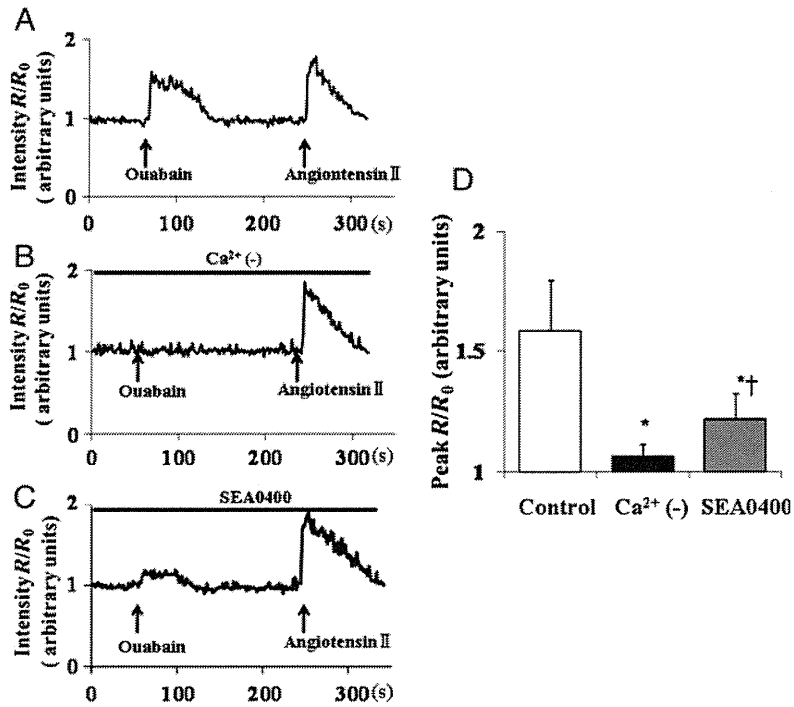

Figure 3 Representative intracellular Ca^{2+} transients in cardiac fibroblasts at control (*A*), Ca^{2+} free condition (*B*), and SEA0400 administration (*C*). The values are expressed as R/R_0 (R: 340/380 nm emission ratio; R_0: R at time 0). Peak R/R_0 was evaluated from 15 cells (3 cells from 5 independent experiments) in each protocol (*D*). Data are presented as mean ± SD. $^*P < 0.05$ vs. control. $^\dagger P < 0.05$ vs. Ca^{2+} (−).

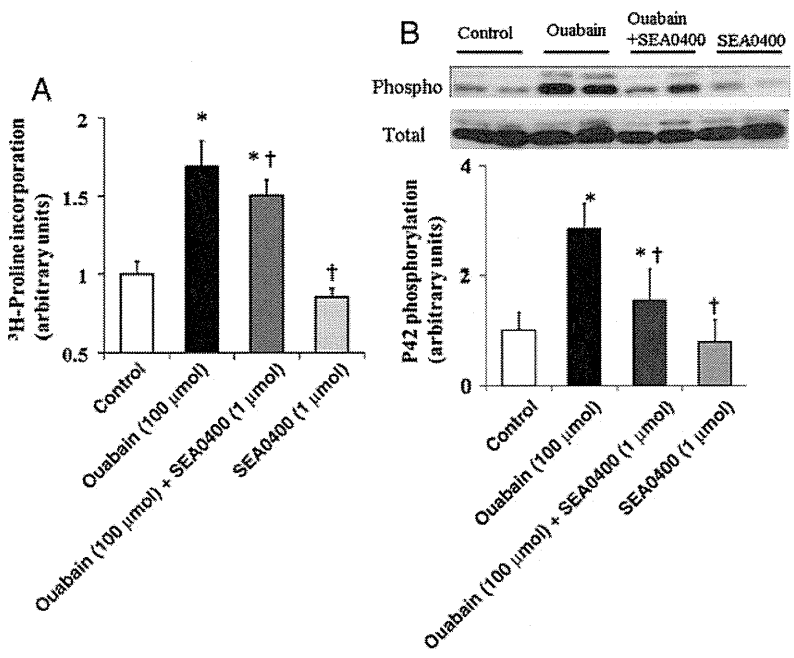

Figure 4 (*A*) Summary data for ^{3}H-Proline incorporation in fibroblasts. (*B*) Representative western blot analysis of phosphorylated p42/44 mitogen-activated protein kinase and total p42/44 mitogen-activated protein kinase, and summary data for p42 phosphorylation. Phosphorylated p42 values were normalized against the total p42 signal and expressed as mean ± SD. $^*P < 0.05$ vs. control; $^\dagger P < 0.05$ vs. ouabain.

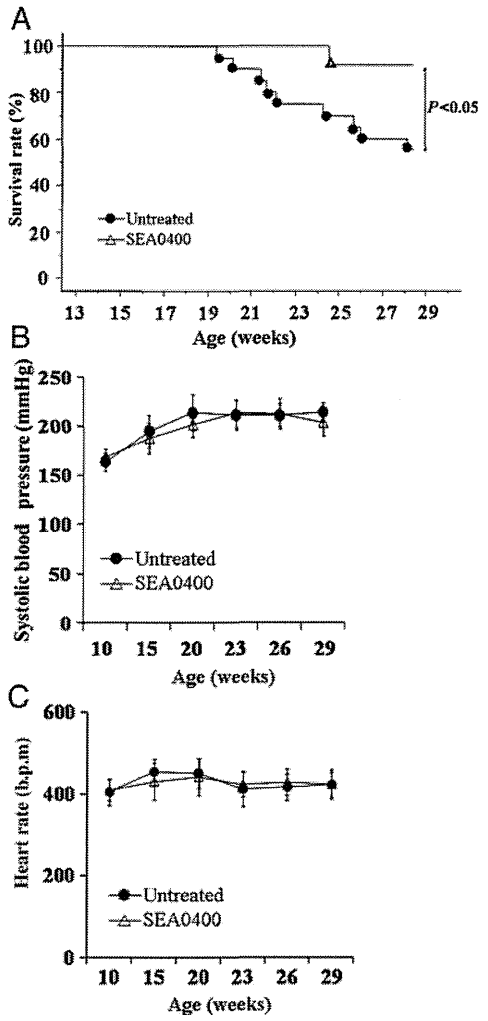

Figure 5 Kaplan–Meier survival curves (*A*) and serial changes in systolic blood pressure (*B*) and heart rate (*C*) in the untreated and SEA groups.

necessarily reproduce the effects of ouabain at its physiological concentration, the current data suggest that DLFs can induce LV fibrosis through the direct pathway in cardiac tissue, and that the blockade of this pathway is a therapeutic strategy for hypertensive HFPEF independent of anti-hypertensive effects.

Previous *in vitro* studies have shown that DLFs promote collagen production in fibroblasts through the non-pumping function of Na^+/K^+-ATPase signalosome,[5,7] and this signalling cascade has been considered to lead to the activation of Src and p42/44 MAPK.[5] Liu *et al.*[6] reported that the activation of p42/44 MAPK is mandatory in transforming growth factor-β-stimulated collagen synthesis in cardiac fibroblast. These studies suggest that DLFs-induced stimulation of non-pumping function of Na^+/K^+-ATPase is responsible for the enhancement of collagen production. Digitalis-like factors also inhibit pumping function of Na^+/K^+-ATPase and increase intracellular Na^+ concentration, which in turn raises $[Ca^{2+}]_i$ through the entry mode of NCX[8]; however, a previous study showed that the increase in $[Ca^{2+}]_i$ was secondary to the activation of p42/44 MAPK in myocytes.[10] If so in fibroblast, the blockade of the entry mode of NCX may not lead to the inhibition of p42/44 MAPK and collagen production. This study showed that the ouabain-induced increase in $[Ca^{2+}]_i$ was due to the influx of extracellular Ca^{2+} through the entry mode of NCX, and that the blockade of the entry mode with SEA0400 attenuated the phosphorylation level of p42/44 MAPK and the collagen synthesis as well as the increase in $[Ca^{2+}]_i$. These results suggest that DLFs activate p42/44 MAPK and promote collagen synthesis in fibroblasts at least partly through the inhibition of pumping function of Na^+/K^+-ATPase and the secondary promotion of the entry mode of NCX. The entry mode of NCX also enhances migration, contraction, and proliferation of fibroblasts.[2,5] Thus, the blockade of the entry mode of NCX can be expected to inhibit tissue fibrosis in the condition with enhanced secretion of DLFs.

This study demonstrated that the secretion of DLFs was enhanced in an animal model of hypertensive HFPEF, and that the chronic administration of SEA0400, an inhibitor of the NCX entry mode,[12] attenuated LV fibrosis and myocardial stiffening, inhibited the elevation of LV filling pressure and pulmonary congestion, and improved the survival rate of this model. Iwamoto *et al.*[8] reported the depressor effects of SEA0400 in Dahl salt-sensitive rats fed on high-salt diet. We used a low dose of SEA0400 to avoid its anti-hypertensive effects and demonstrated that SEA0400 provided the beneficial effects even without the changes in systolic blood pressure. Thus, the blockade of the entry mode of NCX is likely a new therapeutic strategy for the prevention of LV fibrosis, LV stiffening, and HFPEF, and our results suggest that the attenuation of LV fibrosis is provided through the inhibition of collagen synthesis rather than through the enhancement of collagen degradation. Previous studies have suggested that the entry mode of NCX has protective effects on LV systolic function in patients and animals with reduced ejection fraction.[26] However, the administration of SEA0400 did not alter LV endocardial and mid-wall fractional shortenings in HFPEF model rats (*Table 3*), suggesting that the chronic blockade of the entry mode of NCX does not provide adverse effects on systolic function in HFPEF.

Current guidelines for the treatment of heart failure recommend the use of digitalis in patients with reduced ejection fraction to decrease hospitalization for worsening heart failure. Digitalis Investigation Group (DIG) trial showed that digitalis did not reduce mortality of heart failure patients irrespective of ejection fraction but was not inferior to placebo,[27] indicating that digitalis does not provide adverse effects. The current results suggest the adverse effects of digitalis and are likely discrepant from the clinical studies. However, a *post hoc* analysis of DIG trial demonstrated that high serum digoxin concentration was associated with an unadjusted increase in all-cause mortality, whereas low serum digoxin concentration was associated with its decrease.[28] Thus, the adverse effects of DLFs may be exerted in association with the increase in their tissue/serum concentration, which is partly compatible with the current results. Another explanation is that the effects of all of DLFs are not the same.[29] Some of DLFs provide counteractive effects against other DLFs. Thus, the effects of exogenous digitalis may not mimic those of endogenous DLFs.

Table 3 Haemodynamic and echocardiographic parameters at the age of 19 weeks in Study 4 (Dahl salt-sensitive rats)

	Control group ($n = 8$)	Untreated group ($n = 8$)	SEA group ($n = 8$)	*P*-value for NO difference
Systolic blood pressure (mmHg)	128 ± 7	217 ± 12*	211 ± 10*	<0.001
LV end-diastolic dimension (mm)	9.7 ± 0.7	10.6 ± 0.8	10.3 ± 0.7	0.055
LV posterior wall thickness at end-diastole (mm)	1.1 ± 0.1	1.7 ± 0.1*	1.5 ± 0.1****	<0.001
LV endocardial fractional shortening (%)	27 ± 3	28 ± 7	30 ± 6	0.690
LV mid-wall fractional shortening (%)	16 ± 2	14 ± 3	16 ± 3	0.312
LV end-diastolic pressure (mmHg)	5 ± 5	17 ± 7*	9 ± 5**	0.023
Time constant of LV relaxation (ms)	18 ± 2	34 ± 12*	28 ± 3*	0.004
Myocardial stiffness constant	2.9 ± 0.6	7.0 ± 1.4*	5.5 ± 1.4****	<0.001
A ratio of LV mass to tibial length (mg/mm)	21 ± 3	32 ± 2*	31 ± 3*	<0.001
A ratio of lung weight to tibial length (mg/mm)	36 ± 2	84 ± 22*	52 ± 16**	<0.001

Values are expressed as mean ± SD.
*$P < 0.05$ vs. control group.
**$P < 0.05$ vs. untreated group.

