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ORIGINAL ARTICLE

Association between osteopontin promoter variants and diastolic dysfunction in hypertensive heart in the Japanese population

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Heart failure with preserved ejection fraction is recently highlighted as a major health problem, and diastolic dysfunction associated with hypertension has a dominant role in the development of heart failure with preserved ejection fraction. Osteopontin (OPN) is a secreted phosphoprotein, which mediates fibrosis. In animal models, OPN is upregulated in response to pressure overload and is thought to be involved in systolic dysfunction. However, the functional role of OPN in diastolic dysfunction is unknown. The guanine base insertion polymorphism at −156 position of the *OPN* promoter is postulated to upregulate the transcription of *OPN* in human. To investigate whether −156del/G polymorphism of *OPN* promoter is associated with diastolic dysfunction in hypertensive hearts, the patients with hypertension have been genotyped for variants of −156del/G polymorphism by genomic sequencing. Diastolic function of the left ventricle was estimated as the ratio of early to atrial filling (E/A ratio), obtained by pulsed-Doppler derived transmitral flow in echocardiographic analysis. The patients with −156G allele displayed lower E/A ratio compared with those with −156del/del genotype, suggesting exacerbated diastolic function. Notably, in case of the population with diabetes mellitus, the patients with −156G allele showed significant association with lower E/A ratio, compared with −156del/−156del patients. Multiple linear regression analysis revealed that prevalence of −156G allele was an independent factor for lowering E/A ratio. The −156del/G genetic variants of *OPN* promoter were associated with decreased E/A ratio in hypertensive patients. These results suggest that OPN has a functional role in the development of diastolic dysfunction in hypertensive hearts.
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Keywords: diabetes mellitus; diastolic function; osteopontin; polymorphism

INTRODUCTION

Heart failure (HF) is a common cause of cardiovascular death and may occur in the presence of either a normal or decreased left ventricular (LV) function.[1–3] HF with normal ejection fraction makes up nearly 50% of HF, and is a major public health problem of increasing prevalence.[4] To date, no effective therapy has been established for HF with preserved systolic function to improve prognosis.[5]

Left ventricular diastolic dysfunction is thought to be a major cause of HF with preserved systolic function.[6] Although the evidence about its pathophysiology and a reliable therapeutic strategy are still lacking, hypertension is well known to underlie the development of diastolic failure.[4] Aging and diabetes are closely related to diastolic dysfunction as well.[4,7] According to the data from animal model with pressure overload, various biological elements such as neurohumoral factors and growth factors are involved in the pathogenesis of diastolic failure.[8–10] In contrast, there are no established mechanisms regarding diastolic failure in hypertensive patients. Thus, further clinical investigation to dissect the mechanism of diastolic failure is required to improve the prognosis of HF with preserved systolic function.

Osteopontin (OPN) is an extracellular matrix glycoprotein, which displays several functions in different physiological and pathological processes, including bone remodeling, inflammation and cell-mediated immunity.[11,12] More recently, OPN has emerged as an important protein involved in cardiovascular diseases, including post-myocardial infarction (MI) remodeling and atherosclerosis.[13,14] OPN is expressed at low level under physiological condition in heart;

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however, its expression is markedly increased after MI and cardiac hypertrophy.[14,15] Overexpression of OPN in mouse cardiomyocyte induces cardiac dysfunction, dilation and fibrosis in heart,[16] suggesting its expression is closely related to myocardial function and fibrosis.[14] However, the association of OPN with diastolic function remains to be fully elucidated in the patients with diastolic HF.

It is well known that OPN transcription can be activated by various stimuli through transcriptional factors, such as AP1 and Runx2.[17,18] Recently, three functional polymorphisms (−66T/G, −156del/G and −443T/C) on the promoter region of *OPN* gene have been found to affect gene expression by altering transcriptional activity[19] and reported to be associated with several diseases, including pseudoxanthoma elasticum, stroke and chronic hepatitis C.[20–22] The insertion of guanine base at position −156 (−156G allele) on the OPN promoter generates a Runx2 binding site so that the binding of Runx2 factor to the −156G position promotes OPN transcription.[19]

In the present study, we investigated the association between diastolic dysfunction and the −156del/G polymorphism on the OPN promoter in patients with hypertension.

