Pathological impacts of sarcomere gene mutations and haplotype analysis

To investigate the possible pathological roles of the missense variants, their functional impacts were predicted by *in silico* analysis using PolyPhen-2, Mutation Taster and PROVEN. As shown in Table 1, *MYBPC3* Thr1046Met was suggested to be a benign mutation or a polymorphism, as was the case with the *TNNT2* Lys253Arg polymorphism. On the other hand, *MYH7* Glu935Lys and *MYBPC3* Arg160Trp were predicted to be disease-causing mutations. We, then, searched for these two mutations in our HCM cohort consisting of 162 familial and 100 sporadic cases,[10] and found the *MYH7* Glu935Lys in three (two familial and one sporadic) patients and the *MYBPC3* Arg160Trp in two (one familial and one sporadic) patients. Because family samples were available from each one family for the *MYH7* and *MYBPC3* mutations, we investigate the linkage of these mutations with HCM in the multiplex families along with the analyses of microsatellite markers.

As shown in Figure 1, we found that the *MYBPC3* Arg160Trp mutation was not co-segregated with HCM in a multiplex family with HCM originated from Tohoku area, because one subject showing LV hypertrophy and diastolic dysfunction did not carry the *MYBPC3* mutation (Figure 1a). A microsatellite analysis of the *MYBPC3* locus showed that the *MYBPC3* mutation (160Trp allele) was linked to a haplotype composed of allele 314 of MYBPC3-CA and allele 158 of D11S4109 in this family (Figure 1a). We also analyzed the other HCM patient from Kyushu area and two athletes, both from Kanto area, who carried the *MYBPC3* mutation, for the microsatellite markers. It was revealed that they also shared the 160Trp-314-158 haplotype, suggesting a common ancestral origin of the *MYBPC3* mutation (Supplementary Table S1).

On the other hand, a microsatellite analysis of the *MYH7* locus in the other multiplex family from Kyushu area showed a linkage of *MYH7* Glu935Lys with HCM (Figure 1b). Although the parents with the *MYH7* Glu935Lys mutation were cousins,[13] it was found that they carried different *MYH7*-mutation-linked haplotypes (Figure 1b), because both sons, who were homozygous for the *MYH7* mutation, were heterozygous for both MYOI and MYOII loci (Figure 1b). This observation indicated that there were at least two different haplotypes harboring the *MYH7* mutation. By analyzing the other two HCM patients from Kansai area and Kanto area and the athlete from Kanto area, who had the *MYH7* mutation, we obtained the data consistent with that the MYOI (intron 1)-rs2069540 (exon3)-rs2069542 (exon 8)-rs735712 (exon 12)-rs2231126 (exon 12)-rs121913639 (exon 23, Glu935Lys)-rs7157716 (exon 24)-MYOII (intron 24) haplotype of 164-C-C-C-C-935Lys-T-148 was shared by all of them, implying the common ancestral origin of the *MYH7* mutation (Supplementary Table S2).

Clinical phenotype of the athletes carrying the sarcomere gene mutations

The athlete carrying the *MYH7* Glu935Lys mutation was a 19-year-old male who played football. His ECG showed high voltages (voltage of R wave in V5 lead: 33 mm, voltage of S wave in V1 lead: 26 mm) and q waves in II, III, aVF, V5, and V6 leads, indicating a sign of LV hypertrophy, although no LV hypertrophy was evident in his echocardiography. Phenotypes of the other four subjects carrying the *MYBPC3* Arg160Trp or Thr1046Met variant are also shown in Table 2. Of note was that an athlete with the *MYBPC* Arg160Trp mutation was shown to have asymmetric cardiac hypertrophy in his echocardiography, which is a typical cardiac phenotype of HCM.

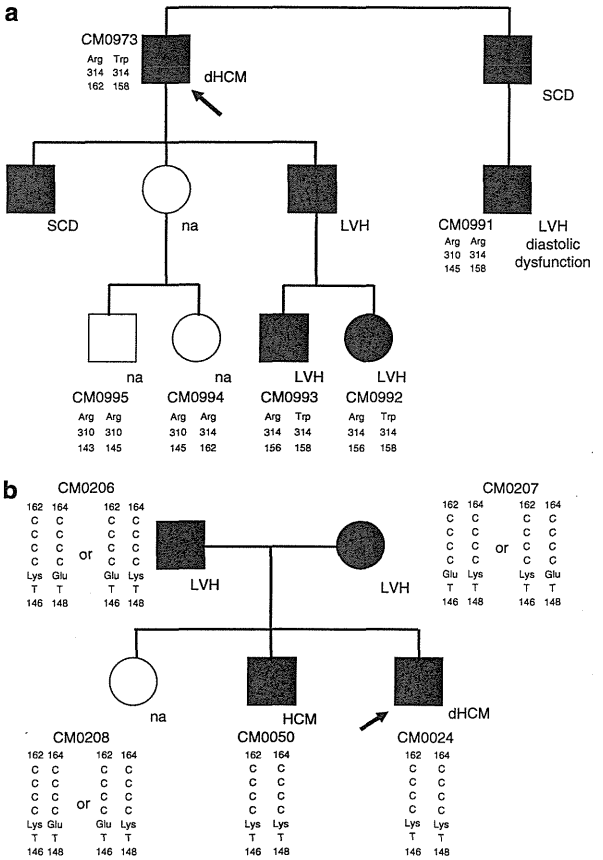

Figure 1 Linkage studies for *MYBPC3* and *MYH7* mutations in multiplex families with HCM. (**a**) Pedigree of a HCM family with the *MYBPC3* Arg160Trp mutation. *MYBPC3* haplotypes composed of rs193068692 (Arg160Trp)-MYBPC3-CA (fragment length)-D11S4109 (fragment length) were deduced from the genotyping data and indicated with ID of subjects. (**b**) Pedigree of a HCM family with the *MYH7* Glu935Lys mutation. *MYH7* haplotypes consisting of MYOI (fragment length)-rs2069540 (C/T)-rs2069542 (C/T)-rs735712 (C/T)-rs2231126 (C/T)-rs121913639 (Glu935Lys)-rs7157716 (C/T)-MYOII (fragment length) were deduced from the genotyping data and indicated with ID of subjects. Owing to the phase ambiguity, haplotypes could not be determined in three subjects. As well, MYOI–MYOII haplotype could not be determined due to the phase ambiguity and indicated tentatively. Arrows indicate proband patients. dHCM, dilated phase HCM; HCM, hypertrophic cardiomyopathy; LVH, ECG findings of LV hypertrophy; NA, no abnormality in cardiac phenotype; SCD, sudden cardiac death.

DISCUSSION

In this study, three sarcomere gene mutations that were reported to be associated with HCM were identified in five trained athletes. It was reported that the prevalence of HCM in elite athletes was significantly less than that in the general population (1 in 500 in the general subjects[3]), suggesting that structural and functional changes associated with HCM might naturally select-out most individuals with HCM from competitive sports.[15] However, a minority of HCM patients showed relatively mild LV hypertrophy and many of these patients are asymptomatic.[16] Actually, a small but substantial subset of elite athletes manifested with a slight increase of LV wall thickness, which defined a gray zone of overlap between the extreme expressions of 'athlete's heart' and a mild HCM form.[4] In addition, the most

Table 2 Clinical findings in young athletes carrying the sarcomere gene mutations

ID	*Gene*	*Mutation*	*Abnormality in ECG*	*Abnormality in echocardiography*	*Cardiomegaly in chest X-ray examination*[a]
Jun-49	*MYH7*	Glu935Lys	LVH (RV5+SV1 = 59 mV), q in II, III, aVf	ND	None
Jun-3	*MYBPC3*	Arg160Trp	LVH (RV5+SV1 = 55 mV)	Asymmetric hypertrophy (IVS = 14 mm, PW = 11 mm)	None
Jun-16	*MYBPC3*	Arg160Trp	LVH (RV5+SV1 = 40 mV), RAD, ST-T change	ND	None
Jun-75	*MYBPC3*	Thr1046Met	LVH (RV5+SV1 = 42.5 mV)	ND	None
Jun-78	*MYBPC3*	Thr1046Met	iRBBB, LA overload	ND	None

Abbreviations: ECG, electrocardiogram; iRBBB; incomplete right bundle branch block; IVS, thickness of intra ventricular septum; LA, left atrium; LVH: left ventricular hypertrophy; ND, not done; PW, thickness of posterior wall; RAD, right axial deviation; RV5, voltage of R wave in V5 lead; SV1, voltage of S wave in V1 lead.
[a]Cardiomegaly was defined by the cardiothorax ratio over 50%.

common cause of SCD in young athletes appears to be HCM.[16,17] These observations suggest the critical importance to clarify whether the increased LV wall thickness would represent a physiological adaptation of heart to athletic training or a pathological condition such as HCM. This ambiguity needs to be solved by applying a number of noninvasive parameters including a genetic testing to prevent athletes from SCD, although the causative role of the sarcomere gene mutations should be carefully considered.

Prevalence of the mutations in nucleotide variations database from healthy individuals including dbSNP, 1000 genomes and human genetic variation databases, description of clinical significance in the ClinVar database, and *in silico* studies would be helpful to predict the pathological significance of identified mutations. In this study, *MYBPC3* Thr1046Met was suggested to be benign and its clinical significance was debatable, although we could not exclude a possibility of its role in promoting cardiac hypertrophy, because the mutation had been reported in patients with elderly onset HCM who might have any confounding factors including mild hypertension.[14] In addition, *MYBPC3* Arg160Trp was not linked to HCM in a multiplex family tested in this study, indicating that it was not the cause of HCM for all the HCM patients in this family. However, a possibility of another disease-causing mutation in the *MYBPC3* Arg160Trp-negative HCM patient could not be excluded and therefore *MYBPC3* Arg160Trp might be a responsible mutation in the other HCM patients (Figure 1a), because *in silico* studies strongly suggested functional alterations by this rare variant.

On the other hand, *MYH7* Glu935Lys was not found in the healthy individuals in dbSNP, 1000 genomes and human genetic variation databases, listed as pathogenic mutation in the ClinVar database, and predicted *in silico* to have functional impacts in this study. We previously reported the phenotypes of *MYH7* Glu935Lys mutation in the Japanese HCM family (Figure 1b).[13] In this family, the proband patient and his elder brother were homozygous for the mutation and showed dilated phase of HCM and severe LV hypertrophy, respectively, and both eventually died at their thirties. In contrast, their parents were heterozygous for the mutation and both showed mild LV hypertrophy in the presence of blood hypertension.[13] Their elder sister was also heterozygous for the mutation, but she was normotensive and did not manifest with LV hypertrophy. These observations suggested a gene dose effect of the mutation on the clinical manifestation of HCM, that is, homozygotes were severer than the heterozygotes, as well as an age- or blood pressure-dependent penetrance of HCM caused by this mutation. Therefore, it can be speculated that the athlete carrying the *MYH7* Glu935Lys mutation in the heterozygous state might develop apparent cardiac hypertrophy or HCM phenotypes in the future, if he would continue the athletic training or when he would develop hypertension. Continuous and careful follow-up will be required for him to evaluate the clinical phenotypes caused by the mutation and/or athletic training. Similarly, careful follow-up is required for two athletes with the *MYBPC3* Arg160Trp mutation, especially for a subject who showed asymmetric cardiac hypertrophy in his echocardiography (Table 2).