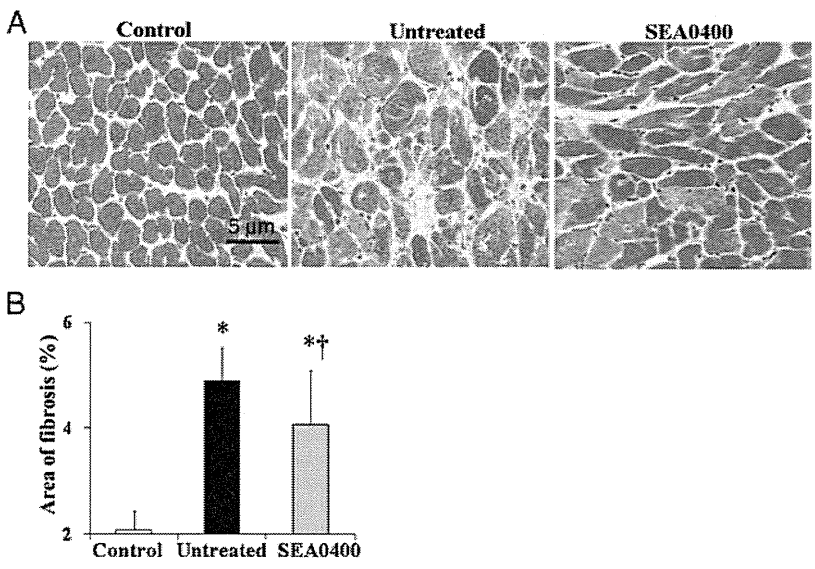

Figure 6 Representative Azan Mallory staining of left ventricle (*A*) and summary data for area of fibrosis (*B*). Data are expressed as mean ± SD. *$P < 0.05$ vs. control; †$P < 0.05$ vs. untreated.

Study limitation

First, the chronic administration of ouabain promoted myocardial fibrosis in Study 2, but we did not directly assess the effects on LV diastolic function. Our previous study showed that myocardial fibrosis proportionally promotes myocardial stiffening,[3] suggesting that ouabain exacerbated diastolic function in Study 2. Elkareh *et al.*[7] demonstrated that marinobufagenin, one of DLFs, induced myocardial fibrosis and diastolic dysfunction. The administration of ouabain did not change indices of transmitral flow velocity curves (*Table 2*); however, they are insensitive to changes in diastolic function in subjects with preserved ejection fraction.[30] Therefore, the results of Study 2 may not contradict our conclusion. Second, the increase in LV stiffness of HFPEF is provided by myocyte stiffening as well as interstitial fibrosis.[13] SEA0400 modulates the amplitude of the intracellular Ca^{2+} transient and myocyte contractility.[31] However, we did not assess the effects of DLFs and SEA0400 on myocyte stiffness, and thus, the beneficial effects of SEA0400 may not be attributed solely to the attenuation of LV fibrosis. Third, we used Dahl salt-sensitive rats fed on high-salt diet as the hypertensive HFPEF model. Exogenous salt loading and an increase in extracellular Na^+ concentration may directly increase intracellular Na^+ concentration and induce the NCX entry mode independent of DLFs. However, the decrease, not the increase, in the extracellular Na^+ concentration elevated

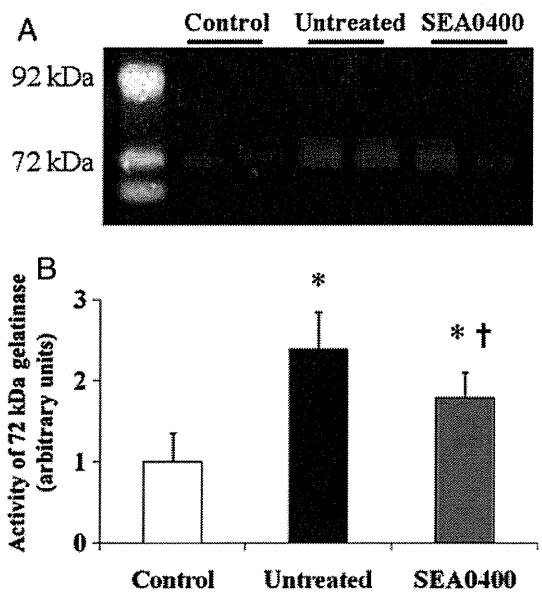

Figure 7 Representative gelatinolytic bands from *in vitro* gelatin zymography of left ventricle (*A*) and summary data for 72 kDa gelatinase activity (*B*). The left line of (A) was a positive control for matrix metalloproteinase-2 (72 kDa) and matrix metalloproteinase-9 (92 kDa). Data are expressed as mean ± SD. $^{*}P < 0.05$ vs. control; $^{\dagger}P < 0.05$ vs. untreated.

$[Ca^{2+}]_i$ through the NCX entry mode.[25] The profibrotic effects of ouabain were not masked by high-salt intake (*Figure 2*). Exogenous salt loading may not directly cause the NCX entry mode. Fourth, SEA0400 has been reported as the most potent and selective inhibitor of NCX.[12] However, SEA0400 depressed Ca^{2+} transients even in the absence of NCX.[32] Therefore, we cannot completely exclude the possibility that *in vivo* efficiency of SEA0400 was provided through mechanisms other than the blockade of DLFs-induced NCX entry mode. Fifth, the administration of SEA0400 was initiated at the compensatory hypertrophic stage without LV fibrosis,[2] and this study showed the preventive, not therapeutic, effects against LV fibrosis. Left ventricular fibrosis is usually highly prevalent in human HFPEF by the time of diagnosis. To assess the therapeutic effects of the blockade of the entry mode of NCX, future studies are required.

Conclusions

The secretion of DLFs was enhanced in the hypertensive HFPEF model. Ouabain activated p42/44 MAPKs and enhanced collagen production with the elevation of $[Ca^{2+}]_i$ via the NCX entry mode. SEA0400, the potent inhibitor of the NCX entry mode, attenuated the development of LV fibrosis and stiffening, and prevented the transition to HFPEF with the improvement of survival rate in the animal models. Thus, DLFs and the subsequently activated the Ca^{2+} entry mode of NCX may play important roles in the development of LV fibrosis and hypertensive HFPEF, and the blockade of the entry mode of NCX may be a new therapeutic strategy for this phenotype of heart failure. This study was conducted in a rat model for hypertensive HFPEF, and future studies in large animal models or humans are awaited to confirm the clinical relevance of the current findings.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

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Distinguishing focal fibrotic lesions and non-fibrotic lesions in hypertrophic cardiomyopathy by assessment of regional myocardial strain using two-dimensional speckle tracking echocardiography: Comparison with multislice CT

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ABSTRACT

Purpose: To distinguish focal fibrotic and non-fibrotic lesions in left-ventricular myocardium (LVM) in hypertrophic-cardiomyopathy (HCM)-subjects, we compared myocardial regional peak-strain values using two-dimensional speckle-tracking transthoracic-echocardiography (TTE) in multislice computed-tomography (CT)-detected fibrotic, non-fibrotic and normal control lesions.

Methods: Twenty subjects (10 consecutive HCM-subjects (8-males, mean 63.4-years), 10 healthy controls (5-males, mean 51.5-years)) underwent speckle-tracking TTE (iE-33), and analysis of regional peak-longitudinal (LS) and radial-strain (RS), and corresponding strain rates in each of 17 LVM segments (American-Heart-Association classification). In HCM-subjects, fibrotic lesions were identified by early-phase defective enhancement and late-phase abnormal enhancement by CT (Light-Speed-Ultra-16). Regional peak LS and RS at basal, mid and apical levels were measured in MSCT-detected fibrotic and non-fibrotic LVM lesions.

Results: In 10 HCM subjects, 143 lesions (84.1%) yielded good tracking on TTE. Twenty lesions showed fibrotic changes in 5 subjects by CT.

Regional peak-LS and RS absolute values were significantly lower in both fibrotic and non-fibrotic lesions in HCM subjects than in controls at basal, mid, apical levels (all $P<0.05$). While peak-LS (%) absolute values were significantly lower in fibrotic than non-fibrotic lesions at basal, mid and apical levels (all $P<0.05$), regional peak-RS absolute values were significantly lower only at mid levels. LS was a more sensitive indicator than the corresponding rate, with better reproducibility.

Conclusions: In HCM, regional peak-LS was significantly lower in fibrotic than non-fibrotic lesions in LVM by CT. Regional peak-LS by speckle-tracking provides useful information noninvasively to distinguish fibrotic from non-fibrotic lesions in LVM in HCM and normal LVM in healthy controls.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a genetic disorder of the myocardium caused by mutations in cardiac sarcomeric proteins [1,2]. The clinical features of this disease are heterogeneous, ranging from asymptomatic throughout life to severe symptoms of heart failure at a young age. While HCM subjects frequently have a favorable prognosis, sudden cardiac death (SCD) occurs in some subjects in the absence of prior previous symptoms [3–7]. The principal mechanism responsible for SCD in HCM subjects is often estimated to be ventricular tachycardia or fibrillation [8].

Focal myocardial fibrosis, myocardial hypertrophy, fiber disarray, and increased loose connective tissue in the left ventricular (LV) myocardium (LVM) have been reported to be the major structural myocardial abnormalities in HCM subjects [9,10]. In several post-mortem studies, myocardial fibrosis was frequently observed in the LVM in HCM subjects who underwent SCD without any prior symptoms [11,12]. Conversely, recent studies have also demonstrated that a large number of asymptomatic or mildly symptomatic HCM subjects who have not suffered SCD, have multiple areas of patchy myocardial fibrosis in LVM [13]. Therefore, even though the relationship between presence of myocardial fibrosis in LVM and occurrence of SCD in HCM subjects in vivo has not yet been established, some such relationship may exist; therefore, risk stratification using the presence of myocardial fibrosis in LVM may be critical for future prediction of the occurrence of SCD in HCM subjects.

Delayed hyper-enhancement (DHE) on gadolinium contrast-enhanced magnetic resonance imaging (MRI) has been reported as a highly sensitive imaging modality to detect focal fibrotic lesions in LVM, including in HCM subjects [14–16]. Several studies have demonstrated that the extent of DHE has been associated with

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severity of myocardial damage and predictive markers of the occurrence of SCD in HCM subjects [13,17–20]. Multislice computed tomography (MSCT), as well as MRI, has also been regarded as an independent useful tool for detection of focal fibrotic lesions in LVM [21] and estimation of prognosis of HCM. Early identification of those HCM subjects with fibrotic lesions in LVM detected by MSCT is of clinical relevance.

During the cardiac cycle, regional deformation of the myocardium occurs in 3 major directions: longitudinally, circumferentially, and radially. Recently, with the development of transthoracic echocardiography (TTE), myocardial strain and strain rate can be quantified to evaluate regional LV function. The physical definition of strain is the relative change in length of a material related to its original length. Strain rate is the temporal derivative of strain and describes the temporal change in strain (rate of shortening or lengthening). These measurements are angle independent, using two-dimensional (2D) speckle tracking imaging (STI) in a rapid, simple, and reliable manner [22,23]; the accuracy of this modality has been confirmed by comparing sonomicrometry and MRI tagging as reference methods. There have been some reports concerning the relationship between regional LV function quantified by 2D STI and focal fibrotic lesions in LVM by MRI in HCM subjects [24], but there are few studies concerning the relationship between regional LV function by 2D STI and focal fibrotic lesions in LVM by MSCT in HCM subjects.

In this study, we sought to distinguish focal MSCT-detected fibrotic lesions from non-fibrotic lesions in LVM detected in HCM subjects and normal control lesions; this was achieved using myocardial regional peak strain values and those strain rates calculated by temporally differentiated strain values using 2D STI.

2. Materials and methods

2.1. Study population

Twenty subjects (10 consecutive HCM subjects (8 males, mean 63.4 years) and 10 healthy controls (5 males, mean 51.5 years)) underwent 2D STI TTE (iE-33, Philips). The diagnosis of HCM was based on the TTE findings of either asymmetric or generalized hypertrophied LVM in the absence of other cardiac or systemic diseases causing LVM hypertrophy, such as aortic valve stenosis or hypertensive heart diseases. The control group consisted of 10 healthy subjects without family histories of HCM, with normal clinical and TTE findings.

