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ORIGINAL ARTICLE
Myocardial Disease

Ubiquitin, p62, and Microtubule-Associated Protein 1 Light Chain 3 in Cardiomyopathy

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Background: The accumulation of ubiquitinated proteins has been detected in diseased hearts and has been associated with the expression of p62 and microtubule-associated protein 1 light chain 3 (LC3), which are related to autophagy. We evaluated differences in ubiquitin accumulation and p62 and LC3 expression in cardiomyopathy using endomyocardial biopsies.

Methods and Results: We studied 24 patients (aged 24–70 years; mean age 55 years) diagnosed with dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), or non-cardiomyopathy (NCM) who underwent endomyocardial biopsy. Biopsied samples were evaluated by microscopy for ubiquitin accumulation and expression of p62 and LC3. Ubiquitin accumulation and p62 and LC3 expression were observed in all patients. Ubiquitin accumulation was higher in DCM than in HCM or NCM; p62 expression was higher in DCM than in HCM. There were no significant differences in LC3 expression among the groups. Ubiquitin accumulation was significantly related to serum N-terminal pro B-type natriuretic peptide concentration and the expression of p62, but not LC3.

Conclusions: Ubiquitin accumulation was more prominent in DCM than in HCM and NCM, which may be due to a relative shortage of clearance, including autophagy, compared with production.

Key Words: Autophagy; Cardiomyopathy; Heart failure; Pathology

Cardiac protein homeostasis is regulated by a protein quality control machinery that detects, repairs, and disposes of cytotoxic proteins using various mechanisms, which include chaperone proteins, the ubiquitin-proteasome system (UPS), and autophagy.[1] Abnormal aggregation and accumulation of ubiquitinated proteins in the cytosol have been detected in human hearts with idiopathic or ischemic cardiomyopathies.[2,3] Autophagy can be detected using electron microscopy, and its dysregulation is associated with several cardiac diseases, including cardiomyopathy.[4] A previous study showed that immunohistochemistry for microtubule-associated protein 1 light chain 3 (LC3) and p62 can be used to detect autophagy.[5] However, there are no precise histological reports on the expression of ubiquitin, LC3, or p62 in cardiac diseases. The aim of this study was to evaluate the accumulation of ubiquitin and the expression of p62 and LC3 in cardiac myocytes in dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and non-cardiomyopathy (NCM).

Methods

The Ethics Committee of Nagasaki University Hospital approved the study protocol (Approval no. 20081714-3), and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patients for the publication of their details.

Between January 2018 and December 2021 (3 years), we evaluated patients (aged 24–72 years) who underwent endomyocardial biopsy owing to clinical suspicion of myocardial diseases, including cardiomyopathy and myocarditis. Of these patients, 24 (aged 24–70 years; mean age 55 years) were included in the present study. The clinical characteristics, laboratory data, and histopathological fea-

