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ORIGINAL ARTICLE

Plasma heparin cofactor II activity is inversely associated with left atrial volume and diastolic dysfunction in humans with cardiovascular risk factors

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Thrombin has a crucial role in cardiac remodeling through protease-activated receptor-1 activation in cardiac fibroblasts and cardiomyocytes. As heparin cofactor II (HCII) inhibits the action of tissue thrombin in the cardiovascular system, it is possible that HCII counteracts the development of cardiac remodeling. We investigated the relationships between plasma HCII activity and surrogate markers of cardiac geometry, including left atrial volume index (LAVI), relative wall thickness (RWT) and left ventricular mass index, and deceleration time (DcT) and the ratio of peak E velocity to early diastolic mitral annulus velocity (E/e' ratio) as surrogate markers of left ventricular diastolic dysfunction measured using echocardiography in 304 Japanese elderly individuals without systolic heart failure (169 men and 135 women; mean age: 65.4 ± 11.8 years). Mean plasma HCII activity in all participants was 95.8 ± 17.0% and there was no difference between the mean plasma HCII activities in males and females. Multiple regression analysis revealed that there were significant inverse relationships between plasma HCII activity and LAVI (coefficient: −0.2302, $P<0.001$), between HCII activity and RWT (coefficient: −0.0007, $P<0.05$), between HCII activity and DcT (coefficient: −0.5189, $P<0.05$) and between HCII activity and E/e' ratio (coefficient: −0.0558, $P<0.01$). Plasma HCII activity was independently and inversely associated with the development of cardiac remodeling, including cardiac concentric change, left atrial enlargement and left ventricular diastolic dysfunction. These findings suggest that cardiac tissue thrombin inactivation by HCII is a novel therapeutic target for cardiac remodeling and atherosclerosis.
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Keywords: diastolic dysfunction; heparin cofactor II; left atrial volume; thrombin

INTRODUCTION

Thrombin acts as a pivotal enzyme for generating fibrin clots and activates platelets, vascular endothelial cells, vascular smooth muscle cells, macrophages and fibroblasts to enhance procoagulation,[1] chemoattraction,[2] mitogenesis[3] and proliferation[4] of these cells. Thrombin exerts its physiological and pathological actions in these cells through the proteolytic processing of specific cell-surface receptors known as protease-activated receptors (PARs).[5,6] Nelken *et al.*[7] demonstrated that PAR-1 was widely expressed in regions where macrophages, cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells and mesenchymal-appearing intimal cells are abundantly present. Thus, excessive PAR-1 activation promotes cardiovascular disorders, including cardiac remodeling, arterial thrombosis and atherosclerosis. From the point of view of PAR-1 modulation in cardiovascular remodeling, the use of antithrombin agents and antagonists of the thrombin receptor might be useful approaches for the treatment and prevention of these disorders. Antithrombin (AT), a major serine protease inhibitor (serpin), inhibits thrombin action at the intravascular lumen and it exerts its optimal AT actions by binding to heparan sulfate and heparan sulfate proteoglycans on the luminal surface of endothelial cells. In addition, heparin cofactor II (HCII), which is also a serpin, is a plasma glycoprotein with a molecular weight of 65.6 kDa that is synthesized by the liver and circulates in plasma at a concentration of 1.0 μmol l^{-1}. HCII potently inhibits thrombin action by forming a bimolecular complex with dermatan sulfate proteoglycans under the endothelial layer in mammalians.[8,9] Although AT inhibits not only the thrombin action, but also the actions of several proteases involved in blood coagulation or fibrinolysis, HCII only inactivates thrombin and has no inhibitory effect on the action of any other proteases. As HCII can counteract the actions of thrombin at injured vascular walls, we, and others, have investigated and confirmed the protective role of HCII

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against atherosclerosis in clinical examinations and studies using HCII-deficient mice.[9–15]

As the development of vascular remodeling, including atherosclerosis, has been shown to be closely associated with cardiac remodeling in humans and experimental animal models, we hypothesized that HCII is involved in the process of not only atherosclerosis, but also cardiac remodeling. In order to clarify this issue, we investigated the relationships between plasma HCII activity and surrogate markers with respect to cardiac remodeling in elderly subjects with cardiovascular risk factors. We found that plasma HCII activity is inversely associated with cardiac remodeling in subjects without systolic heart failure (HF).