We measured and analyzed myocardial regional peak longitudinal (LS) and radial strain (RS) and LS rate (LSR) and RS rate (RSR) in each of 17 segments in the LVM, according to the American Heart Association classification.

After informed consent, MSCT was performed only in HCM subjects to detect fibrotic lesions in LVM as well as to evaluate the condition of the coronary arteries, degree of hypertrophy of LVM, presence of thrombi in LV and LV wall motion in four-dimensional images. Regional peak LS, RS, LSR, and RSR at basal, mid and apical levels were also measured in fibrotic and non-fibrotic lesions in the LVM detected by MSCT.

2.2. Multislice CT

All HCM subjects underwent electrocardiogram (ECG)-gated enhanced MSCT (Light Speed Ultra 16, GE healthcare). Twenty-five seconds (early phase) and 6 min (late phase) after injection of 100 ml of the iodinated contrast material (350 mgI/ml Iomeron, Eisai), retrospective ECG-gated acquisitions were performed with 1.25 mm slice thickness, 0.5 s per rotation, tube voltage 140 kV and tube current 300 mA. After acquisition, end diastolic data were reconstructed at 80% of the R-to-R interval of the ECG.

Fibrotic lesions in the LVM were identified as simultaneous enhancement defects in the early phase and abnormal enhancement in the late phase (Fig. 1).

HCM subjects were divided into two groups, those with or without fibrotic lesions in any LVM segments, compared with healthy normal controls who were assumed not to have any fibrotic lesions in LVM.

2.3. TTE measurements

Standard 2D and pulsed Doppler TTE examination (IiE-33, Philips Medical Systems, Tokyo, Japan) was performed in all subjects with an S5-1 transducer (from 2.4 to 4.2 MHz). TTE parameters were measured according to the recommendations of the American Society of Echocardiography. LV end diastolic and end-systolic volumes, LV ejection fraction (LVEF), and left atrial end-systolic volume were measured from apical imaging planes by a modified Simpson's method. The ratio of the end-diastolic thickness of the interventricular septum (IVS) to that of the LV posterior wall (PW) was calculated, and subjects were classified as asymmetric septal hypertrophy if the ratio of IVS/PW was >1.3. Apical hypertrophy was diagnosed if the ratio of maximal segmental wall thickness of the apex of LV vs that of another LVM site was >1.5. Continuous wave Doppler was used to measure maximum velocity across the LV outflow tract (LVOT) from apical 5- or 3-chambered views. Subjects were classified on the basis of their hemodynamic status as obstructive (resting LVOT gradient ≧30 mmHg) or non-obstructive (resting LVOT gradient <30 mmHg).

2.4. Strain and strain rate measurement by TTE

After high frame rate (60 or more Hz) second harmonic LV apical 4-chamber, 2-chamber, and 3-chamber views from the apex were imaged, LV wall was divided into 17 segments (septal, anterior, lateral, inferior segments at basal, mid, and apical levels, and anteroseptal, posterior segments at basal and mid levels, and apical cap) and strain and strain rate in each segment were individually analyzed using 2D STI TTE.

We analyzed peak LS (%), LSR (1/s = 1/s), RS (%) and RSR (1/s) in each of the 17 segments in the fibrotic and non-fibrotic lesions in the LVM identified by MSCT. As circumferential strain occurs in only 6 of 17 segments analyzed in TTE, circumferential strain was excluded from this analysis.

Global LS, RS, LSR and RSR were calculated by averaging values of all 17 segmental strains and strain rate, respectively. The analysis was obtained via an off-line system using auto-analyzing QLAB software (Phillips Medical Systems) version 7.0 (Fig. 2).

Inter and intra-observer variabilities of regional peak strain values and corresponding rates were evaluated by an experienced physician and a technician.

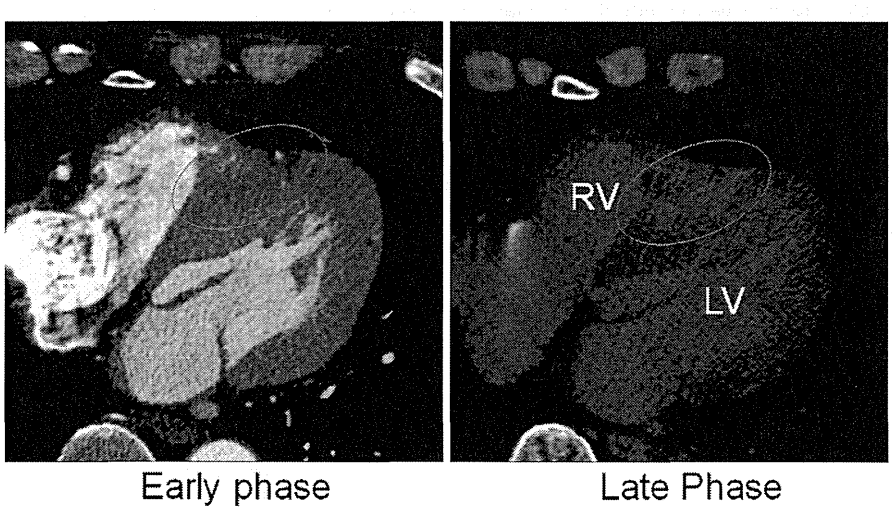

Fig. 1. Axial source images of enhanced multislice computed tomography in a subject with hypertrophic cardiomyopathy (asymmetrical septal hypertrophy type) acquired at 30 s (early phase) and 6 min (late phase) after injection of contrast material. Fibrotic lesion was defined as contrast defect in the early phase and abnormal enhancement in the late phase. Fibrotic lesions (surrounded by red circles) can be observed in hypertrophic inter ventricular septum and apical anteroseptum. RV and LV indicate right and left ventricles, respectively.

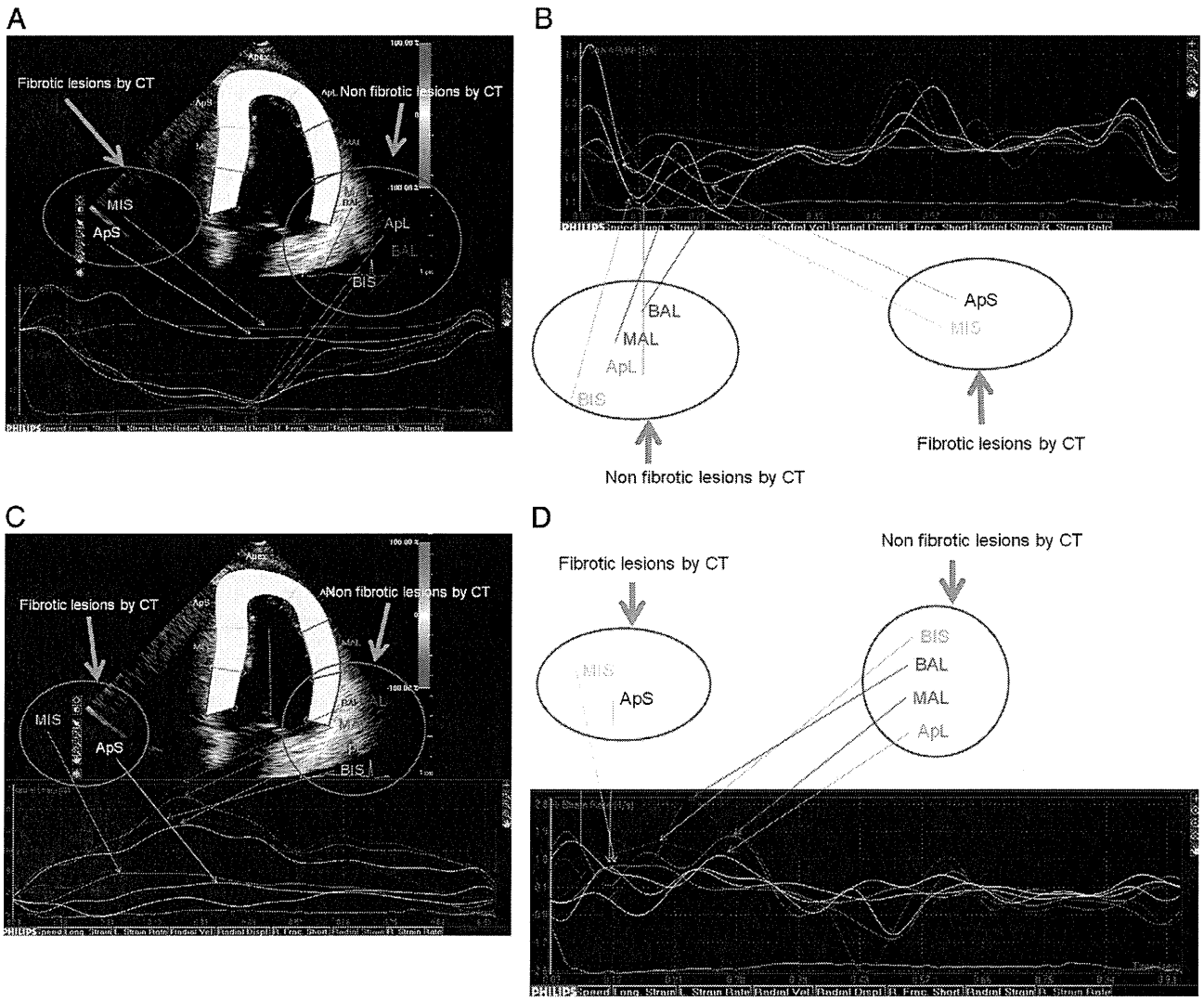

Fig. 2. Color-coded strain images acquired by transthoracic echocardiogram and charts in the same subject with hypertrophic cardiomyopathy (asymmetrical septal hypertrophy type) as that shown in Fig. 1. (A) Upper part represents four chamber views of transthoracic echocardiogram at end diastole. Bottom represents regional longitudinal strain values in each lesion during one cardiac cycle. Absolute values of longitudinal strain were lower in mid interventricular septum (MIS) and apical septum (ApS) in which fibrotic lesions were confirmed on CT as represented in Fig. 1, than in mid anterolateral (MAL), apical lateral (ApL), basal anterolateral (BAL) and basal interventricular septum (BIS) in which fibrotic lesions were not observed. (B) Regional longitudinal strain rates (LSR) in the same subject with HCM. There were non-remarkable differences of regional peak LSR between fibrotic (ApS, and MIS) and non-fibrotic lesions (BAL, MAL, ApL and BIS) on CT (C) Upper part represents four chamber views of transthoracic echocardiogram at end diastole. Bottom represents radial strain values in each lesion during one cardiac cycle. Absolute values of radial strain were lower in MIS and ApS in which fibrotic lesions were observed on CT as represented in Fig. 1, than in BAL, MAL, ApL, and BIS in which fibrotic lesions were not observed. (D) Regional radial strain rates (RSR) in the same subject with HCM. There were non-remarkable differences of regional peak RSR between fibrotic (ApS, and MIS) and non-fibrotic lesions (BAL, MAL, ApL and BIS) on CT.

2.5. Statistical analysis

All values are presented as mean ± standard deviation (SD). TTE parameters and strain and strain rate were compared among three groups (HCM subjects with fibrosis, those without fibrosis, and healthy controls), and among another three kinds of lesions in LVM (fibrotic lesions and non fibrotic lesions on MSCT, and normal control lesions) using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Differences were considered statistically significant if the p value was less than 0.05 with a 95% confidence interval. Inter- and intra-observer variabilities were evaluated by determination of correlation coefficients (CCs) and by Bland and Altman analysis.

3. Results

3.1. Fibrotic lesions in LVM detected on MSCT

In 10 HCM subjects, among a total of 170 lesions, 143 lesions (84.1%) yielded good tracking in TTE. Of these, 20 lesions showed fibrotic changes in five subjects by CT. Baseline characteristics of five subjects in whom fibrotic lesions were observed on CT are shown in Table 1.

3.2. Baseline characteristics

The clinical characteristics and baseline TTE parameters for the three groups, HCM subjects with and without fibrotic lesions, and normal healthy controls, are shown in Tables 2 and 3, respectively.