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Table 1. Patient Characteristics and Data	
Age (years)	58.5 [52–68]
Female sex	6 (25.0)
BMI (kg/m^2)	26.3 [23.3–28.0]
Drinking history	13 (54.2)
Smoking	13 (54.2)
DCM	9 (37.5)
HCM	10 (41.7)
Sarcoidosis	3 (12.5)
Hypertension	13 (54.2)
Untreated hypertension	2 (8.3)
Diabetes	9 (37.5)
CAD	0 (0.0)
COPD	1 (4.2)
Stroke	1 (4.2)
Anemia	2 (8.3)
CKD	7 (29.2)
VT	4 (16.7)
Atrial fibrillation	3 (12.5)
LVAD	1 (4.2)
Valve surgery	2 (8.3)
Laboratory data	
WBC (/μL)	5,950 [4,950.0–7,375.0]
Lymphocytes (%)	32.4 [20.5–37.8]
Hemoglobin (g/dL)	14.7 [13.8–15.4]
Platelets (×10^4/μL)	18.9 [15.8–21.7]
Total protein (g/dL)	7.2 [6.9–7.7]
Albumin (g/dL)	4.2 [4.0–4.5]
Total bilirubin (mg/dL)	0.80 [0.60–1.1]
NT-proBNP (pg/mL)	1,260.0 [388.0–1,966.5]
eGFR (mL/min/1.73 m^2)	62.7 [52.5–73.7]
hs-TNT (ng/mL)	0.016 [0.011–0.033]
HbA1c (%)	6.2 [5.7–6.5]
Creatine kinase (U/L)	80.5 [63.8–162.3]
CRP (mg/dL)	0.09 [0.04–0.19]
Echocardiography	
IVST (mm)	11.5 [9.0–17.3]
PWT (mm)	11.0 [8.3–12.0]
LVDd (mm)	53.5 [41.5–64.3]
LVDs (mm)	33.0 [25.0–56.0]
LVEF (%)	45.0 [27.3–72.8]
E/A	0.95 [0.80–1.8]
LAD (mm)	43.5 [38.0–47.0]
LAVI (mL/m^2)	47.0 [34.0–53.0]
TRPG (mmHg)	25.0 [22.0–31.0]
RVD (mm)	27.0 [23.0–31.0]
Medications	
β-blocker	18 (66.7)
MRA	8 (33.3)
ACEi/ARB	11 (45.8)
ARNI	0 (0.0)
SGLT2 inhibitor	2 (8.3)
Diuretics	9 (37.5)

Values are presented as n (%) or as the median [interquartile range]. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor-neprilysin inhibitor; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; HCM, hypertrophic cardiomyopathy; hs-TNT, high-sensitivity troponin T; IVST, interventricular septal thickness; LAD, left atrial diameter; LAVI, left atrial volume index; LVAD, left ventricular assist device; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro B-type natriuretic peptide; PWT, posterior wall thickness; RVD, right ventricular dimension; SGLT2, sodium-glucose cotransporter 2; TRPG, tricuspid regurgitation pressure gradient; VT, ventricular tachycardia; WBC, white blood cell count.

tures (photomicrography) of these patients were evaluated.

Histopathology

The biopsied myocardium was fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (4 μm) were prepared and were subjected to hematoxylin and eosin staining or immunostaining. The primary and secondary antibodies used were as follows: rabbit polyclonal anti-p62 (MBL, Tokyo, Japan; 1:200 dilution) and goat polyclonal anti-rabbit IgG for p62; mouse monoclonal anti-LC3 (MBL; 1:1,000 dilution) and goat polyclonal anti-mouse IgG for LC3; and mouse monoclonal anti-ubiquitin (GeneTex, Irvine, CA, USA; 1:1,000 dilution) and goat polyclonal anti-mouse IgG for ubiquitin. Samples were incubated at 4°C overnight after microwave treatment in citrate buffer.

To quantify the expression of ubiquitin and p62, image intensity was evaluated using WinROOF software (MITANI Corporation, Fukui, Japan), a multipurpose color image processor. Briefly, images covering most of the biopsy specimen area in one visual field were obtained randomly under a light microscope (magnification ×200; BX-53; Olympus). Raw imaging data were analyzed using WinROOF to quantify ubiquitin and p62 immunopositivity in cardiomyocytes.

The number of LC3-immunopositive dots within cardiac myocytes was counted in 5 randomly chosen high-power fields (magnification ×400).

Statistical Analysis

Statistical analyses were performed for all study variables. Continuous variables are expressed as the mean±SD or as the median and interquartile range, and were compared using Student's t-test or Welch's test, as appropriate. Categorical variables were compared using the χ^2 test or Fisher's exact test.

Correlations between 2 variables were analyzed, with Spearman's rank correlation test was used to measure the degree of association between the 2 variables. Statistical significance was set at $P<0.05$. All analyses were performed using JMP Pro17 (SAS Institute, Cary, NC, USA).

Results

The characteristics of the study population are summarized in **Table 1**. This study included 9 patients with DCM, 10 with HCM, and 5 with NCM (3 with sarcoidosis and 2 with hypertensive heart disease) who were clinically and pathologically diagnosed using endomyocardial biopsy.

