

BMJ Open Association of ACE gene D polymorphism with left ventricular hypertrophy in patients with diastolic heart failure: a case-control study

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ABSTRACT

Objectives: To explore the association between ACE gene insertion/deletion (I/D) polymorphism with left ventricular hypertrophy (LVH) in patients with hypertension who have developed heart failure with preserved ejection fraction (HFpEF). Being a major contributor to the development of diastolic heart dysfunction, the renin angiotensin aldosterone system and its genetic variations are thought to induce LVH in hypertensive hearts apart from haemodynamic factors.

Design: Case control study.

Setting: An Iranian referral university hospital.

Participants: 176 patients with hypertension and a diagnosis of HFpEF on presence of symptoms of heart failure plus Doppler echocardiographic documentation of left ventricular (LV) diastolic dysfunction and/or elevated NT-proBNP levels. Those with significant coronary, valvular, pericardial and structural heart diseases were excluded as well as patients with atrial fibrillation, renal failure and pulmonary causes of dyspnoea. They were divided into two cohorts of 88 cases with and 88 controls without LVH, after determination of LV mass index, using two-dimensional and M-mode echocardiography. The I/D polymorphism of the ACE gene was determined using the PCR method.

Results: The D allele was significantly more prevalent among cases with compared with controls without LVH ($p=0.0007$). Genotype distributions also differed significantly under additive ($p=0.005$, OR=0.53, 95% CI 0.34 to 0.84) and recessive ($p=0.001$, OR=0.29, 95% CI 0.13 to 0.66) models.

Conclusions: In patients with hypertension who develop HFpEF, the D allele of the ACE gene is probably associated with the development of LVH. With the detrimental effects of LVH on the heart's diastolic properties, this can signify the role of genetic contributors to the development of HFpEF in patients with hypertension and may serve as a future risk predictor for the disease.

INTRODUCTION

Hypertension is one of the most important risk factors of atherosclerotic diseases.¹ It is

Strengths and limitations of this study

- In this study, we genotyped ACE insertion/deletion polymorphism in 176 patients with hypertension who had developed heart failure with preserved ejection fraction (HFpEF).
- This study made comparison of genotype distribution between cases with and controls without left ventricular hypertrophy for the first time in a population of patients with HFpEF.
- The study included women as well as men, however, women outnumbered men partly because HFpEF is more prevalent among women.
- A diagnosis of HFpEF was made on robust criteria with measurements of left ventricular diastolic properties carried out using echocardiography, and NT-proBNP levels. The study could thus recruit 176 patients fulfilling the diagnostic criteria.

shown to be associated with the development of heart failure with preserved ejection fraction (HFpEF), a syndrome of heart failure (HF) with evidence of abnormal left ventricular (LV) diastolic function. Formerly known as diastolic HF (DHF), it represents about 50% of patients diagnosed with HF and carries morbidity and mortality risk as high as those associated with HF and reduced ejection fraction.² At 5 years, the cumulative mortality rate is reported to be 65% for patients with HFpEF with an adjusted HR of 1.48 when compared with persons with no HF and a normal LV ejection fraction (LVEF). Besides being associated with a high incidence of systemic hypertension (70–88%), HFpEF is highly prevalent in older and obese patients and in females.³

The diastolic function of the human heart is closely related to haemodynamic and humoral factors. Neurohormonal alterations underlying the development and progression

of hypertension can thus have significant impact on myocardial active relaxation and passive stiffness, the pathophysiological underpinnings of elevated diastolic pressure and abnormal diastolic function.^{4 5} The renin angiotensin aldosterone system (RAS) is one of the key players. Angiotensin II, as the major effector of RAS, exerts powerful vasoconstrictor and trophic effects, and is shown to be involved in mediating deleterious consequences of hypertension.⁶ It is known to participate in profibrotic mechanisms by influencing extracellular matrix composition through attenuating the expression of matrix metalloproteinases (MMPs), enhancing the endogenous tissue inhibitor of MMP-1 and inducing expression of connective tissue growth factor.^{7 8} It can also stimulate myocyte hypertrophy via paracrine release of transforming growth factor and endothelin-1 from fibroblasts.⁹