METHODS

Study subjects

The study subjects consisted of 318 unrelated consecutive hypertensive patients from 40 to 80 years of age who attended to Osaka University Hospital. All individuals were of Japanese ethnic origin. Hypertension was defined as a systolic blood pressure of $\geqslant$140 mm Hg and/or a diastolic blood pressure of $\geqslant$90 mm Hg on repeated measurements or receiving antihypertensive treatment. Diabetes mellitus (DM) was defined according to the American Diabetes Association criteria.[23] Patients with atrial fibrillation were excluded. In addition, the patients with valvular stenosis as well as with severe valvular regurgitation were removed from analyses. The present study was approved by the Institutional Review Board of the Osaka University Graduate School of Medicine, and was executed in accordance with the Declaration of Helsinki. All study subjects provided written informed consent with regard to the study procedures.

Genotyping

Genomic DNA was extracted from samples of peripheral blood leukocytes using the QIAamp DNA Blood Maxi Kit (Qiagen KK., Tokyo, Japan) according to the manufacturer's protocol. Genotyping of −156 del/G polymorphisms was performed by sequencing in combination with PCR using GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Briefly, 333 bp of region of interest in the *OPN* promoter was amplified using the forward (5′-GCTGAATGCCCATCCCGTAA-3′) and reverse (5′-TCAGCTGAATGCAC AACCCAGT-3′) primers and sequenced by ABI PRISM 310 genetic analyzer (Applied Biosystems) using Big Dye Terminator v1.1 Cycle Sequencing Kit following manufacturer's protocol.

Echocardiographic assessment

Echocardiography was performed using an ultrasonic sector scanner with 2.5-and 3.75-MHz transducers with Sonos 5500 Ultrasound system (Philips Medical Systems, Tokyo, Japan). Imaging and Doppler echocardiography were performed in all of the participants in this study. Studies were performed with phased-array echocardiography with M-mode, 2D, pulsed and color-flow Doppler capabilities as previously reported.[24] Left ventricular internal dimension, and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations.[25] Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as described previously.[26] LV mass was calculated using Devereux-modified American Society of Echocardiography cube equation.[27] LV mass was considered an unadjusted variable and was normalized by body surface area and expressed as LV mass index. Left ventricular hypertrophy (LVH) was diagnosed when LV mass index was greater than 132 g m^{-2} in men and 109 g m^{-2} in women patient following previous reports.[28,29] To analyze mitral flow, the pulsed wave beam was positioned in a line parallel to the LV long axis with the sample volume at the level of the mitral annulus. The highest velocity pattern of LV diastolic filling during at least four cardiac cycles was recorded. Peak flow velocities of early filling wave and atrial filling wave were obtained, and the ratio of early to atrial filling waves (E/A) was calculated. The patients with more than 1.0 of E/A ratio concomitant with decreased fractional shortening (<20%) were regarded as exhibiting pseudo-normalization and were excluded from analyses.

Statistical analysis

Normality was evaluated for each variable on the basis of normal distribution plots and histograms and by Shapiro-Wilk test. Statistical comparisons were performed using the Student's *t*-test for continuous variables (if applicable, the *t*-test was modified for unequal variances) and χ^2-test or Fisher's exact test for categorical variables. We performed multiple regression analysis to examine the effect of −156del/G polymorphism adjusted for other variables contributing to E/A ratio by SPSS for windows version 11.0J software (SPSS, Chicago, IL, USA). Values in the tables were displayed as means ± s.d., whereas values in bar graphs were expressed as means ± s.e.m. All *P*-values are two-tailed, and significance was set at the 5% level ($P<0.05$).

RESULTS

We studied 318 consecutive patients with hypertension. The characteristics of all patients are summarized in Table 1. The genotype frequency for −156del/G polymorphism (59.8% for del/del, 35.2% for G/del and 5.0% for G/G) was similar to the expected frequencies in Japanese population and none of genotype distributions differed from Hardy-Weinberg expectations.