In conclusion, we report here for the first time the results of a systematic screening for the HCM-associated sarcomere gene mutations in young athletes with abnormal ECG findings. We identified one and two missense mutations in *MYH7* and *MYBPC3*, respectively, which were reported to be the HCM-associated mutations, in five athletes. Of note was that 4 of them showed an apparent sign of LV hypertrophy in ECG and such abnormality was noted in 62 of 102 athletes, indicating that the sarcomere gene mutations were found in 4 of 62 (6.5%) athletes with ECG sign of LV hypertrophy. This result indicates that the potential application of genetic testing in trained athletes provides a definitive diagnosis of HCM in subjects with early ECG signs of LV wall thickness. Further genetic analyses in the other HCM genes for sarcomere proteins will help understand the genetic factors in young athletes with difficult diagnostic situation or with borderline findings of HCM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Olivetti, G., Cigola, E., Maestri, R., Lagrasta, C., Corradi, D. & Quaini, F. Recent advances in cardiac hypertrophy. *Cardiovasc. Res.* **45**, 68–75 (2000).
2 Lauschke, J. & Maisch, B. Athlete's heart or hypertrophic cardiomyopathy? *Clin. Res. Cardiol.* **98**, 80–88 (2009).
3 Maron, B. J. Sudden death in young athletes. *N. Engl. J. Med.* **349**, 1064–1075 (2003).

4 Pelliccia, A., Maron, M. S. & Maron, B. J. Assessment of left ventricular hypertrophy in a trained athlete: differential diagnosis of physiologic athlete's heart from pathologic hypertrophy. *Prog. Cardiovasc. Dis.* **54**, 387–396 (2012).
5 Maron, B. J. Risk stratification and role of implantable defibrillators for prevention of sudden death in patients with hypertrophic cardiomyopathy. *Circ. J.* **74**, 2271–2282 (2010).
6 Fuller, C. M., McNulty, C. M., Spring, D.A., Arger, K. M., Bruce, S. S., Chryssos, B. E. *et al.* Prospective screening of 5615 high school athletes for risk of sudden cardiac death. *Med. Sc. Sports Exerc.* **29**, 1131–1138 (1997).
7 Corrado, D., Basso, C., Schiavon, M. & Thiene, G. Screening for hypertrophic cardiomyopathy in young athletes. *N. Engl. J. Med.* **339**, 364–369 (1998).
8 Pelliccia, A., Maron, B. J., Culasso, F., Di Paolo, F. M., Spataro, A., Biffi, A. *et al.* Clinical significance of abnormal electrocardiographic patterns in trained athletes. *Circulation* **102**, 278–284 (2000).
9 Otsuka, H., Arimura, T., Abe, T., Kawai, H., Aizawa, Y., Kubo, T. *et al.* Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. *Circ. J.* **76**, 453–461 (2012).
10 Kimura, A. Molecular basis of hereditary cardiomyopathy: abnormalities in calcium sensitivity, stretch response, stress response and beyond. *J. Hum. Genet.* **55**, 81–90 (2010).
11 Richard, P., Isnard, R., Carrier, L., Dubourg, O., Donatien, Y., Mathieu, B. *et al.* Double heterozygosity for mutations in the beta-myosin heavy chain and in the cardiac myosin binding protein C genes in a family with hypertrophic cardiomyopathy. *J. Med. Genet.* **36**, 542–545 (1999).
12 Kubo, T., Kitaoka, H., Okawa, M., Matsumura, Y., Hitomi, N., Yamasaki, N. *et al.* Lifelong left ventricular remodeling of hypertrophic cardiomyopathy caused by a founder frameshift deletion mutation in the cardiac myosin-binding protein C gene among Japanese. *J. Am. Coll. Cardiol.* **46**, 1737–1743 (2005).
13 Nishi, H., Kimura, A., Harada, H., Adachi, K., Koga, Y., Sasazuki, T. *et al.* Possible gene dose effect of a mutant cardiac beta-myosin heavy chain gene on the clinical expression of familial hypertrophic cardiomyopathy. *Biochem. Biophys. Res. Commun.* **200**, 549–556 (1994).
14 Anan, R., Niimura, H., Takenaka, T., Hamasaki, S. & Tei, C. Mutations in the genes for sarcomeric proteins in Japanese patients with onset sporadic hypertrophic cardiomyopathy after age 40 years. *Am. J. Cardiol.* **99**, 1750–1754 (2007).
15 Basavarajaiah, S., Wilson, M., Whyte, G., Shah, A., McKenna, W. & Sharma, S. Prevalence of hypertrophic cardiomyopathy in highly trained athletes: relevance to pre-participation screening. *J. Am. Coll. Cardiol.* **51**, 1033–1039 (2008).
16 Maron, B. J., Pelliccia, A. & Spirito, P. Cardiac disease in young trained athletes. Insights into methods for distinguishing athlete's heart from structural heart disease, with particular emphasis on hypertrophic cardiomyopathy. *Circulation* **91**, 1596–1601 (1995).
17 Erdmann, J., Daehmlow, S., Wischke, S., Senyuva, M., Werner, U., Raible, J. *et al.* Mutation spectrum in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. *Clin. Genet.* **64**, 339–349 (2003).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg).

Supplementary Information

Screening of sarcomere gene mutations in young athletes with abnormal findings in electrocardiography: identification of a *MYH7* mutation and *MYBPC3* mutations

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Supplementary Table S1 Microsatellite analysis of*MYBPC3* locus in HCM patients and athletes carrying*MYBPC3* Arg160Trp mutation

ID	phenotype*	description	Area**	*MYBPC3* Arg160Trp***	MYBPC3-CA	D11S4109
CM0973	dHCM	proband patient	Tohoku	+	314/314	158/162
CM0991	ECG-LVH, Echo diast dysfunctior	affected family relative of CM097	Tohoku	−	310/314	145/158
CM0992	ECG-LVH	affected family relative of CM097	Tohoku	+	314/314	156/158
CM0993	ECG-LVH	affected family relative of CM097	Tohoku	+	314/314	156/158
CM0994	np	unaffected family relative of CM097	Tohoku	−	310/310	143/145
CM0995	np	unaffected family relative of CM097	Tohoku	−	310/314	145/162
CM0007	HOCM, ASH (IVS=25, PW=20)	sporadic HCM case	Kyushu	+	314/316	158/164
Jun-3	ECG-LVH, ASH (IVS=14, PW=11)	athlete with ECG abnormality	Kanto	+	314/314	158/164
Jun-16	ECG-LVH	athlete with ECG abnormality	Kanto	+	310/314	158/164

*; dHCM: dilated HCM, ECG-LVH: LV hypertrophy in ECG, np: no particular finding, HOCM: obstructive HCM, ASH: asynmetric hypertrophy, IVS: thickness of intraventricular septum (mm), PW: thickness of posterior wall (mm)

**; Area in Japan:

***; +: heterozygote of mutation, -: none

Supplementary Table S2 Haplotype analysis of *MYH7* locus in HCM patients and athletes carrying *MYH7* Glu935Lys mutation

ID	phenotype*	description	Area**	MYOI	*MYH7* Glu935Lys***	rs2069540	rs2069542	rs735712	rs2231126	rs7157716	MYOII
CM0024	dHCM, ASH (IVS=26, PW=13)	proband patient	Kyushu	162/164	++	CC	CC	CC	CC	TT	146/148
CM0050	HOCM	affected family relative of CM0024	Kyushu	162/164	++	CC	CC	CC	CC	TT	146/148
CM0206	mild LVH	affected family relative of CM0024	Kyushu	162/164	+	CC	CC	CC	CC	TT	146/148
CM0207	mild LVH	affected family relative of CM0024	Kyushu	162/164	+	CC	CC	CC	CC	TT	146/148
CM0221	np	unaffected family relative of CM0024	Kyushu	162/164	+	CC	CC	CC	CC	TT	146/148
CM0997	HCM, Af, moderate negative T in ECG	sporadic HCM case	Kanto	162/164	+	CC	CC	CC	CC	TT	146/148
CM1245	HNCM	familial HCM case	Kansai	162/164	+	CC	CC	CC	CC	TT	148/152
Jun-49	ECG-LVH	athlete with ECG abnormality	Kanto	160/164	+	CT	CT	CT	CC	CT	146/148

*; dHCM: dilated HCM, ASH: asymmetric hypertrophy, IVS: thickness of intraventricular septum (mm), PW: thickness of posterior wall (mm), HOCM: obstructive HCM, np: no particular finding, Af: atrial fibrillation, HNCM: non-obstructive HCM, ECG-LVH: LV hypertrophy in ECG.

**; Area in Japan:

***; ++: homozygote of mutation, +: heterozygote of mutation, -: none

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The impact of an inverse correlation between plasma B-type natriuretic peptide levels and insulin resistance on the diabetic condition in patients with heart failure

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ABSTRACT

Background. A diabetic state is causally related to heart failure (HF); therefore, there should be a close correlation between the severity of diabetes and HF. However, a direct relationship between these conditions has rarely been reported and remains unclear. This study was designed to precisely examine this relationship, taking into consideration the possible association between natriuretic peptide (NP) levels and insulin resistance.

Material and methods. We examined various hemodynamic parameters and simultaneously performed blood biochemical analyses of consecutive patients who underwent cardiac catheterization at our institution (n = 840).

Results. Simple regression analyses showed that hemoglobin A1c (HbA1c) levels were not significantly changed by the left ventricular end-diastolic pressure (LVEDP) and left ventricular ejection fraction (LVEF), which were correlated with a low cardiac index. Rather, there was a negative correlation between the HbA1c levels and plasma BNP levels as a marker of HF. A multivariate analysis showed no correlations between the HbA1c levels and cardiac functional parameters (LVEDP, LVEF or the plasma BNP levels), suggesting that the trend toward high HbA1c levels in HF cases is likely to be limited for unknown reasons. To search for an explanation of this finding, we examined the potential biological interactions between BNP and insulin resistance. A multivariate analysis revealed that

Abbreviations: AF, atrial fibrillation; ANOVA, one-way analysis of variance; ANP, A-type natriuretic peptide; BMI, body mass index; BNP, B-type natriuretic peptide; CAPD, continuous ambulatory peritoneal; cGK, c-GMP-dependent protein kinase; c-GMP, cyclic guanosine monophosphate; CI, cardiac index; CSA, coronary spastic angina; DBP, diastolic blood pressure; HD, hemodialysis; HIF-1α, hypoxia-inducible factor; HF, heart failure; HOMA-IR, homeostasis model assessment-insulin resistance; HTN, hypertension; IHD, ischemic heart disease; IRI, immunoreactive insulin; Log BNP, logarithmic BNP; Log HOMA-IR, logarithmic HOMA-IR; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; NEP, endopeptidase; NP, natriuretic peptide; RAAS, renin–angiotensin aldosterone system; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SBP, systolic blood pressure; s-Cr, serum creatinine.

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the plasma BNP levels were positively correlated with age, creatinine levels and LVEDP and inversely correlated with the male gender, body mass index and HOMA-IR (homeostasis model assessment-insulin resistance) ($P < 0.001$, respectively), but not HbA1c levels. This analysis indicated a close correlation between plasma BNP levels and insulin effectiveness in HF.

Conclusions. HF and diabetes tend to worsen with each other; however, the appearance of an association between them was likely blunted due to the considerable effect of NP in counteracting insulin resistance, even during the metabolically harmful condition of HF.