METHODS

Subjects for cross-sectional study

We consecutively recruited 304 Japanese subjects (169 males and 135 females) who were outpatients with lifestyle-related diseases and subjects older than 35 years of age were recruited consecutively from the Department of Medicine and Bioregulatory Sciences and Department of Cardiovascular Medicine at Tokushima University Hospital, Tokushima, Japan between April 2007 and September 2009. All subjects underwent a standardized interview and a physical examination. Current smokers were defined as subjects who had smoked within the past year. Body mass index was calculated as an index of obesity. Blood pressure was measured twice and averaged. Hypertensive patients were defined as those with systolic blood pressure (SBP) $\geqslant$140 mm Hg and/or diastolic blood pressure (DBP) $\geqslant$90 mm Hg or those receiving antihypertensive agents. Pulse pressure was calculated as SBP−DBP. Patients who were diagnosed with white coat hypertension were not categorized as having hypertension. Hyperlipidemic patients were defined as those with low-density lipoprotein cholesterol $\geqslant$140 mg $^{-1}$dl and/or triglyceride level $\geqslant$150 mg dl^{-1} or those receiving lipid-lowering agents. Patients were classified as diabetics by their use of insulin and/or oral hypoglycemic agents or by glycosylated hemoglobin A1c (HbA1c) $>$6.5%. In this study, the criteria for cardiovascular risk factor(s) included current smoking, hypertension, hyperlipidemia and diabetes mellitus. The exclusion criteria included subjects with overt left ventricular systolic dysfunction (ejection fraction $<$50%), history of previous episodes of congestive HF, moderate to severe valvular disease and atrial fibrillation, known malignancy, renal failure, liver dysfunction and malnutrition. Our study followed the institutional guidelines of the University of Tokushima and was approved by the Institutional Review Board. Prior informed consent was obtained from all patients according to the Declaration of Helsinki.

Biochemical analyses

Before noon, overnight fasting blood samples were collected from the antecubital vein and were assayed immediately for HbA1c and serum lipid parameters, including low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride level. Serum levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride level were measured using the enzymatic method. HbA1c was measured using high-performance liquid chromatography.

Measurements of plasma HCII and AT activities

Blood was drawn as described above, collected into a tube containing 1/10 volume of 3.8% sodium citrate and centrifuged at 2000×*g* for 20 min. Plasma was stored at −80 °C until use. Plasma HCII and AT activities were measured as previously described.[16]

Echocardiography

The ultrasound instrument used in this study was a Toshiba Aplio 80 with a 2.5-MHz transducer (Toshiba Medical Corporation, Tokyo, Japan). Left atrial size was quantified as left atrial volume (LAV) because LAV is a more accurate estimate of left atrial size than M-mode or 2D left atrial diameters and is a better predictor of cardiovascular events.[17–19] Left atrial (LA) dimensions were measured in three orthogonal planes: parasternal long axis, lateral and superoinferior (Figure 1).[20] Those dimensions were recorded at the end phase of ventricular systole. As suggested by current guidelines,[19] LAV was calculated by using the length diameter ellipsoid method computed at ventricular end-systole by the following equation: V=4π/3×(parasternal long axis/2)×(lateral/2)×(superoinferior/2). Finally, left atrial volume index (LAVI) was indexed by body surface area. Left ventricular mass index (LVMI) was estimated using Devereux formula[21] and was calculated as an index of body surface area. Relative wall thickness (RWT) was calculated as (septal wall thickness+posterior wall thickness in the end-diastolic phase)/(left ventricular end-diastolic diameter). Doppler echocardiographic assessment, including measurements of peak velocities of E and A waves and deceleration time (DcT), was carried out in all patients who underwent tissue Doppler imaging. Spectral pulsed wave Doppler tissue interrogation of longitudinal mitral annular velocity was recorded throughout the cardiac cycle at the septal annulus in the apical four-chamber view. The ratio of peak E velocity to early diastolic mitral annulus velocity (E/e' ratio) was calculated. Other routine echocardiographic examinations, including measurements of left ventricular fractional shortening (FS%) and ejection fraction, were also performed.

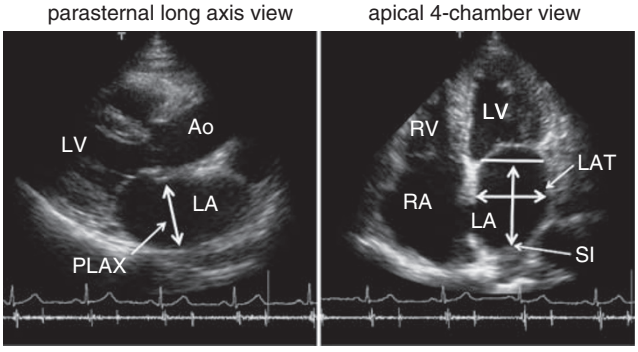

Figure 1 Representative images of measurement of LAV. PLAX was taken in the parasternal long-axis view. The LAT and SI dimensions were both taken from the apical four-chamber view using the inner edge-to-inner edge measurement. LAV was calculated by using the length diameter ellipsoid method, applying the following equation: V=4π/3×(PLAX/2)×(LAT/2)×(SI/2). LAV, left atrial volume, PLAX, parasternal long axis, LAT, lateral, SI, superoinferior.