Only plasma brain natriuretic peptide levels were higher in HCM subjects with than without fibrotic lesions, and also higher than in the normal control group (both $P<0.05$).

In TTE, mean LVEF in HCM subjects with and without fibrotic lesions were 65.0 and 62.0%, respectively and LV systolic function was relatively well preserved. Asymmetric septal hypertrophy (ASH)-type HCM was frequent (the proportion of ASH was 60% in HCM subjects both with and without fibrotic lesions) and conversely there were no

Table 1
Baseline characteristics of five hypertrophic cardiomyopathy subjects in whom fibrotic lesions were observed on multislice computed tomography (MSCT). Ages ranged from 20 to 75 years, mean age 56, median age 67 years and all were males. Patterns of hypertrophy on transthoracic echocardiogram (TTE) were: three subjects with symmetric septal hypertrophy (ASH) and two with concentric.

Patient	Age (yrs)	Gender	Pattern of hypertrophyon TTE	Fibrotic lesions on MSCT
1	75	Male	Concentric	ML, BS, MS, BAS, MAS
2	67	Male	Concentric	Apex
3	20	Male	ASH	BS, MS, ApS, BAS, MAS, ApAS
4	66	Male	ASH	BS, MS, BAS, MAS
5	69	Male	ASH	BS, MS, BAS, MAS

ML = mid lateral, BS = basal septum, MS = mid septum, ApS = apical anteroseptum, BAS = basal anteroseptum, MAS = mid anteroseptum, ApAS = apical anteroseptum.

apical hypertrophy types in either group in this study. Mean maximal LV wall thickness was 24.8 mm in HCM subjects with fibrotic lesions in LVM and 18.6 mm in HCM subjects without fibrotic lesions; both were significantly greater than that in normal controls (both $P<0.05$), but there were no significant differences between values in HCM subjects with and without fibrotic lesions. Similarly, the mean E/Ea values, which are indicators of LV diastolic function, were 12.2 and 12.8 in HCM groups with and without fibrotic lesions, respectively; even though these were not significantly different, both were significantly higher than the value for the healthy control group (both $P<0.05$).

4. Regional strain

4.1. Lesion-based analysis of regional peak LS and RS

Regional peak LS and RS (%) in the three kinds of lesions: 1), 2), fibrotic and non-fibrotic lesions in LVM on CT in HCM subjects and 3), lesions in LVM in normal controls, are shown in Figs. 3 and 4.

Absolute values of regional peak LS and RS were significantly lower in fibrotic and non-fibrotic lesions in HCM subjects than in lesions in normal controls (both $P<0.01$). Furthermore peak LS was significantly lower in fibrotic lesions in HCM subjects than in non-fibrotic lesions ($P<0.05$). However, there were no significant differences of peak RS in fibrotic and non-fibrotic lesions in HCM subjects.

Furthermore all lesions in LVM were divided regionally into three levels, namely basal, mid, and apical and comparisons made in those regions in fibrotic and non-fibrotic lesions in HCM subjects and in lesions in normal controls (Table 4).

Absolute values of regional peak LS and RS in basal, mid and apical levels were all significantly lower in fibrotic and non-fibrotic lesions in HCM subjects than those in normal controls (all $P<0.05$). Furthermore absolute values of regional peak LS in basal, mid and apical levels were all significantly lower in fibrotic lesions in LVM than those in non-fibrotic lesions in HCM subjects (all $P<0.05$). Absolute values of regional peak RS in the mid level only were significantly lower in fibrotic lesions in HCM subjects than those in non-fibrotic lesions in HCM subjects ($P<0.05$), i.e. there were no significant differences of regional peak RS in basal and apical levels between fibrotic and non-fibrotic lesions in such subjects.

Table 2
Characteristics of the three groups: hypertrophic cardiomyopathy (HCM) subjects with fibrosis, without fibrosis on computed tomography (CT), or normal controls. NYHA indicates New York Heart Association. ACE and ARB indicate angiotensin-converting enzyme, angiotensin II receptor blocker, respectively. In the Tukey–Kramer test for post-hoc analysis, plasma brain natriuretic peptide was significantly higher in HCM subjects with fibrosis than in HCM subjects without fibrosis and in normal controls (both $P<0.05$).

	HCM with fibrosis (n=5)	HCM without fibrosis (n=5)	Controls (n10)
Age, mean ± SD (yrs)	59.4 ± 22.3	65.4 ± 6.0	51 .5 ± 1 9.7
Male gender(%)	100%	60%	50%
NYHA class(mean)	1.4	1.2	0
Syncope	20%	0%	0%
Family history of HCM	40%	0%	0%
Plasma brain natriuretic peptide (pg/mi)	152.4 ± 15.1*t	58.1 ± 18.3	17.8 ± 8.0
Non sustained ventricular tachycardia on 24 h dynamic electrocardiogram	40%	60%	0%
Usage of beta-blocker	60%	100%	0%
Usage of calcium channel blocker	60%	20%	0%
Usage of ACE-inhibitor or ARB	40%	60%	0%
Usage of diuretics	20%	0%	0%
Usage of anti-arrhythmias	20%	0%	0%
Usage of implantable cardioverter defibrillator	40%	20%	0%

*p<.005 (HcM with fibrosis vs HCM without fibrosis), t<0.05 (HCM with fibrosis vs controls).

Table 3
Transthoracic echocardiographic findings in the three groups: hypertrophic cardiomyopathy (HCM) subjects with fibrosis, without fibrosis on computed tomography (CT), or normal controls. E/A indicates early filling per atrial filling. E/Ea indicates the ratio of early transmitral velocity to tissue Doppler mitral annular early diastolic velocity and is an indicator of left ventricular diastolic function. TTE parameters were compared among three groups using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Maximum left ventricular wall thickness and E/Ea were significantly greater in HCM subjects with and without fibrosis on CT than in normal controls (both $P<0.05$), but there were no significant differences between HCM subjects with and without fibrosis.

	HCM with fibrosis (n=5)	HCM without fibrosis (n=5)	Controls (n=10)
Left ventricular end diastolic dimension (mm)	42.8 ± 6.8	47.2 ± 4.0	50.1 ± 3.9
Left ventricular end systolic dimension (mm)	30.4 ± 9.9	32.2 ± 4.6	32.7 ± 3.5
Left ventricular ejection fraction (%)	65.0 ± 13.2	62.0 ± 16.3	63.6 ± 4.8
Left atrial dimension (mm)	40.0 ± 5.1	46.2 ± 5.8	36.8 ± 3.1
Left atrial volume index (mi/cm^2)	35.5 ± 7.6	37.8 ± 12.6	24.6 ± 4.1
Maximum left ventricular wall thickness (mm)	24.8 ± 6.9*	18.6 ± 5.0t	9.2 ± 0.9
Asymmetric septal hypertrophy type, n(%)	60%	60%	0%
Diffuse type, n (%)	20%	0%	0%
Apical hypertrophytype	0%	0%	0%
Hypertrophic obstructive cardiomyopathy	20%	40%	0%
E/A	0.70 ± 0.3	0.81 ± 0.6	1.05 ± 0.4
E/Ea	12.2 ± 4.8*	12.8 ± 9.0t	7.0 ± 3.0

*p<.005 (HCM with fibrosis vs controls), t<0.05 (HCM without fibrosis vs controls).

5. Regional strain rate

5.1. Lesion-based analysis of regional peak LSR and RSR

Regional peak LSR and RSR in the three kinds of lesions: 1), 2), fibrotic and non-fibrotic lesions in LVM on CT in HCM subjects and 3), lesions in LVM in normal controls, are shown in Figs. 5 and 6.

Absolute values of regional peak LSR and RSR were significantly lower in fibrotic and non-fibrotic lesions in HCM subjects than those in lesions in normal controls (all $P<0.01$). Furthermore, absolute regional peak LSR values were significantly lower in fibrotic lesions in HCM subjects than in non-fibrotic lesions in such subjects ($P<0.05$); however, there were no significant differences in regional peak RSR in fibrotic and non-fibrotic lesions in LVM in HCM subjects.

All lesions in LVM were divided regionally into three levels, namely basal, mid, and apical and comparisons made in those regions

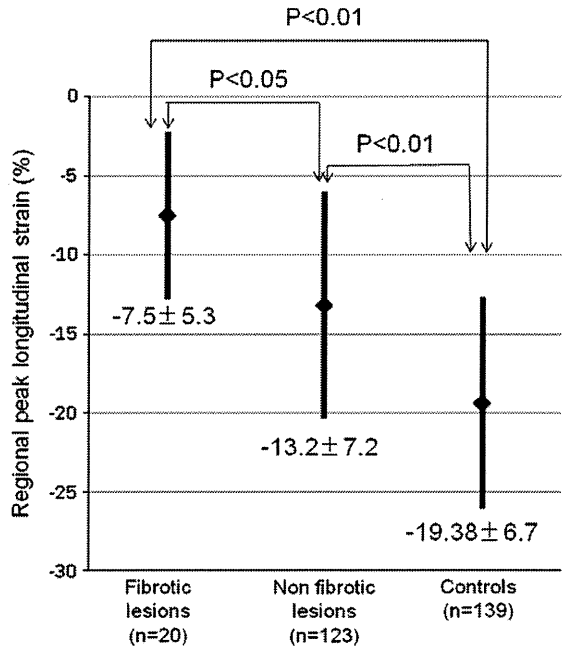

Fig. 3. Lesion-based analysis of regional peak longitudinal strain. Regional peak longitudinal strain (%) is shown in three kinds of lesions: 1), 2) fibrotic and non-fibrotic lesions in left ventricular myocardium (LVM) on computed tomography in HCM subjects and 3) lesions in LVM in normal controls. These strains were compared among three kinds of lesions using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of regional peak longitudinal strain were significantly lower in fibrotic and non-fibrotic lesions in HCM subjects than those in lesions in normal controls (both P<0.01) and values were also significantly lower in fibrotic lesions in HCM subjects than in non-fibrotic lesions (P<0.05).

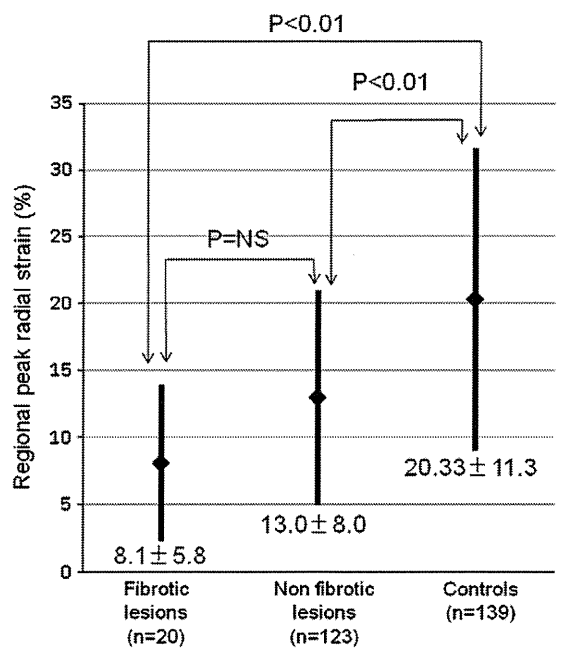

Fig. 4. Lesion-based analysis of regional peak radial strain. Regional peak radial strain (%) is shown in three kinds of lesions: 1), 2) fibrotic and non-fibrotic lesions in left ventricular myocardium (LVM) on computed tomography in hypertrophic cardiomyopathy (HCM) subjects and 3) lesions in normal controls. These strains were compared among three kinds of lesions using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of regional peak radial strain were significantly lower in fibrotic and non-fibrotic lesions in LVM in HCM subjects than in lesions in LVM in normal controls (both P<0.01), but there were no significant differences between those in fibrotic and non-fibrotic lesions in HCM subjects (P = not significant (NS)).