1. Introduction

Diabetes is often associated with hypertension, ischemic heart disease (IHD) and chronic kidney disease, all of which are major risk factors for heart failure (HF) [1]. The presence of diabetes markedly increases the likelihood of HF and results in worse outcomes for patients with HF [2,3]. Furthermore, the Framingham Study firmly established an epidemiological link between diabetes and HF [4], and similar findings have since been noted in a number of other studies [5,6]. Therefore, there is no doubt that diabetes is a critical risk factor for HF in the future. Conversely, the onset of HF is an independent risk factor for developing or worsening diabetes [7].

The pathophysiology of HF is complex and includes a variety of underlying mechanisms. Among these elements, neurohumoral factors, such as the renin–angiotensin aldosterone system (RAAS), sympathetic nervous system and others, deeply contribute to the pathophysiology of HF [8]. Oxidative stress is also important for the progression of cardiac remodeling, which leads to HF. Many studies have recognized that remodeling stimuli, such as mechanical strain and the level of tumor necrosis factor-α, may increase the formation of reactive oxygen species (ROS) in the myocardium [9,10]. On the other hand, the pathophysiology of diabetes is also very complicated, although some of its underlying mechanisms appear to be similar to those of HF. For example, the RAAS, sympathetic nervous system and oxidative stress also each contribute to the pathogenesis of diabetes [11]. Furthermore, cardiac function subsequently deteriorates as a result of increased atherosclerosis and myocardial damage caused by diabetic microangiopathy. Therefore, it is reasonable to speculate that diabetes is associated with the deterioration of HF.

To the best of our knowledge, few reports have shown a close correlation between the HbA1c level and the current degree of HF as evaluated by hemodynamic and other parameters. We suppose that this may be a paradox of the relationship between diabetes and HF in that the relationship between diabetes and the future onset of HF may be different from the relationship between diabetes and the current degree of HF. Importantly, this information would be useful to resolve various questions pertaining to the association between energy metabolism and HF, and there may be compensatory mechanism(s) involved in the pathophysiology of HF that could potentially rescue metabolic abnormalities, including glucose intolerance and reduced lipid catabolic activity.

A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), also known as atrial and brain natriuretic peptides, respectively, are cardiac hormones with a wide range of potent biological effects, including vasodilation, natriuresis and inhibition of the RAAS and sympathetic nervous system. BNP is selectively secreted from the ventricles, and the magnitude of this secretion varies as a function of the severity of HF [12]. We and others have previously shown that ANP and BNP are anti-inflammatory hormones; the infusion of human ANP (carperitide) is useful for improving hemodynamics as well as inhibiting ROS production in patients with HF [13]. Furthermore, an important report using BNP transgenic mice revealed that BNP is a metabolic regulator by demonstrating that the natriuretic peptide (NP)/cyclic guanosine monophosphate (c-GMP)-dependent protein kinase (cGK) cascade promotes muscle mitochondrial biogenesis and lipid oxidation, thus preventing obesity and glucose intolerance [14]. However, the effects of NP on energy metabolism have not yet been clearly proven or elucidated in humans because the only disease related to high NP levels is HF, and many other humoral factors associated with HF may cause the deterioration of glucose and catabolic lipid metabolism. Hence, the favorable actions of NP on energy metabolism may be hidden or outstripped by these other factors.

We continue to believe in the pluripotency of endogenous NP secreted from the failing heart and herein hypothesize that NP counteracts both hemodynamic deterioration and the development of metabolic abnormalities in HF. In this study, we examined the possible hidden actions of NP, especially on glucose metabolism, in patients with cardiovascular disease using common indicators, including hemoglobin A1c (HbA1c) levels, homeostasis model assessment-insulin resistance (HOMA-IR) as a marker of insulin resistance, body mass index (BMI) and plasma BNP levels.

2. Material and Methods

2.1. Study Patients

The study population consisted of 840 consecutive patients admitted to the Jikei University Hospital from February 2012 to July 2014 in whom left heart catheterization, including hemodynamic measurements, coronary angiography (n = 840) and left ventriculography (n = 797), and blood sampling tests of the plasma BNP level were performed and reviewed. The insulin and serum glucose levels were also measured in each patient. Among them, right heart catheterization with a Swan–Ganz catheter was performed in addition to left heart catheterization (n = 218). Individuals requiring an urgent catheter intervention for acute coronary syndrome were excluded from this study because the plasma BNP level rapidly and considerably changes within the acute phase of acute myocardial infarction [15]. We also excluded patients with type 1 diabetes mellitus and those receiving insulin therapy. The subjects' baseline characteristics, including clinical parameters and biochemical data, were collected retrospectively from their medical records, and the

data were analyzed anonymously. This study was approved by the ethics committee of the Jikei University School of Medicine (Study protocol: 24-355[7121]); and we complied with the routine ethical regulation of our institution as follows. This is a retrospective study and the informed consent could not be obtained from each patient. Instead of informed consent from each patient, we publicly posted a notice about the study design and contact information at a publicly-known space in our institution.

2.2. *Underlying Cardiac Diseases*

Within the study population, patients with IHD exhibited newly diagnosed coronary stenosis on angiography; a medical history of coronary artery disease, including myocardial infarction; a post-percutaneous coronary intervention or post-coronary artery bypass grafting status; and newly diagnosed coronary spastic angina (CSA) upon an acetylcholine provocation test or a diagnosis of CSA. In addition, patients with valvular heart disease, cardiomyopathy and/or hypertension were included in this study.

2.3. *Measurement of the Plasma BNP, HbA1c and Insulin Levels*

Whole blood (5 mL) was collected in tubes containing potassium EDTA (1 mg/mL blood). The plasma BNP levels were determined according to an enzyme-linked immunosorbent assay (non-extracted) using an antibody against human BNP (Shionogi, Tokyo, Japan). The HbA1c levels were measured using high-performance liquid chromatography (HPLC) (HLC723-G9, TOSOH, Tokyo, Japan) by a standardized laboratory protocol using a method certified by the National Glycohemoglobin Standardization Program (NGSP), with intra- and interassay coefficients of variation (CsV) of 1%. The immunoreactive insulin (IRI) levels were also measured using HPLC. The HOMA-IR values were calculated according to the formula in a previous report, i.e., HOMA-IR = fasting IRI (μU/mL) × fasting plasma glucose (mg/dL)/405 [16]. Blood sampling was performed immediately before or after cardiac catheterization. BMI was calculated as the body weight (kg) divided by the square of the height (m). Hypertension (HTN), diabetes mellitus and dyslipidemia were defined as previously described [17].

2.4. *Statistical Analysis*

Continuous variables are expressed as the mean ± SD. Comparisons between groups were made using Pearson's chi-square test for categorical variables and the Mann–Whitney U test or Student's t-test for continuous variables, where appropriate. To achieve a normal distribution, the BNP levels and HOMA-IR values were log transformed prior to the analysis. The patients were divided into four groups according to the left ventricular ejection fraction (LVEF) and left ventricular end-diastolic pressure (LVEDP). A multivariate analysis was performed to compare the values among the quartiles using one-way analysis of variance (ANOVA) followed by a Games–Howell post-hoc analysis. A multiple regression analysis was also performed to assess dependent determinants. A P value of <0.05 was considered to be statistically significant, and all data were statistically analyzed using the SPSS software package, version 21.0 (SPSS, Chicago, IL).

3. Results

3.1. *Study Population*

The baseline characteristics of the overall population (n = 840) in the present study are shown in Table 1. The underlying disease was IHD in 559 patients (66.5%), cardiomyopathy in 88 patients (10.5%) valvular disease in 91 patients (10.8%), HTN in 623 patients (74.2%) and diabetes mellitus in 258 patients (30.7%).

Table 1 – Clinical characteristics of the patients.

n = 840	
IHD, n (%)	559 (66.5)
Cardiomyopathy, n (%)	88 (10.5)
Valvular disease, n (%)	91 (10.8)
Congenital heart disease, n (%)	11 (1.3)
AF, n (%)	51 (6.1)
Hypertension, n (%)	623 (74.2)
Diabetes Mellitus, n (%)	258 (30.7)
Dyslipidemia, n (%)	614 (73.1)
Current + past smoker, n (%)	559 (66.5)
HD and CAPD, n (%)	74 (8.8)
Age (yrs ± SD)	65.2 ± 11.1
Male, n (%)	692 (82.4)
Body mass index (kg/m^2 ± SD)	24.1 ± 3.9
BNP (pg/mL ± SD)	177.2 ± 486.1
Log BNP ± SD	1.7 ± 3.3
Fasting plasma glucose (mg/dL ± SD)	111.8 ± 26.0
HbA1c (% ± SD)	6.1 ± 0.9
Fasting IRI (μU/mL ± SD)	7.3 ± 12.8
HOMA-IR ± SD	2.2 ± 3.3
Log HOMA-IR ± SD	0.23 ± 0.28
s-Cr (mg/dL ± SD)	1.58 ± 2.30
Hemoglobin (g/dL ± SD)	13.3 ± 2.0
Albumin (g/dL ± SD)	4.0 ± 0.4
LVEF (% ± SD) (At LVG)	57.0 ± 12.0
LVEDP (mmHg ± SD) (At preLVG)	16.5 ± 5.9
CI (L/min/m^2 ± SD) (n = 218)	2.81 ± 0.78
SBP (mmHg ± SD) (At LVG)	133.4 ± 25.0
DBP (mmHg ± SD) (At LVG)	70.4 ± 15.6
Heart rate (beat/min ± SD) (At LVG)	71.3 ± 12.8
Calcium-channel blockers, n (%)	465 (55.4)
ACE-inhibitors/Angiotensin receptor Blockers, n (%)	507 (60.4)
Nitrates/Nicorandil, n (%)	223 (26.5)
Beta-blockers, n (%)	334 (39.8)
Aldosterone blocker, n (%)	60 (7.1)
Statins, n (%)	474 (56.4)
Fibrates, n (%)	4 (0.5)
Diuretics, n (%)	136 (16.2)
Oral hypoglycemia Agents, n (%)	160 (19.0)

IHD, ischemic heart disease; AF, atrial fibrillation; HD, hemodialysis; CAPD, continuous ambulatory peritoneal; BNP, B-type natriuretic peptide; Log BNP, logarithmic B-type natriuretic peptide; HbA1c, hemoglobin A1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment ratio; Log HOMA-IR, logarithmic HOMA-IR; s-Cr, serum creatinine; LVEF, left ventricular ejection fraction; LVEDP, left ventricular end-diastolic pressure; CI, cardiac index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

