 2.4	144.0 ± 2.3
Potassium (mmol/L)	3.73 ± 0.53	3.68 ± 0.36	3.75 ± 0.41	3.77 ± 0.33
Uric acid (umol/L)	252.7 ± 53.4	218.4 ± 43.4*	277.0 ± 53.0	257.9 ± 48.7*
AST (U/L)	31.3 ± 6.8	29.8 ± 3.7*	31.0 ± 4.1	30.7 ± 5.3
FBG (mmol/L)	6.2 ± 1.4	5.8 ± 1.0*	5.5 ± 1.1	5.3 ± 1.0*
HDL-C (mmol/L)	1.04 ± 0.26	1.18 ± 0.27*	0.91 ± 0.22	1.00 ± 0.24*
LDL-C (mmol/L)	2.61 ± 0.83	2.49 ± 0.92	2.45 ± 0.80	2.56 ± 1.06
TG (mmol/L)	1.58 ± 0.92	1.27 ± 0.63*	1.30 ± 0.70	1.32 ± 0.66

LVH, left ventricular hypertrophy; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI: body mass index; AST, aspartate transaminase; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides. Data are expressed as mean ± standard deviation (SD).

* $P < 0.05$.

haplotypes with blood pressure were analyzed using a linear regression modeling, adjusted by conventional cardiovascular risk factors. A two-tailed P-value of < 0.05 was considered statistically significant. All analyses were performed using SPSS statistical software (Version 13.0; SPSS, Chicago, Illinois, USA).

3. Results

3.1. Baseline characteristics

In total, 647 patients (347 females and 300 males) with essential hypertension were enrolled in this case-control study. There were 289 hypertensive patients with LVH and they were enrolled in LVH group. A total of 358 hypertensive patients without LVH were enrolled in control group. In female subjects, age, heart rate, body mass index, creatinine, plasma sodium, uric acid, aspartate transaminase, fasting blood glucose, and triglycerides were significantly higher while high-density lipoprotein cholesterol was significantly lower in LVH group as compared with control group (Table 1). In males, age, heart rate, body mass index, creatinine, uric acid, fasting blood glucose, SBP and DBP were significantly higher while high-density lipoprotein was significantly lower in LVH group as compared with control group (Table 1). Genotype/allele frequencies for ACE2 tagSNPs rs2074192, rs4646176, rs4646155 and rs2106809 were listed in Table 2. The genotype frequencies of ACE2 SNPs did not deviate from that predicted by Hardy–Weinberg equilibrium (Table 2). The analysis by Haploview showed that rs4646155 and rs4646176 were in strong LD (linkage disequilibrium) ($D' = 0.87$ and $r2 = 0.73$) in the study population. ACE2 rs2074192 and rs2106809 were also in the same LD block ($D' = 0.95$ and $r2 = 0.62$). The haplotype reconstruction using Phase software suggested that there were 2 major haplotypes CCGC and TCGT with frequencies > 0.10.

3.2. Association of LVH with ACE2 tagSNPs and common haplotypes

In female patients, Chi-square test showed that frequencies of rs2074192 and rs2106809 minor allele were significantly higher in LVH group as compared with control group (Table 2). After adjustment for common risk factors including age, SBP, heart rate, fasting blood glucose, low-density lipoprotein, high-density lipoprotein and uric acid by multivariate logistic regression analysis, ACE2 rs2074192 and rs2106809 minor alleles respectively conferred 2.1 and 2.0 fold risks for LVH in females (both $P < 0.05$, Table 3). As for rs4646155 and rs4646176, the analysis did not find any significant association between their genotype/allele distributions and LVH in female subjects (Table 2 and Table 3).

In male subjects, Chi-square test did not found any influence of ACE2 tag SNPs on the risk for LVH (Table 2). We could not observe any significant association between these SNPs and the risk for LVH in males even after adjustment for common risk factors by multivariate logistic regression analysis (Table 3).

As for haplotype analysis, a multivariate logistic regression analysis was performed to analyze the relationship between ACE2 haplotypes and the risk for LVH. In female patients, a common haplotype TCGT significantly increased the risk for LVH in a dominant model or an additive model (Table 3) while another common haplotype CCGC significantly decreased the risk for LVH in an additive model (Table 3). The covariates-adjusted mean left ventricular mass index (LVMI) was 11% greater in female subjects with TCGT haplotype than in those with other haplotypes ($P < 0.001$, ANCOVA). The covariates-adjusted mean SWT were 5% thicker in female subjects with TCGT haplotype than in those with other haplotypes ($P < 0.001$, ANCOVA). In male patients, no statistically significant association between ACE2 haplotypes and the risk for LVH could be observed (Table 3).