Table 4
Regional peak longitudinal and radial strain classified by presence of fibrotic lesions in hypertrophic cardiomyopathy (HCM) subjects in comparison with normal controls. All lesions in left ventricular myocardium (LVM) were divided into three parts regionally: basal, mid, and apical parts; these were compared in fibrotic and non-fibrotic lesions in LVM in HCM subjects and in lesions in normal controls. These strains were compared among three kinds of lesions using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of regional peak longitudinal strain in basal, mid and apical parts were all significantly lower in fibrotic and non-fibrotic lesions in LVM in HCM subjects than those in normal controls (all P<0.05).Furthermore, absolute values of peak longitudinal strain in basal, mid and apical parts were all significantly lower in fibrotic lesions in LVM than those in non-fibrotic lesions in HCM subjects (all P<0.05).Absolute values of regional peak radial strain in basal, mid and apical parts were all significantly lower in fibrotic and non-fibrotic lesions in LVM in HCM subjects than those in lesions in normal controls (all P<0.05). However, these absolute values in the mid part only were significantly lower in fibrotic lesions in HCM subjects than those in non-fibrotic lesions (P<0.05); in contrast there were no significant differences in regional peak strain in basal and apical parts, between fibrotic and non-fibrotic lesions in HCM subjects.

	Fibrotic lesions (n=20)	Non fibrotic lesion (n=123)	Normal controls (n=139)
Regional peak longitudinal stain			
Base, %	−6.3±3.6*‡	−11.1±6.2†	−19.2±8.1
Mid, %	−5.6±2.2*‡	−11.5±5.6†	−19.1±5.2
Apical, %	−5.4±1.5*‡	−12.7±7.3†	−19.9±6.7
Regional peak radial stain			
Base, %	10.0±7.1*	15.3±8.9†	21.3±11.1
Mid, %	7.2±5.2*‡	13.8±8.4†	23.0±11.3
Apical, %	8.9±6.4*	12.4±6.7†	17.1±10.9

*p<.005 (fibrotic segments vs controls).
†<0.05 (non fibrotic segments vs controls).
‡<0.05 (fibrotic segments vs non fibrotic segments).

in fibrotic and non-fibrotic lesions in HCM subjects and in lesions in normal controls (Table 5).

Absolute values of regional peak LSR and RSR in basal, mid and apical levels were all significantly lower in fibrotic and non-fibrotic lesions in HCM subjects than those in normal controls (all P<0.05). Absolute values of regional peak LSR in the mid part only were significantly lower in fibrotic lesions in HCM subjects than those in non-fibrotic lesions in such subjects (P<0.05); however, there were no significant differences in absolute regional peak LSR values in basal and apical levels, nor of regional peak RSR in these three levels, between fibrotic and non-fibrotic lesions.

6. Global strain

6.1. Patient-based analysis of global peak longitudinal strain among HCM subjects with and without fibrosis and in healthy control groups

Global peak LS and RS (%) in HCM subjects with and without fibrotic lesions in LVM on CT and normal controls are shown in Figs. 7 and 8, respectively.

Absolute global peak LS and RS values were both significantly lower in HCM subjects with and without fibrotic lesions, than those in normal controls (both P<0.01); furthermore these values were significantly lower in HCM subjects with fibrotic lesions than those in HCM subjects without fibrosis (P<0.05).

7. Global strain rate

7.1. Patient-based analysis of global peak LSR and RSR

Global peak LSR and RSR in HCM subjects with and without fibrotic lesions in LVM on CT and in normal controls are shown in Figs. 9 and 10.

Absolute global peak LSR and RSR values were significantly lower in HCM subjects both with and without fibrotic lesions than values in normal controls (both P<0.01); however, there were no significant

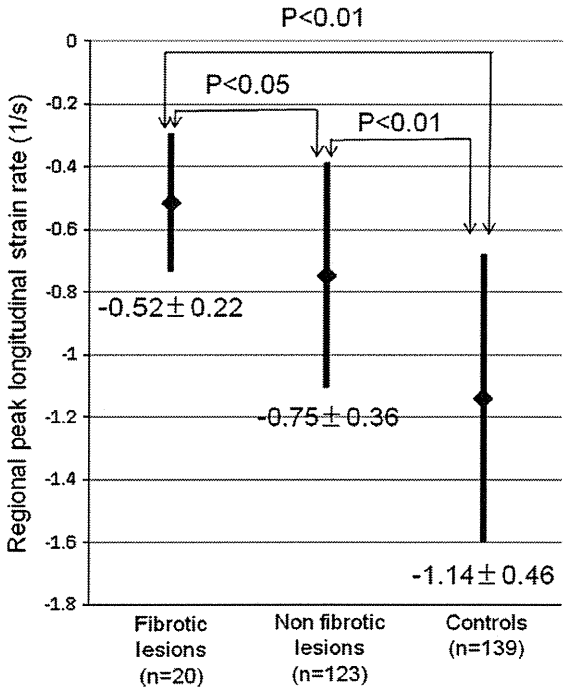

Fig. 5. Lesion-based analysis of regional peak longitudinal strain rate Regional peak longitudinal strain rate (1/s = 1/c) is shown in three kinds of lesions: 1), 2) fibrotic and non-fibrotic lesions in left ventricular myocardium (LVM) on CT in hypertrophic cardiomyopathy (HCM) subjects and 3) lesions in LVM in normal controls. These strain rates were compared among three kinds of lesions using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of regional peak longitudinal strain rate were significantly lower in fibrotic and non-fibrotic lesions in HCM subjects than those in lesions in normal controls (both P<0.01) and furthermore these values were significantly lower in fibrotic than in non-fibrotic lesions in HCM subjects (P<0.05).

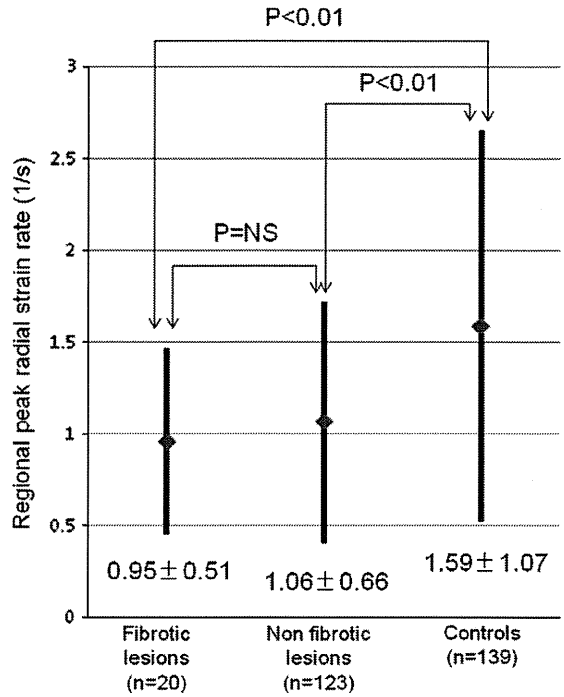

Fig. 6. Lesion-based analysis of regional peak radial strain rate Regional peak radial strain rate (1/s = 1/s) is shown in three kinds of lesions: 1), 2) fibrotic and non-fibrotic lesions in left ventricular myocardium (LVM) on CT in hypertrophic cardiomyopathy (HCM) subjects and 3) lesions in normal controls. These strain rates were compared among three kinds of lesions using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of regional peak radial strain rate were significantly lower in fibrotic and non-fibrotic lesions in LVM in HCM subjects than those in lesions in LVM in normal controls (both P<0.01), but there were no significant differences between these values in fibrotic and non-fibrotic lesions in LVM in HCM subjects (P = not significant (NS)).

differences between those in HCM subjects with fibrotic lesions and those without fibrotic lesions.

7.2. Inter- and intra-observer variabilities of regional peak strain values and corresponding rates

Inter- and intra-observer variabilities of regional peak LS, RS, LSR and RSR, on analysis of 143 sites in total, are shown in Table 6. Inter and intra-observer variabilities of LS were superior to those of RS regarding CC and absolute values of mean differences ± 1.96 SD. Inter-observer variabilities of LSR were superior to those of RSR regarding CC and absolute values of mean differences ± 1.96 SD; however intra-observer variability of RSR was superior to those of RS and LSR regarding CC.

Inter- and intra-observer variabilities of regional peak LS, RS, LSR and RSR, in part-by- part analysis are also evaluated (data were not shown). Inter- and intra-observer variabilities of LS were superior to those of RS regarding CC and absolute values of mean differences ± 1.96 SD in all basal, mid and apical parts. Inter- and intra-observer variabilities of LSR were superior to that of RSR regarding absolute values of mean differences ± 1.96 SD in basal and apical parts; however, intra-observer variability of RSR was superior to that of RS and LSR regarding CC.

8. Discussion

In HCM subjects, even though global LV systolic function, expressed as LVEF, is frequently preserved, regional systolic dysfunction is often observed [25–36]; furthermore, HCM subjects are frequently regarded as exhibiting regional heterogeneity of contractility. This discrepancy may be explained by regional variations in myocardial histopathological changes, such as myocardial disarray, fibrosis, hypertrophy and the occurrence of focal fibrotic lesions detected by MRI or MSCT.

Table 5
Regional peak longitudinal (LSR) and radial strain rate (RSR) classified by presence of fibrotic lesions in hypertrophic cardiomyopathy (HCM) subjects in comparison with normal controls. All lesions in LVM were divided into three parts: basal, mid, and apical parts regionally and compared in fibrotic and non-fibrotic lesions in LVM in HCM subjects and in normal control lesions. These strain rates were compared among three kinds of lesions using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of regional peak LSR in basal, mid and apical parts were all significantly lower in fibrotic and non-fibrotic lesions in LVM in HCM subjects than those in normal controls (all P<0.05). Absolute values of regional peak LSR in the mid part only were significantly lower in fibrotic than in those in non-fibrotic lesions in HCM subjects (P<0.05), but there were no such significant differences in absolute values of regional peak LSR in basal and apical parts. Absolute values of regional peak RSR in basal, mid and apical parts were all significantly lower in fibrotic and non-fibrotic lesions in LVM in HCM subjects than those in lesions in normal controls (all P<0.05). However, there were no significant differences in absolute values of regional peak RSR in basal, mid, and apical parts in fibrotic and non-fibrotic lesions in LVM in HCM subjects (P = not significant).

	Fibrotic lesions (n = 20)	Non fibrotic (n = 139)	Normal controls
Regional peak LSR			
Base, % (1/s)	−0.59 ± 0.23*	−0.70 ± 0.41†	−1.06 ± 0.66
Mid, (1/s)	−0.37 ± 0.14*‡	−0.69 ± 0.31†	−1.17 ± 0.51
Apical, (1/s)	−0.77 ± 0.04*	−0.83 ± 0.35†	−1.09 ± 0.46
Regional peak RSR			
Base, (1/s)	1.13 ± 0.69	1.16 ± 0.70†	1.42 ± 0.72
mid, (1/s)	0.84 ± 0.29*	1.11 ± 0.76†	1.98 ± 1.24
Apical, (1/s)	0.84 ± 0.46	0.95 ± 0.52†	1.38 ± 1.07

*<.005 (fibrotic lesions vs controls)
†<0.05 (non fibrotic segments vs controls)
‡<0.05 (fibrotic lesions vs non fibrotic segments)

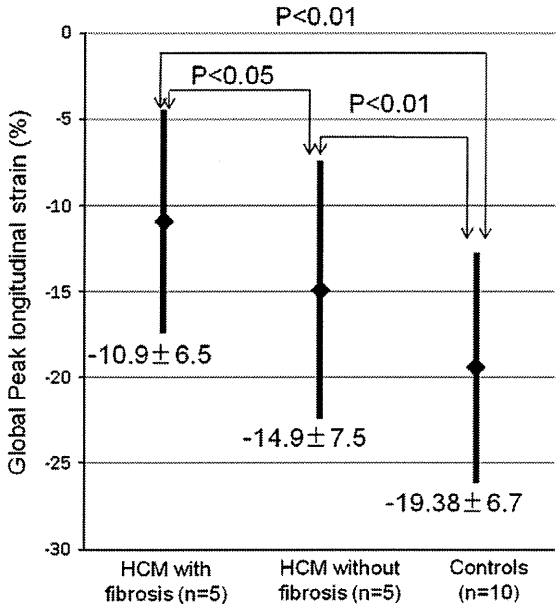

Fig. 7. Patient-based analysis of global peak longitudinal strain. Global peak longitudinal strain (%) is shown in hypertrophic cardiomyopathy (HCM) subjects with and without fibrosis in left ventricular myocardium on CT and normal controls. Theses strains were compared among three groups using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of global peak longitudinal strain were significantly lower both in HCM subjects with and without fibrosis, than those in normal controls (both P<0.01) and furthermore were significantly lower in HCM subjects with fibrosis than those in HCM subjects without fibrosis (P<0.05).