Table 1 Clinical characteristics of patients

Parameter	*All patients (n=318)*
Gender (male/female)	189/129
Age (years)	63.0 ± 9.3
BMI (kg m^{-2})	24.0 ± 3.5
Systolic blood pressure (mm Hg)	157.9 ± 28.0
Diastolic blood pressure (mm Hg)	93.0 ± 16.8
Antihypertensive drug treatment	157 (49.4%)
Calcium channel blockers	105 (33.0%)
ACE inhibitors and/or ARBs	90 (28.3%)
ACE inhibitors	47 (14.8%)
ARBs	50 (15.7%)
β-Blockers	32 (10.1%)
α-Blockers	8 (2.5%)
Diuretics	28 (8.8%)
Diabetes	95 (29.9%)
HbA1c (%)	5.88 ± 1.43
Fasting blood glucose (mg dl^{-1})	113.8 ± 43.0
Hyperlipidemia	167 (52.5%)
Total cholesterol (mg dl^{-1})	205.3 ± 36.8
Triglyceride (mg dl^{-1})	145.6 ± 89.3
HDL cholesterol (mg dl^{-1})	53.5 ± 15.6
Diabetes-related treatment	71 (22.3%)
Lipid-lowering treatment	69 (21.7%)
Coronary heart disease	36 (11.3%)
OPN G-156del (DD/GD/GG)	190/112/16

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockade; BMI, body mass index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; OPN, osteopontin.
Data are expressed as mean ± s.d. or number (percentage) of the subjects.

Table 2 Comparison of characteristics between OPN-156del/del and -156G genotype groups

Variable	*del/del* (n=*190*)	*G/del+G/G* (n=*128*)	P-*value*
Gender (male/female)	114/76	75/53	0.816
Age (years)	63.1 ± 9.3	62.7 ± 9.4	0.712
BMI (kg m^{-2})	24.0 ± 3.6	24.1 ± 3.4	0.853
Systolic blood pressure (mm Hg)	158.7 ± 26.6	156.7 ± 30.2	0.606
Diastolic blood pressure (mm Hg)	93.2 ± 16.4	92.6 ± 17.5	0.771
Antihypertensive drug treatment	91 (48.1%)	66 (51.6%)	0.569
Calcium channel blockers	62 (32.8%)	43 (33.6%)	0.904
ACE inhibitors and/or ARBs	52 (27.5%)	38 (29.7%)	0.704
ACE inhibitors	30 (15.9%)	17 (13.3%)	0.629
ARBs	27 (14.3%)	23 (18.0%)	0.433
β-Blockers	17 (9.0%)	15 (11.7%)	0.452
α-Blockers	6 (3.2%)	2 (1.6%)	0.481
Diuretics	17 (9.0%)	11 (8.7%)	1.000
Diabetes	50 (26.3%)	45 (35.2%)	0.105
HbA1c (%)	5.8 ± 1.4	6.1 ± 1.5	0.110
Fasting blood glucose (mg dl^{-1})	112.7 ± 47.6	115.5 ± 35.5	0.591
Hyperlipidemia	102 (53.7%)	65 (50.8%)	0.648
Total cholesterol (mg dl^{-1})	206.6 ± 37.1	203.4 ± 36.3	0.464
Triglyceride (mg dl^{-1})	144.1 ± 87.1	147.9 ± 92.9	0.716
HDL (mg dl^{-1})	54.2 ± 16.8	52.7 ± 13.7	0.466
Diabetes-related treatment	37 (19.8%)	34 (26.8%)	0.170
Lipid-lowering treatment	37 (19.6%)	32 (25.0%)	0.269
Coronary heart disease	18 (9.5%)	18 (14.1%)	0.212

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockade; BMI, body mass index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; OPN, osteopontin. Data are expressed as mean ± s.d. or number (percentage) of the subjects.