There are several reports that genetic variations in RAS play a major role in the development of LV hypertrophy (LVH) in hypertensive hearts beyond what is expected from the chronic pressure overload alone.^{10–12} Insertion/deletion (I/D) polymorphism of the *ACE* gene is the most extensively studied and has been shown to be associated with increased LV mass in both, normotensive and hypertensive populations,^{13–15} patients with diabetes, patients with chronic kidney disease¹⁶ and those with hypertrophic cardiomyopathy.¹⁷ While genetic determinants of HFpEF are not extensively studied and there is no direct report targeting genetic variations by employing genome-wide association studies (GWAS),¹⁸ genetic variants affecting neurohormonal regulation of blood pressure (BP) and associated LV mass are thus eligible candidates, knowing that LVH is considered as a critical contributor to LV diastolic dysfunction.⁴ Concordantly, Wu has reported that the DD genotype of the *ACE* gene can predispose an individual to DHF in a case-control study of the Chinese population.¹⁹ Furthermore, *ACE* gene I/D polymorphism has been related to baseline muscular strength and power in older adults, indicating its role in overall physical performance and functional capacity, which is seriously limited in patients with HFpEF.²⁰

In patients with a history of hypertension, with detrimental effects of increased LV mass on myocardial stiffness and diastolic heart filling, genetic determinants of RAS function might play a role in progression to HFpEF through development of LVH. This prompted us to test the hypothesis that the D allele of the *ACE* gene is associated with an increased LV mass in patients with hypertension with DHF.

MATERIALS AND METHODS

Study design, setting and participants

This was a single-centre case-control study of patients with a diagnosis of HFpEF from the cardiovascular ward and clinic of Fasa University Hospital. A total of 231 patients with hypertension with clear clinical

presentations of HF and normal or near normal LV systolic function were prospectively identified. A single cardiologist examined all the patients and non-invasive assessment of LV dysfunction during diastole was performed. A diagnosis of HFpEF was made based on the criteria described elsewhere, after ruling out other non-cardiac causes of HF symptoms.²¹ Doppler and tissue Doppler echocardiography was used to measure mitral inflow early rapid filling wave (E) and mitral annular early diastolic (E') velocities, respectively. In cases with $E/E' > 15$, the diagnosis of HFpEF was made regardless of NT-proBNP levels. For those with $8 < E/E' < 15$, NT-proBNP level of more than 220 pg/mL was used to make the diagnosis. Those with a medical history of significant coronary artery disease (CAD; 18 men, 4 women), myocardial infarction (MI; 2 men), significant valvular disease (1 man), secondary hypertension (1 man), hepatic and renal impairment (4 men, 2 women), and atrial fibrillation (9 men, 14 women), were excluded because of impact on the study variables. Finally, 176 patients were selected and divided into two groups: cases with LVH defined as LV mass index (LVMI) $> 115 \text{ g/m}^2$ for men and $\text{LVMI} > 95 \text{ g/m}^2$ for women, and risk factor-matched control group without LVH.²² Demographic data and medical history were recorded at the time of echocardiography, and laboratory data were collected from their medical chart records.

Echocardiography

Measurements of LV end diastolic diameter, LV end systolic diameter, LV septal and LV posterior wall thickness, and left atrial diameter at end diastole were carried out in M-mode parasternal long axis view. Image acquisition was repeated three times and an average was calculated. In cases with suboptimal M-mode acquisition, measurements in two-dimensional views were obtained instead. Measurements of ventricular E wave, peak velocity of late filling wave (A), E/A, E wave deceleration time and mitral annular early diastolic velocity were carried out according to American Society of Echocardiography guidelines²³ with the use of a 1–5 MHz PA transthoracic echocardiography probe, Kontron. LVMI was calculated using the Devereux *et al*²² formulae. LVEF was estimated by an eyeballing method, and Doppler and colour Doppler studies identified patients with at least moderate aortic or mitral stenosis/regurgitation as significant valvular diseases.

