Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Molecular Cardiology

Impact of Renin-Angiotensin System Polymorphisms on Development of Systolic Dysfunction in Hypertrophic Cardiomyopathy

– Evidence From a Study of Genotyped Patients –

Akira Funada, MD; Tetsuo Konno, MD; Noboru Fujino, MD; Akihiko Muramoto, MD; Kenshi Hayashi, MD; Toshinari Tsubokawa, MD; Kenji Sakata, MD; Masa-aki Kawashiri, MD; Yoshiyu Takeda, MD; Hidekazu Ino, MD; Masakazu Yamagishi, MD, PhD

Background: Although the renin–angiotensin system (RAS) can affect the development of left ventricular (LV) hypertrophy, few data exist regarding the relationships between RAS polymorphisms and alteration of LV function. The effect of RAS polymorphisms on LV function in genotyped hypertrophic cardiomyopathy (HCM) was examined in the present study.

Methods and Results: The study group comprised 126 carriers with sarcomere gene mutations from 49 HCM families (64 males, mean age 51±21 years). LV morphology and function were evaluated by echocardiography. In angiotensin-converting enzyme (ACE) insertion/deletion (I/D), the D allele (n=81) exhibited significantly larger LV end-systolic dimension (LVDs) (32±11 mm) and lower ejection fraction (56±15%) than those with the II genotype (28±7 mm and 62±12%, respectively, P<0.05; n=45). Although angiotensin II type 1 receptor (AT_1-R) A/C^{1166} polymorphism did not affect echocardiographic parameters, the presence of the ACE D allele with the AT_1-R C^{1166} allele (n=9) was associated with larger LVDs (37±17 mm) and lower ejection fraction (48±20%) compared with other genotypes (30±9 mm and 58±14%, respectively, P<0.05; n=117). Under these conditions, severe LV hypertrophy was frequently associated with LV wall thinning.

Conclusions: The presence of both the ACE D and AT_1-R C^{1166} allele is associated with LV dilation with systolic dysfunction in genotyped HCM. In addition to the severity of LV hypertrophy, screening for these RAS polymorphisms could contribute to further risk stratification of patients with HCM, although other genetic polymorphisms should be further examined. (*Circ J* 2010; **74:** 2674–2680)

Key Words: Angiotensin-converting enzyme; Hypertrophic cardiomyopathy; Left ventricular remodeling; Renin–angiotensin system; Systolic dysfunction

Hypertrophic cardiomyopathy (HCM) is a primary cardiac disorder that has been defined as a left ventricular (LV) hypertrophy without other cardiovascular disease, and often transmits with heterogeneous clinical and morphological expression. Although many HCM patients experience a relatively benign course, there still remains a high risk of adverse cardiac events, such as sudden death, heart failure and embolism because of atrial fibrillation. Therefore, a major challenge in the management of the broad spectrum of HCM disease has been identifying the subsets of patients predisposed to sudden death and unexpected or heart failure-related death.[1–3] In fact, some patients show progression of systolic dysfunction with and without the LV dilatation that is characteristic of dilated cardiomyopathy,[4–6] although heart failure in HCM is largely the consequence of diastolic dysfunction.[7] However, the predisposing or precipitating causes of developing systolic dysfunction are not fully determined.

Differences in the clinical manifestations of HCM can be related to the presence of different disease-causing genes that encode sarcomeric proteins or different mutations within a given gene. However, the phenotypic expression and clinical course of HCM are also heterogeneous, even within families with an identical etiological sarcomere gene mutation.[8–10] This may be influenced by additional genetic factors, such as renin–angiotensin system (RAS) polymorphisms,

Received May 18, 2010; revised manuscript received July 21, 2010; accepted August 2, 2010; released online October 21, 2010 Time for primary review: 15 days

Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan
Mailing address: Masakazu Yamagishi, MD, PhD, Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medicine, 13-1 Takara-machi, Kanazawa 920-8640, Japan. E-mail: myamagi@med.kanazawa-u.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-10-0482

which can affect both LV hypertrophy and remodeling.[11] It is also intriguing to speculate whether the severity of LV hypertrophy (LVH) itself can be associated with development of LV dysfunction.[4–6] Therefore, the aim of this study was to examine the potential relationship between RAS polymorphisms and development of systolic dysfunction in HCM patients based on a molecular genetic diagnosis.

Methods

Study Population

This study included 49 unrelated patients with HCM exhibiting disease-causing mutations in genes such the beta-myosin heavy chain (*MYH7*), cardiac myosin binding protein-C (*MYBPC3*), cardiac troponin T (*TNNT2*), and cardiac troponin I (*TNNI3*). In addition, their family members were clinically and genetically examined and 77 carriers with the same etiological sarcomere gene mutation as each patient were identified. Thus, total 126 genetically-affected patients (64 males, mean age 51±21 years) comprised the study population. All patients were identified at the Kanazawa University Hospital or its affiliated hospitals between 1998 and 2006.