Statistical analysis

Statistical analyses were performed using the StatView statistical package (StatView 5.0; SAS Institute, Japan Ltd., Tokyo, Japan). Continuous variables were averaged and values were expressed as the mean ± s.d. or as a percentage for categorical parameters. Male gender and the presence of hypertension, diabetes mellitus, hyperlipidemia and current smoking status were coded as dummy variables. The degrees of association between independent variables including sex, age, body mass index, SBP, serum lipid parameters, HbA1c, plasma AT and HCII activities, history of current smoking, hypertension, diabetes mellitus and hyperlipidemia were determined by means of multiple regression analysis.

RESULTS

Characteristics of subjects

The physical and laboratory characteristics of the subjects enrolled in this study are shown in Table 1. The high-density lipoprotein cholesterol levels and plasma AT activity were higher in females than in males. On the other hand, males showed higher levels of serum creatinine and more were current smokers. The mean plasma HCII activity in all of the participants was 95.8 ± 17.0% and there was no difference between the mean plasma HCII activity in males and females. In addition, there were no significant gender differences in the age and body mass index, SBP, pulse pressure, low-density lipoprotein cholesterol, triglyceride level and HbA1c values. No significant gender differences were observed between the prevalences

Table 1 Clinical characteristics of subjects and echocardiographic measurements

Variables	*Total (n=304)*	*Male (n=169)*	*Female (n=135)*	P-*value (male vs. female)*
Age (years)	65.4 ± 11.8	65.1 ± 11.5	65.7 ± 12.3	NS
BMI ($kg\,m^{-2}$)	23.6 ± 3.5	23.8 ± 3.0	23.4 ± 3.9	NS
SBP (mm Hg)	132.4 ± 19.1	132.9 ± 18.4	131.8 ± 19.9	NS
PP (mm Hg)	55.0 ± 12.3	53.7 ± 12.2	56.5 ± 14.4	NS
LDL-C ($mg\,dl^{-1}$)	119.2 ± 35.1	118.2 ± 38.6	120.6 ± 30.3	NS
HDL-C ($mg\,dl^{-1}$)	55.4 ± 19.6	50.1 ± 18.4	62.2 ± 19.1	<0.001
TG ($mg\,dl^{-1}$)	145.4 ± 94.2	150.1 ± 103.7	137.1 ± 80.4	NS
HbA1c (%)	6.0 ± 1.4	6.0 ± 1.4	6.0 ± 1.4	NS
Cre ($mg\,dl^{-1}$)	0.81 ± 0.30	0.92 ± 0.31	0.67 ± 0.21	<0.001
AT (%)	100.3 ± 17.6	98.0 ± 16.1	103.3 ± 19.0	<0.05
HCII (%)	95.8 ± 17.0	94.7 ± 16.8	97.1 ± 17.2	NS
BNP ($pg\,ml^{-1}$)	56.1 ± 78.1	53.2 ± 65.8	60.1 ± 88.7	NS
Current smoking *n* (%)	117 (38.5%)	96 (56.8%)	21 (15.6%)	<0.001
Hypertension *n* (%)	206 (67.8%)	115 (68.0%)	91 (67.4%)	NS
Diabetes mellitus *n* (%)	84 (27.6%)	46 (27.2%)	38 (28.1%)	NS
Hyperlipidemia *n* (%)	155 (51.0%)	87 (51.5%)	68 (50.3%)	NS
Medications				
Aspirin *n* (%)	83 (27.3%)	48 (28.4%)	35 (25.9%)	NS
Statins *n* (%)	60 (19.7%)	32 (18.9%)	28 (20.1%)	NS
Ca blockers *n* (%)	92 (30.3%)	46 (27.2%)	46 (34.1%)	NS
ACEIs/ARBs *n* (%)	102 (33.6%)	59 (34.9%)	43 (31.9%)	NS
β Blockers *n* (%)	26 (8.6%)	12 (7.1%)	14 (10.4%)	NS
Antidiabetes agents *n* (%)	78 (25.7%)	41 (24.2%)	37 (27.4%)	NS
Echocardiographical measurements				
LADI ($mm\,m^{-2}$)	23.2 ± 5.7	21.9 ± 3.9	24.9 ± 7.0	<0.001
LAVI ($ml\,m^{-2}$)	26.3 ± 18.7	24.2 ± 9.1	28.9 ± 26.1	NS
RWT	0.43 ± 0.09	0.43 ± 0.09	0.43 ± 0.10	NS
LVMI ($g\,m^{-2}$)	107.0 ± 35.4	111.2 ± 37.4	101.8 ± 32.2	<0.05
E/A	0.85 ± 0.32	0.86 ± 0.32	0.84 ± 0.32	NS
E/e'	9.78 ± 4.00	9.19 ± 4.08	10.50 ± 3.72	<0.05
DcT (msec)	231.8 ± 65.4	222.2 ± 61.3	243.8 ± 68.6	<0.01
FS (%)	37.9 ± 8.4	36.9 ± 7.7	39.1 ± 9.1	<0.05
EF (%)	64.9 ± 9.1	63.9 ± 9.8	66.1 ± 7.9	<0.05