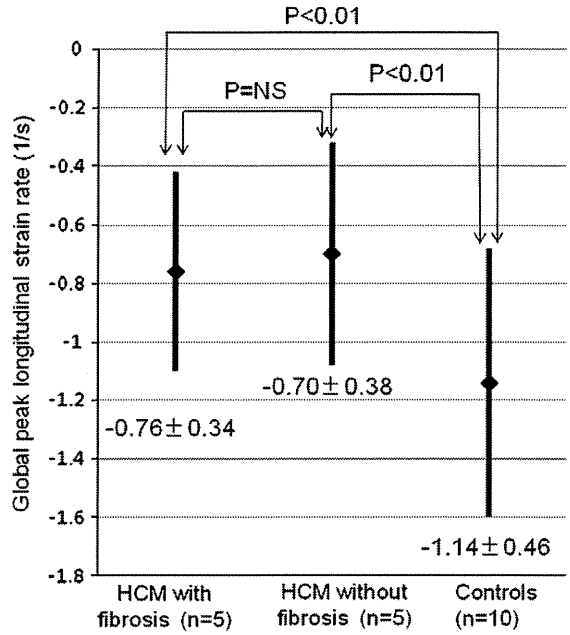

Fig. 9. Patient-based analysis of global peak longitudinal strain rate. Global peak longitudinal strain rate (1/s=1/s) is shown in hypertrophic cardiomyopathy (HCM) subjects with and without fibrosis in left ventricular myocardium on CT and in normal controls. These strain rates were compared among three groups using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of global peak longitudinal strain rate were significantly lower both in HCM subjects with and without fibrosis than those in normal controls (both P<0.01), but there were no significant differences between those in HCM subjects with and without fibrosis (P=not significant (NS)).

In HCM subjects, decreased regional LV systolic function may appear not only in hypertrophied LVM, but also in non-hypertrophied myocardium. This finding may be influenced by the pathologic process, which may be the reason for reduced global strain; thus strain rates were observed not only in fibrotic lesions but also in non-fibrotic lesions in LVM in HCM subjects. Furthermore, myocardial

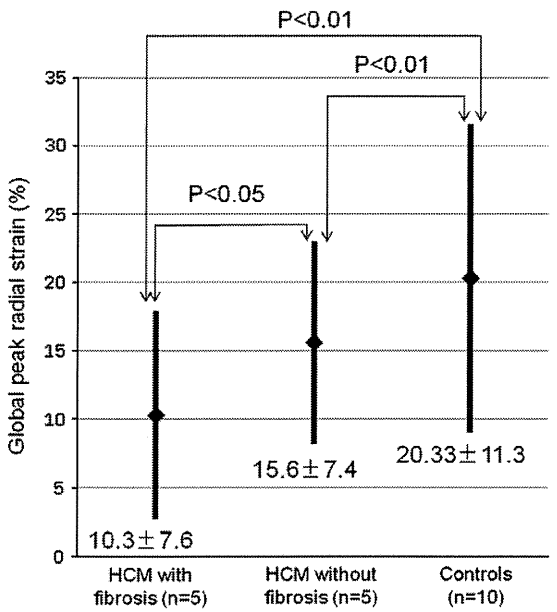

Fig. 8. Patient-based analysis of global peak radial strain. Global peak radial strain (%) is shown in hypertrophic cardiomyopathy (HCM) subjects with and without fibrosis in left ventricular myocardium on CT and normal controls. These strains were compared among three groups using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of global peak radial strain were significantly lower both in HCM subjects with and without fibrosis than those in normal controls (both P<0.01) and furthermore were significantly lower in HCM subjects with fibrosis than those in HCM subjects without fibrosis (P<0.05).

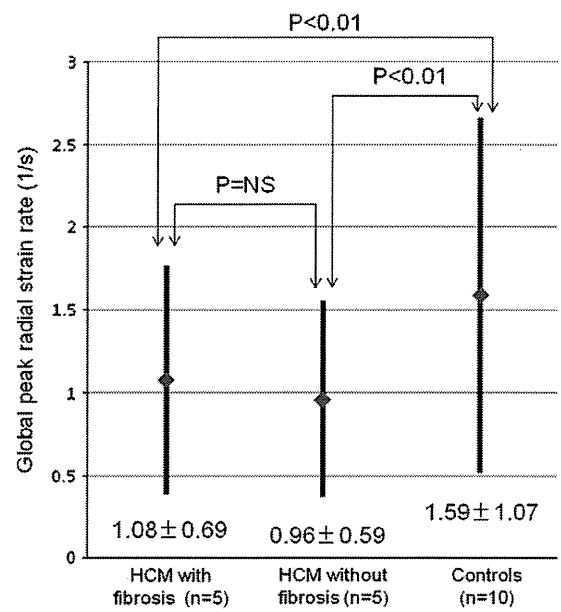

Fig. 10. Patient-based analysis of global peak radial strain rate Global peak radial strain rate (1/s=1/s) is shown in hypertrophic cardiomyopathy (HCM) subjects with and without fibrosis in left ventricular myocardium on CT and in normal controls. These strain rates were compared among three groups using analysis of variance and the Tukey–Kramer test for post-hoc analysis. Absolute values of global peak radial strain rate were significantly lower both in HCM subjects with and without fibrosis than in normal controls (both P<0.01), but there were no significant differences between values in HCM subjects with without fibrosis (P=not significant (NS)).

fibrosis can occur anywhere throughout the myocardial wall, predominantly involving the mid-wall or subepicardium; this suggests that myocardial fibrosis in HCM subjects is not due to the presence of ischemic epicardial coronary artery disease, but from ischemia due to structural abnormalities expressed at intramural level in such disease [9,15,19,22].

The detection of myocardial fibrosis in LVM has mainly been analyzed by MRI [37] and sometimes CT [38–43], but also on occasion using TTE. Weidemann et al. [36] reported that the "double peak sign" in strain-rate imaging tracings appears to be a reliable tool to diagnose focal fibrotic lesions in LVM. However, from our results, strain values themselves may be preferable to corresponding strain rates because of their high detectability in distinguishing fibrotic lesions from their non-fibrotic counterparts in LVM in HCM subjects. Carasso et al. [22] also showed that LS in STI and systolic and early diastolic strain rate obtained from 2D velocity vector imaging were lower in LVM of HCM subjects than in normal controls; on the other hand their circumferential values in STI were higher, former of which is in good agreement with our results.

Regional LV function can also be evaluated by measuring strain or deformation of LVM by TTE and MRI [44–46]. Previous studies have shown that global and regional strain using both TTE and MRI were decreased in HCM subjects compared with normal controls [12,30,33,35,36,45,47]. Tagged cardiac MRI can also measure myocardial strain or deformation in three major cardiac axes, namely LS, RS and circumferential strain [44–46,48]. Kramer et al. [31]used MR tissue tagging to measure the distribution of systolic segmental shortening in HCM subjects. These authors found that circumferential and longitudinal shortening strain were more heterogeneous in HCM subjects with the greatest reduction in the IVS compared with normal controls. Disadvantages of tagged-cardiac MRI include the fact that it is time-consuming and there are also spatial and temporal resolution limitations due to the capability of MRI itself.

Tissue Doppler imaging (TDI)-based strain measurements using 2D STI also provide information on regional LV function [44,45,49]. However, in contrast to STI, TDI-based strain measurements are angle dependent since they employ Doppler effects and simultaneous opposite deformation in the long and short axes. Clinical use of TDI-based strain measurements is also limited because of tethering effects from other regions and cardiac translational artifacts [44].

Table 6
Inter- and intra-observer variabilities of regional peak longitudinal (LS) and radial strain (RS) and LS rate (LSR) and RS rate (RSR), in analysis of 143 sites in total. Inter- and intra-observer correlation coefficients (CC) and absolute values of mean differences ± 1.96 standard deviation (SD), of regional peak strain values and corresponding rates, based upon Bland and Altman analysis, are shown. Inter- and intra-observer variabilities of LS were superior to those of RS regarding CC and absolute values of mean differences ± 1.96 SD. Inter-observer variability of LSR was superior to that of RSR regarding CC and absolute values of mean differences ± 1.96 SD; however, intra-observer variability of RSR was superior to those of RS and LSR regarding CC.

Strain	Variability	Correlation coefficient	P value	Absolute values of mean differences ± 1.96 SD
Longitudinal strain	Inter-observer	0.83	<0.01	1.8 ± 2.2%
Longitudinal strain	Intra-observer	0.87	<0.001	2.6 ± 2.8%
Radial strain	Inter-observer	0.55	<0.01	5.1 ± 5.0%
Radial strain	Intra-observer	0.41	<0.05	7.7 ± 7.9%
Strain rate	Variability	Correlation coefficient	P value	Absolute values of mean differences ± 1.96SD
Longitudinal strain rate	Inter-observer	0.76	<0.001	0.27 ± 0.34(1/s)
Longitudinal strain rate	Intra-observer	0.53	<0.01	0.25 ± 0.24(1/s)
Radial strain rate	Inter-observer	0.44	<0.05	0.42 ± 048(1/s)
Radial strain rate	Intra-observer	0.56	<0.05	0.51 ± 0.59(1/s)

Yang et al. [22] indicated that IVS strain in HCM subjects was significantly reduced, particularly at the midlevel; this finding was also supported by the fact that the extent of this abnormality was directly related to the severity of LV hypertrophy and the degree of asymmetry. Other investigators [47] also demonstrated that values of three components of strain obtained from 2D STI were significantly reduced, despite normal LV function as assessed by standard criteria in HCM subjects. Our results showing that regional LS can distinguish fibrotic lesions from non-fibrotic lesions in HCM subjects at any level, are consistent with these previous reports. However, RS and peak LSR could only distinguish fibrotic lesions from non-fibrotic lesions in HCM subjects at mid level. Furthermore peak RSR could not distinguish fibrotic lesions from non-fibrotic lesions in HCM subjects at any level of LVM in our study.

Popovic et al. [24] also have demonstrated that myocardial fibrotic lesions in LVM are associated with reduced LS in HCM subjects, and that the presence of fibrotic lesions and wall thickening were both multivariate predictors for presence of lower regional LS. These results suggest that myocardial fibrosis affects the function of longitudinal fibers earlier. In normal subjects, there is transmural heterogeneity of LV mechanics, with a decreasing gradient of shortening from endocardial to epicardial layers, and significant stretch along myofibers in the epicardial layers [31,34–36]. These authors report that the differential shortening of subepicardial and subendocardial layers may lead to high subendocardial strain, but an absence of subepicardial strain in HCM subjects. As a consequence, mid-wall circumferential and radial strain, which are influenced by strain throughout the LV wall, are usually lower in spite of normal LVEF. Our results may support this hypothesis.

Another reason for the heterogeneity of decreased RS in the same LV wall may be because the mid-segment comprising the thickest area is the region where most pathologic changes occur. In our population, there were no significant differences in LV wall thickness between HCM subjects with or without fibrotic lesions on CT. However, potential pathological changes in mid-segments may exist and lead to more regional dysfunction in radial shortening. These points support our results indicating that there were no significant differences in LV diastolic dysfunction between HCM subjects with and without fibrotic lesions in LVM. Additionally, differences between LS and RS as a means to distinguish fibrotic and non-fibrotic lesions in HCM have been estimated as follows: LS was calculated as fiber length in systole minus fiber length in diastole per fiber length in diastole; RS was calculated as LVM wall thickness in systole minus LVM wall thickness in diastole per LVM wall thickness in diastole. As in HCM subjects, absolute values of LVM wall thickness and increased LVM wall thickness may influence values of regional peak RS more than the presence of fibrotic lesions in LVM. Therefore detectabilities of regional peak LS in distinguishing fibrotic from non-fibrotic lesions in LVM in HCM subjects were superior and more suitable than those of regional RS. As strain rates are determined by differentiation of each strain value, the same tendency was therefore observed between LSR and RSR in distinguishing fibrotic lesions from non fibrotic lesions in LVM in HCM subjects; furthermore, strain values themselves were more sensitive than corresponding strain rates in distinguishing fibrotic from non-fibrotic lesions.