The diagnosis of HCM was based on echocardiographic findings, such as maximal LV wall thickness ≥13 mm and the absence of any other cause of LVH as specified in the criteria of Maron et al.[12] Patients with systolic dysfunction were also included in this study: patients with (1) progression from typical HCM to systolic dysfunction during follow-up, and (2) typical HCM with an identical sarcomere gene mutation identified in the same family without any other cause of systolic dysfunction. To further examine whether hypertrophy itself can be a risk factor for developing LV dysfunction, the changes in the echocardiographic parameters from initial to last evaluation divided by follow-up period were additionally adopted as a sub-analysis. Written informed consent was given by all patients in accordance with the guidelines of the Bioethical Committee on Medical Research, School of Medicine, Kanazawa University, Kanazawa, Japan.

Genetic Studies

Genomic DNA was purified from patients' white blood cells, after which in vitro gene amplification was performed by polymerase chain reaction (PCR). Oligonucleotide primers were used to amplify all exons and exon/intron boundaries of 4 sarcomere genes, namely, *MYH7*, *MYBPC3*, *TNNT2*, and *TNNI3*, using standard protocols, as described previously.[13–16] Single-strand conformational polymorphism analysis of amplified DNA was then performed. For abnormal single-strand conformational polymorphism patterns, the nucleotide sequences of the cloned PCR products were determined on both strands (bidirectional sequencing) by the dye terminator cycle sequencing method using an automated fluorescent sequencer (ABI PRISM™ 310 Genetic Analyzer, PE Biosystems, Foster City, CA, USA). The sequence variation was confirmed by restriction enzyme digestion.

Determination of RAS-Related Gene Polymorphisms

In this study, we chose the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) and the angiotensin II type 1 receptor (AT_1-R) A/C^{1166} from among the many RAS polymorphisms because these 2 polymorphisms (ie, the ACE D allele (DD and ID genotypes) and the AT_1-R C^{1166} allele (CC and AC genotypes)) are reported to be associated with increased LVH in HCM.[17–19] Gene polymorphisms of ACE I/D were identified by PCR performed with a set of oligonucleotide primers flanking the polymorphic site in intron 16. To avoid mistyping, each sample found to have the DD genotype was subjected to a second round of independent PCR amplification with a primer pair that recognized an insertion-specific sequence, as described previously.[20] The AT_1-R A/C^{1166} polymorphism was determined by PCR and restriction fragment length polymorphisms, as previously reported.[21] PCR was performed to amplify a fragment encompassing the polymorphic site at nucleotide position 1166 in the 3' untranslated region of the human *AT_1-R* gene. PCR products were then digested with restriction enzyme DdeI (Toyobo, Osaka, Japan) and the cleaved products were separated by electrophoresis.

Table 1. Demographic and Clinical Characteristics of the Study Population

n	126
Age (years)	51±21
Male (%)	64 (51)
Echocardiography	
LAd (mm)	40±9
IVST (mm)	15±6
PWT (mm)	10±2
MWT (mm)	16±6
LVDd (mm)	46±8
LVDs (mm)	30±10
EF (%)	58±14
Disease-causing gene (%)	
MYH7	17 (14)
MYBPC3	35 (28)
TNNT2	23 (18)
TNNI3	51 (40)
Renin-angiotensin system polymorphisms (%)	
ACE I/D	
DD	25 (20)
ID	56 (44)
II	45 (36)
AT_1-R A/C^{1166}	
AA	112 (89)
AC	14 (11)
CC	0 (0)
Combination of ACE I/D and AT_1-R A/C^{1166}	
ACE D allele with AT_1-R C allele	9 (7)
Others*	117 (93)

Values are mean±SD unless otherwise shown.
LAd, left atrial dimension; IVST, interventricular septal thickness; PWT, posterior wall thickness; MWT, maximal wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; EF, ejection fraction; *MYH7*, β-myosin heavy chain; *MYBPC3*, cardiac myosin binding protein-C; *TNNT2*, cardiac troponin T; *TNNI3*, cardiac troponin I; ACE, angiotensin-converting enzyme; I, insertion; D, deletion; AT_1-R, angiotensin II type 1 receptor; D allele, DD and ID genotype; C allele, CC and AC genotype.
*Others comprises ACE D allele with AT_1-R AA^{1166} genotype, ACE II genotype with AT_1-R C^{1166} allele and ACE II genotype with AT_1-R AA^{1166} genotype.