DCM, HCM, and NCM were defined as follows. DCM

Table 2. Comparisons of Characteristics and Data Among Groups

	DCM (n=9)	HCM (n=10)	NCM (n=5)	P value		
				DCM vs. HCM	DCM vs. NCM	HCM vs. NCM
Age (years)	58.0 [55.5–64.5]	64 [52.0–70.0]	42.5 [32.3–61.8]	0.5637	0.2007	0.0729
Female sex	2 (22.2)	3 (30)	1 (20.0)	1.000	1.000	1.000
BMI (kg/m^2)	23.9 [19.4–27.9]	25.4 [24.1–27.7]	30.4 [26.4–34.8]	0.3913	0.0117	0.0070
Drinking history	4 (44.4)	6 (60.0)	3 (60.0)	0.6563	1.000	1.000
Smoking	4 (44.4)	5 (50.0)	4 (80.0)	1.000	0.3007	0.5804
Hypertension	4 (44.4)	5 (50.0)	4 (80.0)	1.000	0.3007	0.5804
Diabetes	3 (33.3)	4 (40.0)	2 (40.0)	1.000	1.000	1.000
CAD	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–
COPD	1 (11.1)	0 (0.0)	0 (0.0)	0.4737	1.000	–
Stroke	0 (0.0)	1 (10.0)	0 (0.0)	1.000	–	1.000
Anemia	2 (22.2)	0 (0.0)	0 (0.0)	0.2105	0.5055	–
CKD	3 (33.3)	2 (20.0)	2 (40.0)	0.6285	1.000	0.5604
VT	2 (22.2)	1 (10.0)	1 (20.0)	0.5820	1.000	1.000
Atrial fibrillation	3 (33.3)	0 (0.0)	0 (0.0)	0.0867	0.2582	–
LVAD	1 (11.1)	0 (0.0)	0 (0.0)	0.4737	1.000	–
Valve surgery	2 (22.2)	0 (0.0)	0 (0.0)	0.2105	0.4000	–
Laboratory data						
WBC (/μL)	6,600.0 [5,050.0–7,250.0]	5,450.0 [4,750.0–6,625.0]	8,550.0 [5,100.0–15,700.0]	0.3911	0.6404	0.3263
Hemoglobin (g/dL)	14.6 [13.7–15.6]	14.3 [13.8–15.0]	15.6 [14.3–16.5]	0.4373	0.1815	0.0821
Platelet (×10^4/μL)	18.9 [15.7–22.0]	18.1 [16.3–20.2]	24.6 [11.3–34.3]	0.5544	0.8839	0.6682
Total protein (g/dL)	7.0 [6.1–7.9]	7.1 [6.9–7.5]	7.6 [6.8–7.8]	0.9673	0.2849	0.1013
Albumin (g/dL)	4.1 [3.7–4.5]	4.2 [4.0–4.4]	4.3 [3.5–4.5]	0.7428	0.2744	0.2863
Total bilirubin (mg/dL)	0.90 [0.80–1.2]	0.7 [0.6–1.1]	0.75 [0.48–2.6]	0.1915	0.2539	0.6217
NT-proBNP (pg/mL)	2,662.0 [1,408.0–6,508.5]	671.5 [334.5–1,621.5]	945.5 [187.9–6,026.8]	0.0048	0.0113	0.5815
eGFR (mL/min/1.73 m^2)	52.6 [38.2–62.7]	72.1 [59.1–76.7]	63.4 [46.8–74.2]	0.0333	0.1880	0.6595
hs-TNT (ng/mL)	0.024 [0.013–0.050]	0.014 [0.011–0.020]	0.017 [0.012–0.074]	0.1411	0.4232	0.7123
HbA1c (%)	6.2 [5.7–6.4]	6.1 [5.3–6.7]	6.3 [5.6–6.9]	0.84117	0.9412	0.8539
Creatine kinase (U/L)	78.0 [56.5–136.5]	104.5 [76.0–177.0]	64.5 [50.5–217.3]	0.3475	0.3852	0.0861
CRP (mg/dL)	0.11 [0.040–0.20]	0.065 [0.040–0.14]	0.19 [0.085–3.6]	0.6517	0.3496	0.1406
Echocardiography						
IVST (mm)	9.0 [7.4–10.0]	18.0 [14.75–22]	9.5 [8.8–12.5]	0.0004	0.1247	0.0042
PWT (mm)	8.0 [8.0–10.5]	12.0 [10.0–133.0]	12.0 [11.3–14.0]	0.0187	0.0122	1.000
LVDd (mm)	65.0 [61.0–70.5]	41.0 [34.8–43.8]	60.0 [51.8–72.3]	<0.0001	0.1559	0.0005
LVDs (mm)	56.0 [53.5–62.5]	25.0 [19.0–27.0]	51.5 [34.0–59.0]	0.0036	0.1606	0.0026
LVEF (%)	26.0 [19.5–35.0]	73.0 [69.5–76.5]	36.5 [29.5–57.8]	0.0003	0.0443	0.0119
E/A	1.7 [0.95–3.0]	0.85 [0.78–0.93]	1.4 [1.0–3.2]	0.0558	0.9151	0.1141
LAD (mm)	45.0 [41.5–50.0]	38.5 [36.3–44.3]	46.0 [33.0–47.3]	0.0202	0.2224	0.6112
LAVI (mL/m^2)	48.0 [37.0–54.0]	44.0 [33.0–51.0]	35.0 [20.0–62.0]	0.3640	0.4101	0.7156
TRPG (mmHg)	23.0 [20.5–28.5]	24.0 [21.5–32.0]	28.0 [22.8–38.3]	0.7234	0.1231	0.2134
RVD (mm)	31 [23.3–33.8]	27.0 [21.0–28.5]	28.5 [25.5–30.0]	0.1344	0.6191	0.3098
Medications						
β-blocker	8 (88.9)	1 (11.1)	0 (0.0)	1.0000	0.0030	0.07
MRA	8 (88.9)	0 (0.0)	0 (0.0)	0.0001	0.0030	–
ACEi/ARB	6 (66.7)	4 (40.0)	1 (20.0)	0.3698	0.2657	0.6004
ARNI	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–
SGLT2 inhibitor	1 (11.1)	1 (10.0)	0 (0.0)	1.0000	1.0	1.000
Diuretics	9 (100.0)	0 (0.0)	0 (0.0)	<0.0001	0.0005	–