3.3. Association of blood pressure with ACE2 tagSNPs and common haplotypes

Multivariate linear regression analysis indicated that rs2074192 minor allele T allele significantly increased SBP in females (Table 4). In female subjects, the haplotype analysis suggested that ACE2 CCGC haplotype significantly decreased SBP in both dominant model and additive model ($P < 0.01$, Table 4). Mean SBP in female carrying CCGC haplotype was 3.4 mm Hg lower than mean SBP in females carrying other haplotypes after adjustment for covariates including age, heart rate, low-density lipoprotein, high-density lipoprotein, creatinine, fasting blood glucose, potassium and sodium by analysis of covariance ($P < 0.01$, ANCOVA). In males, multivariate linear regression analysis indicated that CCGC was negatively associated with DBP while TCGT haplotype was positively associated with both SBP and DBP (Table 4). Mean SBP in males carrying CCGC haplotype was 2.4 mm Hg lower while mean SBP in males carrying TCGT haplotype was 2.6 mm Hg higher as compared with males carrying other haplotypes after adjustment for covariates including age, heart rate, low-density

Table 2
Genotype and allele distribution of ACE2 tagSNPs.

Group	Genotype			P[a]	Allele		OR (95% CI)
	MM	Mm	mm		M	m	
rs2106809	CC	CT	TT		C	T	
Females							
LVH (n = 162)	60 (37.0%)	73 (45.1%)	29 (17.9%)	0.41	191 (59.7%)	129 (40.3%)*	1.213 (1.003–1.447)
Control (n = 185)	89 (48.6%)	73 (39.5%)	23 (12.4%)	0.13	251 (67.8%)	119 (32.2%)	
Males							
LVH (n = 127)	–	–	–	–	71 (55.9%)	56 (44.1%)	1.251 (0.944–1.657)
Control (n = 173)	–	–	–	–	112 (64.7%)	61 (35.3%)	
rs4646155	GG	GA	AA		G	A	
Females							
LVH (n = 162)	155 (95.7%)	7 (4.3%)	0 (0.0%)	0.78	317 (97.8%)	7 (2.1%)	0.444 (0.188–1.050)
Control (n = 185)	168 (90.8%)	16 (8.6%)	1 (0.5%)	0.37	352 (95.1%)	18 (4.9%)	
Males							
LVH (n = 127)	–	–	–	–	125 (98.4%)	2 (1.6%)	0.389 (0.082–1.842)
Control (n = 173)	–	–	–	–	166 (97.6%)	7 (2.4%)	
rs4646176	CC	CG	GG		C	G	
Females							
LVH (n = 162)	152 (93.8%)	10 (6.2%)	0 (0.0%)	0.69	314 (96.9%)	10 (3.1%)	0.816 (0.367–1.811)
Control (n = 185)	172 (93.0%)	12 (6.5%)	1 (0.5%)	0.14	356 (96.2%)	14 (3.8%)	
Males							
LVH (n = 94)	–	–	–	–	123 (97.9%)	4 (2.1%)	0.778 (0.233–2.602)
Control (n = 126)	–	–	–	–	166 (97.6%)	7 (2.4%)	
rs2074192	CC	CT	TT		C	T	
Females							
LVH (n = 162)	44 (27.2%)	76 (46.9%)	42 (25.9%)	0.43	164 (50.6%)	160 (49.4%)*	1.186 (1.008–1.397)
Control (n = 185)	69 (37.3%)	78 (42.2%)	38 (20.5%)	0.07	216 (58.4%)	154 (41.6%)	
Males							
LVH (n = 127)	–	–	–	–	69 (53.3%)	58 (46.7%)	1.162 (0.891–1.515)
Control (n = 173)	–	–	–	–	105 (60.7%)	68 (39.3%)	

ACE2, angiotensin converting enzyme 2; LVH, left ventricular hypertrophy; OR, odds ratio; 95% CI, 95% confidence interval. MM = (homozygous) common allele, Mm = heterozygous, mm = (homozygous) minor allele.

[a] P values of Hardy-Weinberg Equilibrium test.