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; AT, antithrombin; BMI, body mass index; BNP, B type natriuretic peptide; Cre, creatinine; DcT, deceleration time; E/A, ratio of peak mitral Doppler inflow velocities; E/e', ratio of peak E velocity to early diastolic mitral annulus velocity; EF, ejection fraction; FS, fractional shortening; HbA1c, hemoglobin A1c; HCII, heparin cofactor II; HDL-C, high-density lipoprotein cholesterol; LADI, left atrial dimension index; LAVI, left atrial volume index; LDL-C, low-density lipoprotein cholesterol; LVMI, left ventricular mass index; PP, pulse pressure; RWT, relative wall thickness; SBP, systolic blood pressure; TG, triglyceride.

of hypertension, diabetes mellitus and hyperlipidemia and the use of medications for cardiovascular treatment in males and females.

Echocardiographic measurements

Table 1 also shows the results of the echocardiographic examinations. Although the left atrial dimension index (LADI) in females was significantly larger than that in males, there was no statistically significant gender difference in LAVI. Males manifested higher LVMI values than females. In contrast, the left ventricular systolic functions indicated by FS% and ejection fraction were both slightly higher in females than in males. Although there was no gender difference in the value of E/A, DcT and E/e' ratio were significantly larger in females than in males.

HCII is an independent and negative determinant of left atrial size

Age and sex-adjusted scatter plots between HCII and LADI and between HCII and LAVI indicated significant linear associations (Figure 2). Multiple regression analysis showed that age was a positive contributor for an increase in LADI and LAVI (Table 2). Conversely, HCII was found to be an independent and negative contributor for an increase in LADI and LAVI (Table 2). The significance of the relationship between HCII and LA size markers was more accurate in LAVI than in LADI (Figure 2 and Table 2).

Plasma HCII activity is inversely associated with concentric left ventricular remodeling

As increased RWT was recognized as a concentric change of the cardiac left ventricle, we evaluated the independent determinants for increasing RWT using simple and multiple regression analyses. Age and sex-adjusted scatter plots between HCII and RWT demonstrated a significant linear relationship as shown in Figure 2. Although age and body mass index were independent contributors for an increase in RWT, HCII was the sole negative contributor for an increase in RWT (Table 3). Although male gender, pulse pressure and the presence of hypertension were independent contributors for increase in LVMI, plasma HCII activity did not have any significant association with LVMI (Figure 2 and Table 3). Taken together, these results suggest that reduced plasma HCII activity is associated with the phenotype of cardiac concentric remodeling without increased LVMI.