Unless indicated otherwise, values presented as n (%) or as the median [interquartile range]. NCM, non-cardiomyopathy. Other abbreviations as in Table 1.

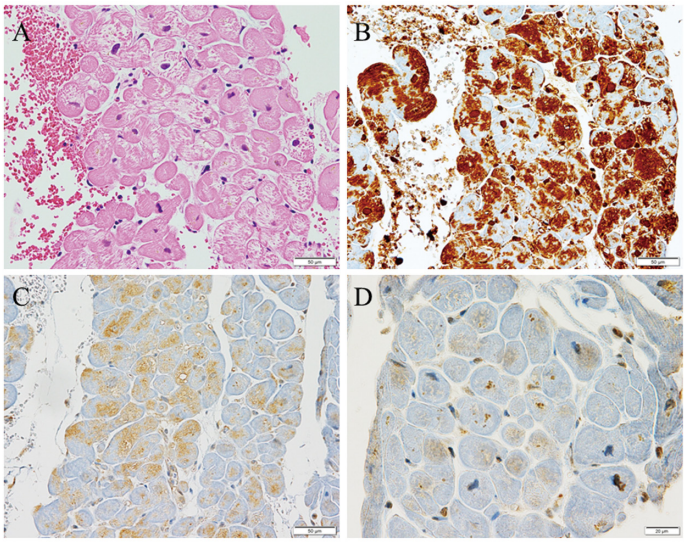


Figure 1. Microphotographs of biopsied myocardium in a patient with dilated cardiomyopathy. (**A**) Hematoxylin and eosin staining; (**B**) immunostaining for ubiquitin; (**C**) immunostaining for p62; and (**D**) immunostaining for microtubule-associated protein 1 light chain 3.