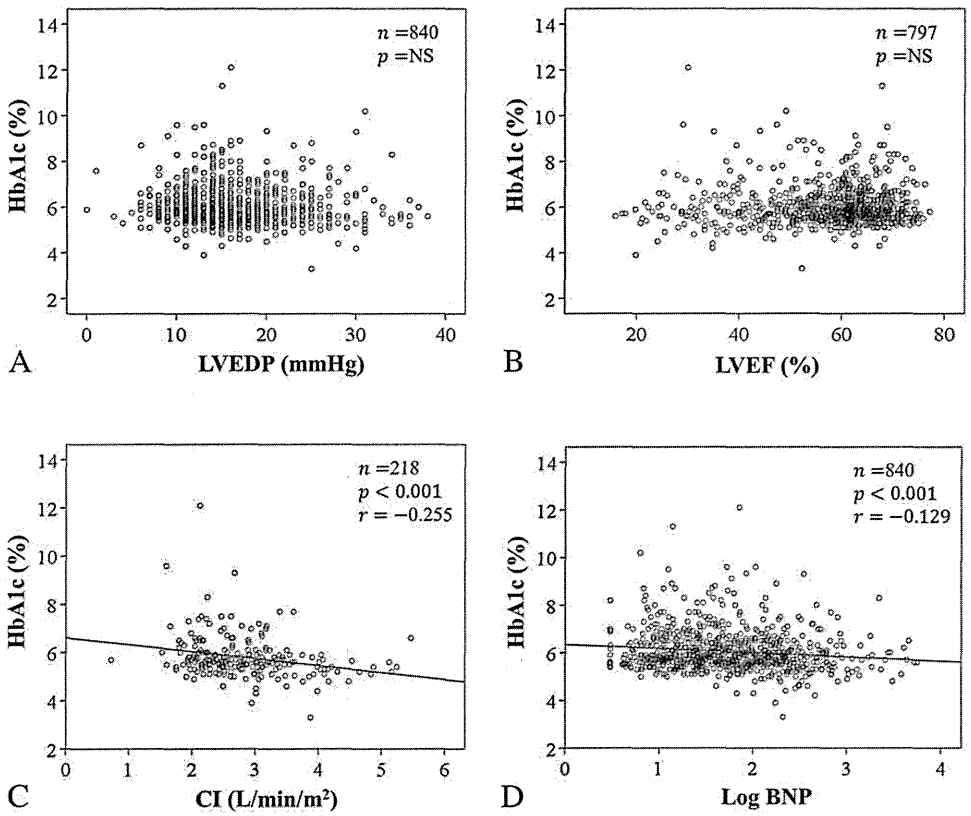

Fig. 1 – HbA1c levels in relation to the severity of heart failure. HbA1c, hemoglobin A1c; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; CI, cardiac index; Log BNP, logarithmic B-type natriuretic peptide. A. Relationship between the HbA1c and LVEDP levels. The HbA1c levels and LVEDP values are represented as scatter plots in the figure. B. Relationship between the HbA1c and LVEF levels. The HbA1c levels and LVEF values are represented as scatter plots in the figure. C. Relationship between the HbA1c and CI levels. The HbA1c levels and CI values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. D. Relationship between the HbA1c and Log BNP levels. The HbA1c levels and Log BNP values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation.

3.2. *HbA1c Levels in Relation to the Severity of HF*

A single regression analysis showed that the HbA1c levels were not correlated with LVEDP and LVEF (P = NS, respectively, n = 840), whereas there was an inverse correlation between the HbA1c levels with the cardiac index (CI) (P < 0.01, n = 218). This suggests that the trend for high HbA1c levels in patients with HF is likely limited for unknown reasons. Interestingly, another single regression analysis showed that the HbA1c levels continued improving in proportion to increasing levels of plasma BNP, which is a sensitive biochemical marker of HF (Fig. 1).

In addition, a multiple regression analysis for determining the HbA1c level was conducted using explanatory factors, including age, gender (male), BMI, dyslipidemia, smoking, serum creatinine (s-Cr), LVEDP, HTN and plasma BNP levels. As shown in Table 2, the plasma BNP levels disappeared as a significant factor (P = NS), but the LVEDP values were not significantly associated with the HbA1c levels (P = NS). Moreover, we performed statistical analysis using LVEF instead of LVEDP; however, the results were similar (P = NS) to those obtained in the analysis using LVEDP (precise data not shown). The multivariate analysis suggests that an increase in HbA1c would be blunted in HF for some reasons.

3.3. *Associations Between the BMI, HbA1c, Log HOMA-IR, Fasting IRI, LVEF and Log BNP Levels*

We performed single regression analyses of two factors among the BMI, HbA1c, Log HOMA-IR, fasting IRI, LVEF and Log BNP levels to provide a helpful perspective. As shown in Fig. 2 (A–D) and Fig. 3 (A–I), there were significantly negative correlations between the Log BNP levels and Log HOMA-IR levels, Log BNP levels and BMI values, Log BNP levels and Fasting IRI levels, and Log BNP levels and LVEF values (P < 0.001, respectively), while significant positive correlations were observed between Log HOMA-IR levels and HbA1c levels, Log HOMA-IR levels and BMI values, Log HOMA-IR levels and fasting IRI levels, BMI values and Fasting IRI levels, BMI values and HbA1c levels (P < 0.001, respectively), and Fasting IRI levels and HbA1c levels (P < 0.01). The LVEF values were not correlated with the Log HOMA-IR, BMI and fasting IRI (P = NS, respectively, n = 797). Among them, significant associations between the Log BNP and Log HOMA-IR and between the Log

Table 2 – Results of the multiple regression analysis of the HbA1c levels in all patients (n = 840).

Explanatory variable	Regression coefficient	Standard regression coefficient	P	95% CI	VIF
BMI	0.040	0.183	<0.001	0.024–0.057	1.286
Age	0.012	0.161	<0.001	0.007–0.018	1.257
Dyslipidemia	0.201	0.104	<0.01	0.070–0.332	1.085
Smoker	0.180	0.099	<0.01	0.051–0.309	1.201
Log BNP	−0.097	−0.072	NS (p = 0.104)	−0.215 to 0.020	1.801
s-Cr	−0.020	−0.055	NS (p = 0.148)	−0.048 to 0.007	1.309
LVEDP	−0.001	−0.006	NS (P = 0.878)	−0.012 to 0.010	1.337
Hypertension	0.014	0.007	NS (p = 0.839)	−0.121 to 0.149	1.111
Gender (male)	0.010	0.004	NS (p = 0.905)	−0.155 to 0.176	1.281

BMI, body mass index; Log BNP, logarithmic B-type natriuretic peptide; s-Cr, serum creatinine; LVEDP, left ventricular end-diastolic pressure.

BNP and BMI (Fig. 2A and B) would have profound meaning in this study.

3.4. *Multivariate Analysis for Determining the HbA1c Levels*

As shown above (Table 2), the trend towards higher HbA1c levels in the HF patients would be limited. To identify hidden mechanisms underlying this finding, we performed a multiple regression analysis of factors determining the plasma BNP levels using the variables age, gender (male), BMI, s-Cr, LVEDP, Log HOMA-IR and HbA1c. As shown in Table 3A, s-Cr, LVEDP and age positively correlated with the Log BNP levels, while BMI, Log HOMA-IR and gender (male) inversely correlated with the Log BNP levels (P < 0.001, respectively). In contrast, HbA1c levels were not correlated with the Log BNP levels (P = NS).

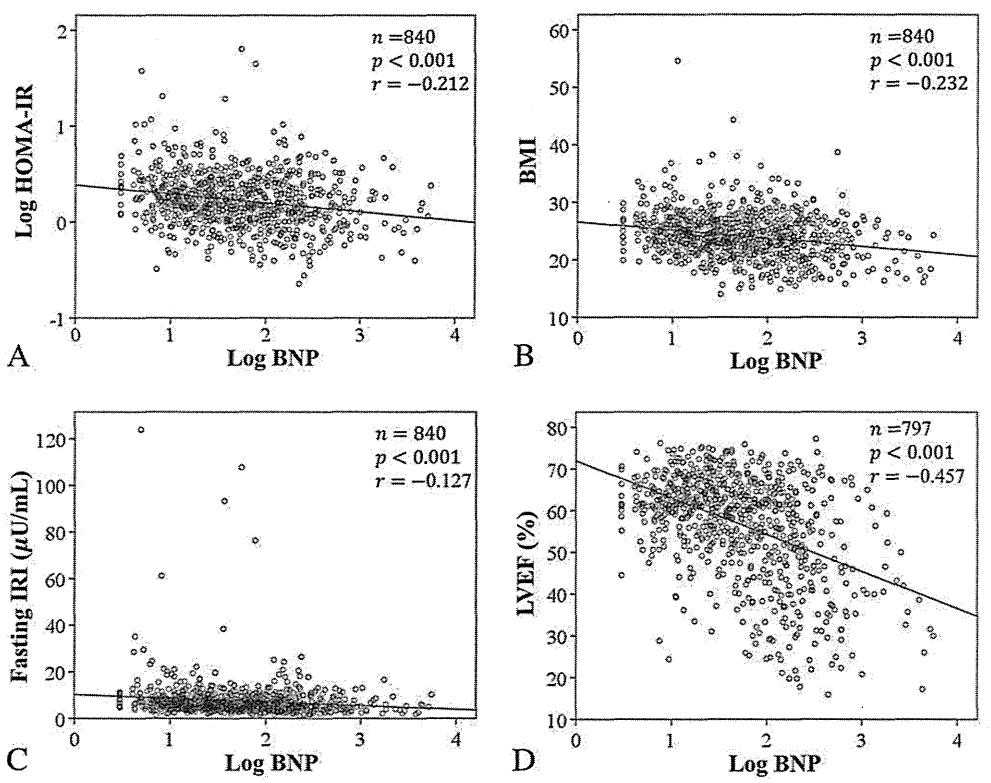

Fig. 2 – Associations between the LogHOMA-IR, BMI, Fasting IRI, LVEF and LogBNP levels. Log HOMA-IR, logarithmic homeostasis model assessment ratio; BMI, body mass index; IRI, immunoreactive insulin; LVEF, left ventricular ejection fraction; Log BNP, logarithmic B-type natriuretic peptide. A. Relationship between the Log HOMA-IR and Log BNP levels. The Log HOMA-IR levels and Log BNP values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. B. Relationship between the BMI and Log BNP levels. The BMI levels and Log BNP values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. C. Relationship between the fasting IRI and Log BNP levels. The fasting IRI levels and Log BNP values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation.D. Relationship between the LVEF and Log BNP levels. The LVEF levels and Log BNP values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation.