Echocardiographic Examinations

Standard transthoracic M-mode and 2-dimensional echocardiographic studies were performed to identify and quantify

Table 2. Differences in Patients' Characteristics Among 4 Sarcomere Gene Mutations

	MYH7 (n=17)	*MYBPC3* (n=35)	*TNNT2* (n=23)	*TNNI3* (n=51)	P value
Age (years)	57±18	52±21	53±19	46±22	0.272
Echocardiography					
LAd (mm)	38±9	38±8	41±8	42±9	0.145
IVST (mm)	16±6	15±3	16±7	13±5	0.128
PWT (mm)	11±3	11±2	10±2	10±2	0.513
MWT (mm)	16±6	16±6	18±7	14±5	0.160
LVDd (mm)	46±9	44±6	48±12	45±7	0.380
LVDs (mm)	30±12	28±7	34±15	30±8	0.184
EF (%)	59±4	61±12	54±4	57±13	0.313
Renin-angiotensin system polymorphisms					
Frequency of ACE I/D polymorphisms					
D allele (%)	12 (86)	16 (46)	15 (65)	38 (75)	0.048
II (%)	5 (14)	19 (54)	8 (35)	13 (25)	
Frequency of AT_1-R A/C^{1166} polymorphisms					
C allele (%)	1 (6)	2 (6)	6 (3)	5 (10)	0.078
AA (%)	16 (94)	33 (94)	17 (97)	46 (90)	
Frequency of combination of ACE I/D and AT_1-R A/C^{1166} polymorphisms					
ACE D allele with AT_1-R C allele (%)	1 (6)	1 (3)	3 (13)	4 (8)	0.521
Others (%)	16 (94)	34 (97)	20 (87)	47 (92)	

Values are mean±SD unless otherwise shown.
Abbreviations as in Table 1.

the morphologic features of the LV. LV end-diastolic dimension (LVDd) and end-systolic dimension (LVDs), and the thicknesses of the interventricular septum (IVST) and LV posterior wall (PWT) were measured at the level of the tips of the mitral valve leaflets. Additionally, we measured the anterior and lateral walls of the LV at the same levels used for measuring the septum and posterior walls. From these measurements, we defined the maximal LV wall thickness (MWT). Ejection fraction (EF) was calculated by Teichholz's method and by modified Simpson's method when LV dilatation or regional decrease of LV wall motion occurred. The left atrial dimension was measured at end-systole.

Statistical Analysis

Values are expressed as mean±SD. Measured values were compared between 2 groups with an unpaired Student's t-test. Comparison between groups was performed using a one-way analysis of variance followed by Scheffé's method. Categorical variables were compared by the chi-square test for independence. A P-value<0.05 was considered statistically significant. Statistical analyses were carried out with the computer software StatView for Windows version 5.0 (Abacus Concepts, Inc, Berkeley, CA, USA).

Results

Genetic Results and Clinical Characteristics of the Study Population

The demographic and clinical characteristics of the study population are presented in Table 1; 15 different mutations were identified in 126 patients and among them, 17 patients had mutations in *MYH7* (Ala26Val, n=4; Arg204His, n=1; Arg858Cys, n=4; Arg870Cys, n=1; Gly733Glu, n=2; Met822Leu, n=2; and Glu935Lys, n=3), 35 patients had mutations in *MYBPC3* (Arg820Gln, n=15; c.2067+1G>A, n=15; and Del593C, n=5), 23 patients had mutations in *TNNT2* (Arg92Trp, n=10; Lys273Glu, n=10; Val85Leu, n=1; and Phe110Ile, n=2), and 51 patients had a mutation in *TNNI3* (Lys183Del; n=51). All of these mutations have been identified and described elsewhere.[22,23]

As for the frequencies of ACE I/D, 36% were II, 44% were ID and 20% were DD. For the AT_1-R A/C^{1166}, 89% were AA and 11% were AC (Table 1); 9 of the 126 patients had both the ACE D and AT_1-R C^{1166} alleles and of these 9 patients 7 were from 7 unrelated kindred with genetic mutations (*MYH7*=1, *MYBPC3*=1, *TNNT2*=1 and *TNNI3*=4), and the other 2 were from a kindred with a *TNNT2* mutation in which 1 was typical HCM and the other had systolic dysfunction.

The differences in the underlying sarcomere gene mutations are shown in Table 2. There were no differences between the 4 genes with respect to echocardiographic parameters. Regarding the relationship between sarcomere gene mutations and RAS polymorphisms, the proportion of the II genotype of ACE I/D polymorphisms was higher than that of the D allele only in association with *MYBPC3* mutation (Table 2).