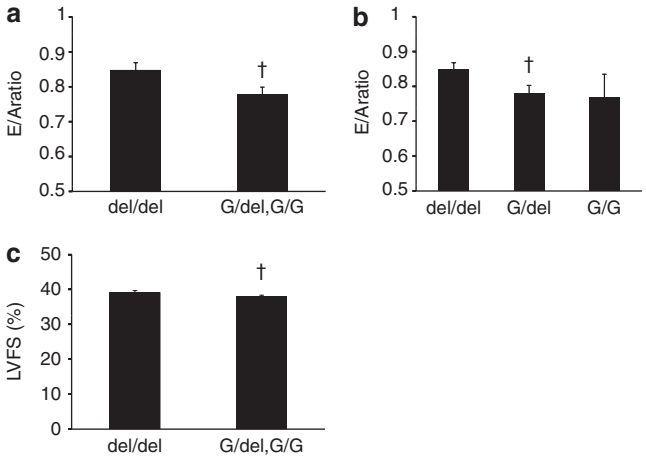

Figure 1 Left ventricular function assessed by echocardiography. (**a**) Ratio of early to atrial filling (E/A ratio) for the patients without −156G allele (del/del, *n*=190) and with −156G allele (G/del+G/G, *n*=128). (**b**) E/A ratio for the patients with −156del/−156del (del/del, *n*=190), −156G/−156del (G/del, *n*=112) and −156G/−156G (G/G, *n*=16) variants. (**c**) Left ventricular fractional shortening (LVFS) for the patients without −156G allele (del/del) and with −156G allele (G/del+G/G). Data are expressed as means ± s.e.m. †$P<0.05$ *vs.* −156del/−156del group.

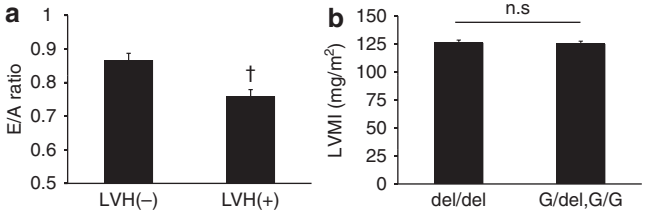

Figure 2 Left ventricular (LV) diastolic function and LV mass assessed by echocardiography. (**a**) Ratio of early to atrial filling (E/A) ratio for the patients with or without left ventricular hypertrophy (LVH). (**b**) Left ventricular mass index (LVMI) for the patients without −156G allele (del/del) and those with −156G allele (G/del+G/G). Data are expressed as means ± s.e.m. †$P<0.05$ *vs.* LVH (−) group.

The comparisons of parameters between the patients with del/del genotype and those with G allele are shown in Table 2. There were no significant differences between the G-allele carriers and the non-carriers in the fundamental parameters such as gender, age, body mass index, blood pressure and prevalences of DM and dyslipidemia. In addition, the antihypertensive drugs for the treatment of hypertension were similar in both groups.

The assessments of cardiac function and LV mass by echocardiography are shown in Figure 1. E/A ratio in the patients with G allele displayed significantly lower values compared with those with del/del genotype, suggesting diastolic function is impaired in the cohort of G carriers (Figure 1a). There were no significant differences between G/del heterozygotes and G/G homozygotes. (Figure 1b). With regard to systolic function, the patients with G allele demonstrated mild, but significantly, lower LV fractional shortening compared with del/del patients (39.4 ± 0.5% *vs.* 37.9 ± 0.5%, P=0.045, Figure 1c). These results suggest that −156G genotype is associated with cardiac dysfunction in hypertensive patients.

As LVH is postulated as a major determinant in developing diastolic dysfunction, we subsequently focused on the correlation LVH, E/A ratio and −156 del/G genotype. Consistent with multiple previous reports, the patients with LVH showed significantly lower E/A ratio than those without LVH (Figure 2a). However, there were no significant differences in LV mass index between two groups, indicating that LV diastolic dysfunction in the patients with −156G allele is independent of cardiac hypertrophy (Figure 2b).

To further dissect the association of −156del/G polymorphism with diastolic dysfunction, we performed stratified analysis for those subjects in association with or without DM. The comparisons of patient characteristics between each group are shown in Table 3. Without DM, the patients with G allele displayed no significant differences in E/A ratio compared with del/del subjects. In contrast, concomitant with DM, the patients with G allele demonstrated significantly lower E/A ratio compared with those with del/del genotype (Figure 3). There were no significant differences in other parameters such as age, gender and treatment for hypertension between two groups with DM (data not shown).