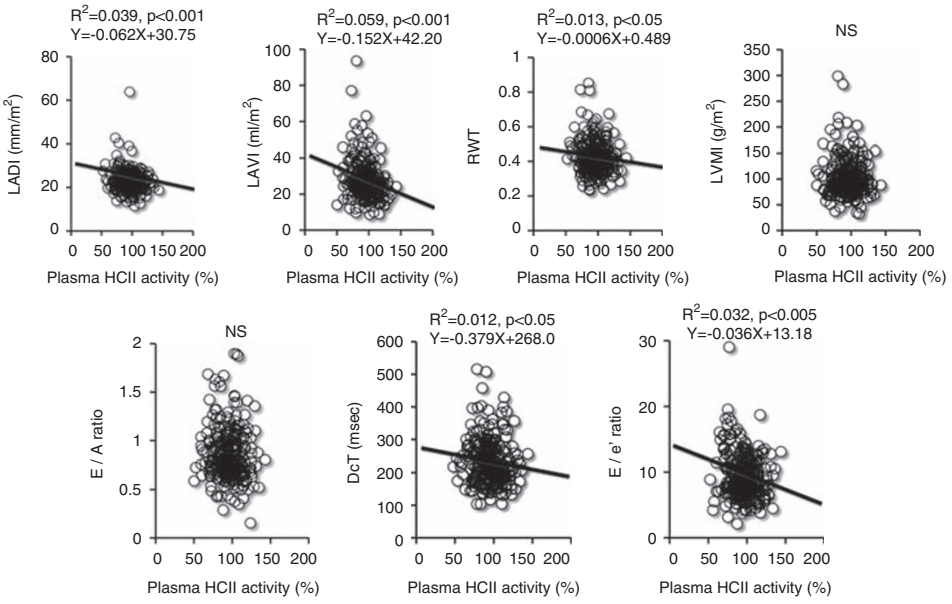

Figure 2 Age and sex-adjusted scatter plots between plasma HCII activity and cardiac geometrical and functional parameters. LADI, left atrial dimension index, LAVI, left atrial volume index, RWT, relative wall thickness, LVMI, left ventricular mass index, DcT, deceleration time.

Table 2 Multiple regression analysis for determinants of left atrial size

	LADI			*LAVI*		
Variables	*Coefficient*	*95% CI*	P-*value*	Coefficient	*95% CI*	P-*value*
AGE (years)	0.1595	0.1108 to 0.2082	<0.001	0.1832	−0.0057 to 0.3665	NS
Male gender	−2.9927	−4.1406 to −1.8450	<0.001	−6.8917	−11.8067 to −1.9768	<0.01
BMI	0.0035	0.0004 to 0.0067	<0.05	−0.0169	−0.6073 to 0.5735	NS
SBP	−0.0378	−0.0884 to 0.0127	NS	−0.1701	−0.3403 to 0.0012	NS
PP	0.0441	−0.0239 to 0.1121	NS	0.1964	−0.0319 to 0.4248	NS
HbA1c	−1.3773	−1.3767 to 0.0012	NS	−1.6265	−3.7859 to 0.5328	NS
LDL-C	0.0128	−0.0053 to 0.0309	NS	0.0168	−0.0441 to 0.0778	NS
HDL-C	−0.0045	−0.0378 to 0.0287	NS	−0.0166	−0.1281 to 0.0949	NS
TG	−0.0011	−0.0086 to 0.0064	NS	−0.0091	−0.0343 to 0.0159	NS
Cre	1.6709	−0.6776 to 4.0194	NS	6.8872	−0.9949 to 14.7695	NS
AT	0.0081	−0.0304 to 0.0464	NS	0.0571	−0.0718 to 0.1859	NS
HCII	−0.5529	−0.0892 to −0.0213	<0.005	−0.2302	−0.3511 to −0.1092	<0.001
Current smoking	0.5983	−0.7553 to 1.9518	NS	1.9213	−2.6218 to 6.4601	NS
Hypertension	1.2537	−0.2001 to 2.7075	NS	5.6799	0.8008 to 10.5591	<0.05
Diabetes mellitus	1.7445	−0.2327 to 3.7217	NS	2.9979	−3.6382 to 9.6342	NS
Hyperlipidemia	−0.2281	−1.6375 to 1.1813	NS	−2.3291	−7.0596 to 2.4014	NS
		r^2=0.2210, P<0.001			r^2=0.1595, P<0.001	

Abbreviations: AT, antithrombin; BMI, body mass index; CI, confidence interval; Cre, creatinine; HbA1c, hemoglobin A1c; HC II, heparin cofactor II; HDL-C, high-density lipoprotein cholesterol; LADI: left atrial dimension index; LAVI: left atrial volume index; LDL-C, low-density lipoprotein cholesterol; PP, pulse pressure; SBP, systolic blood pressure; TG, triglyceride.

Plasma HCII activity is inversely associated with left ventricular diastolic dysfunction

As not only left ventricular systolic dysfunction, but also left ventricular diastolic dysfunction were shown to be closely associated with all-cause mortality in the general population,[22] we evaluated the relationship between plasma HCII activity and left ventricular diastolic function. Although age, SBP and the presence of hypertension were independent contributors for decrease in E/A ratio, plasma HCII activity did not have any significant association with E/A ratio (Figure 2 and Table 4). On the other hand, significant linear associations were found in age and sex-adjusted scatter plots between HCII and DcT, and between HCII and E/e' value as shown in Figure 2. We then evaluated the independent determinants of DcT and E/e' ratio and found that HCII was an independent and negative contributor for increases in DcT and E/e' value (Table 4). These results indicated that HCII is the sole protective factor against left ventricular diastolic dysfunction.