Echocardiographic Parameters and RAS Polymorphisms

Among the ACE I/D polymorphisms, the presence of the dominant D allele (DD and ID genotypes) was associated with significantly decreased PWT (10±2 mm vs 11±3 mm, P<0.05), larger LVDs (32±11 mm vs 28±7 mm, P<0.05) and lower EF (56±15% vs 62±12%, P<0.05) than in those with the II genotype. We demonstrate 2 representative patients who showed entirely different clinical manifestations, despite having an identical mutation of Arg92Trp in *TNNT2*.[24] One had dilated cardiomyopathy-like features with the ACE D

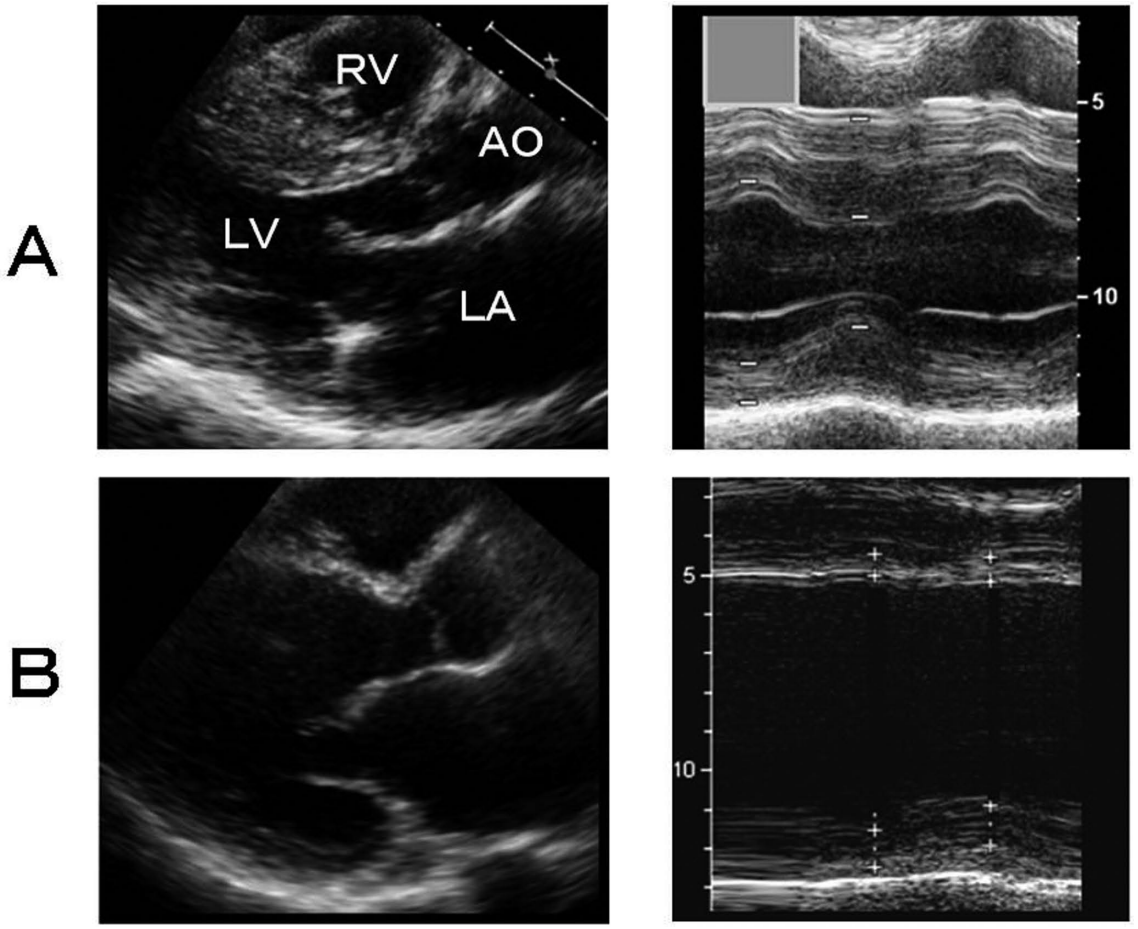

Figure. Two-dimensional (**Left**) and M-mode (**Right**) echocardiography of genotyped patients with the same sarcomere gene mutation, such as Arg92Trp in troponin T, and different renin–angiotensin system gene polymorphisms. (**A**) A 75-year-old female with angiotensin-converting enzyme (ACE) insertion–insertion had been diagnosed as having hypertrophic cardiomyopathy (HCM) at 64 years of age. Eleven years later, echocardiography still showed marked septal hypertrophy represented by thickness of the interventricular septal wall of 20 mm and 11 mm for the posterior wall. Left ventricular (LV) end-diastolic and end-systolic dimensions were 45 mm and 27 mm, respectively, yielding an ejection fraction of 70%. (**B**) A 63-year-old male with ACE deletion allele had been diagnosed with HCM at 51 years of age. Interestingly, during the 12 years since the initial diagnosis, LV systolic dysfunction and dilatation gradually developed. LV end-diastolic and end-systolic dimensions were 65 mm and 57 mm, respectively, representing decreased systolic function with an ejection fraction of 26%. AO, aorta; LA, left atrium; RV, right ventricle.

allele and the other with an ACE II genotype had typical HCM (**Figure**). Interestingly, the presence of the ACE D allele with the AT_1-R C^{1166} allele was associated with significantly larger LVDs (37±17 mm vs 30±9 mm, P<0.05) and lower EF (48±20% vs 58±14%, P<0.05) compared with others (**Table 3**), although AT_1-R A/C^{1166} polymorphism itself did not influence the echocardiographic parameters.