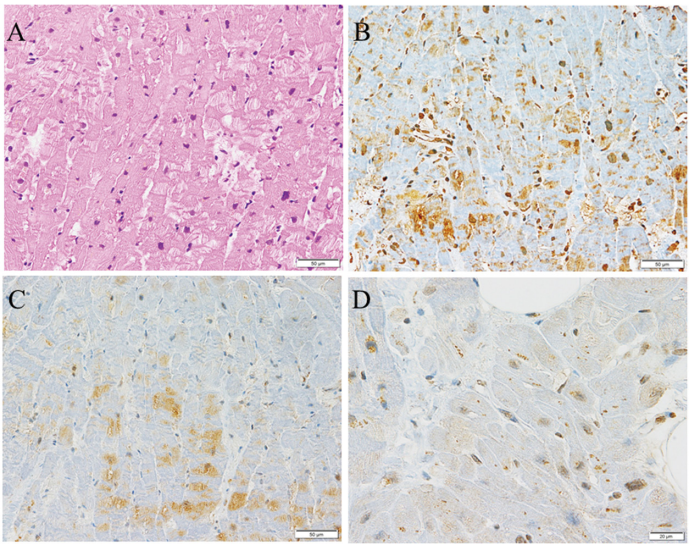


Figure 2. Microphotographs of biopsied myocardium in a patient with hypertrophic cardiomyopathy. (**A**) Hematoxylin and eosin staining; (**B**) immunostaining for ubiquitin; (**C**) immunostaining for p62; and (**D**) immunostaining for microtubule-associated protein 1 light chain 3.

was defined as left ventricular ejection fraction (LVEF) ≤40% and left ventricular (LV) dilatation not explained by coronary artery disease or other causes of global systolic dysfunction, such as abnormal loading conditions, tachycardiomyopathy, cardiotoxicity, or myocarditis. HCM was defined as the presence of increased LV wall thickness that was not solely explained by abnormal loading conditions with characteristic pathological features, including myocardial disarray. NCM was defined as any condition other than cardiomyopathy (primary myocardial disease in the absence of coronary artery disease, hypertension, valvular disease, and congenital heart disease). Parameters were compared between these groups, in addition their evaluation in all patients.

Body mass index was higher in the NCM than DCM and HCM groups (**Table 2**). N-Terminal pro B-type natriuretic peptide (NT-proBNP) concentrations were significantly higher in the DCM than HCM and NCM groups, and the estimated glomerular filtration rate was lower in the DCM than HCM group (**Table 2**). Creatine kinase and high-sensitivity troponin T levels did not differ significantly among the groups (**Table 2**). Transthoracic echocardiography (TTE) revealed that the interventricular septal thickness (IVST) was greater in the HCM than DCM and NCM groups, and that LV posterior wall thickness (LVPWT) was smaller in the DCM than HCM and NCM groups (**Table 2**). The LV diastolic dimension (LVDd) and LV systolic dimension were lower in the HCM group than in the DCM and NCM groups. LVEF was higher in the HCM than DCM and NCM groups, and was lower in the DCM than NCM group (**Table 2**).