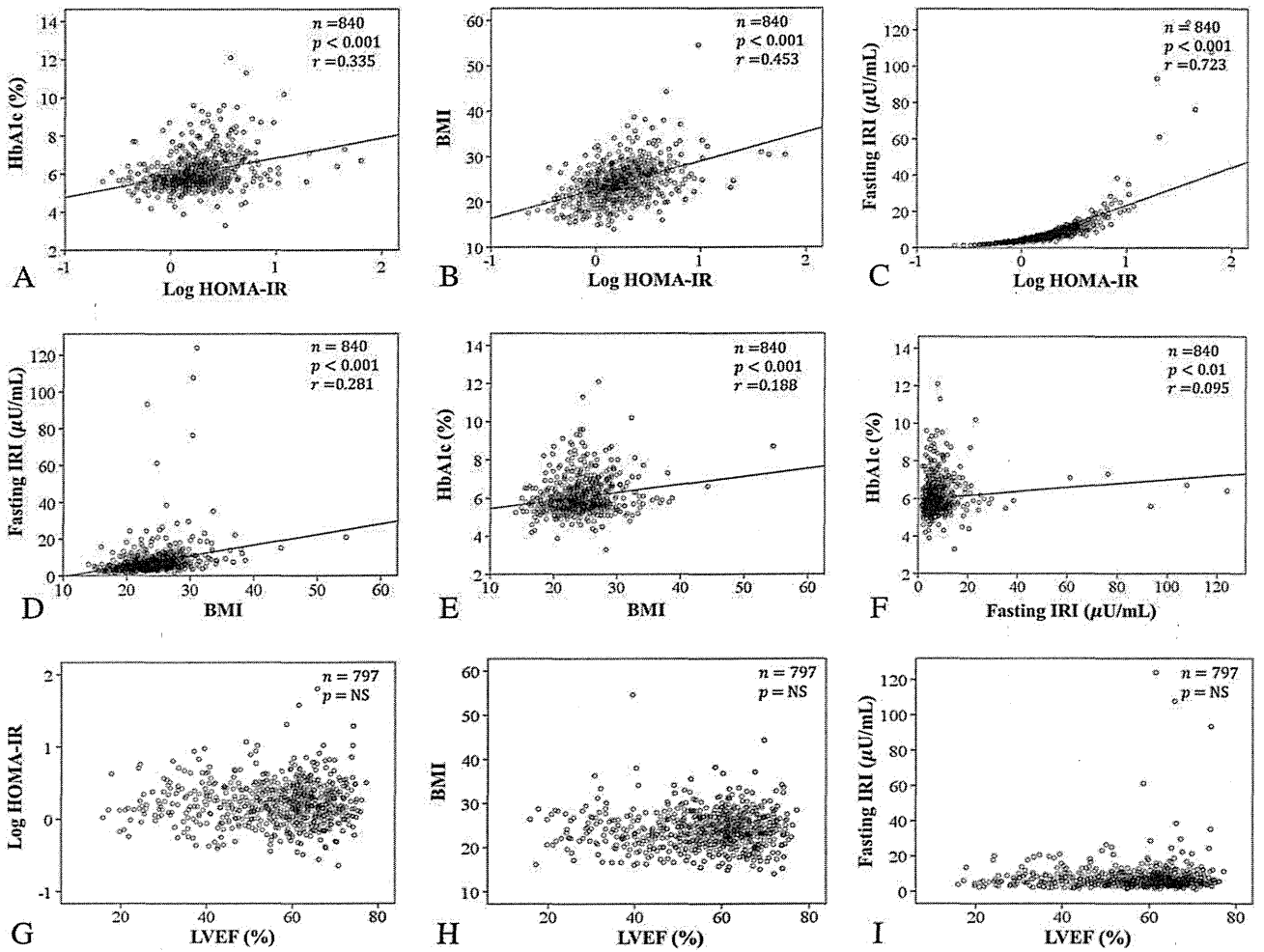

Fig. 3 – Associations between the Log HOMA-IR, BMI, Fasting IRI, HbA1c, and LVEF levels. Log HOMA-IR, logarithmic homeostasis model assessment ratio; BMI, body mass index; IRI, immunoreactive insulin; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction. A. Relationship between the HbA1c and Log HOMA-IR levels. The HbA1c levels and Log HOMA-IR values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. B. Relationship between the BMI and Log HOMA-IR levels. The BMI levels and Log HOMA-IR values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. C. Relationship between the fasting IRI and Log HOMA-IR levels. The fasting IRI levels and HOMA-IR values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. D. Relationship between the fasting IRI and BMI levels. The fasting IRI levels and BMI values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. E. Relationship between the HbA1c and BMI levels. The HbA1c levels and BMI values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. F. Relationship between the HbA1c and fasting IRI levels. The HbA1c levels and fasting IRI values are represented as scatter plots in the figure. The solid black line indicates the regression curve for the logarithmic fitted equation. G. Relationship between the Log HOMA-IR and LVEF levels. The Log HOMA-IR levels and LVEF values are represented as scatter plots in the figure. H. Relationship between the BMI and LVEF levels. The BMI levels and LVEF values are represented as scatter plots in the figure. I. Relationship between the fasting IRI and LVEF levels. The fasting IRI levels and LVEF values are represented as scatter plots in the figure.

We also performed multivariate analysis by using fasting IRI instead of Log HOMA-IR. The multiple regression analysis for the Log BNP levels showed that fasting IRI was a significant factor ($P < 0.01$) as shown in Table 3B.

3.5. *Additional Analysis in Other Subgroups*

There were 160 patients who used anti-diabetic drugs. We excluded these patients and analyzed the remaining 680 patients by the same statistical analysis. As a result, there was no significant correlation between the Log BNP levels and the HbA1c levels by simple regression analysis (P = NS); the Log BNP levels showed a significant correlation with the Log HOMA-IR levels ($P < 0.001$), but not with the HbA1c levels (P = NS), by the multivariate analysis. This analysis clearly showed a close correlation between the plasma BNP levels with insulin effectiveness but not with the diabetic condition in HF.

In patients with a fasting glucose level greater than 140 mg/dl, glucose toxicity would decrease beta-cell function, possibly leading to a different analysis from that with patients

Table 3 – Results of the multiple regression analysis of the Log BNP levels in all patients (n = 840).

Explanatory variable	Regression coefficient	Standard regression coefficient	P	95%CI	VIF
A					
s-Cr	0.097	0.351	<0.001	0.083–0.112	1.073
LVEDP	0.040	0.369	<0.001	0.034–0.046	1.089
Age	0.014	0.238	<0.001	0.011–0.017	1.126
BMI	−0.021	−0.131	<0.001	−0.031 to −0.011	1.464
Log HOMA-IR	−0.264	−0.115	<0.001	−0.400 to −0.128	1.382
Gender (male)	−0.160	−0.096	<0.001	−0.248 to −0.071	1.085
HbA1c	−0.018	−0.025	NS (P = 0.378)	−0.059 to 0.022	1.167
B					
s-Cr	0.096	0.346	<0.001	0.081–0.110	1.069
LVEDP	0.041	0.377	<0.001	0.035–0.047	1.083
Age	0.014	0.240	<0.001	0.011–0.017	1.128
BMI	−0.025	−0.154	<0.001	−0.035 to −0.016	1.312
Fasting IRI	−0.007	−0.088	<0.01	−0.011 to −0.003	1.096
Gender	−0.162	−0.097	<0.001	−0.250 to −0.073	1.087
HbA1c	−0.038	−0.051	NS (p = 0.058)	−0.077 to 0.001	1.076

Log BNP, logarithmic B-type natriuretic peptide; s-Cr, serum creatinine; LVEDP, left ventricular end-diastolic pressure; BMI, body mass index; Log HOMA-IR, logarithmic HOMA-IR; IRI, immunoreactive insulin; HbA1c, hemoglobin A1c.

whose glucose levels were less than 140 mg/dl [18]. We thus performed a multivariate analysis only in the patients whose glucose level less than 140 mg/dl (n = 740). The result remained essentially the same; Log HOMA-IR inversely correlated with the Log BNP levels (P < 0.001), while the HbA1c levels were not significant (P = NS).

The insulin secretory capacity deteriorates in relation to the degree of DM. We divided the study population into two groups, DM (258 patients) and non-DM (582 patients). As a result, the Log BNP levels were inversely correlated with the Log HOMA-IR levels by simple linear regression analysis (r = −0.220, P < 0.001) and also by multivariate analysis (P < 0.001) in the non-DM group. The Log BNP levels were inversely correlated with the Log HOMA-IR levels by simple linear regression analysis (r = −0.235, P < 0.001) and also by multivariate analysis (P < 0.05) in the DM group.

Patients with AF, HD and CAPD were included who had factors that could influence NP. The plasma BNP levels were significantly higher in patients with AF (317.7 ± 638.7 pg/mL), HD and CAPD (938.9 ± 1319.1 pg/mL in all). We also performed the same statistical analysis by using the remaining 718 patients without AF, HD and CAPD. As a result, there was an inverse correlation between the Log BNP levels with Log HOMA-IR by simple linear regression analysis (r = −0.223, P < 0.001) and multivariable analysis (P < 0.001).

There is a possibility that poor appetite associated with severe HF reduces body weight and may also superficially improve the diabetic condition. The condition of nutrition might have been

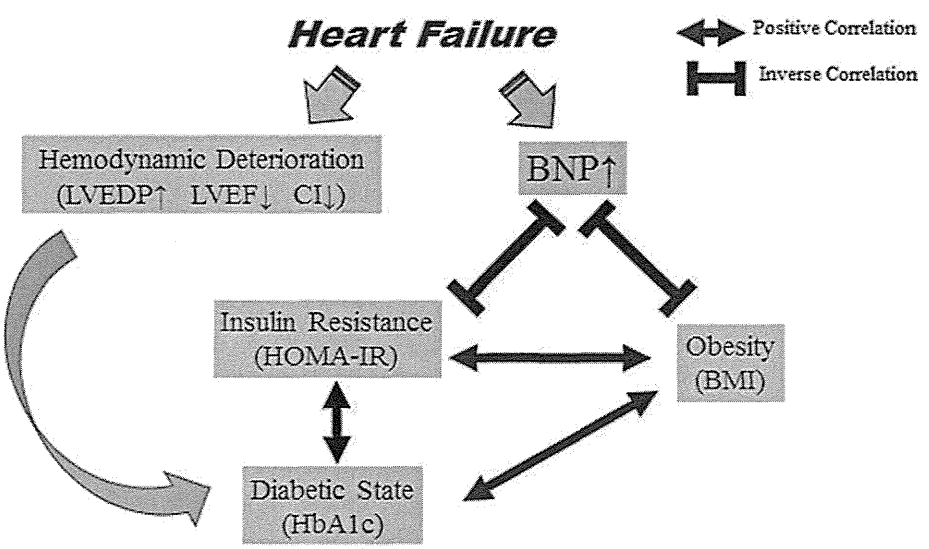

Fig. 4 – Schematic illustration.There are substantial associations between the HOMA-IR, BMI and HbA1c levels. BNP would suppress the BMI and HOMA-IR values.HOMA-IR, homeostasis model assessment ratio; BMI, body mass index; HbA1c, hemoglobin A1c; BNP, B-type natriuretic peptide, CI, cardiac index; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction.

slightly reduced, even in the present study population. However, the serum albumin levels were 4.0 ± 0.4 g/dL (N = 840) and the hemoglobin levels were 13.3 ± 2.0 g/dL (N = 839); the absolute levels of both were almost within normal limits.

4. Discussion

It is well recognized that metabolic abnormalities, such as diabetes, produce cardiovascular disease and HF. In addition, the underlying mechanisms of diabetes and HF partly overlap and may be confounded, as many neurohumoral factors are activated in a similar manner in both diseases. Therefore, a close correlation between HbA1c levels and the degree of HF should be conceivable, although there have curiously been few reports showing a close correlation between these parameters. The present study also showed no sensible correlation between HbA1c levels and the severity of HF in a multiple regression analysis. We took an interest in this phenomenon and hypothesized that there is a hidden, but firm, effect of neurohumoral factors on the onset of metabolic abnormalities in patients with HF. Notably, the multivariate analysis clearly showed that the Log BNP levels inversely correlated with the HOMA-IR values, suggesting that BNP partially halts the progression of diabetes by improving insulin resistance, even under the metabolically harmful conditions of HF.

Considering possible mechanisms, as for the substantial inverse relationship between the plasma BNP level and insulin resistance, it is necessary to discuss high BMI (obesity) and its relationship to plasma BNP levels, as obesity is crucially involved in the development of insulin resistance [19]. In the current study, the statistical analyses revealed an inverse correlation between the plasma BNP levels and BMI. This association matches the findings of pioneering work showing that obese individuals in the cohort of the Framingham Heart Study had considerably lower plasma NP levels than those with a normal weight [20]. We also previously reported that the secretion of NP from the heart is reduced and that the circulating NP levels are decreased in obese patients compared with non-obese patients [21,22]. It is important to discuss the pathophysiological function of adipocytes and their relationship to NP. First, it is probable that NP reduces BMI by improving the form and function of adipocytes. Second, it is likely that unknown factors secreted from adipocytes attenuate the production of NP in the heart and/or augments NP clearance at some sites in the body. Although it is unclear at present which mechanism is correct or predominant, it is conceivable that these pathways are mutually related as follows.