Finally, to address the independency of *OPN* −156del/G promoter variant as a factor associated with E/A ratio, multiple linear regression analysis was performed (Table 4). As a result, it was revealed that *OPN* −156G promoter variants, in addition to age, body mass index and LVH, were independently associated with decreased E/A ratio.

DISCUSSION

In the present study, we first revealed that −156G polymorphism of *OPN* promoter is associated with diastolic dysfunction in patients

Table 3 Comparison of characteristics between OPN-156del/del and -156G genotype groups with or without diabetes

	Non Diabetes (n=*23*)			*Diabetes* (n=*95*)		
Variable	*del/del* (n=*140*)	*G/del+G/G* (n=*83*)	P-*value*	*del/del* (n=*50*)	*G/del+G/G* (n=*45*)	P-*value*
Gender (male/female)	77/63	47/36	0.889	37/13	28/17	0.271
Age (years)	62.8 ± 9.2	61.6 ± 9.8	0.370	64.1 ± 9.7	64.8 ± 8.2	0.690
BMI (kg m^{-2})	24.0 ± 3.6	23.9 ± 3.5	0.823	24.0 ± 3.7	24.4 ± 3.3	0.568
Systolic blood pressure (mm Hg)	162.3 ± ± 25.6	161.9 ± 30.7	0.921	147.9 ± 27.0	144.9 ± 25.9	0.668
Diastolic blood pressure (mm Hg)	95.8 ± 15.7	95.7 ± 17.8	0.976	85.5 ± 16.4	85.3 ± 14.4	0.958
Antihypertensive drug treatment	67 (48.2%)	42 (50.6%)	0.782	24 (48.0%)	24 (53.3%)	0.683
Calcium channel blockers	48 (34.5%)	29 (34.9%)	1.000	14 (28.0%)	14 (31.1%)	0.823
ACE inhibitors and/or ARBs	36 (25.9%)	24 (28.9%)	0.642	16 (32.0%)	14 (31.1%)	1.000
ACE inhibitors	21 (15.1%)	9 (10.8%)	0.422	9 (18.0%)	8 (17.8%)	1.000
ARBs	18 (12.9%)	15 (18.1%)	0.332	9 (18.0%)	8 (17.8%)	1.000
β-Blockers	13 (9.4%)	11 (13.3%)	0.379	4 (8.0%)	4 (8.9%)	1.000
α-Blockers	4 (2.9%)	1 (1.2%)	0.653	6 (3.2%)	2 (1.6%)	1.000
Diuretics	9 (6.5%)	5 (6.1%)	1.000	8 (16.0%)	6 (13.3%)	0.778
Diabetes						
HbA1c (%)	5.0 ± 0.4	5.0 ± 0.4	0.432	7.2 ± 1.4	7.3 ± 1.3	0.651
Fasting blood glucose (mg dl^{-1})	97.4 ± 22.0	98.2 ± 13.4	0.768	152.5 ± 69.0	144.6 ± 41.9	0.510
Hyperlipidemia	75 (53.6%)	42 (50.6%)	0.680	27 (54.0%)	23 (51.1%)	0.838
Total cholesterol (mg dl^{-1})	208.6 ± 37.0	209.3 ± 36.8	0.887	201.1 ± 37.5	193.1 ± 33.4	0.278
Triglyceride (mg dl^{-1})	144.9 ± 73.1	151.3 ± 103.7	0.604	141.8 ± 117.6	142.0 ± 71.3	0.991
HDL (mg dl^{-1})	54.9 ± 17.4	53.9 ± 14.2	0.665	51.9 ± 15.0	50.7 ± 12.7	0.678
Diabetes-related treatment	—	—	—	37 (75.5%)	34 (75.6%)	1.000
Lipid-lowering treatment	22 (15.8%)	18 (21.7%)	0.284	15 (30.0%)	14 (31.1%)	1.000
Coronary heart disease	11 (7.9%)	9 (10.8%)	0.474	7 (14.0%)	9 (20.0%)	0.584

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockade; BMI, body mass index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; OPN, osteopontin.
Data are expressed as mean ± s.d. or number (percentage) of the subjects.