Immunohistochemical staining demonstrated ubiquitin

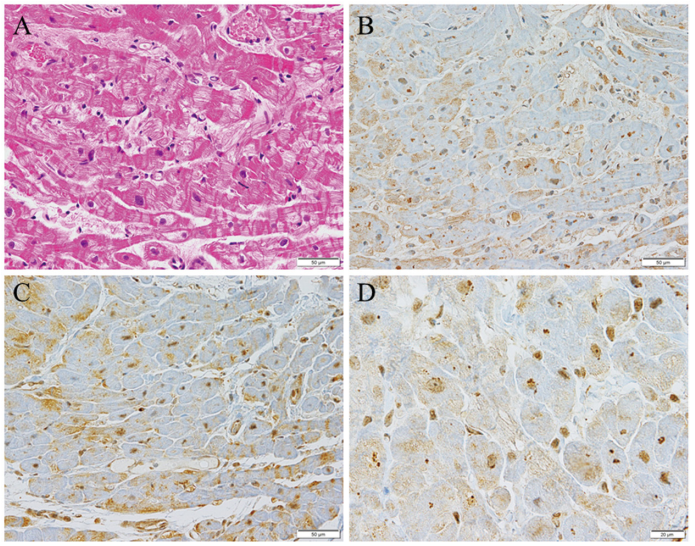


Figure 3. Microphotographs of biopsied myocardium in a patient with hypertensive heart disease. (**A**) Hematoxylin and eosin staining; (**B**) immunostaining for ubiquitin; (**C**) immunostaining for p62; and (**D**) immunostaining for microtubule-associated protein 1 light chain 3.

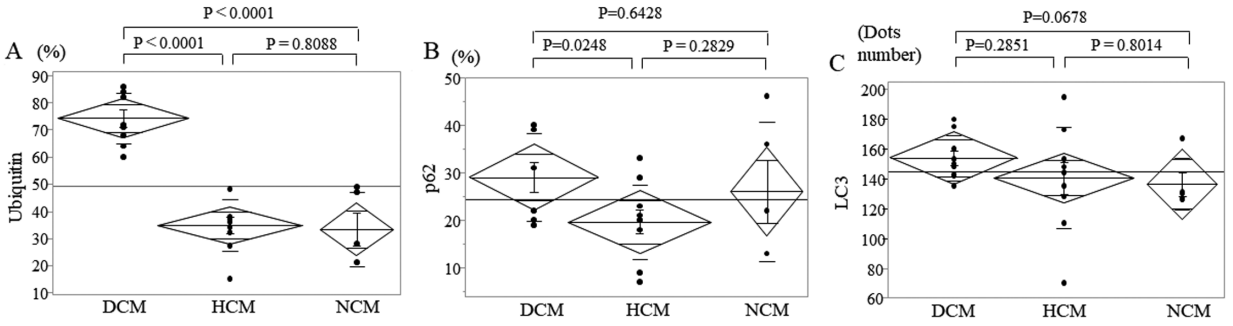


Figure 4. (**A**) Ubiquitin, (**B**) p62, and (**C**) microtubule-associated protein 1 light chain 3 (LC3) expression in the biopsied myocardium of patients with dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and non-cardiomyopathy (NCM).

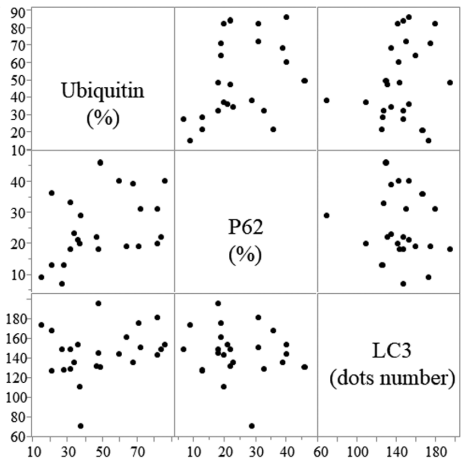
accumulation and p62 and LC3 expression in cardiac myocytes in DCM (**Figure 1**), HCM (**Figure 2**), and NCM (**Figure 3**). Ubiquitin accumulation was higher in the DCM than HCM and NCM groups (74.3±9.5% vs. 34.7±9.6% and 33.2±13.8%, respectively; P<0.0001 for both; **Figure 4A**). The expression of p62 was higher in the DCM than HCM group (29.0±9.2% vs. 19.6±2.7%, respectively; P=0.0284; **Figure 4B**). There was no significant difference in LC3 expression among the three groups (**Figure 4C**). Ubiquitin accumulation was significantly related to p62 expression (ρ=0.4660, P=0.0217) but not to LC3 expression (ρ=0.2492, P=0.2402; **Figure 5**).