DNA extraction and genotyping

After obtaining informed consent, 3–4 mL of venous blood samples of all selected patients were collected in (EDTA) containing tubes.

Genomic DNA was extracted using a salting-out method. Obtained DNA was dissolved in TE (10 mM Tris, 1 mM EDTA, PH=8) and stored at -20°C until PCR analysis. Detection of *ACE* I/D polymorphism was carried out using a PCR method described

previously.²⁴ The primers were as follows: forward 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse 5'-GATGTGGCCATCACATTCGTCAGAT-3'. The PCR reaction was carried out in a total volume of 25 µL containing 50–200 ng of template DNA, 10 µM of each primer, 2.5 µL 10X PCR buffer (Gene Fanavaran, Iran), 2 mM MgCl₂, 200 µM each dNTP and 0.5 units of Taq DNA polymerase (Gene Fanavaran, Iran). The PCR profiles were as follows: initial denaturation at 94°C for 5 min and then 35 cycles of denaturation at 94°C for 40 s, annealing at 60°C for 60 s and extension at 72°C for 60 s followed by a final elongation at 72°C for 5 min. In order to avoid mistyping ID as DD genotype, each sample found to have the DD genotype was reconfirmed by another PCR with insertion-specific primers (forward 5'-TGGGACCACAGCGCCCGCCACTAC-3' and reverse 5'-TCGCCAGCCCTCCCATGCCATAA-3') as previously described.²⁵

Statistical analysis

All continuous variables are presented as means±SD, and differences between groups were determined using Student t test. Pearson's χ^2 tests were applied to test for significance in differences of genotype and allele frequencies between the two groups. A p value of <0.05 (two tailed) was considered to be significant. The Hardy-Weinberg equilibrium was performed using Fisher's exact test. We also analysed the distribution of genotype frequencies under three different genetic models (additive (D/D=0, I/D=1 and I/I=2), recessive (I/D and D/D vs I/I) and dominant (I/I and I/D vs D/D)), using the SNPAssoc package of R V.3.0.1. (<http://www.Rproject.org>).²⁶ All other data were also analysed using R V.3.0.1.

RESULTS

A total of 176 patients were included. Eighty-eight individuals with LVH and 88 without LVH. Demographic and

laboratory data of participants in case and control groups are listed in table 1. There were no significant differences in age, body mass index (BMI), systolic and diastolic BP, diabetes mellitus status, smoking status, serum creatinine, low-density lipoprotein, high-density lipoprotein and total cholesterol levels between cases and controls. Demographic and laboratory data are also presented according to each genotype (see online supplementary table S1). Eighty-three per cent of patients with LVH were woman, compared with 58% of those without LVH ($p<0.001$). Patients with LVH had lower haemoglobin (12.7 ± 1.5 vs 13.3 ± 1.6 , $p=0.03$) and higher fasting blood sugar (145.3 ± 64.1 vs 127.5 ± 68 , $p=0.07$) levels compared with those without LVH. Echocardiography data are shown in table 2. Genotype distribution and allele frequencies differ significantly between the two groups ($p=0.0007$), where the D allele was found to be more prevalent among patients with LVH (table 3). The genotype difference between groups was significant under additive ($p=0.005$, OR=0.53, 95% CI 0.34 to 0.84) and recessive ($p=0.001$, OR=0.29, 95% CI 0.13 to 0.66) models (table 4). Allele frequencies were still significantly different between the two groups after adjustment for age, sex, BMI, and systolic and diastolic BP (table 3).

DISCUSSION

With the shortage of available evidence on the potential contributors to HFpEF, we postulated that genetic factors might impose greater risk of diastolic heart dysfunction in patients with hypertension by mediating development of LVH. In the present study, we showed that the D allele of the *ACE* gene is associated with an increased LV mass in an Iranian population with hypertension and a diagnosis of HFpEF.