DISCUSSION

Enlarged LAD reflects left atrial pressure and volume overload in response to cardiac dysfunction associated with cardiovascular diseases, including atrial fibrillation.[23,24] There is accumulating evidence

Table 3 Multiple regression analysis for determinants of RWT and LVMI

	RWT			*LVMI*		
Variables	*Coefficient*	*95% CI*	*P-value*	*Coefficient*	*95% CI*	*P-value*
Age (years)	0.0014	0.0004 to 0.0024	<0.01	0.3183	−0.0595 to 0.6961	NS
Male gender	0.0049	−0.0216 to 0.0315	NS	10.0476	2.2111 to 17.8841	<0.05
BMI	0.0035	0.0004 to 0.0067	<0.05	0.5971	−0.6014 to 1.7956	NS
SBP	0.0005	−0.0004 to 0.0014	NS	0.1341	−0.2102 to 0.4783	NS
PP	−0.0004	−0.0017 to 0.0008	NS	0.2489	0.0001 to 0.4998	<0.05
HbA1c	−0.0006	−0.0123 to 0.0110	NS	−2.7856	−7.1692 to 1.5981	NS
LDL-C	0.0001	−0.0003 to 0.0004	NS	−0.0317	−0.1554 to 0.0921	NS
HDL-C	0.0002	−0.0004 to 0.0008	NS	−0.1381	−0.3644 to 0.0884	NS
TG	0.0001	−0.0001 to 0.0002	NS	−0.0392	−0.0902 to 0.0118	NS
Cre	−0.0195	−0.0621 to 0.0231	NS	7.5455	−8.4553 to 23.5464	NS
AT	0.0002	−0.0005 to 0.0009	NS	−0.0382	−0.2997 to 0.2233	NS
HCII	−0.0007	−0.0013 to −0.0002	<0.001	−0.0873	−0.3328 to 0.1582	NS
Current smoking	0.0001	−0.0244 to 0.0247	NS	4.3466	−4.8755 to 13.5687	NS
Hypertension	0.0162	−0.0101 to 0.0426	NS	15.3231	6.6293 to 24.0171	<0.001
Diabetes mellitus	−0.0051	−0.0409 to 0.0307	NS	−0.0164	−13.4878 to 13.4549	NS
Hyperlipidemia	0.0056	−0.0199 to 0.0312	NS	0.7756	−8.8272 to 10.3785	NS
		r^2=0.0864, P<0.05			r^2=0.0722, P<0.05	

Abbreviations: AT, antithrombin; BMI, body mass index; CI, confidence interval; Cre, creatinine; HbA1c, hemoglobin A1c; HC II, heparin cofactor II; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVMI, left ventricular mass index; PP, pulse pressure; RWT, relative wall thickness; SBP, systolic blood pressure; TG, triglyceride.

Table 4 Multiple regression analysis for determinants of E/A ratio, DcT and E/e' ratio