It can be supposed that a high BNP level plays a causative role in lowering BMI and improving insulin resistance. In this regard, there is a definitive report using BNP transgenic mice that showed that NP/cGK cascades promote muscle mitochondrial biogenesis and fat oxidation via the upregulation of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) and peroxisome proliferator-activated receptor (PPAR) γ, thus preventing obesity and glucose intolerance [14]. In addition, it has been reported that NP stimulates triglyceride lipolysis in adipocytes, a process also regulated by the sympathetic nervous system [23]. These two pathways promote the uncoupling of mitochondrial respiration and thermogenesis in brown adipocytes [24,25]. It is thus reasonable that an increased BNP level accelerates lipolysis in adipose tissue, resulting in a lower BMI and improvements in insulin resistance. On the other hand, it is conceivable that high BMI plays a causative role in lowering the plasma BNP level. It has been reported in patients with obesity that the heart utilizes fatty acids exclusively as a source of energy, with the concomitant suppression of glucose utilization via the activation of PPARα [26,27]. PPARα has also been reported to suppress BNP production [28]. Hence, it is thus conceivable that increased fatty acid availability, as reflected by elevated plasma free fatty acids, suppresses BNP production, thereby resulting in a lower plasma BNP level. In addition, it has been demonstrated that hypoxia-inducible factor (HIF-1α) plays a crucial role in BNP production [29,30]. Insulin induces the HIF-1α level [31,32] and it is therefore conceivable that BNP production is reduced under conditions of insulin resistance via HIF-1α suppression in obese patients [21].

Adiponectin may play an important role in the link between a low BNP level and insulin resistance. The production of adiponectin is reduced in relation to the severity of obesity [33] and increased by successful therapy for obesity [34]. In addition, the adiponectin level is known to inversely correlate with insulin resistance [35]. Remarkably, NP itself potentially enhances the production of adiponectin in human adipocytes [36]. Therefore, a low NP level is expected to play a causative role in the onset of insulin resistance via low adiponectin production. Moreover, NP itself potentially reduces inflammation [13]. The relatively high activity of inflammation occurring under a low NP level may further reduce the production of adiponectin [37].

Recently, we reported that a low plasma BNP level was associated with patients with stable IHD, which suggests that low BNP plays a causative role in the progression of IHD [38]. Considering that IHD is associated with insulin resistance [39], there may be close links between IHD, insulin resistance and a low BNP level; this topic is anticipated to become a hotly debated subject in the immediate future.

As mentioned above, an increased plasma BNP level is mutually related to a decrease in BMI and improvement in insulin resistance. From the viewpoint of energy metabolism, methods to increase the endogenous NP level may constitute a therapeutic strategy for treating insulin resistance. For example, drugs inhibiting neutral endopeptidase (NEP) activity, the major NP degrading enzyme, may be useful for improving insulin resistance; the exogenous administration of NP is also helpful for this purpose. Upregulating the NP level represents a therapeutic strategy for improving hemodynamics in cases of HF as well as ameliorating metabolic abnormalities, such as obesity and insulin resistance. In addition, there was an inverse correlation between BNP levels and fasting IRI levels. Taking into consideration a cardio-toxic effect of hyperinsulinemia [40], this result would suggest that BNP partly rescues cardiotoxicity by suppressing hyperinsulinemia.

In recent history, the potential of NP to reduce insulin resistance has been reported at several institutions [41,42]. The present study provides a new way of thinking about a formerly inexplicable relationship between a diabetic state estimated according to the HbA1c level and the degree of HF.

Finally, a schematic illustration of our findings is shown in Fig. 4. It is well known that there is a substantial association between the HOMA-IR, BMI and HbA1c levels. We herein demonstrated that BNP suppresses the BMI and HOMA-IR values, although it is difficult to distinguish between the cause and effect in each case.

5. Study Limitation

We speculate that the HbA1c levels would become higher in relation to the severity of HF; this can likely be shown by using a better marker of HF. Unfortunately, we could not find a suitable marker that was firmly associated with HbA1c levels in the current study. There are generally many markers of cardiac dysfunction, such as hemodynamic parameters during cardiac catheterization, echocardiographic measurement values, cardiac scintigraphic measurement values and others. Among the markers, there would surely be a good marker associated with HbA1c levels in HF. CI seems to be highly associated with HbA1c levels compared with LVEDP or LVEF in this study; however, the study patient number was limited and the association remains unclear. In any case, it is interesting to see that the plasma BNP levels, which should be a sensitive biological marker of HF, were never a suitable marker associated with the diabetic condition but rather tended to be inversely associated with the diabetic condition through improved insulin resistance. Furthermore, BMI reflects not only fat accumulation but also body fluid excess in HF. BMI might not be a suitable marker for an analysis of the diabetic condition. Even so, the previous reports using BMI as a potential index of obesity clearly showed a negative correlation between plasma BNP levels and obesity [43,44].

This study was a cross-sectional study, and a further study would be warranted to determine the counter-regulatory effect of natriuretic peptides on insulin resistance in patients with HF.

In conclusion, HF and diabetes tend to worsen with each other; however, the appearance of the association between them was likely blunted because of a considerable effect of NP in counteracting insulin resistance, even during the metabolically harmful condition of HF.

Author Contributions

Conceived and designed the experiments: YI, MK, MY.
Performed the experiments: YI, KM, KO, TN, TO.
Analyzed the data: YI, MK, KM, MY.
Contributed reagents/materials/analysis tools: YI, MK, KM, MY.
Wrote the paper: YI, MK, MY.

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Conflict of Interest

None of the authors have any conflicts of interest to disclose.

REFERENCES

[1] Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. American College of Cardiology; American Heart Association Task Force on Practice Guidelines; American College of Chest Physicians; International Society for Heart and Lung Transplantation. Heart Rhythm Soc Circ 2005;112(12):e154–235.
[2] Iribarren C, Karter AJ, Go AS, Ferrara A, Liu JY, Sidney S, et al. Glycemic control and heart failure among adult patients with diabetes. Circulation 2001;103(22):2668–73.
[3] Kishimoto I, Makino H, Ohata Y, Tamanaha T, Tochiya M, Kada A, et al. Hemoglobin A1c predicts heart failure hospitalization independent of baseline cardiac function or B-type natriuretic peptide level. Diabetes Res Clin Pract 2014;104(2):257–65.
[4] Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol 1974;34(1):29–34.
[5] Bertoni AG, Hundley WG, Massing MW, Bonds DE, Burke GL, Goff Jr DC. Heart failure prevalence, incidence, and mortality in the elderly with diabetes. Diabetes Care 2004;27(3):699–703.
[6] Nichols GA, Gullion CM, Koro CE, Ephross SA, Brown JB. The incidence of congestive heart failure in type 2 diabetes: an update. Diabetes Care 2004;27(8):1879–84.
[7] Bell DS. Heart failure: the frequent, forgotten, and often fatal complication of diabetes. Diabetes Care 2003;26(8):2433–41.
[8] Goldsmith SR. Interactions between the sympathetic nervous system and the RAAS in heart failure. Curr Heart Fail Rep 2004;1(2):45–50.
[9] Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, et al. Stretch-induced programmed myocyte cell death. J Clin Invest 1995;96(5):2247–59.
[10] Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, et al. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor- α and angiotensin II. Circulation 1998;98(8):794–9.
[11] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114(12):1752–61.
[12] Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, et al. Brain natriuretic peptide as a novelcardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J Clin Invest 1991;87(4):1402–12.
[13] Shono M, Yoshimura M, Nakayama M, Yamamuro M, Abe K, Suzuki S, et al. Predominant effect of A-type natriuretic peptide on reduction of oxidative stress during the treatment of patients with heart failure. Circ J 2007;71(7):1040–6.
[14] Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, et al. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes 2009;58(12):2880–92.
[15] Morita E, Yasue H, Yoshimura M, Ogawa H, Jougasaki M, Matsumura T, et al. Increased plasma levels of brain natriuretic peptide in patients with acute myocardial infarction. Circulation 1993;88(1):82–91.
[16] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9.

[17] Komukai K, Ogawa T, Yagi H, Date T, Suzuki K, Sakamoto H, et al. Renal insufficiency is related to painless myocardial infarction. Circ J 2007;71(9):1366–9.
[18] DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism 1989;38(4):387–95.
[19] Beavers KM, Case LD, Blackwell CS, Katula JA, Goff Jr DC, Vitolins MZ. Effects of weight regain following intentional weight loss on glucoregulatory function in overweight and obese adults with pre-diabetes. Obes Res Clin Pract 2014. http://dx.doi.org/10.1016/j.orcp.2014.09.003 [Epub ahead of print].
[20] Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Wilson PW, et al. Impact of obesity on plasma natriuretic peptide levels. Circulation 2004;109(5):594–600.
[21] Mizuno Y, Harada E, Katoh D, Kashiwagi Y, Morikawa Y, Nakagawa H, et al. Cardiac production of B-type natriuretic peptide is inversely related to the plasma level of free fatty acids in obese individuals - possible involvement of the insulin resistance -. Endocr J 2013;60(1):87–95.
[22] Date T, Yamane T, Yamashita S, Matsuo S, Matsushima M, Inada K, et al. Paradoxical clearance of natriuretic peptide between pulmonary and systemic circulation: a pulmonary mechanism of maintaining natriuretic peptide plasma concentration in obese individuals. J Clin Endocrinol Metab 2012; 97(1):E14–21.
[23] Arner P. Human fat cell lipolysis: biochemistry, regulation and clinical role. Best Pract Res Clin Endocrinol Metab 2005; 19(4):471–82.
[24] Bordicchia M, Liu D, Amri EZ, Ailhaud G, Dessì-Fulgheri P, Zhang C, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin Invest 2012;122(3):1022–36.
[25] Collins S. A heart-adipose tissue connection in the regulation of energy metabolism. Nat Rev Endocrinol 2014;10(3):157–63.
[26] Banke NH, Wende AR, Leone TC, O'Donnell JM, Abel ED, Kelly DP, et al. Preferential oxidation of triacylglyceride-derived fatty acids in heart is augmented by the nuclear receptor PPARα. Circ Res 2010;107(2):233–41.
[27] Duncan JG, Bharadwaj KG, Fong JL, Mitra R, Sambandam N, Courtois MR, et al. Rescue of cardiomyopathy in peroxisome proliferator-activated receptor-α transgenic mice by deletion of lipoprotein lipase identifies sources of cardiac lipids and peroxisome proliferator-activated receptor-α activators. Circulation 2010;121(3):426–35.
[28] Liang F, Wang F, Zhang S, Gardner DG. Peroxisome proliferator activated receptor (PPAR)alpha agonists inhibit hypertrophy of neonatal rat cardiac myocytes. Endocrinology 2003;144(9):4187–94.
[29] Luo Y, Jiang C, Belanger AJ, Akita GY, Wadsworth SC, Gregory RJ, et al. A constitutively active hypoxia-inducible factor-1alpha/VP16 hybrid factor activates expression of the human B-type natriuretic peptide gene. Mol Pharmacol 2006;69(6):1953–62.
[30] Weidemann A, Klanke B, Wagner M, Volk T, Willam C, Wiesener MS, et al. Hypoxia, via stabilization of the hypoxia-inducible factor HIF-1alpha, is a direct and sufficient stimulus for brain-type natriuretic peptide induction. Biochem J 2008;409(1):233–42.
[31] Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. J Biol Chem 2002; 277(31):27975–81.
[32] Demidenko ZN, Blagosklonny MV. The purpose of the HIF-1/PHD feedback loop: to limit mTOR-induced HIF-1α. Cell Cycle 2011;10(10):1557–62.
[33] Meyer LK, Ciaraldi TP, Henry RR, Wittgrove AC, Phillips SA. Adipose tissue depot and cell size dependency of adiponectin synthesis and secretion in human obesity. Adipocytes 2013; 2(4):217–26.
[34] Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001;86(8):3815–9.
[35] Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 2001;50(5):1126–33.
[36] Tsukamoto O, Fujita M, Kato M, Yamazaki S, Asano Y, Ogai A, et al. Natriuretic peptides enhance the production of adiponectin in human adipocytes and in patients with chronic heart failure. J Am Coll Cardiol 2009;53(22):2070–7.
[37] Bozcali E, Polat V, Akbulut H, Ferzeyn Yavuzkir M, Karaca I. Serum adiponectin, anemia and left ventricular dimensions in patients with cardiac cachexia. Cardiology 2013;126(4):207–13.
[38] Minai K, Ogawa T, Kawai M, Komukai K, Tanaka T, Ogawa K, et al. The plasma B-type natriuretic peptide levels are low in males with stable ischemic heart disease (IHD) compared to those observed in patients with non-IHD: a retrospective study. PLoS One 2014;9, e108983.
[39] Paternostro G, Camici PG, Lammerstma AA, Marinho N, Baliga RR, Kooner JS, et al. Cardiac and skeletal muscle insulin resistance in patients with coronary heart disease. A study with positron emission tomography. J Clin Invest 1996; 98(9):2094–9.
[40] Shimizu I, Minamino T, Toko H, Okada S, Ikeda H, Yasuda N, et al. Excessive cardiac insulin signaling exacerbates systolic dysfunction induced by pressure overload in rodents. J Clin Invest 2010;120(5):1506–14.
[41] Khan AM, Cheng S, Magnusson M, Larson MG, Newton-Cheh C, McCabe EL, et al. Cardiac natriuretic peptides, obesity, and insulin resistance: evidence from two community-based studies. J Clin Endocrinol Metab 2011;96(10):3242–9.
[42] Chang HR, Hsieh JC, Hsu BG, Wang LY, Yu-Chih Chen M, Wang JH. N-terminal pro-B-type natriuretic peptide is inversely associated with metabolic syndrome in hypertensive patients. Am J Med Sci 2014;348(3):210–4.
[43] Das SR, Drazner MH, Dries DL, Vega GL, Stanek HG, Abdullah SM, et al. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the Dallas Heart Study. Circulation 2005;112(14):2163–8.
[44] Arase S, Kawai M, Nakane T, Ito K, Ogawa K, Minai K, et al. The increasing impact of a higher body mass index on the decrease in plasma B-type natriuretic peptide levels. IJC Metab Endocr 2014;4:39–46.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Clinical and Pathological Impact of Tissue Fibrosis on Lethal Arrhythmic Events in Hypertrophic Cardiomyopathy Patients With Impaired Systolic Function