To the best of our knowledge, our report is the first to show an association between a genetic polymorphism and LVH in a patient population with a diagnosis of

Table 1 Demographic and laboratory data

Variable	All (N=176)	Without LVH (N=88)	With LVH (N=88)	p Value
Age	62.5±12.6	61.70±13.26	63.3± 12.01	0.35
BMI (kg/m ²)	26.41±5.47	26.78±5.52	26.04± 5.43	0.37
Sex (F/M)	124/52	51/37	73/15	<0.001
Smoking (%)	13.5	14	13	0.82
DM (%)	35	30	40	0.15
SBP (mm Hg)	143.2±27.2	141.2±26.9	145.3± 27.4	0.32
DBP (mm Hg)	84.5±11.4	84.5±12.4	84.4± 10.5	0.97
HB (g/dL)	13.0±1.6	13.3±1.6	12.7± 1.5	0.03
Cr (mg/dL)	1.12±0.33	1.09±0.30	1.16±0.35	0.20
FBS (mg/dL)	136.4±66.4	127.5±68	145.3±64.1	0.07
TG (mg/dL)	163.7±85.2	166.6±99.3	160.8±68.7	0.65
LDL (mg/dL)	114.9±32.8	112.9±33.4	117.0±32.3	0.40
HDL (mg/dL)	39.9±8.8	39.9±8.9	39.9±8.7	0.97

BMI, body mass index; Cr, creatinine; DBP, diastolic blood pressure; DM, diabetes mellitus; F, female; FBS, fasting blood sugar; HB, haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LVH, left ventricular hypertrophy; M, male; SBP, systolic blood pressure; TG, triglyceride.

Table 2 Echocardiography data

	All (N=176)	Without LVH (N=88)	With LVH (N=88)	p Value
IVS (cm)	1.21±0.17	1.13±0.17	1.29±0.14	<0.001
LVPW (cm)	1.19±0.17	1.10±0.17	1.27±0.13	<0.001
LVEDD (cm)	4.18±0.67	3.87±0.57	4.50±0.64	<0.001
LVMI (g/m ²)	104.90±35.01	80.34±19.05	129.49±29.69	<0.001
LA diameter (cm)	3.50±0.56	3.38±0.61	3.62±0.48	0.003
LVEF	0.55±0.06	0.56±0.04	0.55±0.08	0.04
E (m/s)	0.64±0.21	0.62±0.18	0.67±0.24	0.20
A (m/s)	0.85±0.21	0.85±0.17	0.86±0.25	0.78
E/A	0.77±0.29	0.74±0.21	0.81±0.35	0.17
Sm (cm/s)	7.41±2.01	7.72±2.05	7.11±1.95	0.045
E' (cm/s)	6.61±1.46	6.77±1.43	6.47±1.48	0.16
A' (cm/s)	10.10±2.16	10.26±1.97	10.21±1.98	0.30
E/E'	10.18±3.79	9.53±3.01	10.83±4.37	0.024
DT (ms)	184.7±63.07	191.81±58.18	177.60±67.20	0.13

A, peak velocity of late filling wave; A', mitral annular late diastolic velocity; DT, deceleration time; E, mitral inflow early diastolic velocity; E', mitral annular early diastolic velocity; IVS, interventricular septum; LA, left atrium; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; LVPW, left ventricular posterior wall diameter; Sm, systolic mitral annular velocity.

HFpEF. Studies targeting genetic contributors of DHF are scarce. While genes contributing to DHF risk in humans still await identification,²⁷ our results may exemplify one such attempt and corroborate the role of genetic factors in susceptibility of hypertensive hearts to develop HFpEF. However, one limitation of this study is that 70% of participants are women. Although HFpEF is more prevalent among women,² there is no epidemiological study reporting the female-to-male ratio of Iranian patients with HFpEF. Therefore, future studies targeting men with hypertension with HFpEF is warranted. Furthermore, as this was a case-control study, determination of the exact duration of hypertension in the patients was not feasible. Although it was their first documentation of HFpEF and symptom presentation, these patients likely has hypertension undiagnosed or untreated over a longer time period. This problem needs to be addressed in a cohort study of healthy individuals without hypertension, with frequent and long enough follow-ups to detect the development of hypertension and consequent HFpEF.