Serum NT-proBNP concentrations were significantly related to ubiquitin accumulation (ρ=0.5470, P=0.0057), but not to p62 (ρ=0.2296, P=0.2804) or LC3 (ρ=0.3121, P=0.1376) expression (**Table 3**). The high-sensitivity troponin level, a marker of myocardial damage, showed no significant relationship with ubiquitin, p62, or LC3.

Among the TTE parameters, IVST and LVPWT were significantly and negatively correlated with ubiquitin accumulation (IVST: ρ=−0.5207, P=0.0091; LVPWT: ρ=−0.6041, P=0.0018) and p62 expression (IVST: ρ=−0.5817, P=0.0029; LVPWT: ρ=−0.4877, P=0.0156; **Table 3**). LVDd was positively correlated with ubiquitin (ρ=0.5472, P=0.0057) accumulation, whereas LVEF was negatively correlated with ubiquitin accumulation (ρ=−0.6374, P=0.0008; **Table 3**).

Discussion

Otsuka et al[6] demonstrated that the area fraction of ubiquitin was significantly higher in DCM hearts than in normal controls, and that it was significantly positively correlated with plasma B-type natriuretic peptide concentrations in DCM hearts. Weekes et al[7] reported that overall protein ubiquitination increased 2-fold in DCM relative to ischemic heart disease hearts and 5-fold relative to donor hearts, although the precise mechanism was unknown. Our data showed that ubiquitin accumulation increased approximately 2-fold in DCM compared with diseased (HCM and NCM) hearts, and that ubiquitin accumulation was positively correlated with NT-proBNP and LVDd and



Variable	vs. Variavle	Rank correlation coefficient (ρ)	P value (Prob>|ρ|)
p62	Ubiquitin	0.4660	0.0217
LC3	Ubiquitin	0.2492	0.2402
LC3	p62	-0.1019	0.6357

Figure 5. Relationship between ubiquitin accumulation and the expression of p62 and microtubule-associated protein 1 light chain 3 (LC3) in the biopsied myocardium. There was a significant relationship between ubiquitin accumulation and p62, but not LC3, expression. There was no correlation between p62 expression and LC3 expression.

Table 3. Correlations Between Parameters

Variable	vs. variable	Rank correlation coefficient ([ρ])	P value
Laboratory data			
NT-proBNP	Ubiquitin	0.5470	0.0057
	p62	0.2296	0.2804
	LC3	0.3121	0.1376
hs-TNT	Ubiquitin	0.1350	0.5292
	p62	0.1989	0.3514
	LC3	0.2223	0.2965
TTE			
IVST	Ubiquitin	−0.5207	0.0091
	p62	−0.5817	0.0029
	LC3	−0.0083	0.9693
LVPWT	Ubiquitin	−0.6041	0.0018
	p62	−0.4877	0.0156
	LC3	−0.1904	0.3728
LVDD	Ubiquitin	0.5472	0.0057
	p62	0.2091	0.3267
	LC3	0.0763	0.7229
LVEF	Ubiquitin	−0.6374	0.0008
	p62	−0.3057	0.1463
	LC3	0.066	0.7592

LVPWT, left ventricular posterior wall thickness; TTE, transthoracic echocardiography. Other abbreviations as in Table 1.

negatively correlated with LVEF. These results are consistent with those of previous studies and suggest that ubiquitin accumulation may be related to cardiac dysfunction, especially in DCM.

We also demonstrated the expression of p62 and LC3 in all patients, indicating the presence of autophagy. The expression of p62 was lower in HCM than DCM, and p62 was negatively correlated with LVPWT, but LC3 showed no significant differences among the groups and no relationship with biomarkers or echocardiographic data for heart failure and cardiac dysfunction.