Global strain and strain rates were derived by averaging regional strain and strain values from 17 segments. Therefore, global strain and strain rates can distinguish HCM subjects both with and without fibrotic lesions from normal controls. However, the accuracy of differentiation of HCM subjects with fibrotic lesions from those without fibrotic lesions is dependent upon how many fibrotic lesions are included in LVM of the study population. Occasionally in this population, differentiation of HCM subjects with fibrotic lesions from those without fibrotic lesions can be achieved using global peak LS and RS. However, this was not the case using global LSR and RSR, because strain rates were less sensitive than corresponding strain

values. Furthermore, concerning inter- and intra-observer variabilities of regional peak strain values and corresponding rates, the reproducibility of LS is better than those of RS, LSR and RSR in analysis of whole sites and in part-by-part analysis.

9. Study limitations

Our study population was small, retrospective and non-randomized in a single center.

Circumferential strain incorporating only 6 segments in TTE, was excluded from this analysis.

Mishiro et al. [50] showed decreased strain in HCM subjects using TDI in both hypertrophied and non-hypertrophied LVM lesions. In this study however, comparison of strain and strain rates in hypertrophic and non-hypertrophic lesion in HCM was not investigated. Even though there were no differences in maximum LV wall thickness between HCM subjects with and without fibrotic lesions, comparison of the incidence of fibrotic lesions on CT between hypertrophic and non hypertrophic sites was not performed; this was because the determination of cut-off LV wall thickness was difficult from the small number of subjects in this study.

Evidence for the presence of myocardial disarray was not directly shown from these results.

In this study, we used MSCT instead of MRI which is recognized as gold standard for detecting myocardial fibrosis, (i) because another principal reason for this examination was for evaluation of coronary arteries and (ii) due to the limited availability of MRI in our Institute. Fibrotic lesions in the LVM were not quantitatively identified using clear cut-off CT attenuation, but qualitatively identified as defects in enhancement in the early phase and abnormal enhancement in the late phase simultaneously. The degree of CT attenuation of LE varied widely, because such LE occurs with necrosis of LVM in acute myocardial infarction, fibrosis in old myocardial infarction, HCM, dilated cardiomyopathy, cardiac sarcoidosis, and focal inflammatory lesions as in acute myocarditis or cardiac sarcoidosis. Therefore definition of LE using clear cut-off CT attenuation is difficult. Furthermore, in the present study population, there were none who exhibited a clinical course of acute myocarditis, cardiac sarcoidosis, acute myocarditis or acute-phase cardiac sarcoidosis. We also evaluated the coronary arteries of HCM subjects to rule out ischemic cardiomyopathy. In 8 HCM subjects, we did not employ invasive coronary angiography, but MSCT revealed no significant luminal stenosis in coronary arteries of these subjects.

The technical limitations of TTE constituted another limitation of our study. Although STI TTE provides accurate and angle-independent measurements, it is still affected by the quality of the TTE data. In HCM subjects, 84.1% of lesions yielded good tracking in TTE, but the remaining 15.9% yielded poor tracking and could not be used for analysis.

Further similar studies using 2D STI are needed in a larger population for quantitative evaluation of LE using MRI with clear cut-off MRI attenuations for detection of fibrotic lesions in LVM.

10. Conclusion

In HCM, regional peak LS was significantly lower in fibrotic than non-fibrotic lesions in LVM by CT. Regional peak LS measurements by STI can provide useful information noninvasively to distinguish fibrotic from non-fibrotic lesions in LVM in HCM, and normal LVM in normal healthy controls.

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The authors of this manuscript have certified that they comply with the principles of ethical publishing in the International Journal Cardiology [51].

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Rationale and design of a study to evaluate effects of pitavastatin on Japanese patients with chronic heart failure
The pitavastatin heart failure study (PEARL study)

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ABSTRACT

Background: HMG-CoA reductase inhibitors (statins) are known to have pleiotropic effects in addition to their lipid-lowering effect. Many studies have suggested cardioprotective effects of statins, however, recent large-scale clinical trials using rosuvastatin, a hydrophilic statin, have failed to show beneficial effects on cardiovascular events in patients with severe heart failure. We have designed the study to evaluate the effects of pitavastatin, a lipophilic statin, on Japanese patients with mild to moderate heart failure.
Methods and results: Five hundred seventy-seven patients with chronic heart failure were enrolled. We used a prospective, randomized, open-label, and blinded-endpoint evaluation (PROBE) design. Patients aged 20–79 years old with symptomatic (NYHA functional class II or III) heart failure and a left ventricular ejection fraction of ≤45% were randomly allocated to either receive pitavastatin (2 mg/day) or not in addition to conventional therapy for heart failure by using the minimization method. Follow-up will be continued until March 2011. The primary endpoint is a composite of cardiac death and hospitalization for worsening heart failure.
Conclusions: The PEARL study will provide important data on the role of pitavastatin in the treatment of Japanese patients with mildly symptomatic heart failure (UMIN-ID: UMINC000000428).

1. Introduction

Heart failure is a complex clinical syndrome that results from structural and functional disorders of the heart associated with a variety of cardiovascular diseases. The number of patients with heart failure has been increasing and heart failure is becoming a major public health problem. Over the past 20 years, there has been considerable progress in the treatment of chronic heart failure (CHF) with appearance of angiotensin-converting enzyme (ACE) inhibitors, angiotensin-II receptor blockers (ARB), β blockers and aldosterone antagonists. However, the number of deaths due to CHF has been increasing steadily and further strategies for CHF are needed.

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, commonly called statins, are the most widely used agents for the treatment of hypercholesterolemia. Clinical trials have shown that treatment with statins significantly reduces the incidence of cardiovascular events in patients with coronary artery diseases [1–4]. In addition to their lipid-lowering actions, statins have also been reported to have various pleiotropic effects, including antiinflammatory effects, antioxidant effects, angiogenic effects, protective effects of endothelial cells, and inhibitory effects on neurohormonal activation [5]. It has been demonstrated that statins inhibit the progression of heart failure in animal models with non-ischemic heart failure [6–8]. These effects of statins suggest its potential to ameliorate components of the complex pathophysiology of CHF and to contribute to a promising treatment for CHF in the future.

Many observational studies and retrospective analyses have suggested that treatment with statins decreases the incidence of heart failure in patients with coronary artery disease and reduces mortality of patients with CHF [9–12]. Prospective small trials have also confirmed the beneficial effects of statins on heart failure [13–17]. On the other hand, recent two prospective randomized large clinical trials have shown that statin treatment has no effect on the clinical outcomes of patients with CHF [18,19]. However, it remains unclear whether treatment with statins on CHF patients would show the same

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Table 1
Inclusion criteria.

1. Age 20–79 years at the time of randomization
2. NYHA functional class II or III
3. Left ventricular ejection fraction as measured by echocardiography of ≤45%
4. Stable NYHA class for 2 or more weeks before the randomization
5. Mild hypercholesterolemia (serum total cholesterol ≤250 mg/dl and/or serum LDL-cholesterol ≤170 mg/dl)

NYHA, New York Heart Association; LDL, low-density lipoprotein.

results with different study designs in relation to the type of statin used, characteristics of the patients, severity of heart failure, and the endpoints examined. Pitavastatin is a lipophilic statin and has longer-acting effects on low-density lipoprotein (LDL)-cholesterol reduction and high-density lipoprotein cholesterol increase. Pitavastatin also has a high bioavailability and is minimally metabolized by the cytochrome P450 system [20–22]. The Pitavastatin Heart Failure (PEARL) study was designed to evaluate the beneficial effects of pitavastatin on the incidence of cardiac death and hospitalization for worsening heart failure in Japanese patients with CHF.

2. Methods

2.1. Study population

We enrolled 577 symptomatic CHF patients with mild hypercholesterolemia between June 2006 and June 2008. The inclusion criteria are: between 20 and 79 years of age; New York Heart Association (NYHA) functional class II or III; left ventricular ejection fraction (LVEF) as measured by echocardiography of ≤45%; stable NYHA class for 2 or more weeks prior to the randomization; mild hypercholesterolemia (serum total cholesterol concentration of ≤250 mg/dl and/or serum LDL-cholesterol concentration of ≤170 mg/dl); and availability of written informed consent of the patient (Table 1). The exclusion criteria are: receiving treatment with a statin prior to the randomization; history of acute myocardial infarction within 3 months prior to the randomization, percutaneous coronary intervention (PCI), or coronary artery bypass grafting, or cardiac resynchronization therapy-pacemaker or -defibrillator implantation performed within 3 months prior to the randomization; malignancy; serious renal or hepatic dysfunction; collagen disease; pregnancy or possible pregnancy; and lack of informed consent (Table 2).

2.2. Study design

The PEARL study is a multicenter, prospective, randomized, open-label, blinded-endpoint (PROBE) trial being carried out in Japan. The eligible patients are allocated randomly to either the pitavastatin group (2 mg/day) or the control group (no statin). As the usual daily dosage of pitavastatin is 1–2 mg in Japan, we used 2 mg of pitavastatin in the present study. Patient allocation was performed by the minimization method on the basis of the following baseline variables: age, gender, serum total cholesterol concentration, LVEF, history of ischemic heart disease, and history of hospitalization for CHF. According to the protocol, all the patients will be followed up for an average of 3 years until March 2011. If any patients in the control group need further reduction of the serum total cholesterol concentration, lipid-lowering agents except statins can be administered (Fig. 1).

2.3. Endpoints

The primary endpoint is a composite of cardiac death and hospitalization due to worsening CHF. The secondary endpoints are all-cause death, cardiac death, hospitalization due to worsening CHF, myocardial infarction, unstable angina, stroke, PCI, and surgical therapy for worsening CHF. Tertiary endpoints include evaluations for cardiac function by echocardiography, NYHA class, incidence of new-onset atrial fibrillation, serum high-sensitivity C-reactive protein, B-type natriuretic peptide (BNP), norepinephrine, tumor necrosis factor-α, interleukin-6, and tenascin C, and urinary 8-isoprostane. We also evaluate left ventricular systolic function, volume and myocardial perfusion by ^{99m}Tc-sestamibi quantitative gated single-photon emission computed tomography (SPECT) and measure surrogate markers for CHF by blood tests.

Table 2
Exclusion criteria.

1. Administration of statins before randomization.
2. Unstable decompensated heart failure at randomization.
3. Acute myocardial infarction within 3 months prior to the randomization.
4. Percutaneous coronary intervention, coronary artery bypass grafting, or cardiac resynchronization therapy-pacemaker or -defibrillator implantation performed within 3 months prior to the randomization.
5. Malignancy.
6. Serious renal or hepatic dysfunction.
7. Collagen disease.
8. Pregnancy or possible pregnancy.
9. Lack of informed consent.
10. Judged by the investigator to be ineligible for inclusion in the study.

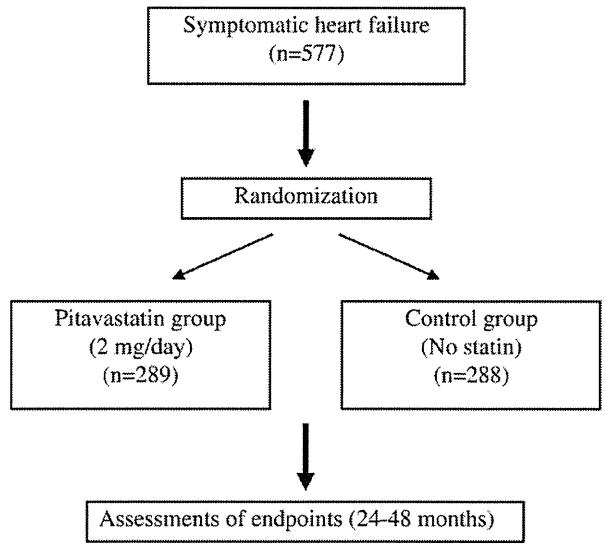

Fig. 1. Study design.