When patients were divided into 3 groups, such as ACE D allele with AT_1-R C^{1166} allele (n=9), ACE D allele with AT_1-R AA^{1166} genotype (n=72) and ACE II genotype with AT_1-R C^{1166} allele or AT_1-R AA^{1166} genotype (n=45), those with the ACE D and AT_1-R C^{1166} alleles showed the largest LVDs dimension (37±17 mm vs 31±10 mm vs 28±7 mm, P<0.05) and the lowest EF (48±20% vs 56±14% vs 62±12%, P<0.05) among the 3 groups.

Effect of LVH on the Relationship Between LV Dysfunction and RAS Polymorphisms

To study the development of LV dysfunction, 59 of the 126 patients underwent serial echocardiography at least 2 years apart. After excluding 15 patients with LV dysfunction such as EF<50% and/or LVDd>55 mm at initial evaluation, the data from the remaining 44 patients (25 males, mean age 48±16 years and mean follow-up 6.7±3.5 years) were analyzed. When patients were divided into 2 groups by median MWT of 19 mm, there were 23 patients with MWT≥19 mm and 21 patients with MWT<19 mm. There was no significant difference between the 2 groups in the frequencies of disease-causing genes and RAS polymorphisms. Interestingly, MWT and IVST decreased by 0.46±1.01 and 0.35±1.14 mm/year, respectively, in patients with MWT≥19 mm, although MWT and IVST increased by 0.43±0.78 and 0.43±0.75 mm/year, respectively, in those with MWT <19 mm (P<0.05, respec-

Table 3. Differences Among Genotypes of RAS Gene Polymorphisms

	ACE I/D			AT_1-R A/C^{1166}			Combination of ACE I/D and AT_1-R A/C^{1166}		
	D allele (n=81)	II (n=45)	P value	C allele (n=14)	AA (n=112)	P value	ACE D with AT_1-R C (n=9)	Others (n=117)	P value
Age (years)	53±20	47±23	0.146	53±18	50±21	0.634	58±18	50±21	0.308
Echocardiography									
LAd (mm)	41±8	39±10	0.412	42±10	40±8	0.579	45±10	40±8	0.113
IVST (mm)	14±6	16±5	0.108	15±7	15±6	0.740	12±6	15±6	0.209
PWT (mm)	10±2	11±3	0.048	10±2	10±2	0.942	11±3	10±2	0.835
MWT (mm)	15±6	16±5	0.223	17±6	15±6	0.471	15±5	16±6	0.606
LVDd (mm)	47±9	44±7	0.057	47±13	46±8	0.677	49±15	45±7	0.157
LVDs (mm)	32±11	28±7	0.025	32±15	30±9	0.489	37±17	30±9	0.043
EF (%)	56±15	62±12	0.021	56±18	58±14	0.612	48±20	58±14	0.042

Values are mean±SD.
ACE D with AT_1-R C=ACE D allele with AT_1-R C allele; other abbreviations as in Table 1.

Table 4. Effect of Degree of LV Hypertrophy on LV Dysfunction

	MWT≥19 (n=23)	MWT<19 (n=21)	P value
Age (years)	50±12	46±20	0.49
Male (%)	13 (57)	12 (57)	0.97
Follow-up (years)	6.9±3.8	6.5±3.3	0.72
Echocardiography at initial evaluation			
IVST (mm)	20±3.7	14±2.6	<0.0001
PWT (mm)	11±2.8	11±2.2	0.32
MWT (mm)	22±3.0	14±2.6	<0.0001
LVDd (mm)	43±5.7	44±3.3	0.36
LVDs (mm)	26±4.5	26±3.6	0.77
EF (%)	65±7.0	66±6.7	0.57
Echocardiography at last evaluation			
IVST (mm)	18±6.5	16±4.3	0.16
PWT (mm)	11±2.7	11±2.4	0.75
MWT (mm)	20±6.2	16±4.5	0.03
LVDd (mm)	46±6.0	45±6.4	0.73
LVDs (mm)	29±6.9	29±7.3	0.86
EF (%)	61±11	61±12	0.93
Annual change in echocardiographic parameters			
ΔIVST/year	−0.35±1.14	0.43±0.75	0.011
ΔPWT/year	−0.06±0.65	−0.05±0.56	0.96
ΔMWT/year	−0.46±1.01	0.43±0.78	0.002
ΔLVDd/year	0.41±1.33	0.09±1.05	0.38
ΔLVDs/year	0.48±1.19	0.28±1.24	0.59
ΔEF/year	−0.67±2.19	−0.57±2.08	0.87

Values are mean±SD unless otherwise shown.
Δ, change from initial evaluation to last evaluation. All abbreviations as in Table 1.

tively) (Table 4). Thus, patients with MWT≥19 mm showed higher annual EF decrease and larger annual LV dimension increase than patients with MWT<19 mm, although results did not reach statistical significance.