	E/A ratio			*DcT*			*E/e' ratio*		
Variables	*Coefficient*	*95% CI*	P-*value*	*Coefficient*	*95% CI*	P-*value*	*Coefficient*	*95% CI*	P-*value*
Age	−0.0145	−0.0174 to −0.0116	<0.0001	1.2596	0.5774 to 1.9418	<0.0001	0.0529	0.01845 to 0.0875	<0.005
Male gender	−0.0188	−0.0948 to 0.0571	NS	−15.8474	−33.8634 to 2.1687	NS	−1.0224	−1.9344 to −0.1104	<0.05
BMI	−0.0101	−0.0192 to −0.0010	<0.05	0.0472	−2.1169 to 2.2114	NS	0.1333	0.0238 to 0.0875	<0.05
SBP	−0.0027	−0.0054 to −0.0001	<0.05	0.3901	−0.2314 to 1.0117	NS	−0.0106	−0.0421 to 0.0208	NS
PP	0.0035	−0.0014 to 0.0085	NS	−0.0136	−0.8506 to 0.8234	NS	0.0177	−0.0246 to 0.0601	NS
HbA1c	0.0035	−0.0298 to 0.0369	NS	−4.9187	−12.8339 to 2.9971	NS	0.2779	−0.1228 to 0.6786	NS
LDL-C	−0.0002	−0.0011 to 0.0007	NS	−0.1817	−0.4051 to 0.0417	NS	0.0064	−0.0049 to 0.0177	NS
HDL-C	−0.0009	−0.0026 to 0.0009	NS	0.2759	−0.1329 to 0.6848	NS	0.0115	−0.0092 to 0.0322	NS
TG	−0.0003	−0.0007 to 0.0001	NS	−0.0195	−0.1117 to 0.0725	NS	0.0041	−0.0005 to 0.0088	NS
Cre	0.1219	−0.0559 to 0.2996	NS	−26.2691	−55.1619 to 2.6237	NS	0.4169	−1.0456 to 1.8796	NS
AT	−0.0002	−0.0041 to 0.0001	NS	0.3135	−0.0001 to 0.0628	NS	0.0053	−0.0186 to 0.0292	NS
HCII	−0.0007	−0.0025 to 0.0012	Ns	−0.5189	−0.9622 to −0.0756	<0.05	−0.0558	−0.0783 to −0.0333	<0.001
Current smoking	−0.0313	−0.1016 to 0.0389	NS	11.4582	−5.1942 to 28.1107	NS	0.1031	−0.7399 to 0.9461	NS
Hypertension	−0.0778	−0.1532 to 0.0023	<0.05	−6.8629	−24.7475 to 11.0218	NS	0.0718	−0.8335 to 0.9772	NS
Diabetes mellitus	−0.0656	−0.1682 to 0.0369	NS	−6.3831	−30.7084 to 17.9422	NS	−0.0591	−1.2904 to 1.1723	NS
Hyperlipidemia	0.0191	−0.0539 to 0.0936	NS	6.1835	−11.1565 to 23.5234	NS	−0.3927	−1.2705 to 0.4850	NS
		r^2=0.3283, P<0.001			r^2=0.1601, P<0.0001			r^2=0.1762, P<0.0001	

Abbreviations: AT, antithrombin; BMI, body mass index; CI, confidence interval; Cre, creatinine; DcT, deceleration time; HbA1c, hemoglobin A1c; HC II, heparin cofactor II; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PP, pulse pressure; SBP, systolic blood pressure; TG, triglyceride.

that the association between cardiovascular disease and LAV/body surface area (LAVI) is stronger than that observed with LAD/body surface area (LADI) after adjustment for age and gender in subjects with sinus rhythm.[17,23–25] Therefore, we assessed the relationships between plasma HCII activity and the LA size parameters. We found a significant inverse correlation between plasma HCII activity and LAVI, as well as LADI even after adjustment for other confounding cardiovascular risk factors, suggesting that HCII independently counteracts LA enlargement. Enlarged LAV has been shown to be associated with inflammation and atherosclerosis.[26,27] As cuff-tube placement around the femoral artery caused increases in the gene expression levels of inflammatory cytokines and chemokines, such as interleukin-1β and -6 and monocyte chemoattractant protein-1 in HCII-deficient mice compared with the levels in wild-type mice,[14] there is a possibility that HCII prevents LA enlargement partly through its anti-inflammatory potency leading to attenuation of left atrial remodeling. As LAV is an indicator of the burden of diastolic dysfunction, even in patients without atrial fibrillation or significant valvular heart disease,[28] our results indicated that plasma HCII activity could be involved in the pathogenesis of left ventricular diastolic dysfunction. Therefore, we estimated association between plasma HCII activity and left ventricular (LV) diastolic function in humans and observed the significant

relationship between plasma HCII activity and ventricular diastolic dysfunction indicated by the DcT and E/e' ratio. As it is well known that prolonged DcT indicates abnormal LV relaxation in patients with early diastolic abnormality,[29] a significant inverse relationship between plasma HCII activity and DcT suggests that HCII has potency to counteract abnormal LV relaxation in subjects without systolic LV dysfunction. Diastolic tissue Doppler velocities reflect myocardial relaxation and, in combination with conventional Doppler measurements, the ratios (transmitral early diastolic velocity/mitral annular early diastolic velocity (E/e' ratio)) have been used to non-invasively estimate LV filling pressure, as well as pulmonary capillary wedge pressure.[30–32] As pulmonary capillary wedge pressure is a prognostic indicator in patients with HF, E/e' value is a similarly powerful predictor of prognosis in patients with various cardiac diseases.[33] In the present study, plasma HCII activity was independently and inversely associated with the value of E/e', suggesting that HCII preserves compliance of the left ventricular wall.