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Background: The natural history of hypertrophic cardiomyopathy (HCM) varies from an asymptomatic benign course to a poor prognosis. Myocardial fibrosis may play a critical role in ventricular tachyarrhythmias (VT/VF); however, the clinical significance of tissue fibrosis by right ventricular (RV) biopsy in the long-term prognosis of HCM patients remains unclear.

Methods and Results: We enrolled 185 HCM patients (mean age, 57±14 years). The amount of fibrosis (%area) was quantified using a digital microscope. Hemodynamic, echocardiographic, and electrophysiologic parameters were also evaluated. Patients with severe fibrosis had longer QRS duration and positive late potential (LP) on signal-averaged ECG, resulting in a higher incidence of VT/VF. At the 5±4 year follow-up, VT/VF occurred in 31 (17%) patients. Multivariate Cox regression analysis revealed that tissue fibrosis (hazard ratio (HR): 1.65; P=0.003 per 10% increase), lower left ventricular ejection fraction (HR: 0.64; P=0.001 per 10% increase), and positive SAECG (HR: 3.14; P=0.04) led to a greater risk of VT/VF. The combination of tissue fibrosis severity and lower left ventricular ejection fraction could be used to stratify the risk of lethal arrhythmic events in HCM patients.

Conclusions: Myocardial fibrosis in RV biopsy samples may contribute to abnormal conduction delay and spontaneous VT/VF, leading to a poor prognosis in HCM patients. (*Circ J* 2015; **79:** 1733–1741)

Key Words: Arrhythmias; Fibrosis; Histopathology; Hypertrophic cardiomyopathy; Prognosis

Hypertrophic cardiomyopathy (HCM) is usually recognized by left ventricular (LV) hypertrophy on echocardiography or a family history of HCM.[1] Histopathological changes, including myocardial hypertrophy, tissue fibrosis, or myocardial disarray,[2,3] may cause a distorted impulse propagation and inhomogeneous refractoriness, a substrate of electrical instability during tachycardia, which can lead to ventricular tachycardia (VT) or ventricular fibrillation (VF) and sudden cardiac death (SCD).

The natural history of HCM patients varies from an asymptomatic benign course to a poor prognosis because of heart failure (HF), lethal ventricular arrhythmias, or SCD.[4] Therefore, risk stratification in HCM patients has been a major issue. A positive late potential (LP) detected by signal-averaged electrocardiography (SAECG) has been used as a marker of electrical instability,[5] although myocardial scarring visualized by cardiac magnetic resonance (CMR) imaging can better predict long-term clinical outcome compared with other risk factors such as syncope and family history of SCD.[6–8] Myocardial fibrosis, as measured by late gadolinium enhancement (LGE) on CMR, was recently found to be an independent predictor of adverse outcome in HCM patients.[9,10] However, there are only a few case reports of the relationship between CMR-LGE and direct fibrotic changes.[11,12] It remains unclear

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whether or not histopathological changes are associated with the risk of VT/VF and SCD in HCM patients.

In this study, we hypothesized that advanced myocardial fibrosis in HCM plays a critical role in lethal arrhythmic events, including VT/VF, implantable cardioverter-defibrillator (ICD) appropriate discharge, and SCD. We therefore quantified the fibrotic change in tissue samples from right ventricular (RV) biopsy and assessed its relevance to the long-term prognosis of HCM patients. This study examined the novel quantitative significance of tissue fibrosis in HCM patients associated with electrophysiological conduction abnormalities that lead to VT/VF and poor prognosis.

Methods

Diagnosis of Patients

We retrospectively surveyed 494 consecutive patients who had undergone RV endomyocardial biopsy at the National Cerebral and Cardiovascular Center between 1996 and 2011. The diagnosis of HCM was made on the basis of typical clinical, echocardiographic, and hemodynamic features according to established criteria,[1] used for a number of years, in the presence of LV wall thickness ≥15 mm without dilated ventricular chambers or any other cardiac or systemic disorders, including aortic stenosis or marked hypertension at the time of clinical diagnosis. In this study, the borderline LV hypertrophy criterion (LV wall thickness 13–14 mm) was not applicable because genetic examinations were not performed in this cohort. Asymmetric hypertrophy was originally applied to patients with conventional septal hypertrophy; however, the pattern or distribution of LV hypertrophy was not taken into account as per the latest recommendation.[1] Thus, asymmetric hypertrophy is determined if the LV thickness ratio of maximum to minimum in the same cross-section exceeds 1.3.

RV Biopsy and Histopathological Analysis

RV endomyocardial biopsy was performed in this cohort because of (1) differential diagnoses for other cardiomyopathies, such as amyloidosis, Fabry's disease, sarcoidosis, or hypertensive heart disease; (2) atypical progression of LV dysfunction; or (3) new-onset HF despite preserved left ventricular ejection fraction (LVEF). We excluded patients younger than 20 years old because myocardial features may change with age. We also excluded male and female patients older than 75 and 80 years old, respectively. Patients with coexisting valvular diseases responsible for cardiomyopathy were also excluded.[2]

A total of 238 patients were clinically diagnosed and pathologically confirmed to have HCM (including 114 HCM with overt LV dysfunction[10] defined as LVEF <50%); 53 patients were excluded because their tissue samples (Masson's staining) had deteriorated over time. Finally, 185 patients (mean age 57±14 years, 62% male) were evaluated. This study was approved by the institutional ethics committee (M24-071).

Biopsy samples were obtained from the endocardium at the right interventricular septum using disposable biopsy forceps (Toyokura Ika Kogyo Co, Ltd, Tokyo, Japan) by the transvenous approach via the femoral vein or the right jugular vein, as described elsewhere.[13] The detailed tissue sample preparation methods are described in **Supplementary File 1**. The extent of tissue fibrosis was automatically calculated by the area of fibrosis (%) in the total area of the Masson's trichrome sample using a digital microscope (Aperio Scanscope, Aperio Technology, Vista, CA, USA) (**Figure S1**), which has been utilized for calculating myocardial fibrosis elsewhere.[12] The degree of myocardial disarray was graded from 0 to 5, as described in **Table S1**). Separate from the quantitative risk assessment, tissue fibrosis was qualitatively classified into 3 degrees: mild (<10% area of fibrosis in specimens), moderate (10–20%), and severe (>20%), as previously reported[14] for further risk stratification, with and without other prognostic factors.

Electrophysiological Analysis

A standard 12-lead ECG was recorded in all patients. The SAECG was recorded from the X, Y, and Z orthogonal leads. LP was defined as present when at least 2 of the following 3 criteria were positive: filtered QRS duration (fQRS) >120 ms; root-mean-square voltage in the terminal 40 ms (RMS40) <18 μV; and duration of the low amplitude signal <40 μV (LAS40) >38 ms. The detailed electrophysiological protocol is shown in **Supplementary File 1**.

Echocardiography

After patients with significant valvular disease were excluded, the echocardiographic measurements were performed as follows: the end-diastolic and end-systolic dimensions were measured on the parasternal view at the level of papillary muscles and the left atrial size was measured on the parasternal long-axis view. Measurement of maximum wall thickness and definition of asymmetric hypertrophy were described above.

Hemodynamic Study

All patients underwent catheterization for hemodynamic evaluation. The LVEF was measured using left ventriculography, CMR imaging, or radio nuclear imaging. All patients were examined by right heart catheterization to assess hemodynamics. Coronary angiography was performed in all patients during their first hospitalization for diagnosis or within the year prior.

CMR-LGE Analysis

Of the 185 total patients, 60 underwent CMR using the gadolinium-enhanced imaging technique. The detailed CMR protocol and its LGE analysis were described previously[15] and are described in **Supplementary File 1**. In brief, CMR was performed on a 1.5-T MR scanner (Magnetom Sonata, Siemens, Erlangen, Germany) and LGE used a segmented inversion-recovery (IR) prepared true-FISP sequence with ECG triggering at 2, 5, 10, and 20 min after the administration of 0.15 mmol/kg of gadolinium-DTPA (Magnevist, Bayer Schering Pharma, Berlin, Germany). For quantification of LV mass, we semi-automatically traced the LV endocardial and epicardial contours at end-diastole in each short-axis slice of 7 sections using customized software (Ziostation2; Ziosoft Inc, Tokyo, Japan). A region of interest (ROI) was selected within the normal remote myocardium to generate the mean and standard deviation (SD) for the various SDs. The mass of LGE (%LGE) was automatically calculated with the same software as regions exhibiting a signal intensity above a predetermined threshold (4 SD above the mean signal intensity of apparently normal myocardium).[12]

Follow-up

Patient follow-up began on the day of biopsy. Patients were tracked through outpatient visits every 1–3 months or were followed at ICD check-ups every 6 months. The endpoint of the study was lethal arrhythmic events defined as sustained VT or VF, ICD appropriate discharge, or aborted SCD during the follow-up period. SCD was diagnosed if the patient underwent a sudden collapse within 1 h of onset of symptoms without any previous cardiac manifestation.