* $P < 0.05$ vs control, analyzed by chi-square test or Fisher's exact test.

Table 3
The odd ratios of ACE2 haplotypes and SNPs for LVH by multivariate logistic regression analysis.

Sex		N (frequency)	Dominant model		Additive model	
			OR (95% CI)	P value	OR (95% CI)	P value
Females						
Haplotype	CCGC[a]	369 (0.53)	0.720 (0.417–1.241)	0.237	0.679 (0.493–0.936)	0.018
	TCGT[a]	241 (0.35)	2.203 (1.340–3.623)	0.002	1.536 (1.084–2.178)	0.016
	TCGC[a]	45 (0.06)	1.188 (0.591–2.388)	0.629	–	–
SNP	rs2074192 T		2.094 (1.249–3.512)	0.005	1.488 (1.072–2.066)	0.017
	rs4646176 G		1.084 (0.434–2.707)	0.862	1.015 (0.428–2.403)	0.974
	rs4646155 A		0.521 (0.199–1.365)	0.184	0.517 (0.202–1.321)	0.168
	rs2106809 T		2.029 (1.235–3.333)	0.005	1.553 (1.100–2.193)	0.012
Males						
Haplotype	CCGC[a]	166 (0.55)	0.991 (0.592–1.660)	0.974	–	–
	TCGT[a]	110 (0.37)	1.184 (0.690–2.031)	0.541	–	–
	TCGC[a]	10 (0.03)	0.391 (0.078–1.950)	0.252	–	–
SNP	rs2074192 T		1.161 (0.689–1.957)	0.575	–	–
	rs4646176 G		0.682 (0.180–2.589)	0.574	–	–
	rs4646155 A		0.319 (0.061–1.670)	0.176	–	–
	rs2106809 T		1.328 (0.782–2.256)	0.294	–	–

ACE2, angiotensin converting enzyme 2; LVH, left ventricular hypertrophy; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval. ACE2 haplotypes and SNPs were considered as a predictor of LVH after adjustment for the influencing factors including age, blood pressure, heart rate, fasting blood glucose, uric acid, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol.

[a] ACE2 rs2074192-rs4646176-rs4646155-rs2106809.

lipoprotein, high-density lipoprotein, creatinine, fasting blood glucose, potassium and sodium by analysis of covariance ($P < 0.05$, ANCOVA).

4. Discussion

Left ventricular hypertrophy is a common form of target organ damage in hypertensive patients. To evaluate the effect of hereditary factors of ACE2 gene on the susceptibility to LVH, we designed and performed a blood pressure matched, case-control study. The present study selected 4 tagSNPs, which captured a total of 25 ACE2 SNPs and reflected the mutation information from 3′ untranslated regions (3′UTR) to intron 16. The analysis defined only one block and two major haplotypes in the study region. The present study is the first study to investigate the relationship between ACE2 genetic variants and

Table 4
The association of blood pressure with ACE2 haplotypes and tagSNPs by linear regression analysis.

Group		Systolic blood pressure				Diastolic blood pressure			
		β[a]	P[a]	β[b]	P[b]	β[a]	P[a]	β[b]	P[b]
Females									
Haplotype	CCGC[c]	−0.166	0.001	−0.143	0.006	−0.024	0.645	−0.059	0.258
	TCGT[c]	0.047	0.370	0.093	0.076	0.001	0.988	0.024	0.648
SNP	rs2074192 T	0.068	0.194	0.141	0.006	0.073	0.160	0.060	0.253
	rs4646176 G	0.081	0.117	0.062	0.230	0.064	0.213	0.066	0.199
	rs4646155 A	0.071	0.173	0.053	0.309	0.016	0.753	0.021	0.684
	rs2106809 T	0.047	0.363	0.089	0.091	0.020	0.706	0.031	0.552
Males									
Haplotype	CCGC[c]	−0.095	0.076	–	–	−0.108	0.049	–	–
	TCGT[c]	0.107	0.049	–	–	0.110	0.046	–	–
SNP	rs2074192 T	0.071	0.192	–	–	0.100	0.071	–	–
	rs4646176 G	0.086	0.111	–	–	0.051	0.358	–	–
	rs4646155 A	0.096	0.072	–	–	0.067	0.221	–	–
	rs2106809 T	0.102	0.058	–	–	0.104	0.058	–	–

ACE2, angiotensin converting enzyme 2; SNP, single nucleotide polymorphism. ACE2 haplotypes and SNPs were considered as a predictor of blood pressure after adjustment for the influencing factors including age, heart rate, creatinine, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fasting blood glucose, potassium and sodium. The β values were standardized in the model.