Discussion

The major findings of this study are that, in addition to severity of LVH, the ACE D allele is associated with development of systolic dysfunction and LV dilatation in genotyped HCM. Furthermore, the presence of the ACE D allele with the AT_1-R C^{1166} allele appears to be a risk factor for further systolic dysfunction and LV dilatation.

The ACE D allele has been reported to be associated with higher plasma ACE levels than the II genotype,[25] and subjects with the AT_1-R C^{1166} allele exhibit higher angiotensin II responses than those with the AA genotype.[26] Therefore, the ACE D and AT_1-R C^{1166} alleles cause an increase in RAS

activity, which is closely related to myocardial hypertrophy and subsequent LV remodeling.[11] Indeed, the ACE D and the AT1-R C1166 alleles have been reported as associated with increased LVH in HCM.[17–19] In previous follow-up studies of the predictors of LV systolic dysfunction in HCM, patients who developed systolic dysfunction had a greater LV wall thickness than patients who maintained normal systolic function at initial evaluation.[4–6] Thus, it is possible that these 2 RAS polymorphisms are associated not only with LVH but also with subsequent LV remodeling that can lead to development of systolic dysfunction and LV dilatation. However, few data exist regarding the relationship between these polymorphisms and the progression of systolic dysfunction in HCM patients because of methodological difficulties in collecting both phenotypic and genetic data from such cases.

In the present study, the ACE D allele was associated with decreased PWT, decreased EF and increased LVDs. In addition, the presence of the ACE D allele with the AT1-R C1166 allele was associated with further decrease in EF and increase in LVDs, both of which reflect LV remodeling. Therefore, one might speculate that the ACE D and AT1-R C1166 alleles are responsible for LVH and subsequent LV remodeling. The present study is the first to demonstrate the novel modifying effects of these RAS polymorphisms as a risk factor for progression of systolic dysfunction and LV dilatation in the clinical setting. RAS polymorphisms partially account for the inter- and intrafamilial variability in the phenotypic expression of HCM.

It was quite interesting that the combination of the ACE D and AT1-R C1166 alleles contributed to the development of systolic dysfunction and LV dilatation. The ACE I/D and AT1-R A/C1166 polymorphisms are known as those that cause LVH. Indeed, studies have revealed a direct relationship between the burden of polymorphisms and the degree of LVH in HCM.[8,9] In a histologic study, the burden of gene polymorphisms related to LVH was associated with the extent of cardiomyocyte hypertrophy in HCM.[27] Those results support the hypothesis of different polymorphic genotypes forming a compound unit, the components of which act in a related manner.[8] In the present study, the presence of the ACE D and AT1-R C1166 alleles was associated with further decrease in EF and increase in LVDs compared with the ACE D allele alone. It is possible that the combined effects of polymorphisms related to LVH are associated with not only LVH but also the resultant further LV remodeling that can lead to greater systolic dysfunction and LV dilatation.

In contrast to the results of previous studies,[17–19] neither the ACE D nor the AT1-R C1166 allele itself was related to the presence of LVH in the present study. This may be explained by the fact that almost all of the previous studies had limited numbers of patients and the inclusion criteria differed from ours. In particular, the patients enrolled in previous studies were diagnosed as HCM only by echocardiography without the genetic analysis that was an important part of the inclusion criteria for the present study. Inclusion of HCM with systolic dysfunction in the present study might also have contributed to the different results. Indeed, 25 of the 126 (20%) patients has decreased EF (<50%). The AT1-R CC1166 genotype was not found in the present study, probably because of the small number of patients with the AT1-R CC1166 genotype in the general Japanese population.[21]

During the follow-up, MWT and IVST in patients with MWT ≥19 mm decreased, although those in patients with MWT <19 mm increased, which suggests that LV remodeling is prominent in accordance with LVH. Indeed, patients with MWT ≥19 mm showed higher annual EF decreases and larger annual LV dimension increases than patients with MWT <19 mm. Under these conditions, RAS polymorphisms were not associated with LVH and development of LV dysfunction, probably because we excluded patients with LV dysfunction at initial evaluation, as they could be highly affected by RAS. It is also possible that LV dysfunction because of hypertrophy reflects remodeling caused by ischemia, myocardial fibrosis, small vessel disease and myocardial disarray, suggesting that other genetic or environmental factors could be involved.[5,6]

Clinical Implications and Study Limitations

It has been recently demonstrated that angiotensin II type 1 receptor blockers decreased myocardial fibrosis in a transgenic mouse model of human HCM,[28] and this class of drug has improved LV diastolic function and prevented progression of LVH in clinical HCM patients.[29,30] In the present study, the presence of the ACE D and AT1-R C1166 alleles was shown to be a possible maker for effective prediction of systolic dysfunction and LV dilatation in HCM. For pharmacological intervention, we suggest that RAS blockade could be effective in patients with these RAS polymorphisms, although the rationale and hypothesis need further investigation.