As one of the end organ damages associated with hypertension, LVH identifies with a poor outcome and strongly predicts MI, stroke and cardiovascular death in patients with hypertension.²⁸ Mohammed *et al*²⁹ studied

autopsy findings and reported that patients with HFpEF have more cardiac hypertrophy, coronary microvascular rarefaction and fibrosis compared with age-matched controls. The observed increased incidence of HFpEF in patients with hypertension is related to LVH and only adequate control of BP reduces progression from hypertension to HF.³⁰ Antihypertensive medications are shown to reduce LV chamber stiffness along with cardiac hypertrophy, which eventually leads to improved LV diastolic filling.^{28 31 32}

An increased LV mass, a common finding in patients with diastolic dysfunction,^{4 29} is a consequence of prolonged pressure overload in patients with hypertension, however, neurohormonal alterations and genetic determinants are also known to be involved.^{33 34} Activation of RAS as a major contributor to the pathophysiology of hypertension leads to vasoconstriction and sodium and fluid retention.³ Angiotensin II, the end product of RAS, has trophic influences on cardiomyocytes³⁵ and increases collagen synthesis as well by means of activating metalloproteases.³⁶ In an animal model of DHF, Yamamoto *et al*³⁷ found that RAS contributes to the transition to DHF through the development of excessive hypertrophy and ventricular fibrosis in hypertensive heart disease. Intracrine mechanisms for Ag II are also

Table 3 Distribution of genotypes and allele frequencies between patients with and without LVH

	Genotype frequencies (%)					Allele frequencies (%)			
Patients	I/I	I/D	D/D	p Value	Adjusted p value*	I	D	p Value	Adjusted p value*
With LVH	9 (12.3)	50 (59)	29 (28.7)	0.007†	0.004†	68 (38.6)	108 (61.4)	0.007†	0.016
Without LVH	25 (35.2)	43 (38.9)	20 (25.9)			93 (52)	83 (47)		

*p Value adjusted for sex, age, BMI, systolic and diastolic blood pressure.

†Considered as significant.

BMI, body mass index; I/D, insertion/deletion; LVH, left ventricular hypertrophy.

Table 4 Analysis of genotype distributions under three genetic models

	Additive (D/D=0, I/D=1 and I/I=2)	Recessive (I/D and D/D vs I/I)	Dominant (I/I and I/D vs D/D)
p Value	0.005*	0.001*	0.12
OR	0.53	0.29	0.60
95% CI	0.34 to 0.84	0.13 to 0.66	0.31 to 1.17
Adjusted p value†	0.012*	0.002*	0.34

*Considered as significant.

†p Value adjusted for sex, age, BMI, systolic and diastolic blood pressure. BMI, body mass index; I/D, insertion/deletion.

described. Baker *et al* showed that intracellular expression of Ag II peptide leads to hypertrophic growth of rat cardiomyocytes without an increase in BP or in serum Ag II levels. The resulting hypertrophy and fibrosis associated with Ag II action then produces a non-compliant LV chamber with diminished ability of active relaxation, which is thought to be the pathophysiological underpinning of DHF.³⁸