Warfarin is a synthetic thrombin inhibitor and is frequently used for the prevention of cardiogenic thrombosis. AT is also an endogenous thrombin inhibitor in the intravascular lumen. As warfarin has never been documented to have a pharmacological effect on anticardiac remodeling and as the present study has shown that AT was not associated with any cardiac remodeling phenotype, thrombin inactivation at the intravascular lumen and/or intracardiac chamber might be unable to attenuate cardiac remodeling. Conversely, as HCII is thought to inhibit thrombin action by forming a bimolecular complex with dermatan sulfate proteoglycans that are deposited at vascular smooth muscle cells and fibroblasts, HCII may also exert tissue thrombin inactivation in cardiomyocytes and/or cardiac fibroblasts in concert with binding to the deposited dermatan sulfate.

PAR-1 is expressed in the heart by cardiomyocytes and cardiac fibroblasts[34,35] and a recent study demonstrated that PAR-1 expression was increased in the hearts of patients with ischemic and idiopathic dilated cardiomyopathy.[36] PAR-1 expression is increased in the LV of a mouse model for chronic HF[37] and Pawlinski *et al.*[38] demonstrated that PAR-1 overexpression by cardiomyocytes induced cardiac hypertrophy in MHC-PAR-1 mice. These hypertrophic changes of cardiomyocytes by PAR-1 activation may be partly explained by the mechanism of cleavage of PAR-1 resulting in activation of Gq, G12/13 and Gi, as well as downstream signaling pathways, including the MAPK pathways ERK 1/2 and ERK5.[39,40] Therefore, activation of PAR-1 in the heart promotes hypertrophic growth and/or influences the survival of cardiomyocytes.[34] These findings are consistent with the assumption that PAR-1 activation in the heart, including cardiomyocytes and cardiac fibroblasts, accelerates cardiac remodeling, leading to reduced elasticity of the left ventricular wall. From these previous observations, we hypothesized that HCII counteracts cardiac remodeling through inactivation of the tissue thrombin–PAR-1 pathway. As there is a possibility that activation and expression of (pro) thrombin and its major receptor PAR-1 axis is modulated according to the condition of cardiac stress in each subject, it is difficult to clarify the exact interplay between HCII and PAR-1 activation in such subjects. However, our results suggest that reduced plasma HCII activity is one of several major causes of LA enlargement and LV diastolic dysfunction regardless of PAR-1 expression levels.

The results of the present clinical study were further corroborated by a study using our HCII-deficient mice.[41] In that study, infusion of angiotensin II prominently accelerated cardiac remodeling, including concentric LV changes, enlargement of LAV and exaggeration of cardiac fibrosis in HCII-deficient mice when compared with littermate wild-type mice.[41] The study using HCII-deficient mice demonstrated that HCII protects against angiotensin II-induced cardiac remodeling through the suppression of the NAD(P)H oxidase–TGF-β1 pathway.[41] Therefore, we speculate that HCII is also capable of attenuating oxidative stress in the human heart, as well as in the murine heart through suppression of the NAD(P)H oxidase–TGF-β1 pathway.

As LV hypertrophy causes LA enlargement and the LA enlargement may impair LV function, it is important to clarify whether HCII primarily influences LA or LV remodeling. Although further examinations are needed to clarify this issue, results of our animal studies using HCII-deficient mice indicate that abnormal changes, including angiotensin II-induced LV concentric change and LA enlargement with acceleration of cardiac fibrosis, seem to occur simultaneously after starting angiotensin II infusion (data not shown in reference 41), and it is therefore possible that HCII directly and independently influences LA enlargement and LV dysfunction.

The results of the present clinical study cannot be extended to the general population because we only enrolled patients with cardiovascular risk factors and we previously reported that subjects without cardiovascular risk factors had higher levels of plasma HCII activity than those in subjects with one or more cardiovascular risk factor.[11] Thus, large-scale investigations and cohort studies are required to assess and clarify the prognostic value of plasma HCII activity for cardiac remodeling in the general population. In addition, it is crucial to compare plasma HCII activities in subjects with and without LV systolic dysfunction for understanding the pathophysiological roles of HCII in cardiac remodeling. Therefore, further examinations focusing on the relationship between HCII and systolic HF are needed. In summary, plasma HCII activity is independently and inversely associated with the development of cardiac remodeling including concentric cardiac changes, LA enlargement and LV diastolic dysfunction. These results suggest that the inactivation of thrombin in cardiac tissue by HCII might be a novel and valuable therapeutic approach to prevent cardiac remodeling and atherosclerosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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