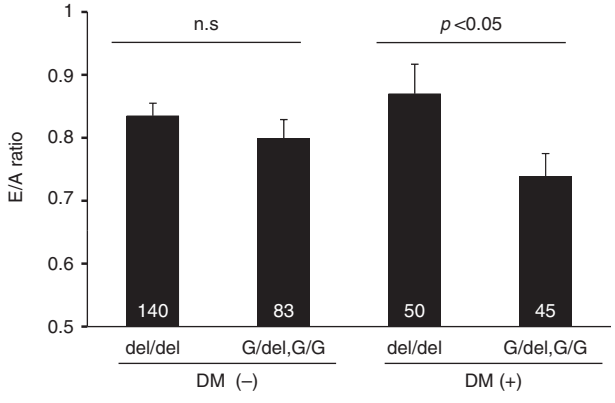

Figure 3 Left ventricular diastolic function (E/A ratio) for the patients without −156G allele (del/del) and with −156G allele (G/del+G/G) in association with diabetes mellitus. Numbers on the bars indicate the numbers of the subjects.

with hypertension. Moreover, we first demonstrated that comorbid DM exacerbates impaired relaxation specifically in −156G allele carriers. To date, pathogenesis of diastolic dysfunction or HF with preserved systolic function remains elusive,[30] especially in diabetic patients.[7] Our results suggest that OPN may have a crucial role in the development of diastolic failure in hypertensive patients associated with DM.

−156G polymorphism on OPN promoter in hypertensive patients

According to the NCBI database (rs1752488 for SNP of −156 site of *OPN* promoter), the frequencies of del allele and G allele are 0.770 and 0.230, respectively, indicating the estimated frequencies of each genotype are 59.3% for del/del, 35.4% for del/G and 5.3% for G/G. None of genotype distributions in the present study differed from the Hardy-Weinberg expectations. Thus, our data indicates that −156del/G polymorphism of *OPN* promoter is not related to the onset of hypertension.

Pressure overload, OPN expression and cardiac function

OPN is associated with various cardiac pathogenesis in heart diseases including post-MI remodeling, cardiac hypertrophy and diabetic cardiomyopathy.[31] The expression of OPN is observed in fibroblasts and macrophages in remodeling heart after MI,[14] whereas cardiomyocytes produce OPN in hypertrophied myocardium caused by pressure overload. *OPN* null mice show decreased cardiac function and dilation after MI, indicating beneficial effect of OPN in post-infarct remodeling.[14] In contrast, *OPN* deletion attenuates cardiac hypertrophy after transverse aortic constriction procedure in mice,[15] suggesting expression of OPN in cardiomyocytes is detrimental in pressure overload. In line with those observation in murine models, cardiac-specific expression of OPN results in dilated cardiomyopathy and premature death with myocyte apoptosis, macrophage infiltration and impaired cardiac conducting system,[16] proposing that cardiac expression of OPN in hypertrophied myocytes mediates systolic and diastolic dysfunction. Consistent with those previous findings in animal models, our results

Table 4 Multiple regression analysis of the factors associated with E/A ratio

	Standardized β-coefficient	P-*value*
Female	−0.017	0.734
Age	−0.415	<0.001
BMI	−0.174	<0.001
Diabetes	0.032	0.534
LVMI	−0.121	0.018
−156 G allele	−0.125	0.013
Adjusted R^2	0.230	<0.001

Abbreviations: BMI, body mass index; E/A ratio, ratio of early to atrial filling; LVMI, left ventricular mass index

indicate that enhanced OPN transcription with −156G allele is closely related to cardiac dysfunction in patients with hypertension. Moreover, our data suggest that the −156G allele is an attributable risk to lowering E/A ratio, surprisingly to the same extent as LVH (Table 4).