Among genetic polymorphisms of different components of RAS, I/D polymorphism of the *ACE* gene is the most extensively studied and is shown to be associated with CAD, MI, stroke and depression. We have previously, in the Iranian population, shown that the D allele is also associated with CAD in depressed patients.³⁹ The observed association of the D allele of the *ACE* gene with LVH in patients with hypertension with HFpEF in this study suggests the role of genetic factors in inducing hypertrophy and diastolic dysfunction. Previous reports have suggested the role of the D allele in the development of LVH in patients with hypertension. Gharavi *et al*¹¹ demonstrated that the D allele of the *ACE* gene, independently of other covariates, is associated with cardiac mass and relative wall thickness in patients with hypertension. Concordantly, Celentano *et al*¹⁴ concluded that the DD genotype is a genetic marker of LVH in systemic hypertension. However, there is conflicting evidence as well debating the association of the D allele with LVH in a large Framingham study population, and in Chinese patients with hypertension.^{12 40}

Being under the influence of genetic determinants, RAS activation may lead to cardiac hypertrophy and the resulting increased LV mass can be viewed as a marker of progression to HFpEF in patients with hypertension. Besides, there are reports that older adult carriers of the D allele of the *ACE* gene have a greater physical performance level in a 6 min walk test compared with those with a II genotype.²⁰ This is consistent with our findings and implicates the muscular hypertrophic role of the D allele in augmenting muscular mass in adults alongside inducing cardiac hypertrophy. It may have implications in clinical assessment of HFpEF and affect the severity of symptoms; an issue that needs to be addressed in future studies.

CONCLUSION

The observed increased likelihood of LVH in carriers of the D allele with hypertension and HFpEF in our study

strengthens the proposition that inheritance of the D allele can increase the risk of developing HFpEF in patients with hypertension. Such genetic determinants could potentially have important therapeutic indications as well as risk stratifying capabilities in future.

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Contributors EB designed the study, performed all the echocardiographic examinations, was involved with data interpretation and supervised the analysis, and wrote and made the final review of the manuscript. MR performed the laboratory work and PCR, and was involved with data interpretation. JJ was involved with the study design, set up the PCR and laboratory work, carried out the interpretation and analysis, and helped in writing the manuscript's methods section. SMM, MZ and AM assisted with clinical data gathering, laboratory work and PCR. NF was involved with the study design, and assisted with analysis and in writing the manuscript.

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Table 1. Demographic and laboratory data in each Genotype

Variable	All (N=176)	DD	ID	II	P-value
Age	62.5 ± 12.6	63.67 ± 13.16	61.4 ± 12.40	64.32 ± 12.6	0.40
BMI (Kg/m ²)	26.41 ± 5.47	26.37 ± 5.2	26.8 ± 5.68	25.19 ± 5.19	0.31
Sex (F/M)	124/52	40/9	61/32	23/11	0.12
Smoking (%)	13.5	8	14	23	0.04
DM (%)	35	38	33	35	0.89
SBP (mmHg)	143.2 ± 27.2	141.1 ± 23.3	145.6 ± 29.0	139.8 ± 27.4	0.45
DBP (mmHg)	84.5 ± 11.4	83.9 ± 9.8	85.0 ± 11.0	83.9 ± 14.8	0.82
HB (g/dL)	13.0 ± 1.6	12.9 ± 1.6	13.0 ± 1.6	13.0 ± 1.7	0.92
Cr (mg/dL)	1.12 ± 0.33	1.1 ± 0.25	1.17 ± 0.37	1.05 ± 0.30	0.17
FBS (mg/dL)	136.4 ± 66.4	125.4 ± 31.3	145.9 ± 82.8	126.19 ± 18.4	0.13
TG (mg/dL)	163.7 ± 85.2	148.6 ± 54.7	172.9 ± 94.9	160.3 ± 91.8	0.26
LDL (mg/dL)	114.9 ± 32.8	114.7 ± 36.6	117.6 ± 29.4	108.0 ± 35.5	0.34
HDL (mg/dL)	39.9 ± 8.8	40.0 ± 9.6	39.9 ± 8.5	39.97 ± 8.81	0.99

BMI: body mass index, DM: diabetes mellitus, SBP: systolic blood pressure, DBP: diastolic blood pressure, HB: hemoglobin, Cr: creatinine, FBS: fasting blood sugar, TG: triglyceride, LDL: low density lipoprotein, HDL: high density lipoprotein