2.4. Sample size calculation

We assumed that the hazard ratio of cardiac events (cardiac death and hospitalization for worsening heart failure) of the pitavastatin group to the control group was 0.57 based on the previous reports [11,23]. On condition that the patients are evenly allocated and followed up for 24–48 months (median 36 months) after the enrollment, at least 200 patients are required for each arm to provide 80% power to detect a hazard ratio of 0.57 using a 2-sided significance level of 0.05 by the log-rank test. After considering maximum 15% for the loss of follow-up or unsuitable for analysis, we have expected that more than 470 patients are needed to be enrolled in this study.

2.5. Statistical analysis

Continuous variables such as laboratory data are expressed by mean ± standard deviation (SD) at each period. Group comparisons between the pitavastatin group and the control group will be calculated by Student t-test, Analysis of Variance (ANOVA), Chi-square test, Fisher's exact test, or the Mann–Whitney U-test, as appropriate. Comparisons within groups will be assessed by paired t-test or the Kruskal–Wallis test. Cumulative event rate for primary and secondary events will be determined by life-table method, and the difference between the groups will be tested by log-rank test or Cox proportional hazards regression model. Univariate predictive variables will be backward eliminated in multivariable models with the likelihood ratio criterion, and hazard ratios with 95% confidence intervals will be reported. All primary analyses will be done by intention-to-treat. Statistical analysis will be performed with SPSS 17.0 and Sample Power (SPSS Inc, Chicago, Il).

2.6. Study management

Data on the primary and secondary endpoints and adverse events will be collected at various time-points and interim analyses will be performed every year after the initiation of the study to evaluate the events and the treatment safety. An independent endpoint committee (Appendix 1) consisting of three members, who are blinded to any information relating to the group allocations, evaluates each event and classifies the results. An independent data and safety monitoring board (Appendix 2) is composed of three members and reviews all reports from the endpoint committee to advise early termination of the study for safety, scientific or ethical reasons. A steering committee (Appendix 3) is responsible for the study design and scientific execution of the study.

2.7. Baseline characteristics

Finally, 577 patients were randomly assigned to two groups. Of the total, 289 were assigned to the pitavastatin group and 288 to the control group. The baseline characteristics of the patients enrolled are presented in Table 3. The mean age was 62.7 years and 18.5% of the patients were female. The mean blood pressure was 119.4/

Table 3
Baseline characteristics (n = 577).

Mean age, years	62.7 ± 11.9
Females (%)	18.5
Mean systolic blood pressure (mmHg)	119.4 ± 17.9
Mean diastolic blood pressure (mmHg)	71.9 ± 11.2
NYHA functional class	
II (%)	89.6
III (%)	10.4
Mean LVEF (%)	35.0 ± 8.5
Mean total cholesterol (mg/dl)	202.3 ± 32.4
Mean LDL-cholesterol (mg/dl)	125.6 ± 30.5
Median (interquartile range) BNP (pg/ml)	126.0 (52.2–288.5)
Medical history	
Ischemic heart disease (%)	28.6
Medication	
ACE inhibitors/ARBs (%)	83.8
Diuretics (%)	74.8
β blockers (%)	72.5
Digitalis glycoside (%)	24.9
Calcium channel blockers (%)	16.0

NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LDL, low-density lipoprotein; BNP, B-type natriuretic peptide; ACE inhibitors/ARBs, angiotensin-converting enzyme inhibitors or angiotensin-II receptor blockers.

71.9 mmHg and 89.6% of the patients were classified to NYHA functional class II. The mean LVEF was 35.0% and 28.6% of the patients had a history of ischemic heart disease. The mean serum levels of total cholesterol and LDL-cholesterol were 202.3 mg/dl and 125.6 mg/dl, respectively. The median serum BNP in the patients was 126.0 pg/ml.

3. Discussion

The PEARL study has been carefully designed to address critical unanswered questions. Does pitavastatin have the beneficial effects on the incidence of cardiac death and hospitalization for worsening heart failure in Japanese patients with CHF? It is well known that treatment with statins significantly reduces the incidence of cardiovascular events in patients at high risk, especially those with coronary artery diseases, irrespective of the baseline cholesterol levels [24]. Several retrospective analyses and large observational studies have suggested that treatment with statins decreases the incidence of CHF and reduces mortality in patients with CHF [9–12]. Furthermore, prospective trials assessing the effects of statins on surrogate endpoints such as biomarkers and echocardiographic parameters demonstrated the beneficial effects of statins on patients with CHF [13–17]. Meta-analyses of statin treatment in randomized clinical trials also confirmed a reduction of cardiovascular mortality in patients with CHF of both ischemic and nonischemic etiologies [5]. These results support the pleiotropic effects of statins demonstrated by basic research and the beneficial effects of statins on patients with CHF.

Recently, the results of two prospective randomized trials have been published [18,19]. The CORONA (Controlled rosuvastatin multinational study in heart failure) study randomized 5,011 patients with symptomatic CHF of ischemic etiology to 10 mg rosuvastatin or placebo [18]. The primary endpoint was a composite of death from cardiovascular causes, nonfatal myocardial infarction, and nonfatal stroke. A daily dose of 10 mg rosuvasatin did not reduce the primary composite cardiovascular outcome, death from cardiovascular causes, nonfatal myocardial infarction, nor nonfatal stroke. The GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza cardiaca)–HF(Heart Failure) trial randomized 4631 patients with symptomatic CHF of both ischemic and nonischemic etiologies to 10 mg rosuvastatin or placebo [19]. The primary endpoints were time to death, and time to death or admission to hospital for cardiovascular reasons. The treatment with rosuvastatin did not affect the primary endpoints in the 2 studies [18,19]. However, the CORONA study showed that there were fewer hospitalization for cardiovascular causes which was one of the secondary endpoints in the rosuvastatin group. It remains unknown at present whether the results of the CORONA study and the GISSI-HF trial can be applied in the different patient selection or in the different kind of statin.

Concerns have been raised about the possible deleterious effects of statins. Circulating lipoproteins have the ability to bind and detoxify bacterial lipopolysaccharide. Because lipopolysaccharide stimulates the release of inflammatory cytokines, statins may increase inflammation in patients with CHF [25]. Moreover, statins decrease the synthesis of not only cholesterol but also of other downstream products in the mevalonate pathway. Coenzyme Q10 (ubiquinone), which is an essential cofactor in the mitochondrial electron transport chain, is one of the downstream products. Because coenzyme Q10 plays an important role in the mitochondrial respiratory chain and has an antioxidant function [26,27], statins may reduce adenosine triphosphate production and aggravate heart failure.

In the PEARL study, we use pitavastatin to evaluate the beneficial effects of statin therapy on Japanese patients with CHF. Pitavastatin is a statin which was developed in Japan and has potent lipid-lowering effect [20–22]. Pitavastatin is a lipophilic agent, while rosuvastatin is a hydrophilic agent. Pitavastatin is distributed selectively to the liver and is excreted in bile without metabolic modification [28]. The renal excretion rate is less than 2% and the terminal elimination half-life of 11 h [29]. It has been reported that the degree of reduction in plasma coenzyme Q10 by lovastatin, pravastatin, simvastatin, and atorvastatin corresponds closely to the degree of reduction of the serum or plasma LDL-cholesterol level [30]. A recent study showed that atorvastatin significantly reduced the plasma level of coenzyme Q10 but pitavastatin did not affect it in Japanese patients with heterozygous familial hypercholesterolemia, although both statins reduced the serum LDL-cholesterol levels by the same degree [31]. Statins potently reduce the serum LDL-cholesterol level by inhibiting the synthesis of mevalonate and inducing the expression of LDL receptors mainly in the hepatocytes. Pitavastatin has been reported to most strongly increase the LDL receptor mRNA expression among all statins [32]. These data indicate that there is minimal likelihood of deleterious effects resulting from inhibition of the mevalonate pathway in case of pitavastatin. As pitavastatin is hardly metabolized through the cytochrome P450-mediated pathway, pitavastatin is expected to have little interaction with other agents metabolized through the P450 pathway [33]. This property may be potentially beneficial for patients with CHF who take several kinds of medicines for the treatment of heart failure.

There are some differences in the characteristics of patients and protocol among the PEARL study, the CORONA study, and the GISSI-HF trial. The ratio of patients with ischemic heart failure was 40% in the GISSI-HF trial, and 100% in the CORONA study, while only 28.6% of patients have ischemic heart failure in the PEARL study. The mean age of the patients was 73, 68, and 63 years in the CORONA, the GISSI-HF, and the PEARL, respectively. The enrolled patients appear to be less symptomatic in the PEARL study (NYHA II 89.6%, III 10.4%, IV 0%) than in the CORONA study (NYHA II 37.0%, III 61.5%, IV 1.5%) and the GISSI-HF trial (NYHA II 62.5%, III 35.0%, IV 2.5%). The differences in severity of CHF patients may affect the results of those trials. Furthermore, all the patients enrolled in the PEARL study are Japanese. There are differences in the responses to therapeutic drugs between Asian and Western populations [34]. Asian patients frequently exhibit high responses to therapeutic drugs [34]. This phenomenon is also recognized in cardiovascular drugs including statins. Pharmacokinetic investigations have demonstrated higher plasma levels of statins in Asians as compared with Caucasians [35]. The high responses of Asians to statins are thought to be related to genetic differences in the metabolism of statins [35]. The kind of statins used in the PEARL study, the CORONA study, and the GISSI-HF trial are also different. Pitavastatin used in the PEARL study has many characteristics different from rosuvastatin used in the other two studies, such as lipophilicity and bioavailability.

The PEARL study is the first study to evaluate the effects and safety of pitavastatin in Japanese patients with moderate CHF. This study is expected to clarify whether pitavastatin might have beneficial effects on the patients receiving current standard therapy for CHF.

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Appendix 1. Independent Endpoint Committee

Yuji Kira (Showa General Hospital); Hiroyuki Morita (The University of Tokyo); Yuji Hashimoto (Kameda General Hospital).

Appendix 2. Independent Data and Safety Monitoring Board

Akira Yamashina (Tokyo Medical University); Tsutomu Yamazaki (The University of Tokyo); Naoki Ishizuka (International Medical Center of Japan).

Appendix 3. Steering Committee

Issei Komuro, Yoichi Kuwabara, and Hiroyuki Takano (Chiba University); Koichi Node (Saga University); Teruo Inoue (Dokkyo Medical University); Hiroshi Ito (Akita University); Toshiharu Himi (Kimitsu Chuou Hospital); Takanobu Tomaru (Toho University Sakura Medical Center); Junji Kanda (Asahi Chuo Hospital); Satoshi Kobayashi (Chibaken Saiseikai Narashino Hospital); Mitsuyuki Shimizu (Jikei University School of Medicine, Kashiwa Hospital); Akira Miyazaki, Toshihisa Inoue, and Akira Aouda (Chiba Cardiovascular Center); Fumitaka Nakamura (Teikyo University Chiba Medical Center); Tatsuji Kanoh (Juntendo University); Youichi Katoh (Juntendo University Urayasu Hospital); Yoshihiko Seino (Nippon Medical School Chiba Hokusoh Hospital); Michiaki Hiroe (International Medical Center of Japan); Junichi Yamazaki (Toho University Omori Medical Center); Kyoichi Mizuno (Nippon Medical School); Toyoaki Murohara (Nagoya University); Masashi Kitakaze (National Cardiovascular Center); Kenji Kawajiri (Matsubara Tokushukai Hospital); Yoshihiko Saito (Nara Medical University); Kengo Kusano (Okayama University); Masakazu Kohno (Kagawa University); Keijiro Saku (Fukuoka University); Katsusuke Yano (Nagasaki International University); Koji Oku (Nagasaki Medical Center); Hisao Ogawa (Kumamoto University); Tetsunori Saika (Oita University).

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Agonist-Independent Constitutive Activity of Angiotensin II Receptor Promotes Cardiac Remodeling in Mice

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