Renin–angiotensin system and OPN expression

Renin–angiotensin system is postulated to have central roles in the development of diastolic dysfunction through both hypertrophy-dependent and -independent mechanisms.[32,33] Recently, it has been reported that OPN expression is regulated by renin–angiotensin system,[34] and OPN emerged as a key regulator in both vascular remodeling and development of atherosclerosis, which could deteriorate diastolic function by disturbing micro/macro coronary circulation. Indeed, modulation of renin–angiotensin system by angiotensin-converting enzyme inhibitors or angiotensin receptor blockade is reported to decrease expression of OPN in vascular smooth muscle cells[35] and could ameliorate diastolic function. However, approximately one-third of patients in each group received angiotensin-converting enzyme inhibitors or angiotensin receptor blockade in the present study and the treatment with those drugs did not affect the trend of E/A ratio obtained from both del/del and G allele groups (data not shown). Presumably, our results might suggest that OPN expression not in vascular smooth muscle cells, but in cardiomyocytes could be a critical regulator in the development of diastolic dysfunction in hypertensive patients.

OPN transcription and −156del/G polymorphism

Runx2 has an important role in the regulation of OPN transcription. The G base insertion at −156 site of *OPN* promoter generates another Runx2-binding site,[19,22] and Runx2 bindings are crucial for regulation of OPN transcription in bone tissue.[36] As discussed above, enhanced expression of OPN in heart potentially causes cardiac dysfunction. Together, we assume that OPN transcription in patients with −156G allele is more susceptible to Runx2-dependent upregulation and that patients carrying −156G allele demonstrated impaired diastolic function compared with −156del/−156del patients. In a previous report, one of three functional polymorphisms on *OPN* promoter (−66T/G) is associated with the onset of Type 1 diabetes in young female patients,[37] suggesting OPN transcription might be related with pathogenesis of type1 DM. As our study includes Type 2 DM patients, but not Type 1, it is reasonable that no statistical association exists between the frequency of −156del/G and incidence of DM in our subjects, though further investigation might be needed to assess the association of *OPN* promoter genotypes with the onset and the progression of Type 2 diabetes.

Hypertension with diabetes and −156G allele-mediated diastolic dysfunction

Diastolic dysfunction is the most prominent characteristics of diabetic cardiomyopathy,[38,39] and HF is the most common cause of death in patients with Type 2 diabetes after their first MI.[40] In combination with hypertension, Fukui *et al*.[41] observed that DM accelerates LV diastolic dysfunction via renin–angiotensin system in hypertensive rats. Taken together, hypertension and DM could synergistically deteriorate diastolic function in human. Kawamura *et al*.[13] previously reported that OPN expression is upregulated in diabetic human and rat vascular walls. In addition, OPN deletion attenuated diastolic dysfunction in heart of streptozotocin-induced diabetic mouse model.[42] Thus, OPN could upregulate in diabetic patients and affect diastolic function. In line with this, our results demonstrated that the diabetic patients with −156G allele displayed significantly lower E/A ratio compared with those with −156del/−156del genotype. In contrast, among the patients without DM, there were no significant differences in E/A ratio between the patients with and without −156G allele. Thus, our results suggest that OPN has a pivotal role in the development of diastolic dysfunction in hypertensive patients, especially with DM.

Study limitations

There are several limitations in our study. First, we did not measure serum OPN levels in the participated patients. Some previous investigators measured serum or urine OPN levels, and evaluated the association with functional polymorphisms in *OPN* promoter.[43,44] Indeed, none of them demonstrated significant differences in OPN levels between respective haplotypes, despite observed association with diseases. Presumably, not serum OPN levels, but local expression of OPN might be important to the onset of those disorders. We also did not evaluate other functional polymorphisms in promoter region of OPN such as −66T/G and −443T/C. Further investigation is needed to analyze the association between diastolic dysfunction and those two polymorphisms on *OPN* promoter.

In conclusion, our results first demonstrated the association of *OPN* promoter polymorphism with impaired cardiac diastolic function in human. As OPN is reported to have a pivotal role in the development of cardiac fibrosis and failure in animal models, it might be a promising therapeutic target for diastolic dysfunction in hypertensive patients, especially carrying −156G allele.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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