There remain several limitations to the present study. First, we did not include all of the disease-causing genes, only screening for the 4 major disease-causing genes because their frequencies are higher than the others and comprise most of the genotyped HCM cases, even in the Japanese HCM population.[22,31] Second, 51 of the 126 (40%) genetically-affected patients had a mutation in *TNNI3*, which has caused less than 6% of HCM in most large series studied to date. Therefore, the distribution of mutations in our population was somewhat different from those previously reported.[22,31]

Third, we studied only 2 gene polymorphisms related to LVH: ACE I/D and AT1-R A/C1166. Therefore, further investigation of the other polymorphisms related to LVH, such as angiotensinogen M235T, an A/G exchange at position-1903 of the cardiac chymase A gene, and a C/T exchange at position-344 in the aldosterone synthase gene, is necessary in future to confirm the association between RAS polymorphisms and the development of systolic dysfunction.[8,9,27] Fourth, there may be familial bias, because gene-specific genotype–phenotype correlations have been reported.[24,32,33] However phenotypic variability of HCM is not completely explained by disease-causing gene mutation alone.[8–10] From that we infer that the final phenotype is likely the consequence of interaction between the disease-causing gene mutation and other gene variants in the genetic background, such as RAS polymorphisms and environmental factors.

Finally, a proportion of the original study population did not undergo serial evaluation. Therefore, we could not precisely demonstrate a relationship between RAS polymorphisms and life-long LV remodeling from hypertrophy to dysfunction. However, it can be speculated that LV dysfunction would be more prominent in patients with previously greater LV wall thickness and this process would be modified by RAS polymorphisms, as shown in the present study. Long-term follow-up of a large number of patients may confirm this hypothesis.

Conclusions

The ACE D allele is associated with systolic dysfunction and LV dilatation in genotyped HCM. Furthermore, the pres-

ence of the ACE D and AT_1-R C^{1166} alleles is closely related to further systolic dysfunction and LV dilatation, although LVH itself may contribute to the development of LV dysfunction. We suggest that, in addition to screening for disease-causing gene mutations, detection of these RAS polymorphisms may assist further risk stratification and better pharmacological therapy in genotyped HCM patients.