[a] Dominant model.

[b] Additive model.

[c] ACE2 rs2074192-rs4646176-rs4646155-rs2106809.

Table 5
ACE2 SNPs selected for study and captured SNPs in linkage disequilibrium.

TagSNPs	Tagged SNPs	Allele	Region	r^2	D′
rs4646155	rs4646140	G:A	Intron 6	1.0	1.0
	rs4646144	G:A	Intron 7	1.0	1.0
	rs4646155	G:A	Intron 8	1.0	1.0
	rs61433707	C:T	Intron 14	1.0	1.0
rs4646176	rs879922	C:G	Intron 11	0.83	1.0
	rs2301692	T:C	Intron 12	1.0	1.0
	rs2301693	G:A	Intron 12	1.0	1.0
	rs4646171	A:G	Intron 14	1.0	1.0
	rs4646174	C:G	Intron 14	1.0	1.0
	rs4646176	C:G	Intron 14	1.0	1.0
	rs1514281	T:C	Intron 16	1.0	1.0
	rs1514282	A:G	Intron 16	1.0	1.0
	rs1514283	A:G	Intron 16	1.0	1.0
	rs4240157	A:G	Intron 16	0.83	1.0
	rs4646174	C:G	Intron 14	0.83	1.0
	rs4830542	C:T	3′UTR	0.83	1.0
	rs1132186	A:C	3′UTR	0.83	1.0
	rs58967744	T:G	3′UTR	1.0	1.0
rs2106809	rs2106809	C:T	Intron 1	1.0	1.0
	rs2285666	T:C	Intron 3	0.90	0.96
	rs4646142	G:C	Intron 7	0.90	0.96
	rs2097723	T:C	3′UTR	0.81	0.99
	rs1356037	G:C	3′UTR	0.81	0.99
rs2074192	rs2074192	C:T	Intron 16	1.0	1.0
	rs714205	C:G	Intron 16	0.81	1.0

SNP, single nucleotide polymorphism; tagged SNPs, captured SNPs by tagSNPs. Data were got from HapMap CHB database and Ensembl project.

the risk for hypertensive left ventricular hypertrophy.

Besides hemodynamic change, endocrine regulation was another important contributor to the pathogenesis of LVH in patients with essential hypertension. The suppression of RAS independently contributed to the magnitude of LVH regression after eliminating the interference of blood pressure [21,22]. Additionally, a previous study suggested that a deletion polymorphism in ACE gene was related with LVH even after considering the interference of blood pressure [23]. Since the above studies showed that RAS had an effect on cardiac structure independent of blood pressure, we assumed that ACE homologue ACE2 was independently associated with LVH. Therefore, we designed this case-control study to evaluate whether hereditary factors of ACE2 gene were associated with the LVH independent of alteration of blood pressure. In order to eliminate the influence of blood pressure, we set a strict inclusion criterion about blood pressure to include blood pressure matched hypertensive patients as a control group. In females, blood pressure in LVH group was comparable to that in control group whereas in males, blood pressure was still slightly higher in LVH group than in its control group. Thus, the interference of BP had been minimized in the present study.

In females, we found that ACE2 rs2074192 and rs2106809 T allele and TCGT haplotype increased the susceptibility to LVH. In fact, two latest studies have reported that ACE2 genetic variants were associated with the alteration of cardiac structure [15,16]. In Uygurs, ACE2 SNPs rs2048683, rs4240157, rs4646156, rs4646188 and rs879922 were linked to heavier LVMI [15]. In North Indian ethnicity, prevalence of ACE2 (7160726 C > G) variant genotypes (CG and GG) was significantly higher in subjects with dilated cardiomyopathy as compared to controls [16]. The above studies suggest that genetic factors in ACE2 gene may be involved in the alteration of cardiac structure in different ethnic groups. ACE2 rs2106809 T allele, a risk marker for LVH in our study, has been found to confer a 1.6-fold risk for hypertension in women [18]. It seems that rs2106809 T allele not only increases the susceptibility to hypertension but also contributes to the target organ damage in hypertensive females.