Table 1. Baseline Characteristics of Patients With HCM

	Total (n=185)	Tissue fibrosis			P value
		Mild (n=58)	Moderate (n=78)	Severe (n=49)	
Age	57±14	59±12	56±13	57±16	NS
Sex (% male)	114 (62)	34 (59)	53 (68)	27 (55)	NS
Family history of SCD, n (%)	16 (9)	4 (7)	6 (8)	6 (12)	NS
Hypertension, n (%)	72 (40)	24 (42)	31 (40)	17 (36)	NS
Diabetes mellitus, n (%)	31 (17)	11 (20)	12 (15)	8 (17)	NS
Atrial fibrillation, n (%)	70 (38)	16 (28)	21 (27)	18 (37)	NS
Syncope, n (%)	41 (22)	9 (16)	18 (23)	14 (29)	NS
Prior NSVT, n (%)	55 (30)	13 (22)	25 (32)	17 (36)	NS
Prior sustained VT/VF, n (%)	26 (14)	5 (9)	9 (12)	12 (24)	NS
Prior hospitalization, n (%)	76 (41)	15 (26)	35 (45)	26 (53)*	0.01 vs. mild*
Echo and hemodynamic parameters					
LVEF, %	47±19	45±19	48±19	47±20	NS
Max. wall thickness, mm	17±6	16±6	17±6	18±6	NS
Wall thickness >30 mm, n (%)	5 (3)	2 (3)	2 (3)	1 (2)	NS
Asymmetric hypertrophy, n (%)	82 (44)	28 (48)	31 (40)	23 (47)	NS
Max. PG >30 mmHg, n (%)	41 (22)	9 (16)	23 (29)	9 (18)	NS
BNP, pg/ml (IQR)	256 (137–506)	221 (116–470)	278 (126–502)	272 (175–613)	NS
PCWP, mmHg	12±7	10±6	12±6	13±7*	0.03 vs. mild*
Pathological parameters					
Myocyte diameter, μm	21±5	20±4	21±5	22±4	NS
Myocardial disarray, grade: 0–5	2.6±1.3	2.4±1.4	2.6±1.1	2.7±1.3	NS
ECG and electrophysiology					
QRS duration, ms	119±30	114±24	121±27	122±39	NS
QTc interval, ms	454±67	452±72	446±52	468±81	NS
LAS40, ms	32±22	28±16	30±17	41±29*	0.03 vs. mild*
fQRS, ms	120±30	114±28	122±30	123±34	NS
RMS voltage, μV	63±64	67±58	64±63	57±71	NS
LP(+) by SAECG, n/total N (%)	30/123 (24)	8/38 (21)	9/53 (17)	13/32 (41)	NS
CMR parameters (n=60)					
LV mass, g	165±54	158±58	179±54	148±45	NS
LGE % LV mass (4SD), %	31±18	32±19	28±18	35±15	NS
Medication, treatment					
ICD/CRT-D at diagnosis, n (%)	5 (3)	0 (0)	3 (4)	2 (4)	NS
β-blocker, n (%)	68 (37)	16 (28)	34 (44)	8 (16)	NS
ACEI/ARB, n (%)	79 (43)	26 (45)	31 (40)	22 (45)	NS
Amiodarone, n (%)	14 (8)	1 (2)	5 (6)	8 (16)*	0.01 vs. mild*
Other antiarrhythmics, n (%)	43 (23)	10 (17)	20 (26)	12 (24)	NS

*Statistically difference between mild and severe. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CMR, cardiac magnetic resonance imaging; CRT-D, cardiac resynchronization therapy with defibrillator; fQRS, total filtered QRS duration; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LAS40, duration of the low amplitude signal <40 μV; LP(+), positive late potential; LVEF, left ventricular ejection fraction; NS, not significant; NSVT, nonsustained ventricular tachycardia; PCWP, pulmonary capillary wedge pressure; PG, pressure gradient in left ventricle; RMS, root-mean-square; SAECG, signal-averaged electrocardiogram; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation.

Statistical Analysis

Continuous variables are expressed as the mean±SD, median (interquartile range of 25–75%), or n (%). Comparison among the 3 groups was made using Tukey's method for continuous variables to adjust multiplicity, applying $P<0.05$ as the significance level. Bonferroni's method was used for categorical variables, applying $P<0.016$ among the 3 groups as the significance level. Survival curves were calculated by the Kaplan-Meier method using the log-rank test for group comparison among the extent of graded tissue fibrosis (<10%, 10–20%, and >20%). All variables with a P-value <0.05 in the univariate analysis were considered candidates for inclusion in the multivariate analysis. Cox proportional hazard regression adjustment was performed to calculate the hazard ratio (HR) in the multivariate analysis. All analyses were performed with JMP version 9 software (SAS Institute Inc, Cary, NC, USA).

Results

Baseline Characteristics

The baseline characteristics of the 185 patients are shown in **Table 1**; 76 (41%) patients had a history of hospitalization for HF or arrhythmia, and 26 (14%) had a history of VT/VF, in which nonsustained VT was not included. ICD or cardiac resynchronization therapy with defibrillator (CRT-D) was undertaken in 5 patients at baseline. The baseline LVEF, pul-

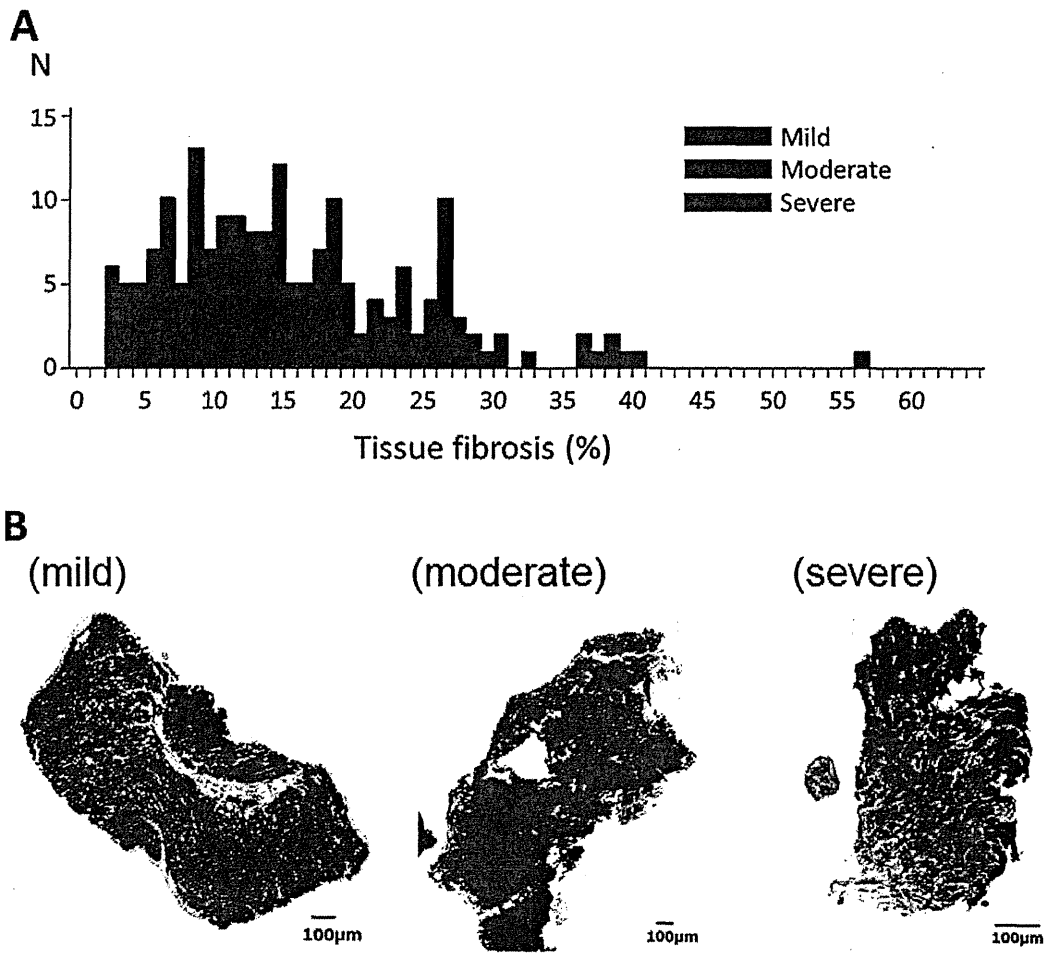

Figure 1. Quantitative fibrotic change in right ventricular (RV) biopsy specimens from hypertrophic cardiomyopathy patients with mild, moderate or severe fibrosis. (**A**) Distribution of fibrosis (%) in all samples. (**B**) Representative RV biopsy specimens stained by Masson's trichrome showing mild (3.9% of area), moderate (18.2%) or severe (31.7%) fibrosis, which represents the fibrosis of the entire area, including endomyocardial thickening and perivascular fibrosis.

monary capillary wedge pressure (PCWP), B-type natriuretic peptide (BNP) concentration, and maximum LV wall thickness were 47±19%, 12±7 mmHg, 256 (IQR: 137–506) pg/ml, and 17±6 mm, respectively, at the time of biopsy.

Clinical and Histopathological Changes

The average tissue area was 2.34±1.38 mm^2. In the tissue sample measurements, the fibrosis ratio (% area) was 15.7±9.8% and the distribution of fibrotic change in all samples is shown in **Figure 1A**. The level of fibrosis was classified as mild (<10%; n=58), moderate (10–20%; n=78), or severe (>20%; n=49). A representative tissue sample of each group is shown in **Figure 1B**.

As shown in **Table 1**, no significant correlation was found among the groups for age, sex, history of hypertension, diabetes mellitus, atrial fibrillation, or other conventional risk factors, including family history of SCD, syncope, maximum wall thickness, and pressure gradient. A history of hospitalization for HF or arrhythmia was more common in patients with severe (n=26, 53%) tissue fibrosis compared with mild (n=15, 26%) tissue fibrosis (P=0.01).

LVEF and plasma BNP were not associated with the degree of fibrosis at the time of diagnosis. On the other hand, PCWP was higher in patients with severe fibrosis compared with mild fibrosis (P=0.03). The mean myocyte diameter (21±5 μm) and degree of myocardial disarray (2.6±1.3) in the total cohort did not differ among the groups.

Conduction Abnormality and Lethal Ventricular Arrhythmias

Figure 2 shows representative tissue samples of mild (6.8%) and moderate (16.6%) fibrotic change in HCM patients. Although LVEF and QRS duration on ECG were comparable in these 2 patients, a longer filtered QRS duration, LAS40, and thus positive LP were detected in the patient with moderate fibrosis. Although not all patients underwent SAECG (n=123), increased fibrosis (%area) was mildly associated with longer LAS40 (r^2=0.07, P<0.01) (**Figure S2**). LAS40 was larger in patients with severe fibrosis compared with mild fibrosis (P<0.05) (**Table 1**).

Next, we compared the degree of tissue fibrosis and the development of lethal ventricular arrhythmias. As shown in **Figure 3A**, the degree of fibrosis at the time of HCM diagno-