References

1. Maron BJ. Hypertrophic cardiomyopathy: A systematic review. *JAMA* 2002; **287:** 1308–1320.
2. Elliott P, McKenna WJ. Hypertrophic cardiomyopathy. *Lancet* 2004; **363:** 1881–1891.
3. Kubo T, Kitaoka H, Okawa M, Hirota T, Hayato K, Yamasaki N, et al. Clinical impact of atrial fibrillation in patients with hypertrophic cardiomyopathy: Results from Kochi RYOMA Study. *Circ J* 2009; **73:** 1599–1605.
4. Harris KM, Spirito P, Maron MS, Zenovich AG, Formisano F, Lesser JR, et al. Prevalence, clinical profile, and significance of left ventricular remodeling in the end-stage phase of hypertrophic cardiomyopathy. *Circulation* 2006; **114:** 216–225.
5. Thaman R, Gimeno JR, Murphy RT, Kubo T, Sachdev B, Mogensen J, et al. Prevalence and clinical significance of systolic impairment in hypertrophic cardiomyopathy. *Heart* 2005; **91:** 920–925.
6. Biagini E, Coccolo F, Ferlito M, Perugini E, Rocchi G, Bacchi-Reggiani L, et al. Dilated-hypokinetic evolution of hypertrophic cardiomyopathy: Prevalence, incidence, risk factors, and prognostic implications in pediatric and adult patients. *J Am Coll Cardiol* 2005; **46:** 1543–1550.
7. Yamamoto K, Sakata Y, Ohtani T, Takeda Y, Mano T. Heart failure with preserved ejection fraction. *Circ J* 2009; **73:** 404–410.
8. Ortlepp JR, Vosberg HP, Reith S, Ohme F, Mahon NG, Schröder D, et al. Genetic polymorphisms in the renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: A study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. *Heart* 2002; **87:** 270–275.
9. Perkins MJ, Van Driest SL, Ellsworth EG, Will ML, Gersh BJ, Ommen SR, et al. Gene-specific modifying effects of pro-LVH polymorphisms involving the renin-angiotensin-aldosterone system among 389 unrelated patients with hypertrophic cardiomyopathy. *Eur Heart J* 2005; **26:** 2457–2462.
10. Marian AJ, Mares A Jr, Kelly DP, Yu QT, Abchee AB, Hill R, et al. Sudden cardiac death in hypertrophic cardiomyopathy: Variability in phenotypic expression of beta-myosin heavy chain mutations. *Eur Heart J* 1995; **16:** 368–376.
11. Wang JG, Staessen JA. Genetic polymorphisms in the renin-angiotensin system: Relevance for susceptibility to cardiovascular disease. *Eur J Pharm* 2000; **410:** 289–302.
12. Maron BJ, Gottdiener JS, Epstein SE. Patterns and significance of distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy: A wide angle, two dimensional echocardiographic study of 125 patients. *Am J Cardiol* 1981; **48:** 418–428.
13. Carrier L, Bonne G, Bährend E, Yu B, Richard P, Niel F, et al. Organization and sequence of human cardiac myosin binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. *Circ Res* 1997; **80:** 427–434.
14. Jaenicke T, Diederich KW, Haas W, Schleich J, Lichter P, Pfordt M, et al. The complete sequence of the human β-myosin heavy chain gene and a comparative analysis of its product. *Genomics* 1990; **8:** 194–206.
15. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, et al. α-Tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: A disease of the sarcomere. *Cell* 1994; **77:** 701–712.
16. Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M, et al. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997; **16:** 379–382.
17. Lechin M, Quiñones MA, Omran A, Hill R, Yu QT, Rakowski H, et al. Angiotensin-I converting enzyme genotypes and left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. *Circulation* 1995; **92:** 1808–1812.
18. Doolan G, Nguyen L, Chung J, Ingles J, Semsarian C. Progression of left ventricular hypertrophy and the angiotensin-converting enzyme gene polymorphism in hypertrophic cardiomyopathy. *Int J Cardiol* 2004; **96:** 157–163.
19. Osterop AP, Kofflard MJ, Sandkuijl LA, ten Cate FJ, Krams R, Schalekamp MA, et al. AT1 receptor A/C1166 polymorphism contributes to cardiac hypertrophy in subjects with hypertrophic cardiomyopathy. *Hypertension* 1998; **32:** 825–830.
20. Ogimoto A, Hamada M, Nakura J, Miki T, Hiwada K. Relation between angiotensin-converting enzyme II genotype and atrial fibrillation in Japanese patients with hypertrophic cardiomyopathy. *J Hum Genet* 2002; **47:** 184–189.
21. Takami S, Katsuya T, Rakugi H, Sato N, Nakata Y, Kamitani A, et al. Angiotensin II type 1 receptor gene polymorphism is associated with increase of left ventricular mass but not with hypertension. *Am J Hypertens* 1998; **11:** 316–321.
22. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, et al. Hypertrophic cardiomyopathy: Distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003; **107:** 2227–2232.
23. CardioGenomics. http://cardiogenomics.med.harvard.edu/home (accessed 27 April, 2010).
24. Fujino N, Shimizu M, Ino H, Okeie K, Yamaguchi M, Yasuda T, et al. Cardiac troponin T Arg92Trp mutation and progression from hypertrophic to dilated cardiomyopathy. *Clin Cardiol* 2001; **24:** 397–402.
25. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half of the variance of serum enzyme levels. *J Clin Invest* 1990; **86:** 1343–1346.
26. van Geel PP, Pinto YM, Voors AA, Buikema H, Oosterga M, Crijns HJ, et al. Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increase response to angiotensin II in human arteries. *Hypertension* 2000; **35:** 717–721.
27. McLeod CJ, Bos JM, Theis JL, Edwards WD, Gersh BJ, Ommen SR, et al. Histologic characterization of hypertrophic cardiomyopathy with and without myofilament mutations. *Am Heart J* 2009; **158:** 799–805.
28. Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. *Circulation* 2001; **103:** 789–791.
29. Araujo AQ, Arteaga E, Ianni BM, Buck PC, Rabello R, Mady C. Effect of Losartan on left ventricular diastolic function in patients with nonobstructive hypertrophic cardiomyopathy. *Am J Cardiol* 2005; **96:** 1563–1567.
30. Yamazaki T, Suzuki J, Shimamoto R, Tsuji T, Ohmoto-Sekine Y, Ohtomo K, et al. A new therapeutic strategy for hypertrophic nonobstructive cardiomyopathy in humans: A randomized and prospective study with an angiotensin II receptor blocker. *Int Heart J* 2007; **48:** 715–724.
31. Kimura A. Molecular etiology and pathogenesis of hereditary cardiomyopathy. *Circ J* 2008; **72:** A-38–A-48.
32. Niimura H, Bachinski LL, Sangwatanaroj S, Watkins H, Chudley AE, McKenna W, et al. Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. *N Engl J Med* 1998; **338:** 1248–1257.
33. Kokado H, Shimizu M, Yoshio H, Ino H, Okeie K, Emoto Y, et al. Clinical features of hypertrophic cardiomyopathy caused by a Lys183 deletion mutation in the cardiac troponin I gene. *Circulation* 2000; **102:** 663–669.