The present study could not provide a pathophysiological explanation for the observed association. However, a study by Chen found that females carrying rare alleles of ACE2 rs2106809 and rs2074192 (T allele) had lower circulating levels of Ang-(1-7) as compared with non-carriers [19]. We speculate that rs2106809 or rs2074192 or another SNP in the same LD block would down-regulate the ACE2 expression and thereby decrease level of Ang-(1-7). The lack of antihypertrophic effects of Ang-(1-7) will contribute to myocardial hypertrophy. Since rs2106809 and rs2074192 are in strong LD block with many other SNPs (Table 5), it is still unknown which is the functional SNP. According to Ensembl project, rs2285666 may be a strong candidate for the functional SNP because it is located in a splice region of ACE2 gene. Another candidate would be rs2106809 because the bioinformatics analysis indicated that intronic SNP rs2106809 might create an intronic exonic splicing enhancer (ESE) site to influence the splicing efficiency of ACE2 [24].

In the present study, ACE2 major haplotypes CCGC and TCGT were associated with blood pressure, which was consistent with the study by Zhao [25]. Zhao found that two major haplotypes TTCGCCCGGT and

TTTCCCCGGC of ACE2 SNPs rs1514283-rs1514282-rs2074192-rs714205-rs4646176-rs4646174-rs879922-rs4646155-rs4646140-rs2285666 were associated with SBP response to low-sodium intervention in a Chinese population [25]. In fact, haplotype TTCGCCCGGT in Zhao's study and haplotype CCGC in our study are probably identified as the same haplotype in most of the subjects while TTTCCCCGGC and TCGT are probably identified as the same one. Although the selected SNPs were different between Zhao's and our study, the LD relationship in ACE2 gene could be helpful to identify their relationship. According to the LD relationship (Table 5) and the same ethnicity in Zhao's and our's study, ACE2 rs1514283, rs1514282, rs4646174, rs879922 and rs4646176 were in complete linkage. ACE2 rs4646155 and rs4646140 were also in complete linkage. In addition, ACE2 rs2285666 and rs2106809 were in strong LD block (Table 5). Therefore, we speculate that the two blood pressure-related haplotypes in Zhao's study and the major haplotypes in our study are likely identified as the same haplotypes in most of the subjects. These two independent studies suggested that there were two blood pressure-related haplotypes in ACE2 gene in Chinese Han ethnicity.

We observed significant associations of ACE2 SNPs and haplotypes with the risk for LVH in females. We believed the results were reliable because we had used a blood pressure matched population as a control group and further analyzed the data using a multivariate regression model to adjust the other confounding variables including blood pressure. In addition, the study population was homogenous with a similar living environment, similar dietary habits (Cantonese dishes with characteristics of low-salt diet) and the same ethnicity (Chinese Han population). However, the association could not be observed in males, which was partly due to the mismatch of blood pressure between LVH group and control group in males. Another plausible explanation was that ACE2 gene was located in X chromosome so each male only had one allele and one haplotype while each female owned two. Therefore, with the same sample size, males would own only one half of the number of allele and haplotype as compared with females, which would decrease the test efficiency in males. The final possibility was that this effect did only exist in females but not in males because sexual differences in RAS had been confirmed in previous study [26,27].

There are several limitations should be stated. Firstly, we did not provide evidences for the pathophysiological explanation for the effects of ACE2 haplotypes and SNPs in the present study. Secondly, the sample size of male subjects should be enlarged to confirm the absence of the effect in males. Thirdly, the present study could not accurately estimate the intake of salt and just exclude the high-salt intake patients by rough estimate. Fourthly, since a large percentage of the subjects with essential hypertension are elderly, part of the study population in our research is geriatrics.

5. Conclusion

In conclusion, ACE2 tagSNPs rs2074192 and rs2106809 as well as major haplotypes CCGC and TCGT are associated with blood pressure and LVH, suggesting 4 risk markers for target organ damage in hypertensive patients.

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Author contributions

Zhimin Fan, Guihai Wu, Minghui Yue and Xuerui Tan conceived and designed the research; Zhimin Fan, Jianfeng Ye, Yequn Chen, Bayi Xu, Zhouwu Shu, Jinxiu Zhu and Nan Lu performed the experiments; Zhimin Fan, Xuerui Tan and Yequn Chen analyzed the data; Zhimin Fan and Zhouwu Shu contributed reagents/materials/analysis tools; Zhimin Fan wrote the paper. All authors read and approved the final manuscript.

Conflict of interest

The authors declared no conflict of interest.

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