LC3 is a component of autophagy vacuoles, and previous studies have shown an increase in LC3 in cardiac myocytes in the hearts of transgenic DCM mouse models,[8] as well as in patients with ischemic cardiomyopathy or DCM compared with control hearts.[9,10] Mechanical

unloading of the failing human heart by an LV assist device (LVAD) decreases autophagy markers, including LC3, suggesting that autophagy may be an adaptive mechanism in the failing heart and that this phenomenon is attenuated by LVAD support.[11] Kostin et al[3] reported that cytosolic ubiquitin-positive aggregates linked to increased levels of autophagy were observed in DCM. It has been also reported that LC3 protein levels were higher in HCM septal myectomies than in non-failing control hearts.[4] Taken together, our LC3 data are compatible with those of previous studies, suggesting that the levels of autophagy activation may be similar among DCM, HCM, and NCM patients with heart failure.

Previous reports have shown an increase and/or accumulation of p62 in cardiac myocytes in DCM and genetic cardiomyopathy.[8,12,13] However, there are no precise reports on p62 expression in HCM. p62 is a multidomain, multifunction protein involved in autophagy and a series of signaling processes.[14,15] p62 is a ubiquitin- and LC3-binding protein that is essential for the clearance of ubiquitinated and non-ubiquitinated proteins by autophagy.[16] p62 itself is degraded during autophagy, and the suppression of autophagy leads to the accumulation of p62.[17] The transcription of p62 is modulated by oxidative stress (nuclear factor erythroid 2-related factor 2 [Nrf2]), the Ras/mitogen-activated protein kinase pathway, the JNK/c-Jun pathway, and some chemical compounds.[18] The UPS is indispensable for the degradation of most proteins, including myofibrillar proteins, and the ubiquitination of target proteins is required for degradation by the UPS.[19] Proteasome inhibition and other stressors, such as starvation, can also induce p62 synthesis.[20] Thus, the expression of p62 may depend on the balance between its production by certain factors, including UPS inhibition, and degradation by autophagy.

Although misfolded protein accumulation and aggresome formation characterize DCM hearts, aggresomes fail to trigger the autophagy lysosomal pathway, with consequent accumulation of both p62 and dysfunctional mitochondria.[12] p62 is upregulated in mouse proteinopathic hearts. p62 promotes aggresome formation and autophagy activation and protects cardiomyocytes against proteotoxic stress.[21] Disruption of cardiomyocyte autophagy has been reported in cardiac DCM, as well as in aging, diabetes, and heart failure.[22–24]

Previous studies have shown that excessive cardiac autophagy leads to cardiac hypertrophy and heart failure.[25,26] A previous pathological study showed that numerous cardiomyocytes had ubiquitin-positive inclusions, and ultrastructural analysis indicated that cardiomyocytes contained typical autophagic vacuoles in the biopsied myocardium of a patient in the transition stage from HCM to heart failure.[27] Moreover, autophagy is activated during ventricular hypertrophy to maintain cellular homeostasis, although excessive autophagy eliminates essential cellular elements and possibly provokes cell death, which, together, contribute to hypertension-related heart disease.[28] Thus, prompt autophagy activation may be related to the lower expression of p62 in HCM without severe heart failure than in DCM.

Recently, other autophagy mechanisms have been shown to be independent of ubiquitin-binding receptors, including p62.[29,30] Thus, mechanisms other than p62 may be related to differences in p62 expression between DCM and HCM.

This study had some limitations. First, this was a single-center retrospective study, and the number of cases was small and may not be enough to demonstrate significant differences between groups for some data. We did not examine autophagy or mitophagy levels in the myocardium using electron microscopy. We also did not have cardiac magnetic resonance imaging data, including late gadolinium enhancement, for the evaluation of autophagic cell death.

Conclusions

In conclusion, ubiquitin accumulation was more prominent in DCM than in HCM and NCM, which may be due to a relative shortage of clearance, including autophagy, compared with production.

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Disclosures

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IRB Information

This study was approved by thee Ethics Committee of Nagasaki University Hospital (Approval no. 20081714-3).

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